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TAIPEI MEDICAL UNIVERSITY
Graduate Institute of Biomedical Materials and Tissue Engineering

中華民國生醫材料及藥物制放學會
Chinese Taipei Society for Biomaterials and Controlled Release

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2023 ISOMRM & BCRS
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Keynote Speech

September 1 (Friday)	
K-1	*Keynote Lecture: Understanding mechano-drivers of ageing and developing methods to slow it down!* **Prof. Justin John Cooper-White, The University of Queensland, Australia**
K-2	*Keynote Lecture: Novel Hydrogels-based Strategies for Regenerative Medicine and Drug Delivery* **Prof. Ki-Dong Park, Ajou University, Korea**
K-3	*Keynote Lecture: Multifunctional bandages as potential strategy for chronic skin wound management* **Prof. Sabine Szunerits, University of Lille, France**
K-4	*Keynote Lecture (Online): Biodegradable polymer scaffolds and their composites for biomedical applications* **Prof. Guo-Ping Chen, National Institute for Materials Science, Japan**
September 2 (Saturday)	
K-5	*Keynote Lecture: Mechano-epigenetic engineering for cell reprogramming* **Prof. Song Li, University of California, United States**
K-6	*Keynote Lecture: Semiconductor Nanotheranostic Systems: A Panacea Tool for Cancer Treatment* **Prof. Hsin-Cheng Chiu, National Tsing Hua University, Taiwan**
K-7	*Keynote Lecture: Biocompatibility Issues for the Tissue Engineered Products for Commercialization* **Prof. Gilson Khang, Chonbuk National University, Korea**
K-8	*Keynote Lecture: Platelet lysates and their Extracellular vesicles in regenerative medicine* **Prof. Thierry Burnouf, Taipei Medical University, Taiwan**

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Understanding mechano-drivers of ageing and developing methods to slow it down!

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Abstract:

By 2050, many countries will have more than 25% of their people over 60 years of age. The impending substantial socio-economic impact, resulting from increases in age-related diseases and reduced participation in society, is one of the biggest risks to future economic growth and productivity, a 'Demographic time bomb'! To develop novel solutions to extending health span as our population ages, it is essential to understand the biology of ageing and the fundamental mechanisms underlying the reduced capacity to maintain functional tissue as we age, and furthermore, to develop viable solutions to ideally modulate these outcomes. Ageing is a complex, multifactorial evolutionarily conserved process, and whilst there are many intrinsic and extrinsic drivers, increased tissue stiffness is associated with a diverse array of age-related pathologies, including cancer, cardiovascular disease, chronic kidney disease, liver/lung fibrosis, and diabetes. Changes in cell and tissue mechanics are accepted hallmarks of specific pathological states, but most recently, it is becoming clearer that tissue stiffening (through fibrosis) may precede disease development, and these altered mechanical cues can drive its progression and persistence. We have recently developed a novel iPSC-based model incorporating an inducible ageing cassette to study the onset and persistence of aged-related loss and gain of function in a range of tissues within the neural, cardiovascular, urinary, and musculoskeletal systems. As an example, we have recently applied this model to provide new insights into the underlying ageing drivers and relative contributions of resident tissue cell types, when aged, on the onset of fibrosis and functional deficits in engineered human aged cardiac tissue. Our recent investigations into aged pericytes have also shown critical changes in their biology as they age, resulting in a loss of functional engagement with endothelium, highlighting potentially a previously ignored key contributor to age-dependent impairment of angiogenesis and novel therapeutic targets. To offer a pathway to therapeutic intervention, we have developed a new gene delivery system that is able to target the cells responsible for the fibrotic onslaught within these ageing tissues and show that in delivering anti-fibrotic mechano-miRs, we can substantially reduce fibrosis *in vitro* and *in vivo* and recover tissue morphology, mechanics, and function.

KEYWORDS: ageing, iPSC-based models, engineered living systems, mechanomedicine, nanoparticles

2023 ISOMRM & BCRS
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Novel Hydrogels-based Strategies for Regenerative Medicine and Drug Delivery

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Abstract:

Reactive oxygen and nitrogen species (RONS) are generated in cellular metabolism and have been indicated as critical modulators for various therapeutic applications, such as the treatment of vascular disorders, wound healing, and cancer treatment. However, the short half-life and "double-edged sword" functions of RONS in living systems are great challenges to its clinical applications. Therefore, the development of efficient carriers to deliver RONS at physiological conditions to the targeted sites is highly desirable. Recently, injectable hydrogels have been widely used as bioactive materials for the controllable and local delivery of therapeutic agents for tissue regeneration, owing to their extracellular matrix mimicking properties, tunable mechanical properties, and minimally invasive surgical procedures. In our lab, we developed injectable gelatin hydrogels that can control RONS (H2O2, NO) release for a wide range of possible applications, including wound healing, vascular disorders, and anti-infection [1-3]. In our approach, phenol moieties were conjugated onto the gelatin backbone to enable crosslinking of them for hydrogel formation via enzymatic crosslinking reaction of horseradish peroxidase (HRP). The physico- chemical properties of hydrogels, including gelation time, mechanical strength, and degradation rate are easily controlled by varying concentrations of HRP and H2O2. Glucose oxidase (GOx) or copper nanoparticles (Cu NPs) were incorporated into hydrogels matrices to produce H2O2 and NO, respectively. The in vitro release studies demonstrated that the release behavior of H2O2 and NO from the hydrogel matrices can be precisely controlled in a wide range of concentrations (from nano- to micromol). The hydrogels with optimal conditions enhanced the cellular activities of endothelial cells (proliferation, migration, and tube formation), facilitated neovascularization, or inhibited bacterial growth. Our results suggest that RONS-releasing gelatin hydrogels can be utilized as advanced materials for tissue regenerative medicine and 3D bioprinting applications.

KEYWORDS: Bioactive hydrogels, ROS and RNS release, Controlled stem cell function, Tissue regeneration, Drug delivery

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Multifunctional bandages as potential strategy for chronic skin wound management

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Abstract:

Wound management can be a complex task and finding appropriate treatments to overcome the different factors contributing to delayed healing can pose a significant challenge. The evaluation and healing time of wounds are usually performed by visual inspection, especially according to the TIMERS (Tissue, Infection/Inflammation, Moisture, wound Edge, Repair/Regeneration, and Social) assessment guidelines. Recently, molecular approaches that allow determining the healing stage of the wound can unveil alternative therapeutic approaches and provide unique support to clinicians. Thanks to the development of biosensors integrated into wound bandages, wound control without qualified personnel might become reality (Figure 1).

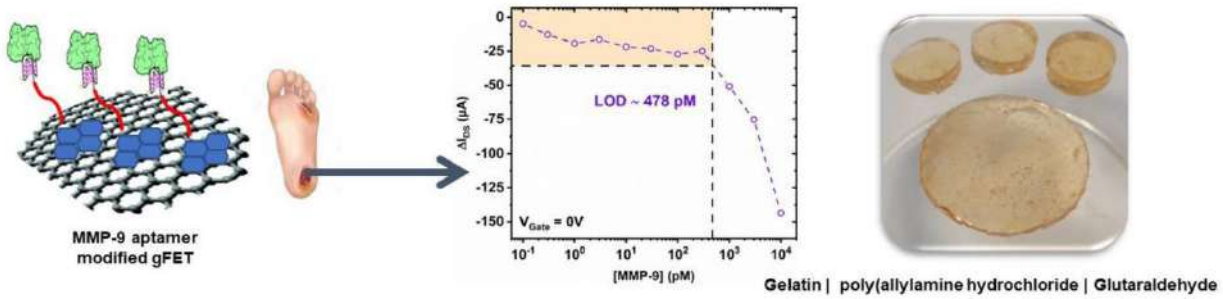

Figure 1: From Sensing to Wound therapy using graphene field effect transistors modified with aptamers and different hydrogels.

In our latest efforts in the direction of wound therapy, we have explored the use of graphene-based field effect transistors (gFET) for sensing zinc-dependent endoproteinases, known as metalloproteinases (MMPs), as presented here. MMPs, such as MMP-9, are upregulated in non- healing wounds and their levels were found to vary between 1.5-912 pM (0.1-60.8 ng mL-1) [1]. Here, we present the successful evaluation of an aptamer based gFET for the selective and sensitive monitoring of matrix MMP-9 in wound fluids from non-healing wounds, benchmarked against an antibody-based ELISA analysis format, and will discuss this development [2].

We also explored the value of a range of hydrogels loaded with platelet-derived extracellular vesicles. This was done along the line of the therapeutic approach of wound healing shown in Figure 1.

KEYWORDS: wound healing, graphene, field effect transistor, hydrogels

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Biodegradable polymer scaffolds and their composites for biomedical applications

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Abstract:

Porous scaffolds of biodegradable polymers can serve as not only templates to control cell functionsfor regeneration of functional tissue and organs, but also carriers to load therapeutic drugs and nanoparticles for therapeutic applications. We designed and prepared a few types of porous scaffolds frombiodegradable polymers and extracellular matrices for biomedical applications. The first type is porous scaffolds prepared by using pre-prepared ice particulates. The method could precisely control the porous structures of scaffolds of natural polymers. Scaffolds with open and interconnected porous structures wereprepared to facilitate cell seeding and migration. The method was also used to create the microppattened pore structures in scaffolds. The micropatterned porous scaffolds were used for muscle tissue engineering.The second type of scaffolds is composite scaffolds of synthetic polymers and natural polymers. Collagensponges or microsponges were incorporated in the pores or openings of mechanically strong PLLA or PLGA porous skeletons to form the composite structures. The PLLA or PLGA skeletons provided high mechanical strength, while the collagen sponges and mircosponges facilitated cell interaction. The third type is biomimetic ECM scaffolds prepared by using cell culture technology. The method was used to prepare ECM scaffolds from different types of cultured cells. The composition of the ECM scaffolds wasdependent on the cell type and phenotype that were used to prepare the scaffolds. The method was also used to prepare autologous ECM scaffolds that were prepared from patients' own cells. The autologous ECM scaffolds had excellent biocompatibility. Furthermore, stepwise tissue-mimicking ECM scaffolds were prepared by controlling the stepwise differentiation of stem cells. Matrices and scaffolds mimicking the stepwise osteogenesis, chondrogenesis and adipogenesis were prepared. These porous scaffolds were used for 3D culture of fibroblasts, myoblasts, chondrocytes and bone marrow-derived MSCs for tissue engineering of dermis, muscle, cartilage and bone. The fourth type is photothermal scaffolds prepared by hybridization of photothermal agents such as gold nanoparticles and black phosphorus nanosheets with biodegradable polymers. The photothermal scaffolds possessed high photothermal conversion capacity andcould ablate breast cancer cells under near infrared laser irradiation. The photothermal scaffolds also facilitated adipogenic differentiation of human mesenchymal stem cells. The photothermal scaffolds should be useful for both photothermal ablation of breast cancer cells and breast tissue engineering.

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Mechano-epigenetic engineering for cell reprogramming

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Abstract:

Our research focuses on cell and tissue engineering, with the goal of utilizing the potential of stem cell differentiation, cell reprogramming, and synthetic biology to improve regenerative medicine and disease therapy. At this seminar, I will discuss our work on mechanoepigenetic engineering for cell reprogramming. While cell reprogramming allows for the transformation of cells into a different lineage, the impact of biophysical factors on this process is not yet fully understood. Our work has revealed that micro-structured materials can influence the shape of cell nuclei and epigenetic state, leading to improved cell reprogramming efficiency. Furthermore, reducing intracellular tension and cell adhesion increases chromatin accessibility and enhances cell reprogramming. We have also discovered that applying active forces, such as a millisecond squeeze on the cell nucleus using microfluidic devices, can overcome epigenetic barriers and facilitate cell reprogramming. These findings have significant implications for the field of mechano-genomics and may lead to the creation of new cell engineering technologies.

KEYWORDS: Epigenetic state, cell reprogramming, biophysical factors

2023 ISOMRM & BCRS
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Semiconductor Nanotheranostic Systems: A Panacea Tool for Cancer Treatment

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Abstract:

Cancer is a major cause of morbidity and mortality worldwide, causing nearly 10 million deaths despite the extensive explorations on the treatment and diagnosis of cancer. The advancements in nanomedicine have broadened our understanding of malignant tumors at the subcellular and molecular levels, developing well-designed nanoplatforms, including semiconductor nanomaterials. The semiconductor heterostructure is usually made up of inorganic materials and possesses distinct physical and chemical properties as well as versatile morphologies and sizes, rendering themselves of particular interests in biomedical applications. The modern understanding of semiconductors depends on quantum physics, which explains the movement of the electrons and holes. When a semiconductor absorbing energy such as light, temperature, ultrasound and radiation, the thermal movement of valence electrons intensifies, leading to formation of free electrons and holes. Electrons can then jump from valence band to conduction band and induce various reactions like reduction, oxidation, photothermal effect and so on. The synergistic interactions between noble metals and a high dielectric semiconductor could produce extraordinary photoelectric properties. Inspired by plasmon-enhanced photocatalysis of noble metal/semiconductor hybrid nanomaterials, our first work has constructed a plasmon- semiconductor nanotheranostic system comprising gold nanostars/graphene quantum dots (AuS/QD) hybrid nanoparticles loaded with BNN6 and surface modified with pyrene-PEG for the photo-triggered hyperthermia effect and NO production as the dual modality treatment against orthotopic triple-negative breast cancer. The hybrid nanodevice has shown enhanced plasmonic energy transfer from localized surface plasmonic resonance of Au nanostars to QD semiconductor that activates the BNN6 species loaded on QD surfaces, leading to the effective NO production and therapy in addition to the photothermal response. The plasmonic resonance property of the Au nanostars in the AuS@QDBNPEG NPs also impart the device the prominent photoacoustic imaging contrast when used in vivo. To further explore the photocatalytic tumor therapy, narrow- bandgap semiconductor bismuth sulfide@carbon nanorods (Bi2S3@C NRs) were also developed as the second part of this speech. The semiconductor was further decorated with palladium (Pd) nanoparticles in situ to form a hetero nanostructure system (Bi2S3@C/Pd HNSs) that promotes catalytic performance under NIR irradiation at 808 nm. In this nanocatalytic system, the Pd nanoparticles help to capture photoexcited electrons from the Bi2S3@C NRs while hydrogen sulfide (H2S/HS-) overexpressed in colon tumor can undergo splitting reaction into hydrogen (H2) with the catalytic response of holes. The latter not only reduces the H2S concentration in tumor, but also greatly improves the charge-separation efficiency. Both significantly promote the hydrogen therapy and the hyperthermia effect. Hydrogen therapy focuses mainly on the mitochondria damage and thus ATP reduction intracellularly. The ATP shortage impairs the expression of heat-shock

protein and thus alleviates cellular photothermal resistance. Moreover, high Z metals, including Bi and Pd, can be used as promising elements for constructing high- performance CT imaging. Inspired by the H2S splitting and antitumor efficiency of Bi2S3@C/Pd HNSs, the last part of this speech involved the development of H2S scavenging Cu2−xSe NPs,
which is an important p-type semiconductor with a relatively high hole concentration and exhibits strong LSPRs in the NIR II window. In general, chemodynamic therapy (CDT) alone is not sufficient to inhibit colon cancer because of the strong reducing microenvironment by high expression of intratumoral H2S. To overcome this limitation, a new strategy has been realized to re-model colon tumor microenvironment by depleting endogenous H2S and synergistically elevating CDT and PTT (photothermal therapy). The Cu2+ ions released from Cu2-xSe NPs oxidize glutathione (GSH) to GSSG. The Cu+ ions released from NPs catalyzes H2O2 to hydroxyl radical for CDT. Cu2-xSe can effectively scavenge endogenous H2S in colon tumors, leading to the enhanced antitumor effects of the photothermal and chemodynamic combination therapy.

KEYWORDS: Metal-semiconductor nanomaterials, photothermal therapy, gas therapy, chemodynamic therapy, theranostics

2023 ISOMRM & BCRS
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Biocompatibility Issues for the Tissue Engineered Products for Commercialization

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Abstract:

Around 1992 as 20 years ago, Advance Tissue Science Co (USA), now merged to Smith & Nephew Co., USA, had been submitted to approve to USA FDA for first cartilage TEMPs as autologous chondrocyte/polyglycolic acid (PGA) nonwoven scaffold. At that time, no one had doubted to approve cartilage TEMPs since PGA was already approved by FDA in human clinical trial and chondrocyte was used autologous primary cell. At last, this product has been still retard up to approve FDA. Main reason might be in terms of safety. Implanted TEMPs have been reported to induce sequential events of immunologic reactions in response to injury caused by implantation procedures and result in acute inflammation marked by a dense infiltration of inflammation-mediating cells at the materials-tissue interface. Prolonged irritations provoked by implanted biomaterials advance acute inflammation into chronic adverse tissue response characterized by the accumulation of dense fibrotic tissue encapsulating the implants.

In this lecture, we will discuss (1) recent advances for the commercialization trends for the tissue engineered products (TEMPS) including regenerative medicinal products, (2) scaffolds in terms of biocompatibility and safety issue, (3) smart scaffold for the application of clinical trial including improved biocompatibility and the reduction of host response, and (4) biocompatibility issue for the natural and synthetic polymers.

2023 ISOMRM & BCRS
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Platelet lysates and their extracellular vesicles in regenerative medicine

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Abstract:

Platelets, the small anucleated blood cells, have emerged as a pragmatic alternative avenue to mesenchymal stromal cells for discovering new therapeutic applications in cell therapy and regenerative medicine. Recognized for their crucial role in hemostasis, allogeneic platelet concentrates (PCs) sourced from healthy donors are utilized by hospitals to manage bleeding in patients with thrombocytopenia or platelet dysfunctions. In addition to their hemostatic function, platelets contribute to innate and adaptive immunity, inflammation, wound healing, and tissue repair. Within platelets, alpha-granules, dense granules, and lysosomes serve as biological compartments harboring a wide range of trophic factors, enzymes, and signaling molecules. Furthermore, platelets release extracellular vesicles (p-EVs), containing a diverse biomolecular cargo into the bloodstream, that facilitates communication between cells.

The unique functional capabilities of platelets and p-EVs have sparked interest in exploring allogeneic PCs as a clinical-grade source for developing innovative biotherapies. These biotherapies hold promise in addressing the needs of cell therapy, regenerative medicine, and targeted drug delivery. Pooled human platelet lysates (HPLs), derived from allogeneic PCs no longer suitable for transfusion, were initially developed to replace fetal bovine serum in growth media used for expanding therapeutic human cells in vitro for transplantation. This breakthrough paves the way for the gradual utilization of virally-inactivated small-pool or large-pool allogeneic HPLs and HPL-derived p-EVs as biotherapies for ocular surface disorders and potentially for neurodegenerative diseases, osteoarthritis, and wound care. Additionally, allogeneic platelets are increasingly regarded as an easily accessible source of cells and EVs that can be harnessed for targeted drug delivery.

This presentation explores the realistic emerging translational applications of allogeneic platelet biotherapies, emphasizing their advantages and limitations in the realm of regenerative medicine and cell therapies.

Invited Talk

September 1 (Friday)	
I-1	*Invited Lecture: Biomaterials-based delivery of signaling molecules and cells for tissue engineering and regenerative medicine* **Prof. Heungsoo Shin, Hanyang University, Korea**
I-2	*Invited Lecture: Core-Shell Microgels: A Versatile Platform for Driving the Chondrogenic Differentiation of Mesenchymal Stem cells* **Prof. Chien-Wen Chang, National Tsing Hua University, Taiwan**
I-3	*Invited Lecture (Online): Physicochemical Design of Cell Microenvironments for Fat Tissue Engineering* **Prof. Michiya Matsusaki, Osaka University, Japan**
I-4	*Invited Lecture: The interplay between hemostasis and immune response in biomaterial development for osteogenesis* **Prof. Yin Xiao, Griffith University, Australia**
I-5	*Invited Lecture: Living Cell Intracellular polymerization for immunotherapy, tissue engineering, synthetic biology, and drug delivery* **Prof. Che-Ming (Jack) Hu, Academia Sinica, Taiwan**
I-6	*Invited Lecture: Solutions for Corneal Blindness Using Functionalized PEG-based Hydrogel Films* **Prof. Gregory Dusting, Centre for Eye Research Australia, Australia**
I-7	*Invited Lecture: Analysis Of Silk-Based Construct For Bone-Tendon Integration in Anterior Cruciate Ligament Reconstruction* **Prof. Cho-Hong (James) Goh, National University of Singapore, Singapore**
I-8	*Invited Lecture: The Development And Evolution Of Biomaterials In Cartilage Tissue Engineering: Malaysian Perspective* **Prof. Tunku Kamarul Zaman, University of Malaya, Malaysia**
I-9	*Invited Lecture: Joint Cartilage Engineering* **Prof. Feza Korkusuz, Hacettepe University, Turkey**
I-10	*Invited Lecture: The Symphony via Photoelectrochemical for Alzheimer's Disease* **Prof. Jung-Chih (George) Chen, National Yang Ming Chiao Tung University, Taiwan**
I-11	*Invited Lecture: Tissue- material interface and biocompatibility evaluation of medical devices/biomaterials* **Prof. Sabareeswaran Arumugam, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), India**
I-12	*Invited Lecture: Understanding the cellular forces that control cell fate and disease progression* **Prof. Yongsung Hwang, Soonchunhyang University, Korea**
I-13	*Invited Lecture: The synthesis of magnetofluorescent carbon dots for bioimaging of mammary carcinoma cells* **Prof. Wen-Tyng Li, Chung Yuan Christian University, Taiwan**

I-14	*Invited Lecture (Online): Photoresponsive drug delivery for the treatment of cancer and eye diseases* **Prof. Wei-Ping Wang, The University of Hong Kong, Hong Kong**
I-15	*Invited Lecture (Online): Next-generation materials for lyophilized RNA-lipid nanoparticles* **Prof. Akon Higuchi, National Central University, Taiwan**
I-16	*Invited Lecture: Organic/Inorganic hybrid nano structure design for NIR-excited photo dynamic therapy* **Prof. Kohei SOGA, Tokyo University of Science, Japan**
I-17	*Invited Lecture: Nanomedicine approaches for precise tumor cell-targeting radiotherapy and metastasis inhibition* **Prof. Tse-Ying Liu, National Yang Ming Chiao Tung University, Taiwan**
I-18	*Invited Lecture: A Self-powered Smart Dressing for Active Infection Prevention and Accelerated Wound Healing* **Prof. Zong-Hong Lin, National Taiwan University, Taiwan**
I-19	*Invited Lecture: Engineered Perfluorocarbon Dual-Layered Drug Nanocarriers Provide Effective Photoimmunotherapy against Colorectal Cancer* **Prof. Yu-Hsiang Lee, National Central University, Taiwan**
I-20	*Invited Lecture: Bio-inspired zwitterionic polymeric chelating assembly for treatment of copper-induced cytotoxicity and triggered-release drug delivery* **Prof. Chun-Jen Huang, National Central University, Taiwan**
I-21	*Invited Lecture: Biological nanoparticle, RBC-derived vesicles (RDVs), for biomedical applications* **Prof. Dong-Ming Huang, National Health Research Institutes, Taiwan**
I-22	*Invited Lecture: Catalase mimicking manganese oxide nanozymes as anti-cancer and anti-inflammation nanotherapeutics* **Prof. In-Kyu Park, Chonnam National University, Korea**
I-23	*Invited Lecture: Rabies Virus Glycoprotein-Mediated T Cell Infiltration to Brain Tumor By Magnetoelectric Gold Yarnballs* **Prof. Shang-Hsiu Hu, National Tsing Hua University, Taiwan**
I-24	*Invited Lecture: New concept of cancer therapy using engineered macrophage (Mactrigger)* **Prof. Yoshiki Katayama, Kyushu University, Japan**
I-25	*Invited Lecture: TAK1 blockade as a therapy for ocular neovascularization* **Prof. Guei-Sheung Liu, Centre for Eye Research Australia, Australia**
I-26	*Invited Lecture: Novel Drug Delivery System Using Nano-Prodrugs* **Prof. Hitoshi Kasai, Tohoku University, Japan**
I-27	*Invited Lecture: Cell-assembling collagen microgel for stem cell therapy in critical limb ischemia* **Prof. Sangheon Kim, Korea Institute of Science and Technology, Korea**
I-28	*Invited Lecture: How hair follicle stem cells interact with the environment* **Prof. Sung-Jan Lin, National Taiwan University, Taiwan**

I-29	*Invited Lecture: Engineering Different Scaffold-Free 3D Culture Systems of Adipose-Derived Stem cells for Tissue Regeneration* **Prof. Nai-Chen Cheng, National Taiwan University Hospital, Taiwan**
I-30	*Invited Lecture: Infrapatellar fat pads-derived stem cells is a favorable cell source for articular cartilage tissue engineering: A study based on 3D organized self-assembled biomimetic scaffold* **Prof. Chen-Chie Wang, Taipei Tzu Chi Hospital, Taiwan**
I-31	*Invited Lecture: Stem Cells in Disease Modelling, Drug Discovery and Therapeutic Development* **Prof. Thamil Selvee Ramasamy, University of Malaya, Malaysia**
September 2 (Saturday)	
I-32	*Invited Lecture: 3D printed PCL/HAp implant in in vivo application of segmental bone defect of femoral shaft* **Prof. Vasif Hasirci, Acibadem University, Turkey**
I-33	*Invited Lecture: Microfluidic Chips for Cell Spheroids Culture* **Prof. Chia-Hsien Hsu, National Tsing Hua University, Taiwan**
I-34	*Invited Lecture: 3D Bioprinted pectin and gelatin skin grafts containing fibroblasts and bioactive agents* **Prof. Nesrin Hasirci, Middle East Technical University, Turkey**
I-35	*Invited Lecture: In vitro Spermatogenesis Platforms* **Prof. Petek Korkusuz, Hacettepe University, Turkey**
I-36	*Invited Lecture: Multiscale design of 3D hydrogel bioink with ROS scavenging and retina tissue regeneration* **Prof. Jiashing Yu, National Taiwan University, Taiwan**
I-37	*Invited Lecture: Biologically inspired scaffolds for neural tissue regeneration* **Prof. Sing Yian Chew, Nanyang Technological University, Singapore**
I-38	*Invited Lecture: Ear mesenchymal stem cells (EMSCs): an good in vitro model of primary cells to study regenerative medicine and molecular biomedicine* **Prof. Dinh-Toi Chu, Vietnam National University, Vietnam**
I-39	*Invited Lecture: Selective and rapid proliferation of stem cells on growth factor-tethered surfaces* **Prof. Koichi Kato, Hiroshima University, Japan**
I-40	*Invited Lecture: Cell chirality in tissue morphogenesis* **Prof. Ting-Hsuan (Cecil) Chen, City University of Hong Kong, Hong Kong**
I-41	*Invited Lecture: Platelet-derived Biomaterials for Xenogenic Application of Cartilage Repair* **Prof. Ming-Fa Hsieh, Chung Yuan Christian University, Taiwan**
I-42	*Invited Lecture: Cartilage tissue engineering and osteoarthritis therapy: mesenchymal stem cells, perivascular stem cells, and platelet-derived extracellular vesicles* **Prof. Pan-Pan Chong, University of Malaya, Malaysia**

I-43	*Invited Lecture: Temperature-responsive polymeric reagents for extracellular vesicle isolation and analysis* **Prof. James Lai, National Taiwan University of Science and Technology, Taiwan**
I-44	*Invited Lecture: Extracellular vesicles from human right atrial appendage stromal cells are cardioprotective* **Prof. David Lundy, Taipei Medical University, Taiwan**
I-45	*Invited Lecture: Structured soft polymers as functional biomaterials* **Prof. Liam Grover, University of Brimingham, United Kingdom**
I-46	*Invited Lecture: Preparation of sub-100-micron calcium-alginate microspheres using nitrogen flow focusing: dependence of spherical shape on gas streams* **Prof. Jin-Jia Hu, National Yang Ming Chiao Tung University, Taiwan**
I-47	*Invited Lecture: A Biomaterial Prospective on Gasotransmitter-Induced Therapeutic Angiogenesis* **Prof. Subramaniam Sadhasivam, Bharathiar University, India**
I-48	*Invited Lecture: Biotribology of Biomaterials: Studies from Total Joint Implant to Orthokeratogy* **Prof. Hsu-Wei Fang, National Taipei University of Technology, Taiwan**
I-49	*Invited Lecture: 3D/4D Printing of Composite/Hybrid Structures for Tissue Engineering* **Prof. Min Wang, The University of Hong Kong, Hong Kong**
I-50	*Invited Lecture: Monodisperse cell-laden microgel droplets for cartilage tissue engineering* **Prof. Hsia-Wei Liu, Fu Jen Catholic University, Taiwan**
I-51	*Invited Lecture: Antioxidant nanoparticles that scavenge the intestinal ROS lead to health* **Prof. Yukio Nagasaki, University of Tsukuba, Japan**
I-52	*Invited Lecture: Modularized microfluidic-based bioreactor for multiplex cell stimulation* **Prof. Chia-Wen Tsao, National Central University, Taiwan**
I-53	*Invited Lecture:EPIGENETIC REGULATIONS OF ADIPOSE-DERIVED STEM CELLS DURING* *SPHEROID FORMATION AND PERIPHERAL NERVE REGENERATION* **Prof. Chia-Ching (Josh) Wu, National Cheng Kung University, Taiwan**
I-54	*Invited Lecture: Bioanalytical Applications of Engineered Intrinsically Disordered Proteins* **Prof. Gabriel Lopez, The University of New Mexico, United States**
I-55	*Invited Lecture: Nanobioanalytical investigations of Extracellular vesicles from secretome of macrophages - Possible implication in the treatment of fibrosis* **Prof. Wilfrid Boireau, FEMTO-ST Institute, France**
I-56	*Invited Lecture: Application of a genetically-engineered human macrophage cell to investigate cellular responses to nano/microplastics* **Prof. Masaya Yamamoto, Tohoku University, Japan**
I-57	*Invited Lecture: Using Functional Photoacoustic Imaging to Understand Pancreatic Tumor Hypoxia Dynamics during Treatment* **Prof. Lun-De Liao, National Health Research Institutes, Taiwan**

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Biomaterials-based delivery of signaling molecules and cells for tissue engineering and regenerative medicine

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Abstract:

For several decades, tissue engineering has garnered significant attention for its potential toreplace or repair damaged or diseased tissues and organs by harnessing the body's own natural repair mechanisms. While cell-based therapies have shown promise, challenges remain in delivering cells to the site of injury, and promoting their survival and integration into host tissue.Biomaterials may offer a viable solution by creating a supportive microenvironment for cell growth, differentiation, and integration. Specifically, biocompatible materials can be tailored to present signaling molecules, such as growth factors, cytokines and small molecules, in a controlled manner to promote tissue regeneration. Various techniques, including physical and chemical immobilization, have been developed for this purpose. In parallel, multi-cellular spheroids, which mimic tissue and organ structure, have been extensively studied as a 3D assembly module for engineering organ-like architectures. However, spheroids present issues including hypoxia caused by intense cell-cell interactions, a lack of precise control over cell functionality, and limited ability to replicate the spatial organization of native 3D tissue structures.To address these challenges, biomaterials can also be utilized to create spheroids with enhanced properties and to support them to recapitulate complex tissue architecture. This presentation details our approaches to using biomaterials for surface-mediated delivery of signaling moleculesand their integration with spheroids for musculoskeletal tissue regeneration.

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Core-Shell Microgels: A Versatile Platform for Driving the Chondrogenic Differentiation of Mesenchymal Stem Cells

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Abstract:

In this study, we propose a novel approach to promote the chondrogenic differentiation of human mesenchymal stem cells (hMSCs) for cartilage repair. Co-culturing hMSCs with allogenic or xenogeneic chondrocytes has shown promise in enhancing chondrogenic differentiation [1,2]; however, challenges remain regarding cell separation and distance between the two cell types. To address these challenges, we developed a core-shell microgel co-culture system using a microfluidic device. The core-shell microgels were designed to spatially separate hMSCs and porcine chondrocytes (pCHs) while maintaining a short distance to facilitate the exchange of growth factors. The formation of stem cell aggregates in the core area of the microgel promoted the differentiation ability of hMSCs. The core-shell structure was easily regulated by adjusting the flow rate ratio of each solution, and the encapsulated hMSCs exhibited high viability within the microgel. To create the miniaturized stem cell-chondrocyte co-culture system, hMSCs and pCHs/polymer solutions were injected into the microfluidic device, followed by soaking the formed microgels in a crosslinking medium for alginate shell gelation. The morphology of the encapsulated stem cells was observed using Actin/Hoechst staining, confirming their dispersion in the core area initially, subsequent formation of a single cell aggregate, and gradual growth into aggregates. To evaluate the efficacy of the core-shell microgels co-culture system, we performed biochemical assays, immunohistology staining, and qPCR analysis. The results demonstrated that co-encapsulation of pCHs significantly enhanced the chondrogenic differentiation of hMSCs. This finding suggests that the core-shell microgels can facilitate the generation of stem cell spheroids and their subsequent differentiation, leading to improved efficacy in chondrogenic differentiation for animal or clinical applications. In conclusion, this miniaturized stem cell- chondrocyte co-culture system holds promise for cartilage repair, offering a potential solution to overcome existing challenges in stem cell-based cartilage regeneration.

KEYWORDS: core-shell microgels, chondrogenesis, stem cells, microfluidics, cell therapy

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Physicochemical Design of Cell Microenvironments for Fat Tissue Engineering

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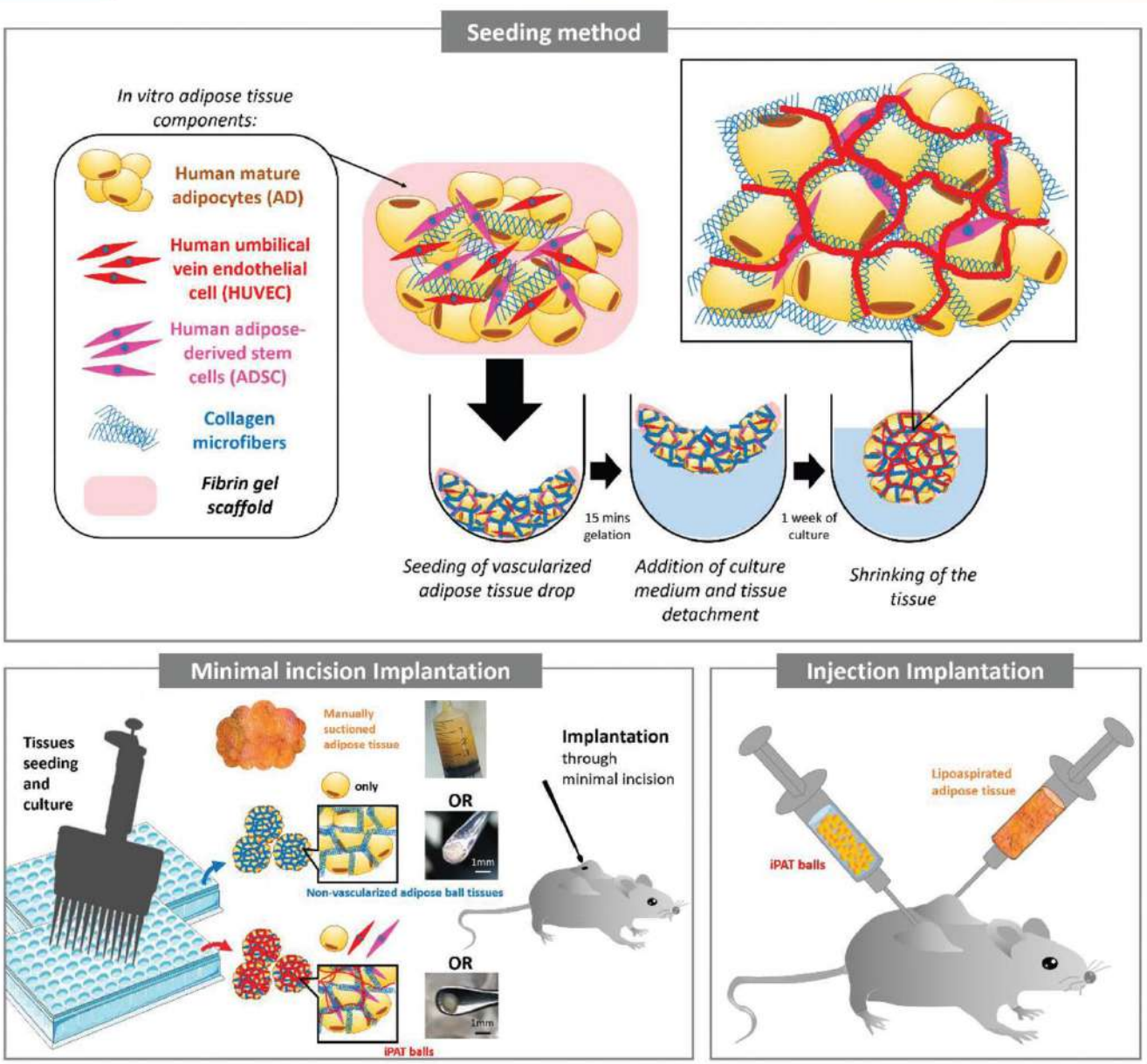
Abstract:

Adipose tissue regeneration for filling soft tissue defects has wide clinical application, affecting patients not only cosmetically, but also their well-being and quality of life, such as after tumor resection, following trauma, or for the treatment of high-grade burns. While there are many possible methods of adipose reconstruction, from synthetic implants to autologous fat transplantation through liposuction or deep inferior epigastric perforator flap methods, only natural components or the use of the patient's own adipose tissue currently lead to acceptable results. One of the reasons is the inefficient blood supply to the fat graft, due to an inadequate supportive vasculature regeneration. Thus, one of the current strategies is to add adipose stem cells before transplantation (stem cell enrichment grafting like cell-assisted-lipotransfer). There are some reports of positive outcomes with respect to the improved graft retention and higher capillary density found in the graft.

To fully reconstruct mature state adipose tissues, we recently developed collagen microfiber (CMF) tissues, which improved the maintenance of mature adipocyte viability and functions [1,2]. Unlike classical collagen gel using dilution, these dispersed collagen microfiber scaffolds allowed the attainment of a final physiological concentration closer to the one found in adipose tissue, while protecting the fragile mature adipocytes from shear stress. The endothelial cells were also found to be able to attach to these collagen microfibers, helping the vasculature construction through integrin-induced adhesion [3]. The innovative purpose of this study was then to construct injectable prevascularized adipose tissues (iPAT) as in vivo-likemature adipose ball-shaped tissues (**Figure 1**) that could be subcutaneously injected in mice and showing improved survival outcomes compared with the implantation of adipose ball tissues without prevascularization or the classic liposuctioned adipose tissue [4]. The key advantage of this method relied on the higher cell engraftment, survival, and tissue volume maintenance, probably due to a stronger host-vasculature connection also involving the lymphatic and neural vasculature networks. Another benefit of this technology was also the ability to store the iPAT balls made from only one liposuction operation, allowing the later noninvasive reinjection of iPAT if needed for final patient's graft volume correction.

KEYWORDS: Adipose tissue, collagen microfiber, vascularization, tissue engineering, iPAT



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The interplay between hemostasis and immune response in biomaterial development for osteogenesis

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Abstract:

Treatment of large bone defects, particularly bone non-union, remains a clinical challenge. The gold-standard bone substitute continues to be an autologous bone graft, which is difficult to be replaced with synthetic biomaterials. Considering these aspects, strategies should be formulated to develop advanced materials for functional bone regeneration. Recent studies have revealed that hematoma (the first tissue structure formed at the bone injury site) plays an essential role in bone healing. Hematoma consists of a fibrin clot, infiltrated immune cells, and tissue progenitor cells. It bridges the bone defect and provides a microenvironment for the interplay between hemostasis and the immune systems. Moreover, an ideal fibrin structure with appropriate fiber thickness and density could facilitate bone regeneration, and biomaterial implantation could affect fibrin structure. Meanwhile, immunoregulation plays an essential role in bone healing. In particular, materials inducing a shift from inflammatory to anti-inflammatory phenotypes in immune cells show enhanced osteoinductivity. More importantly, the interaction between hemostasis and the immune system should play a vital part in bone regeneration by determining both fibrin structure and bone healing microenvironment. Coagulants-triggered inflammation could, in turn, facilitate coagulation cascades, which form positive feedback to amplify both processes. Meanwhile, anti-coagulants neutralize coagulation and inhibit inflammation and thereby control the coagulation and inflammation to prevent thrombosis. The balance between coagulation–inflammation and anti-coagulation–anti-inflammation plays a determinant role in the fibrin structure and fibrinolysis process. The inflammation could be “quenched” gradually during this process, whereby a highly effective microenvironment for bone regeneration can be generated. Presently, there are limited biomaterial studies targeting the bone-healing hematoma, particularly the hemostasis–immune interplay. Considering this, this review summarizes the current materials for hemostasis and immunomodulation, and the critical role of the hemostasis–immune interaction in bone regeneration. It also proposes potential strategies to develop materials with the capacity to generate a highly effective bone healing hematoma, by modulating the hemostasis–immune interplay to maintain the balance between coagulation–inflammation and anti-coagulation–anti-inflammation.

KEYWORDS: Bone regeneration, Bone substitutive biomaterials; Coagulation; Fibrinolysis, Osteoimmunomodulation

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Living Cell Intracellular polymerization for immunotherapy, tissue engineering, synthetic biology, and drug delivery

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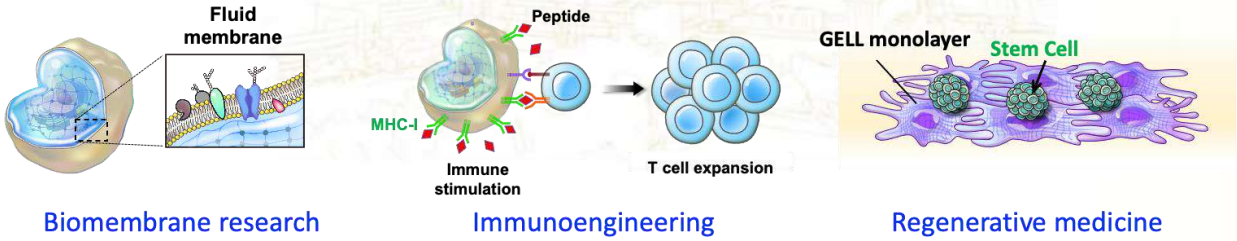
Abstract:

We developed a rapid photochemistry that enables intracellular hydrogel polymerization of living cells for the generation of a new class of cell-gel hybrid material known as polymerized cells. The polymerized cells retain the integrity of its plasma membrane bilayer and mobile surface proteins for biological engagement[1], and their polymerized interior confers extraordinary stability and long-term storability. We demonstrate the applicability of the polymerization chemistry to a large number of cells types, including fragile and fastidious primary cells derived from murine and human hosts. The presentation covers recent utility of the polymerized cellular system in biomedical applications, including immune cell therapy[2], regenerative medicine[3], synthetic biology[4], and drug delivery. The robust, cell-like biomaterial offers new engineering opportunities for constructing biomimetic devices.

KEYWORDS: intracellular polymerization, radical polymerization, biomimicry, immunotherapy, tissue engineering, biomembrane.



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Solutions for Corneal Blindness Using Functionalised PEG-based Hydrogel Films

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Abstract:

Eye disease leading to corneal damage is a major cause of loss of vision throughout the world. There is also a growing shortage of donor corneas available for transplantation, especially in Asia and the developing world. Our research focusses on ocular tissue engineering and cell therapy in which chemical engineers, cell biologists, and ophthalmologists collaborate in an integrated manner to cure blinding eye disease [1]. Here we describe a biodegradable PEG-based hydrogel used as a device to facilitate the difficult but preferred surgical procedure known as Descemet's Membrane Endothelial Keratoplasty (DMEK): this is implantation of a monolayer, cellular graft to repair the corneal endothelium. Secondly, we address the use of a tissue- engineered corneal endothelium to replace donor tissue used for corneal endothelial keratoplasties.

Standard transplantation of cadaveric donor corneal tissue accounts for a third of the total corneal transplantation performed in the world. However, the monolayer cellular graft used for DMEK is extremely delicate and difficult to handle even by highly skilled ophthalmologists, resulting in low adoption of DMEK with most surgeons opting for other surgical approaches that suffer inferior visual outcomes and higher rejection rates. To address this, we invented a hydrogel scaffold (HYGELIX) which is an ultra-thin polyethylene glycol (PEG)-based, biodegradable hydrogel support that binds to donated endothelial tissue. HYGELIX supports and protects the tissue, minimises tissue scrolling and makes the positioning of the tissue, during surgery, much easier. We transplanted HYGELIX constructs in sheep. Corneal opacity was induced by denuding the endothelium in the left eye of anaesthetized sheep (with the right eye as control), followed by insertion of the constructs into the anterior chamber.

Previously, using our hydrogel as scaffold, we demonstrated safety and efficacy of a tissue-engineered endothelial keratoplasty in sheep. However, human corneal endothelial cells (hCEC) are more difficult to culture. Recently we have functionalised our PEG-based hydrogel film with either carboxylic acid or a peptide with the arginine-glycine-asparigine-(RGD) signalling sequence to improve hCEC culture outcomes.

B4G12, (an hCEC line) was cultured on our hydrogel (non-functionalised control), RGD- and carboxylic acid functionalised hydrogel, as well as on standard tissue culture plastic (positive control). After culture for 14 days, cells were fixed in 4% PFA and immunofluorescence for CEC marker zonula occuludens-1 (ZO-1) was performed. Morphometrics including confluence, mean individual cell area, and cell circularity was performed on immunofluorescent images using ImageJ. Cell density (/mm2), familiar to eyebankers, was calculated from cell area and confluence. On all surfaces B4G12 cells expressed ZO-1 and maintained polygonal morphology (high circularity). Mean results (confluence, area, calculated cell/mm2, circularity): non- functionalised hydrogel (50 %, 317 µm2, 1577 cell/mm2, 0.74), RGD functionalised hydrogel (93%, 249 µm2, 3735 cell/mm2, 0.71), carboxylic

acid functionalised hydrogel (86 %, 263 µm2, 3270 cell/mm2, 0.73), tissue culture plastic (100 %, 291 µm2, 3436 cell/mm2, 0.69). A donor corneal endothelium had a circularity of 0.76. Eyebanks typically require donor tissues to have greater than 2500 cell/mm2.

Thus we demonstrated that HYGELIX is a safe and effective technology to deliver DMEK grafts to the anterior chamber - without the difficulty of graft scrolling. Improvement of corneal endothelial cell culture on our PEG-based hydrogel, functionalised with either carboxylic acid or peptide, bring us closer to a tissue-engineered endothelial keratoplasty. Given the global shortage of corneas for transplantation, if tissue-engineered endothelial keratoplasty were adopted, then more donated corneas would be available to treat other indications for keratoplasty, restoring vision to millions.

KEYWORDS: blindness, corneal endothelium, hydrogel, keratoplasty, ophthalmologists, tissue-engineered corneal endothelium

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Analysis Of Silk-Based Construct For Bone-Tendon Integration in Anterior Cruciate Ligament Reconstruction

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Abstract:

The fibrocartilaginous type enthesis interfacial tissue provides the strength to secure ligament to bone. In surgical reconstruction of ACL with tendon graft, optimal healing of the tendon graft within the bone tunnel is not adequately achieved. Therefore, the proposed solution involves the use of a silk fibroin (SF)-based sheath, loaded with nanoparticles of low crystallinity hydroxyapatite (nHA), and sleeved onto tendon autografts to complement their use and promote enthesis formation. However, biocompatibility data has shown that the SF/nHA scaffold has a moderate irritant to the surrounding bone tissues in a 12-week implantation study in rabbits. It is unclear whether the immune response was pro-inflammatory or anti-inflammatory. Since the scaffold is made of degradable materials, the local irritancy effects caused by the degradation process cannot be ruled out. It was hypothesized that during the degradation process, the SF/nHA scaffold degradation products can contribute to the polarization of macrophage phenotypes from proinflammatory M1 to anti-inflammatory M2 to facilitate tissue repair and regeneration. In order to evaluate the inflammatory response, SF/nHA and SF scaffolds were fabricated and subsequently subjected to protease XIV degradation in-vitro for 24 days. Thereafter, the immune response towards the degradation products of the scaffold was characterized by culturing THP-1 derived macrophages with the degradation products. It was found that as the SF/nHA and SF scaffolds were degraded in protease, the initial degradation products had elicited a proinflammatory response while the latter degradation products had elicited an anti-inflammatory response, suggesting that the degradation products could contribute to the polarization of macrophages from pro-inflammatory M1 to anti-inflammatory M2 phenotype. Additionally, it was found that the macrophages expressed higher anti-inflammatory cytokines (IL-10, TGF-β1) when cultured with the degradation products from SF. This could be due to the higher glycine, alanine, and serine amino acids concentration in the degradation solution which could exert a greater anti-inflammatory effect on the macrophages.

Consequently, a porcine ACL reconstruction model was used to evaluate safety and efficacy of the silk fibroin (SF)-based sheath. The sheath was sutured onto both ends of the tendon graft and pulled through both femoral and tibial bone tunnels. All animal experiments were approved by the respective IACUC. The enhanced integration of tendon autograft to bone was evident within the femoral and tibial ends of the graft from as early as 1-month post reconstruction. Continuous host integration and bone remodeling were observed through the 9 months period, with significant bone tunnel narrowing in the test groups observed by the end of the study. Mechanical study showed that the sheaths enhanced graft tensile strengths. The SF-based sheath serves as a delivery platform for cellular and bioactive components. Progenitor cells attracted from the host into the porous sheath could have reconstituted the native cellular]

environment of the enthesis by differentiating into chondrocytes and osteoblasts. Consequently, there was enhanced graft-to-host integration progressively over the 9 months implantation period, which resulted in overall mechanical properties closer to that of the native bone-ACL-bone construct.

KEYWORDS: Silk fibroin and nanohydroxyapatite construct, Anterior Cruciate Ligament Reconstruction, inflammatory response, tendon graft to bone integration.

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The Development And Evolution Of Biomaterials In Cartilage Tissue Engineering: Malaysian Perspective

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Abstract:

Injuries to the cartilage remain a challenging problem to treat due to its intrinsic inability to undergo spontaneous repair or regeneration. Previous attempts to repair damaged cartilage using conventional surgeries have resulted in limited repair outcomes, especially in the long term, prompting the need for a more robust method of managing this difficult to treat condition. One such strategy involves the use of biological repair approach, such as tissue engineering. The method involves the custom design of tissue-like material *in vitro*, and later to be implanted via a surgical procedure, that has the potential to replace the loss or damaged cartilage. Classically, tissue engineering involves the use of 3 main elements: cell source, growth factors and biomaterials. In each of these, the effectiveness and appropriateness need to be met while demonstrating high levels of compatibility and usability for its intended function. Whilst cell sources and growth factors have inherent issues that needs to be dealt with, these are not as intense nor challenging as compared to be task of designing the right biomaterial. In addition to the need of being biocompatible, reliable, safe and structurally resilient; biomaterials for cartilage replacement need to have special properties that is inherent to the tissue itself, such as a narrow Young's modulus that mimics the natural tissue. Furthermore, there appear to be the need for biomaterials to perform beyond its natural state to achieve a successful surgical repair process, such as being flexible-viscoelastic, and yet still undergo a controlled biodegradation phasic process that would ultimately appear as a natural creeping substitution like change. These and many other issues have prompted several developments and processes to happen over several decades, with each development contributing toward improving the previous biomaterial design thereby bringing us one step closer to meeting the cartilage tissue engineering agenda.

In the present lecture, the efforts conducted in Malaysia over the past 2 decades to meet this challenge shall be presented and discussed. These will cover issues such as the sources of biomaterials, either as natural or artificial, to design specifications and feats such as the exploration of different compounds, coating and imprinting techniques, as well as manufacturing technologies such as biomimetic, functional, nanotechnology, finite element modeling, additive manufacturing, 3D printing, and drug delivery eluting materials. Also worth discussing is the need to meet regulatory requirements, of which Malaysia holds a very tight and firm regulation incorporating both the needs of US FDA and Europe's EMA. In an area where transdisciplinary research is required, the topic on the development and evolution of biomaterials for cartilage repair or replacement would serve as one of the most interesting topics in medical sciences, where engineering concepts will cross borders with the needs of biological and medical experts in order to solve a long standing untreatable disease.

KEYWORDS: Cartilage, Tissue Engineering, Biomaterial, Cartilage Regeneration, Cartilage Repair.

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Joint Cartilage Engineering

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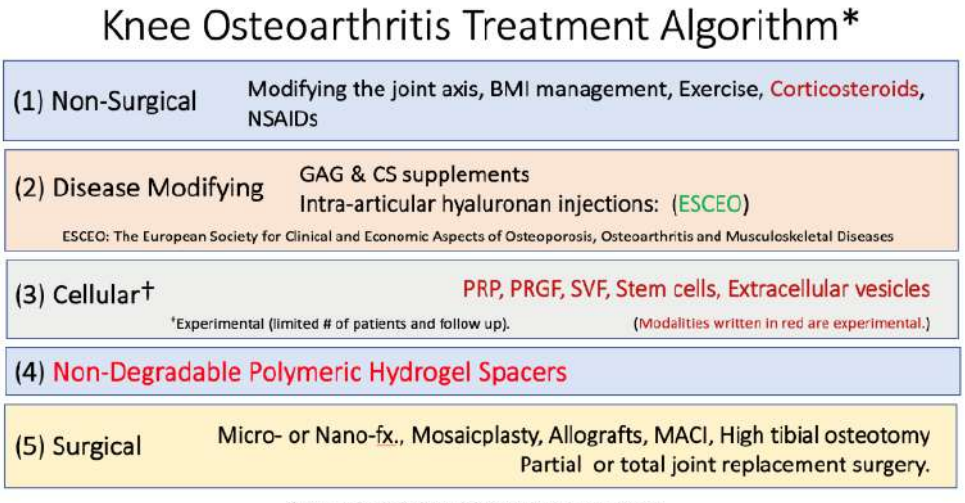
Abstract:

Joint cartilage engineering is an emerging and demanding research field as cartilage of joints have limited regenerative capacity. Non-surgical treatment approaches, such as body mass management, exercise and non-steroidal anti-inflammatory medicines, often yield suboptimal outcomes, leading to disability and decreased quality of life for affected individuals [1].

Degeneration of the joint cartilage may occur secondary to trauma in a young age or by mechano-biological factors with ageing. Joint cartilage engineering focuses on replacing and regenerating the original tissue by cells, biomaterials and signaling molecules when disease modifying supplements and hyaluronan injections fail. Insight on injectable modalities for osteoarthritis treatment will be delivered [2]. Key aspects of cellular replacements will be discussed to highlight the complexity of cartilage engineering [3]. Strategies for biomaterials will be explored. Scaffold-based methods involve the use of biomaterials as a framework to support cell attachment, proliferation and differentiation. A recent approach for decreasing synovial inflammation in knee joint are the non-degradable polymeric hydrogel spacers (Figure 1). Scaffold-free techniques on the other hand rely on cell self-assembly to form tissue-like structures. Emerging technologies, such as 3D bioprinting offer promising perspectives for cartilage engineering. Cell sources for cartilage regeneration will be examined, including chondrocytes, mesenchymal stem cells and induced pluripotent stem cells. The role of growth factors, signaling molecules, and biomechanical stimulation techniques in promoting tissue maturation and functionality will be discussed.

Translation of joint cartilage engineering approaches from bench to bedside is explored. Challenges related to regulatory approval, scalability and long-term efficacy are examined.

KEYWORDS: Joint cartilage, engineering, regenerative medicine, biomaterials, stem cells



*Modified from the OARSI Guidelines.

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

The Symphony via Photoelectrochemical for Alzheimer's Disease

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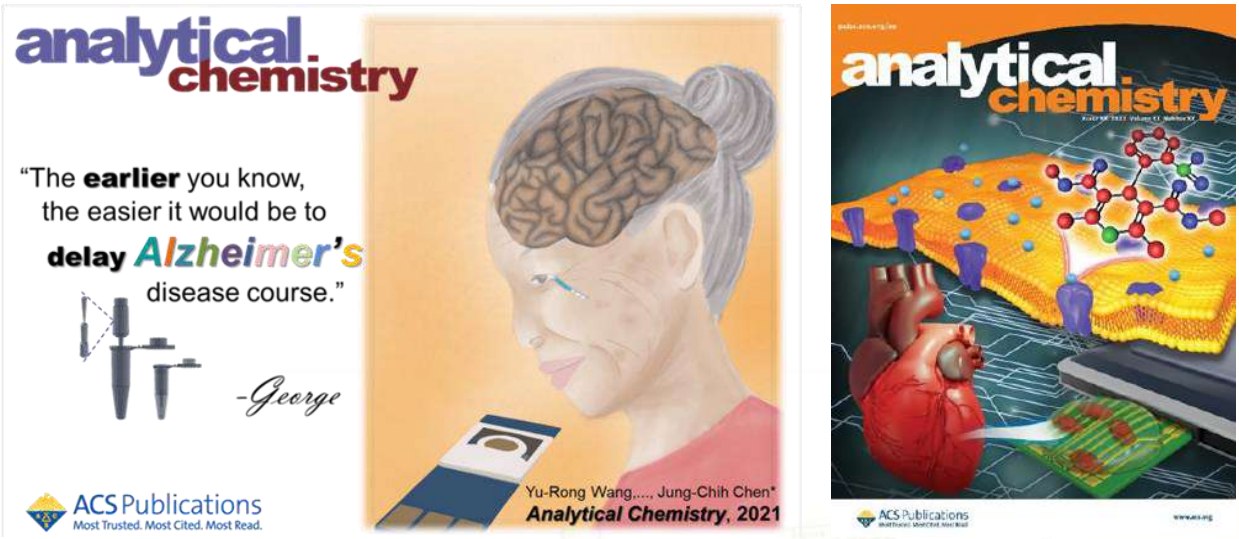
Abstract:

Three major medical issues that humans should take very seriously in the future are nothing else but malignant tumors, the aging population, and impaired fertility. On the issue of aging population, elderly dementia has become a global problem and is costly to society (about 52 trillion NTD per year). Although many countries in the world now have dedicated to related research, it is usually too late to have effective treatment after the confirmed diagnosis. Consequently, early diagnosis should not be regarded as an effective way to control dementia, while proper treatment and good diagnostic techniques are required to go side by side to be an effective strategy. Among them, Alzheimer's disease (AD) is the most common type of dementia, accounting for 60-70% of all dementia cases. Alzheimer's disease dementia is caused by the degradation and atrophy of brain cells, which causes memory and other brain functions to decline. The amyloid-like hypothesis is the most accepted pathogenic mechanism of AD currently. The study pointed out two major related pathological features. One is the amyloid precursor protein (APP) and the other is the Tau protein. In this proposal, we will focus on the study of one of the most common dementia symptoms, AD. Advanced scientific methods will be used to highlight the importance of simple and rapid screening. However, at the same time, it is a violation of clinical rules if the advanced technology can only delay mental deterioration. Therefore, our integrated project also raises prospects of several new AD treatments and we believe the impact of combing with advanced screening technology to AD will be invaluable.

As an example, to see how the ancient bioelectrochemical plus photo technology assists in the detection of β-amyloid without taking CSF as the sample. Use the idea of electrochemical dephosphorylation to achieve phosphorylated Tau plaque removal, and then use AI technology to analyze PET images (AV45, FDG, and C-PiB), that can be detected and treated early under the cooperation of medicine and engineering. Therefore, our team will use peripheral body fluids for early screening and rapid detection of Alzheimer's disease. In addition, AI analyzes the correlation between medical images and peripheral body fluids as early AD diagnosis. Both the medical and academic communities understand the need for early screening and rapid detection of AD, not only to delay deterioration but

also to contribute to early treatment. Through the appropriate bioreactors that have been designed and developed by our laboratory, and the completion of electrochemical and/or computer science cross-discipline integrated real-time detection platforms such as Biophotonics-Optogenetics and Bionanomaterials-UCNP technology developed through this research, a new analysis with bionanomaterials technology is bound to be created

KEYWORDS: AD Rapid Screening and Electrochemical Therapy, Biomedical AI, Biophotonics - Optogenetics, Bionanomaterials-UCNP



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Tissue-material interface and biocompatibility evaluation of medical devices/biomaterials

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Abstract:

Medical implants perform, augment, or replace a natural function in the human body. They vary greatly in complexity and application, ranging from simple devices to complex devices. They account for a multi-billion-dollar global industry with a lot of opportunities for continuous growth due to the development of the healthcare sector. Preclinical safety evaluation of medical devices offers the opportunity to investigate and study the intended use of devices and materials. Preclinical evaluation programmes are designed to determine the safety, efficacy, and biocompatibility of biomaterials and medical devices. Material scientists are currently paying more attention to the process of making smart and biocompatible materials. Biologists are trying to understand the cellular and molecular events taking place in the tissues adjacent to the material in vitro and in vivo. Vascular stents, mechanical heart valves, vascular grafts, pacemakers, orthopaedic nails and joints, intraocular lenses, shunts, and other implants play an important role in biomedical technology. However, implant biocompatibility is determined by both material and tissue parameters; their use has exploded and has affected virtually every surgical specialty; and their research and development has become a major topic in science and technology. Recent developments in cell biology and material processing have increased the likelihood of tissue-engineered materials being the new and unstoppable wave in implant manufacturing in the next decades. The tissue reaction to implanted materials includes inflammation, hyperplasia, metaplasia, and, on rare occasions, neoplasia. The reaction would be determined by the implant material's physicochemical properties as well as host parameters such as soft tissue, hard tissue, and the location of implantation. When an implant is firmly linked to the host and stays functional, such as a prosthetic heart valve or vascular graft, the link at the tissue-material interface is nothing more than a smooth mechanical linkage by fibrous connective tissue and not a molecular union. Thus, the biological evaluation performed as part of the device's risk assessment assists us in defining the device's toxicity and compatibility. Any medical device's success is determined on the tissue-material interface in all tissues.

KEYWORDS: Biocompatibility, osseointegration, injury, inflammation, wound healing.

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Understanding the cellular forces that control cell fate and disease progression

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Abstract:

Although the biomechanical properties of native extracellular matrix (ECM) and its contribution to the cell-cell and cell-matrix interactions play a critical role in maintaining tissue homeostasis and disease progression, an efficient method to understand these biological events is still a daunting task. Thus, in this talk, I will describe the dynamic role of cell-cell/cell-matrix interactions of various cells, which cultured on biomaterials with various matrix stiffnesses, in regulating lineage/tissue-specific differentiation of stem cells and disease progression by employing traction force microscopy, intracellular microscopy, and monolayer stress microscopy to understand the cell-generating forces and their subsequent contribution to the disease progression. Such a cell culture platform can offer novel strategies to understand the pathophysiology of various diseases.

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The synthesis of magnetofluorescent carbon dots for bioimaging of mammary carcinoma cells

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Abstract:

Carbon dots are nanoscale particles with diameters smaller than 10 nm, which have properties such as photoluminescence, photostability, ease of surface modification, and low toxicity. Metastatic cancers express high levels of hyaluronic acid (HA), CD44 receptor, and HA synthase in the tumor cell microenvironment. Folic acid (FA) is one of the cancer cell-targeted biomolecules with highly overexpressed receptors on the surface of tumor cells. Both HA-doped carbon dots (HACDs) and FA-doped carbon dots (FACDs) were synthesized via the microwave- assisted method previously. As prepared HACDs and FACDs exhibited red fluorescence, good photostability, and low cytotoxicity. However, the optical imaging for deep tissue visualization is limited by the low depth of light penetration. Here, HA-doped magnetofluorescent carbon dots (HMCDs) and FA-doped magnetofluorescent carbon dots (FMCDs) were prepared by mixing HACDs or FACDs with the solution containing the precursors for iron oxide nanoparticles via microwave synthesis. Both HMCDs and FMCDs emitted red fluorescence under green light excitation. The particle size of HMCD was 10.6 nm, while that of FMCD was 17.1 nm, as measured by transmission electron microscopy. X-ray diffraction and Fourier transform infrared spectrum analysis showed that the HMCDs had the crystal plane of iron oxide and characteristic absorption spectra of iron oxide and HA. The FMCDs also had characteristic peaks of iron oxide and FA. According to the results of dynamic light scattering and agarose gel electrophoresis, both HMCDs and FMCDs carried negative surface charges. Cell viabilities of 4T1 mouse mammary carcinoma cells remained approximately 100% after 24 h of incubation with HMCD at a concentration of 200 μg/mL. While FMCD at a concentration of 100 μg/mL exhibited cell viabilities greater than 70% for 4T1 cells. Prussian blue staining and the competitive binding assay of ligands found that FMCD and HMCD could be effectively taken up by receptor-mediated endocytosis by 4T1 cells, respectively. The results demonstrate the successful synthesis of FMCD and HMCD, which possess fluorescence and magnetism, and targeting capabilities to specific receptors, indicating their potential application of cell-specific targeting probes for imaging of metastatic mammary carcinoma.

KEYWORDS: Magnetofluorescent carbon dot, mammary carcinoma cell, bioimaging, hyaluronic acid, folic acid

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Photoresponsive drug delivery for the treatment of cancer and eye diseases

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Abstract:

The photoresponsive drug delivery strategy offers the potential to enhance drug accumulation at targeted sites where light is applied, thereby increasing therapeutic efficacy while minimizing side effects. Light serves as a versatile and easily adjustable external stimulus, offering spatiotemporal control over the process. However, the strategy currently faces challenges in clinical applications, such as limited light penetration depth in biological tissues. In this talk, I will discuss the two primary mechanisms of photoresponsive drug delivery systems: phototriggered targeting (phototargeting) of drug nanocarriers and phototriggered drug release (photorelease) from drug nanocarriers. I will also present strategies for increasing excitation wavelength and the applications of these systems in treating cancer and eye diseases.

KEYWORDS: Photoresponsive drug delivery, ocular drug delivery, retinoblastoma, age-related macular degeneration, nanomedicine

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Next-generation materials for lyophilized RNA-lipid nanoparticles

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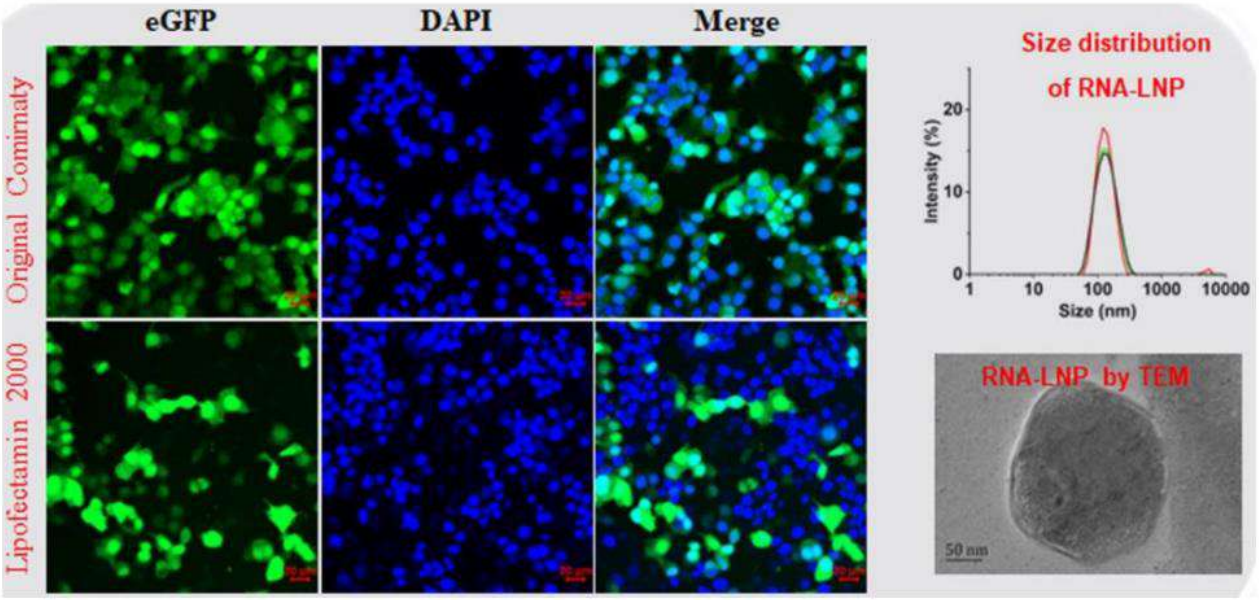
Abstract:

RNA, including mRNA, siRNA and miRNA, is part of a new class of patient treatments that prevent and treat several diseases. As an alternative to DNA therapy using plasmid DNA, RNA functions in the cellular cytosol, avoiding the potential risks of insertion into patient genomes. RNA drugs, including mRNA vaccines, need carrier materials for delivery into the patient's body. Several delivery carriers of mRNA, such as cationic polymers, lipoplexes, lipid-polymer nanoparticles and lipid nanoparticles (LNPs), have been investigated. For clinical applications, one of the most commonly selected types of RNA delivery carrier is LNPs, which are typically formed with (a) ionizable lipids, which bind to RNA; (b) cholesterol for stabilization; (c) phospholipids to form the LNPs; and (d) polyethylene glycol-conjugated lipids to prevent aggregation and provide stealth characteristics. Most RNA-LNP research has been devoted to achieving highly efficient RNA expression in vitro and in vivo. It is also necessary to study the extended storage of RNA-LNPs under mild conditions. One of the most efficient methods to store RNA-LNPs for a long time is to prepare freeze-dried (lyophilized) RNA-LNPs. Future research should include investigating LNP materials for the development of freeze-dried RNA-LNPs using optimal lipid components and compositions with optimal cryoprotectants. Furthermore, the development of sophisticated RNA-LNP materials for targeted transfection into specific tissues, organs or cells will be a future direction in the development RNA therapeutics. We developed RNA-LNP using lipids, which were used in the BNT162b2 (Comirnaty) vaccine produced by BioNtech/Pfizer. The BNT162b2 (Comirnaty) is formed with ALC-0159 (PEGylated lipid), cholesterol, DSPC, and ALC-0315 (((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2- hexyldecanoate)) with a weight ratio of 1.6:42.7:9.4:46.3. We optimized composition of each lipid together with optimal amount of cryoprotectant (sucrose) and developed lyophilized RNA-LNPs, which will be targeted for the treatment of patients having ocular diseases.

KEYWORDS: Lipid nanoparticles, mRNA, lyophilization, cryoprotectant, gene therapy, transfection



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Organic/Inorganic hybrid nano structure design for NIR-excited photo dynamic therapy

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Abstract:

Near infrared (NIR) light is known to have minimal loss as the electromagnetic wave with wavelength around visible light [1]. The authors have worked on the NIR biophotonics since 2005. In bio and medical scenes, the NIR fluorescence with nano probe has been utilized for not only a simple "imaging" to give contrast to show the existence of the probe, but photo dynamic therapy (PDT) [2, 3] and temperature sensing [4, 5].

Roughly speaking, the size of the probes matters for controlling the distribution of the probe in a body. As for the normal cellular response, a size less than 100 nm is required for efficient uptake of the probes by endocytosis, while phagosomes such as macrophage less response to a matter with a size less than 100 nm. As a result, once injected to blood vessel, the probes will be quickly trapped to liver or spleen due to the phagocytosis of the macrophage in them. Those with a size a size less than 100 nm can get a chance to circulate in blood circulation. If the size is less than 10 nm, the probes meet renal excretion through kidney.

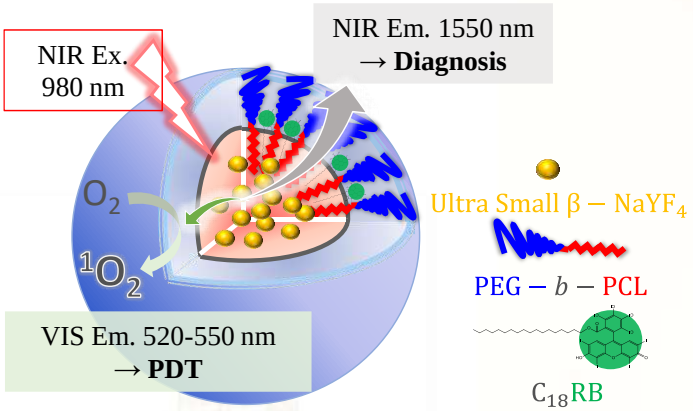
Even if one prepares a probe with a proper size, it cannot keep the size without surface modification. Hydrophilic nano matters in pure water can be dispersed by the same surface charges of the matters. However, under an ionic condition such as physiological saline, the surface charge is cancelled by the surrounding ions and they cannot disperse anymore for minimizing the surface energy. In blood, more serious enemy of the dispersion is proteins. Various kinds of proteins normally have positively and negatively charged parts, as well as hydrophobic parts. Nano matters are easily capture by proteins to form a large agglomerate with a size more than 200 nm. For keeping the size in biological conditions, the only effective way is the introduction of hydrophilic and neutrally charged polymer on the surface of them expecting its steric repulsion [Springer].

As fluorescent agent, organic dyes, quantum dots, carbon nanotubes and rare-earth-doped ceramic nanoparticles (RED-CNPs) are known to efficiently emit light under a NIR excitation [Springer]. Once excitation energy comes in, the energy will be finally converted into heat or light. For having more light, suppression of heat generation is important. In case of the visible fluorescence, it is easier than the case of the NIR fluorescence because energy level separation is wide enough (>12,500 cm^{-1}). Normal molecular vibration frequency can be roughly converted to 1,000～4,000 cm^{-1}. The probability to generate heat depends on the number of thermal quanta to be emitted. The less the number is, the more probable to generate. The smaller separation of the energy levels for emitting light makes more difficult to suppress the heat generation.

As listed above, we may have to deal with inorganic and organic matters together in the nanostructure for a probe. The authors have formulated the thermal generation in organic/inorganic

hybrid nano structures [6, 7]. RED-CNPs are known to emit both visible and NIR photo emission by NIR excitation. They are quenched when the particle size is less than several tens nm in aqueous solution. On the other hand, for renal excretion, as discussed above, a size less than 10 nm is required to RED-CNPs. By applying the strategy for the organic/inorganic hybrid nano structures, we decided to use a micelle structure with hydrophobic polymer core and hydrophilic shell. The core polymer was carefully selected not to cause the quenching of the ultrasmall RED-CNPs with a size of 8 nm. Poly(caprolactone) (PCL) was selected as the core polymer. As shell polymer, one of the most popular biocompatible polymer, poly(ethylene glycol) (PEG) was chosen. The RED-CNPs were modified with oleic acid to be well-dispersed in hydrophobic solutions. The block copolymer, PEG-*block*-PCL properly formed micelle structure by self-assembly process to contain ultra small RED-CNPs to be fluorescent both for the visible fluorescence by an upconversion process, and the NIR fluorescence through normal luminescence process.

As photosensitive dye, a hydrophobized rose bengal (RB) derivative, C_{18}RB, was set on the border of the hydrophobic and hydrophilic structures. RB itself is known to be toxic as it is. However, in the structure, RB is hidden in the PEG brush so that it is not exposed to the cells. Also, the core material, PCL, is known to be biodegradable. After the decomposition of the PCL, the RD-CNPs are expected to have renally excretable size. The nanostructure properly generated singlet oxygen for killing cancer cells without having obvious toxicity when the excitation light is not irradiated.



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Nanomedicine approaches for precise tumor cell-targeting radiotherapy and metastasis inhibition

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Abstract:

Despite being a widely used cancer treatment, radiotherapy often results in severe side effects. While techniques like image-guided radiotherapy and intensity modulation radiotherapy have shown promise in minimizing radiation-induced damage to healthy tissues, achieving precise tumor cell-targeted radiotherapy remains a significant challenge. Boron neutron capture therapy (BNCT) has demonstrated superior tumor cell-targeting capabilities, but its implementation is limited due to the need for specialized facilities such as atomic reactors or accelerators. Consequently, there is a pressing need to develop a tumor cell-targeting radiotherapy approach that can be readily implemented using available clinical resources.

In our research, we have focused on developing strategies to achieve tumor cell-targeting radiotherapy, with the aim of further reducing side effects associated with linear accelerator-based radiotherapy. Our approach encompasses two distinct phases. In the first phase, we have devised targeted sensitization enhanced radiotherapy (TSER) utilizing inorganic nanoparticles. These nanoparticles exhibit targeted-sensitization properties, enabling enhanced delivery of radiation to tumor cells while concurrently stimulating an immune response specifically against the cancer. This approach aims to maximize the effectiveness of radiotherapy while minimizing damage to healthy tissues. In the second phase, we have explored the potential of inorganic nanoparticle- based materials to inhibit tumor metastasis and radiation-induced fibrosis. Metastasis poses a significant challenge in cancer treatment, as it involves the spread of cancer cells to other parts of the body. Through the development of nanoparticle-based materials, our objective is to prevent or impede the metastatic process. Additionally, we aim to address the issue of radiation-induced fibrosis, which can lead to tissue scarring and dysfunction as a consequence of radiotherapy. By integrating these two complementary approaches, we strive to improve the outcomes of radiotherapy by enhancing tumor cell targeting and inhibiting metastasis.

Our research endeavors ultimately contribute to the advancement of more effective and safer cancer treatment strategies.

KEYWORDS: Radiotherapy, nanomedicine, metastasis

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A Self-powered Smart Dressing for Active Infection Prevention and Accelerated Wound Healing

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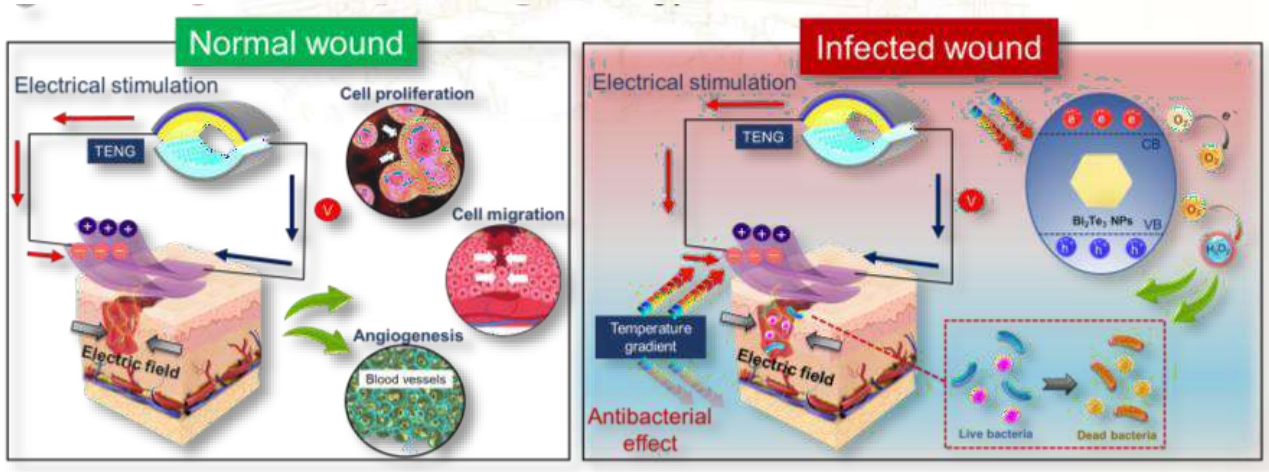
Abstract:

Interruption of the wound healing process due to pathogenic infection remains a major healthcare challenge. The existing methods for wound management require power sources which hinders their utilization outside of clinical settings. Here, a next generation of self-powered wound dressing with wearable capability is developed that can be activated by diverse stimuli from the body motions and provide on-demand treatment for both normal and infected wounds. The highly tunable dressing is composed of thermocatalytic bismuth telluride nanoplates (Bi_2Te_3 NPs) functionalized onto carbon fiber fabric electrodes and triggered by the surrounding temperature difference to controllably generate hydrogen peroxide to effectively inhibit bacterial growth at the wound site. The integrated electrodes are connected to a wearable triboelectric nanogenerator (TENG) to provide electrical stimulation for accelerated wound closure by enhancing cellular proliferation, migration and angiogenesis. The reported self-powered dressing holds great potential in facilitating personalized and user-friendly wound care with improved healing outcomes.

KEYWORDS: Smart dressing, self-powered system, wound healing, infection prevention



Graphic abstract

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Engineered Perfluorocarbon Dual-Layered Drug Nanocarriers Provide Effective Photoimmunotherapy against Colorectal Cancer

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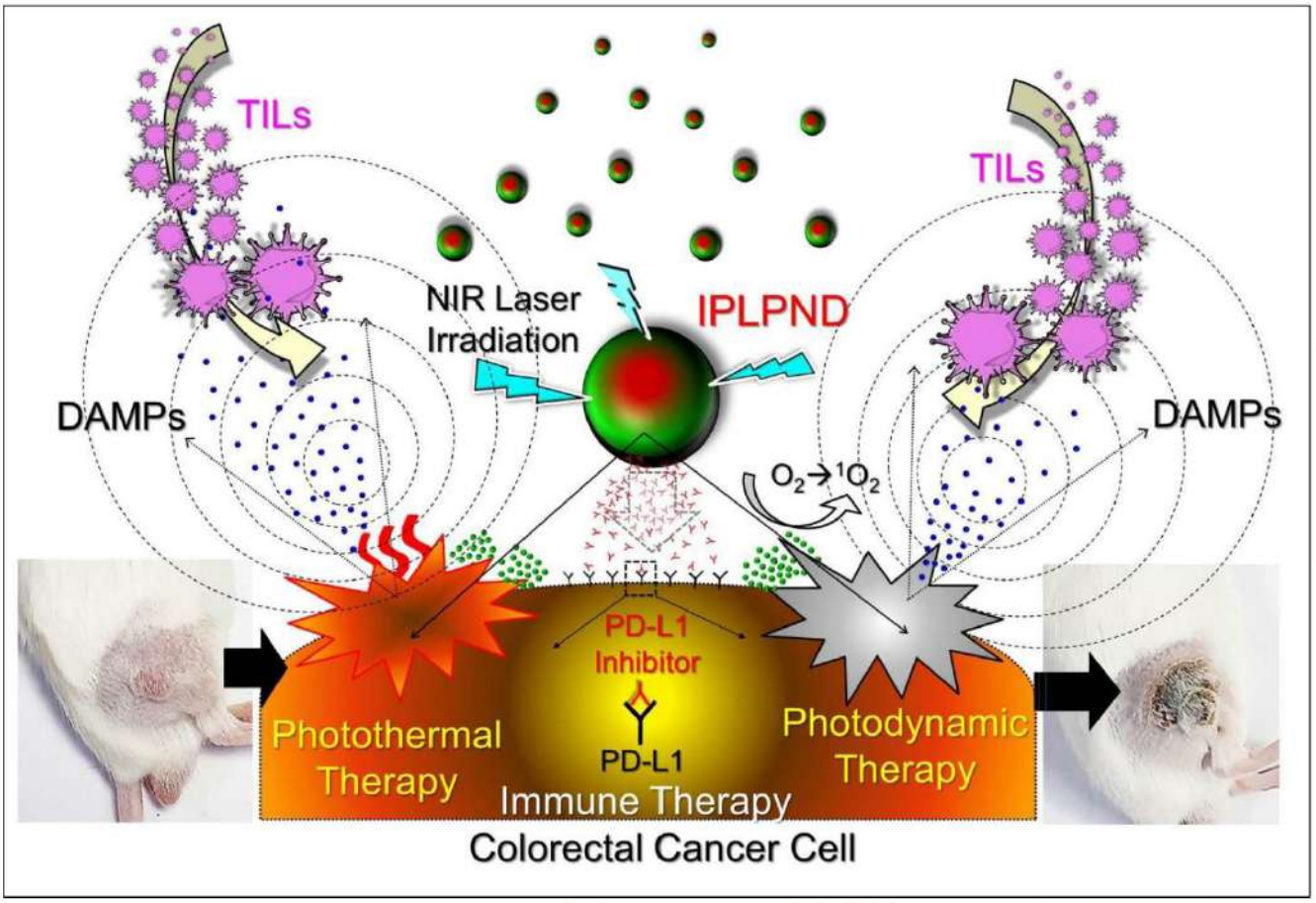
Abstract:

Colorectal cancer (CRC) ranks the 3rd most common neoplastic disease and is the 2nd leading cause of cancer death worldwide, showing approximately two million cases were diagnosed globally and one million patients were died yearly according to the World Health Organization statistics. Survival of CRC is highly dependent on the stage of disease, with > 90% of 5-year cumulative survival for those who diagnosed at stage I compared to about 14% at stage IV [1]. Unfortunately, CRC is often detected at advanced stages where dissemination of cancer cells has occurred, at which chemo- and targeted therapies can only provide a limited efficacy for increase of survival due to serious drug resistance of metastatic CRC as reported previously [2-4]. Such unfavorable circumstances, together with another fact that global prevalence of CRC has been notably increasing over the last decades [5], indicate that development of an effective strategy for CRC treatment is still urgently needed nowadays.

In this study, an emerging indocyanine green (ICG) and anti-programmed cell death ligand 1 monoclonal antibodies (αPD-L1) co-loaded perfluorocarbon double-layer nanodroplets named IPLPNDs were developed for photoimmunotherapy of CRC. The IPLPNDs are able to stabilize the αPD-L1 in the nanocarriers and generate hyperthermia as well as significantly enhanced production of singlet oxygen compared to equal dose of free ICG upon near infrared (NIR) irradiation. Furthermore, the IPLPNDs + NIR can dose-dependently eradicate CT26 cells and subsequently inhibit PD-L1 bioactivity of the survived cells in vitro. Meanwhile, expression levels of HMGB1 and CRT, the two immunogenic cell death (ICD) markers, from the survived cells were elevated 24 h after NIR exposure. Through the animal study, we further demonstrated that the IPLPNDs containing 20-μM ICG and 3-μg/mL αPD-L1 in combination with 1-min NIR irradiation can effectively arrest the growth of CT26 tumor in the mice without generating organ damage, by which the tumor size was merely increased by 56% while that without drug treatment can be tremendously expanded by 15 folds after 10 days. Moreover, the remained tumors treated by IPLPNDs + NIR indeed showed the least PD-L1 and highest CD8 expressions compared to all the other settings, illustrating the significance of immunogenicity of tumor microenvironment on anticancer efficacy. We reason that such tumor inhibition was carried out by phototherapy followed by ICD-enhanced immunotherapy, a two-stages anticancer process in vivo. Taken together, we anticipate that the developed IPLPND is highly applicable for use in the clinical CRC treatment.

KEYWORDS: Colorectal cancer, Photoimmunotherapy, Perfluorocarbon, Immunogenic cell death, Nanodroplet



Graphic abstract

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Bio-inspired zwitterionic polymeric chelating assembly for treatment of copper-induced cytotoxicity and triggered-release drug delivery

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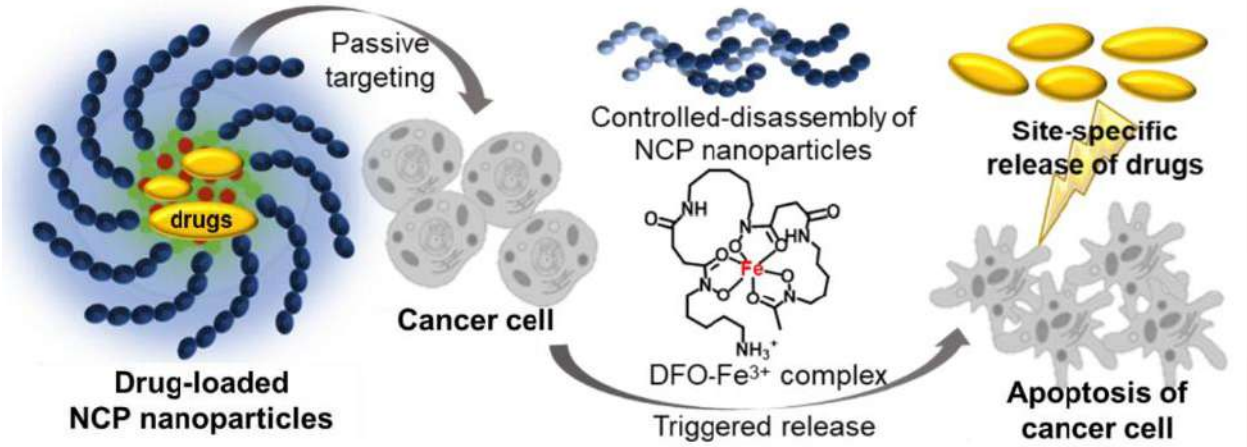
Abstract:

In this presentation, a novel nanoscale coordination polymer using the diblock copolymer poly(2-methacryloyloxyethyl phosphorylcholine)-block-poly(serinyl acrylate) (PMPC-b-PserA) will be presented. Its uses for trigger/release of a hydrophobic drug to induce breast cancer cell apoptosis in vitro and for elimination of toxic free copper ions by chelation. The zwitterionic PMPC block was inspired by the antifouling structure of cell membranes, and the PserA block was inspired by the amphoteric amino acids of proteins. Functional PMPC-b-PserA was synthesized via reversible addition-fragmentation chain transfer (RAFT) polymerization. A mixture of the polymer and Fe3+/Cu2+ self-assembled into nanoparticles via complexation of metal ions with PserA, with the hydrophilic PMPC block at the particle surface. For the drug delivery, Curcumin, a natural water- insoluble polyphenol used to enhance the effects of chemotherapeutics, was encapsulated in the particles as an oil-in-water emulsion. Triggered release of curcumin was achieved by adding deferoxamine, an FDA-approved Fe3+ chelating agent; curcumin release efficiency increased at higher deferoxamine concentrations and lower pH. Triggered release of curcumin induced apoptosis in human triple-negative breast cancer cells. In addition, for detoxification of PMPC-b- PserA, a PserA core with multiple coordination “bridges” between polymers and Cu2+ was formed, leading to self-assembly of core-shell polymer-metal nanoparticles in order to remove free Cu2+. The formation of self-assembled polymer-Cu2+ nanoparticles enables high cell viability and low hemolysis in the presence of Cu2+. Consequently, the hemocompatible, bio-inspired, multivalent, polymeric-chelating agent of PMPC-b-PserA offers its excellent potential in medical uses.

KEYWORDS: Zwitterionic polymer, RAFT, diblock copolymer, Self-assembled nanoparticles, metallic coordination



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Biological nanoparticle, RBC-derived vesicles (RDVs), for biomedical applications

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Abstract:

The development of novel nanoparticles as nanocarriers of labeling molecules, therapeutic drugs, and nucleic acids for cellular imaging, drug targeting, and gene delivery respectively, is one of the most remarkable applications in nanomedicine. However, the concerns about potential hazards associated with xenogeneic nanomaterials are largely pending.

Red blood cells (RBCs) with many biological advantages have been explored as carriers of different bioactive substances. However, the micrometer size of these carrier RBCs limits the accessibility of carrier RBCs with their cargos to extravascular target for intracellular delivery. Interestingly, in the aging process of RBCs in the circulatory system, their hemoglobin and membrane components can be diminished via the vesiculation by the spleen to generate RBC- derived vesicles (RDVs) at ultrasmall size. Also, vesicles similar to circulated RDVs can be generated from RBCs by in vitro osmotic and oxidative stress. Therefore, we have been developing osmotic stress-generated RDVS in vitro for being a novel carrier/delivery system for biomedical applications.

Ultrasmall superparamagnetic iron oxide (USPIO) particles are very useful for the intracellular labeling of cells for cellular magnetic resonance imaging (MRI) to maximize the therapeutic benefit of transplanted stem cells in vivo, which plays a crucial role for the development of successful stem cell therapy. RDVs can efficiently deliver USPIO particles into human bone marrow mesenchymal stem cells (hMSCs) for cellular MRI in vitro and in vivo. Moreover, they are extremely biosafe to their autologous hMSCs, with only trifling effects on cell viability, differentiation, and gene expression. The data demonstrate the potential of RDVs as an ideal delivery system for stem cell tracking [1].

Photodynamic therapy (PDT) has emerged as a potential treatment involving light energy and photosensitizers (PSs), in conjunction with molecular oxygen to elicit cell death. Many nanocarriers for PSs have been developed to solve the problems of limiting the clinical utility of PDT; however, few carriers capable of supplying oxygen have been reported. RDVs can efficiently deliver protoporphyrin IX (PpIX) into Huh7 cells in vitro. Upon irradiation, PpIX delivered by RDVs can induce more apoptotic and necrotic cell death than free PpIX treatment. RDVs have potential as delivery vehicles for efficient PDT due to the oxygen supply capacity.

Nanoparticles as drug delivery systems (DDSs) offer much promise for an effective and specific chemotherapy in cancer management. The above studies suggest that RDVs possessing sufficient stability and biocompatibility could be a facile, effective and biological DDS for systemically administered cancer therapy. Therefore, a novel nanocarrier composed of RDVs' surface-linked with doxorubicin (Dox) using glutaraldehyde (glu) to form Dox-gluRDVs was developed for improved cancer therapy. Dox-gluRDVs can exert superior in vitro cytotoxicity on a panel of cancer cell lines and enhance in vivo anticancer activity in subcutaneous melanoma B16F10-bearing mice through intravenous administration. A novel lysosomal-mitochondrial axis- dependent cell death mechanism is revealed, which is responsible for superior anticancer activity of Dox-gluRDVs in vitro and in vivo. RDVs show the great potential to serve a biological DDS of Dox for systemic administration to improve conventional cancer chemotherapeutics [3].

KEYWORDS: cellular magnetic resonance imaging (MRI), photodynamic therapy (PDT), cancer chemotherapy, nanoparticle

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Catalase mimicking manganese oxide nanozymes as anti-cancer and anti-inflammation nanotherapeutics

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Abstract:

Biocompatible catalytic nanoparticles are recently investigated for advanced nano-therapeutics as they mimic enzyme-like activities such as peroxidase-like, oxidase-like, catalase-like, and superoxide dismutase (SOD)-like activities. Manganese oxide nanoparticles are highly efficient catalase-mimicking nanoparticles. Manganese oxide nanoparticles quickly react with H2O2 and release oxygen and water as the byproducts. Site-specific oxygen production properties of manganese nanoparticles can be beneficial to treat hypoxic tumors where oxygen levels are extremely low. In addition, peroxide scavenging is one of the major advantages to reduce excessive oxidative stress in inflammatory diseases. To exploit these properties for therapeutic purposes, we have developed different types of manganese oxide nanoformulations. Firstly, we have synthesized hydrophobic manganese oxide nanoparticles loaded with reactive oxygen species (ROS) responsive thioketal-linked amphiphilic nano-assembly (MTS@HMO). As a multi-purpose reactive oxygen species (ROS)-catalytic nanozyme, MTS@HMO converted a radiotherapy (RT) resistant hypoxic tumor microenvironment to RT-susceptible one by increasing onsite oxygen production through catalase-like activity. The combination of MTS@HMO with RT enhanced the RT effect and suppressed tumor growth. To explore the anti-inflammatory effect, we have encapsulated the hydrophobic manganese oxide nanoparticle inside ROS-responsive nanomicelle (PTC-M) [1]. the results revealed that PTC-M ameliorated the acute kidney injury in the renal ischemia-reperfusion injury (IRI) model. PTC-M nanomicelle destabilized due to thioketal bond cleavage by ROS oxidants in an inflammatory environment and released the manganese oxide nanoparticles. The released nanoparticles effectively attenuated inflammation and apoptosis in the kidneys of IRI mice and protected against H2O2-induced cellular injury in human proximal tubular epithelial cells. Secondly, we have developed biomineralized manganese dioxide nanoparticles by using bovine serum albumin protein as the template. A mannosylated cationic polymer decorated biomineralized nanoscavenger (mSPAM) targets macrophages and depleted inflammatory signals in LPS induced endotoxemia model [2]. We have observed that mSPAM nanoassembly suppressed HIF1α expression by scavenging H2O2 and suppressed the inflammatory cell infiltration. Moreover, the inflammatory signal suppression prevented IBA-1 immune-positive microglial cell activation and cognitive damage. In another study, we developed an indomethacin-loaded biomineralized manganese dioxide nanoparticle (BIM). A combination of COX2 inhibitor and manganese dioxide nanoparticles in BIM nanoformulation, inhibited neutrophil forward migration and induced neutrophil reverse migration in the zebrafish model [3]. Effective neutrophil migration block further suppressed the inflammatory response in the MSU-induced gouty arthritis model. BIM nanoparticle scavenged ROS and suppressed M1 macrophage activation in peritoneal and air pouch

animal models. Overall, the current strategy of employing biomineralized nanoscavengers for arthritis and sepsis demonstrates clinical significance in the dual blocking of peroxides and COX2 to prevent the influx of inflammatory cells into the sites of inflammation. Taken together, manganese oxide nanoparticles are potent therapeutic options to investigate in pre-clinical and clinical stages for the treatment of multiple inflammatory diseases and cancers.

Keywords: Manganese oxide nanoparticle, Anti-inflammation, Anti-cancer, ROS scavenging, catalase-mimicking

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Rabies Virus Glycoprotein-Mediated T Cell Infiltration to Brain Tumor By Magnetoelectric Gold Yarnballs

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Abstract:

T lymphocyte infiltration with immunotherapy potentially suppresses most devastating brain tumors. However, local immune privilege and tumor heterogeneity usually limit the penetration of immune cells and therapeutic agents into brain tumors, leading to tumor recurrence after treatment. Here, a rabies virus glycoprotein (RVG)-camouflaged gold yarnball (RVG@GY) that can boost the targeting efficiency at brain tumor via dual hierarchy- and RVG- mediated spinal cord transportation, facilitating to perish tumor heterogeneity for T cell infiltration, is developed. Upon a magnetoelectric irradiation, the electron currents generated on the GYs activates the electrolytic penetration of palbociclib-loaded dendrimer (Den[Pb]) deep into tumors. In addition, the high-density GYs at brain tumor also induces the disruption of cell-cell interactions and T cell infiltration. The integration of the electrolytic effects and T cell infiltration promoted by drug loaded RVG@GYs deep in the brain tumor elicits sufficient T cell numbers and effectively prolongs the survival rate of mice with orthotopic brain tumors.

KEYWORDS: Drug delivery, functional polymer, controlled release

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

New concept of cancer therapy using engineered macrophage (Mactrigger)

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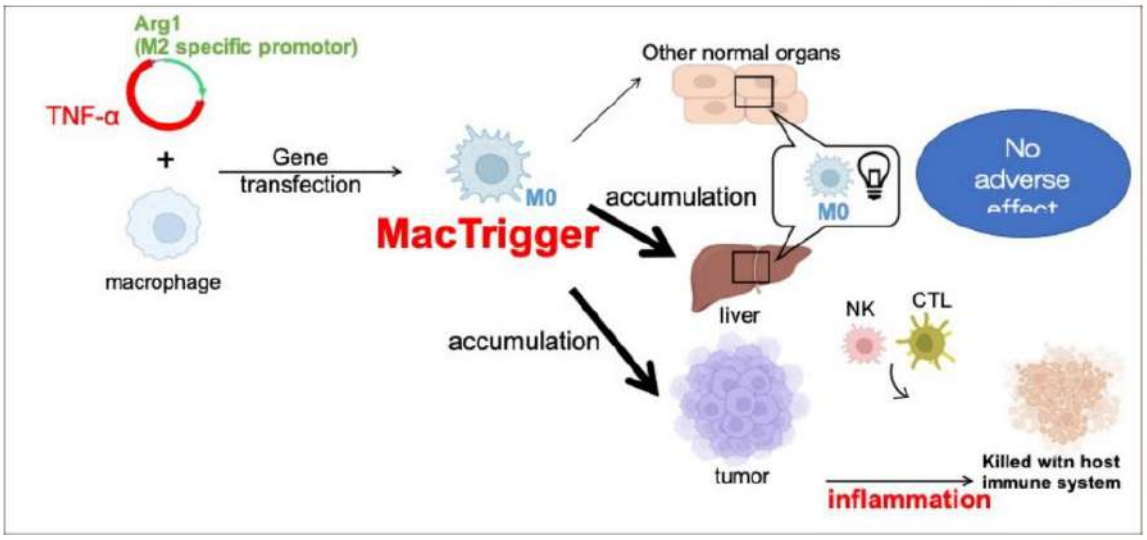
Abstract:

Solid tumors are mature tissues that use a variety of strategies to suppress attack from immune cells. [1]. In particular, cancer attracts macrophages and converts them to the anti- inflammatory type (M2) to protect itself. Therefore, antibody drugs cannot exert their effector actions, and can only use partial functions such as suppressing cancer functions by binding to their antigens, which makes cancer treatment effective. If this immunosuppressive function can be disrupted, the cancer can be effectively attacked by host immune system. One such strategy is immune checkpoint inhibitors. However, they also affect immune checkpoints in normal tissues and can cause devastating side effects. Here we propose a completely new strategy to destroy this immune evasion in cancer tissue [1].

Utilizing the fact that macrophages accumulate in cancer cells and are converted to the M2 type, we have developed macrophages (Mactrigger) that release TNF-α, which triggers inflammation, only when they are converted to the M2 type. Thus the TNF-α gene was inserted downstream of the mouse M2-type macrophage-specific promoter and transfected into RAW264.7. When the Mactriggers were administered through the tail vein of mice, they accumulated in cancer and liver. However, while it was converted to the M2 type in cancer tissues, the conversion to the M2 type was not observed in the liver and remained the M0 type. As a result, its single administration significantly reduced cancer volume and dramatically decreased proliferation markers. In addition, infiltration of natural killer cells and cytotoxic T cells into cancer tissues was observed, suggesting that this anticancer effect is due to accumulated immune cells. The engineered macrophages themselves were also found to die within a few days, making it clear that the long-lasting anticancer effects observed here are not due to this Mactrigger. In other words, the Mactriggers act as a trigger to cause acute inflammation in cancer, and the induced inflammation activated the host immune cell attack to the cancer.

In this study, we proposed a new concept that kills cancer by destroying the immune evasion ability only in cancer tissue and inducing inflammation in the cancer. This methodology has the potential to be very effective in combination with CAR-T therapy, other immunotherapies, or antibody drugs, and we plan to investigate this in the future.

KEYWORDS: Cancer immunotherapy, immune-checkpoint, macrophage, inflammation



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TAK1 blockade as a therapy for ocular neovascularization

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Abstract:

Ocular neovascularization, or pathological angiogenesis in the eye, is a leading cause of blindness in developed countries. Transforming growth factor-β-activated kinase 1 (TAK1) is a mitogen- activated protein kinase kinase kinase (MAPKKK) activated by TGF-β1 and other proinflammatory cytokines. TAK1 is also a key mediator of proinflammatory signals and plays an important role in maintaining vascular integrity upon proinflammatory cytokine stimulation such as TNFα. However, its role in pathological angiogenesis remains unclear, particularly in ocular neovascularization. Here, we discover the involvement and regulatory role of TAK1 in ocular neovascularization. Using TAK1 knockout human endothelial cells subjected to inflammatory stimuli, transcriptome analysis revealed that TAK1 is required to activate inflammatory signaling and mediates its downstream gene expression related to angiogenesis. Pharmacological inhibition of TAK1 by 5Z-7-oxozeaenol significantly attenuated angiogenic responses in the rodent models of retinal neovascularization. Furthermore, topical administration of the gelatin-nanoparticles-encapsulated 5Z-7-oxozeaenol extends the retention of 5Z-7-oxozeaenol in the cornea and led to significant suppression of angiogenic responses in a mouse model of corneal neovascularization. Our study shows the potential of TAK1 as a therapeutic target for pathological angiogenesis in the cornea and retina. The gelatin nanoparticle coupled with 5Z-7-oxozeaenol as a promising new eyedrop administration model in the treatment of neovascularization on the ocular surface.

KEYWORDS: Ocular neovascularization, TAK1, 5Z-7-oxozeaenol, gelatin particle

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Novel Drug Delivery System Using Nano-Prodrugs

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Abstract:

In this presentation, we propose a new concept, termed "pure nano-drugs" (PNDs), which are comprised of drug ingredient and are delivered into cells in a carrier-free state without using polymer. As the model of PNDs, the nanoparticles of SN-38 cholesterol succinate which is the derivatives of SN-38（Fig. 1）having the high anticancer activity were fabricated with less than 100 nm in size (Fig.2) by the reprecipitation method[1] developed at our laboratory[2]. Aqueous dispersion of the nanoparticles has been shown to exhibit an extremely effective anti-cancer activity not only *in vitro* experiment but also *in vivo* experiment (Fig. 3), when compared to irinotecan, a prodrug of SN-38 and a widely used water-soluble anticancer monomer[3]. In addition, interestingly, unlike conventional polymer micelle nanodrugs, our nanodrug has a hydrophobic surface, so it was found that the nanodrug penetrated into cancer cells and exerted its pharmacological effect[4].

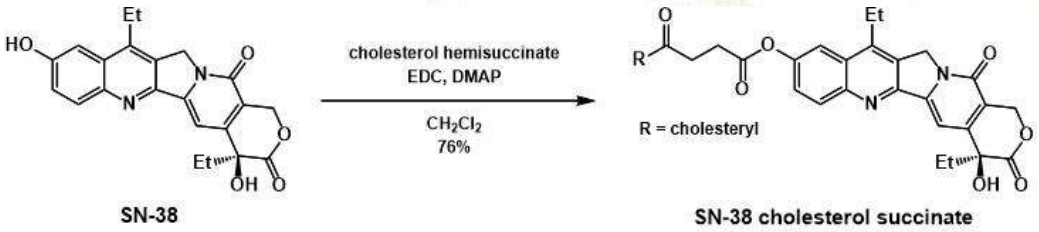

Figure 1 Synthesis of SN-38 cholesterol succinate

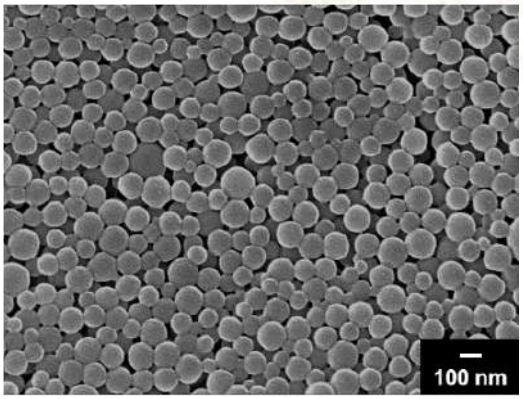

Figure 2 SEM photograph of nano-prodrugs for anti-cancer.

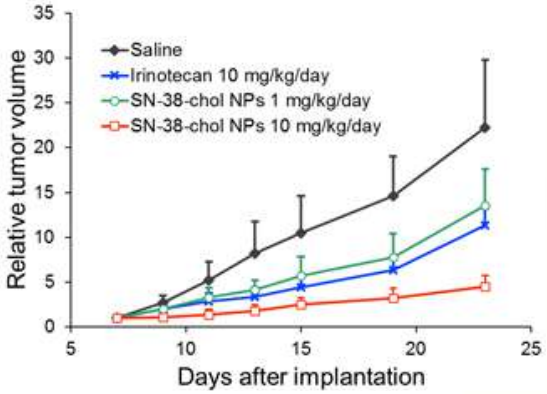

Figure 3 *In vivo* antitumor activities when saline, irinotecan and our nano-prodrugs were added into tumor-bearing mouse.

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Cell-assembling collagen microgel for stem cell therapy in critical limb ischemia

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Abstract:

Critical limb ischemia is a devastating disease characterized by the progressive blockade of blood vessels. Stem cell therapy has emerged as an angiogenic therapy for CLI, as it showed promising therapeutic potential via the paracrine effect of growth factors [1]. However, the therapeutic efficacy of stem cell therapy is very limited, due to the extremely poor cell survival in harsh microenvironment of ischemic region. An approach to deliver stem cells that can enhance cell survival and engraftment is a key for successful therapeutic outcome. In this study, we hypothesized that presence of hyaluronic acid (HA) would cause thinning of collagen fibers such that bulk collagen gel could be easily fragmented into micro-sized particles upon mechanical stress. Collagen microgel (CMG), 30~40 mm, was fabricated from polyionic complex of collagen and hyaluronic acid as a novel scaffold to deliver cells for improving transplantation efficiency.

A novel collagen microgel (CMG) was fabricated from polyionic complex of high- concentrated collagen and HA by mechanical fragmentation. Size distribution of CMG particles were analyzed using an automated Mophologi G3 optical microscope (Malvern Panalytical Ltd). human adipose-derived stem cells (hASCs: S. Biomedics, Seoul) were expanded in CEFOgro media (CEFO Co., Seoul) in a humidified chamber set to 37 oC and 5% CO2. All experiments were performed at fifth passage (P5) of hASCs.We characterized CMG assembly as carrier for cell delivery and investigated the microstructure, mass transfer, cell viability, and angiogenic functions of CMG assembly as therapeutics for CLI treatment. Finally, therapeutic efficacy of CMG assembly was demonstrated in mouse CLI model.

CMG assembly with hASCs increased viscous and elastic property compared to CMG alone. hASCs and cell were uniformly distributed across the 3D assembly. Physiological interaction of hASCs with CMG resulted in injectable 3-dimensional (3D) cell construct (CMG-hASCs) which was inhibited by integrin beta1 blocking antibody and Rho kinase inhibitor. CMG-hASCs assembly was an architecture with increased microporosity in a manner dependent on the increase in the dose of the CMG. Glucose uptake and hypoxic state in CMG-hASCs assembly increased and decreased, respectively, depending on the amount of CMG. We verified had CMG-hASCs assembly improved survival of hASCs compared to hASCs 3D construct. Further analysis with heatmap revealed that genes related to actin contraction, mass transfer, anti-apoptosis, and angiogenesis were upregulated in CMG-hASCs assembly (4:1 in the ratio of CMG:hASCs), compared to hASCs 3D construct and 2D cultured hASCs. CMG-hASCs was verified to have high angiogenic potential in vitro and ex vivo angiogenesis assay. Immunofluorescent images of thigh muscles collected from CMG-hASCs injected mice CLI model showed muscle regeneration and angiogenesis with enhanced survival of transplanted hASCs. Taken together, we propose that CMG-hASCs is a potential therapeutics to treat CLI and CMG is a novel scaffold platform to fabricate 3D cell construct for regenerative medicine.

KEYWORDS: Regenerative medicine, stem cell therapy, collagen microgel, 3D cell culture, critical limb ischemia

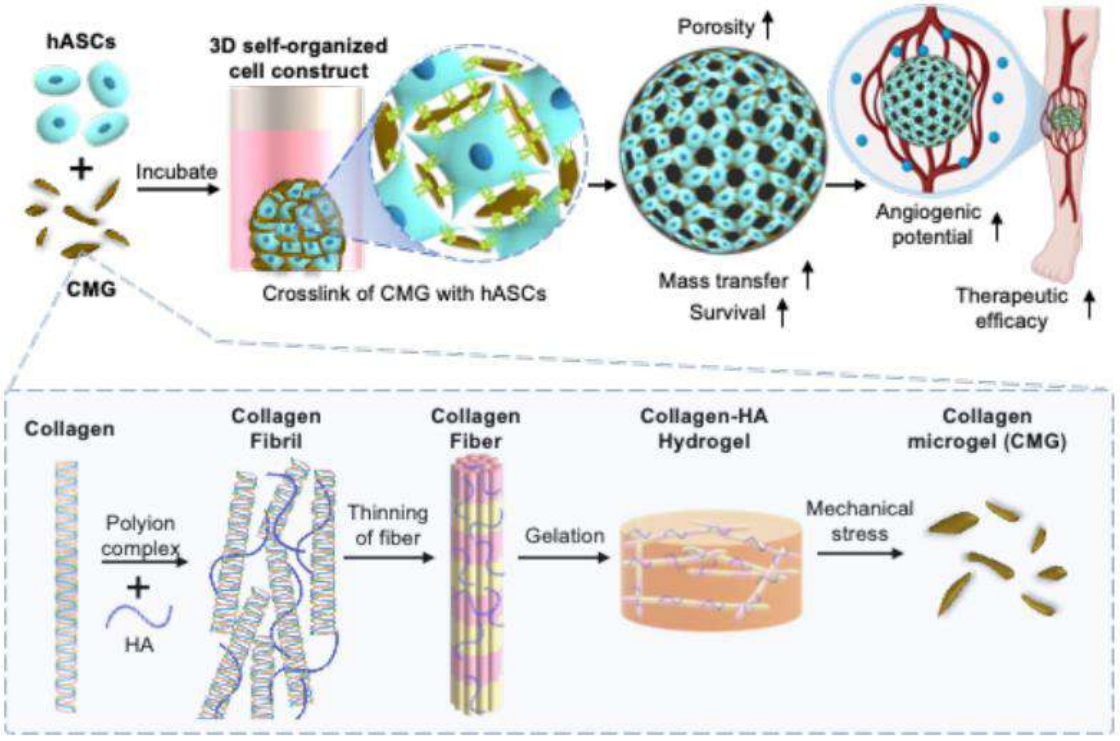

Figure1. CMG-hASCs assembly for treatment of critical limb ischemia

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How hair follicle stem cells interact with the environment

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Abstract:

For most mammals, hair serves as the first barrier that protects and insulates our body from external insults. When skin is injured or the hair coat is impaired, prompt transition from the resting phase to the growing phase to regenerate new hair enables timely recovery of this important protective barrier. Hair follicle regeneration is powered by hair follicle stem cells (HFSC) whose activity is subject to non-cell-autonomous regulation from their niche. In adaption to the ever-changing external environment and cutaneous status, HFSC niche must be endowed with the ability to detect these changes and responds adaptively to modulate HFSC activity for organismal needs. In this talk, I will talk about how the HFSC niche enables HFSCs to detect external light and low temperature for hair follicle regeneration. I will also talk about how inflammation triggered by skin irritation modulates HFSC metabolism to promote hair growth. Targeting these pathways can be new strategies for treatment of hair loss.

KEYWORDS: Hair follicle, stem cells, environment, hair loss, regeneration

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Engineering Different Scaffold-Free 3D Culture Systems of Adipose-Derived Stem Cells for Tissue Regeneration

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Abstract:

Adipose-derived stem cell (ASC) is a valuable source of cell therapy, and we aimed to explore the regenerative capabilities of ASC in scaffold-free three-dimensional (3D) culture conditions. We manipulated spheroid formation of human ASCs by culturing them on fabricated biomaterial films, and we also stimulated extracellular matrix (ECM) secretion of ASCs and fabricated cell sheets by treatment with ascorbate 2-phosphate (A2-P) [1,2].

Enhanced expression of stemness markers Sox-2, Oct-4 and Nanog was noted in ASCs within cell spheroids and sheets, with significantly enhanced neurogenic and hepatogenic transdifferentiation capabilities relative to monolayer ASCs. Meanwhile, adipogenic and osteogenic differentiation capacities of ASCs were still maintained. However, previous reports have found decreased expression of vascular endothelial growth factor (VEGF) in ASC sheets.

RNA-sequencing analysis revealed that upregulation of angiogenesis-related genes was found only in ASC spheroids. Hence, we further integrated ASC spheroids into ASC sheets to enhance the angiogenic capability of cell sheets. The stimulating effect of spheroid formation on ASCs toward endothelial lineage was demonstrated by enhanced CD31 expression, which maintained after ASC spheroids were seeded on cell sheets. Relative to ASC sheets, enhanced expression of VEGF and hepatocyte growth factor was also noted in ASC spheroid-sheets, and conditioned medium of ASC spheroid-sheets significantly enhanced tube formation of endothelial cells in vitro. Moreover, chick embryo chorioallantoic membrane assay showed a significantly higher capillary density with more branch points after applying ASC spheroid-sheets, and immunohistochemistry also revealed a significantly higher ratio of CD31-positive area.
In the spheroid-sheet construct, ASC spheroids can augment the pro-angiogenesis capability of ASC sheets without the use of exogenous biomaterial or genetic manipulation. The strategy of this composite system holds promise as an advance in 3D culture technique of ASCs for future application in angiogenesis and regeneration therapies.

Keywords: adipose-derived stem cell, 3D culture, cell spheroid, cell sheet, angiogenesis

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Infrapatellar fat pads-derived stem cells is a favorable cell source for articular cartilage tissue engineering: A study based on 3D organized self-assembled biomimetic scaffold

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Abstract:
Backgrounds: Adipose tissue–derived stem cells (ADSCs) are a promising cell source for tissue regeneration. However, in articular cartilage tissue engineering, ADSCs isolated from different anatomic parts have various cell characterizations and differentiation potential.
Methods: We compared the chondrogenic potential of ADSCs isolated from infrapatellar fat pads (IPFPs) and subcutaneous fat pads (SCFPs) in 3D highly organized honeycomb-like gelatin scaffold.
Results. The IPFP-ADSCs differentiated chondrocytes had higher ACAN, COL2A1, SOX6, SOX9, COL10, ChM-1, and MIA-3 secretion and lower VEGF and COL1A1 levels than the SCFP-ADSCs in gelatin scaffold. The difference in mRNA level may have attributed to activation of the akt, rhoa, p38, and JNK signaling pathways in the IPFP-ADSCs. The IPFP-ADSCs differentiated chondrocytes had higher COL type II and glycosaminoglycan production and less polymerization of β-actin. In an ex vivo mice model, MRI unveiled a shorter T2 relaxation time which indicated that IPFP-ADSCs/scaffold construct can secret more extracellular matrix than SCFP-ADSCs group. Histological staining showed that the IPFP-ADSCs/scaffold construct had better chondrogenicity of new cartilage tissue generation as evident in S-100 staining and collagen type II.
Conclusion. ADSCs isolated from IPFP are a better candidate for cartilage regeneration with the potential of cartilage tissue engineering using the IPFP-ADSCs/scaffold technique.

KEYWORDS: Adipose tissue–derived stem cells (ADSCs), Infrapatellar fat-pad, cartilage tissue engineering, scaffold

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Stem Cells in Disease Modelling, Drug Discovery and Therapeutic Development

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Abstract:

The development of regenerative strategies to treat degenerative diseases and ageing-associated disorders is a major challenge in healthcare settings. This requires tremendous efforts across the research spectrums on investigating both cell-based and cell-free strategies including understanding stem cell behaviour in disease states and harnessing the therapeutic potential of stem cells and their derivatives. In our lab, we have successfully developed stem cell-derived organoids or 3D cultures of the miniature liver, brain, and cartilage along with cancer stem cell models to explore many aspects of biology- cellular and molecular mechanisms, drug testing systems and transplantable cellular resources. From the perspective of regenerative medicine, stem cell-based models are imperative to study both degenerative diseases and unleash the therapeutic value of stem cells as single and combination therapy as potential treatment modalities for various degenerative conditions. In order to make stem cell transplantation a success, we have been developing rejuvenation strategies for ageing stem cells and expanding their therapeutic value by improving the stem cell bioprocessing pipeline. While one arm of the lab is focusing on regenerative medicine, the other arm of my research focuses on modelling cancer stem cells using stem cell biology knowledge to unravel key regulatory targets through integrated multi-omic bioinformatics analysis and drug repositioning that will enable the targeting of the resistant population in a tumour, therefore eradicating cancer. In this talk, I will highlight how we can leverage stem cell biology to understand disease mechanisms, model the diseases and unleash their therapeutic potential.

KEYWORDS: Stem Cells, Cancer Stem Cells, Tissue Engineering, Bioinformatics, Drug Discovery and Repositioning, Disease Modelling, Therapeutic Development

2023 ISOMRM & BCRS
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3D printed PCL/HAp implant in in vivo application of segmental bone defect of femoral shaft

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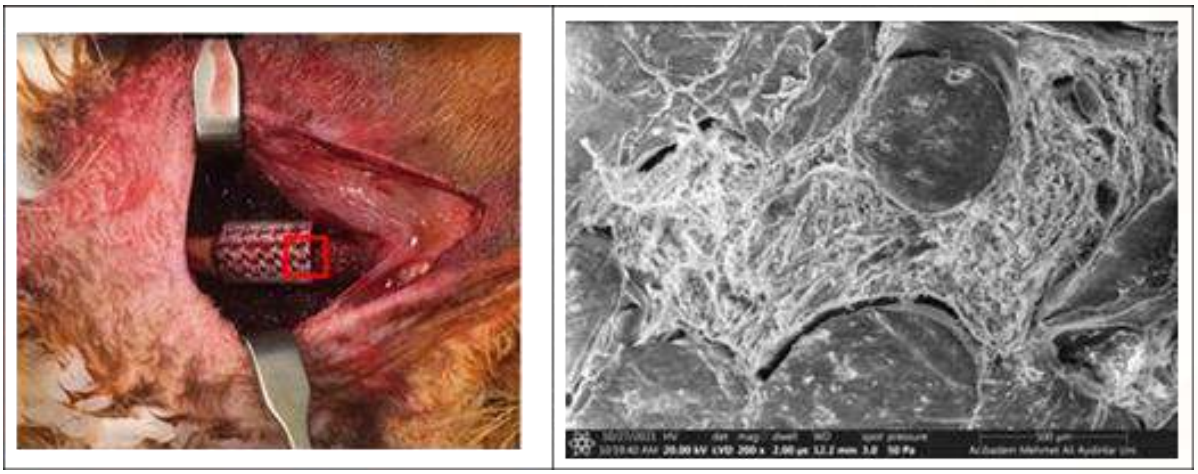
Abstract:

Treating segmental bone defects is a clinical challenge as they often exceed the self healing capacity of the body. Autografts are considered the gold standard treatment, but their limited availability is a restricting factor. In order to address the need for a strong load bearing structure and to mimic the actual repair of such bone defects, a biodegradable cylindrical implant composed of nanohydroxyapatite (nHAp) and medical grade polycaprolactone was 3D printed for segmental application. In vivo studies involved 12 male New Zealand male white rabbits divided into two groups, one of which received the PCL-nHAp implant while the second group received autogenous segmental bone graft after being reversed in direction. Two more rabbits were used as the untreated group. The test was terminated on week 6. Histological and SEM examination were conducted to study the level of healing and tissue ingrowth. Mechanical testing of the implant carrying femoral bone was performed with 3-point bending to determine stability after tissue ingrowth. Radiological studies (X-Ray and CT) were conducted on weeks 2, 4, and 6 for implant, autograft and control groups. Mechanical testing showed that even though the implant (57±5N) and autograft (93±56N) groups demonstrated significantly lower ultimate load compared to the control group (245 ±5N), there was no significant difference in between the implant and autograft groups. The closeness of the ultimate loads of the two test groups and shortness of the implantation duration, indicates a distinct integration of the implant and the bone at the end of the 6 week test period. Further analysis through histology, SEM and EDS showed that this application allowed for tissue ingrowth. Histological findings indicated that in both implant and autograft groups, new bone tissue formation was seen; however, there was a sizeable callus formation in the autograft groups. SEM revealed a very close contact between newly formed bone and the implant and complete penetration into the porous structure of the implant indicated the appropriateness of the implant design.

In conclusion, the biodegradable cylindrical implant nHAp carrying PCL presented tissue growth comparable to the autograft with bone integration. The implant was able to satisfy the need for bone grafting by exhibiting comparable bone healing and biomechanical stability thus it can serve as a viable alternative when no autograft suitable for the treatment of large segmental bone defects is available.

KEYWORDS: Segmental bone defect, 3D printing, PCL-Hydroxyapatite



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2023 ISOMRM & BCRS
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Microfluidic Chips for Cell Spheroids Culture

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Abstract:

Cell spheroids are three-dimensional spherical cellular aggregates that can better mimic the in-vivo cellular microenvironment compared to the traditional two-dimensional monolayer cell cultures. Cell spheroids can be formed from many different types of cells including embryonic stem cells (ESCs) which when cultured in a 3D culture condition can spontaneously aggregate into a three-dimensional sphere called embryonic body (EB), which can form all three germ layers of endoderm, mesoderm, and ectoderm, and can be induced to differentiate into different cell types for fundamental and application research of tissue engineering and regenerative medicine. The formation of EBs can be achieved by several 3D cell culture approaches including suspension culture in bacterial-grade dishes low or vessel bioreactor, culture in methylcellulose semisolid media, culture in spinner flask and more recently, microfabricated devices containing microchannel and microwells. For generating small quantities of EBs, the hanging drop method is most widely used due to it is easy to perform in the laboratory and requires minimal equipment and materials. However, the hanging droplet method requires manual operation for each individual droplets and is limited by the difficulty of exchanging medium in the droplets. To address these problems, we have developed of a microfluidic chip-based method for hanging drop culture of cells. Our method uses microchannel with opening wells to form large numbers of hanging droplets without needing to pipette the droplets individually. The utilities of this technology in 3D cell spheroid culture of embryonic stem cells, cancer cells, and the co-culture of spheroids of these two cell types will be presented.

KEYWORDS: Microfluidics, lab-on-a-chip, cell spheroid, in-vitro model

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3D Bioprinted pectin and gelatin skin grafts containing fibroblasts and bioactive agents

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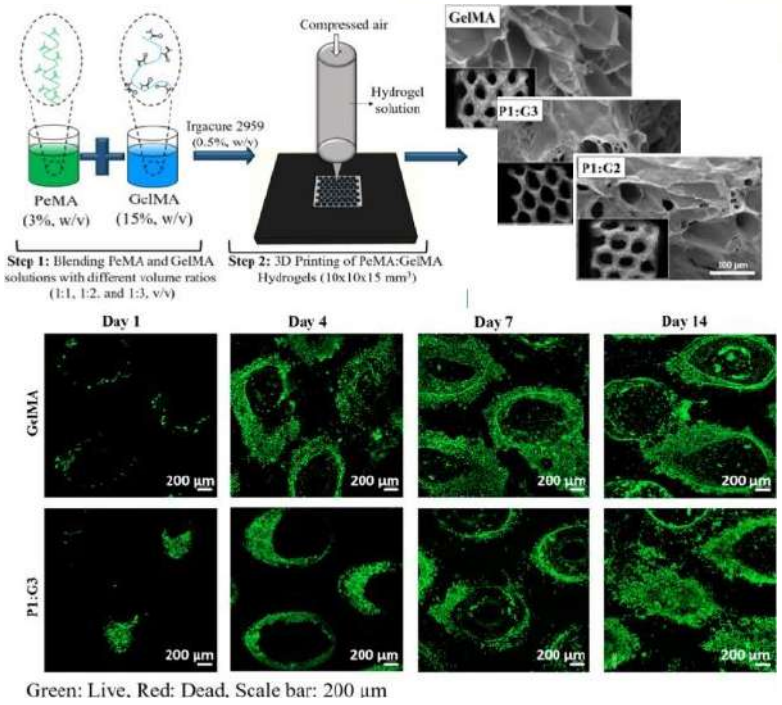
Abstract:

Skin is the largest organ of the body which protect rest of the organs and acts as a shield against environmental influences. In case of chronic wounds, diabetic ulcers or high degree of burns, regeneration of skin tissue may take months or even may not occur at all. Autografts, allografts or xenografts may be the solutions, but all have some disadvantages as lack of donor site or immune rejection. Therefore, synthetic skin grafts, especially hydrogels are the best choices for the treatment skin damages. Hydrogel grafts, containing antimicrobial chemicals, bioactive agents and cells, are capable to prevent infection, promote healing, provide moist environment and act as a regenerative scaffold for the newly forming tissue [1].

In this study, multifunctional scaffolds were developed by 3D bioprinting using methacrylated pectin (PeMA), methacrylated gelatin (GelMA) and L929 fibroblast cells. Vitamin- C (as cell protector) and curcumin (as antibiotic) were loaded into the hydrogels. The gels were prepared in different compositions of PeMA and GelMA (as PeMA/GelMA=1/1, 1/2, 1/3) and characterized [2]. Due to higher mechanical stability, cell viability and in situ degradation kinetics, PeMA/GelMA=1/3 was chosen as the ultimate composition. Curcumin (100 µg/mL or 150 µg/mL) and Vitamin-C (0.25mM or 0.75 mM) separately were added into the optimized hydrogels. In the cases when Vitamin-C was at higher concentration, the 3D printed hydrogels demonstrated higher cell viability and collagen deposition for L929 cells. Meantime, the in vitro drug release studies of curcumin demonstrated a pH sensitive release with higher rates in basic media, and it also had an effective antimicrobial activity against Gram positive S.aureus strains and Gram negative E.coli strains. In conclusion, 3D printed (with Vitamin-C and curcumin) and 3D bioprinted (with L929 cells, Vitamin-C and curcumin) hydrogels can be considered as effective skin graft candidates and as a new option for the treatment of full-thickness chronic skin wounds.

KEYWORDS: 3D bioprinting, skin graft, hydrogel, pectin, gelatin, curcumin, Vitamin-C



Green: Live, Red: Dead, Scale bar: 200 µm

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In vitro Spermatogenesis Platforms

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Abstract:

The gonadotoxicity of childhood cancer treatment modalities causes permanent infertility/sub-infertility in nearly half of male patients [1]. The current clinical and experimental approaches are limited to cryopreservation of prepubertal testicular strips [2] and *in vitro* spermatogenesis which are inadequate to achieve the expanded spermatogonial stem/progenitor cells (SSPC) and spermatogenesis in vitro (Fig. 1). *In vitro* spermatogenesis platforms have emerged as innovative and promising tools addressing the challenges associated with male infertility. These platforms offer a controlled environment that simulates the complex process of spermatogenesis [3] and enables the generation of spermatozoa or round spermatids from spermatogonial stem/progenitor cells. By recapitulating the native physiological conditions required for spermatogenesis, these platforms allow for the study of critical aspects such as meiosis and haploid cell formation. Bone marrow derived mesenchymal stem cells (BMSC) bearing a close resemblance to Sertoli cells, improved spermatogenesis in animal models.

Recently, we reported 3 testicular culture platforms, microfluidic device (MFD) [4], hanging drop (HD) [4] and air-liquid interphase (ALI) [5], which provide *in vitro* spermatogenesis in prepubertal C56BL/6 mice with varying performances. Dynamic PDMS-based MFD with an insert for cellular secretome was designed (#PCT/TR2022/050188) as spermatogenesis-on-chip platform. We aimed to evaluate the efficacy of those setups with additional BMSC therapy in terms of self-renewal of stem/progenitor cells, spermatogenesis and structural and functional maturation of seminiferous tubules in vitro by measuring the expansion of SSPCs, *in vitro* spermatogenesis and functional maturation of testis by histochemical, flow cytometric and chromatographic techniques. One-way ANOVA and Kruskal Wallis tests analyzed parametric and nonparametric correlative outputs, respectively. Hacettepe University Ethical Board approved the studies (#52338575-109, #52338575–96). BMMSC conditioned medium-based testis-on-chip microfluidic platform supported the maintenance of spermatogonial stem/progenitor cells, progress of spermatogenesis and improvement of testicular maturation for 42 days while HD and ALI were limited to 28 days. Our findings established the efficacy of syngeneic BMSCs on the survival and expansion of the SSPC pool and differentiation of spermatogonia to round spermatids during *in vitro* culture of prepubertal mice testes for 42 days. The Scientific and Technological Research Council of Turkey (#218S421) and Hacettepe University Scientific Research Projects (BAP) Coordination Unit (#TYL-2018-17531) funded the studies.

Those BMSC therapy-based microfluidic and static culture platforms might be helpful in providing alternative cures for male fertility by supporting *in vitro* differentiated spermatids that can be used for round spermatid injection (ROSI) to female oocyte in animal models. Our findings demonstrate that a

novel BMSC-based microfluidic testis-on-chip device and static culture platforms supporting the maintenance of SSPCs and spermatogenesis in prepubertal mice in vitro. This new, cell therapy-based microfluidic platform may contribute to a safe, precision-based cell and tissue banking protocols for prepubertal fertility restoration in future. In vitro spermatogenesis platforms also hold promise for clinical applications, including fertility preservation for prepubertal boys facing gonadotoxic treatments, restoration of fertility in adult cancer survivors, and the generation of patient-specific spermatozoa for assisted reproductive technologies. In conclusion, in vitro spermatogenesis platforms represent a cutting-edge approach in reproductive biology research, offering valuable insights into the fundamental processes of spermatogenesis and presenting potential clinical applications. Further advancements in the field hold the potential to revolutionize male fertility preservation, diagnostics, and therapeutic interventions, providing hope for individuals facing infertility-related challenges.

KEYWORDS: In vitro spermatogenesis, male infertility, testis culture platforms, microfluidics

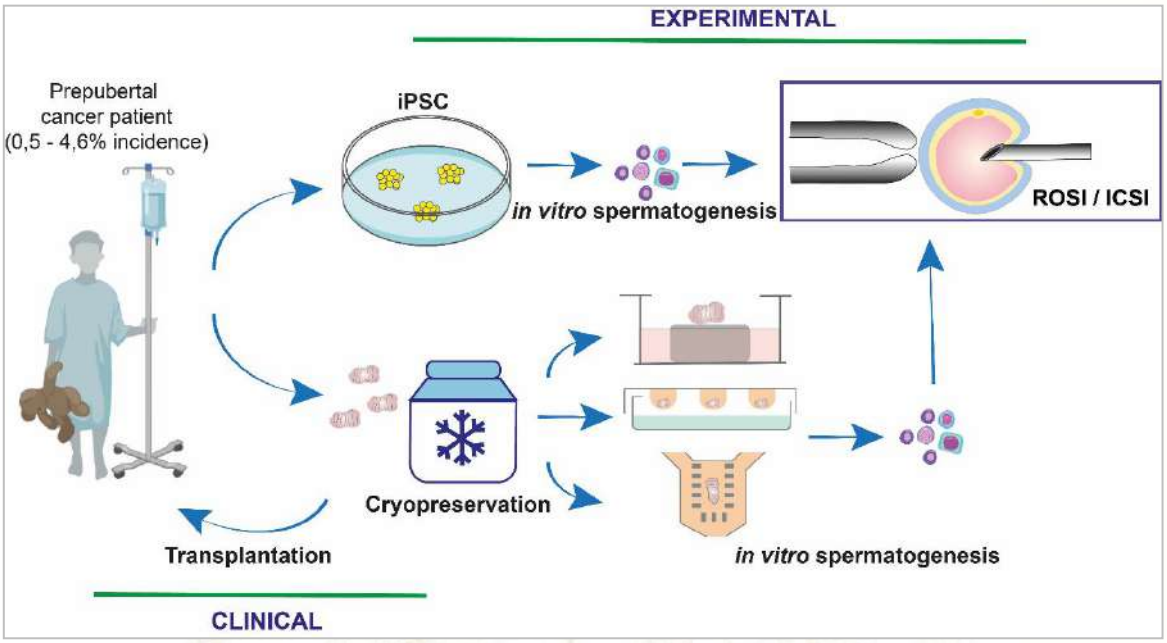

Figure 1. The current clinical approach and experimental studies for male cancer patients' fertility preservation are given.

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Multiscale design of 3D hydrogel bioink with ROS scavenging and retina tissue regeneration

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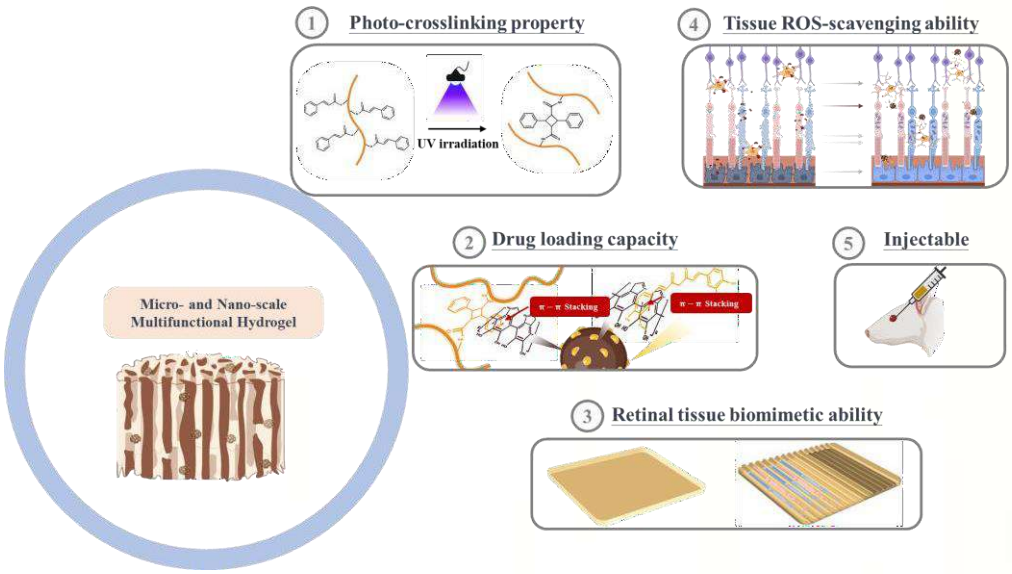
Abstract:

Retinal oxidative stress damage, associated with conditions such as glaucoma, macular degeneration, and diabetic retinopathy, poses a significant challenge for the development of targeted and efficacious therapeutic interventions. In this study, we present the design and synthesis of a functional hydrogel, nitrocinnamic esterified gelatin (GelCA), which addresses this challenge through an innovative drug delivery system. GelCA is synthesized by grafting cinnamic acid onto natural gelatin polymers, facilitating photo-crosslinking and enhancing pi-pi stacking drug adsorption functionality.

To augment the antioxidant properties of the hydrogel, we employed polydopamine nanoparticles (PDA NPs) as drug carriers, with the natural antioxidant curcumin(Cur) adsorbed onto their surfaces. This combination leverages the known antioxidant capabilities of both polydopamine and curcumin to create a more effective treatment option. The drug-laden nanoparticles were subsequently encapsulated within the GelCA hydrogel matrix, ensuring a controlled and localized release of the therapeutic agents.

Experimental results demonstrated that the multifaceted Cur@PDA NPs adsorbed GelCA hydrogel exhibits excellent injectability, biocompatibility, and antioxidant capabilities. The innovative hydrogel design allows for effective drug delivery and targeted treatment, potentially leading to improved patient outcomes across various retinal disorders.

KEYWORDS: antioxidative, ROS, hydrogel, retina tissue regenerative, bioink



Graphic abstract

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EPIGENETIC REGULATIONS OF ADIPOSE-DERIVED STEM CELLS DURING SPHEROID FORMATION AND PERIPHERAL NERVE REGENERATION

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Abstract:

The neuropathogenesis was triggered after nerve injury. We are interested to discover the potential therapeutic approach for the regeneration of the peripheral nervous system (PNS). In adipose-derived stem cells (ASCs), we discovered the sphere formation which is important for morphological changes in adult stem cells and material modification using biomaterials. We found the fibroblast growth factor receptor was significantly increased during neural lineage cells (NLC) induction. Further analysis of ASC-derived spheres discovered the involvement of histone deacetylase (HDAC) 5 nucleus translocation during NLC induction. The HAT activities were decreased and the trimethylation of H3K4 and H3K9 were increased during spheroid formation. The supplement of FGF9 during NLC induction facilitated the Schwann cells (SCs) fate commitment via the FGF9-FGFR2-Akt phosphorylation pathway. The fate committed SC can participate in the myelin sheath formation during nerve regeneration. We also investigated the epigenetic changes of different HDACs after PNS injury. The HDAC inhibitor (HDACi) sodium phenylbutyrate (PBA) was discovered to reduce SCs inflammation and improve sciatic nerve regeneration after injury. The PBA inhibited nuclear factor kappaB (NFkappaB)-p65 phosphorylation and translocation by regulating the HDAC3 expression and activity. Taken together, the epigenetic regulation on neural spheroid formation in ASCs and the cellular responses of Schwann cells in the microenvironment after PNS injury play important roles for nerve repair and regeneration.

KEYWORDS: adipose-derived stem cell, chitosan, epigenetic modification, neural differentiation

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Ear mesenchymal stem cells (EMSCs): a good *in vitro* model of primary cells to study regenerative medicine and molecular biomedicine

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Abstract:

Ear mesenchymal stem cells (EMSCs) have been investigated to differentiate into adipocytes, chondrocytes, osteocyte, and muscle cells in vitro. This stem cell population is easy to be collected, cultured and differentiated, especially from the animal models such as mice and rat. We have used EMSCs to differentiate into adipocytes to study the molecular mechanisms of lipid metabolism, fat expansion, adipogenesis and fat cells' functions [1, 2]. This type of stem cells has been also using to study auricular cartilage regeneration [3]. Here, we aim to disuse the possibility of EMSCs to be used as an *in vitro* model to find the molecular mechanisms and biomaterials for regenerative medicine as well as biomedicine in general. Additionally, we will present some our data using EMSCs to investigate adipocytes' development and fat metabolism.

KEYWORDS: *Ear mesenchymal stem cells; in vitro model; regenerative medicine; biomedicine*

Acknowledgements

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Selective and rapid proliferation of stem cells on growth factor-tethered surfaces

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Abstract:

Advances in stem cell biology have enabled us to consider novel regenerative approaches for various diseases and traumatic injuries. In fact, a lot of clinical studies are currently undertaken to validate the safety and efficacy of such therapeutic interventions. However, there are still many challenges to be addressed before the clinical applications of stem cell-based regenerative therapies. Among various challenges, a technology that permits efficient expansion of stem cells is of top importance. This is mostly because the number of stem cells we can obtain is usually quite limited, while the enormous number of cells are required for the structural and functional recovery of damaged tissues.

With such a background, we have been involved in developing bioreactors that allow us to selectively and rapidly expand various types of stem cells in a closed system [1]. In a series of our studies, we have focused on two aspects of bioreactor systems: One aspect is stem cell carriers with a large specific surface area, such as microgel carriers. We have been studying how stem cells can be appropriately expanded on the carriers, while keeping their stem cell state. The other aspect is concerned with molecular design of cell carrier surfaces so as to optimize surface microenvironment for the adherent culture of stem cells. This paper is mainly directed to introduce our previous attempts to molecularly design growth factor-tethered surfaces for the selective and raid expansion of neural and mesenchymal stem cells.

Somatic stem cells are known to reside within a biological niche where various biomacromolecules serve to maintain the stem cell functions. Inspired by such a stem cell niche, we have been concerned with designing materials surfaces on which stem cells are selectively and rapidly proliferate in response to surface-tethered growth factors, while maintaining their undifferentiated state.

In the case of neural stem cells (NSCs) isolated from rat fetal brains, they were found to specifically express a receptor for epidermal growth factor (EGF). Based on this information, hexahistidine-tagged engineered EGF was tethered to the Ni-bound substrate surface [2]. Thanks to the stable chelation of hexahistidine peptide to the Ni(II) ions, EGF was stably tethered to the surface [3] and facilitated to capture selectively neural stem cells from a heterogenous NSC population [4]. The EGF–EGF receptor interactions served to activate NSC proliferation to yield highly homogenous population of NSCs within a reasonable period [4–6]. It was further shown that a similar strategy was feasible for human neural progenitor cells [7] and NSCs derived from mouse induced pluripotent stem cells [8].

On the other hand, a substrate onto which basic fibroblast growth factor (bFGF) was tethered through chelation between hexahistidine peptide and surface-bound Ni(II) ions was found to capture human bone marrow-derived mesenchymal stem cells to promote the proliferation of the cells [9]. Interestingly, in situ refolding of partially denatured bFGF on the surface was found to enhance the

promotive effect, indicating that the efficiency of the effect was highly depend on the structural integrity and hence biological activities of bFGF after surface tethering.

All these results described above demonstrate the feasibility of our bioinspired surface design for the efficient expansion of stem cells. The growth factor-tethering will be a promising method for developing stem cell carriers effective for their expansion.

KEYWORDS: Regenerative medicine, stem cell, genetic engineering, growth factor, growth factor receptor, proliferation

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Cell chirality in tissue morphogenesis

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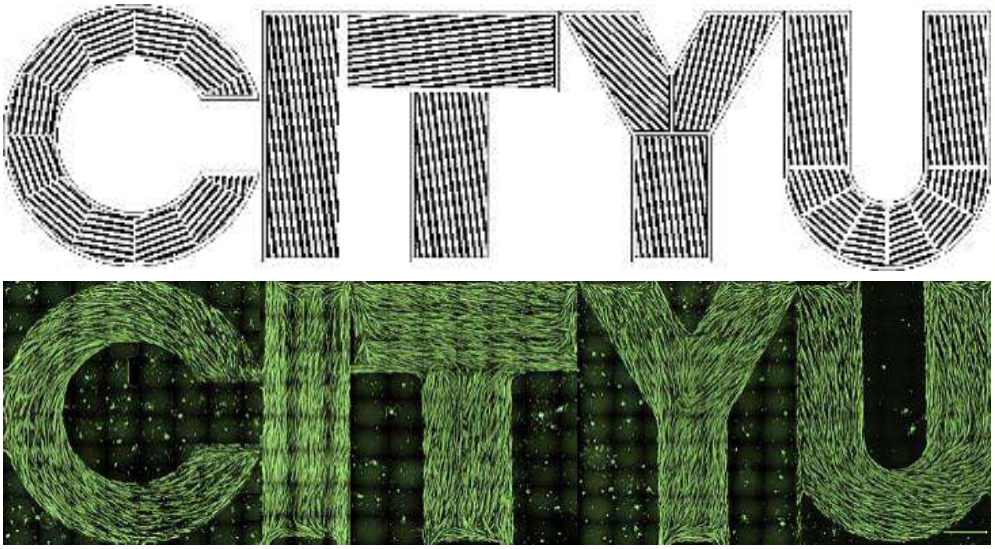
Abstract:

Cell chirality can be seen as cellular motions with intrinsic left-right (LR) bias. Examples are found in embryonic cells that establish LR axis of animal body plan, visceral distribution, and overall handedness of organ orientation. For specialized adult cells derived from somatic tissue, footprints of cell chirality can be also seen by their ability of generating cellular torque, migration with LR bias, or forming specific alignment in the multicellular level. In this talk, we report a series of approaches to characterize cell chirality and its role in tissue morphogenesis. First, we developed a nanowire magnetoscope that reveals a rotating force – torque – exerted by cells. Internalized ferromagnetic nanowires were used to reflect the rotational force of cells, and we found that such cellular torque is biased with clockwise (CW) or anticlockwise (ACW) direction depending on cell types1. Second, we applied micropatterned substrate and automated image processing to investigate the remnant effects of culture density on cell chirality2. Next, using human mesenchymal stem cells (hMSCs) on micropatterned substrate as a model system, we report an early committed cell chirality during lineage specification. hMSCs exhibited an ACW-biased nucleus rotation on circular micropatterns, and such chirality was reversed to CW bias after adipogenic induction. Remarkably, adipogenic differentiation is up-regulated by forcing the formation of CW-biased actin filament, adipogenic differentiation was up-regulated3, suggesting the role of cell chirality in engaging the lineage commitment. Finally, combining our understanding of cell chirality in single cells and multicellular organization, we synergize microtopographic cues and chiral nematics of cells to guide the formation of skeletal myotubes into scalable and controlled patterns of myotubes with enhanced length, diameter, and contractility4. Together, our studies indicate that cell chirality should not be misinterpreted as a random, noisy event that causes unexpected and undesired phenomenon. Instead, it is an essential factor enabling the LR tissue morphogenesis, and should be embraced when developing engineering strategy that leads to stronger and more functional tissue.

KEYWORDS: Cell chirality, Tissue morphogenesis, Biofabrication, Pattern formation



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Platelet-derived Biomaterials for Xenogenic Application of Cartilage Repair

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Abstract:

The autologous platelet-derived therapies such as platelet-rich plasma is widely used in the human, but allogeneic and xenogeneic therapies are currently limited. Platelet lysates depleted of antigens such as blood cells are a potential solution for allogeneic or xenogeneic applications. In this study, porcine platelet lysates containing undetectable antigens such as blood cells and complement were developed. Porcine platelet lysate was injected in the joint cavity and the osteochondral defects of rabbits for the evaluation of biocompatibility and the repair capability of the defects. The tissue sections in the joints cavity of the rabbits showed very mild inflammatory reactions, while the injection of platelet lysate in the osteochondral defects was found to effectively inhibit the cartilage arthritis in the peripheral of the defects. This study demonstrates the therapeutic potential of xenogeneic platelet lysate.

KEYWORDS: Porcine, platelet lysate, xenogeneic, cartilage, osteoarthritis

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Cartilage tissue engineering and osteoarthritis therapy: mesenchymal stem cells, perivascular stem cells, and platelet-derived extracellular vesicles

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Abstract:

Cartilage damage, which can potentially lead to osteoarthritis, is a leading cause of morbidity in the elderly. This is mainly due to the ability of cartilage to undergo self-repair following an injury being limited, resulting from the limited proliferative ability of adult chondrocytes, lack of vascularization and innervation, slow matrix turnover, and low numbers of progenitor cells. Although these issues may be resolved with the presence of progenitor cells within the tissue, it is well-established that cartilage is deficient in undifferentiated stem cells. Furthermore, the principal cells in mature cartilage (chondrocytes) have limited proliferative ability due to the physical restrictions in the surrounding environment. To overcome this, the potential of biological therapies for cartilage regeneration has recently gained much interest in the challenging arena of repairing damaged joint cartilage.

Our research focuses on isolating and expanding adult mesenchymal stem cells **(**MSCs) derived from bone marrow and peripheral blood, followed by inducing chondrogenic differentiation *in vitro* (Chong et al., 2012). The study further utilized RNA microarray to reveal up-regulated and down-regulated genes during chondrogenic induction. These MSCs and MSC-driven chondrocytes were embedded in biocompatible alginate scaffolds and transplanted into surgically created cartilage defects in knee joints of animal models (Dashtdar et al., 2011; Tay et al., 2012). In addition, our animal study demonstrated that perivascular stem cells (PSCs) have similar levels of stemness and chondrogenic ability to MSCs. We have also determined the interplay of hyaline cartilage loss and subchondral bone changes in patients with established knee osteoarthritis, as well as assessed the biosynthesis of isolated osteoarthritic chondrocytes in response to varying dynamic compressive strain and loading duration (Fig. 1) (Chong et al., 2020).

The promising finding arising from the animal and human studies has now been translated into clinical service involving suitable screened patients with grade I and II knee osteoarthritis to undergo repair through platelet-derived extracellular vesicles (PEV) treatment. The result from ultra-structural and nanoparticle tracking analysis demonstrated a highly heterogeneous size and number of isolated PEVs, with a particle size range of 80-500 nm. *In vitro*, PEVs are found to stimulate chondrocyte proliferation. Pre-stimulation with IL-1β induced distinct shrinkage of chondrocytes but did not compromise the cell number. The condition is reversed by PEVs that induce chondroprotection and promote proliferation. The autologous PEVs were processed in the Good Manufacturing Practice (GMP) laboratory (Fig. 2), and the intra-articular injection was performed at the orthopaedic clinic. The

prospective cohort showed patients who received PEV exhibited the most improvement among other control groups. Our existing technology and facilities have developed an affordable biological treatment for early-stage osteoarthritis patients.

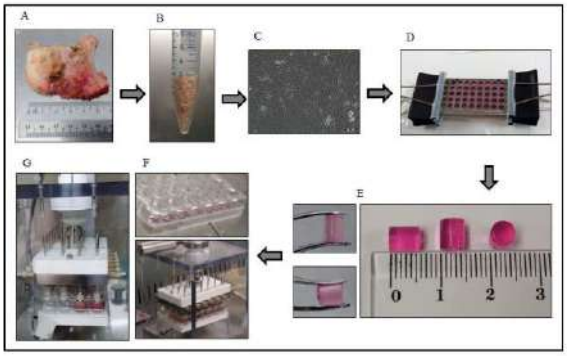

Figure 1: Dynamic compression of chondrocytes embedded in the scaffold. (A) Osteoarthritic proximal tibial cuts were collected during total knee replacement surgery. (B) Fine pieces of cartilage tissue. (C) Culture of chondrocyte cells in a 2D environment. (D) Mould of agarose seeded chondrocytes cells scaffold. (E) The size of each scaffold was 5mm x 5mm (diameter x height). (F, G) Bioreactor for the dynamic compression of the chondrocyte-embedded scaffolds.

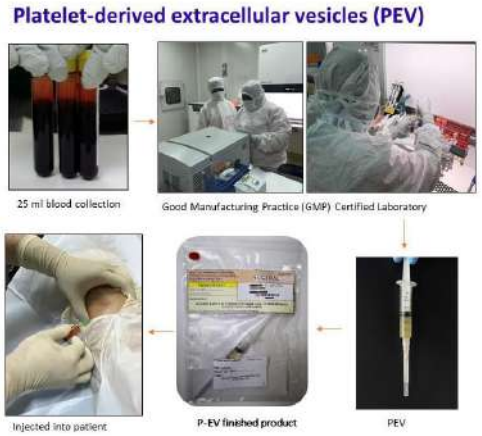

Figure 2: Isolation and intra-articular platelet-derived extracellular vesicles (PEV) injection.

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Temperature-responsive polymeric reagents for extracellular vesicle isolation and analysis

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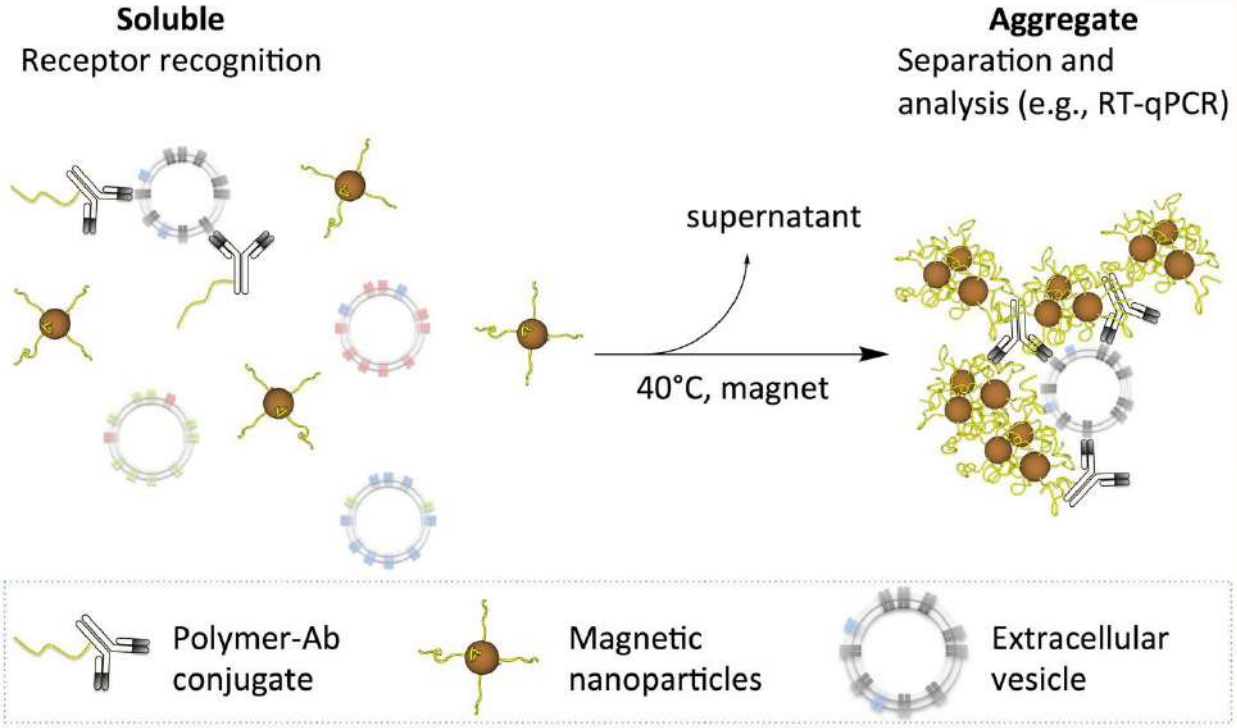
Abstract:

Extracellular vesicles (EVs) are secreted by cells, including disease cells, to mediate intercellular communication; therefore, they can potentially be utilized for delivering therapeutics, diagnosing diseases, and facilitating life science research. Characterization and isolation methods serve as the foundation for the EV technology development. However, current mainstream separation methods such as ultracentrifugation might involve complex procedure, are time consuming, exhibit limited capacity, and result in moderate purity as well as yield[1]. To enable efficient EV isolation to facilitate the downstream analyses, we have developed a temperature- responsive polymeric binary reagent, consisting of temperature-responsive magnetic nanoparticles and polymer-antibody conjugates that can transition from hydrophilic nanoscale reagents to microscale aggregates in response to temperature stimuli[2-4]. The reagent system decouples the recognition component (polymer-antibody conjugate) from the separation component (magnetic nanoparticles) to leverage the diffusion and instantaneous binding advantages of small nanoscale reagents. Then, transitions to micron-sized aggregates with high magnetophoretic mobility for the rapid separation.

EVs from human semen were used as a model analyte by targeting tetraspanin markers CD81, CD9, and CD63[2]. We assessed the efficiency of EV purification by quantifying microRNAs (let-7b and miR-29b) known to be carried by EVs from semen by RT-qPCR and normalized to the levels of spike-in control. Compared to an equivalent volume of untreated EVs or EVs subjected to the isolation protocol using control antibodies which do not specifically select for EV, let-7b and miR-29b were detected at 32- and 178-fold higher concentrations, respectively, in the specifically selected sample. The reagent system was also utilized in conjunction with transmembrane immunoglobin and mucin domain-4 (TIM-4) to isolate EVs from bovine milk by target phosphatidylserine (PS). To evaluate the isolation process, let-7b, found in high abundance in bovine milk EV, was used. An equivalent volume of unprocessed milk was also analyzed to estimate capture efficiency. Ct values for let7-b were normalized to the miR-cel-39 spike-in control, added before RNA extraction of the captured EVs. Compared to the control group, mouse IgG1 κ isotype, TIM-4 construct isolation led to more let-7b, with 4 – 13 folds increase. The EV isolation efficiency was estimated to be 51 – 77%. Taken together, these results indicate that the binary reagent system with anti-tetraspanin antibodies or PS selectively isolated EVs. Compared to the Dynabeads®, which requires a 2-day separation process, the temperature-responsive binary reagent system isolated EV rapidly via 4-step within 90 minutes.

KEYWORDS: bioprocessing, biomarkers, therapeutics, smart polymer, magnetic separation



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Title: Extracellular vesicles from human right atrial appendage stromal cells are cardioprotective

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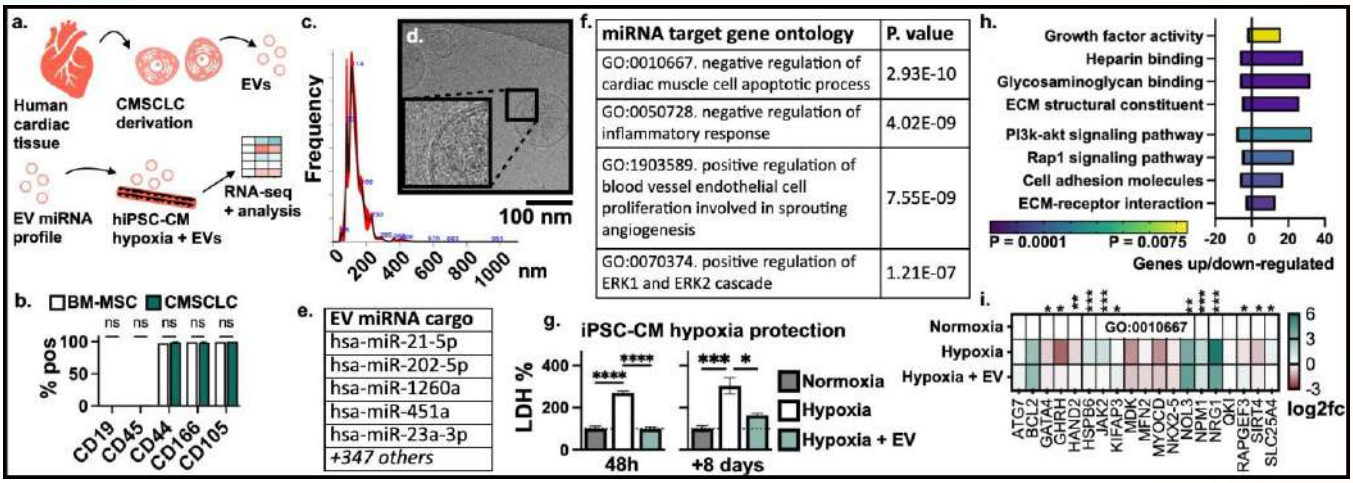
Abstract:

Introduction: Extracellular vesicles (EVs) from bone marrow (BM), adipose, umbilical and other mesenchymal stromal cells (MSCs) have been explored for use as therapeutics in myocardial infarction (MI). We isolated an MSC population from the right atrial appendage (RAA) of human heart tissues and previously showed their efficacy in mouse MI (Czosseck, 2022 j.jconrel.2022.10.057). Here we evaluate the cardioprotective effects of their EVs on hypoxic human cardiomyocytes, and examine their cargo and mechanisms of action.

Materials and Methods: Cardiac MSC-like cells (CMSCLCs) were isolated from the RAA (N = 6 separate donors) and cultured in aMEM with FGF2. Flow cytometry was used to confirm surface marker expression. EVs were isolated by ultracentrifugation of conditioned culture media for 100,000 g for 16 hours at 4 ˚C. EV count and size was quantified by nanoparticle tracking analysis (NTA) and cryoEM. EVs were temporarily added (67ng/µl, 48h) to hypoxic (1% O2), human induced pluripotent cell-derived cardiomyocytes (iPSC- CMs) to assess their protective effects. LDH release was used to measure the degree of iPSC-CM injury at early (48h) and late (8d post-reoxygenation and EV removal) timepoints. CMSCLC EV miRNA content was profiled using Qiagen miRNome and RNA-seq was used to compare hypoxic EV-treated iPSC-CMs against hypoxic controls. IRB approval was obtained from Taipei Medical University, protocol N201910027.

Results and Discussion: A summary of the experimental design is shown in (a). CMSCLCs were isolated from human donor cardiac tissues (a). Cells were positive for CD44, CD166 and CD105, and negative for CD19 and CD45, comparable to BM-MSCs (b). EVs were isolated and profiled. NTA (c) showed a mean particle size of 104.7 ± 11.2 nm, and cryoEM (d) showed spherical vesicles with lipid bilayer membranes, confirming EVs. miRNA cargo was analysed (e), revealing several highly expressed miRNAs including miR-21-5p, 202-5p, 1260a and others. miRNA target gene pathway analysis (f) revealed several highly significant pathways including reduction of cardiomyocyte apoptosis, anti-inflammation, ERK1/ERK2 activation, and angiogenesis. Hypoxic human iPSC-derived CMs incubated with EVs showed strong preservation of viability, with no increase in LDH after 48h hypoxia, or 8 days after reoxygenation injury (g). EVs were removed after the hypoxia period. RNA-seq of iPSC-CMs showed several significantly affected biological pathways (h, upper) and KEGG pathways (h, lower) with EV incubation compared to hypoxia and vehicle control. Analysis of GO:0010667 (i) confirmed that EVs preserved more normal expression of CM survival/apoptosis-related genes, including higher GATA4, HAND2 and NOL3.



a, experiment design; b, CMSCLC surface markers; c, example NTA of CMSCLC EVs; d, EV cryoEM. e, top 5 highest miRNAs; f, highly significant predicted miRNA target pathways; g, LDH release from normoxic, hypoxic or hypoxic + EV iPSC-CMs after 48h hypoxia and 8d following reoxygenation ($n \geq 8$ replicates per group); h, selected highly significant biological pathways (BP) and KEGG pathways between hypoxia and hypoxia + EV groups; i, selected genes in GO:0010667 ns, not significant, * $p \leq 0.05$, *** ≤ 0.01 *** ≤ 0.001, **** ≤ 0.0001. $n \geq 3$ for all miRNA/RNA-seq.

Conclusions: The significant preservation of iPSC-CM viability observed in our study suggests that these EVs could be a promising therapeutic strategy for treating MI in the future. We are currently confirming activation of downstream anti-apoptotic, pro-survival pathways, and examining methods for EV delivery.

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Structured soft polymers as functional biomaterials

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Abstract:

Hydrogels have been used as biomaterials for the delivery of therapeutics for decades now. They find use as wound dressings, contact lenses and more recently have been employed for the delivery and retention of biologically active entities such as cells into the body. The complexity of the polymers from which the majority of hydrogels are formed, in addition to the potential for structuring on multiple length scales means that there is still significant potential to innovate, tuning mechanical properties, biodegradation and even controlling biological environments [1,2]. This talk will discuss how we have taken polymers with known toxicity profiles and modified them through physical and chemical processes in order to produce materials that can deliver drugs over a sustained period of time (>6h on the surface of the eye) [3] or even act as sinks for growth factors or as surface lubricants. I will discuss how we have used shear-structuring to develop gellan-based materials for the alleviation of severe dry eye and the prevention of scarring following microbial keratitis [4]. In addition, I will report how we have created composite materials that not cover a larger surface area than existing sprays [5], but also exhibit muco-adhesive properties. These sprays have been used to prevent viral infection [6] and also to mediate inflammation and reduce scarring [7] in the mouth of patients with epidermolysis bullosa. Notably, all of the technologies in this presentation have been manufactured in a way that has enabled them to reach first-in-human trials, with two of the technologies being currently available commercially.

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Preparation of sub-100-micron calcium-alginate microspheres using nitrogen flow focusing: dependence of spherical shape on gas streams

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Abstract:

We developed a miniature gas-liquid coaxial flow device using glass capillary tubes, with the aim of producing sub-100 micron calcium alginate microspheres with it. However, at different collection distances and flow rates of nitrogen and alginate solutions, we obtained several calcium alginate particles with different shapes. We found that monodisperse, spherical particles (microspheres) can only be obtained within a certain range of gas flow rates and corresponding collection distances. For particles of sub-100 micron size, gas flow rate and collection distance are critical for spherical shape formation. We reasoned that for a droplet of this size, surface tension would dominate the flow of the droplet into the calcium chloride solution, rather than gravity, upon contact with the liquid surface. We also used acetaminophen (the active ingredient of Pronaton) as a model drug microsphere as a drug carrier as an example of the application of this microsphere. We used two devices to prepare large (~150 μm) and small (~70 μm) drug-loaded microspheres. We complexed drug-loaded microspheres with chitosan of different molecular weights to study the effect of microsphere size and chitosan molecular weight on their in vitro drug release.

KEYWORDS: calcium-alginate microspheres, microencapsulation, Gas-liquid coaxial flow, gelation, polyelectrolyte complex, droplet impingement

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A Biomaterial Prospective on Gasotransmitter-Induced Therapeutic Angiogenesis

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Abstract

Gas transmitters viz., NO, CO and H2S, have materialized as strategic players in the regulation of various pathophysiological functions and have stimulated the development of gas therapy for various pathogenesis. The lack of gasotransmitters production have been implicated in various diseases such as hypertension, endothelial dysfunction, myocardial infarction, ischemia and impaired wound healing as they are involved in the regulatory action of angiogenesis. A clear insight on the regulatory mechanisms and breakthroughs in gasotransmitter therapy has sparked new hope for treatment of vascular impairment. However, the unstable nature and poor target specificity of gas donors limit the complete efficacy of drugs. In this regard, biomaterials' alluring properties such as biocompatibility and porosity make them superlative candidates as drug carriers to tunably deliver the gas transmitters for therapeutic angiogenesis. In general, the gas molecules exhibit beneficial effects at relatively low concentrations, whereas the sudden release of high-dose molecules can produce toxicity. Therefore, preferably polymeric biomaterials are used that can regulate the controlled release of the gaseous molecules in order to avoid the cellular dysfunctions. Despite the remarkable development in therapeutic gas transmitters, the development of novel and potent drug delivery approaches for the gas transmitters will facilitate a better understanding of the signaling molecule and eventually accelerate its clinical application.

Keywords: Gas therapy; Angiogenesis; Wound healing; Nitric oxide (NO); Polymeric Carriers

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Biotribology of Biomaterials: Studies from Total Joint Implant to Orthokeratogy

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Abstract:

There are many biotribological systems in the human body, such as the biotribology between the knee joints, the cornea and the eyelid, different oral organs, or the muscle and tendon during exercise. When biotribological system is damaged due to sickness or aging, artificial biomaterials or devices are required for replacement. For example, damaged knee joints are replaced with artificial joints, contact lenses/orthokeratology lenses are needed for vision correction, artificial tears are applied for patients with dry eye symptom, or artificial saliva is required for Xerostomia patients. When a biomaterial is implanted into or combined with biological system, several characteristics of biomaterials need to be considered, including the physico-chemical properties, the interface reaction as well as the mechanical changes between the surface of the biomaterial and the contact tissue. It is common to observe that the lubrication of implanted biomaterials decreases resulting in infection or tissue damaged followed by the failure of implantation. Therefore, the evaluation of biotribological characteristics of biomaterials is required before implantation. My research has been focused on investigating the biotribology of biomaterials in artificial joints, contact lens, and artificial saliva. The results showed that proteins play a critical role for influencing the biotribological properties of implanted biomaterials. By studying the relationship between protein deposition and the friction coefficient of implanted devices, we could screen some biomaterials that can increase the lubrication and decrease the friction between implanted devices and tissues resulting in the reduction of biological reactions and prolong the lifespan of implanted devices.

KEYWORDS: biotribology, friction, lubrication, total joint implant, orthokeratogy

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3D/4D Printing of Composite/Hybrid Structures for Tissue Engineering

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Abstract:

Additive manufacturing, popularly known as "3D printing", provides a powerful manufacturing platform for many industries and can fabricate complicated 3D objects, nonporous or porous, by depositing material/materials in a layer-by-layer manner. 3D printing has been increasingly used in biomedical engineering, particularly in the tissue engineering field [1]. 3D printing technologies comprise an array of technologies: liquid-based, filament- or paste-based, and powder-based technologies. Using smart materials and with innovative designs, 4D printing uses 3D printing technologies to produce dynamic structures that can change their shape, property, and/or function under external stimulus/stimuli during their service time. Using inks that contain living cells, 3D/4D bioprinting creates living structures for different purposes (cancer tissue models, tissue engineering, organ-on-a-chip, etc.) in the biomedical field. Since its emergence more than 30 years ago, tissue engineering has been dominated by the scaffold-based tissue engineering approach, with biodegradable porous scaffolds providing conducive microenvironments for cells and playing vital roles for cell adhesion, proliferation and differentiation and also new tissue formation. Compared to other fabrication techniques, 3D printing has many advantages for scaffold production, such as control of pore shape, size, porosity, etc. It can use patients' own medical imaging data to produce personalized products for individuals, as illustrated in Fig.1. 3D printing technologies greatly improve our ability to fabricate a variety of complex and customized biomedical products accurately, efficiently, economically and with high reproducibility. However, finding or developing suitable biomaterials appears to be a bottleneck for the advancement of 3D/4D printing in tissue engineering [1].

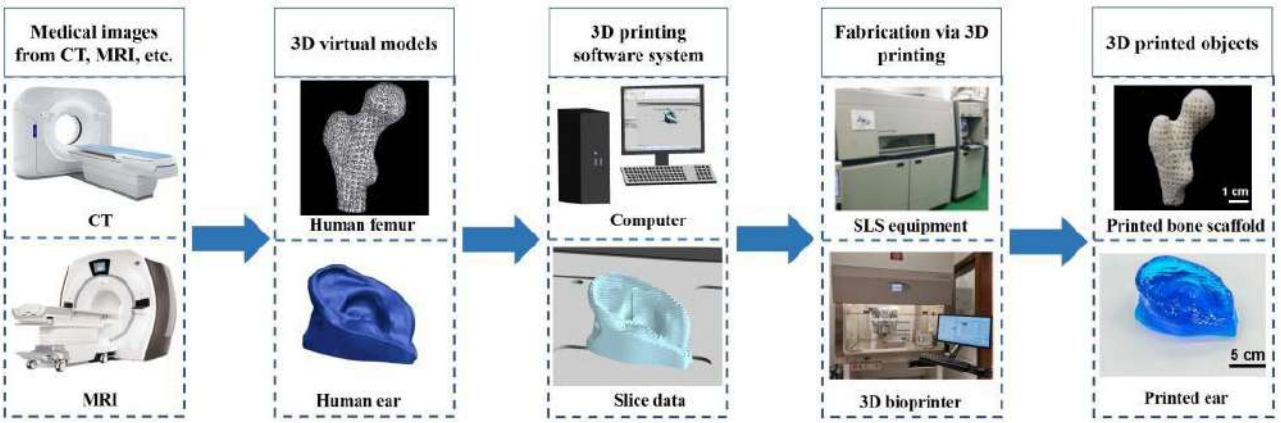

Fig.1 3D printing in tissue engineering

Different 3D printing technologies have different requirements for the materials/inks to be used, and in most situations these requirements are highly demanding. The requirements for 3D printing materials/inks in tissue engineering include printability, biocompatibility, biodegradation properties, and mechanical properties of printed products. Biocompatibility is of paramount importance for a material in tissue

engineering applications but it becomes highly important only when the material can be 3D printed into useful and usable structures. We have investigated / are investigating several 3D printing technologies, such as selective laser sintering (SLS), cryogenic extrusion 3D printing, and digital light projection (DLP), for fabricating advanced tissue engineering scaffolds and cell/scaffold constructs for the regeneration of bone, osteochondral tissue, blood vessel, etc. For example, for 3D printing of bone tissue engineering scaffolds via SLS, bioactive calcium phosphate (Ca-P)/poly(hydroxybutyrate–co-hydroxyvalerate) (PHBV) nanocomposite was developed [2]. Also for bone tissue engineering, nano-biphasic calcium phosphate bioceramic (BCP: a blend of hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP)) was processed into 3D scaffolds via DLP 3D printing [3]. For cryogenic extrusion 3D printing of scaffolds for osteochondral tissue regeneration, graded scaffolds involving β-TCP/poly(lactic-*co*-glycolic acid) (PLGA) nanocomposite was printed [4]. For shape-morphing bone tissue engineering scaffolds, β-TCP/poly(D,L-lactide-*co*-trimethylene carbonate) (PDLLA-*co*-TMC) nanocomposite was made and printed [5]. To obtain complex shape-morphing structures, alginate (Alg) and methylcellulose (MC) blends were investigated; and new mechanisms for changing scaffolds shapes were developed [6]. For blood vessel regeneration, self-folding bilayer scaffolds were 4D printed [7]. This talk will present some of our work on 3D/4D printing of composites/hybrids for tissue engineering. It will provide design guidelines and practical approaches in developing composites/hybrids for printing, as well as their 3D/4D printing into 3D structures.

Keywords: 3D printing, 4D printing, bioprinting, composite, scaffold, graded, shape-morphing

Acknowledgements: Min Wang's research in 3D printing and tissue engineering has been supported by research grants awarded by Hong Kong's Research Grants Council (RGC), National Natural Science Foundation of China, and The University of Hong Kong (HKU).

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2023 ISOMRM & BCRS
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Monodisperse cell-laden microgel droplets for cartilage tissue engineering

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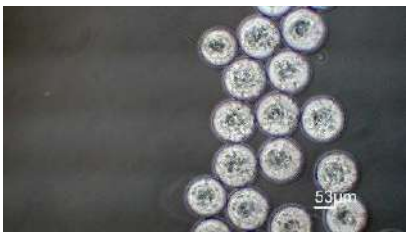
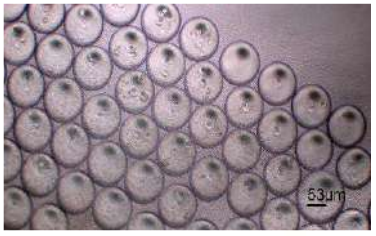
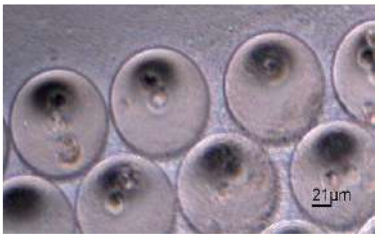
Abstract

Droplet-based microfluidics can produce monodisperse droplets with precisely controlled size and tailor the internal structure for cell encapsulation in a three-dimensional microgels, thus mimicking the i*n vivo* microenvironments for various biological applications, especially tissue engineering. Microfluidics provides safe, accurate, reliable, and cost-effective methods for encapsulating different stem cells, gametes, biomaterials, biomolecules, reagents, genes, and nanoparticles inside picoliter-sized droplets or droplet-derived microgels for different applications. The encapsulation of cells in specifically designed aqueous phase of a microfluidic system can provide profound understand of cell to cell and cell to extracellular matrix interactions, also can be used to regulate various cell behaviors. Herein, we describe droplet-based microfluidic platforms in which cells are grown in aqueous microcompartments separated by an inert perfluorocarbon carrier oil. Thereafter, the viability of the encapsulated human chondrosarcoma cells (SW1353) is assessed by incubating and monitoring cell growth. A premixed cell suspension is made consisting of 5 % gelatin in aqueous cell media. The gelatin droplets were polymerized by cross-linking with transglutaminase to form solid hydrogel microparticles. Cell viability is an important concern in encapsulation systems. To assess this, the cell-laden gelatin droplets are incubated for 1 weeks. The results show that the system in the configuration presented in this application note is well suited for use in cartilage tissue engineering. The cells are shown to survive well the encapsulation process, with no visible adverse effect suggesting no material incompatibility.

Keywords: Droplet, Microfluidic, Cell Encapsulation, 3D Cell Culture, Cartilage Tissue Engineering







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Antioxidant nanoparticles that scavenge the intestinal ROS lead to health

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Abstract:

Reactive oxygen species (ROS), which are inevitably produced by oxygen respiration for life to gain energy, are essential signaling molecules at normal levels but overproduced ROS oxidizes cellular proteins, lipids, and genes and causes various diseases. Natural antioxidants such as vitamins C and E and various synthetic antioxidants have been developed to reduce the involvement of ROS in diseases, but none of them achieved a remarkable medicinal effect. We have designed amphiphilic block copolymers, in which antioxidant moieties were covalently introduced in the hydrophobic segment. The obtained block copolymer spontaneously forms polymer micelles in an aqueous solution. We avoided toxicity and suppressed inflammation by covalently introducing low molecular weight antioxidants into a molecular assembly using polymer micelles as a platform (Fig.1). Since the designed nanoparticle type antioxidant (abbreviated as RNP) significantly reduces their toxicity, it shows a remarkable therapeutic effect on various oxidative stress-related diseases such as cancer, Alzheimer's disease, and ulcerative colitis. Here, we have found that antioxidant nanoparticles localized in the gastrointestinal tract ameliorated stress-induced depression and athletic performance by eliminating gastrointestinal ROS by oral administration. For example, in stress-induced depression model mice, intense inflammation of the gastrointestinal tract was induced, but it was strongly suppressed by the oral administration of RNP. As a result, the increase in stress hormones in the blood and the level of neurologically-related proteins in the brain were suppressed, and the condition of depression was improved (Fig. 2). When mice were made to run on the treadmill until they could no longer run, their GI tract was damaged significantly. After oral administration of RNP, the intestinal damage was recovered significantly and the running time was extended by 40-50% compared to normal mice . Therefore, we conclude that the effective elimination of ROS that is overproduced in the GI tract is extensively involved in maintaining health. The depression experiments have been done

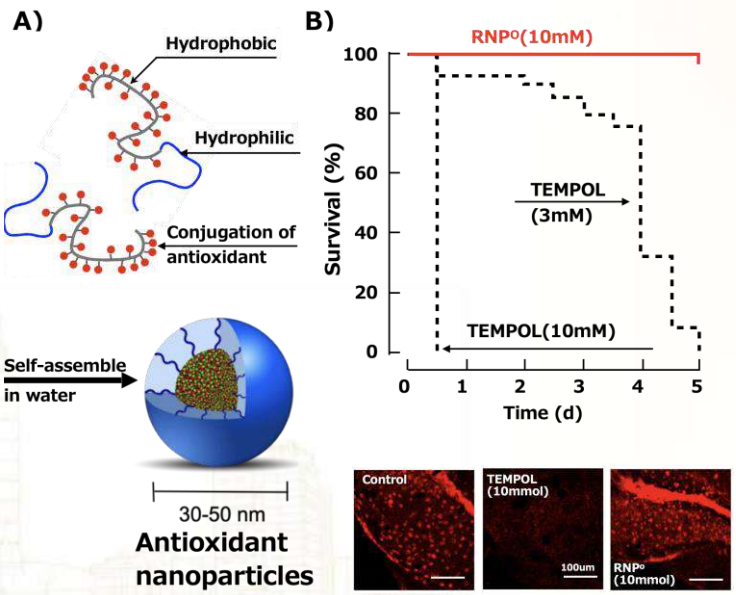

Fig. 1 Design of a new antioxidant polymer that selectively removes bad active oxygen species. A) Covalently conjugates antioxidants (●: TEMPO) to a hydrophobic segment and self-assembles in water, with a size of several tens of nm (abbreviated as RNP). B) Toxicity to zebrafish. Survival rate on the top, mitochondrial staining photograph on the bottom. (Since nanoparticles are difficult to enter normal cells, RNP is completely non-toxic in areas where zebrafish are highly toxic with low molecular weight antioxidants, avoiding the previously problematic mitochondrial damage of antioxidants.) (***Bioconjugate Chem***., 20, 1792(2009)) & ***Molecular Pharm.,*** 13, 3091(2016).

by Mr. Naoki Saigo and Dr. Yutaka Ikeda. The exercise experiments were done by Dr. Takuto Toriumi. The author appreciates their collaboration.

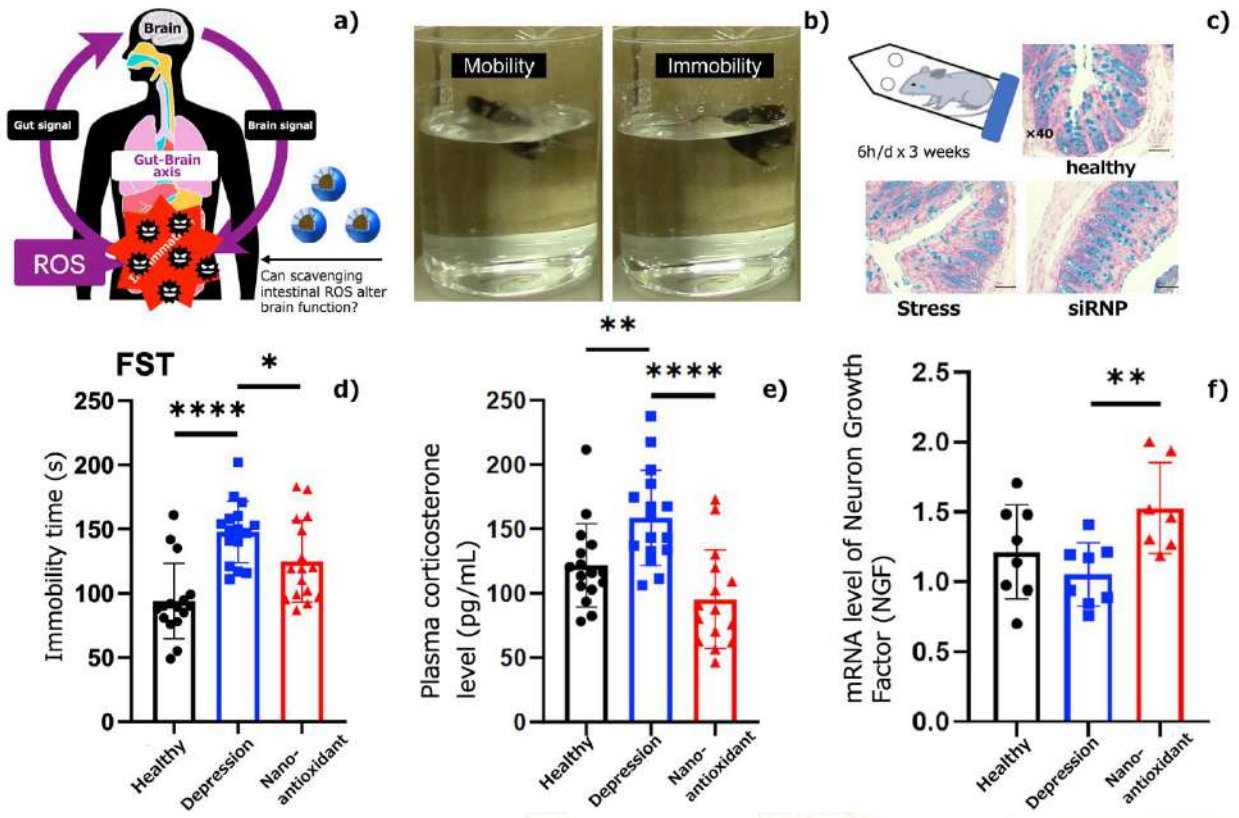

Fig.2. Oral administration of antioxidant nanoparticles (RNP) in chronic stress-induced depression model (CRS) mice. (a) Our hypothesis to recover depression by orally-administered antioxidant nanoparticles via the gut-brain axis. b) Photos of the forced swimming tests for evaluation of depression behavior. c) Preparation of CRS mice and alcian blue staining of colon tissue (The CRS group strongly caused damage in the colon, while the RNP group did not.) (d) Immobility time by forced swimming test (The CRS group increased immobility time affected by the depression, but the RNP group decreased it). (e) Blood corticosterone level (increased in the CRS group but not in the RNP group). f) The level of Newron growth factor (RNP group increased compared with the CRS group.) (Biomaterials, 295, 122053(2023))

KEYWORDS: Oxidative stress, Reactive oxygene species (R0S), Self-assembling polymer micelle, Nanopartice antioxidants, Depression, Gut-brain axis, Exercise-induced gastrointestinal syndrome (EiGS)

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MODULARIZED MICROFLUIDIC-BASED BIOREACTOR FOR MULTIPLEX CELL STIMULATION

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Abstract

We develop a bioreactor providing multiplex electrical and tensile stretching stimulation to the cells. The modularized microfluidic-based bioreactor design (Figure 1) consists of an microfluidic-based chip and an external actuation module. The microfluidic-based chip was assembled by three layers. Where cover layer is designed for cell culture wells, stretchable conductive PPy/PDMS layer is used for physical stretching and electrical stimulations to the cells, and bottom layer with microchannels. The electric stimulation can be simply achieved by applying the electric potential to the copper electrode in the chip (Figure 1). To further apply both electrical and stretching stimulations to the cells simultaneously, PPy/PDMS composite layer was used. As shown in Figure 2 (top), the conductive polypyrrole (PPy) was coated on a corrugated PDMS surface. Thus, the conductive PPy layer remains intact under stretching. During stretching, as displayed in Figure 2 (bottom), as the microchannels in the bottom layer vacuum to a negative pressure by a pump, the PPy/PDMS layer were sucked into the microchannel and case stretching stimulation to the cells (stage I to II & III). Once the vacuum pump is off, the PPy/PDMS layer returns to original stage and complete on stretching cycle. The PPy/PDMS tensile stretching% with different microchannel depth was shown in Figure 3. The tensile stretch can be controlled by the microchannel depth, with the 1.6 mm depth, the tensile stretch is 11.9 to 14. 0%. After stimulation, the bioreactor was placed in the oven for cell culture to evaluate the performance for multiplex cell stimulations.

KEYWORDS: Bioreactor, Electrical stimulation, Tensile stretching stimulation, polypyrrole conductive layer

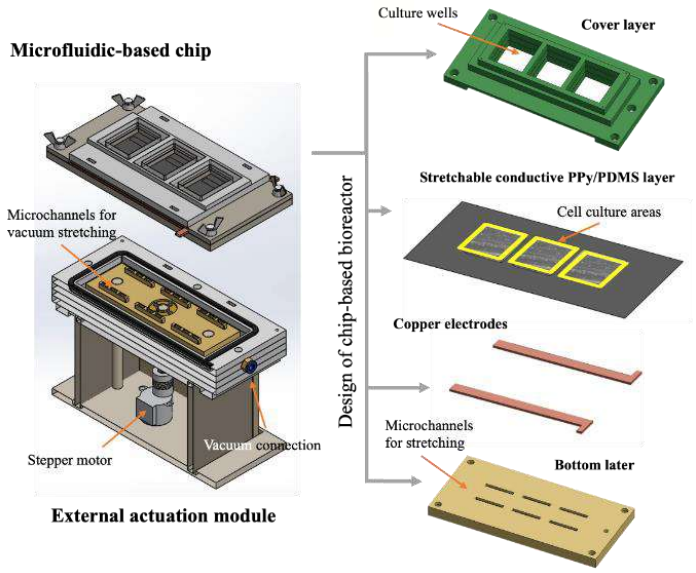

Figure 1: Modularized microfluidic-based bioreactor design

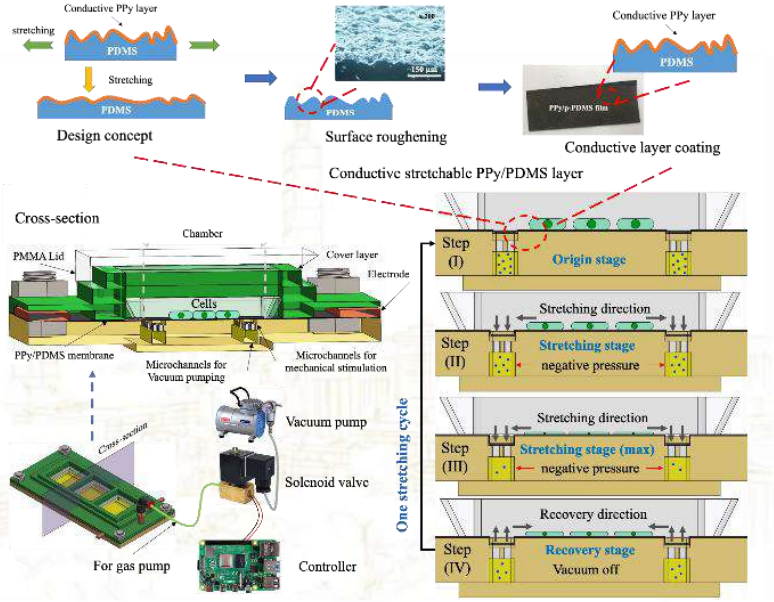

Figure 2: Illustration of multiplex electrical and stretching stimulations in microfluidic-based bioreactor.

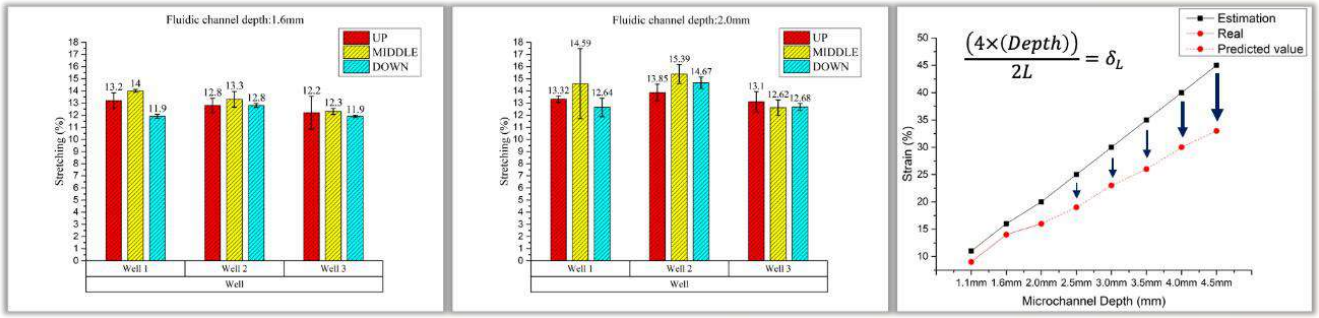

Figure 3: PPy/PDMS tensile strain % for different culture wells with 1.6 (left) and 2.0 (middle) mm depth microchannel. And tensile strain with 1.1~4.5 mm depth microchannel (right). Difference between stretching% between experiment and theoretical model are observed. Which is presumable due to the unsmooth microchannel surface causing gas leakage during pumping.

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Biologically inspired scaffolds for neural tissue regeneration

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Abstract:

The microenvironment that surrounds cells plays critical roles in dictating cell fate and tissue regeneration. Biophysical signals, such as extracellular matrix architecture and compliance affect cellular response. In combination with biochemical signals from drugs, growth factors, nucleic acids and/or cells, synergistic effects on directing cell phenotypic changes and tissue regrowth are often seen. Here, we will present our approaches towards designing scaffolding constructs that recapitulate important characteristics of the extracellular microenvironment to understand and direct cell fate. We will also share our findings of combining the appropriate biophysical and biochemical signals into tissue-engineered scaffolds to promote nerve regeneration and remyelination in the central nervous system.

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Bioanalytical Applications of Engineered Intrinsically Disordered Proteins

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Abstract:

Intrinsically disordered proteins (IDPs) serve myriad regulatory functions in cells across the kingdoms of life, many of which are associated with collective properties of ensembles of IDPs such as liquid-liquid phase separation (LLPS) and formation of chemically-specific compartments.

Recombinantly expressed, engineered IDPs have been explored for a wide range of bioanalytical applications including preparative and analytical separations. We focus on the use of one particular class of IDPs –the elastin-like polypeptides (ELPs) and their fusion proteins—as a particularly useful bioanalytical reagents. ELPs exhibit lower critical solubility temperatures in aqueous and their temperature-triggered LLPS can be easily tuned, predicted and exploited in a wide variety of applications. We have developed ELPs for biodetection applications in which the LLPS behavior of these IDPs are used either in sample preparation (extraction, preconcentration) or transduction of biomolecular recognition. This talk will present our fundamental studies of LLPS of ELPs as a model class of recombinant engineered IDPs, the use of ELPs in extraction of medically important bioanalytes from complex samples, and the use of ELPs in the formation of molecular assemblies that change their visual properties in the presence of important biomarkers (transduction). Emphasis is on development of bioanalytical methods that enable low cost, pointof- care, medical diagnostics.

KEYWORDS: Intrinsically-disordered proteins, elastin-like polypeptides, biosensing, sample preparation, transduction

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Nanobioanalytical investigations of Extracellular Vesicles from secretome of macrophages - Possible implication in the treatment of fibrosis

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Abstract:

Keloids are a fibrotic and inflammatory skin disease characterized by an important accumulation of matrix tissue at the dermal level. They appear following a dysregulation of the healing process, itself controlled by many immune cells such as macrophages[1]. To solve keloids, we have launched the bioproduction of secretome from polarized macrophage subtypes (M1 & M2) and used it to evaluate the ability of macrophages to regulate the fibrotic phenotype of fibroblasts. The functional respectively anti-fibrotic and pro-fibrotic effects of M1 and M2 secretomes observed *in vitro* require biochemical and biophysical characterizations of these secretomes to establish their composition in proteins and extracellular vesicles (EVs).

EVs domain represents a promising field of interest due to their functional biological activities, especially in the cell-to-cell communication. However, many (pre)-analytical challenges must be faced in order to overcome the difficulty of measuring their inherently complex properties, such as polydispersity in size, their phenotype, dynamics of their release and uptake in recipient cells.

We have established an in-house NanoBioAnalytical (NBA) platform which can assist in the characterization of EVs subpopulations[2]. This platform brings sensitive detection in complex media of EVs in a multiplex format, based on Surface Plasmon Resonance Imaging (SPRi) and Atomic Force Microscopy (AFM), which magnify us their metrology, size distribution and morpho-mechanical properties. The tunable property of NBA platform offers further possibilities of biophysical characterizations, particularly the opportunity to couple with Raman spectroscopy by using an original gold biochip as a new core of this platform[3].

By developing a dedicated biochip and a specific analytical pathway to the exploration of the extracellular vesiculome of macrophages, we aim to decipher their role in the pro-inflammatory and pro-fibrotic properties mediated by macrophages subtypes.

KEYWORDS: Extracellular vesicles, Macrophages, Fibrosis, Surface plasmon resonance, Atomic force microscopy.

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2023 ISOMRM & BCRS
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Application of a genetically-engineered human macrophage cell to investigate cellular responses to nano/microplastics

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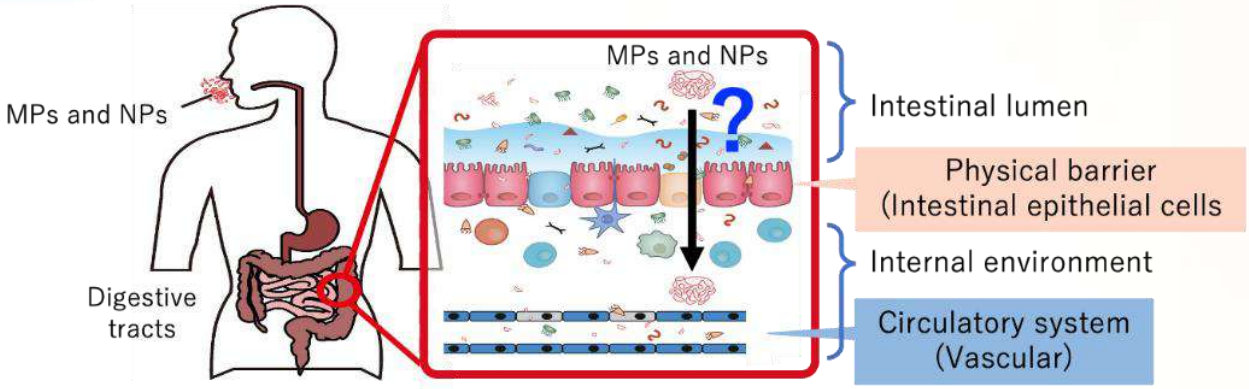
Abstract:

Microplastics (MPs) pollution is a global environmental problem. Potential risks have been considered regarding the effects of MPs on humans through the food chain. To understand the negative impacts of MPs on humans, it is essential to investigate the response of human cells to model plastic particles that mimic environmental MPs. Moreover, due to their small size, nanometer-sized plastic fragments have the undeniable potential to be uptaken into the body and distributed to a specific organ as seen in nanocarriers as a drug delivery system. Nanoplastics (NPs) with a small quantity and size, however, have limitations in collecting from oceans. To date, most studies on NPs have been conducted using polystyrene nanoparticles with a defined size and surface property to understand the body distribution and adverse effects in small organisms and fishes. In this study, we employed a genetically-engineered human macrophage (THP-1 cells) to investigate cellular responses to MPs and/or NPs of poly(ethylene terephthalate) (PET) and isotactic polypropylene (iPP) fragments. A chemiluminescent tag HiBiT® was introduced into the downstream of interleukin-1b (IL-1b) using CRISPR/Cas9 genome editing. Several methods for oxidation and mechanical pulverization have been conducted to obtain plastic fragments. Photo-oxidation and ultrasound pulverization or accelerated oxidation reaction decreased the particle size of PET and iPP fragments, making them irregularly shaped, and increased their crystallinity. In addition, an increase in the carboxyl group introduction ratio and negative surface potential were observed, suggesting surface oxidation. Both PET and iPP fragments induced a higher level of IL-1b secretion on M1-polarized macrophages than non-oxidized ones. Moreover, iPP fragments with a nanometer size were uptaken by human intestinal epithelial cell lines (Caco-2 cells) probably due to endocytosis. In conclusion, several oxidation and mechanical pulverization techniques enable the efficient preparation of model plastic particles with different sizes, shapes, and surface and bulk properties, and are expected to provide new insights into the environmental threat of NPs and MPs on humans.



Acknowledgment: This work was supported by JST, CREST Grant Number JPMJCR21L6, Japan.

KEYWORDS: Genome Editing, Macrophage, Nano/Microplastics, Immune Response

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Using Functional Photoacoustic Imaging to Understand Pancreatic Tumor Hypoxia Dynamics during Treatment

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Abstract:

Photoacoustic (PA) imaging combines the advantages of both optical and ultrasound (US) imaging to enable high resolution *in vivo* imaging with increased imaging depth [1]. We developed a handheld dual-modality real-time US/PA imaging system (HARP) and used phantoms to assess its imaging capabilities *in vitro*. Next, we tested *in vivo* imaging of the contrast agent indocyanine green and cerebral functional imaging with electrical stimulation of the rat forepaw. In addition, we demonstrated that HARP could be used to image tissue oxygenation (SO_2) in a controlled hypoxia challenge. Hypoxia has been shown to promote tumor cell invasiveness, resistance to chemotherapy and radiotherapy, and immunosuppression and is an important factor in cancer treatment [2]. Therefore, we used HARP to monitor changes in the hypoxic tumor microenvironment during treatment of Pan02 and BxPC3 pancreatic tumor models. Tissue SO_2 was compared between untreated tumors and tumors treated with Irinotecan, Onivyde, Gemcitabine, and a combination of Gemcitabine and microbubble-mediated sonoporation. Decrease in hypoxia is a mechanism that can improve tumor therapy. Changes in tumor SO_2 can potentially be used to guide treatment or as an early indicator of tumor response to the treatment.

KEYWORDS: photoacoustic imaging, pancreatic tumor, hypoxia, tissue SO_2, Onivyde, microbubble, sonoporation

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Oral

September 1 (Friday)	
O-1	*Multi -Target Stem Cell Therapy in Companion Animal* **DVM. Min Koo, Konkuk University, Korea**
O-2	*Fabrication of Surface Modified Micro/Nano Spherical Hydrogel as 3D Cell Culture Scaffolds* **Prof. Chun-Yen Liu, National Cheng Kung University, Taiwan**
O-3	*Photo-crosslinked gelatin methacryloyl (GelMA)/hyaluronic acid methacryloyl (HAMA) composite scaffold using anthocyanidin as photo-initiator for bone tissue regeneration* **Ms. Susaritha Ramanathan, National Taipei University of Technology, Taiwan**
O-4	*Gastric models with biomimetic mechanical, topographical and fluid dynamic properties by 3D printing for drug development* **Prof. Ming-Hua Ho, National Taiwan University of Science and Technology, Taiwan**
O-5	*Enhancing NSCLC survival prediction with multi-modal deep radiomics* **Prof. Nguyen Quoc Khanh Le, Taipei Medical University, Taiwan**
O-6	*Exploration and Development of New Decellularized Matrix Hydrogels for Pulmonary Delivery in Rats* **Prof. Chen-Yu Kao, National Taiwan University of Science and Technology, Taiwan**
September 2 (Saturday)	
O-7	*Bioprinting of stimulus-responsive auxetic scaffold for enhanced cartilage regeneration under cyclic tensile force* **Mr. Yen-Hong Lin, China Medical University, Taiwan**
O-8	*Application of ultrahigh frequency transcutaneous electrical nerve stimulation for alleviation of neuropathic pain and neuroinflammation modulation in rat sciatic nerve chronic constriction injury* **Dr. Yu-Wen Lin, National Cheng Kung University Hospital, Taiwan**
O-9	*Effects of Photobiomodulation on Migration of Adipose-derived Stem Cells* **Mr. Mamadi Colley, Taipei Medical University, Taiwan**
O-10	*Comparison of the difference between orthokeratology cleaning solution with added polysaccharides and commercially available products* **Mr. You-Cheng Chang, National Taipei University of Technology, Taiwan**
O-11	*Investigation of Factors Altering Rheological Properties of Poloxamer-Based Thermo-Sensitive Hydrogel* **Prof. I-Cheng Chen, National Taipei University of Technology, Taiwan**
O-12	*Effects of different barbed suture implantation methods on facial tissue tension and displacement in minimally invasive medical cosmetic surgery* **Mr. Chia-Hsien Hsieh, National Taipei University of Technology, Taiwan**

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Multi -Target Stem Cell Therapy in Companion Animal

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Abstract:

Recently, animal hospitals have introduced stem cell therapy in a wide range of areas of chronic diseases such as vertebral disease[1], arthritis[2,3], chronic kidney disease, chronic gastrointestinal inflammation, keratoconjunctivitis sicca, and chronic oral inflammation. The animal stem cell therapy market is expected to grow by an average annual growth rate (CAGR) of 5.2%[4].

Cell therapy offers the possibility of treatment for diseases with limited therapeutic effects compared to conventional therapy. Early diagnosis, prevention, and symptomatic treatment have been the primary methods for treating incurable diseases or various chronic diseases that are caused by aging. Although the main mechanism of cell therapy has shown some positive results, the proper mechanism has not yet been fully revealed[5].

Stem cell therapy for pets does not always have good therapeutic effect, which depends on various conditions. Different stages of the diseases (i.e., early, mid, and late) and the nature of stem cells can influence the outcome. It is generally known that stem cell therapy is effective in the early to mid-stage of the disease, and the effect is limited when administered at the late stage of the disease.

In addition, since standardization of cell therapy for pets has not yet been established, the dosage and quality of stem cells administered may vary, which is insufficient to be recognized as standard treatment[5]. The development of cell therapy is difficult, and the only world-approved pet stem cell treatment is Stem Cure® of Sumitomo Dainippon Pharmaceutical[6].

As conventional treatments for the medical sign of a certain disease commonly focuses on single therapeutic drugs for a specific symptom, patients with multiple diseases are concerned about the side effects of the treatments. Stem cells and its therapeutic effects offer a rational alternative for such patients.

We are currently collecting more pet patient cases of stem cell therapy to establish a new standard for multi-target therapy, which will cover the way for alternative options, and eventually a new paradigm, to deal with complicated pet patients with multiple diseases.

KEYWORDS: Companion animal Chronic disease, Inflammation, Stem-cell therapy, Multi- Target Therapy

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Fabrication of Surface Modified Micro/Nano Spherical Hydrogel as 3D Cell CultureScaffolds

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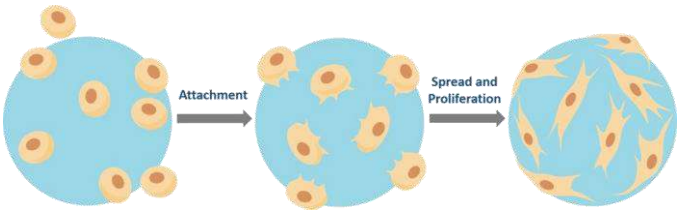
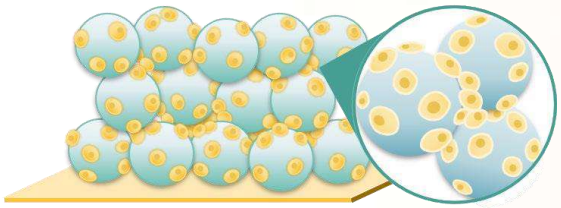
Abstract:

In view of high hydrophilicity, poly(HEMA) can absorb water or biological fluid which areused in a variety of applications such as soft contact lenses, carriers for enzyme and protein immobilization, in controlled drug delivery, cell implants, and so on. Besides that, poly(HEMA) is biocompatible and nontoxic. In order to enhance the adhesion of cell, we also introduce positively charged monomer DEAEMA. Besides that, chitosan coating is believed to be able to increase the interaction between poly(HEMA) particle and cell due to high biocompatibility. Furthermore, nanoparticle is commonly used in biomedical application, especially for drug delivery. Based on microparticles advantage where small particles only require small space to obtain monolayer cultures due to large surface-to-volume. We try using nanoparticle which has smaller size and higher surface area than microparticles for obtaining 3D tissue.

Microspheres have diverse applications in the field of tissue engineering, including the use of microcarriers to deliver drugs, cell encapsulation, fabrication of three-dimensional (3D) porousconstructs by sintering or solubility, and aggregation with cells to obtain 3D tissues. Self-assemble hydrogel microparticles are suitable for developing a scaffold for in vitro tissue model construction. This study demonstrates the synthesis of poly(2-hydroxyethyl methacrylate) (PHEMA) microparticles as scaffolds to construct 3D artificial tissues in vitro. Microsphere hydrogel scaffolds were synthesized via suspension polymerization. The synthesized average diameter of microspheres were around 500 μm. To realize the toxicity and biocompatibility of the synthesized micrometer-sized particles, Normal human dermal fibroblast (NHDF) cells were seeded onto microsphere scaffolds. **Figure 1** shows the cell growth process on microparticle scaffolds. To promote cell adhesion, cationic charged monomer [3-(methacryloylamino)propyl]trimethylammonium chloride (MPTC) was introduced into the microspheres. In addition, enhancement of cell growing via the surface coating of gelatin and chitosan onto the microspheres was investigated. Increase of MPTC content enhances the thermal stability and cell attachment obviously. The fluorescent images indicated that both cationic chargecontent and gelatin-coating elevated the growth of NHDF cells on microparticles. In terms of results, PHEMA microspheres with an optimal positive charge content of 2% MPTC demonstratehigh cell viability and proliferation rate in 3D scaffolds. Furthermore, gelatin-coated PHEMA microspheres with MPTC demonstrate better cell proliferation rate in 3D scaffolds than PHEMAmicrospheres only with MPTC. Based on the results, NHDF cell culture in 3D space was achievedusing PHEMA microsphere scaffolds in vitro.

KEYWORDS: Tissue engineering, microsphere, surface modification, cationic monomer, gelatin





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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Photo-crosslinked gelatin methacryloyl (GelMA)/hyaluronic acid methacryloyl (HAMA) composite scaffold using anthocyanidin as photo-initiator for bone tissue regeneration

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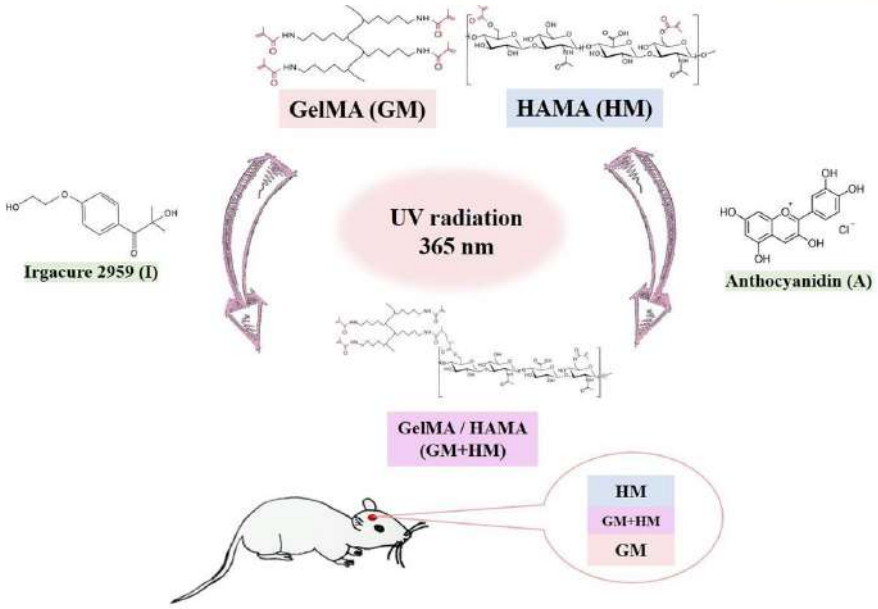
Abstract:

Gelatin and hyaluronic acid are natural biopolymers that are important components of soft tissues in the human body, and both have been used in several biomedical applications [1]. However, further functionalization is required to achieve certain properties, such as tunable mechanical properties and controllable degradation. To increase the applicability of gelatin and hyaluronic acid, methacrylic acid and a photoinitiator have been used to modify photo- crosslinkable gelatin methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA). GelMAand HAMA have been widely used in bone regeneration and tissue applications because of their controllable mechanical properties and ability to fabricate scaffolds [2]. One of the most commonly used photoinitiators is Irgacure 2959 (I2959); however, free radicals released from I2959 are cytotoxic. Therefore, two different photo-initiators, I2959 and anthocyanidin, are used in this study for scaffold fabrication to compare the structural properties of the scaffolds. In vitroand in vivo studies are performed to evaluate the biocompatibility of the scaffolds and their ability to promote the restoration of osteochondral defects. Additionally, other tests, such as fluorescence, degradability, bioactivity, and intracellular alkaline phosphatase (ALP) assays, are also performed.

KEYWORDS: Methacrylic gelatin, Methacrylic hyaluronic acid, Photo-cross-linking agents,Bone Regeneration, Anthocyanidin



Graphic abstract

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2023 ISOMRM & BCRS
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Gastric models with biomimetic mechanical, topographical and fluid dynamic propertiesby 3D printing for drug development

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Abstract:

To evaluate drug effectiveness before costly and time-consuming animal experiments and clinical trials, in vitro analysis is a required step in the development of drugs. However, 2D culture on traditional culture dishes is highly different from the real environment of the human tissues or organs, resulting in deviations in drug effectiveness. Thus, the in vitro biomimetic system was proposed to simulate the digestive tract environment of humans or the dynamic movement of substances in the digestive tract. However, the current in vitro digestive systems often lack the structural cues of human organs and cannot present all the biomimetic properties, such as mechanical, topographical, and fluid dynamic characteristics, at the same time.

In this study, a gastric simulation system was assembled with a stereoscopic gastric model. The gastric rugae structures appeared on the surface of gastric models by applying photo-curing 3D printing. The biomimetic mechanical strength was achieved by using photoresin composed ofcis-1,4 polyisoprene and several photo-reactive diluents. We optimized the resin composition in this study to balance the model stability, resolution, and printing efficiency besides gastric-mimicsoftness. H. pylori was cultured on 2D and 3D-printed models with a fluid flow similar to the emptying behavior of gastric juice, demonstrating the good biocompatibility of models. The results support that the rugae structures decreased the effects of antibiotics, the model softness influenced cell and bacterial attachment, and the fluid dynamic reduced the duration of medicinein the stomach. This study revealed that the novel 3D model was able to simulate the human stomach better than a convectional 2D model in the analysis of drug effectiveness.

KEYWORDS: Gastric model, 3D printing, Biomimetic Properties, Topographical Properties, Mechanical Strength, Photoresin

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Enhancing NSCLC survival prediction with multi-modal deep radiomics

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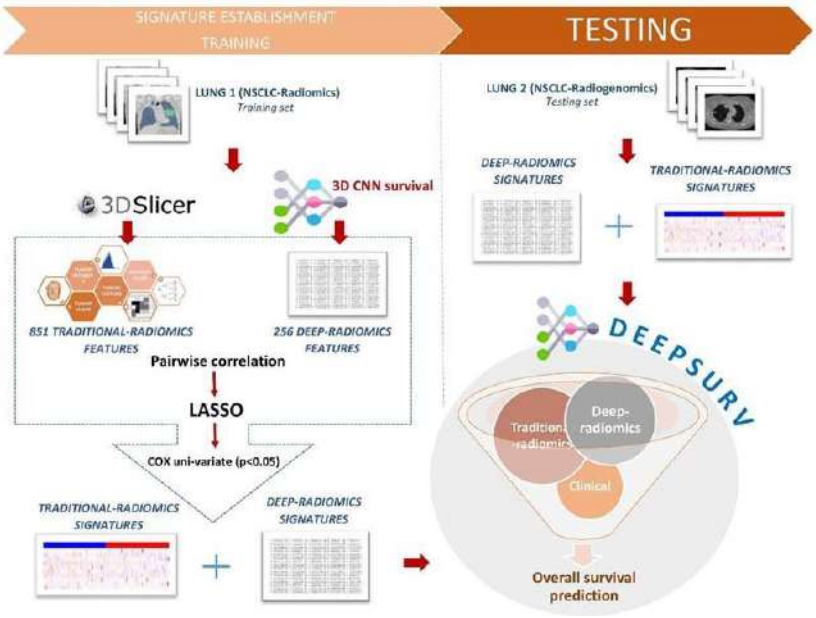
Abstract:

This study endeavors to employ a multi-modal deep learning approach for predicting survival outcomes in non-small-cell lung cancer (NSCLC) patients using CT-based radiomics. Data from two public repositories within the Cancer Imaging Archive (TCIA) were extracted, comprising 420 NSCLC patients in the Lung #1 training set and 516 in the Lung #2 testing set. A3D convolutional neural network (CNN) was implemented to extract 256 deep-radiomics featuresfrom CT scans for each patient. Through rigorous feature selection, radiomics signatures highly correlated with overall survival were identified. These deep-radiomics signatures, in conjunctionwith traditional-radiomics and clinical parameters, were input into the DeepSurv neural network.The Concordance Index (C-index) assessed model performance.

The composite model, integrating both traditional and deep-radiomics, outperformed single-parameter models in the Lung #1 training set. The most efficacious models integrated all three markers (traditional-radiomics, deep-radiomics, and clinical parameters), yielding a C-indexof 0.641 for the Cox proportional hazards (Cox-PH) model and 0.733 using the DeepSurv approach. The Lung #2 test set further corroborated these findings, with the tri-modal model achieving a C-index of 0.746 for Cox-PH and 0.751 with the DeepSurv approach. Notably, the DeepSurv method consistently surpassed the Cox-PH in predictive accuracy, with the highest performance observed in models integrating all three parameters, registering C-index values of 0.733 and 0.751 for training and testing datasets, respectively.

The DeepSurv CT-based deep-radiomics approach demonstrates superior prognostic capabilities compared to traditional Cox-PH models in predicting survival for NSCLC patients. The integration of multi-modal parameters further enhances model efficiency.

KEYWORDS: Non-small-cell lung cancer, Deep learning, 3D convolutional neural network, Survival prediction, Multi-modal approach, CT-based radiomics



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Exploration and Development of New Decellularized Matrix Hydrogels for Pulmonary Delivery in Rats

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Abstract:

At present, there is no established treatment for mitigating the acute respiratory distress syndrome (ARDS) caused by the Covid virus. However, one promising supplementary therapy gaining recognition in clinics and clinical trials involves the utilization of mesenchymal stem cells (MSCs). Both MSCs and extracellular matrix (ECM) materials possess immunomodulatory properties. Based on this, it can be postulated that ECM hydrogel derived from porcine organs holds potential as a viable candidate for creating a scaffold, enabling stem cells to reside in the lungs for a sufficient duration to exert their immunomodulatory effects on immune cells. This approach could be further explored as an adjuvant therapy for patients with COVID-19.

The basement membrane and lamina propria of pig bladder was delaminated, as previously described [1], and was referred to as UBM in this context. The other rest of the bladder was then referred to as sECM thereafter. The whole bladder, UBM, and sECM were decellularized as previously described [1,2]. The decellularization efficiency of decellularized tissues was confirmed by using dsDNA Quantification, DAPI Staining, Histological Staining, and DNA Electrophoresis. The amount of collagen and sGAG retention was studied using the assay kits. The protein composition of ECM was identified by LC-MS/MS. In vitro cytotoxicity and immunomodulatory of the ECM materials were evaluated by ELISA assay, and qPCR assay [3]. In vivo evaluations of the anti-inflammatory and regenerative effects of the dECM hydrogels and dental pulp stem cells (DPSCs) will be evaluated by using a rat acute lung injury model.

Various ECM hydrogels were prepared from the urinary bladder matrix (UBM) and a subtype ECM (sECM), and a whole bladder ECM(B-ECM). In the in vitro studies, all the hydrogels possessed nearly the same biochemical effects toward L929 viability and C2C12 differentiation. ECM degradation products and a co-culture system of MSCs and macrophages were developed to study the immunomodulatory properties of ECM and MSCs under septic conditions. The results showed that B-ECM degradation products could decrease pro-inflammatory and increase anti- inflammatory cytokines from macrophages. In an in vivo mimicking co-culture system, MSCs cultured on B-ECM hydrogel exhibited immunomodulatory properties at both gene and protein levels. Both B-ECM degradation products and MSC conditioned medium supported the wound healing of alveolar epithelial cells.

These results could preliminarily indicate that the use of sECM should no longer be ignored, and B-ECM could be a promising substitution for UBM hydrogels, eliminating the need for a time-costing delamination process, as well as increasing the possibility for mass production. Also, the results from the study could offer a basis for the investigation of immunomodulation by ECM and MSCs before conducting in vivo experiments, which could later be applied in regenerative medicine. Therefore, it can be hypothesized that ECM hydrogel from porcine organs can be served as a promising adjuvant therapy for COVID-19 patients.

KEYWORDS: Extracellular Matrix, Decellularization, Hyrdogels, Pulmonary Delivery, Immunomodulatory Properties

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Bioprinting of stimulus-responsive auxetic scaffold for enhanced cartilage regenerationunder cyclic tensile force

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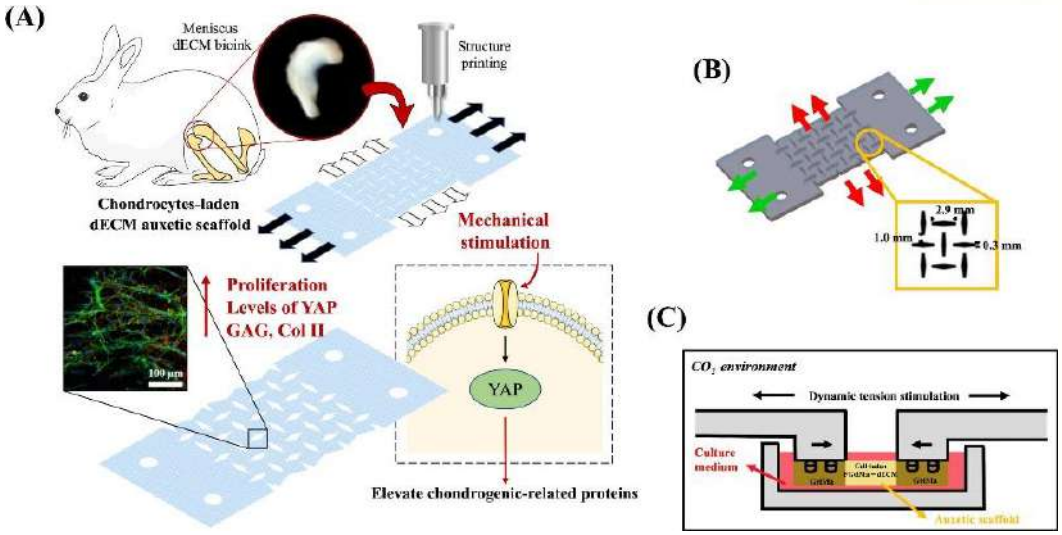
Abstract:

Meniscal damage results in the loss of structural stability and can lead to joint stiffness, impaired mobility, and potential disability. The treatment of meniscal defects remains challenging due to the avascular nature of meniscal cartilage, which hinders nutrient transport and limits the self-repair capacity of chondrocytes [1]. Tissue engineering, combined with advancements in biotechnology, offers a viable solution for meniscal repair or replacement. This study aims to develop a bioink composed of photopolymerizable gelatin methacryloyl (GelMA) and decellularized extracellular matrix (dECM) derived from rabbit menisci, and 3D print an auxeticscaffold with human chondrocytes, which will be subjected to cyclic tensile mechanical stimulation to enhance the secretion of cartilage-related markers. The bioink was prepared by mixing GelMA with different concentrations of dECM at 4°C under light-avoiding conditions. The bioink was then combined with chondrocytes and used for auxetic scaffold 3D bioprinting. Material analysis revealed that dECM had an average content of 75±3.2% of cartilage markers (collagen II and glycosaminoglycans) and showed no presence of DNA, indicating successful decellularization. Fourier-transform infrared spectroscopy confirmed the presence of relevant functional groups in both materials. The fabricated bioink (GelMA/dECM) exhibited favorable mechanical strength, enabling sustained cyclic tensile stimulation. Cell proliferation was enhanced, and the expression of integrin α2β1 was found to be concentration-dependent. Following tensile stimulation, the expression of Yes-associated protein (YAP) increased, accompanied by upregulated cartilage-related growth factors such as collagen II andglycosaminoglycans. The inhibition of YAP through a YAP inhibitor confirmed its involvement in cartilaginous matrix secretion, suggesting that the addition of dECM and mechanical stimulation can accelerate cell growth, enhance cell adhesion, and promote extracellular matrix secretion. By drawing upon the aforementioned, this design strategy holds potential for the futuredevelopment of meniscus tissue engineering applications.

KEYWORDS: Auxetic, tensile stimulation, Decellularized matrix, Chondrogenic



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Application of ultrahigh frequency transcutaneous electricalnerve stimulation for alleviation of neuropathic pain and neuroinflammation modulation in rat sciatic nerve chronic constriction injury

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Abstract

Introduction: Neuropathic pain remains a challenging complication in patients of peripheral compressive neuropathy. Excessive neuroinflammation and pain related neuropeptide acumination at the nerve injury site contributes to exaggerative neuropathic pain and functional loss. Currently physical therapy such as transcutaneous electrical nerve stimulation has demonstrated therapeutic potential, providing noninvasive, safe and promising outcomes. However, the underlying regulatory molecular mechanism remains complex and unexplored.

Methods: In the present study, we aimed to validate the therapeutic effect of ultrahigh frequency transcutaneous electrical nerve stimulation (UHF-TENS) in chronic constriction injuryof adult rat sciatic nerve. The efficacy and safety of UHF-TENS were investigated along with the following mechanistical exploration. Behavioral responses were evaluated using von Frey test. Pain related neuropeptide and inflammatory signals were analyzed in the injured site dorsal root ganglion neurons by immunofluorescent staining.Furthermore, the gene expression profile of neuroinflammatory pathway were explored by RNA sequencing for exploration of the regulating molecular mechanisms.

Results: By applying UHF-TENS, alleviation of mechanical allodynia was achieved and last for3 days for one session of therapy, without additional damage on the myelinated axon structure. Significant reduction of pain related neuropeptide and inflammatory signals were observed in injured dorsal root ganglion neurons. RNA sequencing of differentialgene expression of the sensory neurons revealed significant downregulation in lipid andcarbohydrate metabolism, autophagy and NF-κB pathway.

Conclusion: UHF-TENS provided promising outcome of alleviating neuropathic pain effectively and safely without introducing additional nerve damage. The therapeutic benefit resulted from reduced production of pain related neuropeptide and inflammatorysignals within dorsal root ganglion neurons.

Possible molecular mechanism by UHF- TENS might come from modulation of NF-κB complex, toll-like receptor-7 andPI3K/Akt signaling in sensory neurons.

Keywords: Neuropathic pain, chronic constriction injury, compression neuropathy, transcutaneous electrical nerve stimulation, ultrahigh frequency,neuroinflammation

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Effects of Photobiomodulation on Migration of Adipose-derived Stem Cells

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Abstract:

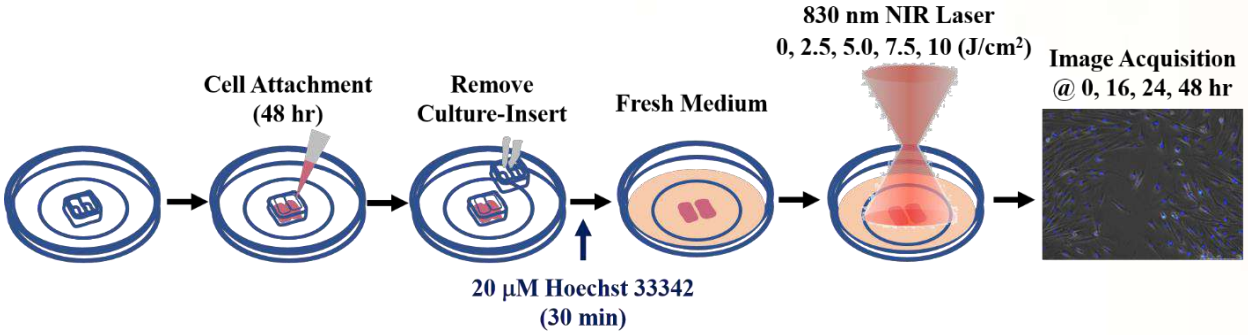
Cell migration is a crucial biological process that plays a vital role in the growth, development, and repair of tissues in living organisms. Adipose-derived stem cells (ADSCs) are a type of mesenchymal stem cell that possesses remarkable properties such as self-renewal, differentiation, and immunomodulatory functions. Photobiomodulation (PBM), also known as low-level laser therapy (LLLT), is a noninvasive and safe technique that has been shown to stimulate cell migration, differentiation, and proliferation in various cell types [1].

Previously, PBM was proposed for the investigation of the modulatory effects on mitochondrial membrane potential (ΔΨm), reactive oxygen species (ROS), and vesicle transportin single-living human adipose mesenchymal stem cells (hADSCs) [2]. The outcomes demonstrated that after 30 minutes of PBM treatment, a fluence of 5 J/cm^2 of PBM greatly improved the (ΔΨm), ROS, and vesicle transport phenomena compared to the control group (0 J/cm^2). These findings demonstrated the effectiveness of PBM in controlling (ΔΨm), ROS, and vesicle trafficking, which have the ability to promote cell growth, migration, and differentiation in cell-based systems. These gave us the motivation to use the same phenomenon on the PBM effects on ADSC cell migration.

The experiment was conducted with PBM at wavelength 830 nm and doses 0, 2.5, 5.0, 7.5, and 10 J/cm^2 to stimulate ADSC migration. The ADSCs were cultured, seeded into a 2-well culture insert at a density of 2x10^5, and removed from the culture insert about 48 h later. The investigation revealed that ADSC wound closure and cell survival were rapid at fluence 5 J/cm^2 in 24 h. ADSCs can migrate toward injured sites with the aid of PBM. Understanding the effectsof PBM on ADSC migration may therefore provide valuable insights into the molecular mechanisms underlying cell migration and tissue repair.

In conclusion, PBM has a significant effect on ADSC migration, which is critical for cell therapy in regenerative medicine. PBM-induced ADSC migration could accelerate the healing process of injured tissues, leading to more effective therapies. Thus, PBM could be a valuable toolin the development of cell therapy using ADSCs for various diseases.

KEYWORDS: Cell migration, Differentiation, Proliferation, Photobiomodulation, Wound healing



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Comparison of the difference between orthokeratology cleaning solution with added polysaccharides and commercially available products

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Abstract:

Orthokeratology (ortho-k) lenses are made of rigid gas-permeable material by the design ofreverse geometry to temporarily reduce myopia during sleep, resulting in having normal vision during the day and in being used for myopia control in children [1-2]. Although the safety of ortho-k lenses has been proven, infection and corneal staining are two major clinical complications. Tear film components contain proteins, lipids, and enzymes, and are easilyadsorbed onto the lens. The possible cause of infection might due to lack of eye movement thus less lysozyme could be spread over the eye surface resulting in making eyes be more susceptibleto the infection if there are bacterial colonization [3]. In addition, immune reactions may also occur if adsorbed tear film components are not removed from the lens completely [4]. Incompleteremoval of adsorbed tear film components from the ortho-k lens may also cause discomfort duringrapid eye movement, and corneal damage may occur when the lens is difficult to be removed fromthe eye due to the deposition [5]. Therefore, how to reduce the risk of infection and corneal damage is a critical issue for ortho-k lens manufacturers.

The previous studies have shown that non-rubbing or rubbing with the commercial care solutions could not remove adsorbed tear film components from the ortho-k lenses [6-7]. We haveshown that polysaccharides could reduce deposition of tear lysozyme and albumin over time, andweakly prevent cholesterol adsorption [8]. In this study, we investigated the ability of removing absorbed tear proteins of polysaccharides-containing care solution by an in vitro protein deposition analysis. The result showed that polysaccharides-containing care solution couldeffectively remove adsorbed lysozyme and albumin resulting in less protein deposition than the commercial care solution.

We also established an in vitro friction testing of ortho-k lens, and the result demonstrated that polysaccharides-containing care solution provided more lubrication compared with thecommercial care solution. In addition, tear film components would increase friction coefficient ofortho-k lenses. The result also showed that polysaccharides-containing care solution would reducethe high friction coefficient caused by tear film components. Our results provided a potential effective ortho-k lens care solution that can more effectively remove tear film components from ortho-k lenses and can provide more lubrication, and may potentially reduce the risk of infectionand corneal damage.

KEYWORDS: Polysaccharides, orthokeratology lens, tear film components, lubrication, care solution

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Investigation of Factors Altering Rheological Properties of Poloxamer-BasedThermo-Sensitive Hydrogel

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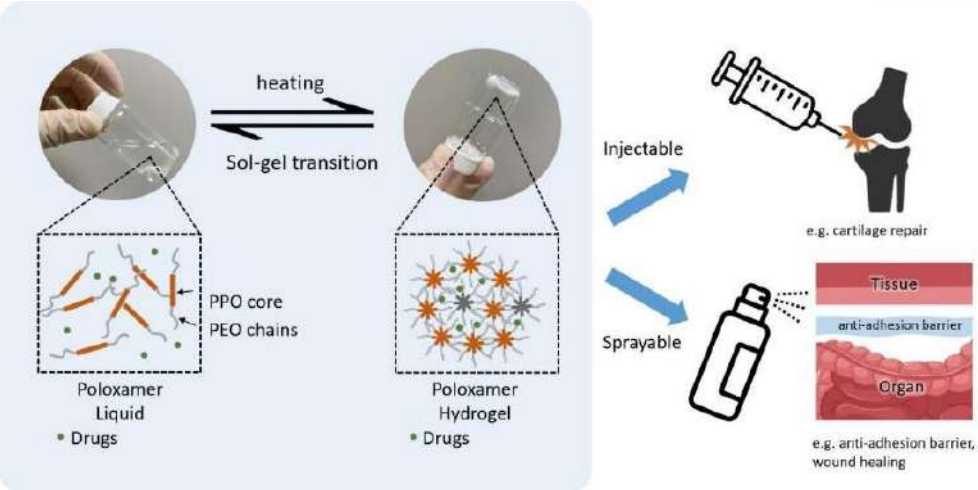
Abstract:

Hydrogels are widely studied biomaterials for biomedical and pharmaceutical applications, including drug-controlled delivery, postoperative anti-adhesion, wound dressings, biosensors, and tissue engineering due to their biodegradability, biocompatibility, low immunogenicity and ease of usage. Among them, thermo-sensitive hydrogels have become the best-studied polymer systems for their controllable responses with external environmental changes. Poloxamers are negatively temperature-sensitive hydrogels and their hydrophilic groups interact with water molecules at lower temperatures (liquid phase) while their hydrophobic groups interact more strongly with increases in temperature causing gelation. The thermo-reversible properties can bemanipulated by adjusting polymer composition, molecular weight and concentration at physiological temperatures.

Our study has investigated the factors affecting the rheological properties of poloxamers and the optimized formulation was converted into a sensitive hydrogel using binary poloxamer P407/P188. Rheological studies proved the formation of gel consistency at physiological temperature. There was a clear trend of decreasing gelling temperature/time when P407 was at higher concentration, and the addition of P188 enhanced the gelling temperature regardless of poloxamer concentration. Addition of polysaccharides enhanced the mechanical property of the binary poloxamer and promoted cell proliferation. This study investigated the intriguing characteristics of poloxamer-based hydrogel, providing useful information to compounding an ideal and desired injectable/sprayable hydrogel for wide use of clinical applications.



KEYWORDS: thermo-sensitive hydrogel; poloxamers; binary poloxamers; polysaccharides;sol-gel transition; biomaterial

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Effects of different barbed suture implantation methods on facial tissue tension anddisplacement in minimally invasive medical cosmetic surgery

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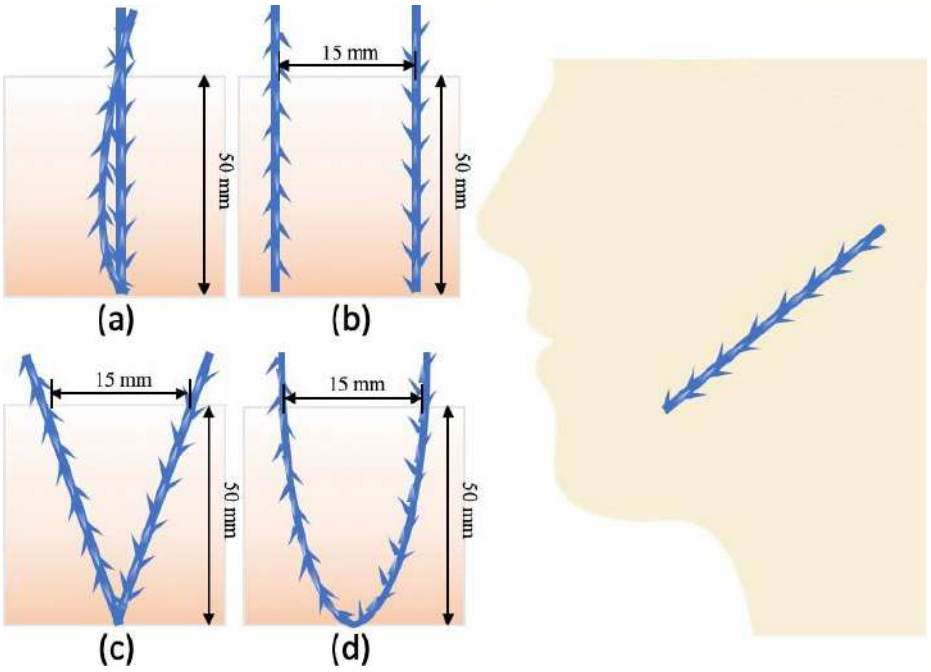
Abstract:

In recent years, minimally invasive surgeries have become increasingly prevalent due to their lower complication rates. The use of barbed sutures in cosmetic surgeries has also become common, as they can lift sagging facial tissues to improve signs of aging. However, despite the various barbed suture options available [1,2], there has been limited research on their mechanicalproperties for facial suspension and no suitable data available on the impact of barbed suture tension, attachment force, and insertion method on facial tissues. As a result, the final appearanceof barbed suture use seems to depend solely on the surgeon's technique and experience. Therefore,establishing reference data and guidelines for the use of barbed sutures in medical aesthetics couldassist surgeons in planning and designing the appropriate tension, quantity, and surgical methodsfor their use.

This study utilized an in vitro tensile testing model developed by previous research [3] to simulate commonly used surgical techniques and investigate the relationship between different suture placement methods, surgical tension, and displacement. Using the synthetic material polydimethylsiloxane (PDMS) to mimic human skin, the tensile strength of barbed sutures was evaluated using a universal material testing machine. The test samples were inserted into PDMS to a fixed depth of 5 cm, and the effect of different suture placement methods, including single, double, V-shaped, and U-shaped, on the distance of facial tissue displacement caused by the applied tension was studied. The influence of barbed suture attachment force on the postoperativefixation of facial tissues was also examined.

The results showed that, at the same tension force (0.3 kgf), the V-shaped and U-shaped samples achieved the maximum displacement distance of 2 mm. Interestingly, the single-strand sample, on the other hand, was able to achieve the same distance as the V-shaped and U-shaped samples with a tension force of less than 0.3 kgf. In terms of the support performance of facial tissues, theV-shaped and U-shaped samples will affect the range of PDMS destruction after unhooking, due to the different angles. These results can provide valuable information for physicians, enablingmore precise design of the techniques for facelift surgery.

KEYWORDS: PDMS; barbed suture; holding capacity; pull-out strength; thread lift.



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Young Investigator Competition (YIC, BCRS)

September 1 (Friday)	
YIC-1	*Fabrication of dynamic covalently crosslinked alginate hydrogels for biomedical applications* **Prof. Yi-Cheun Yeh, National Taiwan University, Taiwan**
YIC-2	*Natural-compound-derived multifunctional nanomedicines for cancer theranostics* **Prof. Chih-Sheng Chiang, China Medical University, Taiwan**
YIC-3	*Monocyte-mediated drug carriers targeting cancer spheroids in a 3D microfluidic cell culture that reconstitute tumor microenvironment* **Prof. Bill Cheng, National Chung-Hsing University, Taiwan**
YIC-4	*Electric stimulation preserves regenerative microenvironment of denervated neuromuscular junction by satellite cell activation and differentiation* **Prof. Yuan-Yu Hsueh, National Cheng Kung University, Taiwan**
YIC-5	*Revolutionary Dual Single Molecule Detection Frameworks: Empowering Nanomedicine and Extracellular Vesicle Research* **Prof. Chi-An (Annie) Cheng, National Taiwan University, Taiwan**
YIC-6	*Unveiling the Synergistic Potential of Chemo-Immunotherapy in Triple-Negative Breast Cancer through Tumor-Microenvironment-on-Chip Technology* **Prof. Jen-Huang Huang, National Tsing Hua University, Taiwan**
YIC-7	*Highly heterogenicity lung-cancer spheroid-based physiological model to recapitulate the microenvironment and the drug response for precision medicine* **Prof. Ming-You Shie, China Medical University, Taiwan**
YIC-8	*Thermoresponsive gold nanohuts Simultaneous targeting to cancer cells and tumor associated macrophages for enhanced Synergistic photoimmunotherapy* **Dr. Hung-Wei Cheng, National Yang Ming Chiao Tung University, Taiwan**
YIC-9	*An Iron Oxide-based Photocrosslinkable Ink for the Applications of Magnetic-Responsive Bioactuators* **Prof. Yi-Chen (Ethan) Li, Feng Chia University, Taiwan**
YIC-10	*Hafnium-doped bioceramic nanoparticles for use as radiosensitizers in cancer treatment* **Prof. Min-Hua Chen, Chung Yuan Christian University, Taiwan**
YIC-11	*Using clinical porous gelatin sponge to establish a 3D Multilayered Intervertebral Disc Degeneration Model* **Prof. Chi-Yun Wang, Ming Chi University of Technology, Taiwan**

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Fabrication of dynamic covalently crosslinked alginate hydrogels for biomedicalapplications

Ke-Han Shen[1], Ting-Hsiang Chiu[1], Ying-Yu Yeh[1], Kuang-Chih Teng[2], Reuben Wang[3],Jiashing Yu[2], and Yi-Cheun Yeh[1,*]

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Abstract:

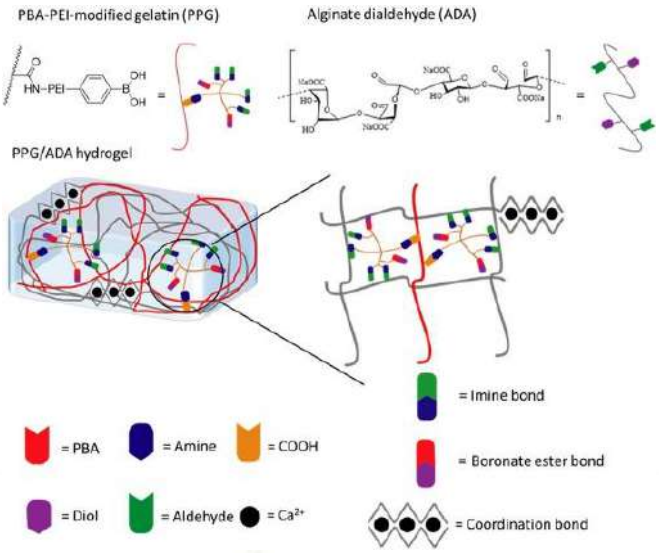
Alginate is one of the popular natural biopolymers to be widely utilized in the biomedical fields (e.g., drug delivery and tissue engineering) with its low cost, biocompatibility, biodegradability, and the ability to be easily crosslinked with non-toxic calcium (Ca^{2+}) ions to form hydrogels. However, using Ca^{2+} ions to crosslink alginate provides limited properties and functions to alginate hydrogels, restricting their advanced utility.

This presentation will focus on describing the developments of the chemistry-based strategy to construct versatile alginate hydrogels with definable structures and properties to expand their biomedical applications. In our design, phenylboronic acid-functionalized polyethyleneimine (PBA-PEI) was synthesized to introduce two orthogonal dynamic covalent crosslinks in the alginate hydrogels, where PBA-PEI was used to crosslink alginate dialdehyde (ADA) through imine bonds and boronate ester bonds. The grafting degree of PBA in the PEI structure was applied to fine-tune the properties (i.e., rheological property, mechanical strength, swelling behavior, and antibacterial activity) of PBA-PEI/ADA hydrogels [1].

Furthermore, gelatin was introduced to the alginate hydrogel by preparing PBA-PEI-modifiedgelatin (PPG) as crosslinkers. PPG was used to crosslink ADA through imine bonds and boronateester bonds, and then Ca^{2+} ions were added to introduce the third calcium-carboxylate crosslinkingin the network to form the triple-crosslinked PPG/ADA+Ca^{2+} hydrogels. Given the three types ofdynamic bonds in the network, PPG/ADA+Ca^{2+} hydrogels possessed a self-healing manner, stimuli-responsiveness, and better mechanical properties compared to single- or double- crosslinked hydrogels. The controlled release capability of PPG/ADA+Ca^{2+} hydrogels was also demonstrated, showing the encapsulated molecules can be rapidly released from the hydrogel network in the presence of hydrogen peroxide while the release rate can be slowed down at acidic pH. Furthermore, PPG/ADA+Ca^{2+} hydrogels presented selected cytotoxicity and drug delivery to cancer cells due to the regulated degradation by the cellular microenvironment [2].

To prepare alginate hydrogels for sensing application, a new type of luminescent alginate hydrogels was constructed through the double network of PPG/ADA along with the europium (III) (Eu^{3+}) ions. PPG/ADA+Eu^{3+} hydrogel was prepared by the combined methods of chemical crosslinking and physical freeze-thawing to present superior mechanical properties. With the pH-responsive characteristics, PPG/ADA+Eu^{3+} hydrogel was used as a red-emitting sensor for volatile solvent vapors and bacteria growth.

Taken together, we have developed a series of alginate hydrogels through the design of the chemical crosslinkers and the use of lanthanide ions. PBA-PEI/ADA hydrogels possess tunable properties and multiple stimuli-responsiveness, PPG/ADA+Ca^{2+} hydrogels with multiple desirable properties and dynamic features perform controlled molecule release, and PPG/ADA+Eu^{3+} hydrogels are used for bacterial sensing. These new types of alginate hydrogelsare potential candidates to expand the usage of alginate hydrogels for advanced biomedical applications.



KEYWORDS: Alginate, hydrogels, dynamic covalent chemistry, antibacterial, controlled release

K.-H. Shen, Y.-Y. Yeh, T.-H. Chiu, R. Wang and **Y.-C. Yeh***: Dual dynamic covalently crosslinked alginate hydrogels with tunable properties and multiple stimuli-responsiveness. ACS Biomater. Sci. Eng., 8(10), 4249-4261 (2022)

K.-H. Shen, T.-H. Chiu, K.-C. Teng, J. Yu and **Y.-C. Yeh***: Fabrication of triple-crosslinked gelatin/alginate hydrogels for controlled release applications. Int. J. Biol. Macromol. (under revision)

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Natural-compound-derived multifunctional nanomedicines for cancer theranostics

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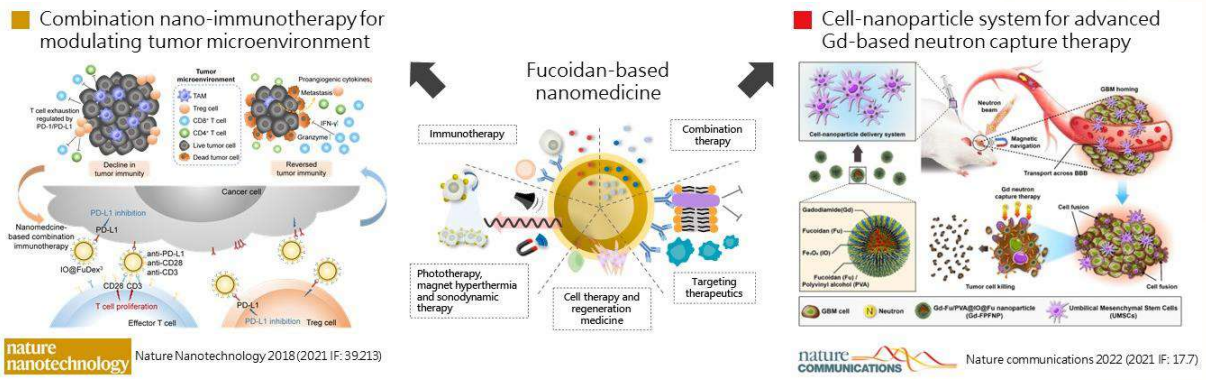
Abstract:

Engineered nanomedicine possesses the capability of overcoming biological barriers and precisely accumulating at the tumors, and thus has been attempted to deliver chemotherapeutics,biologics, and even cell therapy for augmenting the therapeutic index. However, the major composition of nanomedicine is excipient, which would cause toxicity when given at high dose or repeated administration. To address the issue, our team developed a series of nanomedicines composed of natural compounds including fucoidan, quercetin, and astaxanthin [1-3]. These compounds are highly biocompatible and possess biologically therapeutic effects, allowing the derived nanomedicines to target tumor, alleviate tumor progression, activate immune system, andmodulate the tumor microenvironment. Among them, fucoidan-based nanoparticle (FuNP) with immune modulation ability is a promising candidate for drug delivery, self-adjuvant nanomedicineand cancer vaccine. The immunomodulators, inorganic nanoparticles [4], targeting moieties or chemotherapeutics can further be coupled to the FuNP to achieve theranostic application (as shown in graphical abstract). The nano-bio interaction of FuNP demonstrates distinct effects andis worth exploring. Specifically, FuNP can simultaneously target cancer cells and immune cells for tumor inhibition and immune activation, opening the opportunity to enhance the therapeutic index by revealing the crosstalk between systemic and tumor microenvironment [3]. More, strategically combining cells and FuNP in a single system allows the integration of the advantagesfrom both sides. For instance, gadolinium-loaded FuNP was intracellularly delivered to stem cellsto form a stem cell-nanoparticle system (SNS) for neutron capture therapy (NCT). SNS possessesthe nature of stem cell to penetrate blood-brain barrier (BBB) and fuses with brain tumor, while FuNP can stably encapsulate gadolinium to prevent the toxic effect on stem cells. With the effective tumor homing effect, SNS presents a high tumor-blood ration and tumor-normal-tissue ratio at the brain tumor tissue, eliminating the glioblastoma multiform (GBM) to prolong survivalwhile limiting the systemic adverse effect [5]. The strategy of using natural compound such as fucoidan to form inherently therapeutic nanoparticle potentially paving an avenue for the development of a new family of nanomedicines for advanced tumor therapy.

KEYWORDS: fucoidan-based drug delivery system, combination nano-immunotherapy, cell-nanoparticle system, tumor microenvironment, translational science



Fucoidan-based nanoparticle (FuNP) can be eqipped with immunomodulators, inorganic nanoparticles, targeting moieties or chemotherapeutics for multiple applications including combination nano-immunotherapy [3] and cell- based neutron capture therapy [5].

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Monocyte-mediated drug carriers targeting cancer spheroids in a 3D microfluidic cellculture that reconstitute tumor microenvironment

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Abstract:

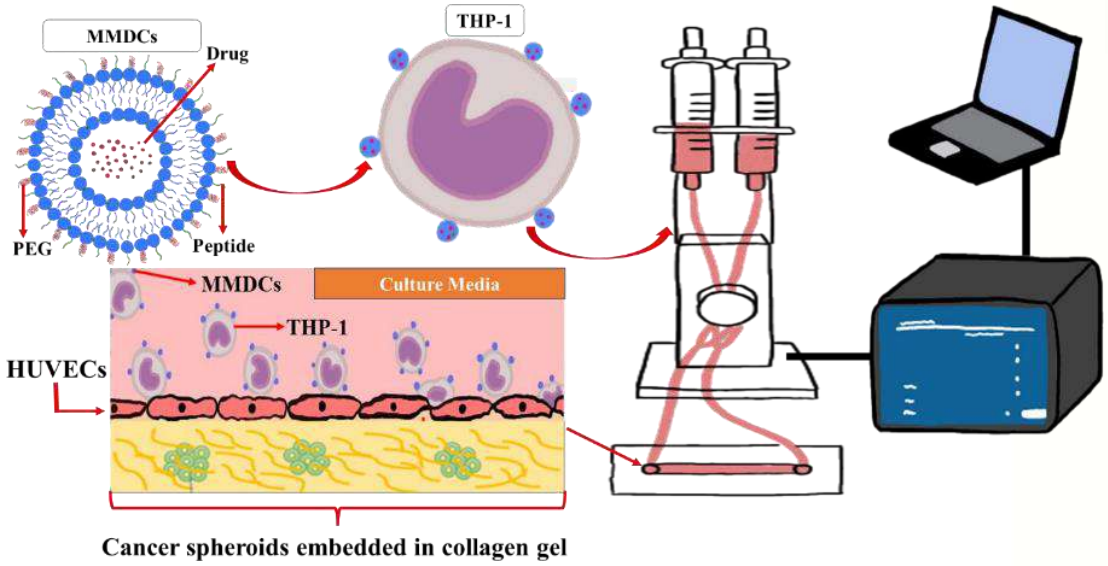
It has become apparent that the reliance on the enhanced permeability and retention (EPR) effectis a significant reason behind most of the clinical failures of nanoparticle-based targeted therapy in patients with cancer. It was revealed that in patients with cancer, the EPR effect only providesless than 2-fold increases in delivery to tumor tissue compared to normal organs and that side effects are often noticed due to off-targeting[1]. Therefore, there is an urgent need to develop a novelnanoparticle-based targeted therapy that can facilitate drug delivery in an EPR-effect-independent manner, thus improving the therapeutic outcome in patients with cancer. Studies have revealed that the number of circulating monocytes in cancer patients is significantly higher than in healthypeople[2]. Most of these circulating monocytes are recruited to tumors directly and are known to extravasate into tumor tissues without relying on the EPR effect[2].

We have developed monocyte-mediated drug carriers (MMDCs) that could deliver therapeutics to the targeted sites without depending on the EPR effect. The MMDCs were synthesized with FDA-approved biomaterials and could be scaled up for large-scale production. Using a commercially available microfluidic system, the MMDCs were demonstrated to hitchhikeonto circulating monocytes (THP-1) under physiological flow rates successfully. In contrast, theclassical formulation of PEGylated liposomes failed to show any interactions with THP-1. The targeting specificity of MMDCs was further demonstrated in a 3D microfluidic cell culture thatreconstituted some of the critical features of the tumor microenvironment. The 3D culture consisted of human breast cancer (MDA-MB-231) spheroids embedded in a collagen matrix, withhuman endothelium (HUVECs) established on top. MMDCs and THP-1 circulated in the 3D cellculture at 37˚C for 4 hours. Fluorescence imaging and flow cytometric analysis revealed that theMMDCs showed strong binding for circulating THP-1 and not for HUVECs MMDCs were shown to undergo trans-endothelial migration through monocyte hitchhiking.
In contrast, MMDCs could not undergo trans-endothelial in the absence of monocytes. Furthermore, either circulating THP-1 or MMDCs could not undergo trans-endothelial migration when the collagen matrix was embedded with HEK293, not MDA-MB-231. This indicated in our 3D microfluidic cell culture that circulating monocytes could only undergo trans-endothelial migration in the presence of cancer spheroids. The MMDCs could only target cancer spheroids and not non-cancer cells.

Our finding demonstrated that MMDCs only interacted with circulating monocytes and not human endothelium. The MMDCs could only undergo trans-endothelial migration via monocyte hitchhiking. The transmigration effect can only be seen in the 3D microfluidic cell culture with cancer spheroids, not non-cancer cells embedded in the collagen matrix. Thus, we have successfully demonstrated the targeting

efficiency of the MMDCs and developed an in vitromodel that could be used to study drug delivery.

KEYWORDS: Monocytes, EPR effect, Drug Delivery, Microfluidic



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Electric stimulation preserves regenerative microenvironment of denervated neuromuscular junction by satellite cell activation and differentiation

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Abstract:

Neuromuscular junction (NMJ) dysfunction can occur after nerve injury, particularly injuries that affect the peripheral nervous system. Following nerve injury, the NMJ undergoes a series of changes that can contribute to dysfunction, including loss of synaptic architecture, neurotransmitters and maintaining mechanism of postsynaptic microenvironment of denervated skeletal muscle. Electroceuticals, also known as bioelectronic medicine or neural engineering, refer to the use of electrical stimulation to modulate the function of the body's neural system for therapeutic purposes. Electroceuticals aim to treat various health conditions by interfacing with the body's nervous system, including the brain, spinal cord, and peripheral nerves[1]. Distal electric stimulation (E-stim) on interphase of neuromuscular junction has been validated as an effective and innovative approach for denervated muscle injury from our previous publication[2]. Electric pulse on affected cells has direct influence on intracellular environment and protein translation. However, the underlying mechanism and regulatory microenvironment are still unexplored. The main purpose of this study is to investigate the regulatory microenvironment and molecular mechanism at the neuromuscular junction of denervated muscle after electric stimulation.

Critical gap (10mm) injury at sciatic nerve of adult Sprague-Dawley rat was created, followed by immediate distal Estim for 30 minutes. Distal E-stim enhanced innervated muscle regeneration, functional gait recoveries and NMJ reinnervation at 6 weeks after injury. Further investigation revealed that distal E-stim ameliorated denervated muscle atrophy, single muscle fibrillation and NMJ degradation at the first 2 weeks. Single cell RNAseq of denervated muscle at day3 demonstrated that the satellite cell cluster had the highest number of differential expressing gene, indicating that the cell type was the most significantly affected by the distal E- stim. As the quantitative analysis showing, Myod1 and Myog were up-regulated in E-stim group, indicating that satellite cell was activated to proliferate and differentiate. On top of that, the gene set enrichment analysis revealed the genes involved in vessel remodeling pathway having a significantly higher expression level in E-stim group than control group, such as Aqp1, Myog, Myod1, Pax7, Cdh5, are known as associated with angiogenesis and vasculature development pathway.

In this short report, we had demonstrated that the satellite cell might be the main cell type contribute to regenerative microenvironment within interphase of neuromuscular junction, responding to distal E-stim. In addition, we had also identified the genetic regulatory network within activated

satellite cells. Via activation such regenerative microenvironment of denervated NMJ, distal E-stim provide promising benefits for improving neuromuscular interface regeneration and resultant functional recoveries in peripheral neuromuscular injuries.

Keywords: electrical stimulation (E-stim), peripheral nerve injury, neuromuscular junction (NMJ), satellite cell, denervated muscle injury, single cell RNAseq

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Revolutionary Dual Single Molecule Detection Frameworks: Empowering Nanomedicine and Extracellular Vesicle Research

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Abstract:

Despite the attractive attributes of nanotechnologies in cancer therapy, their translation to clinical settings has been challenging, hindering their full potential to be realized. Preclinical in vivo studies evaluating the efficacy of cancer nanomedicine primarily rely on tumor size and animal survival metrics, which offer limited insightinto the nanomedicine's mechanism of action. To address this limitation, we have developed a novel integrated framework called nanoSimoa, which combines an ultrasensitive protein detection technique (single-molecule array, Simoa) with cancer nanomedicine. The preliminary results suggest that nanoSimoa has the potential to guide the development of cancer nanomedicines and predict their behavior in vivo. If its generalizability is confirmed, nanoSimoa could become a valuable tool for preclinical testing and accelerate the development of precision medicine.

Extracellular vesicles (EVs) are membrane-encapsulated particles secreted byall cells in the body, carrying nucleic acids and proteins from their parental cells. EVs have transitioned from being considered "garbage bags" to playing a crucial role in liquid biopsy, as they are closely associated with diseases such as cancers, cardiovascular diseases, and infectious diseases. Liquid biopsies, such as blood and urine, provide a non-invasive means of acquiring EVs. However, the detection limitsof existing analytical techniques often prevent the comprehensive study of promising EV biomarkers, as most of them are present at low levels. Furthermore, analyzing circulating tumor-derived EVs (ctEVs) is particularly challenging due to their minute fraction in the total pool of circulating EVs and their tremendous diversity in size and protein content. Additionally, EV-associated proteins are distributed across different spatial compartments, requiring distinct strategies to characterize proteins from each compartment. Many of these proteins are also soluble in the blood, complicating the isolation and study of ctEVs. Consequently, there is a lack of robust tools for identifying and evaluating ctEV-associated biomarker proteins. To overcome these obstacles, we have established a framework called eSimoa, which leverages two key technological innovations for high-resolution profiling of EVs and validation of ctEV biomarker proteins. Firstly, we devised an ultra-sensitive, high-throughput systemthat combines target biomarker pulldown with Simoa quantification to screen for ctEV-associated surface proteins. Secondly, we implemented a Simoa-based strategy to analyze ctEV-associated luminal proteins. This platform allows us tovalidate candidate ctEV proteins in clinical samples with unparalleled resolution.

During this presentation, I will provide an introduction to Simoa technology, which currently stands as the most sensitive protein detection platform, surpassing conventional ELISA by 1000-fold sensitivity. Subsequently, I will present the dual integrated frameworks base on Simoa technology. Lastly, I will discuss the potential application of these dual Simoa frameworks in addressing challenges encountered in the fields of nanomedicine and EV research.

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Unveiling the Synergistic Potential of Chemo-Immunotherapy in Triple-Negative BreastCancer through Tumor-Microenvironment-on-Chip Technology

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Abstract:

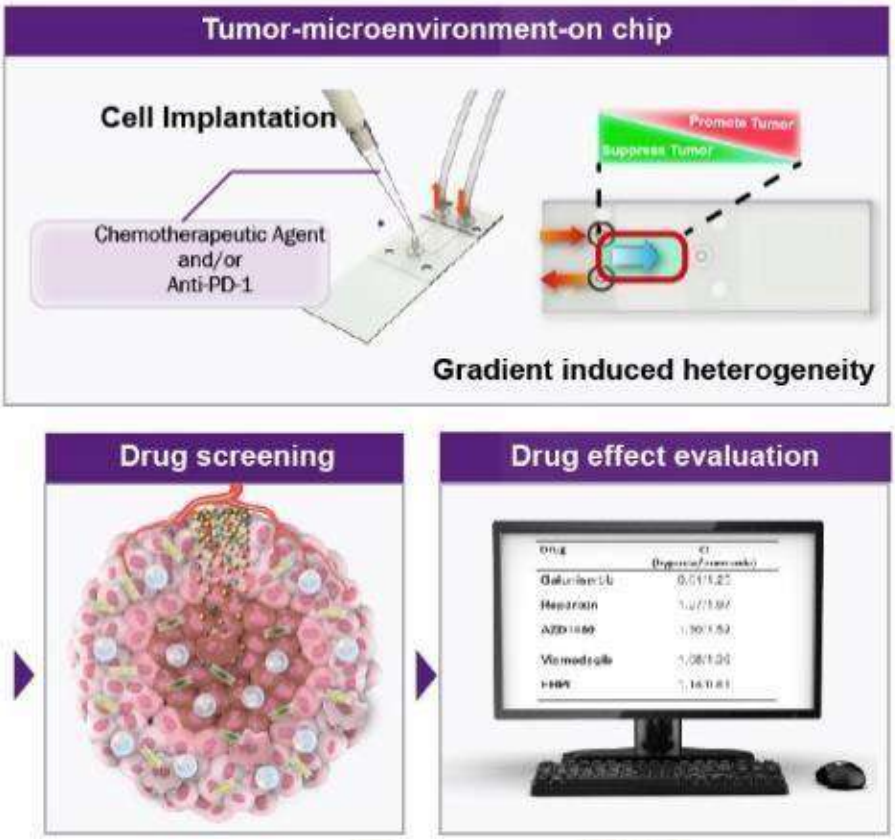
Chemo-immunotherapy offers a promising solution to overcome the challenges associated with immune checkpoint inhibitor (ICI) therapy in triple-negative breast cancer (TNBC). However, identifying the optimal chemotherapy agents that synergize with immunotherapy remains a formidable task, with only a fraction of patients exhibiting favorable responses to ICI treatment. To address this limitation, alternative platforms such as 3D spheroids and organ chips have been explored for drug screening purposes. Nevertheless, these platforms still lack the abilityto accurately assess combination therapy and investigate the intricate interplay between immune cells and cancer cells.

In a previous study, we developed a microfluidic-based drug selection system capable of culturing patient-derived circulating tumor cells on a chip, enabling the evaluation of drug sensitivity [2]. Additionally, we demonstrated the feasibility of assessing multiple drug toxicitieswithin a single device [3]. Building upon these findings, we present a novel design: the tumor- microenvironment-on-chip (TMoC), specifically tailored for the screening of chemotherapeutic agents that synergize with ICIs to enhance T cell-mediated cytotoxicity against tumors. The TMoCcomprises a circulation system and a strip-like 3D tissue culture area, incorporating physiologicaloxygen and nutrient gradients. This unique setup allows for spatial infiltration of cytotoxic CD8+T cells into the tumor microenvironment, thereby preserving its inherent complexity and heterogeneity. Notably, the TMoC offers a rapid and accurate chemo-immunotherapy drug screening platform, with results obtainable within a 36-hour timeframe.

Through the flow cytometry analysis, we assessed the synergistic effects of five potential immunotherapy-promoting drugs and identified galunisertib in combination with an anti-PD-1 antibody as the most effective regimen for inhibiting T cell exhaustion and suppressing tumor growth, as validated in both the TMoC and TNBC mouse models. Importantly, this evaluation process, which typically spans several months in patient-derived xenograft (PDX) models, can now be expedited using the TMoC drug evaluation model. This groundbreaking approach significantly enhances our understanding of the intricate crosstalk between immune cells, tumor microenvironment, and therapeutic agents.

In conclusion, the tumor-microenvironment-on-chip represents a transformative tool for unraveling the untapped potential of chemo-immunotherapy in the context of triple-negative breast cancer. By providing an advanced platform for rapid and accurate drug screening, the TMoC paves the way for further insights into the complex dynamics of immune-cell-cancer-cell interactions and holds immense promise for accelerating the development of tailored treatment strategies.

KEYWORDS: tumor microenvironment; drug screening; synergic effect; immunotherapy; oxygen gradient



Graphic abstract. The systematic identification of chemotherapy synergising with immunotherapy against breast cancer remains challenging because current methods lack specific treatment failure mechanisms (e.g. oxygen gradient, tumor heterogeneity). In this study, we report the design and performance of a tumor-microenvironment-on-chip (TMoC) for the screening of chemotherapeutic agents thatsynergize with immunotherapy, anti-PD-1, to promote T cell-mediated cytotoxicity against tumors.

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Highly heterogenicity lung-cancer spheroid-based physiological model to recapitulate the microenvironment and the drug response for precision medicine

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Abstract:

Lung cancer remains a major health problem despite the considerable research into prevention and treatment methods. Through a deeper understanding of tumors, patient-specific exvivo spheroid models with high specificity can be used to accurately investigate the cause, metastasis, and treatment strategies for lung cancer. This study established a facile route to construct a sophisticated ex vivo lung tumor spheroid model with essential factors in terms of ECM components, cell phenotypes, and vascular barriers for considering patient-specific therapies. We present biofabricate lung tumors consisting of patient-derived tumor spheroids, endothelial cells, and lung decellularized extracellular matrix (LdECM). The composition and physicochemical properties of LdECM provided native-tissue-mimetic physicochemical cues thatsupport the organization of spheroids with a hypoxic signature from either cell lines or primary tumor cells, as well as a vasculature barrier from HUVEC, without altering their susceptibility tovarious anti-cancer drugs. We also demonstrate that the developed lung-cancer spheroid model reproduces patient responses to chemotherapeutics and targeted therapy in a co-clinical trial, with85% accuracy, 86.7% sensitivity, and 80% specificity. RNA sequencing analysis confirm the success of the lung cancer preclinical spheroid model. Particularly, this preclinical examination can be used in the point-of-care analysis because the timeline is within 12 days. We expect that this strategy should help to develop various disease or tumor models and ultimately guide clinicaldecisions.

KEYWORDS: Lung cancer, spheroid, decellularized extracellular matrix, drug screening,precision therapeutics

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Thermoresponsive gold nanohuts Simultaneous targeting to cancer cells andtumor associated macrophages for enhanced Synergistic photoimmunotherapy

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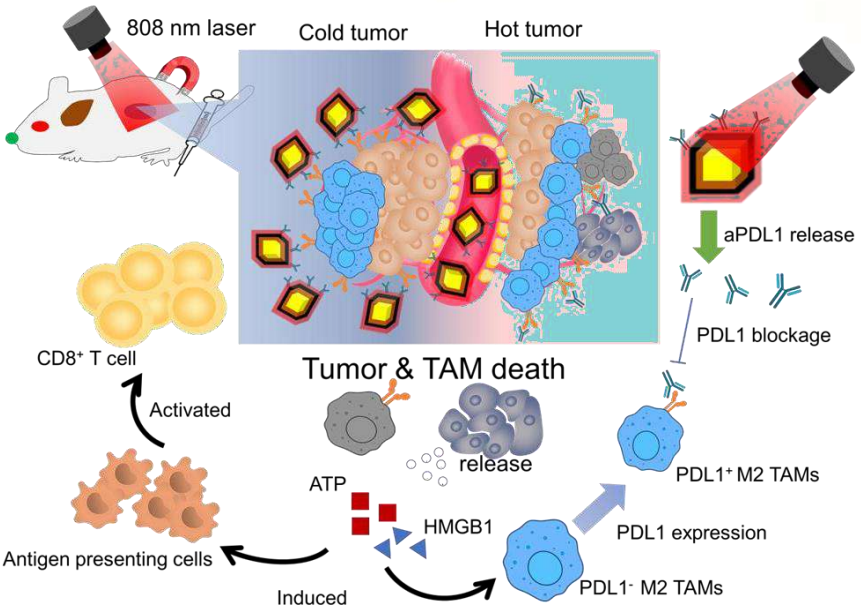
Abstract:

Combining photothermal therapy (PTT) and immune checkpoint inhibitors (ICIs) has been increased the response rate of immunotherapy through "cold" tumor turn into "hot" tumor [1], but tumor associated macrophages (TAMs) was simultaneously upregulated expression of PDL1, leading to the immunosuppression tumor microenvironment. [2] Herein, we integrate PTT and ICIson a Gold nanohut (LbL-AuNH) to optimize the spatiotemporal immune interaction between of the tumor immune microenvironment (TIME) and systematic organs. The LbL-AuNH has a coreof gold nanocages (AuNCs) coated with the layer-by-layer (LbL) shells composed of fucoidan, magnetic iron oxide nanoparticles (IONPs), heat-sensitive betanin and further immobilized with anti-PD-L1 on the surface (Ab-LbL-AuNH). At the near-fired (NIR) irradiation, the local tumor and $PDL1^+$ TAMs were eliminated at the suitable hyperthermia window to promote induction ofimmunogenic cell death (ICD) and reduce the $PDL1^+$ TAMs, facilitating cold tumor turning intohot tumor. Simultaneously, the degradation of betanin released the anti-PD-L1 to block the PDL1on the restored expression $PDL1^+$ TAMs, leading to the activated $CD8^+$ T cells perform the function. Ab-LbL-AuNH significantly enhanced the tumor-localized immunity and the survival of the Hep55.1c-bearing mice under external magnetic field (eMF), demonstrating magnetic- reinforced photoimmunotherapy is the promising strategy to optimize spatiotemporal immune responses.

KEYWORDS: Photothermal therapy, immune checkpoint inhibitor, tumor associated macrophage, gold nanocage, tumor immune microenvironment



Graphic abstract. The structure of temperature-sensitive immune-nanoparticles and its therapeutic strategy.

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An Iron Oxide-based Photocrosslinkable Ink for the Applications of Magnetic-Responsive Bioactuators

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Abstract:

Bioactuators offer a versatile platform for various applications due to their design flexibility and adaptability to different environments. In recent years, there has been increased interest in the development of bioactuators with multifunctionality by combining 3D printing technology and stimuli-responsive materials. In this study, we aimed to fabricate bioactuators with enhanced functionalities by hybridizing magnetic-responsive nanoparticles with photocrosslinkable biopolymers. This hybrid ink enabled us to regulate the stiffness and adjust the actuation ability of the bioactuators. By employing a 3D printing technique, we successfully constructed biomimetic structures resembling those found in natural organisms using the magnetic-responsive photocrosslinkable ink. Moreover, through careful structural design, the printed bioactuators exhibited reversible transformation, mimicking the movements observed in natural creatures. Ourfindings demonstrate the potential of the hybrid magnetic-responsive photocrosslinkable ink for rapid additive fabrication of biomimetic constructs, enabling non-contact magneto-actuation.

KEYWORDS: 3D printing technique, stimuli-responsive materials, bioactuators.

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Hafnium-doped bioceramic nanoparticles for use as radiosensitizers in cancer treatment

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Abstract:

Recently, new pharmaceutical agents containing high atomic metallic ions have been regarded as attractive materials, based on interacting with ionizing radiation to generate free radicals for deeper cancer treatment. Using ionizing radiation, such as X-rays and gamma rays, the tissue penetration depth can easily reach the range of 8–14 cm. Exposed to ionizing radiation,these metallic materials can interact with ionizing radiation to produce Auger electrons, Comptonelectrons, secondary electrons, and photoelectrons, which in turn interact with water molecules toproduce free radicals and trigger the quantities of reactive oxygen species (ROS) in cells. Nowadays, a new inert material named NBTXR3 is being developed to replace metallic nanoparticles with the composition of hafnium oxide (HfO2). HfO2 nanoparticle has a high electron density and possesses photo-luminescent properties. In the preclinical studies, it has been shown that HfO2 nanoparticles could enhance the effect of radiation therapy to kill cancer cells while reducing the damage of side effects to healthy tissues. Nevertheless, despite these advances,HfO2 nanoparticles with inert properties have been regarded as easily excluded from cells throughexocytosis, causing reduced cellular uptake and decreasing a curative effect compared to biodegradable materials. Thus, in this study, we use bioceramics as the host material with the doping of Hf ions into the ceramic structure and ask whether this Hf-doped nanoparticle could enhance ionizing radiation treatment.

Hf-doped bioceramic nanoparticles were synthesized by wet chemical reaction with various doping concentrations of Hf^{4+} relative to Ca^{2+} in the bioceramic host material. The cancer cell line(B16-F10 or/and A549) was employed as the model to assess the impact of ionizing radiation onHf-doped particles.

The results show that the Hf-doped particles could be successfully doped with Hf ions, forming nano-sized and with pH-dependent solubility. The 2',7'-dichlorofluorescein diacetate (DCFH-DA) results reveal that after exposure to gamma rays, Hf-doped bioceramic nanoparticlescould significantly lead to the formation of ROS in cells. Both cell viability and cytotoxicity assayshow consistent results that cancer cell lines are damaged with changes in the cells' ROS level. The in-vivo studies further demonstrate that tumor growth is inhibited owing to the cells' apoptosiswhen particles are bombarded with ionizing radiation. This finding offers a new therapeutic
method of interacting with ionizing radiation and demonstrates the potential of Hf-doped bioceramic nanoparticles in tumor treatment.

KEYWORDS: hafnium, bioceramics, ionizing radiation, radiosensitizer

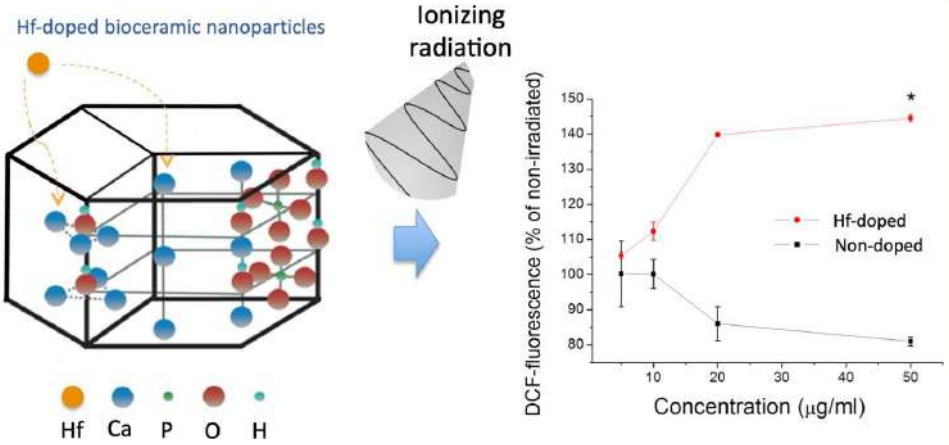

Illustration for the potent generation of ROS when Hf-doped bioceramic nanoparticles arebombarded with ionizing radiation.

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Using clinical porous gelatin sponge to establish a 3D Multilayered IntervertebralDisc Degeneration Model

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Abstract

For intervertebral disc degeneration (IVDD), due to the calcification of endplate, diminished oxygen and nutrients, as well asaccumulated lactate, are present in the microenvironment of the nucleus pulposus (NP) [1]. The disadvantage of 3D layered culture is uneven oxygen and nutrient gradient [2, 3]. Here, to mimic the in vivo microenvironment of the nucleus pulposus, 5-layered 3D culture was constructed using clinical hemostatic gelatin sponge and developed as an IVDD model. Then, in this 5-layered NP cell-loaded sponges model, cell distribution, gradient mRNA expression of decreased NP chondrogenic markers, including glycosaminoglycans (GAGs), COL2A1 and ACAN, as well as an increased degeneration marker, such as MMP3, were clarified from the top to the bottom layer. However, in a single NP cell-loaded disc model, the chondrogenic potency in the middle or bottom layer is better than that in the top layer. To further study the mechanism underlying the degeneration of NP cells in this IVDD model, we examined the contribution of secreted metabolites. Lactate was identified in the supernatant to modulate GAGs and MMP3 expression. Inhibition of lactate influx by MCT-1 inhibitor, AZD3965, reversed the effect of lactate on GAGs and MMP3 expression and further improved the NP cell degeneration in the IVDD model. Thanks to the homogenous expression of lactate in the model, we further identified that the combination of lactate andhypoxia enhanced MMP3 expression. Taken together, multilayered cell-loaded sponges with oxygen and nutrient gradient and lactate accumulation can be a 3D multilayered IVDD model for exploring potential agents for IVDD.

Keywords: Intervertebral disc degeneration, 3D cell culture, hemostatic gelatin sponge, chondrogenic-like nucleuspulposus differentiation

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Young Investigator Award (YIA, ISOMRM)

9/2 (Saturday)	
YIA-1	*Development of Hyaluronic Acid Coated with Kaempferol Nanoparticles for the Treatment of knee Osteoarthritis in Rats* **Prof. Ching-Yu Lee, Taipei Medical University, Taiwan**
YIA-2	*Immunofoam: foam-based immunotherapy for metastatic peritoneal cancer* **Prof. Yen-Liang Liu, China Medical University, Taiwan**
YIA-3	*Improving the Prognosis of Advanced Hepatocellular Carcinoma (HCC): Development and Evaluation of 125I-Labeled GPC3 Antibody Micelle* **Ms. Tzu-Chuan Ho, Kaohsiung Medical University, Taiwan**
YIA-4	*Development of Kaempferol-Loaded Platelet-Derived Extracellular Vesicles for Choroidal Neovascularization Treatment* **Mr. Huai-An Chen, Taipei Medical University, Taiwan**
YIA-5	*(Online) Porous scaffolds with microwell structures for 3D culture of pancreatic beta cells* **Mr. Huajian Chen, National Institute for Materials Science, Japan**
YIA-6	*Optogenetic Technique Advancement for Modeling Neuro-Cardiac Diseases* **Prof. Yen-Ling Sung, Taipei Medical University, Taiwan**
YIA-7	*Evaluation of the sustained-release carrier incorporating novel protein-drug of rhTMD2/3 for the spinal interbody fusion in a rat model* **Prof. Yan-Jye Shyong, National Cheng Kung University, Taiwan**
YIA-8	*Cerium oxide nanoparticles and hyaluronic acid hydrogel for early osteoarthritis treatment* **Dr. Hsuan-Yu Chen, National Taiwan University Hospital, Taiwan**
YIA-9	*The Use of preconditioned Mesenchymal Stromal Cell-Secretome as a Protective Measure for the Kidneys and Liver in an Acute on Chronic Liver Failure Animal Model* **Ms. Ya-Lin Huang, Universidad del Desarrollo, Chile**
YIA-10	*Multifunctional CuO/Cu2O Truncated Nanocubes as Trimodal Image-Guided Near-Infrared-III Photothermal Agents to Combat Multi-Drug-Resistant Lung Carcinoma* **Ms. Munusamy Shanmugam, National Tsing Hua University, Taiwan**
YIA-11	*Using Functional Photoacoustic Imaging to Monitor Tumor Hypoxia Dynamics during Treatment with Microbubbles and Gemcitabine* **Ms. Yuhling Wang, National Health Research Institutes, Taiwan**
YIA-12	*Microfluidic Devices and AIoT Animal Physiological Sensors for a New Compounds Screening System* **Dr. Weilun Sun, Pythia Biotech, Taiwan**

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of Hyaluronic Acid Coated with Kaempferol Nanoparticles for the Treatment of knee Osteoarthritis in Rats

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Abstract:

Current clinical guidelines for the treatment of knee osteoarthritis (OA) remain controversial regarding intra-articular (IA) injections of corticosteroid, hyaluronic acid (HA) or platelet-rich plasma. We need to continue to develop IA injectable drugs or formulations to achieve optimal injectable treatment of knee OA. Kaempferol (KM) has been established to be anti-inflammatory. KM's solubility often is criticized for its clinical applications. Nanoparticles are often engineered as a scaffolding system to facilitate the drug delivery, to control the release rate, to protect the drug against enzymatic or chemical degradation and to enhance bioavailability. HA is used to coat the nanoparticles because of its ability to interact with the CD44 receptor of chondrocytes as a targeting effect. Here, we encapsulate KM in a specialized HA-coated gelatin nano-system (HA- KM GNPs), circumventing the solubility problem and allowing potential-controlled release advantage to treat knee OA.
This study is to load kaempferol in hyaluronic acid coated gelatin-nanoparticle for intra- articular drug delivery and the hypothesis of this study is that the novel nano-encapsulated kaempferol can inhibit inflammation and enhance cartilage regeneration in knee OA.

We engineer hyaluronic acid coated gelatin nanoparticles with kaempferol encapsulation (HA-KM GNPs), whose characters will be assessed by the transmission electron microscope (TEM), the dynamic light scattering analyzer (DLS), and the high-performance liquid chromatography (HPLC). To evaluate biocompatibility of HA-KM GNPs, the viability of rat chondrocytes will be assessed by CCK-8 assay. Then, we use a rat model of anterior cruciate ligament (ACLT)-induced right knee OA to investigate the preclinical therapeutic of HA-KM GNPs. Micro-CT imaging will be used to assess subchondral bone mineral density. Histology and immunohistochemical evaluation will be performed

on full-thickness coronal sections of the articular cartilage in the medial component of right knee to assess cartilage preservation in treated/untreated knee OA.

This nano-encapsulated kaempferol particle is a mono-dispersive, colloidal system with a spherical core-shell, which was assessed by TEM. The average size of the particles is about 120.56±11.13 nm and the zeta potential is 30.16±2.17 mV, measured by DLS for each sample. The encapsulation efficiency was 99.75±0.22%, which was quantified by HPLC. In the rat ACLT- induced model of knee OA, IA injection of HA-KM GNPs can most prevent sclerosis change in subchondral bone of rat knee OA in micro-CT image analysis, reduce levels of inflammatory cytokines in the treatment of rat knee OA, and best improve cartilage erosion and preserve cartilage thickness of rat knee OA compared with PBS, free KM and HA GNPS.

This study has developed a novel nano-encapsulated kaempferol therapeutic system to ameliorate knee OA in rats.

KEYWORDS: nanoparticle, kaempferol, intra-articular injection therapy, knee osteoarthritis

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Immunofoam: foam-based immunotherapy for metastatic peritoneal cancer

Ya-Han Shen[1], Chun-Yi Chiang[1], Ulziijargal Sukhbat[2], Hsin-Chin Li[1], Yen-Chi Tsao[1], Pei-Chi Chiu[3], Yi-Ju Lin[4], Chih-Sheng Chiang[2], Shao-Chun Wang[2,5], Yen-Liang Liu[1,2,5*]

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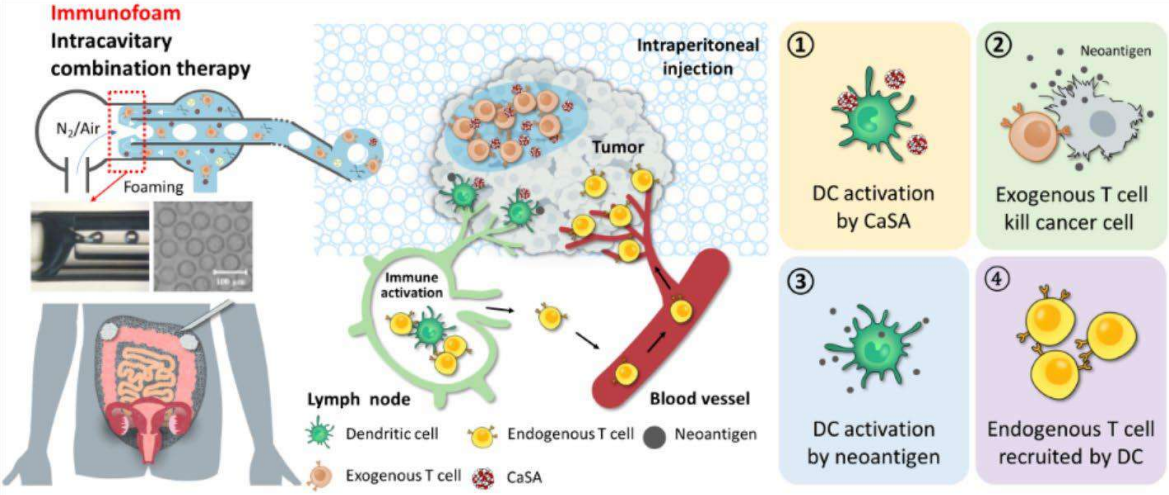
Abstract:

While peritoneal carcinomatosis is a common complication of late-stage cancers with drug resistance, current best practice is limited to palliation or chemotherapy with median survival lastthan one year [1]. An ideal intervention should exhibit therapeutic efficacy and maintain or improve patients' quality of life. Here, we developed a foam-based drug and cell delivery systemnamed Immunofoam. Immunofoam is injectable liquid foam capable of carrying combination therapies, including chemo drugs, immune adjuvants, nano/micro-particles, and immune cells. The foam formulation enables drug carriers to conform to the tissue surface and immerse the
cancer cells in therapeutic agents, extending the drug-contact time. Therefore, the feature of Immunofoam makes it an ideal drug delivery system for intracavitary combination therapy. Thisstudy demonstrated a successful intraperitoneal administration of immune cells through the foaming device, and the results exhibited the biocompatibility of Immunofoam and the cell survivability during the foaming method.
As Stimulator of interferon genes (STING) is emerging as a promising target for cancer immunotherapy [2], we developed Calcium carbonate-encapsulated STING Agonist (CaSA) microparticles which effectively stimulated antigen-presenting cells (APCs) for antitumor immunity and avoided STING-induced cytotoxicity to T-cells. Our results demonstrated that CaSA significantly increased the antitumor immunity of APCs which orchestrated the T-cell mediated cytotoxicity to cancer cells. Combining STING agonist and dendritic cells improved cytotoxic T-cell activity in both in vitro and in vivo models. CaSA was combined with Immunofoam for intraperitoneal injection with APCs and cytotoxic T cells, achieving remarkable therapeutic efficacy with minute doses of STING agonists in murine tumor models: subcutaneousand peritoneal cancer mouse models. Our study demonstrated Immunofoam is a promising therapeutic innovation for intraperitoneal immunotherapy for metastatic peritoneal cancer.

KEYWORDS: foam, immunotherapy, cell therapy, STING agonist, peritoneal cancer



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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Improving the Prognosis of Advanced Hepatocellular Carcinoma (HCC): Developmentand Evaluation of ^{125}I-Labeled GPC3 Antibody Micelle

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Abstract:
Raising the prognosis of advanced hepatocellular carcinoma (HCC) has been a challenge. Selective internal radiation therapy (SIRT) is one of the recommended approaches to treat advanced HCC. Glypican-3 (GPC3) is a specific glycoprotein marker on the cell surface of HCC.Iodine 125 (^{125}I) seed implantation is a safe and effective treatment for cancer. Our aim was to develop ^{125}I-labeled GPC3 antibody (^{125}I-GPC3-Ab) and study the effectiveness of this micelle in the treatment of HCC. ^{125}I-GPC3-Ab was prepared through iodination. The radioactivity and stability of ^{125}I on the GPC3 antibody remained at 85% up to 7 days after iodination. The ^{125}I-GPC3-Ab significantly bound to HCC cell lines compared to non-HCC cell lines at 6, 24, 48, and 96 hours post-incubation ($p<0.01$ or <0.001). Proliferation of HCC cell lines wassignificantly inhibited by incubation with 10μCi of ^{125}I-GPC3-Ab for 48 hours (non-treated cells:100% vs. treated cells: 36.3% to 55.5%, $p<0.001$). Cell death also significantly occurred in the above-mentioned treated HCC cell lines (non-treated cells: 0% vs. treated cells: 56.5% to 70.45%, $p<0.001$). Immunofluorescence staining showed higher expression of DNA damage-related proteins (γ-H2AX and Mre11) and cell cycle-related protein (cyclin B2) in ^{125}I-GPC3-Ab-sensitive HCC cells compared to low-sensitive HCC cells. Based on our results, the potential anti-cancer abilities of ^{125}I-GPC3-Ab was demonstrated. Selective internal radiation therapy (SIRT) is based on the sphere to deliver the radionuclide. HA-polycaprolactone (HA-PCL) has been shown to increase drug accumulation in tumors. We will further use HA-PCL to coat the ^{125}I-GPC3-Ab and explore the effectiveness of this micelle in an HCC-bearing mouse model.

KEYWORDS: Hepatocellular carcinoma (HCC), Radioimmunotherapy, Radioimmune-micelle

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of Kaempferol-Loaded Platelet-Derived Extracellular Vesicles for Choroidal Neovascularization Treatment

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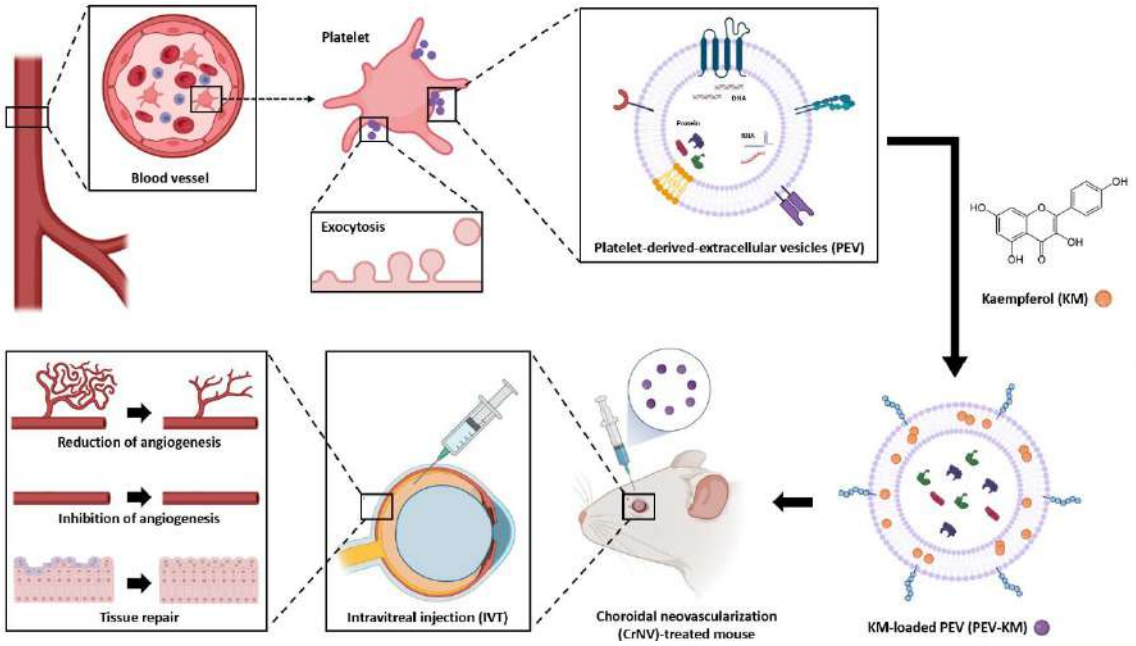
Abstract:

Choroidal neovascularization (CrNV), the excessive growth of abnormal blood vessels from the choroidal vasculature to the neurosensory retina, is a common pathologic lesion that may occur in many ocular diseases and often causes severe vision loss [1]. Currently, the mainstream treatment for CrNV is anti-VEGF therapy, which is monthly intravitreal injections of anti-vascular endothelial growth factor (VEGF) inhibitors, such as Aflibercept and Ranibizumab [2]. Despite the current anti-VEGF drugs demonstrating favorable anti-angiogenic effects in the clinic, challenges still exist, including the high costs, difficulties of manufacturing [3], short-term effects owing to the short half-lives [4], and the risk of some adverse effects, such as poor epithelial healing and corneal thinning [5]. In addition, frequent and repeated intravitreal injections may also lead to various complications, such as inflammation, vitreous hemorrhage, and retinal detachment. Here, we developed a novel nanomedicine (PEV-KM) by combining the anti-angiogenic agent, kaempferol (KM), and platelet-derived extracellular vesicles (PEV). KM is a natural flavonoid with wide pharmacological properties, including anti-angiogenic, anti-inflammatory, antioxidant, anticancer, and neuroprotective properties [6]. PEV, the most abundant extracellular vesicles with a variety of growth factors in body circulation [7], are analogs of physiological nanoparticles released from platelets. Due to the lipid bilayer membrane structure and nano sizes, PEV can be easily uptaken by cells, thereby increasing the bioavailability of therapeutic agents. In this work, we evaluated the anti-angiogenic effect of PEV-KM by In Vitro anti-angiogenic experiments and In Vivo laser-induced choroidal neovascularization model. The results indicated that PEV-KM effectively reduces the area of CrNV, even more effectively than EYLEA, a prescription medicine made of aflibercept. Based on the results, we believed that PEV-KM could be used in clinical applications in the future for treating pathological vascular diseases in the posterior of the eyes. The experimental design is shown in the graphical abstract. PEV were isolated from the blood and loaded with KM to synthesize PEV-KM. After confirming the anti-angiogenic effect via In Vitro experiments, PEV-KM were then injected into CrNV-treated mice by intravitreal injection. Finally, the anti-angiogenic effect of PEV-KM was evaluated by related analyses.

KEYWORDS: Choroidal neovascularization treatment, anti-angiogenic therapy, kaempferol, extracellular vesicles, platelet-derived extracellular vesicles



Graphic abstract

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Porous scaffolds with microwell structures for 3D culture of pancreatic beta cells

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Abstract:

Type 1 diabetes mellitus (T1DM) as a chronic disease seriously impedes patients' life by dysregulation of blood glucose homeostasis and other serious complications. Until now, injectionof synthetic insulin is the most common strategy for relieving symptoms of T1DM. But lifetime injection of insulin is inevitable and inconvenient and T1DM still needs to be cured. It has been well recognized that the malfunction of endocrine cells in pancreatic islets causes T1DM,especially insulin-secreting pancreatic beta cells. Therefore, transplantation of pancreatic beta cells has been expected as a novel cell therapeutic strategy that can treat T1DM once and for all.However, it remains a challenge to maintain cell-cell interaction and to promote functions of pancreatic beta cells.

To address these issues, a gelatin-PLGA mesh based porous microwell scaffold was preparedfor 3D culturing of pancreatic beta cells to promote aggregation and function of cells (Figure 1). Ice particulate templates were used to form microwell array on the surface of gelatin-PLGA meshscaffolds. Templates were prepared by depositing water droplets on hydrophobic surface and freezing in a deep freezer. The sizes of ice particulates could be adjusted by moisture exposure time in a humid environment. PLGA mesh immersed with 5 % (wt%) gelatin solution was pre- cooled in a low temperature chamber and covered by ice particulate templates of four types of different sizes of ice particulates to prepare flat scaffold, small, middle and large microwell scaffolds. The ice particulate templates were removed by freeze-drying and microwell scaffolds were cross-linked by amidation reaction for further use.

The sizes of microwells were confirmed by scanning electron microscopy. Microwellstructures of different sizes were formed on the surfaces of the small, middle and large microwellscaffolds. The microwells had a semi-concave morphology separated by thin ridges. High magnification images showed that the wells of the microwells had dense ultra-small pores. However, there was no microwell structure on the surface of flat scaffold. RIN-5F cells were seeded on the porous microwell scaffolds and cultured for 7 days to investigate the effects of microwell scaffolds on promoting aggregation and function of beta cells. Live/dead staining wasconducted after cell culture for 1 day and 7 days. Results showed that RIN-5F cells had high viability after 1 day and 7 days of culture. The morphology of RIN-5F cells was changed from monodispersed to aggregates on all scaffold. The cells cultured in the small, middle and large microwell scaffolds formed small aggregates and the small aggregates further formed grape-like large aggregates. However, the cells on the flat scaffold formed flake-like clusters. Grape-like aggregates in the microwell scaffolds had strong cell-cell interaction while flake-like cell clustersshowed loose junction under observation of confocal laser scanning microscopy. DNA amount ofRIN-5F cells in the scaffolds increased after culture for 7 days. Insulin secretion of RIN-5F cellsin the scaffolds were investigated by ELISA and compared with 2D culture of RIN-5F cells. Theresults showed that insulin secretion of RIN-5F cells in the microwell scaffolds after 7 days culturewas significantly higher than that in 2D culture. These results indicated that the gelatin-PLGA mesh based porous microwell scaffolds could promote functions of pancreatic beta cells.

KEYWORDS: Diabetes mellitus, ice particulate template, microwell scaffold, beta cell aggregates, insulin secretion.

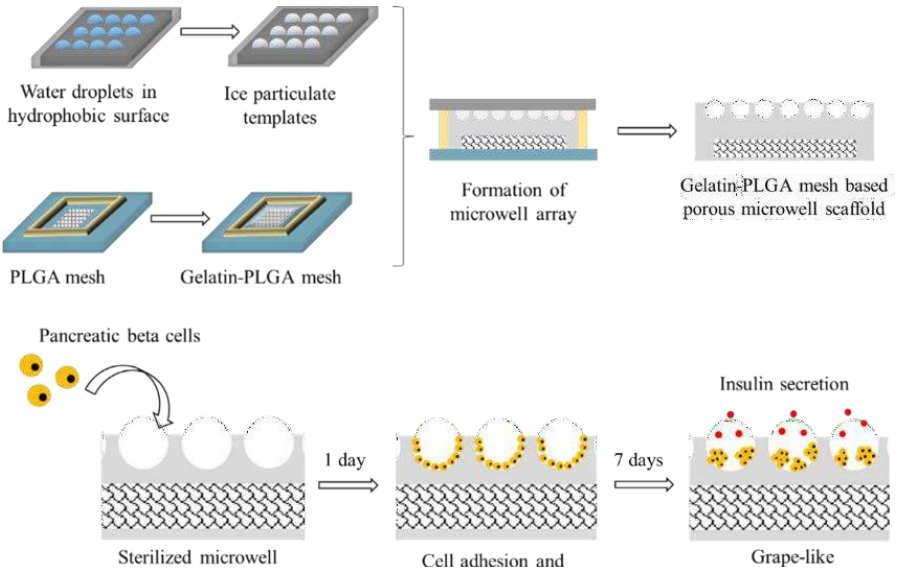

Figure 1 Preparation scheme of microwell scaffolds and 3D culture of pancreatic beta cells

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Optogenetic Technique Advancement for Modeling Neuro-Cardiac Diseases

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Abstract:

Cardiovascular disease remains the leading cause of mortality worldwide, with coronary heart disease, particularly heart failure (HF), being a major contributor. HF induced by the sympathetic nervous system (SNS) has been shown to disrupt the delicate balance between SNS and vagal nerve (VN) interactions, leading to alterations in receptor activation that can profoundlyaffect cardiac structure and function.

Current therapies for HF, such as beta-receptor blockade to inhibit sympathetic drive, have become standard practice. Additionally, electric stimulation techniques have been developed to selectively modulate sympathetic and vagal nerve activity, aiming to restore autonomic balance in HF. However, the understanding of differential responses between the right and left stellate ganglia, as well as the underlying neurochemical and molecular mechanisms contributing to thesedifferences in HF, remains limited. Furthermore, the role of the parasympathetic system in HF andits potential antiarrhythmic effects have not been thoroughly investigated.

Optogenetics, an emerging field for restoring neuronal function in neurological diseases, holds immense potential for modulating cardiac function. However, its application in neural modulation of cardiac function is still in its early stages. The objective of this study is to developa novel optogenetic therapy strategy for sympathoinhibition through dual optogenetic stimulation.In this paper, we employ the optogenetic technique to comprehensively explore the intricate roles of the sympathetic and parasympathetic systems in modulating cardiac function, addressing crucial clinical challenges.

By elucidating the neurophysiological mechanisms involved in autonomic imbalance and investigating the therapeutic potential of optogenetics, this research aims to pave the way for innovative approaches in managing neuro-cardiac diseases. The findings have the potential to enhance our understanding of HF pathophysiology, identify novel therapeutic targets, and ultimately improve patient outcomes.

KEYWORDS: Cardiovascular disease, Heart failure, Neural modulation, Optogenetics

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Evaluation of the sustained-release carrier incorporating novel protein-drug of rhTMD2/3 for the spinal interbody fusion in a rat model

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Abstract:

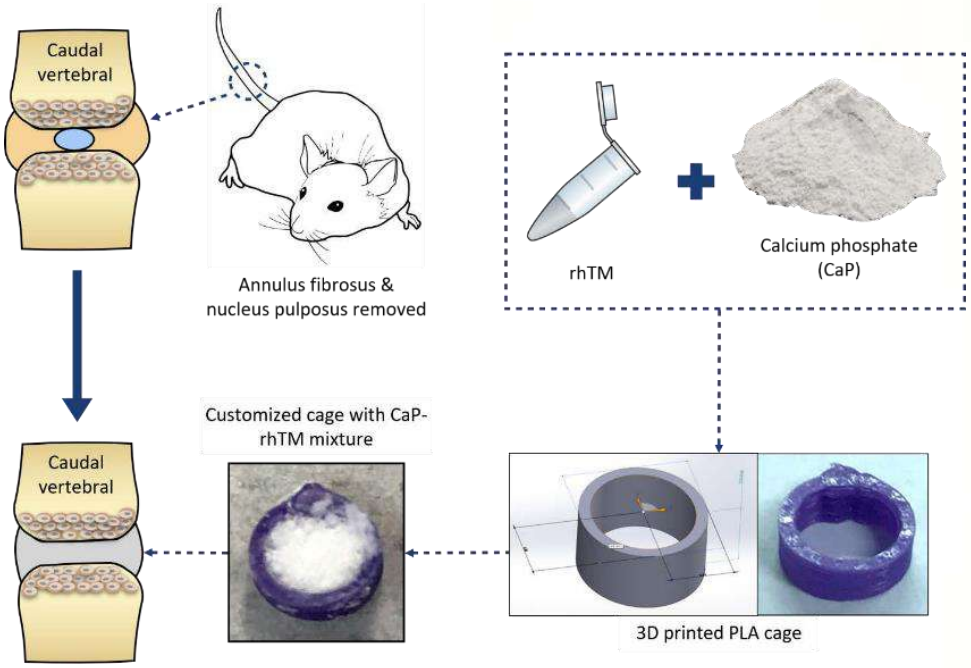
Recombinant human thrombomodulin (rhTM) is a transmembrane glycoprotein, and EGF- like, Ser/Thr-rich domain (rhTMD2/3) can induce osteoblasts proliferation. Because rhTMD2/3s has high potency, controlling delivery of rhTMD2/3 is crucial to play as effects and reduce its side effect. Calcium phosphate (CaP) is a biodegradable, non-toxic biomaterial with good osteoconductive properties. In this study, we used CaP as the carrier to incorporate rhTMD2/3 for slow release. We evaluated the effects of sustained-release rhTMD2/3 on spinal interbody fusion in a rat model.

For in vitro study, pre-osteoblast cell line MC3T3-E1, originally from mouse calvaria, is used as a cellular model to determine the influence of rhTMD2/3 with various concentrations on proliferation and differentiation. As for the in vivo study, a rat model was introduced to implement interbody fusion surgery on an animal scale. Discectomy was conducted on rat caudal vertebrae to create a good site for recording spinal interbody fusion for different periods (8, 16, and 24 weeks) after hybrid cage implanting (cage-only, CaP, and CaP-rhTMD2/3). Micro-computed tomography (μCT) records and analyzes the fusion status for each group (n=5). Further qualification was presented and conducted by osteological histochemical staining and mechanical testing to validate the whole process.

The proliferation rate of MC3T3-E1 shows a hook effect with rhTMD2/3, ranging from 5 to 1000 ng/ml. The 50 ng/ml group is the most significant value compared with a placebo. Then, ALP staining reveals that rhTMD2/3 facilitates a differentiated phase with a significant colorimetric difference and activity compared with the control. A 3D trabecular bone microarchitecture of an intervertebral region illustrates that those treated with CaP-rhTMD2/3 exhibit ossification and trends to fusion. Histological observation validates that rhTMD2/3 facilitates interbody fusion by boosting initial angiogenesis and calcium deposition. Finally, we also demonstrated higher mechanical strength of the rhTMD2/3 group to prove a good fusion quality of the rat caudal vertebrae.

Although the interaction between rhTMD2/3 and osteogenesis remains uncertain, current results have provided promising aspects and potential therapeutics for further clinical study.

KEYWORDS: Recombinant human thrombomodulin (rhTM), Calcium phosphate (CaP), Spinal interbody fusion



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2023 ISOMRM & BCRS
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Cerium oxide nanoparticles and hyaluronic acid hydrogel for early osteoarthritis treatment

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Abstract:

Osteoarthritis (OA) is the most common type of joint disease, accompanied by varying degrees of functional limitation, reducing life quality of patients, especially knee osteoarthritis.
Hyaluronic acid (HA) joint injections and pain relievers are efficient treatments for early- stage osteoarthritis of the knee whereas patients have to knee replacement surgery in the later stages of osteoarthritis. After injected directly into the cavity around the knee joint, HA works by acting like a lubricant and shock absorber in the joints, eases the pain and helps the joints to work properly. However, for the decomposition by hyaluronidase and free radicals in knee joint, HA injection treatment has limited effect time. HA injection shows low benefits to patients suffer from knee and joint osteoarthritis.

The cerium oxide nanoparticles (CNPs) have been reported that a long time free radical scavenger. CNPs combined with HA expected that extending HA decomposition time and having positive effect to osteoarthritis therapy. CNPs were synthesis by salt hydrolysis at room temperature. This study has divided four parts: 1. to analyze for physical property of CNPs. 2. To exam the free radicals are removed by CNPs. 3. The in vitro assays of CNPs. 4. To exam the CNPs/HA for early protection of chondrocyte.

CNPs had successful synthesized by salt hydrolysis with particle size about 120 nm, showed good dispersibility in culture medium and good antioxidant ability. CNPs are biocompatible under the ratio of 6.7 ng/ 1x104 cells. The OA model can be established by the cell treated with H2O2 for 30 min, which ratio to 1x104 cells is 0.03 mmol. The H2O2 damage effect could be resisted when chondrocyte cultured with CNPs /HA after 1 day. The results suggest that CNPs /HA would be a potential candidate for early-stage osteoarthritis treatment.

KEYWORDS: osteoarthritis, cerium oxide nanoparticles, free radical, hyaluronic acid.

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

The Use of preconditioned Mesenchymal Stromal Cell-Secretome as a Protective Measure for the Kidneys and Liver in an Acute on Chronic Liver Failure Animal Model

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Abstract:

Acute on Chronic Liver Failure (ACLF) is a syndrome involving sudden decline in liver function in patients already suffering from either a compensated or decompensated chronic liver disease. When Acute Kidney Injury (AKI) accompanies ACLF, it often leads to a severely diminished survival rate for affected patients [1]. Mesenchymal Stromal Cells (MSCs) therapy is emerging as a potential solution for complicated conditions like ACLF. MSCs produce a wide variety of trophic and immunomodulatory molecules, referred to as MSC-secretome. Remarkably, pre-conditioning these MSCs in vitro can enhance their secretion of anti-inflammatory and trophic factors [2].

Our study sought to examine if intravenous administration of pre-conditioned human MSC-derived secretome could prevent liver failure and AKI in a severe ACLF rat model.

We pre-conditioned human MSCs obtained from adipose tissue in vitro using TNF- α/INFγ. To simulate severe ACLF, we used an i.p. administration of porcine serum for 11 weeks (representing chronic liver disease), followed by D-gal/LPS administration to induce acute liver failure in rats [3]. The first group was given an intravenous injection of 200 µl saline solution (ACLF group), while the second received concentrated MSC-secretome derived from 1x106 MSCs (ACLF-sec group).

By administering MSC-secretome, we observed a significant reduction of ACLF, with the survival rate improving from 40% in the ACLF group to 85% in the ACLF-sec group. This improvement was associated with an increased rate of hepatocyte proliferation (PCNA expression) and decreased hepatocyte apoptosis rate (TUNEL) in the ACLF-sec group. Additionally, the liver's histological structure was preserved with secretome administration.

Increased expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, Cinc-1 and MCP-1) was seen in both the ACLF and ACLF-sec groups. However, a rise in anti- inflammatory molecules (IL-4, IL-5 and TGFβ1) was observed in the ACLF-sec group.

Similarly, renal histopathological evaluation of the ACLF group showed tubular damage, elevated apoptosis rate, and upregulation of Kim-1, HMGB-1, and IL-6 expression. Remarkably, MSC-sec administration improved renal tubular lesions, decreased apoptosis rate, and reduced injury marker expression.

Our results highlight the potential application of MSC-sec in managing multi-organ failure related to ACLF.

KEYWORDS: Acute on Chronic Liver Failure (ACLF), Acute Kidney Injury (AKI), Mesenchymal Stromal Cells (MSCs), Secretome, Cell free therapy.

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Multifunctional CuO/Cu2O Truncated Nanocubes as Trimodal Image-Guided Near- Infrared-III Photothermal Agents to Combat Multi-Drug-Resistant Lung Carcinoma

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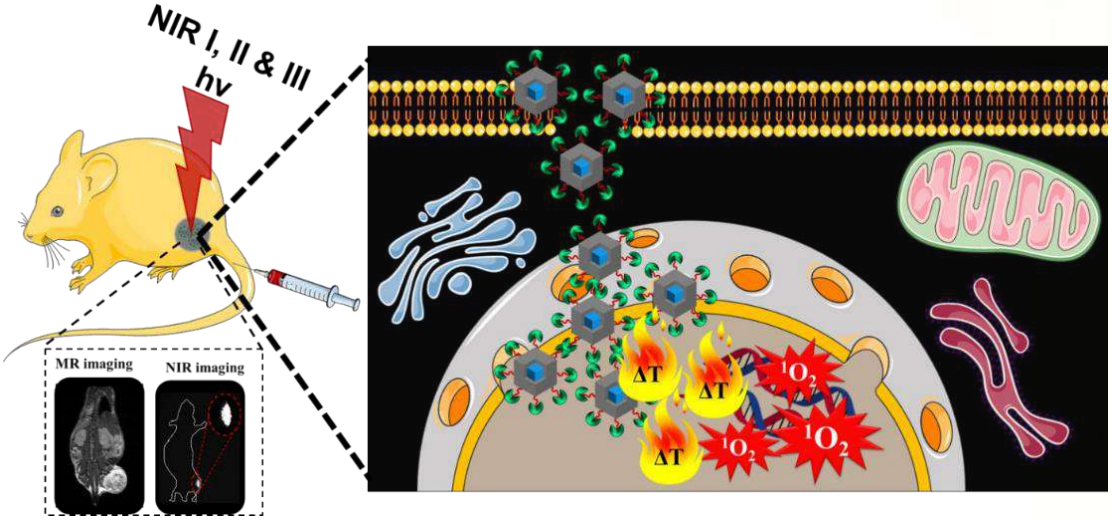
Abstract:

Despite the development of various therapeutic modalities to tackle cancer, multidrug resistance (MDR) and incomplete destruction of deep tissue-buried tumors remain as long- standing challenges responsible for tumor recurrence and low survival rates. In addition to the MDR and deep tissue photoactivation problems, most primary tumors metastasize to the lungs and lymph nodes to form secondary tumors. Therefore, it leaves a great challenge to develop Theranostic approaches to combat both MDR and deep tissue photoactivation problems. Herein, we develop a versatile plasmonic CuO/Cu2O truncated nanocube-based theranostic nanomedicine to act as a triple modal near-infrared fluorescence (NIRF) imaging agent in the biological window II (1000−1500 nm)/ photoacoustic imaging (PAI)/T1-weighted magnetic resonance (MR) imaging agents, sensitize the formation of singlet oxygen (1O2) to exert nanomaterial-mediated photodynamic therapeutic (NIR-II NmPDT), and absorb long NIR light (i.e., 1550 nm) in the biological window III (1500−1700 nm) to exert nanomaterial-mediated photothermal therapeutic (NIR-III NmPTT) effects for the effective destruction of multi-drug-resistant lung tumors. We found that H69AR lung cancer cells do not create drug resistance toward plasmonic CuO/Cu2O TNCs-based nanomedicines.

KEYWORDS: plasmonic nanostructures, NIR-II fluorescence imaging, NIR-III photothermal therapy, multiple-drug resistance, nanotheranostics



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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Using Functional Photoacoustic Imaging to Monitor Tumor Hypoxia Dynamics duringTreatment with Microbubbles and Gemcitabine

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Abstract:

Hypoxia is present in many solid malignant tumors. Hypoxia has been shown to promote tumor cell invasiveness, resistance to chemotherapy and radiotherapy, and immunosuppression and is an important factor in cancer treatment [1]. Oxygenated and de-oxygenated hemoglobin are intrinsic biological optical contrast agents that can be used to monitor hypoxia in the tumor microenvironment using photoacoustic imaging [2]. In this study, a photoacoustic imaging system was setup to image oxy- and deoxy-hemoglobin using 750 nm and 850 nm laser light. The system performance was assessed using a controlled hypoxia study where carbogen was given to induce hypoxia while monitoring tissue oxygenation (SO_2) in the rat hindlimb. Next,we were able to monitor tissue oxygenation using photoacoustic imaging of a pancreatic mouse tumor model and observe increase in hypoxia over time. In order to monitor hypoxia and tumor vascular response to treatment, tumor cells were treated using a combination of Gemcitabine and microbubble-mediated sonoporation. Microbubbles and ultrasound have been used to increase chemotherapy drug penetration into solid tumors [3]. Functional photoacoustic imagingwas used to longitudinally monitor changes in tissue SO_2 and tumor vascularity throughout treatment. Changes in tumor SO_2 can potentially be used to guide treatment or as an early indicator of tumor response to the treatment.

KEYWORDS: photoacoustic imaging (PAI), microbubble, sonoporation, pancreatic tumor,hypoxia, tissue SO_2

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Microfluidic Devices and AIoT Animal Physiological Sensors for a New CompoundsScreening System

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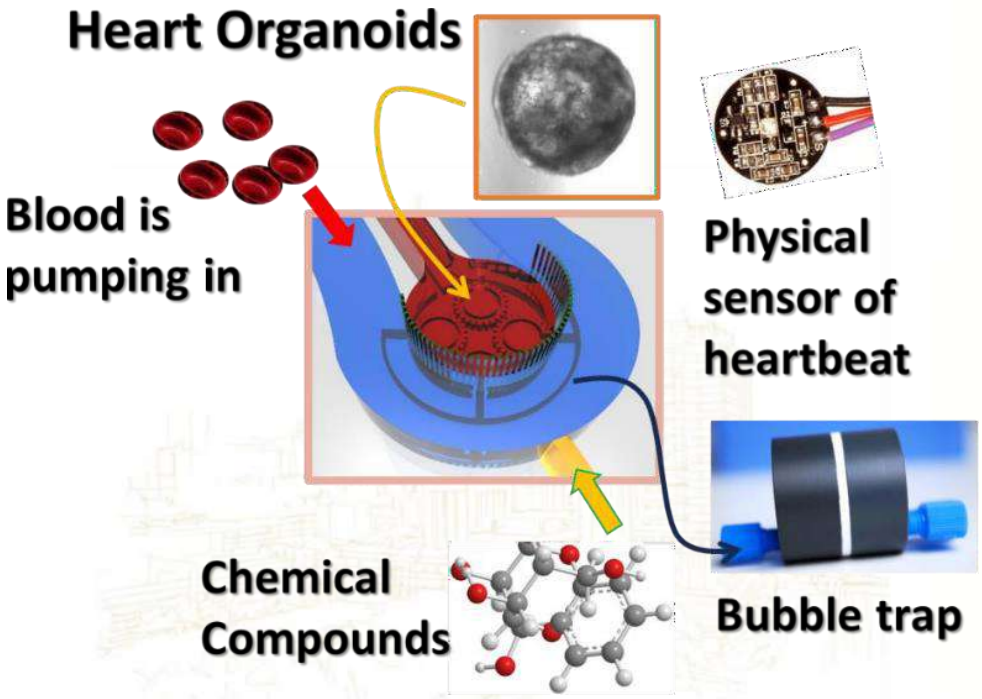
Abstract:

This project will generate a new compounds screening system from a small molecule drug NG13-1 testing by using organ-on-chip, organoids culturing, AI bioinformatics (Genenet Technology) to real-time monitoring of culturing process to obtain cardiotoxicity test report. A small molecule drug, NG13-1 from Vasodynamics, is expected to reduce alopecia from chemotherapy, chemoradiotherapy, and targeted/immunotherapy. However, the effects on the heart, lung, and liver from this new small molecule drug has not been studied. The data from NG13-1 from cardiac organoids on organ-on-chip will be compared with an animal study (Homesuit Biomed Technology) to confirm the efficacy and efficiency of engineered cardiac organoids in organ-on-chip.

Cardiac organoids will be provided from Genenet Technology. The culture condition will be monitored by an AI system to get real-time information. The prototype of cardiac organ-on-chip system will be transformed from a previous model, tumor microenvironment-chip which constructs three circle areas for spheroids and a belt surrounding them. The cardiac organoids willbe implanted in the area from spheroids and the belt will be pumped in with continuous perfusionof medium or blood flow. The pharmacokinetic system will be constructed by connecting heart, lung, and liver-chips to see the metabolic of NG13-1 and the effects on each organ. The difficultiesof connecting organ-on-chips including the common culture medium, pressure, air bubbles, and data collecting. For animal study, Homesuit Biomed technology will develop an algorithm to monitor the rates of heart beats and breathing from animals. The sensors can be implemented in the cadge or under the surgical pad. Via Bluetooth, the caretaker can see the beating rate and frequency of breathing on the app of a cellphone.

This project will validate a new drug screening platform, organ-on-chip, to reduce and replace the use of experimental animals. The alternative for animal study is urgently developing.Since 2007 the European Union (EU) has already stopped using animal tests for cosmetics and in2019 the EU has developed a standard protocol for all organ-on-chips. EU expect within 20 years,we won't use animal for experiment anymore. Further, in 2022, FDA modernization Act 2.0 allowsto use alternative testing methods to animal testing of a new drug. This act expands the horizon inorgan-on-chips and will accelerate the development of a new compound in treatment.

KEYWORDS: organ-on-chip, microfluidics, cardiac organoids, animal physiological sensors, small molecule drug, AI, AioT



Student Oral

September 1 (Friday)	
S-1	*Development and application of a drug carrier platform for probiotic lysate extract delivery* **Yong-Xin Ta, National Yang Ming Chiao Tung University, Taiwan**
S-2	*A chemoimmunotherapy nanogel enables efficient delivery of interleukin-2 and induction of immunogenic cell death for effective cancer therapy* **Yen-Nhi Ngoc Ta, National Tsing Hua University, Taiwan**
S-3	*Magnetolytic therapy-mediated antigen capture and T cell infiltration in lung metastasis by biomimetic zwitterionic copolymer coated magnetic nanoparticles* **Thi My Hue Huynh, National Tsing Hua University, Taiwan**
S-4	*Bridging the Gap Between In Vitro and In Vivo the Chick Embryo Model and Its Potential Applications in Nanomedicine* **Cong-Kai Lin, Taipei Medical University, Taiwan**
S-5	*Calcium carbonate-encapsulated STING agonist for anti-cancer immunotherapy* **Yi-Ju Lin, China Medical University, Taiwan**
S-6	*Development of temperature and reactive oxygen species responsive gelatin-PNIPAM-based hydrogels for drug delivery* **Hsin-Ho Chen, National Taiwan University of Science and Technology, Taiwan**
S-7	*Spheroids-laden hydrogel with spatially confined delivery of signaling molecules for engineering 3D complex tissue* **Eunjin Lee, Hanyang university, Korea**
S-8	*Nanocomposite hydrogels containing immunomodulatory strontium-tannic acid nanoparticles for vascularized skin tissue regeneration* **Yujin Han, Hanyang university, Korea**
S-9	*Fabrication of a collagen-based bioink for 3D bioprinting* **Po-Hsun Chen, National Taiwan University, Taiwan**
9/2 (Saturday)	
S-10	*A Biomimicking and Multiarm Self-Indicating Nanoassembly for Site-Specific Photothermal-Potentiated Thrombolysis Assessed in Vessel-on-a-Chip Device and in vivo Models* **Kuan-Ting Liu, National Taiwan University, Taiwan**
S-11	*Programmed T Cells Infiltration into Lung Metastases with Harnessing Dendritic Cells in Cancer Immunotherapies by Catalytic Antigen-Capture Sponges* **Min-Ren Chiang, National Tsing Hua University, Taiwan**
S-12	*A Novel Peptide Assembling Nanoparticle as Eye Drop for Treating Choroidal Neovascularization* **Yu-Yi Wu, Taipei Medical University, Taiwan**
S-13	*Direct Thermal Growth of Gold Nanopearls on 3D Interweaved Hydrophobic Fibers as Ultrasensitive Portable SERS Substrates for Clinical Applications* **Li-Chia Lu, National Tsing Hua University, Taiwan**

S-14	*Bi2S3@C/Pd-BSA hetero-nanostructures for photocatalysis-mediated hydrogen sulfide splitting and hydrogen production for colorectal cancer therapy* **Arjun Sabu, National Tsing Hua University, Taiwan**
S-15	*Three-dimensionally cultured adipose derived stem cell exosomes for diabetic wound healing* **Edgar Quiñones, National Taiwan University, Taiwan**
S-16	*Platelet-derived biomaterial with hyaluronic acid alleviates temporal mandibular joint osteoarthritis: clinical trial from dish to human* **Wen Tsao, Taipei Medical University, Taiwan**
S-17	*The synergistic in vitro and in vivo antitumor effect of combination therapy with iron oxide nanoparticles and fucoidan against lung adenocarcinoma* **Thi-Luu Ho, Taipei Medical University, Taiwan**
S-18	*One-step Bone Graft: The Efficiency of Bone Regeneration with 3D Culture Wharton's Jelly Mesenchymal Stem Cells* **Chia-Chun Hsu, Changhua Christian Hospital, Taiwan**
S-19	*Disruption of CCL2 in mesenchymal stem cells as an anti-tumor approach against prostate cancer* **Quoc Thang Bui, Taipei Medical University, Taiwan**
S-20	*Isolation of Ovarian Cancer Cells to Establishing Cancer Stem Cell Lines Using Membrane Filtration Method* **Yi-Shuo Su, National central university, Taiwan**
S-21	*Development of A New Prime Editing System for Efficient Genome Editing* **Quyen Thuc Dang, National Tsing Hua University, Taiwan**
S-22	*Nanoparticle-Based Dopaminergic Neuron Differentiation Approach - in vitro Model Derived from Human Induced Pluripotent Stem Cells* **Cai-Jhen Wu, Taipei Medical University, Taiwan**
S-23	*Establishing a drug screening platform based on cardiomyocytes derived from human induced pluripotent stem cells for analyzing the effects of nanosized heart failure medications* **Tzu-Yun Yeh, Taipei Medical University, Taiwan**
S-24	*A high-throughput lung air-blood barrier neutrophil transmigration system for drug dose-dependent study* **Liang-Hsin Chen, Georgia Institute of Technology, United States**
S-25	*Polymerized magnetic cells amenable to synapse formation enable selective capture of antigen-specific T lymphocytes* **Chung-Yao Hsu, Academia Sinica, Taiwan**
S-26	*Combination of Platinum-doped CaCO3 and Amylopectin-based Gel to Synergize with Radiotherapy for High-grade Glioma* **Jason Lin, National Taiwan University, Taiwan**
S-27	*(Online) 4D Printed Multilayered Scaffolds with Enhanced Performance for Bone Regeneration* **Jizhuo Chen, The University of Hong Kong, China**

S-28	*(Online) Influence of hydrogel stiffness on adipogenic differentiation of mesenchymal stem cells with controlled morphology* **Chengyu Lu, University of Tsukuba, Japan**
S-29	*(Online) Composite scaffolds for magnetic hyperthermia of breast cancer and reconstruction of adipose tissue* **Rui Sun, University of Tsukuba, Japan**
9/3 (Sunday)	
S-30	*Multi-functional hydrogels incorporating mineral-coated composite nano fibers with magnetic nanoparticles for photothermal therapy and bone tissue regeneration* **Taeyeon Hwang, Hanyang University, Korea**
S-31	*Changing Behavior of C2C12-GFP on Linear Groove Polydimethylsiloxane (PDMS) Substrate* **Yhusi Karina Riskawati, Universitas Brawijaya, Indonesia**
S-32	*Administration of self-assembly mRNA nanomedicine augmented calvarial defect healing by endochondral ossification* **Cheng-Hsin Wu, China Medical University, Taiwan**
S-33	*Sebacoyl Dinalbuphine Ester-Loaded Nanostructured Lipid Carriers in Gel for Postoperative Pain on Spine Surgery* **Yi-Lian Li, National Cheng Kung University, Taiwan**
S-34	*Antiretroviral-Drugamer In-situ Forming Subcutaneous Injectables with Tunable Drug Release* **Shin-Tian Chien, University of Washington, United States**
S-35	*Effect of Magnetic Field Strength on the Controlled Release Behavior of Magnetic Nanogel Drug Delivery Systems* **Chia-Ke Tsou, Chung Yuan Christian University, Taiwan**
S-36	*Constructing Heart-specific Exosome Profile to Enable Research of Cardiovascular Disease* **Rosie Kao, National Taiwan University of Science and Technology, Taiwan**
S-37	*Enabling Rapid Extracellular Vesicle Isolation from Cell Culture Media by Osmosis* **Casey Huang, National Taiwan University of Science and Technology, Taiwan**
S-38	*Assessment of the neuroprotective and neuro-regenerative potentials of extracellular vesicles isolated from platelet concentrates in Parkinson's disease and traumatic brain injury models* **Liling Delila, Taipei Medical University, Taiwan**
S-39	*Diffusion-tensor imaging and dynamic susceptibility contrast MRI improve radiomics-based machine learning model of MGMT promoter methylation status in glioblastomas* **Tran Nguyen Tuan Minh, Taipei Medical University, Taiwan**
S-40	*Concentrates Urinary Biomarkers Via the Osmosis Processors* **Chia-Yu Lee, National Taiwan University of Science and Technology, Taiwan**
S-41	*Immobilization of lysozyme on chitosan modified nanofiber membrane: Antibacterial Assessment* **Thi-Tam-An Tran, Ming-Chi University of Technology,**
S-42	*Temperature-Responsive Polymer-Antibody Conjugate for Biomarker Separation* **Maggie Shen, National Taiwan University of Science and Technology, Taiwan**

S-43	*H2S-responsive copper selenide Cu2-xSe@BSA nanoparticles for photothermal and chemodynamic combination therapy in colon cancer* **Manoj Kandel, National Tsing Hua University, Taiwan**
S-44	*Novel of Hydroxyapatite Nanoparticle-Loaded Hydrogel Scaffold for Bone Regeneration* **Yi-Chieh Hsu, Taipei Medical University, Taiwan**
S-45	*Anti-aging biomaterial sturgeon chondroitin sulfate chelates biological functions to reprogram stem cell senescence and ameliorate aging to prolong longevity* **Abhinay Kumar Singh, Taipei Medical University, Taiwan**
S-46	*PEO-PLA/PEO core-shell structured fibers fabricated by coaxial electrospinning for controlled drug release* **Ji-Feng Wang, National Yang Ming Chiao Tung University, Taiwan**
S-47	*Oxidation-mediated scaffold engineering of hyaluronic acid-based microcarriers enhances corneal stromal regeneration* **Huan-Lun Ting, Chang Gung University, Taiwan**
S-48	*Highly retina-permeating and long-acting resveratrol/metformin nanotherapeutics for enhanced treatment of macular degeneration* **Chia-Jung Yang, Chang Gung University, Taiwan**
S-49	*Photocrosslinkable carboxymethyl cellulose/collagen/plate-rich plasma hydrogel for wound dressing* **Wei-Chieh Chang, National Taiwan University, Taiwan**
S-50	*The Development of Functionalized Oxidized Bacterial Cellulose-Based Hemostats* **Chonlachat Jaihao, Taipei Medical University, Taiwan**
S-51	*Methacrylate silatrane: Newly synthesized building block for advancement of surface silanization and functional polymers* **Van-Truc Vu, National Central University, Taiwan**
S-52	*Modification of plant-based starch powders for surgical anti-adhesion applications* **Tzu-Shan Fang, National Taipei University of Technology, Taiwan**
S-53	*Liquid foam as carrier of immune cells and anti-cancer agents for intraperitoneal immunotherapy* **Ulziijargal Sukhbat, China Medical University, Taiwan**
S-54	*Human pluripotent stem cell culture on dendrimer surface grafted with ECM-derived peptides* **Wen-Hui Chao, National Central University, Taiwan**
S-55	*GVHD treatment utilizing several types of stem cells cultivated on biomaterials* **Chang-Yen Tsai, National Central University, Taiwan**
S-56	*Culturing and Differentiation of Human Pluripotent Stem Cells on hydrogel mixture of E-cadherin- and ECM-derived peptides* **Zhao-Yu Hong, National Central University, Taiwan**
S-57	*Premixed calcium silicate bone cement with rapid setting and washout resistance* **Yi-Huei Huang, Chung Shan Medical University, Taiwan**
S-58	*3D printing of PEGDA-CAP-rhTM intervertebral cage increased stability on rat spinal fusion model and long-acting release protein drugs to promote intervertebral disc fusion* **Wei Huang, National Cheng Kung University, Taiwan**

2023 ISOMRM & BCRS
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Development and application of a drug carrier platform for probiotic lysate extract delivery

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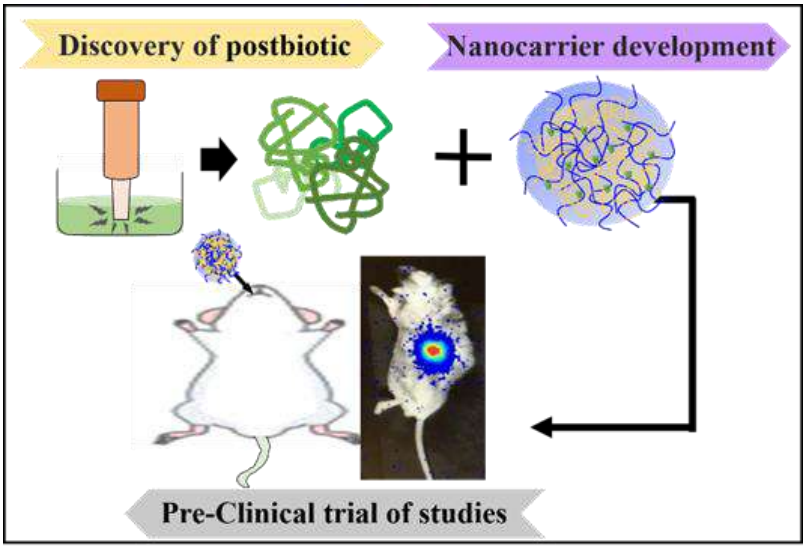
Abstract:

Cancer is a leading cause of global mortality, ranking among the top in terms of mortality rates and annual new cases, as the World Health Organization reported in 2020. The management of advanced cancer often involves chemotherapy. However, prolonged exposure to chemotherapy drugs may lead to the development of drug resistance resulting in the failure of standard treatment [1]. Probiotics, defined as live bacteria that are beneficial to human health, have been widely used in gastrointestinal health care and are highly accepted by the market [2]. They show promising effects in enhancing apoptosis or inhibiting proliferation in various cancer treatments. Postbiotics, including dead bacteria, cell lysate extracts, and supernatants of specific probiotic strains, also demonstrated safety and efficacy for medical purposes [3]. Our research team obtained the lysate extracts of probiotic strains, and successfully screened out lysates of probiotics that have the potential for cancer treatment. However, the efficacy and safety of these lysates can be affected by their protein structure. To address this, we developed an innovative oral therapeutic system by combining lysate extracts with a drug carrier platform. This platform is designed to protect the active lysates from degradation and denaturation, preserving their therapeutic potential during the delivery process and enhancing the overall bioavailability. It demonstrated good anti-cancer efficiency, biocompatibility, controlled drug release properties, and effectively accumulate on targeted tumor sites. This combination not only promoted the development of new drugs but also provided a potent drug delivery method to achieve effective treatment of cancer or related diseases.

KEYWORDS: *cancer, probiotics, postbiotics, lysate extracts, nanocarrier*



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A chemoimmunotherapy nanogel enables efficient delivery of interleukin-2 and induction of immunogenic cell death for effective cancer therapy

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Abstract:

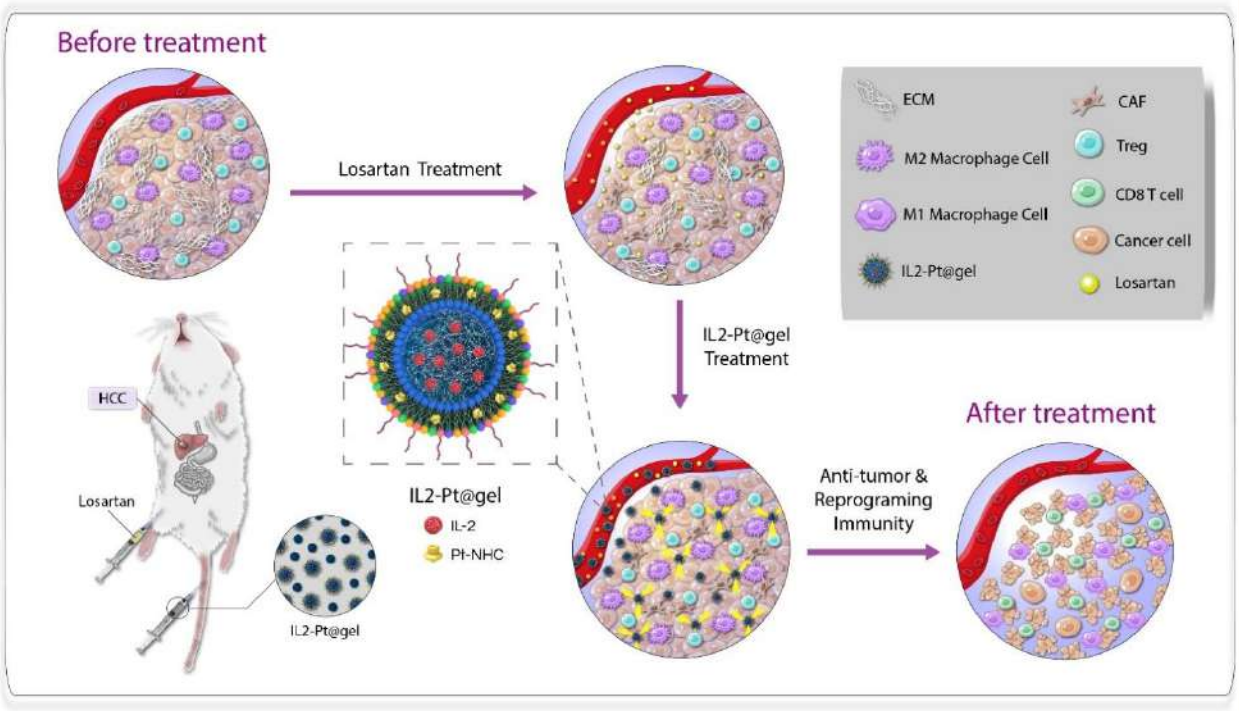
Immunotherapy utilizing recombinant cytokines has emerged as a promising approach for cancer treatment. The cytokine interleukin 2 (IL-2), a notable FDA-approved immunotherapeutic, is a potent activator of both natural killer (NK) and T cells, resulting in $CD8^+$ T-cell proliferation and differentiation [1]. However, the clinical application of IL-2 immunotherapy is limited by its short half-life in circulation, systemic toxicity, and limited accumulation in tumors. In addition, the immunosuppressive tumor microenvironment plays adverse role in hindering the IL-2 effectiveness [2].

Chemo-immuno-therapeutic combinations have demonstrated superior therapeutic efficacy compared to either chemotherapy or immunotherapy single treatment in various cancers. Certain chemotherapy agents could induce immunogenic cell death (ICD), which activates immune cells and establishes long-term anticancer immunity by triggering the release of damage-associated molecular patterns (DAMPs) from cancer cells, thereby improving the clinical outcomes of immunotherapy [3]. Here, we report a new chemoimmuno-nanogel (IL2-Pt@Nanogel) for the delivery of IL-2 and type II immunogenic cell death (ICD) inducer (Pt-NHC). The nanogel could improve the stability and tumor accumulation of IL-2 and Pt-NHC *in vivo* due to its nano-ranged size and sustained cargos release. The incorporation of ICD inducer could trigger the induction of ER-localized ROS from cancer cells, resulting in DAMP production, TAMs repolarization from immunosuppressive M2 toward immunostimulatory M1 phenotype, and reduction of the Treg population in tumors, thereby achieving potent anticancer immunity in combination with IL-2 immunotherapy.

The desmoplastic microenvironment in hepatocellular carcinoma (HCC) with advanced liver fibrosis hampers the efficacy of immunotherapies. Losartan, an angiotensin II receptor blocker, could effectively reduce type I collagen and hyaluronic acid levels in the stroma, and thus reduce desmoplasia [4]. Combining losartan that remodels the desmoplastic tumor microenvironment, IL2-Pt@Nanogel could further improve T-cell infiltration and activation in tumor, and suppress tumor progression as well as lung metastasis in orthotopic HCC models with associated underlying liver fibrosis. In summary, our study presents a promising strategy for IL-2-based chemoimmunotherapy for clinical translation in the treatment of solid tumors, especially the immunosuppressive and desmoplastic HCC with advanced liver fibrosis.

KEYWORDS: Interleukin 2 (IL-2), type II immunogenic cell death inducer, desmoplasia, hepatocellular carcinoma (HCC).

Figure 1: Schematic of the mechanism by which the chemoimmunotherapy nanogel and losartan suppress HCC progression in mice



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Magnetolytic therapy-mediated antigen capture and T cell infiltration in lung metastasis by biomimetic zwitterionic copolymer coated magnetic nanoparticles

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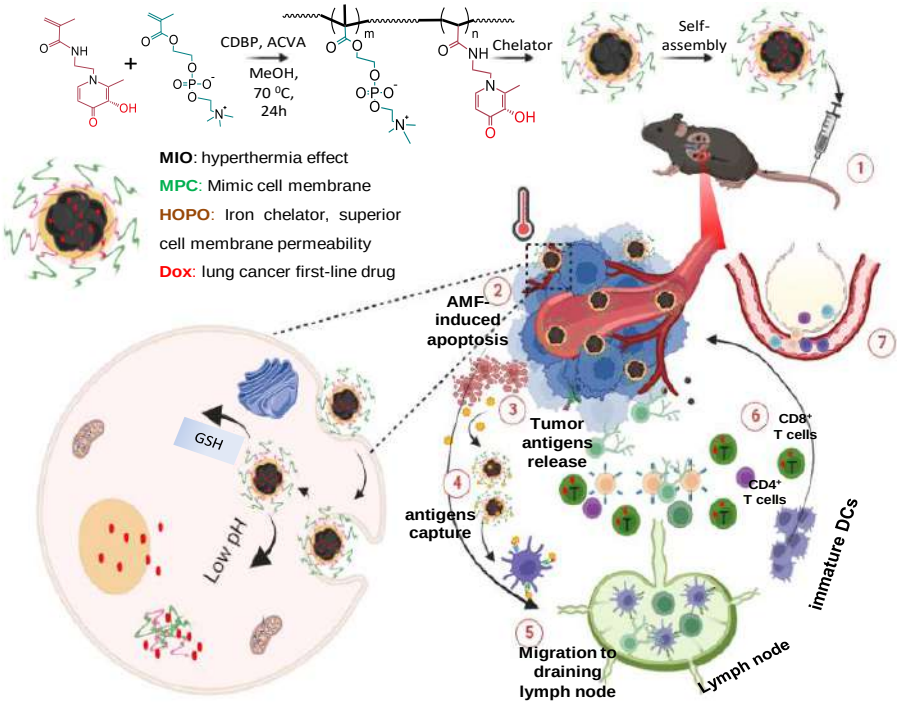
Abstract:

Cancer immunotherapy that can stimulate the activate immunity and enhance the tumor immunogenicity has offered outstanding advantages in cancer therapy. Single-mode immunotherapy, however, is usually ineffective due to some critical challenges, such as the autoimmune reactions, the low immune response and tumor infiltration. Recently, the combination of immunotherapy with other therapeutic treatments to specifically target cancer-inducing at mutilple simultaneous to enhance the therapeutic efficacy has emerged as a powerful strategy to improve the therapeutic effect. Herein, we develop a novel nanoparticle coordination polymer based on 3-hydroxypyridin-4-one (3, 4-HOPO) coated core-shell iron oxide nanoparticles (IONP) with a zwitterionic 2-methacryloyloxyethyl phosphorylcholine (MPC) surface. In particular, the using of IONP as platforms for the hyperthermia therapy, which is synergized immnotherapy including immunogenic cell death (ICD) and reversing the immunosuppressive tumor microenvironment. The zwitterionic MPC block was inspired by the antifouling structure of cell membranes, and the 3, 4-HOPO block was inspired by chelating agent. Our findings demonstrate that copolymer MPC-HOPO coated core-shell IONP can enhance cancer immunotherapy and support the potential of biomimetic engineering for lung metastasis-targeting treatments.

KEYWORDS: Biomimetic, zwitterionic, immunotherapy, iron oxide nanoparticle



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Bridging the Gap Between In Vitro and In Vivo: the Chick Embryo Model and Its Potential Applications in Nanomedicine

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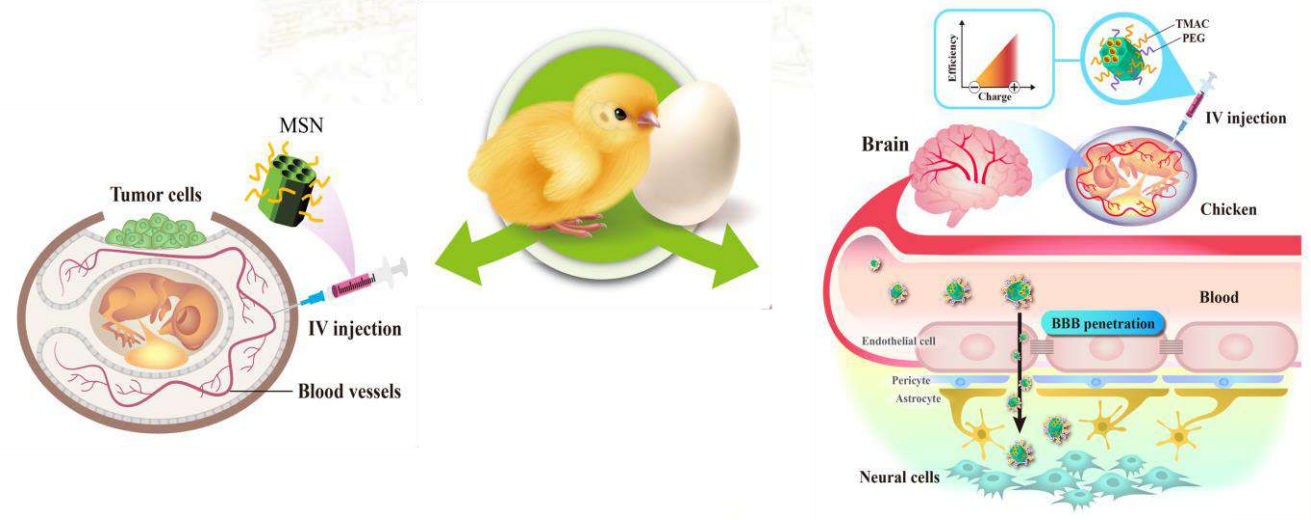
Animal studies are essential in medical research, especially for evaluating therapeutic effects. The chicken chorioallantoic membrane (CAM) has emerged as an in vivo model that bridges the gap between in vitro and in vivo, providing an opportunity for human disease studies. Instead of the mouse model, we aim to demonstrate the benefits of chick CAM and its potential applications in combination with nanotherapeutics. In this study, we develop mesoporous silica nanoparticles (MSNs), which allow to overcome the blood-brain barrier (BBB) and off-target tumor, followed by the drug delivery and release successfully. Similarly to the mouse model, the assessment of nanoparticles crossing the BBB and tumor-related studies in tumor xenograft chick CAM, can be carried out. MSNs with BBB penetration and EPR effect enable the treatment of difficult brain diseases and cancer. Overall, the chick embryo model offers an attractively alternative animal model with a multitude of opportunities for medical research.

KEYWORDS: mesoporous silica, drug delivery, chick embryo chorioallantoic membrane, blood–brain barrier, EPR effect.



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Calcium carbonate-encapsulated STING agonist for anti-cancer immunotherapy

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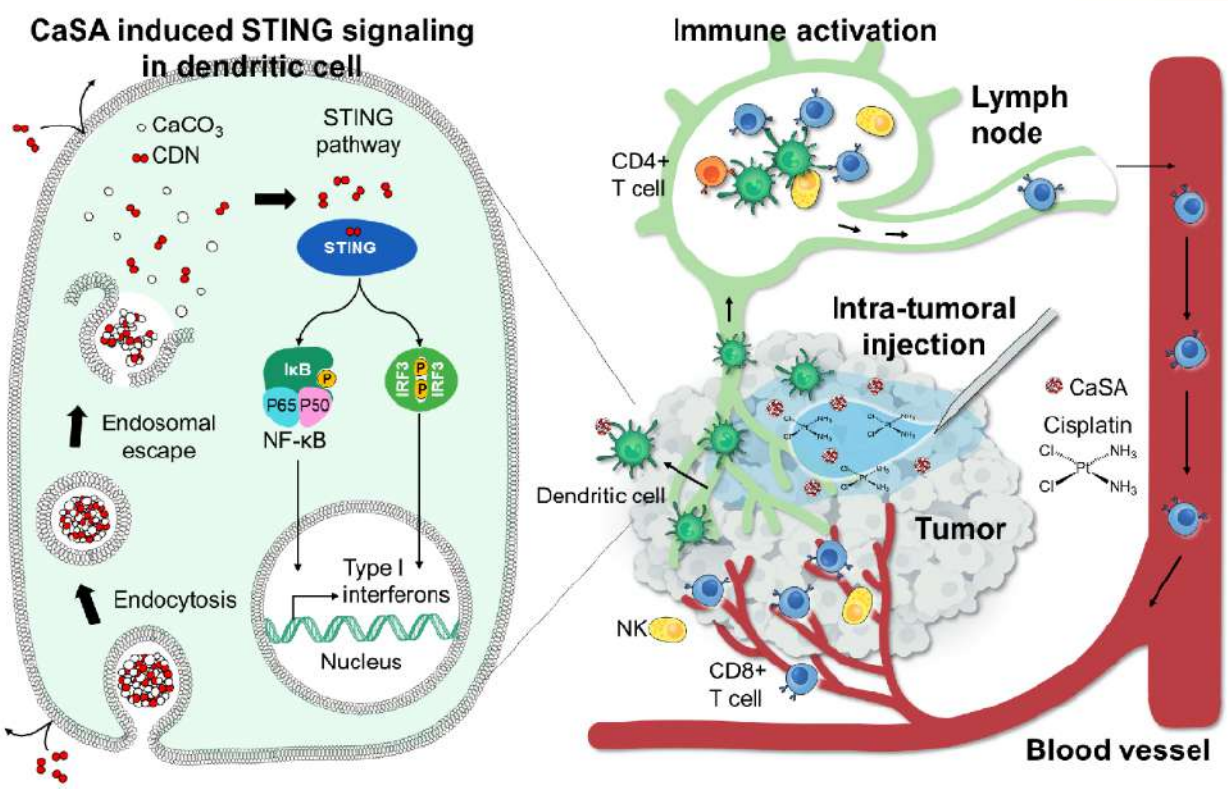
Abstract:

Stimulator of interferon genes (STING) remains a promising target for cancer immunotherapy [1]. Still, several small-molecule STING agonists, such as cyclic dinucleotides, showed limited efficacy and dose-limiting toxicity owing to their rapid enzymatic degradation and clearance and off-target toxicity. Here we developed a **Ca**lcium carbonate-encapsulated **S**TING **A**gonist (CaSA) microparticle, which effectively stimulated antigen-presenting cells (APCs) for antitumor immunity and avoided STING-induced cytotoxicity to T-cells. By manipulating particle size and surface charge, we achieved selective phagocytosis of APCs to CaSA, and the engulfed CaSA enabled the endosomal escape of STING agonists from acidic lysosomes for effective STING activation. We found that large CaSA microparticles (~3 μm of diameter) significantly enhanced phagocytosis and immunogenicity in APCs than small CaSA microparticles did (~1 μm of diameter).

Our results demonstrated that CaSA significantly increased the antitumor immunity of APCs which orchestrated the T-cell mediated cytotoxicity to cancer cells. The levels of pro-inflammatory cytokines, INF-β, TNF-α, IL-6, increased in the medium of CaSA-treated BMDC. The coculture of BMDCs and CD8+ T cells with the treatments of CaSA enhanced secretion of the T-cell-secreting cytokine INF-γ. In addition, we identified that the synergy of STING and calcium signalings boosted the pro-inflammatory response. This study combined CaSA with a thermoresponsive hydrogel for intratumoral injection and chemotherapy drugs, achieving remarkable therapeutic efficacy with minute doses of STING agonists in a murine tumor model. Overall, the CaSA offers a new approach to topical cancer treatments, and this study highlights the promising potential of nanomedicine for immunotherapy.

KEYWORDS: STING agonist, calcium, immunotherapy, antigen-presenting cell



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Development of temperature and reactive oxygen species responsive gelatin-PNIPAM-based hydrogels for drug delivery

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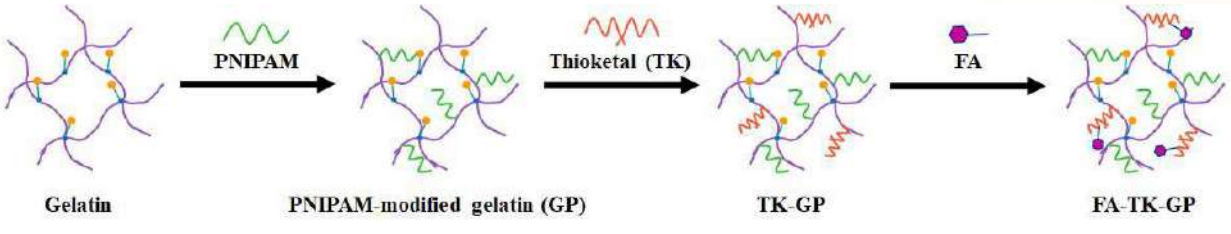
Abstract:

Critical limb ischemia (CLI) is the most severe form of peripheral artery disease (PAD) and occurs due to blockage in the arterial supply of the lower limbs[1]. Oxidative stress plays a critical role in the progression of PAD and can cause inflammation and vascular endothelial cell damage[2-4]. Ferulic acid (FA), a natural phenolic compound, possesses antioxidant, anti-inflammatory and angiogenesis properties[5]. Poly(N-isopropyl acrylamide) (PNIPAM) is an amphiphilic polymer and has a lower critical solution temperature[6]. Thioketal (TK) linker can be selectively cleaved by reactive oxygen species to release the conjugated drug from the host materials. In the study[7], FA was covalently linked to the gelatin-PNIPAM (GP) via a TK bond. The developed FA-grafted GP hydrogels with temperature and ROS responsive properties could provide the sustain drug release under the normal physiological condition and release drug immediately in the oxidative stress environment. The synthesis processes of FA-grafted GP hydrogels were characterized by nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy and ninhydrin assay. The microstructure, rheological behavior, in-vitro biocompatibility, in-vitro drug release study and antioxidant activity of developed hydrogels were analyzed. Therapeutic effects of developed hydrogels were demonstrated in the cell model using human umbilical vein endothelial cells. The rescuing effects of optimized FA-grafted GP hydrogels were investigated by mRNA gene expression, apoptosis and cell viability. The results suggested that this newly developed FA-grafted GP hydrogels with dual-responsive properties show promising potentials to provide a minimally invasive treatment option for PAD.

KEYWORDS: peripheral arterial disease, thermosensitive hydrogel, oxidative stress



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Spheroids-laden hydrogel with spatially confined delivery of signaling molecules for engineering 3D complex tissue

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Abstract:

The use of hydrogel and spheroids is being investigated to mimic osteochondral tissue, which is hierarchically composed of two layers, bone and cartilage tissue, with each layer having different characteristics. The combined regeneration of 3D complex tissue remains a challenge because of heterogeneous distribution of spheroids, limited cell-sprouting, inefficient delivery of inductive factors, de-differentiation, and delamination of the 3D complex construct. Here, human adipose-derived stem cells (hADSCs) spheroids-encapsulated gelatin methacryloyl hydrogel with optimal spheroid size and mechanical strength is prepared, in which homogeneous spheroid distribution and effective cell sprouting were achieved. In particular, two types of composite spheroids incorporating poly-ι-lactic acid nanofibers coated with osteo- or chondro-inductive growth factors are positioned within each layer of bilayer hydrogel to mimic hierarchical osteochondral tissue. hADSCs were differentiated into osteoblasts and chondrocytes, respectively, in each layer due to the spatially confined delivery of inductive factors. Moreover, the interface of bilayer hydrogel is successfully integrated by sequential cross-linking of hydrogel and cell-matrix interaction without delamination. In vivo transplantation of bilayer hydrogel onto mouse subcutaneous tissue results in that encapsulated cells differentiate osteogenic or chondrogenic lineages in each layer without de-differentiation, and attract host-cell infiltration through ECM remodeling capacity. The present approach can be an effective platform to address remaining challenges and to engineer 3D complex tissue.

KEYWORDS: 3D complex tissue, osteochondral tissue, hydrogel, spheroid encapsulation, tissue engineering

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Nanocomposite hydrogels containing immunomodulatory strontium-tannic acid nanoparticles for vascularized skin tissue regeneration

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Abstract:

Skin tissue can be regenerated by normal healing process when acute damage is occurred. However, repeated trauma or infection could induce chronic inflammation, which impairs the regeneration process by inhibiting angiogenesis and proliferation. Hydrogels have been used to treat skin tissue defects and to deliver signaling molecules due to their excellent physical and chemical properties. However, the design of hydrogels should be carefully considered by controlled delivery of appropriate signals and coordinated degradation of the hydrogels to regulate inflammation with promotion of tissue regeneration.

In this study, we developed multi-functional nanocomposite gelatin methacryloyl (GelMA) hydrogels which can control both inflammation and angiogenesis. We first prepared the tannic acid-strontium nanoparticles (TSrPs), and incorporated them into the GelMA hydrogel (G-TSrP), crosslinked under UV exposure. We confirmed that the composite hydrogels had no cytotoxicity to human epithelial cells and endothelial cells, and mouse macrophages. We revealed an immunomodulatory effect of G-TSrP using lipopolysaccharide treated M1 macrophage model and interleukin-4 treated M2 macrophage model. In addition, G-TSrP increased both migration and tube formation ability of endothelial cells. Also, G-TSrP induced recruitment and MMP expression of macrophages, allowing enhanced cell infiltration and biodegradation of GelMA hydrogel. Finally, we found that the composite hydrogels accelerated tissue remodeling and regeneration through immunomodulatory and angiogenic effect via *in vivo* mouse subcutaneous implantation model and full-thickness skin wound healing model. In conclusion, we expect that this multi-functional hydrogel would be a feasible material for skin tissue regeneration.

KEYWORDS: Tannic acid, Strontium, GelMA hydrogel, Biodegradation, Immunomodulation, Angiogenesis, Skin regeneration, Wound healing

Fabrication of a collagen-based bioink for 3D bioprinting

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Abstract:

3D bioprinting has recently emerged as a popular biofabrication method. A bioink is an essential part of 3D bioprinting. A bioink is cross-linked or stabilized during or immediately after bioprinting into an architecture of the intended tissue construct. Collagen, the most abundant protein in vertebrates, plays a vital role in their particular biological functions. However, collagen is known for its water-insoluble fibrous structure of great strength but could not be dissolved in neutral or physiological buffers. Therefore, collagen is rarely applied to 3D bioprinting. This study aimed to develop water-soluble photo-cross-linkable collagen for 3D bioprinting applications. First, type I collagen was modified with maleic anhydride, which shifts collagen pI to acidic pH, allowing modified collagen to be soluble in neutral buffers and bringing collagen cross-linkable alkene groups for cross-linking. Modified collagen could be photocrosslinked by UV illumination in the presence of a photoinitiator. Generally, there are three goals for 3D bioprinting: (1) high printability during filament depositing; (2) excellent cell viability within the architecture to facilitate cell proliferation; (3) efficient and simple way to fabricate bioinks and then process. To maximize printability and cell viability, an alteration of extruding pressure, needle inner diameter, bioink concentrations, and temperatures was performed. The printed structure of the 3D scaffold was well defined and remained stable. Cells could be loaded into the modified collagen hydrogel and then printed via the bioprinter. The fibroblast (L929) remained alive and proliferative. Our results showed that the collagen modified with maleic anhydride is excellent as a bioink.

Keywords: 3D printing, collagen maleate (ColME), printability, cell viability, photo-cross-linking.

A Biomimicking and Multiarm Self-Indicating Nanoassembly for Site-Specific Photothermal-Potentiated Thrombolysis Assessed in Vessel-on-a-Chip Device and in vivo Models.

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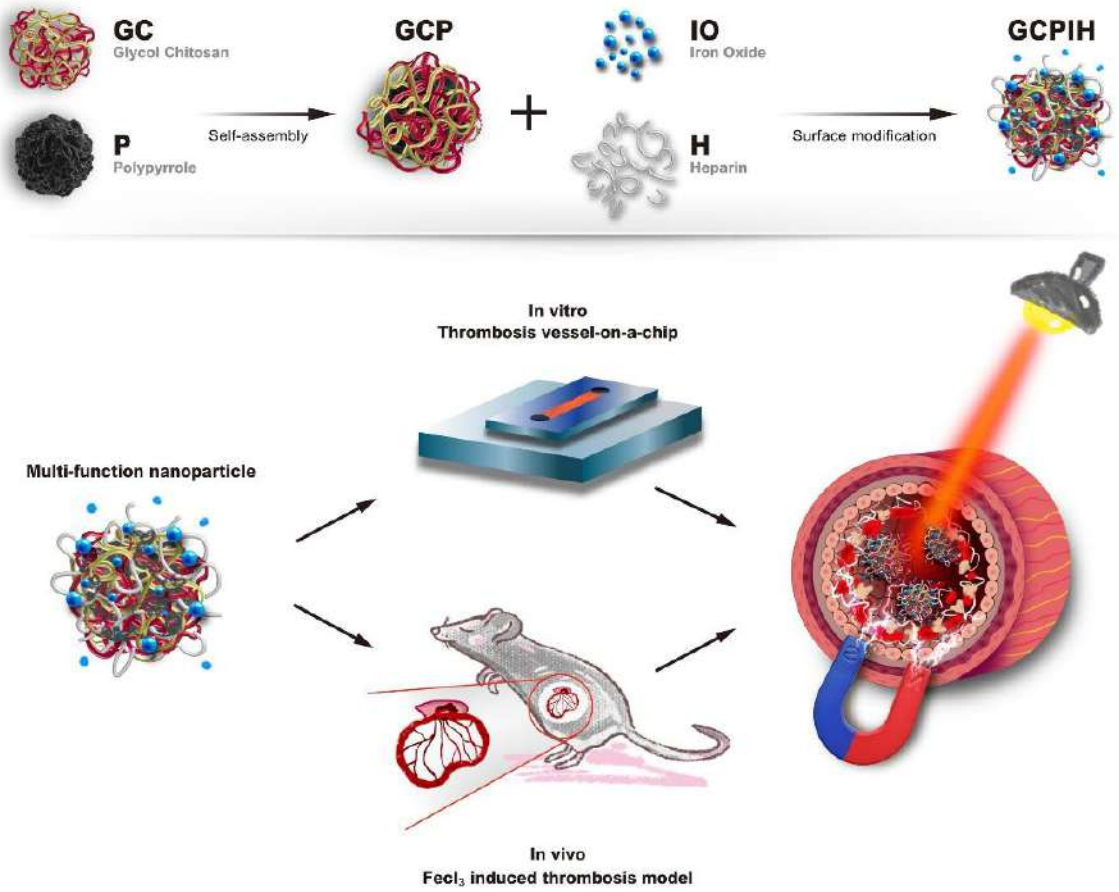
Abstract:

Clinical use of thrombolytic or antithrombotic therapy is constrained by its short circulation time and high off-target hemorrhagic risks. Herein, we propose that these problems can be addressed by precisely integrating a thrombus-homing strategy with phototherapy. Inspired by glycol chitosan and heparin binding to activated thrombi and iron oxide for active magnetic navigation, we report biomimicking, multiarm glycol chitosan-polypyrrole-iron oxide-heparin (GCPIH) nanoparticles for thrombus-specific thrombus delivery and targeted thrombolysis. After self-assembly of this bioengineering formulation, a thrombus-targeting polysaccharide-decorated nanoassembly of polypyrrole (a photothermal probe) and iron oxide (a therapeutic agent for magnetic guidance and enhancement of heparin's anticoagulant effect) was prepared for thrombus-homing delivery. The intricately bioengineered nanoassembly revealed multiple features, including biocompatibility, thrombus-multiple-targeted accumulation with a self-indicating function, and photothermal-potentiated thrombus thrombolysis with augmented therapeutic efficacy. Furthermore, we proposed a purpose-created microfluidic model that was able to simulate targeted thrombolysis of phototherapeutic (GCPIH) nanoparticles with the potential to predict the dynamics of thrombolysis in realistic pathological scenarios. Assessments with human blood confirmed its highly precise homing to activated thrombus microenvironments and efficient near infrared-phototherapeutic effects at thrombus lesions under a physiological flow environment *ex vivo*. Its clot lysis performance in a microfluidic system was comparable to that of a free tissue plasminogen activator and *in vivo* model experiment. This combined experimental, microfluidic, and *in vivo* work confirms the potential of (GCPIH) nanoparticles for thrombus therapy and presents a promising platform to develop thrombolytic nanomedicine.

KEYWORDS: photothermal thrombolytic, thrombosis vessel-on-a-chip, nanoparticle, animal use alternatives (3Rs), thrombosis therapy

Schematic illustration:



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Programmed T Cells Infiltration into Lung Metastases with Harnessing Dendritic Cells in Cancer Immunotherapies by Catalytic Antigen-Capture Sponges

Min-Ren Chiang[1], Wei-Ting Shen[1,2], Pin-Xuan Huang[1], Shang-Hsiu Hu[1,*]

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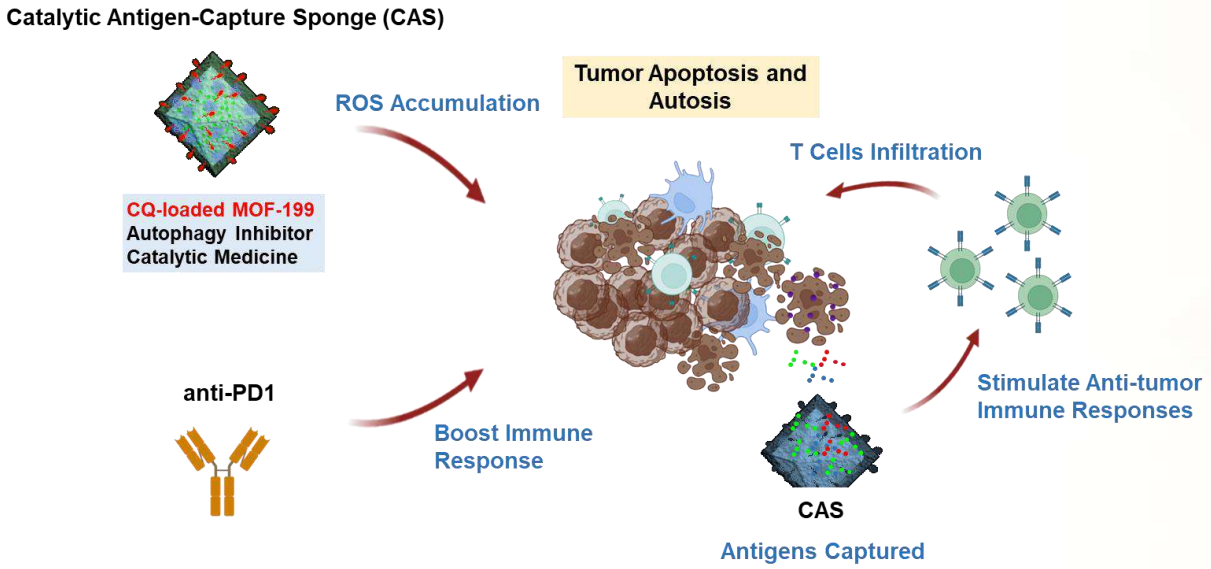
Abstract:

T lymphocytes served as immune surveillance to suppress metastases by physically interacting with cancer cells. Whereas tumor immune privilege and heterogeneity protect immune attack, it limits immune cell infiltration into tumors, especially in invasive metastatic clusters [1, 2]. Here, a catalytic antigen-capture sponge (CAS) containing the catechol-functionalized copper-based metal organic framework (MOF) and chloroquine (CQ) for programming T cells infiltration is reported. The intravenously injected CAS accumulates at the tumor via the folic acid-mediated target and margination effect. In metastases, Fenton-like reaction induced by copper ions of CAS disrupts the intracellular redox potential, i.e., chemodynamic therapy (CDT), thereby reducing glutathione (GSH) levels. With the assistance of chloroquine (CQ), autophagy is inhibited through lysosomal deacidification, which destroys the self-defense mechanism and further enhances cytotoxicity. The treatments facilitate the release of tumor-associated antigens, including neoantigens and damage-associated molecular patterns (DAMPs) [3]. Then, the catechol groups on CAS act as antigen sponges and deliver the autologous tumor-associated antigens to dendritic cells, achieving sustained immune stimulation. The *in-situ* forming CAS lung metastasis as a CDT-induced antigen reservoir causes the infiltration of immune cells in metastatic clusters and inhibited the metastases.

KEYWORDS: Immunotherapy, Metal organic framework, Autophagy inhibition, Antigen capture, Immune checkpoint blockage

Graphic abstract



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A Novel Peptide Assembling Nanoparticle as Eye Drop for Treating Choroidal Neovascularization

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Abstract:

Choroidal neovascularization (ChNV) is the most common seen diseases in ophthalmology. ChNV normally causes by some serious diseases, like diabetic retinopathy (DR) and age-related macular degeneration (AMD) which may causes blood vessel proliferation in choroidal segment [1]. The treatment for posterior eye disease nowadays is by intravitreous injection, but 15–30% of patients shown poor response [2]. Neovascularization occurred when vascular endothelial growth factor (VEGF) angiogenesis factor got up-regulated [3]. In our research, we had discovered a novel peptide (pep) that can distribute VEGF signaling to inhibit angiogenesis. Hyaluronic acid (HA) is a negatively charge material that normally presence in cornea and highly-biocompatibility. By self-assembling mechanism of HA to form positively charge nanoparticles (HA-pep NPs), we combined the advantage of its great capacity and retard the distribution of drug on the eye surface by surface charge attraction to investigate its efficiency.

HA-pep NPs had been characterized by DLS, TEM, NTA, FTIR, and DSC to make sure the size, intensity, zeta potential, functional group, and the strength of structure. The release ratio and encapsulation efficiency of peptide in nanoparticles had also been measured by drug release and protein assay. For the in vitro measurements, human umbilical vein endothelial cells (HUVECs) were used to observed the uptake of nanoparticles into cells in the beginning and the viability of cells were tests by CCK-8 assay. Then, the investigation of whether novel peptide has the anti-angiogenesis function of vessel cell growth was measured by cell migration and tube formation. In animal trial, C57BL/6J mice were used to construct the angiogenesis of ChNV model by laser injury to the retina and assess the therapeutic effect by quantifying its neovascularization leakage area and protrusion degree in the choroid by FFA and OCT. Moreover, we had test for the retention time and condition by IVIS and confocal. The angiogenesis pathway was ensured by proteome. The histological examination had been observed by H&E staining.

HA-pep NPs was prepared with the size of 268.4 ± 7.1 nm, and zeta potential was 18.0 ± 0.8 mV. We also confirmed that the encapsulation rate of peptide in the NPs was around 93.5 ± 0.7 % and TEM images showed that these particles had spherical morphology and peptide separate evenly in the nanoparticles. NTA result showed the concentration of nanoparticles was 1.39 *10^10 ± 4.7*10^8 /1 mL. The structure of the functional groups and the combination of HA-pep NPs were characterized by FTIR and DSC. Result of drug release test showed the slow and stable release ratio of HA-pep NPs. Moreover, by the uptake intensity and figure indicated that HA-pep

NPs had good uptake property, than confirmed the most appropriate drug given concentration by cell viability test. The effect of blood vessel inhibition had been proved by cell migration and tube formation assays. At last, we combined FITC fluorescent on peptide to prepare HA-pep NPs on the surface of mice eyeball to track the drug retention on the ocular surface and posterior segment. Result of H&E staining showed the curation of HA-pep NPs. The diameter of HA-pep NPs showed similar in DLS, TEM, and NTA. It has highly encapsulation efficiency due to the self-assembling mechanism. The result of cell migration and tube formation showed the potential of HA-pep NPs in appropriate concentration of peptide loaded nanoparticles. At last, the result of in vivo neovascularization animal model echoes with in vitro tests. Proteome analysis showed the down-regulation of VEGFA-VEGFR2 signaling pathway. H&E staining of tissues also showed that the integrity of HA-pep NPs group was closest to normal cornea.

We had successfully prepared HA-pep NPs with stable properties. Both in vivo and in vitro results had shown a good angiogenesis inhibition. This study points out the therapeutic effect of HA-pep NPs in choroidal angiogenesis.

KEYWORDS: nanoparticles, peptide, hyaluronic acid, choroidal neovascularization

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2023 ISOMRM & BCRS
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Direct Thermal Growth of Gold Nanopearls on 3D Interweaved Hydrophobic Fibers as Ultrasensitive Portable SERS Substrates for Clinical Applications

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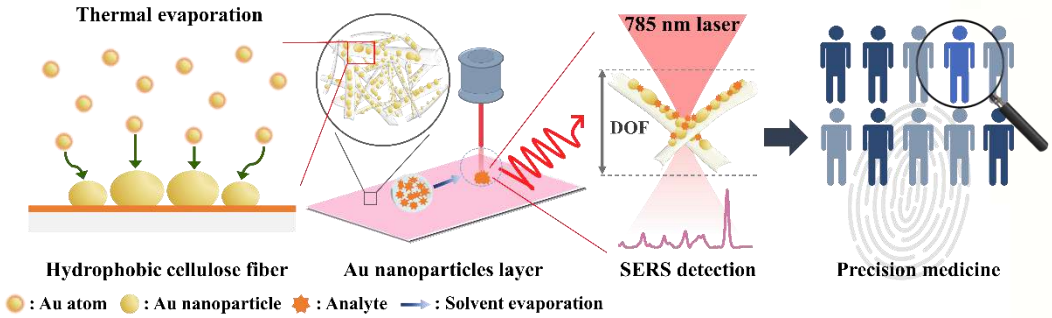
Abstract:

Surface-enhanced Raman spectroscopy (SERS)-based biosensors have attracted much attention for their label-free detection, ultrahigh sensitivity, and unique molecular fingerprinting. In this study, we prepared a wafer-scale, ultrasensitive, highly uniform, paper-based, portable SERS detection platform featuring abundant and dense gold nanopearls with narrow gap distances, deposited directly onto ultralow-surface-energy fluorosilane-modified cellulose fibers through simple thermal evaporation by delicately manipulating the atom diffusion behavior. The as-designed paper-based SERS substrate exhibited an extremely high Raman enhancement factor (3.9×10^{11}), detectability at sub-femtomolar concentrations (single-molecule level), and great signal reproductivity (relative standard deviation: 3.97%), even when operated with a portable 785-nm Raman spectrometer. We used this system for fingerprinting identification of 12 diverse analytes, including clinical medicines (cefazolin, chloramphenicol, levetiracetam, nicotine), pesticides (thiram, paraquat, carbaryl, chlorpyrifos), environmental carcinogens (benzo[a]pyrene, benzo[g,h,i]perylene), and illegal drugs (methamphetamine, mephedrone). The lowest detection concentrations reached the sub-ppb level, highlighted by a low of 16.2 ppq for nicotine. This system appears suitable for clinical applications in, for example, (i) therapeutic drug monitoring for individualized medication adjustment and (ii) ultra-early diagnosis for pesticide intoxication. Accordingly, such scalable, portable, ultrasensitive fibrous SERS substrates open up new opportunities for practical on-site detection in biofluid analysis, point-of-care diagnostics, and precision medicine.[1]



KEYWORDS: surface-enhanced Raman spectroscopy, plasmonic biosensor, single molecule detection, biofluid analysis, pesticide intoxication, therapeutic drug monitoring, point-of-care diagnostics.

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Bi_2S_3@C/Pd-BSA hetero-nanostructures for photocatalysis-mediated hydrogen sulfide splitting and hydrogen production for colorectal cancer therapy.

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Abstract:

Colorectal cancer (CRC) is the third most common cancer worldwide. The tumor microenvironment (TME) in CRC is characterized by the presence of the gaseous transmitter, endogenous hydrogen sulphide (H_2S) (0.3 to 3.4 $mmolL^{-1}$). Under the physiological environment (pH = 7.4, 37°C), 40% of the sulphides within unsaturated H_2S solution are in the form of H_2S, while the rest exist in the form of HS−.The overproduced endogenous H_2S itself is involved in a diverse set of biological activities to promote cancer invasion and progression, for example, DNA repairment, tumor cell metabolism, and angiogenesis. Although the use of H_2S-involved nanotherapeutics has been gradually acknowledged, metal oxides that can deplete H_2S and undergo sulfidation into corresponding NIR activable metal sulphides (PTT agent) is one of the promising strategies to treat CRC. By the precise irradiation of NIR and efficient photothermal conversion of the sulfurized nanomaterials like CuS, MoS_2, Bi_2S_3 have been used for PTT. However, it is difficult for PTT alone to completely kill tumor cells due to the high expression of heat shock protein (HSP), leading to tumor recurrence. However, the inhibition of glycolysis and mitochondrial metabolism could significantly reduce ATP production and down-regulate HSP expression, which would reduce tumor cells heat resistance and improve PTT therapeutic effect. It is well proved that the main target of hydrogen molecule is mitochondrion and thus hydrogen gas can alter the cellular energy metabolism, which could downregulate the expression of HSP and enhance PTT. Therefore, we hypothesize that the synchronous decomposition of endogenous H_2S and production of hydrogen combined with photothermal treatment will act as an all-round strategy to kill the CRC cells effectively.

Considering these facts, we designed and developed a drug-free Bi_2S_3@C/Pd hetero-nanostructures (HNSs), which are capable for splitting intertumoral endogenous H_2S/HS- into molecular hydrogen upon NIR irradiation (808nm) via photocatalysis (Fig. 1). The disadvantage of Bi_2S_3 being used as a photocatalyst is that it is easily photo corroded by NIR irradiation and here we overcome it by a few nm thick layer of carbon coating on the surface. As a narrow band gap (1.2–1.7 eV) n-type semiconductor, Bi_2S_3 upon irradiation with NIR 808nm, the electrons will be excited from the valance band (VB) to the conduction band (CB) (Eq. 1). These electrons on the CB further transfer to the surface of the semiconductor and reduces protons into molecular hydrogen.[1] (Eq.2)

$$\text{Photocatalyst} + h\nu \rightarrow h^{+}_{VB} + e^{-}_{CB} \quad \text{(Eq. 1)}$$

$$2H^{+} + 2e^{-} \rightarrow H_2(g)\ \Delta E^{0} = 0\ V \quad \text{(Eq. 2)}$$

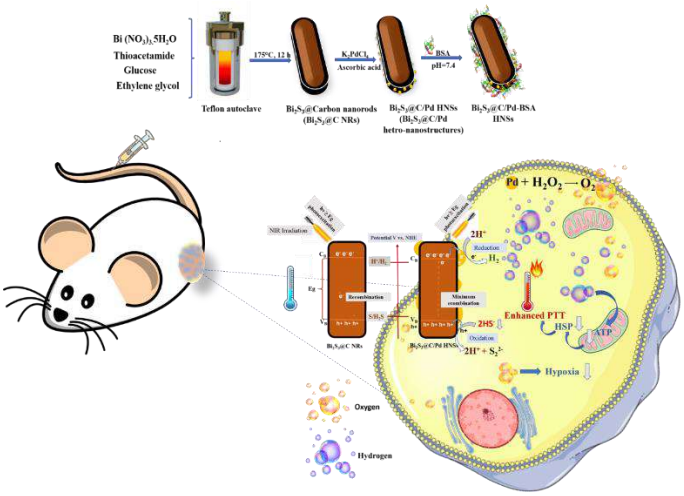

Fig 1: Schematic representation of Bi_2S_3@C/Pd-BSA hetero-nanostructures for photocatalysis-mediated colorectal cancer therapy.

However, effectively suppressing the recombination of electrons and holes to meet the continuous generation of H_2 is also still a factor to be considered for enhancing the performance of photocatalytic reactions. Therefore, it is necessary to find a way to adjust the band gap of the Bi_2S_3 and reduce the recombination of electrons and holes to improve its photocatalytic as well as photothermal performances. Plasmonic metals like palladium[2], palladium NPs are effective for regulating Bi_2S_3 band gaps. Moreover, Pd nanoparticles can catalyze the overexpressed hydrogen peroxide (H_2O_2) in the TME to generate oxygen (O_2) and the photoexcited holes also have the ability to oxidize H_2O_2 to generate O_2, which is beneficial to alleviate the degree of the tumor hypoxia. Overall, under the irradiation of NIR, the electrons in the VB of Bi_2S_3 transit to the CB and generate the electron-hole pairs. Then some electrons will transfer from CB of Bi_2S_3 to the Pd which can effectively prolong the carrier lifetime through preventing the recombination of electron and holes, thus improving the 1) photothermal efficiency 2) hydrogen production 3) H_2S depletion and 4) oxygen production from H_2O_2.

KEYWORDS: Photocatalysis, H_2S splitting, H_2 generation, O_2 evolution, photothermal tumour therapy.

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Three-dimensionally cultured adipose derived stem cell exosomes for diabetic wound healing

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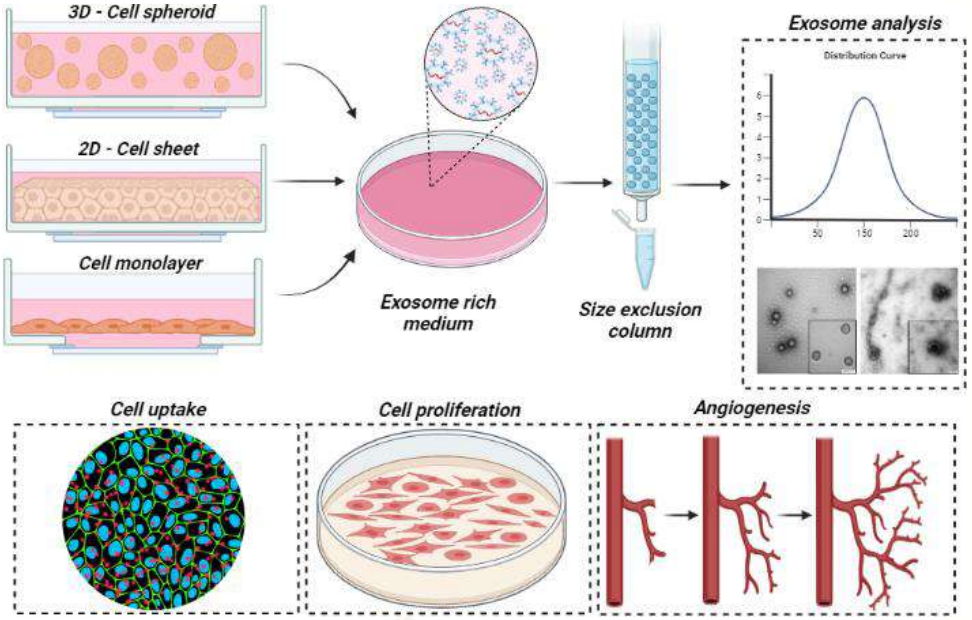
Abstract:

Wound healing is a very complex process subdivided in a variety of steps and stages involving countless mediators, reactions and growth factors. Due to its complexity, a variety of factors and conditions can affect its normal process [1]. One of such conditions is diabetes mellitus, a chronic health condition in which the production and/or effectiveness of insulin are compromised. This decrease of insulin induces a lack of sustained growth for productive granulated tissue, reduced tissue oxygenation and ultimately decrease in the self-repairing capacity of diabetes patients. Hence the development of an effective treatment for chronic wound healing is an imperative step to provide effective care for diabetic patients.

Stem cell manipulation is a promising field of study, in particular their impact on the enhancement of wound healing and other angiogenesis related processes, in which unique and novel approaches can be used to solve complex issues. Of the many different stem cells use for research, adipose derived stem cell (ASC) are one of the leading candidates for all types of cell related therapies. However, transplantation of ASCs into injured tissues may be associated with uncontrolled cell differentiation [2]. Thus research in the use of extracellular microvesicles derived from stem cells as a potential cell-derived therapeutic option free from the translational complications is appealing. The aim of this study is to enhance the regenerative potentials of ASC-MVs by three-dimensional (3D) culture techniques for biomaterial-mediated clinical application in wound healing. In comparison, to traditional monolayer-based culture techniques for exosomes, three-dimensional culture is capable of enhancing both yield and effectiveness of exosomes when applied to wounds. A throughout comparison of monolayer, two-dimensional and three-dimensional culture of ASC has revealed a substantial increase in the overall production of exosomes, as seen by the nano-tracking, protein concentration and other quantitative assays. While various experiments in vitro, such as endothelial cell proliferation, endothelial tube formation and fluorescent imaging of exosome uptake have shown the angiogenesis and wound healing enhancing properties of the three-dimensional cultured exosomes. Additionally, the incorporation of said exosomes in a cross-linked gelatin hydrogel was research, as viable carrier for long term delivery of the exosomes. Based on the above results, we believed that our three-dimensional-cultured derived exosomes can substantially augment instances of cell angiogenesis and wound healing, while eliminating the inherent risk associated with stem cell application.

To obtain the desired exosomes human adipose derived stem cells were cultured using three different culture methods (monolayer, cell sheet and cell spheroid). After the cells reach an appropriate confluence their medium is removed and substituted with a free-exosome medium. This medium is then collected and run through an ultracentrifuge to remove any large molecules or cell that may be present. Following this, the refined medium is passed through a size exclusion chromatographer to separate and select the desired range for the microvesicles. These exosomes are then incorporated into the mTG-crosslinked gelatin hydrogel or used in their free form for the in vitro experiments.

KEYWORDS: Adipose derived stem cell (ASC), exosomes, diabetes wound healing, gelatin hydrogel.



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Platelet-derived biomaterial with hyaluronic acid alleviates temporal mandibular joint osteoarthritis: clinical trial from dish to human

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Abstract:

Background

Bioactive materials have now raised considerable attention for the treatment of osteoarthritis (OA), such as knee OA, rheumatoid OA, and temporomandibular joint (TMJ) OA. TMJ-OA is a common disease associated with an imbalance of cartilage regeneration, tissue inflammation, and disability in mouth movement. Recently, biological materials or molecules have been developed for TMJ-OA therapy; however, ideal treatment is still lacking. In this study, we used the combination of a human platelet rich plasma with hyaluronic acid (hPRP/HA) for TMJ-OA therapy to perform a clinical trial in dish to humans.

Method

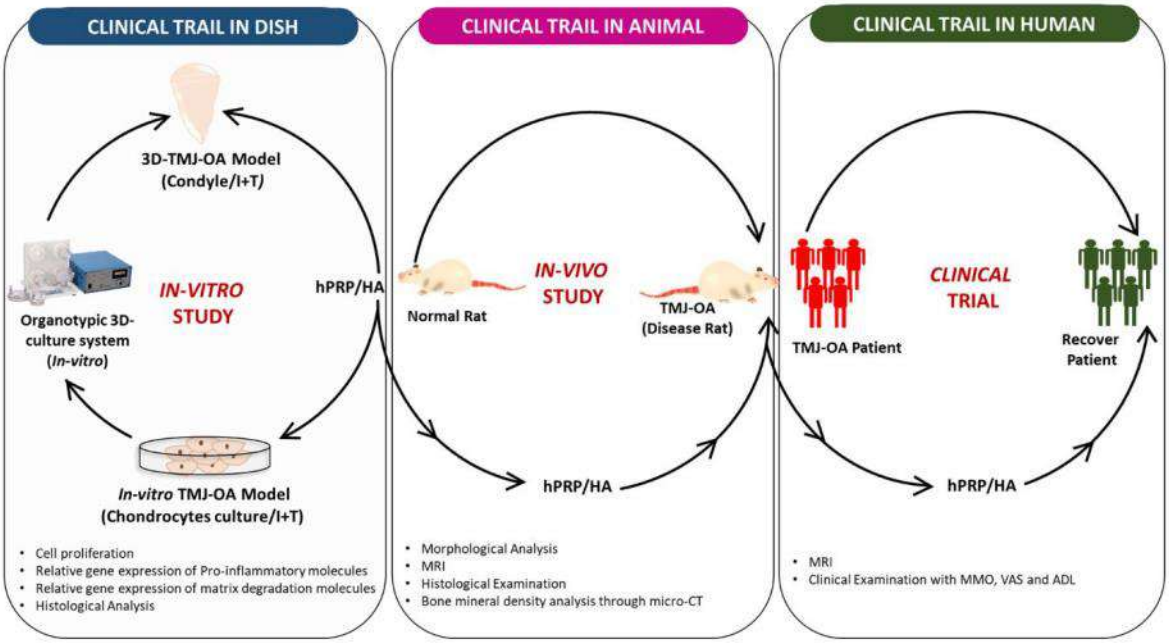
Herein, hPRP was prepared, and the hPRP/HA combined concentration was optimized by MTT assay. For the clinical trial in dish, pro-inflammatory-induced in-vitro and in-vivo mimic 3D TMJ-OA models were created, and proliferation, gene expression, alcian blue staining, and IHC were used to evaluate chondrocyte regeneration. For clinical trial in animals, complete Freund's adjuvant (CFA) was used to induce the TMJ-OA rat model, and condyle and disc regeneration were investigated through MRI. For the clinical trial in humans, 12 patients with TMJ-OA who had disc displacement and pain were enrolled. The disc displacement and pain at baseline and six months were measured by MRI, and clinical assessment, respectively.

Results

Combined hPRP/HA treatment ameliorated the proinflammatory-induced TMJ-OA model and promoted chondrocyte proliferation by activating SOX9, collagen type I/II, and aggrecan. TMJ-OA pathology–related inflammatory factors were efficiently downregulated with hPRP/HA treatment. Moreover, condylar cartilage was regenerated by hPRP/HA treatment in a proinflammatory-induced 3D neocartilage TMJ-OA-like model. During the clinical trial in

animals, hPRP/HA treatment strongly repaired the condyle and disc in a CFA-induced TMJ-OA rat model. Furthermore, we performed a clinical trial in humans, and the MRI data demonstrated that after 6 months of treatment, hPRP/HA regenerated the condylar cartilage, reduced disc displacement, alleviated pain, and increased the maximum mouth opening (MMO). Overall, clinical trials in dish to human results revealed that hPRP/HA promoted cartilage regeneration, inhibited inflammation, reduced pain, and increased joint function in TMJ-OA

KEYWORDS: Platelet-derived biomaterial, TMJ-OA, Hyaluronic acid, Clinical trial



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The synergistic in vitro and in vivo antitumor effect of combination therapy with iron oxide nanoparticles and fucoidan against lung adenocarcinoma

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Abstract:

Due to the unique properties of the magnetic field and contrast image, iron oxide nanoparticles have become an attractive candidate for biomedical applications among biomaterials. Furthermore, fucoidan, a P-selectin ligand expressed on the surface of cancer cells, has emerged as a strategy for targeting specific tumors with chemotherapeutic drugs. Therefore, a novel nano-platform - Fucoidan-coated magnetic iron oxide nanoparticles (Fu-IO NPs) was created to enhance the anticancer effect via P-selectin-mediated cancer delivery and to work as a contrast agent to track cancer cell metastasis.

In this study, Fu-IO NPs were developed by an electrostatic method. The MTT assay was used to test for the biocompatibility of Fu-IO NPs on HEL 299 cells. The systemic cytotoxicity was evaluated on mice. The in vitro and in vivo cellular uptake of the Fu-IO NPs was observed using Prussian Blue staining, TEM images, and MR images. In vitro and in vivo P-selectin expression and ROS generation were examined with fluorescence microscopy. Furthermore, the combination of IO NPs and Fu produced a synergistic anticancer effect on lung adenocarcinoma, which was tested both in vitro and in vivo.

The results showed that Fu-IO NPs were successfully fabricated with a size of around 200 nm. Fu-IO NPs had high biocompatibility in in vitro and no significant systemic toxicity toward mice. MRI results revealed that Fu-IO NPs could precisely target cancer cells while also increasing MRI contrast efficacy. The combination of IO NPs and Fu was enhanced cellular ROS level and the GSH depletion inside lung cancer cells. Moreover, Fu-IO NPs could efficiently accumulate in lung cancer cells than normal healthy cells in both in vitro and in vivo. In the in vitro study for anticancer efficacy, A549 cell survival dropped below 50% after 48 hours of treatment with Fu-IO NPs. Moreover, the in vivo results revealed that Fu-IO NP had the most potent antitumor efficacy, with virtually no tumor recurrence in the treated mice until 14 days later.

Therefore, the combination of IO NPs and Fu may provide us with a new approach to the theranostics of lung adenocarcinoma.

KEYWORDS: the combination of iron oxide nanoparticles and fucoidan, amplification of P-selectin and oxidative stress

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One-step Bone Graft: the Efficiency of Bone Regeneration with 3D Culture Wharton's Jelly Mesenchymal Stem Cells

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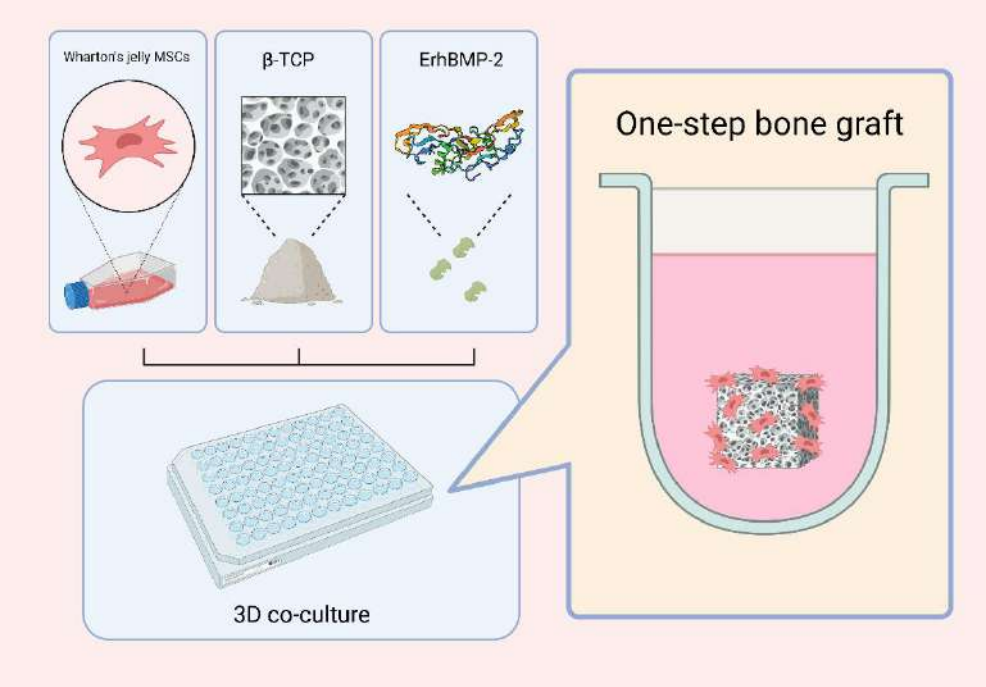
Abstract:

Tissue engineering in bone regeneration has been studied for decades, including choices of scaffold materials, gradient spatial and temporal growth factors releasing, computer-assisted designed three-dimensional printing technology, and co-culture scaffold with cells etc. Among the biomaterials, biphasic calcium phosphate (BCP) consists of beta-tricalcium phosphate (β-TCP) and hydroxyapatite (HA) is the most used bone tissue engineering scaffolds.[1] Studies have proven the biocompatibility, biodegradability, and osteoconductivity of BCP bone grafts in animal models, and currently, there are several commercialized products of BCP for clinical applications. Bone morphogenetic protein-2 (BMP-2) is one of the most studied osteogenesis molecules for bone regeneration. *Escherichia coli*-derived recombinant human bone morphogenetic protein-2 (E-rhBMP-2) is a more efficient method of manufacturing rhBMP-2 because of the ability of E. coli to grow rapidly and could be manufactured as a productive farming protocol.[2] Wharton's jelly mesenchymal stem cells (WJ-MSC) isolated from the donor umbilical cord are a class of stem cells with high differentiative potential, including osteogenesis and angiogenesis.[3] Thus implantation of cells within the scaffold might be a solution to increase the healing process. The current study proposed a protocol of 3D culture WJ-MSC with β-TCP and bone morphogenetic protein-2 (BMP-2) 3D culture for one-step bone graft.

KEYWORDS: Bone regeneration, bone graft, tissue engineering, 3D culture, Wharton's jelly mesenchymal stem cell, beta-tricalcium phosphate (TCP), bone morphogenetic protein-2 (BMP-2)



Graphic abstract (not a mandatory requirement)

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Disruption of CCL2 in mesenchymal stem cells as an anti-tumor approach against prostate cancer

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Abstract

Backgrounds: The C-C motif chemokine ligand 2 (CCL2) plays a key role in tumor progression and is abundant in the prostate cancer microenvironment. In addition to tumor cells, mesenchymal stem cells (MSCs) also secrete CCL2, which is involved in immunomodulatory functions, such as the anti-inflammatory polarization of macrophages, facilitating tumor progression. Therefore, we hypothesized that genetically engineered MSCs with CCL2 disruption could be a novel anti-tumor therapy. Materials and Methods: In this study, we utilized the murine bone marrow-derived MSCs (BM-MSCs) which were confirmed by MSC-associated surface markers and their differentiation properties (osteogenic and adipogenic differentiation). CCL2 disruption was performed by CRISPR/Cas9 for genetic knockout (KO) in MSCs. The biological functions (e.g. proliferation and migration activities) of the derived MSCs (e.g., WT vs. CCL2 KO) were examined by several functional assays. To study the effects of CCL2 KO MSCs in the tumor microenvironment, we selected a tumor cell line (TRAMP- C2) derived from the transgenic adenocarcinoma mouse prostate (TRAMP) model and monitored the tumor growth in syngeneic, and thus immune-competent, mice. The key cell populations associated with anti-tumor effects were analyzed in tumors co-injected with the MSCs. Results: We confirmed that tumor cell-derived CCL2 was crucial for tumor growth and MSC migration. CCL2 KO MSCs inhibited migration of the monocyte/macrophage but not the proliferation of tumor cells in vitro. However, mice co-injected with tumor cells and CCL2 KO MSCs exhibited anti-tumor effects, compared with tumor cells alone and with control MSCs, partly due to increased infiltration of $CD45^{+}CD11b^{+}Ly6G^{-}$ mononuclear myeloid cells. Discussion: The role of MSCs in prostate cancer is still uncertain. Our findings support the idea that knock-out CCL2 derived from MSCs promotes anti-tumor activity. It suggests that CCL2 knockout MSCs promote anti-tumor responses in vivo via mononuclear myeloid cell-mediated pathways. More research is required to investigate this issue. Conclusions: In an immune-competent syngeneic mouse model for prostate cancer, disrupting MSC-derived CCL2 strengthens anti-tumor functions.

Keywords: *CCL2; MSCs; Prostate cancers; CD11b+Ly6G−;TRAMP; Anti-tumor*

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Isolation of Ovarian Cancer Cells to Establishing Cancer Stem Cell Lines Using Membrane Filtration Method

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Abstract:

Abnormal cell growth and multiplication in female ovaries or fallopian tubes resulted in ovarian cancer. This condition is primarily caused by cancer stem cells (CSCs) or cancer- initiating cells (CICs), which constitute a small percentage of the total tumor cell population and are responsible for tumor generation, proliferation, and metastasis. Purification of CSCs or CICs is crucial for both the development and screening of anticancer drugs that are tailored to individual patients. We are developing a membrane filtration method to isolate CICs (CSCs) from ovarian cancer cell line using nylon mesh filter membranes with pore sizes of 11 μm as well as poly(lactide-co-glycolic acid)/silk screen (PLGA/SK) porous membranes with pore sizes ranging from 20-30 μm. The aim of this study is to establish a reliable and effective method for enriching CICs (CSCs) of ovarian cancer cells via membrane filtration.

We investigated the permeation of ovarian cancer cell line (ES-2) through several membranes by the membrane filtration method (Figs. 1 & 2). Over 85% of the cells were collected in the permeation fraction. We found that the permeation fraction of the cells decreased and the recovery fraction of the cells enhanced when the pore size of the membranes is getting small. This discovery suggests that the membrane filtration is potentially an effective method for purifying and isolating CSCs. We also evaluated CSC markers of CD44 and CD133 in each fraction after permeation through several membranes.

The cells showed the highest expression of CD133 in the migrated fraction, followed by the permeation fraction and the recovery fraction. On the other hand, high levels of expression on CD44 were found in any fractions without a noticeable trend. Taken together, the results indicated that the cells in the migrated fraction have higher percentage of ovarian CSCs compared to those in other fractions.

Efficient isolation and purification of CSCs can support the development of effective treatments of the patients having ovarian cancer, because CSCs are crucial in tumor initiation, metastasis, and recurrence. Membrane filtration method is a promising technique for isolation and purification of CSCs of ovarian cancers.

KEYWORDS: Ovarian carcinoma, cancer stem cells, nylon membrane, membrane filtration method, poly(lactide-co-glycolic acid)/silk screen.

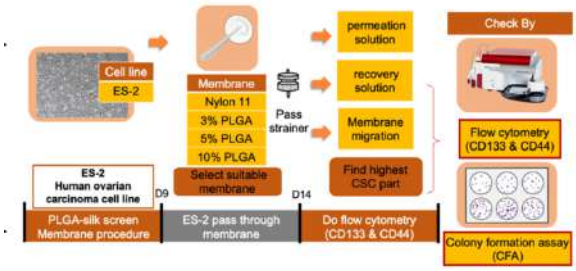

Figure 1 Experimental design of membrane filtration method.

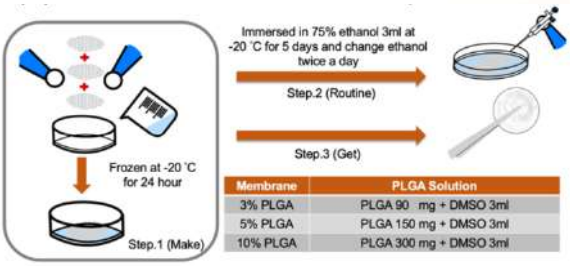

Figure 2 Preparation of PLGA/SK membranes

Reference

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Development of A New Prime Editing System for Efficient Genome Editing

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Abstract:

The advancement of genome engineering technologies has provided a diverse toolbox to manipulate DNA with extraordinary flexibility and precision for correcting and treating genetic diseases [1]. Prime Editor (PE) facilitate mutation correction of virtually all types of mutations, including base transversion, insertion and deletion [2]. However, the current prototypic PE platform has met with the inherent off-target editing induced by SpCas9n and low editing efficiency, which may compromise its transition into clinical settings for genetic disease attenuation or correction. Therefore, in this study, we aim to develop an improved PE platform with higher specificity through the utilization of highly specific HiFiCas9 (HiFiPE) and eSpCas9 (ePE) variants. We showed HiFiPE achieves comparable editing efficiency with higher specificity. We also explored the improvement of editing efficiency by means of HiFiPE architecture optimization (HiFiPEmax) and pegRNA structure engineering (epegRNA). Ultimately, we demonstrated the feasibility of HiFiPE in patient-derived human iPSCs, potentiating its translation into the clinical settings.

KEYWORDS: CRISPR, Prime Editing, iPSC

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Nanoparticle-Based Dopaminergic Neuron Differentiation Approach - *in vitro* Model Derived from Human Induced Pluripotent Stem Cells

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Abstract:
Human induced pluripotent stem cells (hiPSCs) have significantly impacted regenerative medicine and disease modeling due to their unique capacity to differentiate into any cell type in the human body. Dopaminergic neurons (DAns), one of the many potential differentiation targets, have become a focal point due to their implication in Parkinson's disease (PD) [1].
In the present study, we introduce an innovative nanoparticle-based technique for driving the differentiation of hiPSCs into DAns. Capitalizing on the minuscule size and extensive surface area of nanoparticles, we have engineered these particles to interact at a molecular level with biological systems. To instigate the differentiation process, we have specifically encapsulated the transcription factor, Lmx1a, within Polyethylenimine (PEI) and chitosan nanoparticles. Contrasting with viral vectors, nanoparticles can accommodate larger payloads, offer regulated delivery, and provide enhanced stability and reproducibility. Our study utilizes PEI to concentrate the Lmx1a expression plasmid, subsequently coating it with Chitosan to construct a novel nano-delivery vector termed CDP27. With a size of approximately 100 nm and a mildly positive surface charge, CDP27 has shown promising results in delivering the dopaminergic neural chemoattractant gene Lmx1a to stem cells, effectively guiding them toward becoming neural progenitor cells and ultimately differentiating into dopaminergic neurons [2].
The unique nanoparticle-mediated delivery system we developed allows for precise control over the expression of cellular transcription factors, thereby enhancing the efficiency and specificity of neuronal differentiation. Leveraging this technique, we successfully established an in vitro model of DAns derived from hiPSCs. This model

has been used to study the pathophysiological impacts of the neurotoxic compound 6OHDA in PD [3]. Furthermore, with its inherent advantages over viral vectors, our innovative nanoparticle-based dopaminergic neuron differentiation method can be readily applied to drug screening and cell therapy. This opens up novel possibilities in the realms of stem cell research and regenerative medicine [4].

KEYWORDS: human induced pluripotent stem cells, dopaminergic neurons, nanoparticle, regenerative medicine

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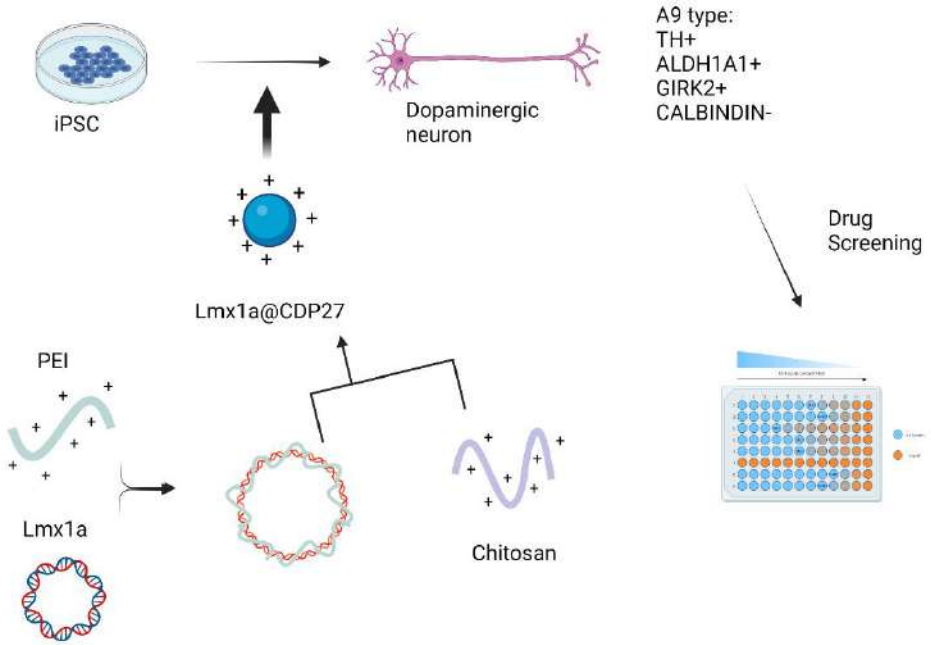

Establishing a drug screening platform based on cardiomyocytes derived from human induced pluripotent stem cells for analyzing the effects of nanosized heart failure medications

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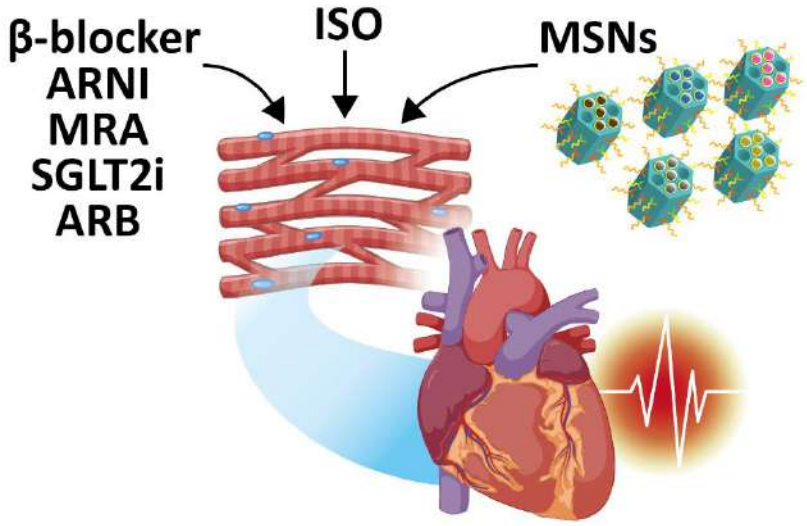
Abstract:
In this study, we innovatively utilize a heart failure model derived from human induced pluripotent stem cells (hiPSCs) to assess the effects of commonly used clinical heart failure therapeutics such as β-blockers, ACEI, ARB, MRA, ARNI, and SGLT2i [1]. Initially, we differentiate hiPSCs into cardiomyocytes and induce a heart failure state using Isoproterenol, thereby establishing a pathological model. Subsequently, we examine the impact of these drugs on cardiomyocytes, evaluating various biological indicators including cell survival rate, cardiomyocyte function (contractile force and speed), and changes in pathological markers such as NT-ProBNP [2]. The structure of Mesoporous silica nanoparticles (MSNs) are stable, and the nanostructure, size and properties will not be changed in organic solution, so it is convenient for different surface physical and chemical modifications, which is beneficial for drug loading and drug delivery [3]. we explore the encapsulation of drugs in MSN via nanotechnology, and assess their sustained-release effects in a cardiac organoid model. MSN has high biocompatibility and potential and feasibility as a biomedical application. Our results indicate that this heart failure model derived from hiPSCs serves as a potent tool for assessing the efficacy of heart failure drugs and investigating the direct impact of nanodrugs on cardiomyocytes. This approach facilitates the development and testing of future therapeutic strategies for heart disease.

KEYWORDS: human induced pluripotent stem cells, cardiomyocyte, nanodrug, heart failure



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A high-throughput lung air-blood barrier neutrophil transmigration system for drug dose-dependent study

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Neutrophil transmigration has been emphasized for airway inflammation study due to its important role in pulmonary disease progression [1]. However, current microphysiological systems such as microfluidic lung-on-a-chip models are low-throughput and unable to collect sufficient number of transmigrated neutrophils for further biological characterization [2]. Transwell-based neutrophil transmigration models, on the other hand, are either composed of only epithelium or modeled in 12- or 24-well plates [3]. In order to introduce high-throughput neutrophil transmigration approaches for in-depth inflammation study, we developed a Transwell-96-based air-blood barrier neutrophil transmigration system incorporated with flow cytometric analysis of transmigrated neutrophils for drug dose-dependent study.

Previously, we developed a high-throughput distal lung air–blood barrier model including both human epithelium and endothelium [4]. Acquiring the optimal size, the model is small enough for efficient dose-dependent study due to less required volume of clinical samples but is big enough to include sufficient neutrophil number for flow cytometric analysis. In this study, the model is used to achieve high-throughput analysis of *in vitro* transmigrated neutrophil number, phenotypes, and air-blood barrier function. With the recruitment of neutrophil-related chemoattractant Interleukin 8 (IL-8) and Leukotriene B4 (LTB4), the system shows a dose-dependent curve on the number of neutrophils that transmigrated through the endothelium then lung epithelium. Moreover, donor dependency occurs when defining the half-maximal inhibitory concentration (IC50) of a JAK-STAT inhibitor, Baricitinib, against the recruitment of transmigrated neutrophils by IL-8. We are also able to create disease model using this system. After exposed to cystic fibrosis (CF) patient sputum-derived airway supernatant (ASN), the system shows CF-related neutrophil phenotype and significant decrease in barrier electrical resistance. Baricitinib also presents an inhibition against neutrophil transmigration and activation

by CF ASN. The system obtains great potential on drug screening and disease investigation for precision medicine in the future.

KEYWORDS: high-throughput, pulmonary, inflammation, neutrophil.

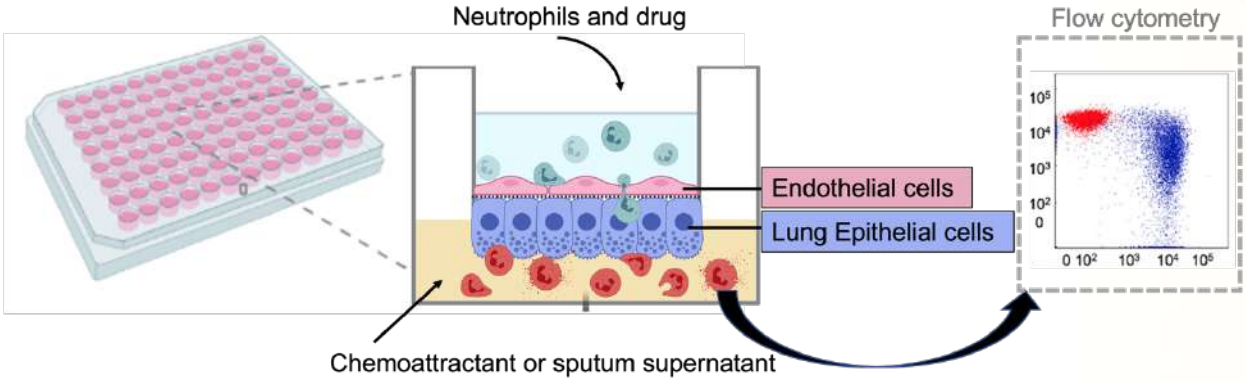

Figure 1 A high-throughput air-blood barrier neutrophil transmigration system.

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Polymerized magnetic cells amenable to synapse formation enable selective capture of antigen-specific T lymphocytes

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Abstract:

Isolation of antigen-specific T lymphocytes by has immense biomedical values in antiviral and anticancer immunity research, T cell receptor (TCR) sequencing, and adoptive cell therapy (ACT) (*1*), yet establishing durable bonding with T cell targets can be challenging given the interactions between peptide-MHC (pMHC) complexes and TCR has low affinity and a fast off-rate. Although multimerization of pMHC has been widely adopted to improve probe sensitivity against target T cells, the passive approach of ligand oligomerization is associated with the inevitable tradeoff between sensitivity and specificity (*2*). In biology, prolonged T cell engagement with cognate antigen presenting cells (APCs) is triggered by a serial process involving receptor clustering, co-stimulator signaling, and actin cytoskeleton reorganization, which selectively proofread and amplify weak interaction events. This serial engagement model at the cellular interface gives rise to the extraordinary selectivity of T cells and serves as an inspiration for T cell isolation strategies. Herein, we develop a magnetic, hydrogel-crosslinked APC (mag-gAPC) for biomimetic T cell engagement and isolation. The inanimate mag-gAPCs are readily tailorable with desired antigen targets for stable presentation, and they elicit immunological synapse formation, T cell actin clustering, and trogocytosis in the presence of cognate T cells. We demonstrate superior antigen-specific T cell isolation efficiency by mag-gAPCs as compared to pMHC-functionalized magnetic probes. In addition, we improvement of adoptive T cell therapy and enhancement of endogenous neoantigen-specific T cell detection following mag-APC-mediated T cell capture. The work provides a novel design principle for T cell capture and usher in new methodologies for T cell research.

Keywords: Antigen-specific T cell capture, Gelated cells, Immunological synapse.

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Combination of Platinum-doped $CaCO_3$ and Amylopectin-based Gel to Synergize with Radiotherapy for High-grade Glioma

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Abstract:

High-grade glioma (HGG) poses a formidable challenge clinically with poor prognosis and high recurrence rate. Nowadays, standard treatment of chemoradiotherapy is shown incurable but merely improve the median survival, because it still failed to tackle the problem of infiltrating nature of tumor, which causes the tumor relapse within several months inevitably [1].

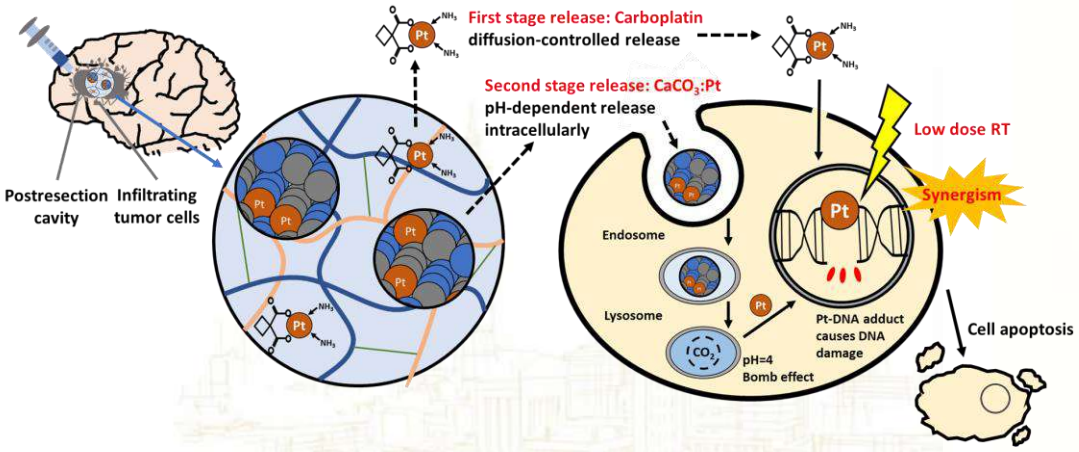
Systemic drug delivery of Temozolomide (TMZ) is able to pass through blood-brain barrier, but it fails to reach an effective concentration at tumor bed and also inevitably causing dismay systemic side effects [1]. On the other hand, Gliadel® (BCNU) wafer, a type of wafer that controlled release BCNU locally, however, was shown to be limited in drug penetration and the drug release of BCNU is only within 7 days, which still causes the recurrence of tumor [2, 3].

The time interval between postresection and radiotherapy is usually 3 weeks for standard treatment of HGG, so it is crucial to block tumor infiltration during this time interval and prevent from recurrence. Accordingly, this study is aim to design a long-term drug delivery system (DDS) for local treatment and further synergize with radiation. The combination of Platinum-doped $CaCO_3$ ($CaCO_3$:Pt) and Carboplatin-loaded Amylopectin-based gel (CPG) is treated locally and divided into 2 releasing stages. Firstly, Carboplatin, released initially from gel by diffusion, is able to cause anti-proliferation effect of cancer cells. Secondly, $CaCO_3$:Pt could cause apoptosis of glioma cells via endocytosis by releasing platinum from $CaCO_3$:Pt intracellularly based on acidic degradation in endosome-lysosome complex, where alkylating agent, platinum could cause cancer cell apoptosis by crosslinking onto DNA.

As for material analysis, Spherical microparticles $CaCO_3$:Pt is synthesized as vaterite form and it was validated by TEM, XRD. The drug release profile of the designed DDS is measured by ICP-MS and was shown to release alkylating agents up to 21 days. As for *in-vitro* test, both the WST-1 assay and live/dead cell staining assay results indicate the CPG and $CaCO_3$:Pt are both cytotoxic to ALTS1C1 glioma cells.

Furthermore, the clonogenic assay validates that both $CaCO_3$:Pt and radiation could inhibit glioma cells from proliferation and both of which could reach a synergistic effect by calculating the combination index. As for *in-ovo* test, the combination of CPG and $CaCO_3$:Pt with radiation was shown to be effective and could suppress the tumor growth in the chicken chorioallantoic membrane model. Lastly, as for *in-vivo* study, glioma cells are grafted intracranially in mice brain, and the CPG combined $CaCO_3$:Pt is intratumoral delivered locally, where the tumor growth was assessed by IVIS imaging system and there were shown to be decreasing in tumor size for treatment groups. As a whole, the combination of CPG and $CaCO_3$:Pt were shown to cause apoptosis of glioma cells and could synergize with radiation so as to block the tumor infiltration, which makes it a potential co-treatment with radiation in addition to TMZ/RT.

Keywords: High-grade glioma, Local drug delivery, Carboplatin, $CaCO_3$, Synergistic effect



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4D Printed Multilayered Scaffolds with Enhanced Performance for Bone Regeneration

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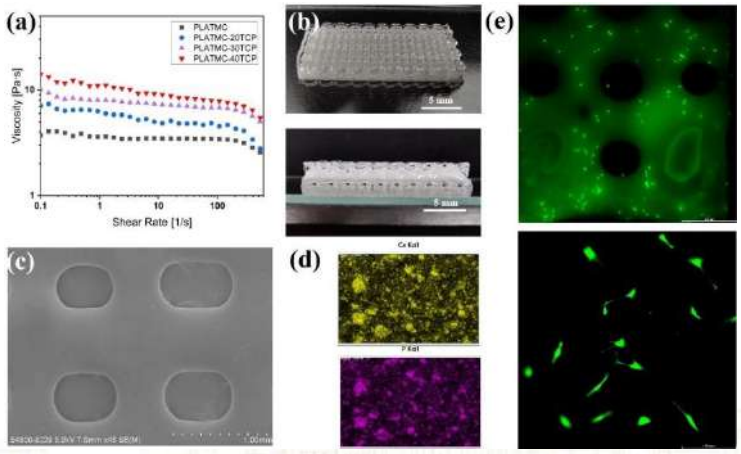
Abstract:

Bone defects caused by injury or surgical removal of tumor significantly deteriorate the structural integrity and functionality of bone and hence can affect the quality of life for patients both short-term and long-term. Tissue engineering (TE) provides a promising way to treat bone tissue damage or loss though the regeneration of bone at the original defect site. So far, TE strategies for bone mainly use scaffold-based TE, which involves construction of porous scaffolds using metal, ceramic, polymer, and/or composite materials [1]. While metal or ceramic-based scaffolds have advantages in mechanical strength, it is difficult or impossible to incorporate bioactive agents into metallic or ceramic scaffolds owing to harsh manufacturing conditions for them such as high temperature sintering. Polymer-based scaffolds use synthetic and/or natural polymers and employ facile fabrication techniques with mild manufacturing conditions. Bioactive biomolecules may be directly incorporated in polymer-based scaffolds whose local release will promote tissue regeneration. Natural polymers usually have weaker mechanical strength while synthetic polymers are usually less biocompatible [2]. Among natural polymers for TE, collagen is often considered for scaffold fabrication. Various techniques have been used to produce collagen scaffolds, including surface coating, electrospinning, solution casting, and 3D dispensing [3]. But 3D printing has shown to be a powerful tool for constructing TE scaffolds with controlled pore characteristics effectively and efficiently. It can also relatively easily incorporate nanoparticles, nanofibers, or bioactive molecules into printed structures. However, very few studies have investigated 3D printing of collagen owing to its low solubility, poor printability, and susceptibility to denaturing. Also, the mechanical performance of collagen scaffolds is poor [4].

In this study, multilayered scaffolds were made via 3D/4D printing for treating bone defects. Briefly, bioactive and biodegradable tricalcium phosphate (β-TCP) nanoparticles were dispersed in the solution of shape memory polymer (SMP) poly(D,L-lactide-co-trimethylene carbonate) (PDLLA-co-TMC, "PTMC" in short) to make printing inks. The inks then were 4D printed into nanocomposite scaffolds as the core structure. The ink made of a solution of collagen with Poloxamer 407 thickener was 3D printed into thick layers of scaffolds on top and bottom surfaces of β-TCP/PTMC nanocomposite scaffolds, attempting to achieve improvements in mechanical and biological performance for multilayered scaffolds. β-TCP/PTMC inks exhibited shear-thinning behavior which is required for printing inks, and increased β-TCP nanoparticle amounts led to increased viscosity and dynamic modulus for the inks. β-TCP/PTMC inks showed good

printability for the 4D printing process. The printability of collagen was also greatly improved with the addition of thickener Poloxamer 407. Cross-sectional SEM and EDX analyses showed that β-TCP nanoparticles were uniformly distributed within 4D printed PTMC struts. Young's modulus and tensile strength of β-TCP/PTMC nanocomposite scaffolds were enhanced with the addition of β-TCP nanoparticles and reached their maximum values at the 30% w/w β-TCP content level; and these properties were compromised when the β-TCP content was increased to 40% w/w. The PTMC SMP made multilayered scaffolds shape morphable with a temperature changed from 20 to 37°C. Cell culture experiments using rat bone marrow mesenchymal stem cells (rBMSCs) indicated good biocompatibility of multilayered scaffolds. rBMSCs adhered, grew and proliferated well on the scaffolds. These results suggest the high potential of multilayered scaffolds for bone tissue regeneration application.



Characterization and evaluation of inks and 3D/4D printed scaffolds: (a) rheological analysis of printing inks, (b) different views of a printed scaffold, (c) morphology of a porous scaffold observed under SEM, (d) EDX mapping on a scaffold cross-section showing Ca and P distribution, respectively, and (e) rBMSC cells cultured on a multilayered scaffold.

Keywords: 4D printing, nanocomposite, collagen, scaffolds, multilayer, bone tissue engineering

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Influence of hydrogel stiffness on adipogenic differentiation of mesenchymal stem cells with controlled morphology

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Abstract:

Recently, many studies have shown that stem cell differentiation fates can be affected by cell morphology and cellular microenvironment. However, it is barely studied how the single stem cell senses the microenvironmental stiffness with controlled morphology. Thus, in this study, the effect of hydrogel stiffness on adipogenic differentiation of human bone-marrow-derived mesenchymal stem cells (hMSCs) with controlled morphology was investigated (figure 1).

Cell morphology was controlled by micropattern techniques. Briefly, the photoreactive PVA was synthesized and coated on tissue culture polystyrene (TCPS) plates and micropatterned by photolithography. Micropatterns of different size (Φ30, 40, 60, and 80 μm) and elongation (Φ60 μm circle with different aspect ratio: 1:1, 2:1, 4:1, 8:1) were prepared. The morphology of the prepared micropatterns was characterized by atomic force microscope. Then, hMSCs were seeded on these micropatterns. After 24 h of culture, the cells with controlled morphology were embedded in agarose hydrogels of different stiffness (w/v, 0.5%, 1.0%, and 3.0%). The bulk stiffness of hydrogels was measured by a static compression test. Then, the cells embedded in hydrogels were cultured in adipogenic medium for 14 d. The rearrangement of hMSCs actin filaments was checked on 3 d, and the adipogenic differentiation in different groups was measured by Oil Red O staining and quantified by counting the Oil Red O positive cells [1].

The spreading area and elongation of hMSCs cultured on the micropatterns followed the geometry of the underlying micropatterns. The stiffness of hydrogels ranging from low to high was 2.3 ± 0.4, 7.0 ± 0.4, and 81.1 ± 2.1 kPa, respectively. The cells showed a different level of adipogenic differentiation that was dependent on both hydrogel stiffness and cell morphology. Adipogenic differentiation became strong when the cell spreading area decreased and hydrogel stiffness increased. Adipogenic differentiation did not change with cell elongation. Therefore, cell spreading area and hydrogel stiffness could synergistically affect adipogenic differentiation of hMSCs, while cell elongation did not affect adipogenic differentiation. A change of cell morphology and hydrogel stiffness was accompanied by actin filament alignment that was strongly related to adipogenic differentiation. The results indicated that cell morphology could affect cellular sensitivity to hydrogel stiffness.

KEYWORDS: hydrogel stiffness, micropattern, cell morphology, spreading area, aspect ratio, adipogenic differentiation, mesenchymal stem cells

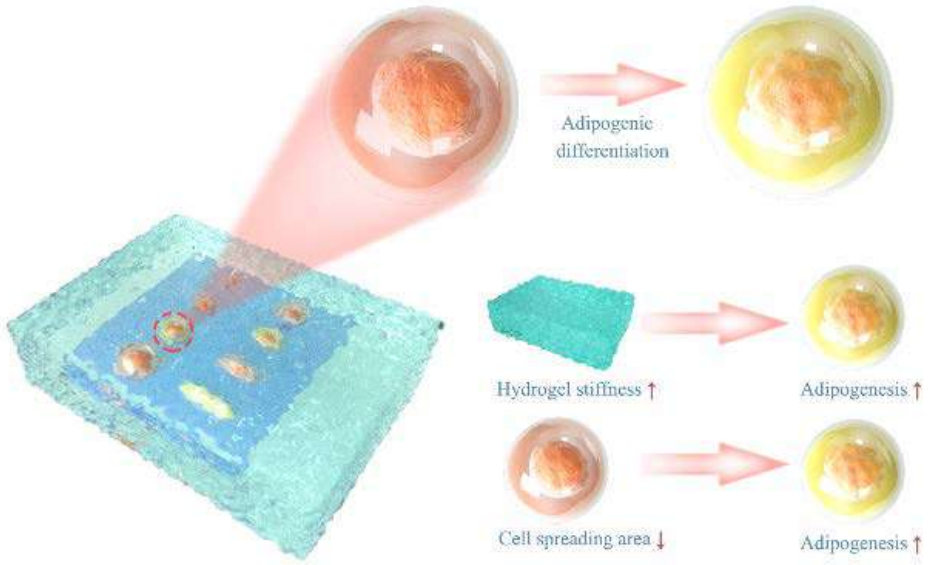

Figure 1 Graphic abstract of micropatterned hMSCs embedded in agarose hydrogels and adipogenic culture

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Composite scaffolds for magnetic hyperthermia of breast cancer and reconstruction of adipose tissue

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Abstract:

Breast cancer is the most common cause of cancer-related death in women worldwide. There remains a challenge in completely eradicating breast cancer cells and reconstructing the tumor-initiated breast defect after surgical intervention. Hyperthermia therapy has been broadly investigated as an attractive modality for therapeutic intervention against breast cancers. In recent years, Fe_3O_4 nanoparticles (NPs), due to its low cytotoxicity, unlimited tissue penetration depth and FDA approval, have been extensively explored as heat mediators for magnetic hyperthermia to achieve maximal ablation of breast cancer cells.

In addition to magnetic hyperthermia in breast cancer, adipose tissue regeneration is necessary to repair cancer-initiated defective breast. Three-dimensional (3D) scaffolds are increasingly considered as a platform to provide necessary spaces and microenvironments for the regeneration of adipose tissue. Based on the above considerations, we developed alternating magnetic field (AMF)-responsive composite scaffolds for in situ magnetic hyperthermia-based breast cancer therapy and reconstruction of adipose tissue (Figure 1) [1].

Fe_3O_4 NPs were synthesized from Fe (II) and Fe (III) precursors in polyol solvent and modified with citric acid. Gelatin was conjugated with folic acid (FA) to obtain FA-gelatin. The citrate-modified Fe_3O_4 NPs were hybridized with the FA-gelatin solution to fabricate the composite scaffolds by using ice microparticles as porogen material. The pore structures of composite scaffolds were investigated by SEM. Their ability to capture FA receptor-expressing breast cancer cells was evaluated in vitro. Their magnetic thermal property and anticancer efficacy under AMF were studied both in vitro and in vivo. In addition, the composite scaffolds were used for in vitro 3D culture of human bone-marrow derived mesenchymal stem cells (hMSCs) to explore their capacity to guide the adipogenic differentiation of hMSCs.

The composite scaffolds had well-interconnected spherical pores that allowed cell migration and infiltration. Due to the presence of folic acid, composite scaffolds could capture FA receptor-expressing breast cancer cells. Composite scaffolds possessed a high magnetic-thermal conversion property and could ablate breast cancer cells under AMF irradiation during in vitro cell culture and in vivo animal experiments. In addition, culture of

hMSCs in the composite scaffolds showed that the composite scaffolds not only supported the adhesion and proliferation of hMSCs, but also promoted lipid droplet formation and up-regulated the expression of adipogenesis-related genes (FASN, FABP4, CEBPA and LPL) when AMF was off. Overall, the results suggested that the composite scaffolds possessed both the magnetic thermal ablation effect to breast cancer cells and the promotive effect for adipogenic differentiation of hMSCs.

KEYWORDS: Fe_3O_4 nanoparticles, composite scaffolds, cancer therapy, magnetic hyperthermia, adipose tissue regeneration

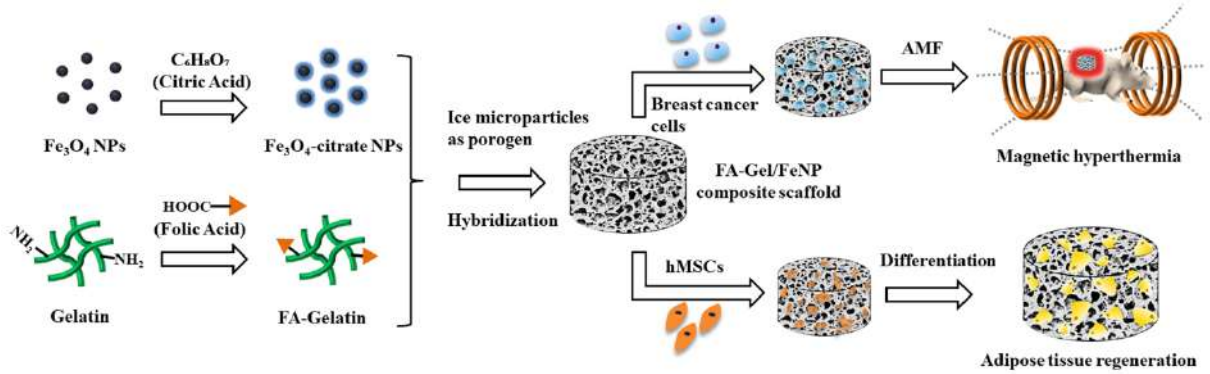

Figure 1. Dual-functional composite scaffold of Fe_3O_4 NPs and gelatin for magnetic hyperthermia and adipose tissue regeneration.

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Multi-functional hydrogels incorporating mineral-coated composite nano fibers with magnetic nanoparticles for photothermal therapy and bone tissue regeneration

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Abstract:

Photothermal therapy (PTT) is a promising non-invasive cancer treatment that uses light-absorbing agents to convert light energy into heat, inducing local hyperthermia that can kill cancer cells. In recent years, PTT has been explored as a potential therapy for bone tumors, but the challenge of promoting bone regeneration after tumor ablation remains. Moreover, reactive oxygen species (ROS) generated by bone tumors exacerbate bone tissue damage and inhibit bone regeneration, which needs to be controlled at an appropriate level. Development of multi-functional biomaterials enabling both cancer-therapeutic and tissue-regenerative outcomes may solve these problems. In this study, we developed a multi-functional composite hydrogels incorporating mineral-coated magnetic nanofibers for PTT and bone regeneration. First, we prepared epigallocatechin gallate (EGCG)-mineral-coated poly-L-lactic acid (PLLA) nanofibers loaded with magnetic nanoparticles (mMF). Then, hydrogels incorporating mMF (G-mMF) were prepared, which lead to more than 80% cell death for osteosarcoma (MG-63). In addition, G-mMF effectively removed hydrogen peroxide (H_2O_2), one of the ROS, to induce upregulation of anti-apoptotic genes and downregulation of pro-apoptotic genes. Meanwhile, G-mMF enhanced the osteogenic differentiation of human adipose-derived stem cells (hADSCs) by up-regulation of osteogenic genes. In conclusion, it is expected that the G-mMF would be multi-functional biomaterials with PTT, ROS scavenging effects and bone regeneration properties.

KEYWORDS: Multi-functional composite hydrogels, Photothermal therapy, ROS scavenging, Osteogenesis

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Changing Behavior of C2C12-GFP on Linear Groove Polydimethylsiloxane (PDMS) Substrate

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Abstract:

Skeletal muscle regeneration poses a significant clinical challenge, necessitating a comprehensive understanding of cell behavior in translational research [1]. Tissue engineering approaches have emerged as promising strategies to address this challenge, and the choice of suitable biomaterials plays a crucial role in the success of these approaches [2]. Polydimethylsiloxane (PDMS) has gained attention in tissue engineering due to its advantageous properties and the incorporation of linear grooves on PDMS substrates offers specific benefits for skeletal muscle tissue engineering [3, 4].

This study aims to investigate the dynamic behavior of C2C12-GFP cells on a linear groove of PDMS substrate, with potential implications for tissue engineering applications. C2C12, a mouse skeletal muscle cell line with GFP (Green Fluorescent Protein) was the culture on the top of the linear groove pattern PDMS for 7 and 14 differentiation days and harvested for protein concentration analysis. The single-layer microchannels, were fabricated by a general PDMS fabrication process; it was sterilised with autoclaved and ultraviolet overnight and either directly seed on the 12-well plate or coated with gelatin. The image was captured by fluorescence microscope. All sample was in 3 biological replicates, and data was performed in mean with standard deviation. And the statistical analysis using a t-test.

After 7 and 14 days of differentiation, C2C12 cell growth on the linear groove PDMS substrate exhibited a significant decrease in protein concentration compared to the control group (8.5 ± 3.2 mg/ml; 3.7 ± 2.1 mg/ml, p=0.15) on differentiation day 7 and (7.9 ± 1.4 mg/ml; 3.1 ± 1.3 mg/ml, p=0.02) respectively. Fluorescence imaging revealed notable changes in behavior, including the clustering of myotubes within the linear grooves.

To further understand the underlying mechanisms, a literature review was also conducted by systematic search using PubMed, MDPI, and Stem Cell Research & Therapy databases. The

inclusion criteria were the original articles published between 2015 and 2023 that focused on the use of PDMS in skeletal muscle tissue engineering. A total of 10 articles were selected for this review. The selected articles demonstrated that PDMS-based scaffolds have shown great potential in promoting skeletal muscle regeneration and improving functional outcomes [5].

This study sheds light on the changing behavior of C2C12-GFP cells on a linear groove PDMS substrate, offering new perspectives on cell behavior and its interaction with specific biomaterial features. The presence of linear grooves in PDMS substrates may offer advantages in skeletal muscle tissue engineering, potentially promoting cell alignment and enhancing tissue regeneration. However, there are still challenges that need to be addressed, such as the optimization of mechanical properties and the need for better integration with host tissues. Further research is warranted to explore the full potential and advantages of linear groove PDMS substrates in skeletal muscle tissue engineering applications, with the ultimate goal of advancing effective and successful tissue engineering strategies.

KEYWORDS: Skeletal muscle, Regeneration, C2C12-GFP, Cell Behavior, Polydimethylsiloxane (PDMS)

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Administration of self-assembly mRNA nanomedicine augmented calvarial defect healing by endochondral ossification

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Abstract:

Large-area craniofacial defect remains a challenge for orthopaedists, hastening the need to develop a facile and safe tissue engineering strategy; osteoconductive material and the combination of optimal growth factors and microenvironment should be considered. Face the unmet need; we proposed that abundant cytokines and chemokines would be secreted from the bone defect, provoking the infiltration of endogenous stem cells to assist bone regeneration. We could provide a potent mRNA medicine cocktail to promptly initiate the formation of bone template, osteogenesis, and subsequent bone matrix deposition through endochondral ossification, which may retard the rapid fibroblast infiltration and prevent the formation of atrophic non-union. We explored the mutual interaction of BMP2 and TGFβ3 mRNA, both potent chondrogenic factors, on inducing endochondral ossification, examined the influence of in vitro transcribed polyA tail length on the mRNA stability, prepared the mRNA nanomedicine using a PEGylated polyaspartamide block copolymer, loaded in a gelatin sponge, grafted in a critical-sized calvarial defect, and evaluated the bone regeneration by histological and μCT examination. The BMP2 and TGFb3 composite mRNA nanomedicine resulted in over 10-fold new bone volume (BV) regeneration in 8 weeks than the BMP2 mRNA nanomedicine administration alone, demonstrating the TGFb3 mRNA nanomedicine synergistically enhances the bone formation capability induced by BMP2 mRNA nanomedicine. Our data demonstrated that mRNA medicine-mediated endochondral ossification provides an alternative cell-free tissue engineering methodology for guiding craniofacial defect healing.

KEYWORDS: mRNA medicine; polyplex nanomicelle; calvarial defect; endochondral ossification; tissue engineering

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Sebacoyl Dinalbuphine Ester-Loaded Nanostructured Lipid Carriers in Gel for Postoperative Pain on Spine Surgery

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Abstract:

Operation on the vertebra often leads to serious pain after surgery. Although intravenous administration of opioids has a strong analgesic effect, it often leads to serious systemic adverse effects[1,2]. In this study, we design a local analgesic with extended-release effect. We combine thermally-sensitive hydrogel Poloxamer 407 with nanostructured lipid carriers (NLC) to be a carrier of the hydrophobic opioid, Sebacoyl Dinalbuphine Ester (SDE)[3], the prodrug of NA. Different from other common gel materials, hydrogel Poloxamer 407 can be administrated on the tissue by injection at low temperature and transit to gel phase in a short time. With these properties, it can be applied on minimally invasive surgery and deliver SDE locally. However, the hydrophilic hydrogel can't dissolve SDE well. As the result, we use NLC to encapsulate SDE and enhance the extended-release effect.

The particle sizes of the NLCs loaded with 5% SDE is nano and uniform in size, which confirms that all SDE was encapsulated in NLCs without precipitation. The thermal-sensitive hydrogel Poloxamer 407 incorporates with NLCs can be injected at low temperature and transit to gel form in a few minutes at 37 °C. HPMC, as thickener, can prolong the erosion duration and enhance the sustained release effect of the formulations[4]. The *in vitro* release profiles revealed that the NLCs incorporated Poloxamer 407 hydrogel can prolong the release of SDE up to 5 days, which can improve the disadvantage of short-acting NA.

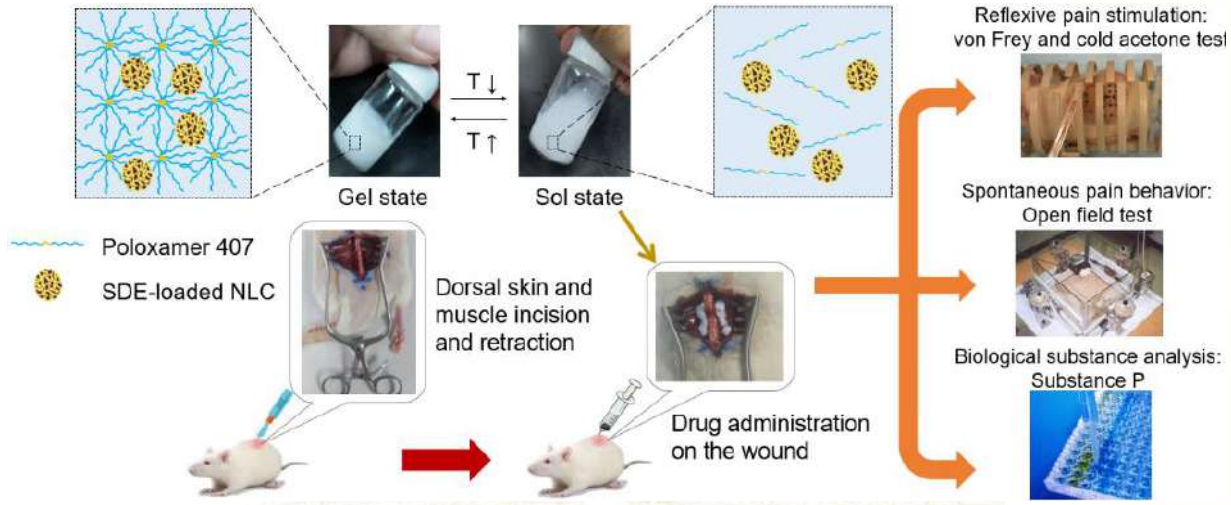
In the animal study, the rats were incised on the back and muscle-retracted for an hour as spine surgery model, and gel formulations are applied to the wounds. Commercial SDE oil streaks (Naldebain®) and Blank NLC in situ gel were used as positive and negative control groups. To confirm the local and extended-release effect of our formulation, SDE dosage of our formulation is set at about 10-20% of the positive control dosage. In von Frey and cold acetone test, SDE-loaded NLC in Poloxamer 407 gel showed similar analgesic effect to the positive control group with less dosage in a week after surgery, about 15% of positive control. SDE-loaded suspension in Poloxamer 407 gel also showed an ideal analgesic effect, however, only lasted three to four days. NLC in gel has a better extended-release effect than suspension in gel, corresponding to the *in vitro* release result. As for substance P analysis in blood, both SDE-loaded NLC and suspension

in Poloxamer 407 gel can effectively lower the substance P after surgery, which means the postoperative pain can be alleviated with the local SDE delivery system we designed.

SDE-loaded NLC in Poloxamer 407 gel can release SDE locally in a sustained release rate and solve the problems of intravenous opioids including serious systemic adverse effects. In addition, the formulation reduces the dosage and the frequency of administration of analgesic. This complex formulation effectively improves the safety, efficiency, and convenience of analgesic administration after surgery.

KEYWORDS: sustained release, thermal-sensitive hydrogel, locally delivering, postoperative pain, Sebacoyl dinalbuphine ester (SDE)

Graphic abstract



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Antiretroviral-Drugamer *In-situ* Forming Subcutaneous Injectables with Tunable Drug Release

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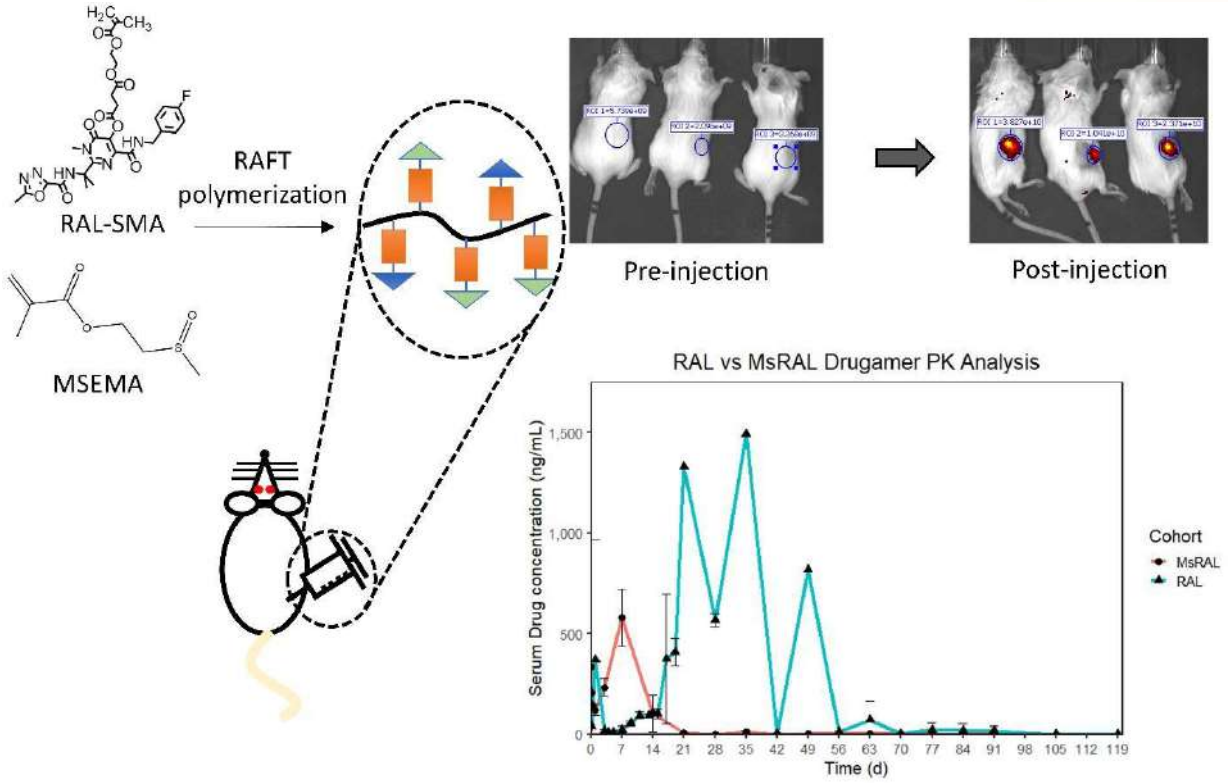
Abstract

Drug delivery formulations that extend duration of action of antiretrovirals serve as an effectively address the problem of poor adherence in the treatment and prophylaxis of HIV[1]. Inthis study, the HIV integrase inhibitor Raltegravir (RAL) is modified into a prodrug methacrylate monomer (RAL-SMA) by esterification with an acyl chloride. RAL-SMA is subsequently polymerized by Reversible Addition Fragmentation chain Transfer (RAFT) polymerization to obtain well-defined polymeric prodrugs with very high drug content. RAFT is an advantageous method to synthesize polymer-drug conjugates by yielding well-defined polymers with controlled molecular weight in mild conditions free of toxic metal salts[2]. In particular for the use of polymers in drug delivery, molecular weight needs to be considered in designing for optimal drug release, thus RAFT polymerization offers an advantage with the low dispersity under mild conditions[3]. By polymerizing RAL-methacrylate prodrugs, we obtain a polymer of drugs, or “drugamers”. Drugamers are subcutaneously injected in the flank of female BALB/cJ mice and forming a drugamer depot, wherein the drug is hydrolytically cleaved to release the drug systematically.

In this effort we have successfully polymerized a family of Raltegravir (RAL)-drugamersas homopolymers and random copolymers with monomer conversion rates of 80% and 85% witha drug loading of 72wt% and 55 wt%, respectively. RAL-SMA was copolymerized with 2-(methylsufinyl)ethyl methacrylate (MSEMA), a biocompatible DMSO-analogue methacrylate monomer[4]. Faster release was exhibited in the random copolymer, which contains hydrophilic and biocompatible monomers, and thus greater water penetration into the organic solvent phase and greater depot dissolution. In particular, the alkyl ester linker yielded relatively rapid release (up to 100x) in RAL-drugamers versus drugamers of tenofovir alafenamide (TAF)[5]. We attributed the difference of hydrolysis to the due to the low pK_a of the ionization of 5,6- dihydroxtpyrimidine-4-carboxamide ring. This study demonstrates selection of linker only partlycontrols the drug release when using the drugamer platform, rather that release may be a function of multiple variables together, such as hydrophilicity of the drugamer and chemical structure of the parent drug. This study emphasizes the tunability of drug release from the drugamer platformby polymer architecture design, and the consideration that must be taken when translating the drugamer platform to different drugs.



Graphical Abstract

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2023 ISOMRM & BCRS
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Effect of Magnetic Field Strength on the Controlled Release Behavior of Magnetic Nanogel Drug Delivery Systems

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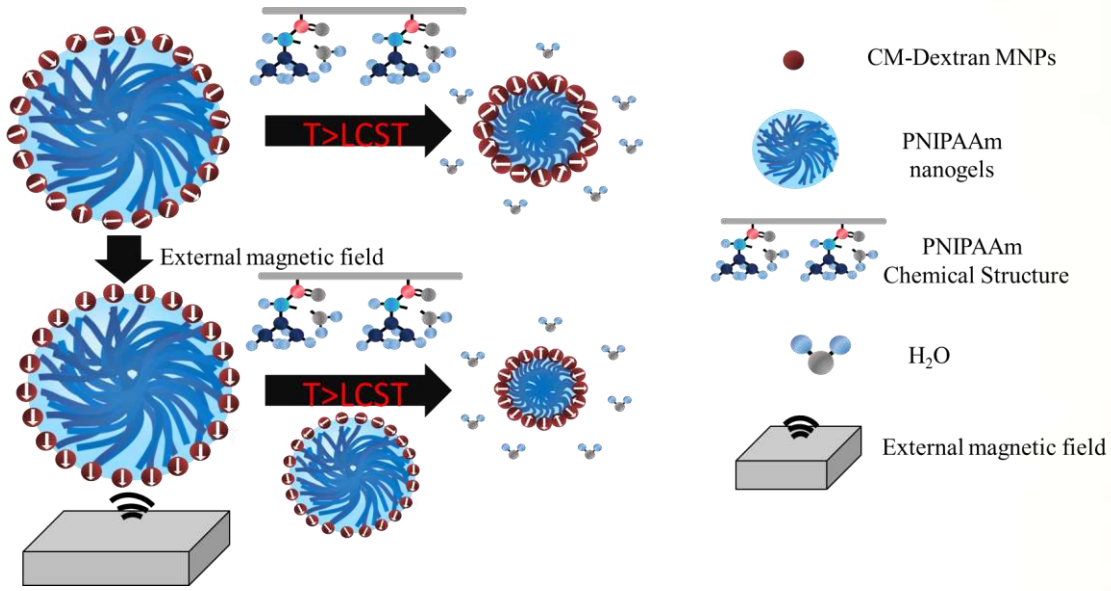
Abstract:

Extensive research has been conducted in the field of biomedical research to explore the development and utilization of drug delivery systems[1]. The chemical synthesis, biocompatibility, and drug delivery properties of temperature and magnetic-responsive magnetic micelles were investigated in this research. Magnetic nanogels were synthesized by combining Poly NIPAAm and magnetic nanoparticles. These nanogels were then utilized as carriers to encapsulate the Hesperetin drug. The physicochemical properties of magnetic nanogel was characterized by Fourier transform infrared spectroscopy (FT-IR), Transmission electron microscope (FEG-TEM), dynamic light scattering (DLS), low critical solution temperature (LCST) superconducting quantum interference device (SQUID) and the thermogravimetric analysis (TGA). The drug release characteristics of magnetic nanogels were investigated by varying the temperature and applying an external magnetic field. In the results, as the concentration of magnetic nanoparticles increases, the lower critical solution temperature (LCST) of the magnetic nanogel also increases[2]. This, in turn, leads to an increase in the drug loading capacity of the nanogel. Furthermore, higher concentrations of magnetic nanoparticles result in an enhanced drug release amount.The application of an external magnetic field allows for precise control over the magnetic moment orientation of magnetic nanoparticles embedded within nanogels. This magnetic responsiveness enables extensive investigations into drug release mechanisms and possibilities. The manipulation of magnetic nanoparticles and magnetic fields has demonstrated the ability to enhance drug release quantities, highlighting the promising potential of magnetic nanogels as localized delivery systems in upcoming times.

KEYWORDS: PolyNIPAAm, magnetic nanoparticle, drug delivery



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Constructing Heart-specific Exosome Profile to Enable Research of Cardiovascular Disease

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Abstract:

Exosomes, a specific type of extracellular vesicles (EVs), play a significant role in both disease progression and diagnosis. They achieve this by facilitating the transfer of exosomal cargoes from one cell to another [1]. An illustrative example involves the contribution of miR-320, which is found in exosomes derived from cardiomyocytes, to the increased risk of atherosclerosis in diabetic patients. The presence of miR-320 inhibits the regeneration of myocardial endothelial cells, rendering the blood vessels susceptible to damage caused by elevated glucose levels in the bloodstream [3].

Identifying exosomes for specific diseases and/or tissues is very challenging. For example, exosomes from cardiomyocyte cell culture media and/or plasma have been utilized to study cardiovascular disease [4]. However, the exosomes from one single cell culture cannot represent the heart tissue complexity. On the other hand, exosomes in plasma originate from various tissues, which lead to convoluted analysis [5, 6]. To address the challenges, explant models, such as placental perfusion, have been employed to generate tissue-specific exosomes. Explants offer the advantage of preserving tissue integrity and functionality, making them suitable for investigating transport mechanisms and related studies [7].

We proposed that the explant method, specifically heart perfusion, could be employed to generate heart-specific exosomes with a more comprehensive profile, thus supporting studies related to cardiovascular disease. For our research, we utilized mice as the animal model. After the collection, the heart perfusate is concentrated through osmosis. And then, exosomes were isolated using ExoQuick-TC. BCA assay showed that there was 50-150 μg protein per milliliter sample; the particle size was around 120 nm (CI: 92 - 146 nm), measured by DLS. In addition, western blot demonstrated CD9 detection in the particles collected from the perfusate, and RT-PCR was employed to characterize the miRNA cargoes. The exosomes will be utilized to treat hear-related cells to investigate the functional difference by comparing to other sources, including cell culture media and plasma.

KEYWORDS: Extracellular vesicles, exosomes, cardiovascular disease, organ explant

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Enabling Rapid Extracellular Vesicle Isolation from Cell Culture Media by Osmosis

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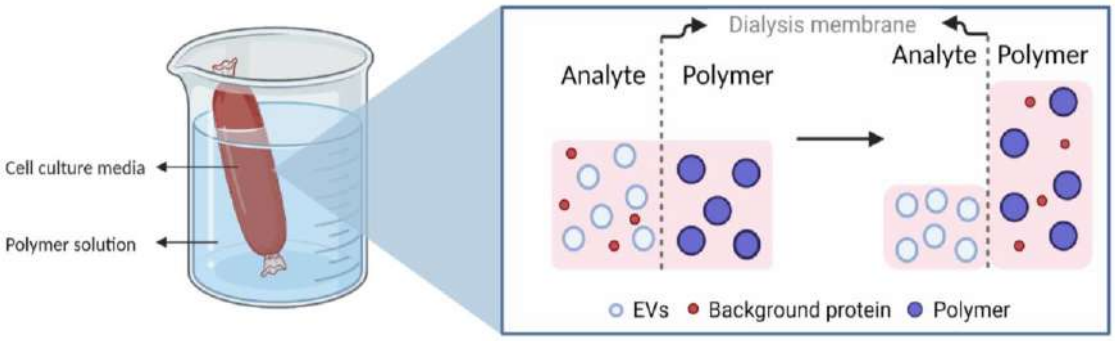
Abstract:

In order to realize the potential of exosomes in biotechnology and clinical fields, there is a need for isolation approaches that can purify exosomes from various solutions, including culture medium and human body fluids (plasma, serum, urine) [1]. For therapeutic and life science research, extracellular vesicles (EVs) are isolated from conditioned cell culture medium due to relatively higher consistency and purity. The commonly used methods to isolate EVs are precipitation, ultracentrifugation, ultrafiltration, and size exclusion chromatography (SEC). However, these have several disadvantages such as time-consuming, protein containmiation, low purity etc. For example, ultracentrifugation, the gold standard for EV isolation, takes 140-600 mins to process and results can vary between operators [2]. Ultracentrifugation may lead to vesicle damage due to the extremely strong centrifuge force and repetitive steps. Therefore, there's a need to develop a novel method to address the challenges.

We have previously demonstrated a simple device to improve biomarker detection limits nearly 100-fold *via* osmosis [3]. Here we hypothesized that osmosis can be utilized as an efficient method for EV isolation from cell culture supernatant, requiring less time and less user steps than ultracentrifugation. The osmosis can also be scaled up for a larger specimen volume. To achieve this, cardiac-derived cells were cultured in EVs-depleted medium for 3 days and the supernatant was harvested for EVs isolation. The osmosis utilized the cellulose ester (CE) membrane with 1000 kDa molecular weight cutoff as a semi-permeable layer to retain EVs while removing excessive soluble proteins and substances.

After a 2-hour osmosis, the sample volume was reduced ~50-fold, and protein concentration increased ~10-fold. The average particle sizes are 150 ± 22, and 131.3 ± 9.2 nm for osmosis and ultracentrifugation respectively. Furthermore, the protein and particle recovery efficiencies for osmosis were 20.45% and 23.72%, while for ultracentrifugation, they were 0.8 % and 0.6 %, respectively. The results clearly indicate that the osmosis resulted in significantly higher EV recovery. Additionally, osmosis is a gentle process, which reduces the possibility of exosome damage compared to ultracentrifugation's strong centrifugal force. Therefore, the process can potentially be utilized for manufacturing EV to facilitate life science research.

KEYWORDS: Exosome, isolation, osmosis



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Assessment of the neuroprotective and neuro-regenerative potentials of extracellular vesicles isolated from platelet concentrates in Parkinson's disease and traumatic brain injury models

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Abstract:

Pre-clinical assessment conducted in our laboratories have established the neuroprotective effect exerted by a purified, heat-treated human platelet lysate (referred to as "HPPL"), engineered for brain administration, in cellular and animal models of Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and traumatic brain injury (TBI). This HPPL is enriched in various trophic factors and contains also extracellular vesicles (EVs). As, EVs play an instrumental role in cell-cell communication and as a cargo of functional biomolecules, and as EVs released from platelet (PEVs) contain their specific mix of neurotrophins, cytokines, and antioxidants, it became much valuable to determine the contribution of PEVs to neuroprotective activity.

In this study we aimed at determining the neuroprotective activity of a PEV preparation, isolated directly from platelet concentrates (PC), using cellular and animal model of PD and TBI.

Apheresis PCs were obtained from the Taipei Blood Center (Guandu, Taiwan). They were centrifuged at 3,000 x g for 30 mins within 6 days after collection to remove the platelets. The platelet-free plasma supernatant was centrifuged at 6,000 x g for 10 mins at 25°C ± 2°C to remove residual cell debris. PEVs were pelletized by high-speed centrifugation at 25,000 x g for 90 mins at 18°C ± 2°C. The surface of the pellet was washed carefully to remove residual plasma and re-

suspended in PBS. PEVs population size and number were characterized using dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and tunable resistive pulse sensing (TRPS). The growth factors content and protein composition of PEVs were assessed by ELISA and liquid chromatography-tandem mass spectrophotometry (LC/MS-MS), respectively. Preclinical *in vitro* assessment of the neuroprotective activity of PEVs in the PD cell model was done using a dopaminergic human Lund human mesencephalic (LUHMES) neuronal cells exposed to erastin neurotoxin, an inducer of ferroptosis cell death. The neurorestorative activity of PEVs in a TBI model was done using a scratch assay of differentiated SH-SY5Y neuroblastoma cells. Moreover, BV-2 microglia cells stimulated with LPS were used to assess the anti-inflammatory activity of PEVs. Negative and positive controls included untreated cells and treatment with our neuroprotective HPPL were used in our *in vitro* studies. Animal tests were performed using a CCI (controlled cortical impact)-TBI model to assess the capacity of PEVs administered intranasally to exert an anti-inflammatory action in mice. The gene expression of selected inflammatory markers were assessed by RT-PCR. The neuroprotective effect of intranasal PEVs was also examined in the MPTP mice model of PD with assessment of expression of tyrosine hydroxylase (TH) positive cells in the substantia nigra by immunohistochemistry (IHC) and of actimetry behavioral test.

We found that the PEVs size ranged from 70 to 350 nm, with a main population at ~200 nm. The PEV concentration of PEVs was in the range of 10^{10}-10^{11}/mL. ELISA and proteomics analysis identified the presence of growth factors, cytokines and antioxidants. A dose of ~1x10^{10} PEVs did not exert any cytotoxic effect and provided a robust significant neuroprotective effect, similar to that of HPPL, in the PD model of LUHMES cells subjected to the erastin neurotoxin. PEVs facilitated the restorative healing of the SH-SY5Y cells in the scratch assay. Additionally, PEVs significantly decreased the expression of inflammatory cytokine Tnf-α when exposed to BV2 cells stimulated with LPS. PEVs delivered intranasally exerted anti-inflammatory function in the TBI mouse model. Gene expression of inflammatory markers Tnf-α, GFAP, Ccl4, Tlr2, and Trem2, which was upregulated by CCI, showed an obvious downtrend upon PEVs treatment. PEVs protected TH expression by dopaminergic neurons of the substantia nigra and improved the rearing motor activity in the MPTP mouse model. These findings support the concept that PEVs exert valuable functional activities for treating neurological brain pathologies and could potentially be used as a biotherapy of CNS neuroinflammatory disorders.

Keywords: platelet extracellular vesicles, neuroprotection, Parkinson's disease, traumatic brain injury, intranasal

Diffusion-tensor imaging and dynamic susceptibility contrast MRI improve radiomics-based machine learning model of MGMT promoter methylation status in glioblastomas

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Abstract:

Glioblastomas (GBM), also known as WHO grade IV gliomas, account for approximately 60% of all adult brain tumors [1]. O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status in patients with glioblastoma is a predictor of response to standard radiotherapy treatment. Magnetic resonance imaging (MRI)-based radiomics studies have used machine learning models to predict MGMT methylation status [2]. There is a need, however, for more studies to utilize radiomic features extracted from diffusion-tensor imaging (DTI) and dynamic susceptibility contrast scans (DSC). This study aimed to build an effective model for predicting MGMT methylation status using radiomics features derived from conventional and advanced MRI scans, including DSC and DTI.

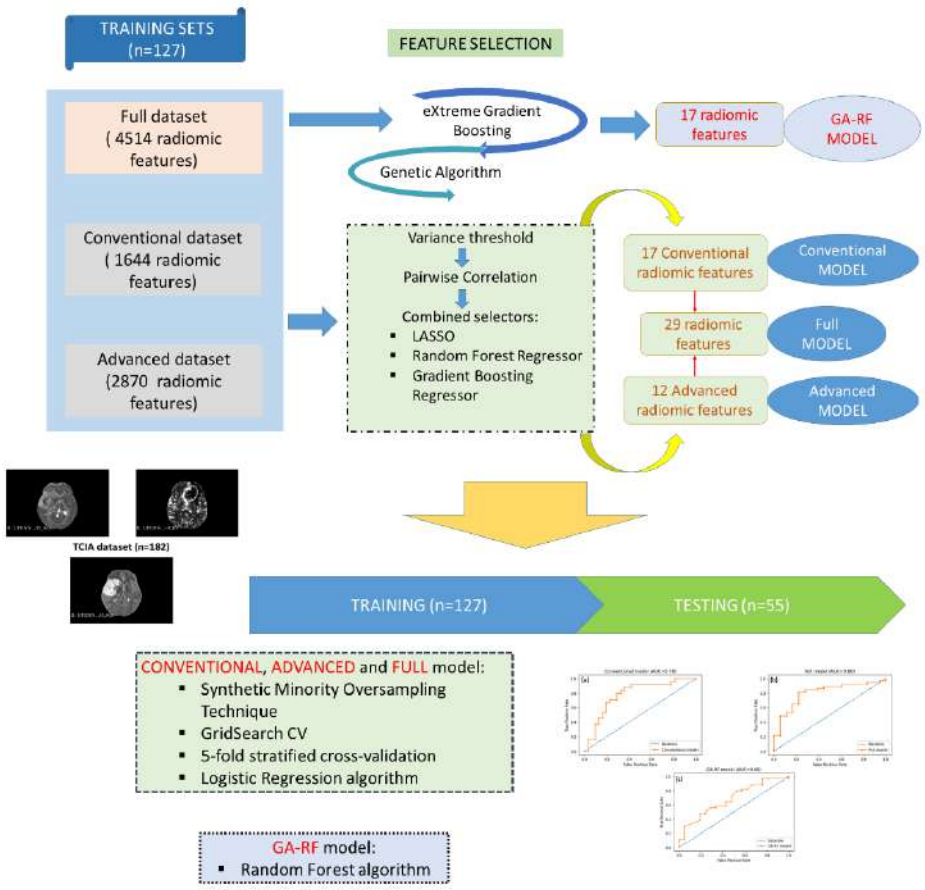
We enrolled 182 GBM patients whose data were obtained from The Cancer Imaging Archive (TCIA). From the initial 4,514 radiomic features, we divided them into two groups of conventional and advanced features, then used a two-step feature selection method to select the top features. The conventional, advanced, and full models were developed from the logistic regression classification algorithm using each group of top features and both of them. We evaluated the performances of these models in three testing sets. Besides, we compared them with the performance of previous state-of-the-art models built by re-running their flowcharts which showed the highest machine learning model's performance in predicting MGMT methylation status [3].

The conventional model achieved a sensitivity of 0.71, a specificity of 0.78, and an accuracy of 0.75. These results of the advanced model were 0.67, 0.74, and 0.71, respectively. Based on the same dataset, the previous state-of-the-art model performed modestly with a sensitivity of 0.65, a specificity of 0.57, and an accuracy of 0.62. Our full model outperformed those models with sensitivity, specificity, and accuracy of 0.76, 0.78, and 0.76, respectively. As for the AUC value, the full model had the highest value at 0.80 compared to the conventional (0.78) and previous (0.68) models.

Our study demonstrated that DTI and DSC imaging might confer a significant advantage in predicting the MGMT methylation status of GBM patients.

KEYWORDS: O6-methylguanine-DNA methyltransferase, glioblastoma, radiomic features, machine learning model, diffusion-tensor imaging, dynamic susceptibility contrast

Graphical abstract:



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Concentrates Urinary Biomarkers Via the Osmosis Processors

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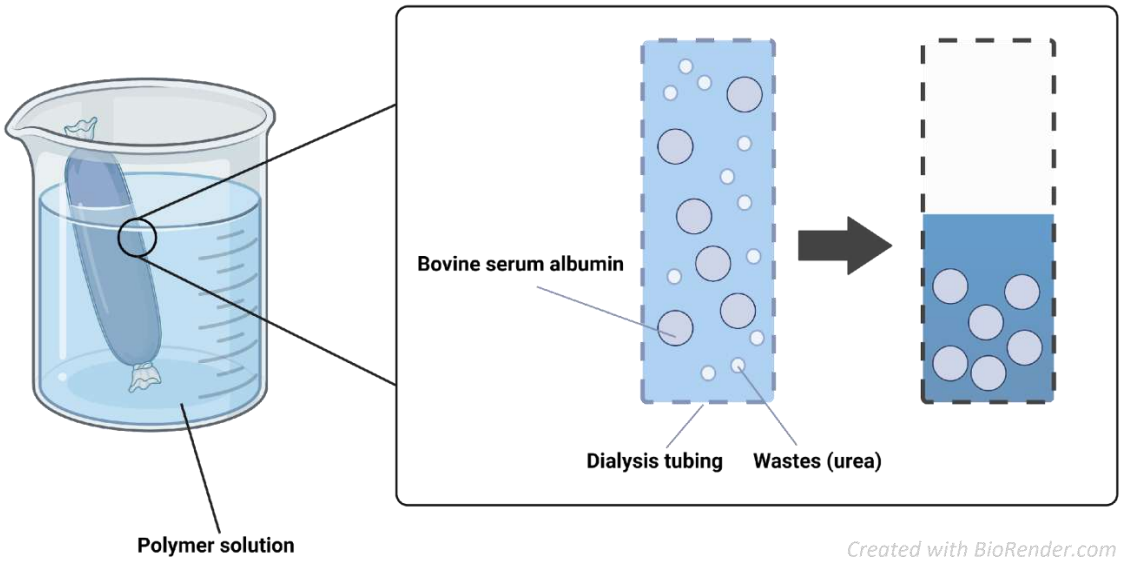
Abstract:

Urine is a type of biospecimen that is excreted from humans through the urinary system and urinary tract as a byproduct of metabolism. It contains metabolic waste products and other substances that can provide information about a person's overall health. Some research have been reported that there are various biomarker in the urine. For example, urothelial carcinoma (UC) is a cancer that begins in the urothelial cells, which is related to the urinary system. A couple of urine-based tests have been approved by Food and Drug Administration (FDA), but these tests suffer from low sensitivity and specificity [1]. Furthermore, urine composition has various substances, such as urea, protein, vitamins, electrolyte salts and ions which can be affected by different physiological variations, health conditions, as well as food consumption [2]. In addition, urine is a very diluted liquid which has low concentration of protein analytes. Therefore, it's necessary to purify and concentrate the specimen before using these biomarkers.

To address these issues, we develop an Osmosis Processor to concentrate urinary protein, purify the urine by removing the small molecules e.g., urea and reconditions the urine environment by spontaneous osmosis reaction. Urea exists in urine with relatively high concentration and is a chaotropic agent, which disrupts the solvating properties of water and potentially interfering with immunoassays. In this study, utilizing our device can easily remove 93% of the solution which original capacity is 15 mL, decrease urea concentration, adjust the suitable pH environment to enable immunoassay (e.g., ELISA) and enrich 10 times protein concentration. Polyethylene glycol was applied to drive osmotic pressure by using different molecular weight and mass concentration [3].



KEYWORDS: biomarker concentration, osmosis, polymers, biospecimen processing, urine

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Immobilization of lysozyme on chitosan modified nanofiber membrane: Antibacterial Assessment

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Abstract:

This study evaluates the antibacterial efficacy of nanofiber membranes, with E. coli DH5-α as the bacterial model. An electrospinning process was used to create a polyacrylonitrile (PAN) nanofiber membrane, which was then used for the modification process based on these previous publications [1][2]. After the alkaline hydrolysis process, chitosan molecules were bonded onto the ion-exchange nanofiber membrane (P-COOH) to create a chitosan-modified nanofiber membrane (P-COOH-CS). Finally, lysozyme was coupled onto the surface of the modified-nanofiber membrane to form the P-COOH-CS-Lys. The optimal conditions for the modification process were evaluated. The effect of the alkaline hydrolysis process was highest when the membrane was incubated for 20 minutes, while the suitable conditions for the attachment of chitosan molecules to the membrane were pH 5, molecular weight 50 kDa, and 1 mg/mL of concentration. The optimal pH and initial lysozyme concentration were 5 and 1 mg/mL, respectively. The antibacterial effects of lysozyme are mostly attributed to the hydrolysis of 1,4-linkages in peptidoglycan between N-acetylmuramic acid and N-acetyl-D-glucosamine residues [3]. Based on the outstanding antibacterial properties of lysozyme and chitosan, it is hypothesized that when these two different biological compounds are combined, the nanofiber membrane would enhance their antibacterial activity [4]. The bacterial inhibitory efficacy gradually increased through the different membrane modification steps, with P-COOH-CS-Lys showing the highest antibacterial efficacy (98.33 ± 1.84%). The reusability of the P-COOH-CS-Lys membrane could maintain very good antibacterial efficacy after 5 repetitions since all the AE values were not significantly different (p-value > 0.05). The cytotoxicity assay of the membrane showed high biocompatibility of P-COOH-CS-Lys (85.11 ± 3.23%), making it a promising material for application in many fields, especially biomedical.

KEYWORDS: Nanofiber membrane; chitosan; lysozyme; Antibacterial efficacy; *E. coli*.

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Temperature-Responsive Polymer-Antibody Conjugate for Biomarker Separation

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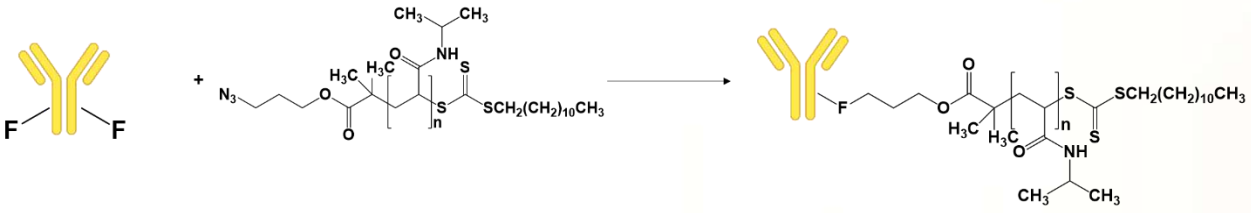
Abstract:

Detecting analytes in the biofluids has been utilized in various biomedical applications. For the existing technology, magnetic beads separation has proven to be convenient tool for selective separation [1]. Magnetic beads with surface immobilized antibodies, the mainstream technology, have been utilized for analyte isolation to enable biomarker detection.

Magnetic beads offer significant advantages in bioseparation due to their large particle diameters, typically around 10 micrometers. However, the use of micrometer-sized particles introduces poor diffusion characteristics. To overcome this limitation, our approach involved the utilization of nanoscale polymer materials. By employing these nanoscale polymers, we aimed to enhance the diffusion properties and improve the overall performance of the bioseparation process.

Here, we developed to conjugate temperature-responsive polymer with antibody, and the difference between magnetic beads and temperature-responsive polymer is particle dimension. To address the challenge, we developed temperature-responsive polymer-antibody conjugates.

To conjugate polymer with antibody, we use poly(n-isopropylacrylamide) which is temperature responsive polymer and is azide terminated to do click chemistry with linker that is DBCO-TFP ester to binding with antibody. The polymer-antibody conjugation utilized click chemistry. Specifically, the antibody was modified with DBCO-dPEG$_4$-TFP ester, and then grafted with azido-poly(N-isopropylacrylamide).



However, the dimension of polymer particle is around nanometer, so we believed that our polymer in solution would be higher efficient to diffusion. The conjugates' hydrodynamic radius are 100-time smaller than the magnetic beads. Therefore, the conjugates can rapidly diffusion to drive real-time analyte recognition.

KEYWORDS: temperature-responsive polymer, biomarker separation, click chemistry

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

H_2S-responsive copper selenide $Cu_{2-x}Se$@BSA nanoparticles for photothermal and chemodynamic combination therapy in colon cancer

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Abstract:
Colorectal cancer has become the most occurring neoplasm in Taiwan. More than 10,000 people were diagnosed with the cancer and approximately 5000 people die of colorectal cancers or related causes in Taiwan annually. Surgery is the most frequently adopted approach for the cancer treatment meanwhile chemotherapy and/or radiotherapy is often used as adjuvant therapy. Nevertheless, it has been found that colon cancer cells are usually resistant to chemotherapy with several mechanisms such as high endogenous H_2S concentration. Currently available cancer treatments present a combination of surgical resection of a tumor along with radiation therapy and/or chemotherapy, which still exhibit unsatisfactory clinical benefits and severe side effects. Thus, novel and effective therapeutic strategies targeting tumor and TME-responsive treatments are needed for better outcomes.

The intracellular level of endogenous hydrogen sulfide (H_2S) was found to be closely related to drug resistance, cell migration and metastasis, and evasion of apoptosis (for example, by autophagy)[1]. Recently, emerging nanotherapeutics have been developed for tumor microenvironment (TME)-responsive chemodynamic therapy (CDT). However, CDT alone fails to suppress colon cancer, which has a strongly reducing microenvironment owing to high expression of H_2S. To overcome this limitation, a new strategy based on $Cu_{2-x}Se$ nanoparticles is proposed to remodulate colon TME by consuming endogenous H_2S and to synergistically elevate the efficacy of CDT and photothermal therapy (PTT).

In this project, we report hexagonal copper selenide ($Cu_{2-x}Se$) nanoparticles, which can effectively eliminate excess H_2S and simultaneously increase the photothermal and chemodynamic properties. Multifunctional $Cu_{2-x}Se$ nanoparticles with excellent catalytic property was prepared using a one-step hydrothermal process (Figure 1A). The Cu^{2+} in $Cu_{2-x}Se$@BSA can react with H_2S to deplete endogenous H_2S and simultaneously achieve enhanced photothermal effect in near-infrared II (NIR-II) region. Cu^{2+} can also deplete the overexpressed GSH via redox reactions to generate Cu^+ ($Cu^{2+} + 2GSH \rightarrow Cu^+ + GSSG + 2H^+$), which mediates Fenton-like reactions to elicit CDT (Figure 1B). We anticipate that copper-based nanoparticles containing both Cu^{2+} and Cu^+ are capable of manipulating the TME and be of great importance in synergistic tumor therapy[2]. Owing to the remarkable H_2S scavenging ability, $Cu_{2-x}Se$@BSA nanoparticles developed herein hold great potential for effective treatment of colorectal cancer.
Collectively, $Cu_{2-x}Se$ nanoparticles exhibit desirable antitumor activity *in vitro* via the synergy effect of PTT, CDT, and H_2S depletion. *In vitro* and *in vivo* efficacy of the developed

nanotherapeutic agent has also been evaluated on a subcutaneous CT26 tumor-bearing mouse model for H_2S-responsive combination therapy against colorectal cancer. In our study the developed, BSA@$Cu_{2-x}Se$ NPs served as a multifunctional therapeutic nanomedicine to scavenge endogenous H_2S from the cancer cells by simultaneously increasing CDT and PTT.

Keywords: Copper selenide, cancer therapy, chemodynamic therapy, photothermal therapy, colorectal cancer

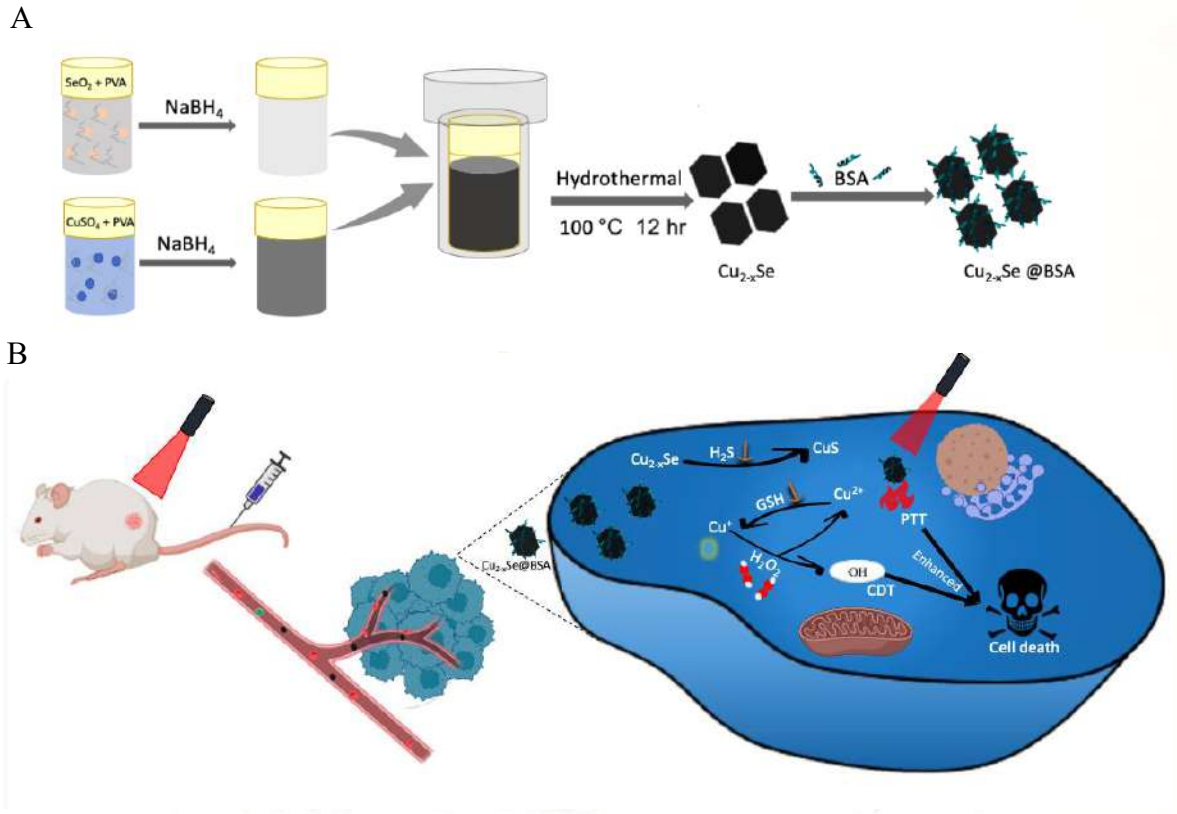

Figure: Schematic illustration of (A) Preparation procedure of $Cu_{2-x}Se$@BSA, (B) therapeutic mechanism of $Cu_{2-x}Se$@BSA nanoparticles.

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Novel of Hydroxyapatite Nanoparticle-Loaded Hydrogel Scaffold for Bone Regeneration

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Abstract:

1.Introduction:

Bone defects caused by trauma, disease, or aging can significantly affect the quality of life of affected individuals. The current treatment options for bone defects, such as bone grafting, have several limitations, including the risks of immune rejection or infection due to the use of donor tissue. Bone tissue engineering has emerged as a promising approach to overcome these limitations and promote bone regeneration. Among the various types of scaffolds used for bone tissue engineering, hydrogel scaffolds have received considerable attention due to their high biocompatibility and ability to mimic the extracellular matrix of bone tissue. In this study, we aimed to develop a novel drug-loaded hydrogel scaffold for bone regeneration, which combines the benefits of hydrogel scaffolds and hydroxyapatite nanoparticles to promote bone tissue regeneration. Additionally, the hydrogel scaffold was modified by natural ingredients to enhance its mechanical strength and biocompatibility. Finally, a new type of drug-loaded hydrogel scaffold was fabricated by photo crosslinking.

2.Materials and Methods:

To create the nanoparticle-hydrogel system, we used polyethylene glycol (PEG, Mw:400, SIGMA) and polyethylene glycol diacrylate (PEGDA, Mw:700, SIGMA) as the hydrogel matrix. Hydroxyapatite (<200nm, SIGMA) nanoparticles is modified by 1% saponin with the purpose of changing surface character to carry drug. The irgacure 2959 (SIGMA) was induced photo crosslinking to UV irradiation at 405 nm. The physical and mechanical properties of the powder were observed to evaluate the effect of the powder on photocuring behavior, including rheology measurements, FTIR and hardness test. Rheology measurements were performed to evaluate the viscosity of the material and determine the optimal concentration of hydroxyapatite nanoparticles for stereolithography. Fourier transform infrared (FTIR) spectroscopy was used to analyze the chemical composition of the scaffold and confirm successful modification of hydroxyapatite nanoparticles for drug loading. Tablets hardness tester is used to measure hydrogel scaffold's physical character, for hardness and mechanical strength. This study combined the drug with the nanoparticles, and the rug release were evaluated the sustained release from the scaffold. Evaluation of cell viability and

cytotoxicity is conducted by CCK-8 and LDH assay, with using bone cell to observe cell for 24 and 48 hours respectively.

3.Result & Conclusion:

In this study, we demonstrated that as the concentration of hydroxyapatite nanoparticles increased, the solution became more suitable for stereolithography, when the hydroxyapatite concentration is higher than 5%. The hydrogel-nanoparticle system has significant potential as a drug delivery platform for tissue engineering applications. The use of hydroxyapatite nanoparticles as a carrier for drug provides several advantages, including improved stability and bioavailability. The hydrogel matrix offers a biocompatible environment for tissue regeneration, and the controlled release of drug ensures sustained therapeutic effects. FTIR test demonstrated that the hydroxyapatite nanoparticles had successfully carried the drug. Our findings from the hardness test indicate that a reduction in the percentage of PEGDA results in a corresponding decrease in hardness. Additionally, the incorporation of 5% hydroxyapatite produces the highest stress level. The survival rate of the hydrogel scaffold to the bone cell was displayed a safety performance at 24 and 48 h, respectively.

As mentioned above, the use of LCD techniques provides a simple and efficient method for creating the hydrogel-nanoparticle system. In conclusion, the hydrogel-nanoparticle system developed in this study has significant potential as a drug delivery platform for tissue engineering applications, particularly in bone regeneration. The results of this study provide a promising foundation for further research into the development of effective and efficient drug delivery systems for tissue engineering applications.

KEYWORDS: Hydrogel scaffold, Liquid crystal technique, Drug release, Bone regeneration

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Anti-aging biomaterial sturgeon chondroitin sulfate chelates biological functions to reprogram stem cell senescence and ameliorate aging to prolong longevity

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Abstract:

Aging is a process of tissue and cell potential dysfunction characterized by hallmarks, including stem cell senescence and alteration in their extracellular matrix microenvironment; a major risk factor for various human chronic conditions, including obesity disorders, osteoarthritis, cardiovascular disorders, and neurodegenerative disorders (e.g., Alzheimer's and Parkinson's diseases) [1]. It is also related to many physiological processes, including regenerative development and wound healing. According to the WHO report, by 2050, the world population over 60 years is expected to increase by 22% and accelerate several age-related diseases [1, 2]. It is clear that urgent need to identify or develop new anti-aging drugs to increase the healthy lifespan. However, biomaterials that reprogram the senescent cells and act as a geroprotector is still need to be investigated.

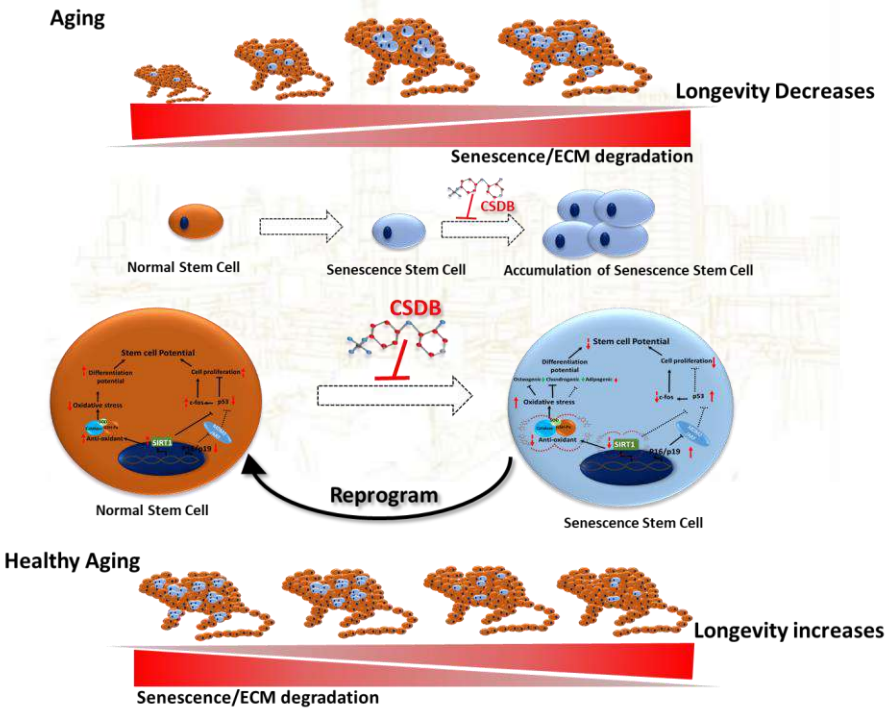
The ECM contains chondroitin sulfate (CS), an essential biomaterial that widely spread on the cell surface in the form of proteoglycan and helps to regulate the homeostasis of cells and tissue [3]. As we know, ECM of normal cell and tissues, contain chondroitin sulfate (CS) that chelates the biological function to maintains the tissue homeostasis. Although CS is widely used as scaffold, hydrogel or drug carrier for various pathological disease, CS have not yet been used as chelating biomaterial for oral drug/pill to ameliorate the certain features of senescence and aging.

In this study, CS was derived from sturgeon cartilage as a biomaterial by enzymatic treatment, and structure was characterized by HPLC, FTIR, UV-Visible absorbance and ^{1}H-

NMR as well as diffusion-ordered spectroscopy (DOSY-NMR) used to investigate molecular weight of CS. The results revealed that chondroitin-sulfate-derived biomaterial (CSDB) from sturgeon was a low molecular weight and consisted of 59 % 4-sulfated CS (CS-4), 23% 6-sulfated CS (CS-6). Here, CSDB was used to investigate their effect on senescence in stem cells from non-genetically modified senescence-accelerated mouse prone-8 (SAMP8) rodent mice, and found that CSDB with the capacity to chelates the biological effect, ameliorates cell proliferation, decreases adipogenesis and oxidative stress. Oral CSDB treatment in SAMP8 mice restores the aging-like phenotype such as bone mineral density, skin morphology to retard aging in terms of recovering stem cells potential. Additionally, CSDB increases the Sirt1 gene expression to inhibit oxidative stress and thus attenuation of expression of the senescence marker genes p16 and p19. Taken together, CSDB has the potential to retards aging by chelating the biological effect as demonstrated in SAMP8 mice, implying that it can be pharmacologically targeted to retards aging-like phenotypes.

Keywords: Chondroitin sulfate; Anti-aging; Anti-oxidant; Stem cell senescence.

Graphical Abstract:



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

PEO-PLA/PEO core-shell structured fibers fabricated by coaxial electrospinning for controlled drug release

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Abstract:

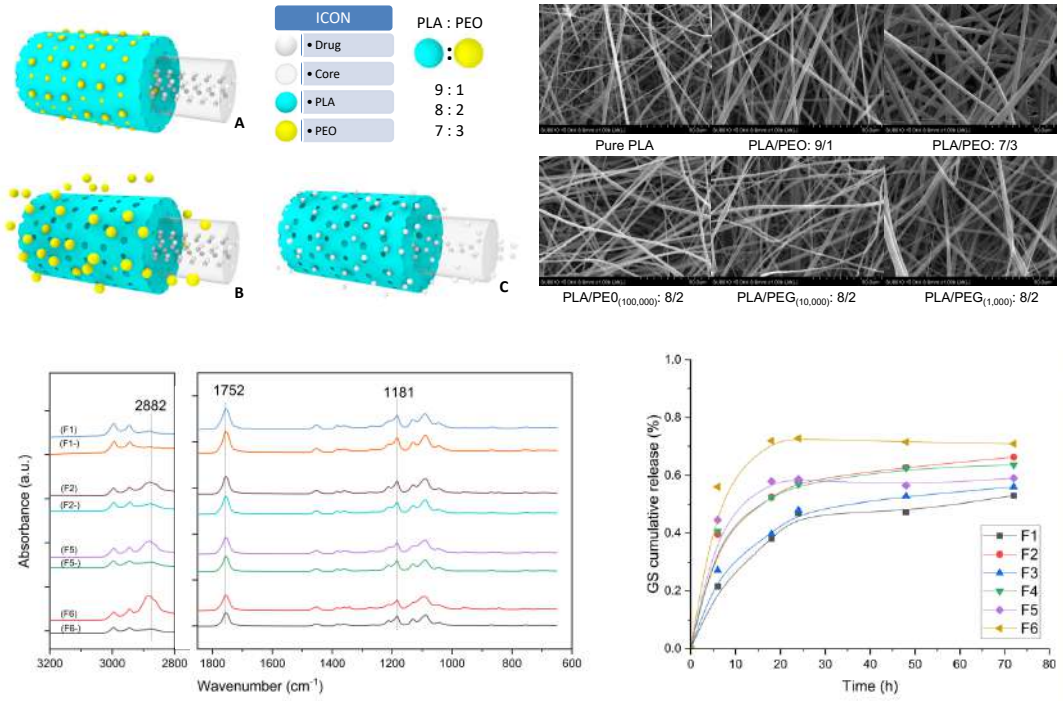
Electrospun wound dressing materials produced using electrostatic spinning have wide applications in the field of wound healing. The precise control of drug delivery rates in wound dressing materials is a prominent research topic. In this study, we successfully developed a coaxial electrospun fiber membrane using polylactic acid (PLA) and polyethylene glycol (PEG) as shell layer materials, and PEG and Gentamicin as core layer model drugs. The electrospun fiber membrane enables accurate control of drug release rates. By adjusting the mass ratio between the shell layer PLA and polyethylene oxide (PEO), as well as the molecular weight of the shell layer PEG, we generated varying quantities and sizes of PEG dissolved molecular-level pores in the shell layer during drug release, thus controlling the rate at which the core layer drugs pass through the shell layer.

Characterization studies of the fiber membrane demonstrated distinct core-shell structures and stable fiber diameters. Fourier-transform infrared spectroscopy (FTIR) analysis indicated that as the mass ratio of PEO in the shell layer increased relative to PLA, the peak intensity of PLA gradually decreased while that of PEO increased slowly. Additionally, increasing the molecular weight of PEG in the shell layer resulted in increased PEG peak intensity. Water solubility tests demonstrated the relatively rapid release of PEG from the fiber membrane, and post-solubility FTIR analysis showed varying degrees of reduction in PEG peak intensities. Gentamicin drug release studies indicated successful control of drug release within the time range of 12 hours to 5 days. In the PLA/PEG shell layer, increasing the mass ratio of PEO accelerated the release rate of Gentamicin, while reducing the molecular weight of PEG decreased the release rate of Gentamicin.

By adjusting the content and molecular weight of PEG in the membrane, different drug release rates can be achieved. This achievement holds promise for future applications in developing wound dressing materials that meet the specific drug release rate requirements during different stages of wound healing. It is worth mentioning that due to the incomplete precipitation of PEG from the fibers during the early stage of drug release, our film can effectively inhibit drug burst release.

KEYWORDS: drug controlled release, coaxial electrospinning, core-shell structured fibers,

sacrificial components.



Graphic abstract (not a mandatory requirement)

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Oxidation-mediated scaffold engineering of hyaluronic acid-based microcarriers enhances corneal stromal regeneration

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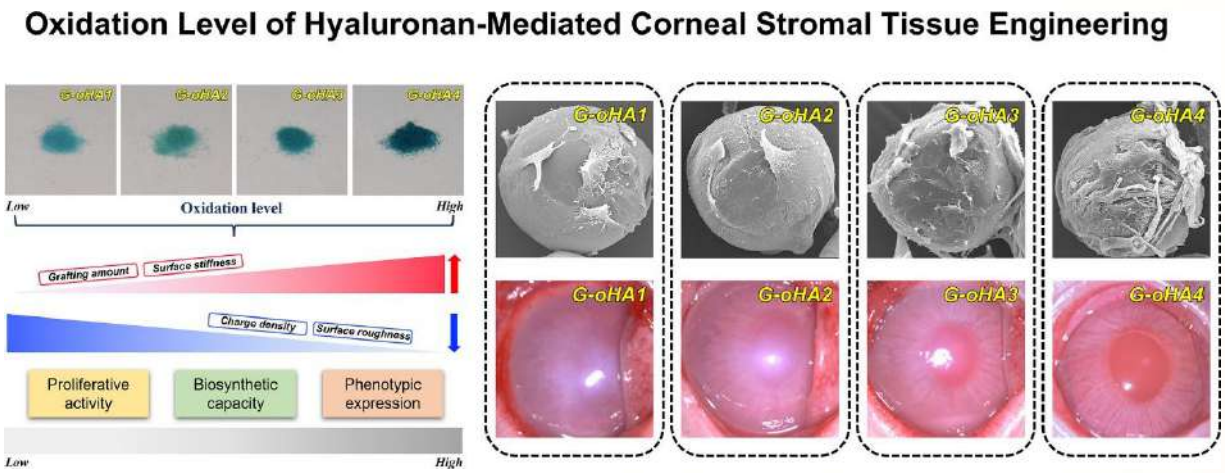
Abstract:

The functional design of scaffolding biomaterials with potent capabilities of promoting cell adhesion and proliferation is critically important for tissue repair and regeneration. Here, we exploit the effects of oxidation level of aldehyde hyaluronic acid (oHA) on gelatin microcarriers for repairing corneal injuries. Specifically, high oxidation levels can endow the microcarrier surface with large oHA grafting amount, smooth topography, and strong stiffness, consequently formulating biocompatible scaffolding materials with superior affinities for keratocyte attachment and growth. In a rabbit model of corneal alkali burn injury, single intracorneal injection of keratocytes/functionalized microcarriers with an appropriate oxidation level could effectively reduce corneal swelling (~62-fold improvement), recover ~94% collagen production and ~89% keratocan expression, and repair disordered collagenous stromal architecture after 4 weeks. These findings on the oxidation level effects of the aldehyde polysaccharide show a great potential use in the development of advanced scaffolds for efficient tissue engineering.

KEYWORDS: Corneal stromal regeneration, Hyaluronic acid, Injectable keratocyte scaffold, Microcarrier-based tissue engineering, Oxidation level effects



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Highly retina-permeating and long-acting resveratrol/metformin nanotherapeutics for enhanced treatment of macular degeneration

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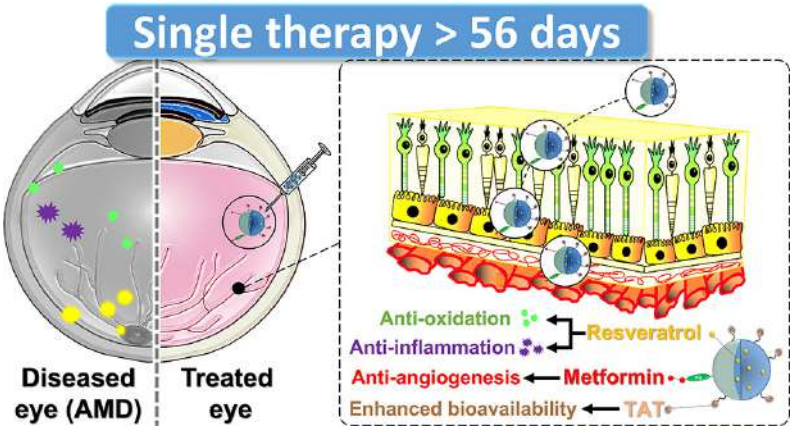
Abstract:

The development of therapeutics for effective treatments of retinal diseases is significantly constrained by various biological barriers. We herein report a nanomedicine strategy to develop nanotherapeutics featured with not only high retinal permeability but also sustained bioactive delivery. Specifically, the nanotherapeutics are rationally designed via aminolysis of resveratrol-encapsulated polycaprolactone nanoparticles (R@PCL NPs), followed by the formation of amide linkages with carboxyl-terminated transacting activator of transcription cell penetrating peptide (T) and metformin (M). The R@PCL-T/M NP nanotherapeutics are demonstrated in vitro to possess persistent drug release profiles, good ocular biocompatibility, and potent bioactive activities for targeting prevailing risk factors associated with retinal diseases. In vivo studies indicate that single-dose intravitreal administration of the R@PCL-T/M NPs can effectively improve retinal permeability (~15-fold increase), prevent loss of endogenous antioxidants, and suppress the growth of abnormal vessels in the retina with macular degeneration for 56 days. This high treatment efficacy can be ascribed to the enhanced retinal permeability of the nanotherapeutics in conjunction with the sustained pharmacological activity of the dual drugs (R and M) in the retinal pigment epithelial region. These findings show a great promise for the development of pharmacological nanoformulations capable of targeting the retina and thereby treating complex posterior segment diseases with improved efficacies.

KEYWORDS: Ocular nanomedicine, therapeutic delivery system, enhanced retinal permeation, sustained drug release, macular degeneration



2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Photocrosslinkable carboxymethyl cellulose/collagen/plate-rich plasma hydrogel for wound dressing

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MOST 111-2221-E-002-021

Abstract:

The process of wound healing is lengthy and intricate. To ensure successful healing, it is crucial to keep the wound moist, protect it from external factors, and prevent infection. Hydrogels are considered ideal wound dressings due to their exceptional ability to absorb water and maintain structural stability, as well as their three-dimensional structure that mimics the extracellular matrix. Additionally, hydrogels promote excellent cell adhesion and exhibit outstanding biocompatibility.

In this research, we utilized methacrylic anhydride to modify carboxymethyl cellulose (CMCMA)[1], which has alkenyl bonds suitable for polymerization, resulting in the formation of a hydrogel. To improve cell adhesion in the CMC hydrogel, we added type I collagen modified with maleic anhydride (ColME)[2], which underwent copolymerization with the CMCMA. The hydrogel was initiated to gel by UV light with the presence of a photoinitiator and had excellent skin adhesion. Additionally, platelet-rich plasma (PRP)[3] was added to the above-mentioned hydrogel system. We hope that the growth factor, releasing from the platelet, can accelerate cell growth and enhance the wound healing rate. Levofloxacin was incorporated into the CMCMA/ColME/PRP hydrogel solution, and after 6 hours, it was released, exhibiting an antibacterial effect on both Gram-positive and Gram-negative bacteria based on a bacterial colony assay. Furthermore, the viability of L929 fibroblasts confirmed the biocompatibility of the drug-loaded hydrogel. Our findings suggest that CMC-based hydrogel holds significant potential as a wound dressing material.

KEYWORDS: Wound dressing, Photo-crosslinking hydrogel, carboxymethyl cellulose (CMC), type I collagen, platelet-rich plasma (PRP)

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2023 ISOMRM & BCRS
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The Development of Functionalized Oxidized Bacterial Cellulose-Based Hemostats

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Abstract:

Hemostat or hemostasis is a process in which blood flow is stopped. It can usually occur automatically as a natural reaction that an organism's body has to defend itself. Besides the natural process, many other methods and materials can be used to stop the bleeding. In some severe trauma injuries, medical conditions, surgical procedures where bleeding is difficult to stop, or on the battlefield where conventional hemostasis may not be sufficient, hemostatic materials are therefore important in promoting hemostasis of blood [1-3].

There are a large number and variety of commercial hemostasis products on the market such as thrombin, fibrin, gelatins, collagen, mineral powder, polysaccharides, and oxidized cellulose. Each type of hemostatic material has a different reaction mechanism depending on the raw material used, which also affects performance, functionality, and price [4, 5], but an ideal hemostatic agent must have several key parameters: high hemostasis efficiency, safety, and availability class. For this reason, bacterial cellulose (BC) is an attractive alternative due to its outstanding as an eco-friendly, non-cytotoxic, non-genotoxic, highly biocompatible material, and biodegradable, attracting increasing interest in development for use as hemostasis material. This study was intended to develop a hemostatic polymer from bacterial cellulose by using TEMPO solution to modify chemical structures. Derived oxidized bacterial cellulose was further treated with Calcium and Zinc ions to enhance their blood clotting properties. There are 3 steps of preparation used in this research; the first step is Urea/NaOH treated for decreased crystallinity of bacterial cellulose. The second step is TEMPO oxidization to change the functional group from -OH to -COOH, and the last step is metal ions treated to enhance the efficacy of hemostasis function. From the XRD test, results revealed that Urea/NaOH treatment decreased the crystallinity of BC film and increased oxidation efficiency by 40% the degree of oxidation BC film. After supplementing with calcium acetate hydrate 0.5 mmole/g sample, 76% calcium ions were detected, and 65% zinc ions were detected in the sample supplement with 1.0 mmole/g sample of zinc acetate dihydrate. Based on these results, it can estimate that oxidized BC can be developed for use in the field of hemostatic polymers efficiently.

KEYWORDS: Bacterial cellulose (BC), oxidization, hemostat, oxidized bacterial cellulose

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Methacrylate silatrane: Newly synthesized building block for advancement of surface silanization and functional polymers

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Organosilicons are popular for surface functionalization due to their simple preparation, availability, and efficient modification across different interfaces [1]. Particularly, 3-(Trimethoxysilyl) propyl methacrylate (TMSPMA) is widely used for the construction of hybrid polymers via radical polymerization for a variety of applications [2]. However, the structural stability and chemical processability of conventional silanes are burdened by their susceptibility to hydrolysis and aggregation, leading to the degradation of their functionalities and implementations [3]. In this study, chemically stable methylacrylate silatrane (MAST) was invented by combining a tricyclic caged structure and a transannular N → Si dative bond for excellent processability, hydrolysis resistance and surface functionalization. The thin, smooth, and highly ordered coating of MAST on silicon wafer was confirmed by using ellipsometry, atomic force microscopy, X-ray photoelectron microscopy and Fourier-transform infrared spectroscopy. In consequence of its unique chemical structure, the accomplished molecular homogeneity and orientation of the MAST layers are attributable to strong intermolecular hydrogen bonds between urea groups, and controlled silanization on oxide surfaces. In addition, zwitterionic monomer of 2-methacryloyloxyethyl phosphorylcholine (MPC) was undergone co-polymerization reactions with MAST or TMSPMA to afford macromolecular modifiers of p(MPC_9-*co*-$MAST_1$) and p(MPC_9-*co*-$TMSPMA_1$), respectively, for functionalized interfaces and antifouling features on silicon substrates. After co-polymerization, p(MPC_9-*co*-$MAST_1$) showed the preservation of silatrane reactivity, whereas the silane groups of p(MPC_9-*co*-$TMSPMA_1$) were hydrolyzed during the process, resulting in aggregation of polymer chains. Hence, p(MPC_9-*co*-$MAST_1$) film on surfaces exhibited excellent wettability, grafting density, and fouling resistance compared to p(MPC_9-co-$TMSPMA_1$). Accordingly, we visualize the huge potential of MAST as a building block for the development of functional hybrid polymers, well-defined polymeric thin films, and nanomaterials.

KEYWORDS: Functional silatrane, controlled silanization, antifouling materials, zwitterionic polymers, surface chemistry.

(Option)

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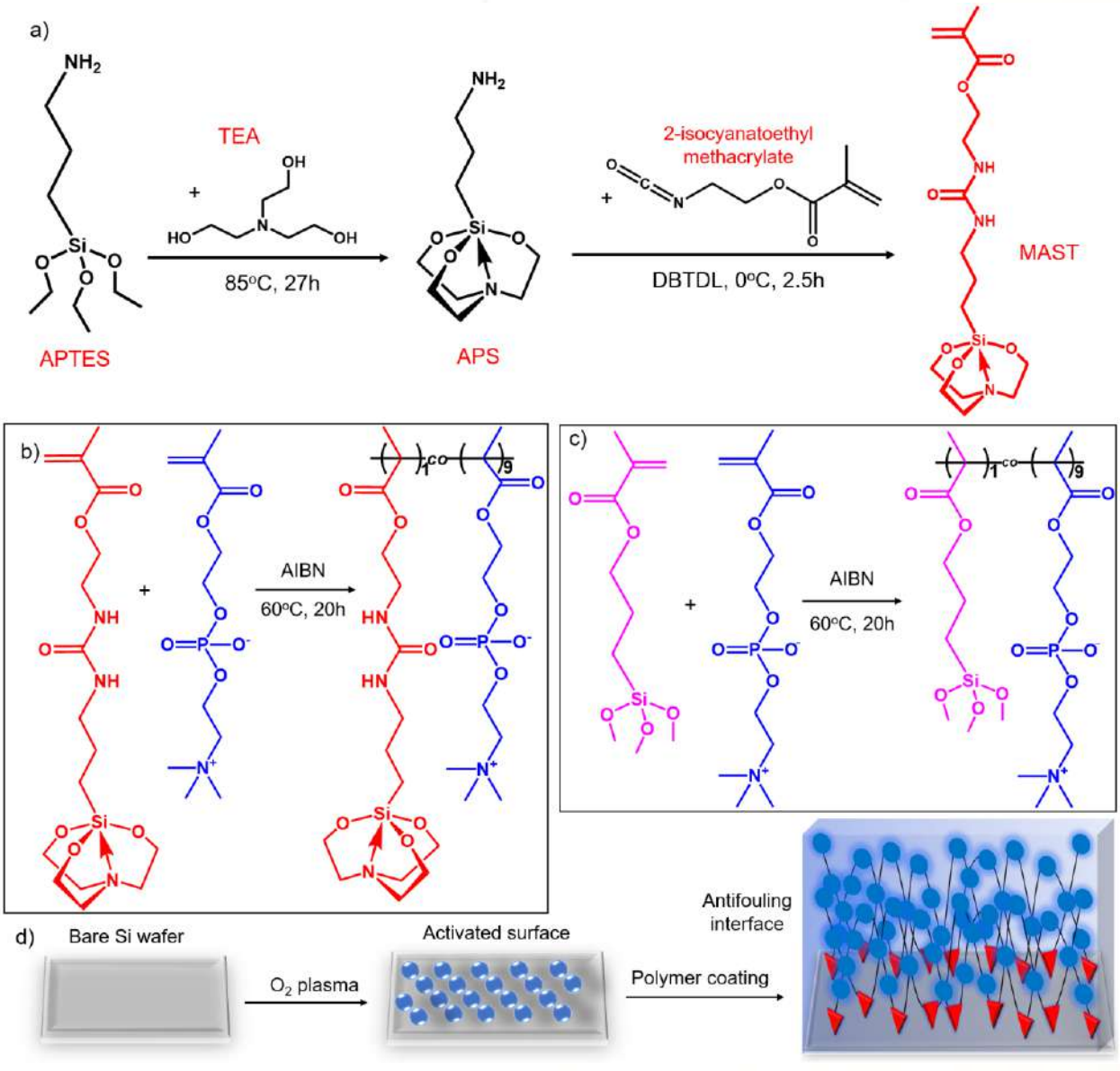

Modification of plant-based starch powders for surgical anti-adhesion applications

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In recent years, there has been much research and development of anti-adhesion materials of powder particles, which can be easily sprayed near the wound to achieve the purpose of anti-adhesion during surgery.

In this study, the plant-based starch was firstly screened, and the suitability of different starches as anti-adhesion materials was discussed and compared. Moreover, different salts were used to make anti-adhesion starch in the emulsification method of particle modification, and the material properties were compared, including the size and morphology of the powder particle, water absorption efficiency, and viscosity. We compared a variety of edible starches on the market, acetylated distarch phosphate has the highest water absorption efficiency of about 598%. In this study, we grafted surfactant on the surface of starch particles by the emulsification method to increase the hydrophilicity of the material and added different salts in the emulsification method.

The results showed that the water absorption efficiency of the acetylated distarch phosphate modified by sodium chloride (NaCl) could be further improved to 1328.3%, the starch modified with potassium chloride (KCl) can be improved to 1131.6%, and the starch modified with calcium chloride ($CaCl_2$) can be improved to 1096.9%. Experimental results indicate that the higher water absorption leads to better anti-adhesion ability.

According to the results, it is later discussed in the study about the possible mechanisms of high-water absorption of the acetylated distarch phosphate and the

effects in the emulsification process and different salts on water absorption. It is seen in the report that the acetylated distarch phosphate was gelatinized from the potato starch, which was heated in acetic acid, therefore changed its molecule structure by attaching more hydrogen bonds onto the starch. These hydrogen bonds then attach more water molecules and improves its water absorption ability. In the emulsification process, it is suspected that because the starch became smaller when stirring and salts were attached to the starch in the emulsification process, therefore its total starch surface increases and further increases the water absorption.

In the future, we would like to further study more variations in the emulsification process on the starch water absorption, particle size, and so on. To become a certified medical advice, we need to investigate the biocompatibility and the efficacy on the modified starch powder.

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Liquid foam as carrier of immune cells and anti-cancer agents for intraperitoneal immunotherapy

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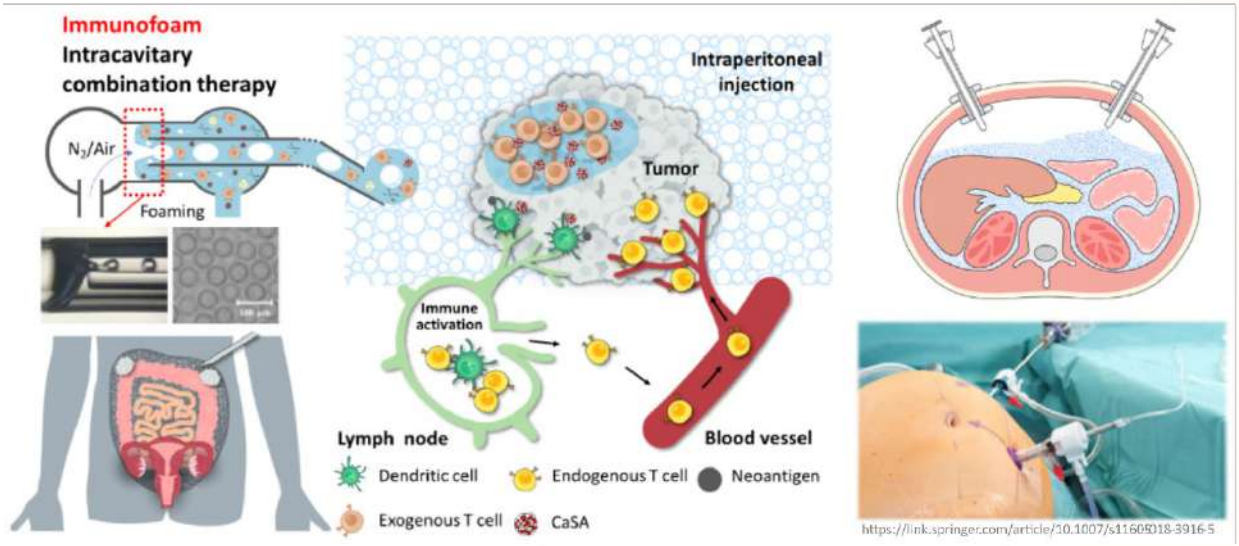
Abstract:

Peritoneal metastases (PM) refer to cancer that has spread to the peritoneum from other organs, including ovarian, gastric, colorectal, appendicular, or pancreatic cancers. As cancer spreads from other organs, PM is often considered an advanced disease associated with poor prognosis. A current best practice is limited to palliation or chemotherapy with median survival last than one year [1]. The combination of cytoreductive surgery and Hyperthermic IntraPEritoneal Chemotherapy (HIPEC) has remained the gold standard for the treatment of PM. Long-term survival has been reported for different disease entities when combining cytoreductive surgery and HIPEC [2, 3]. However, a complete macroscopic cytoreduction is decisive for the prognosis[4], which requires a complex operation with high morbidity and mortality. The multimodal procedure is, therefore, only suitable for a few highly selected patients.

An ideal intervention should exhibit therapeutic efficacy and maintain or improve patients' quality of life. Here, we developed a liquid foam-based drug delivery system named Immunofoam. Immunofoam is injectable liquid foam capable of carrying combination therapies, including chemo drugs, antibodies, and immune cells. Foam offers several feasible properties for intraperitoneal treatment compared to its liquid version, including higher viscosity to prolong drug-tissue contact time, more homogenous drug distribution, and better drug penetration into the interstitium. Foam exhibits higher viscosity than liquid with the same composition. Therefore, the feature of Immunofoam makes it an ideal drug delivery system for intracavitary combination therapy. This study successfully demonstrated an intraperitoneal administration of immune cells through the foaming device, and the results exhibited the biocompatibility of Immunofoam and cell survivability during the foaming method. In addition, the combination of STING agonist and dendritic cells greatly improved cytotoxic T-cell activity, which indicates that Immunofoam innovation is promising for intraperitoneal immunotherapy for carcinomatosis.

KEYWORDS: foam, peritoneal metastases, intraperitoneal therapy, immunotherapy, STING agonist



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Human pluripotent stem cell culture on dendrimer surface grafted with ECM-derived peptides

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Abstract:

Human pluripotent stem cells (hPSCs) can be divided into human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), which have the ability to differentiate into the cells derived from three germ layers, such as endoderm, ectoderm and mesoderm. To maintain the pluripotency of hPSCs, we explored the cell culture biomaterials. We developed peptide-grafted PVA-IA (poly (vinyl alcohol-co-vinyl acetate-co-itaconic acid)) hydrogels with optimal elasticity. We found the hydrogels grafted with specific laminin-β4 (LMN) and vitronectin-derived oligopeptides were the most preferable for hPSC proliferation. In a previous study, we could culture hPSCs successfully on the hydrogels grafted with specific laminin-β4 (LMN) and vitronectin-derived oligopeptides. However, the concentration of oligopeptide solution needed for grafting was much higher (typically 1000 μg/mL) than the concentration of the ECM-coated surface solution (typically 5-10 μg/mL). To improve this point, we created new design with a new approach (**Figure 1**) involving a dendrimer-based peptide-grafted surface. PAMAM dendrimer having generation 3, which was composed of many branched subunits of amide and amine groups, was used to be immobilized on the hydrogel surface, which is expected to have high biocompatibility for hPSC culture and differentiation.

We first reacted the terminal surface groups of the dendrimers with PEG4-SPDP crosslinker. Then, we grafted functional oligopeptides on a dendrimer-grafted surface via PEG4-SPDP crosslinker using the thiol group provided by cysteine in the sequence. The PAMAM dendrimer of generation 3 has 32 terminal surface groups of amines, providing many branching points that can be conjugated with oligopeptides. This PAMAM dendrimer on the hydrogels allowed us to graft oligopeptides at a lower concentration with successful culture of hPSCs.

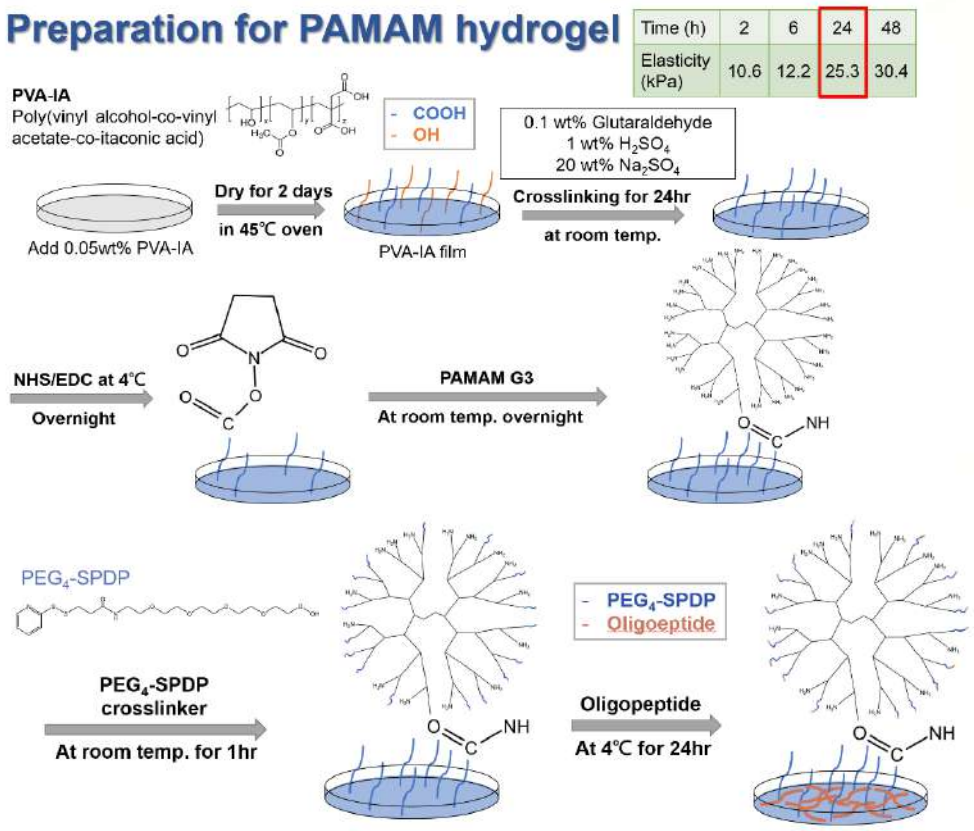

Figure 1. preparation for PAMAM G3 hydrogel

KEYWORDS: Human pluripotent stem cells (hPSC), Mesenchymal stem cells (MSCs), RGD peptide, PAMAM dendrimer

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GVHD treatment utilizing several types of stem cells cultivated on biomaterials

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Abstract:

Graft-versus-host disease (GvHD) occurs when the immune system of the transplanted bone marrow or stem cells recognizes the recipient's tissues as foreign materials and subsequently attacks them. Mesenchymal stromal (stem) cells (MSCs) have been shown to have immunosuppressive activity and can modulate the immune system, which makes them a promising candidate for the treatment of GvHD. By reducing inflammation and modulating the immune response, MSC transplantation may be able to prevent or alleviate the symptoms of GvHD in patients undergoing bone marrow or human MSCs (hMSCs) transplantation. We evaluate (1) which hMSCs are effective to suppress GvHD. We used several types of hMSCs such as human amniotic fluid stem cells (hAFSCs), adipose-derived stem cells (ADSCs) and human pluripotent stem cells (hiPSCs, HPS0077)-derived MSCs in this study. We also investigated (2) which extracellular matrix (ECM) protein-coated surface support GvHD treatment by hMSCs.

In the study, we attempted to differentiate MSCs into osteoblasts on various types of ECM protein-coated surface. After 28 days of osteogenic differentiation, we successfully obtained osteoblasts on the dish. The next step involved seeding the target cells (MSCs or co-cultured cells) onto the dishes to evaluate immune tolerance from mononuclear cells. Mononuclear cells isolated from blood were added directly onto the target cells, and the cells were co-cultured for 2 days in targeting cell media. Finally, the mortality of the targeting cells was determined by live and dead staining assay. Differentiated cells which co-culture with hMSC and mononuclear cells are expected to suppress the inflammation compared to the cells without hMSCs. Furthermore, we would like to evaluate the cell culture biomaterials (ECM-coated dishes), which can support more immunosuppressive effects of MSCs. These immunosuppressive effects of MSCs co-cultured with differentiated cells might also apply to other immune-related diseases.

KEYWORDS: extracellular matrix, biomaterial, graft versus host disease, mesenchymal stem cells, human amniotic fluid stem cells, adipose-derived stem cells.

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2023 ISOMRM & BCRS
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Culturing and Differentiation of Human Pluripotent Stem Cells on hydrogel mixture of E-cadherin- and ECM-derived peptides

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Abstract:

Human pluripotent stem cells (hPSCs) have the potential to differentiate into any of the cell types derived from the 3 germ layers. Xeno-containing Matrigel is the most commonly used substrate for culturing and differentiating hPSCs. Matrigel is a basement membrane extracellular environment composed of multiple components, including collagen IV, laminin-111, etc. Hydrogels grafted with single ECM-derived oligopeptides have been utilized for the culture and differentiation of PSCs. On the other hand, E-cadherin promotes cell-cell adhesion and colony formation of hPSCs. Considering that Matrigel contains multiple components, we designed a hydrogel grafted with E-cadherin-derived oligopeptides mixed with ECM-derived oligopeptides. E-cadherin is a Ca^{2+}-dependent cell-cell adhesion molecule binding of cadherin proteins at different binding sites on hPSCs and it enhances their differentiation, adhesion and proliferation.

We prepared poly(vinyl alcohol-co-itaconic), PVA-IA, hydrogels grafted with vitronectin-derived peptide (VN2CK; GCGGKGGPQVTRGDVFTMP) and laminin β4-derived peptide (LB2CK, GCGGKGGPMQKMRGDVFSP) using NHS/EDC chemistry. In addition, we added amino acids (Lysine, K) on the first of the sequences. Interestingly, the insertion of positively charged amino acids on the first sequence of the oligopeptide significantly enhances the surface grafting density of the peptide-grafted hydrogel and also increases the zeta potential of the hydrogel. Furthermore, E-cadherin emits Akt signaling, which enhances the pluripotency of hPSCs [1]. In the future, we aim to utilize hydrogels grafted with a combination of E-cadherin-derived oligopeptides and ECM-derived oligopeptides to culture hPSCs, enabling them to exhibit enhanced proliferative capacity and the ability to differentiate into low-adherent cells.

KEYWORDS: human pluripotent stem cell, E-cadherin, mixed oligopeptide grafted hydrogel, xeno-free condition, biomaterials, pluripotency

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Premixed calcium silicate bone cement with rapid setting and washout resistance

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Abstract:

Conventional water-mixed bone cement has been an essential biomaterial in orthopedics for bone repairing. Before bone cement is transferred into human body, water and powder should be mixed beforehand. However, the water-mixing process makes surgical procedures more complicated and time-consuming. Hence, there is a demand for premixed bone cement that does not contain an aqueous liquid but absorbs water by itself. The premixed bone cement is injected into the bone defect of the human body, and the water-free liquid is gradually replaced with body fluid, which then promotes the hardening of the cement. Calcium phosphate cement is the first choice of bioactive bone cement material, and premixed calcium phosphate cement has been well developed. Calcium silicate (CS) is another potential bone cement material with better anti-bacterial ability and bone regeneration [1-2]. Additionally, premixed CS bone cement is less developed and has some weaknesses that need to be improved. Long setting time and insufficient washout resistance are the critical problems of premixed CS cement. Hence, the objective of this study was to develop premixed CS bone cement with rapid setting and washout resistance.

CS powder was made by the sol-gel method and then manually mixed with carboxymethyl cellulose (CMC), polyethylene 400 (PEG400), and lactic acid (LA). PEG400, CMC, and LA were water-free liquid, gelling agent, and setting accelerator, respectively. In addition to exploring the effect of LA concentration, the liquid/powder ratio (L/P), which plays an important role, was also the focus of analysis in this study. The results showed that the cement group with low L/P ratio had better washout resistance and shorter setting time. The excellent washout resistance of premixed CS cement may be the CMC effect. The setting time of premixed cement with L/P ratio of 0.3 was less than 7 min, while those with 0.4 and 0.5 L/P ratio had setting time longer than 90 min. It is worth noting that the long setting time of 90 min was greatly reduced to 3 min with higher LA concentration in cement groups. Moreover, cement groups with the highest LA concentration showed the best diametral tensile strength among all groups. PEG, as the main liquid component in all experimental groups, is reported as an antifouling material in a previous study [3]. Undoubtedly, the addition of PEG to alkaline CS cement significantly improved the antibacterial ability of bone cement. In conclusion, premixed CS cement with appropriate contents of PEG400, LA, and CMC could be a potential bone cement biomaterial with rapid setting, washout resistance, and anti-bacterial property.

KEYWORDS: premixed cement, calcium silicate, rapid-setting, anti-washout

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3D printing of PEGDA-CAP--rhTM intervertebral cage increased stability on rat spinal fusion models and long-acting release protein drugs to promote intervertebral disc fusion

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Abstract:

Degeneration of the vertebral disc is a common disease that often requires orthopedic surgery in the form of intervertebral fusion using implants. This involves a cage to hold the implant in place to increase the stability of the implant during the fusion process. Consequently, extensive research has been conducted on the use of both metallic and polymeric materials for a cage, with both types being commercially developed and used. While metal cages carry allergy risks and can interfere with image signals post-healing, polymeric implants exhibit limited hardness and inferior cell adhesion.

The emergence of 3D printing technology has revolutionized allowing for unprecedented levels of precision and customization to fit the shape of the patient's intervertebral disc. Unlike thermal 3D printing which uses heated to melt metal or polymer, polymer 3D printing via photopolymerization has the potential to carry drugs due to the low temperature in the manufacturing process. Light curing technology employs a vat of liquid photopolymer resin that is cured by a digital light projector layer by layer to form a solid structure. The object is then flushed and can be post-cured with UV light to improve its mechanical properties to a highly precise level that can be tailored to the patient's disc shape. This technology can not only increase polymer hardness to meet clinical needs, but also able to carry osteogenic drug and materials to enhance bone cell adhesion.

PEGDA (polyethylene glycol diacrylate) is a commonly used light-curing biocompatible material in 3D printing applications. Upon exposure to UV light, the photoinitiator molecules in PEGDA undergo a photochemical reaction and cross-react with the acrylate groups in the material to form a stable three-dimensional polymer chain network. The hydrolytic stability of PEGDA's polyester backbone allows the PEG hydrogel remains intact in the short term. However, once the PEG diol is aromatized, the introduced ester linkage is prone to slow

degradation in vivo, which can take several months to years. In the case of disc fusion, the long-term degradation of PEGDA can provide initial stability of the cage until new osteogenesis occurs.

Osteogenic drugs and growth factors, such as BMP-2, are commonly used in conjunction with implants, but the single-dose administration required for intervertebral fusion can result in issues such as rapid drug release. Our previous study utilized rhTM, a protein-based drug, combined with CaP powder as a carrier to fill the implant. This approach demonstrated significant bone skeletogenesis after eight weeks in a rat spinal fusion model. Additionally, recent studies have shown that simvastatin can effectively promote osteogenesis.

In this study, we sought to improve and combine drug printing in cage design. To ensure accurate sizing and fit of the cage, pre-surgical CT images were utilized as the basis for model design, allowing for high levels of dimensional customization to fit the intervertebral disc space and avoid slippage due to size incompatibility. Polymer stereolithography (SLA) printing was employed to reduce issues with overpowering image signals, while the incorporation of drug-banded CaP particles into the printing resin allowed for slow and controlled release of the drug.

KEYWORDS: 3D printed drug, degradable fusion cage, spinal fusion, sustain drug release.

Poster

September 1 (Friday)	
P1-1	*Enhancing Prediction of Cardiology Clinical Features of Coronary Heart Diseases Using Ensemble Learning Approaches* **Po-Yin Chang, National Quemoy University, Taiwan**
P1-2	*Melanoma Lesion Detection Enhancement Using Deep Hybrid Segmentation* **Po-Yin Chang, National Quemoy University, Taiwan**
P1-3	*Design of biodegradable silk fibroin neural probe, for deep-brain chemical sensing and electrical stimulation* **Hung-Yu Hsu, National Yang Ming Chiao Tung University, Taiwan**
P1-4	*Application of the Unet model for cell segmentation and cell migration evaluation* **Yi-Yong Chong, Taipei Medical University, Taiwan**
P1-5	Multilabel Object Detection To Predict Breast Cancer Lesions On Mammograms **Quang-Hien Kh, Taipei Medical University, Taiwan**
P1-6	*A deep learning algorithm to diagnose pediatric forearm fracture based on AO/OTA classification* **Le Nguyen Binh, Taipei Medical University, Taiwan**
P1-7	*Identification and validation of novel hypoxia-and immune-related gene signature for gastric cancer prognosis* **Mai Hanh Nguyen, Taipei Medical University, Taiwan**
P1-8	*Optimizing Embryo Selection in IVF through AI-Based Non-Invasive Ploidy Prediction: Integration of Time-Lapse and Clinical Data* **Thi-My-Trang Luong, Taipei Medical University, Taiwan**
P1-9	*Concentrates Urinary Biomarkers Via the Osmosis Processors* **Chia-Yu Lee, National Taiwan University of Science and Technology, Taiwan**
P1-10	*Fucoidan microneedles with adjuvant effect for effectively enhancing antigen-specific immune responses* **Yen-Chin Chen, National Cheng-Kung University, Taiwan**
P1-11	*Investigating the effects of 830 nm low level lasers on ROS and melanin in melanocytes by single-cell analysis and evaluating their potential in the treatment of gray hair* **Liang-Chen Pan, Taipei Medical University, Taiwan**
P1-12	*Machine learning for drug response prediction in lung cancer cell lines* ***Thi-Oanh Tran*, Taipei Medical University, Taiwan**
P1-13	*ddPCR test of AI automated counting for COVID-19* **Wei-Lun Liang, Industrial Technology Research Institute, Taiwan**
P1-14	*Detection of Blood by Utilizing the Surface Enhanced Raman Spectroscopy Technique with The Help of Gold Nanorods and Silver Nanoparticles* **Uğur Köroğlu, Hacettepe University, Turkey**

P1-15	*Bacteriophage grafted superparamagnetic nanoparticle for detection of Shiga-toxin Escherichia coli (E.coli O157:H7)* **Jen-Yu Liao, National Taipei University of Technology, Taiwan**
P1-16	*An ultra-thin soft electrode combined with portable multi-channel EEG acquisition system* **Wei-Han Huang, National Yang Ming Chiao Tung University, Taiwan**
P1-17	*Using Multifunctional Hydrogel Conductivity to Detect Antibacterial Activity* **Hsin Cheng, National Taipei University of Technology, Taiwan**
P1-18	*Using a FET biosensor to measure the binding affinity between resveratrol and the serotonin 5-HT2A receptor* **Mei-Wen Tseng, Chung Yuan Christian University, Taiwan**
P1-19	*Development of an easy-to-use solid phase nucleic acid extraction device for next step isothermal amplification and CRISPR detection of target sequences* **Yun-Hsien Chung, National Tsing Hua University, Taiwan**
P1-20	*Establish isothermal reverse transcriptase recombinase polymerase amplification (RT-RPA) for quick and highly sensitive nucleic acid analysis on a microfluid chip* **Tao-Yun Yen, National Tsing Hua University, Taiwan**
P1-21	*Development of a microfluidic device for high resolution liquid biopsy screening with an isothermal control* **Wei-Chun Lan, National Tsing Hua University, Taiwan**
P1-22	*Silver Nano-island Arrays Deposited on Cicada Wings for Raman Enhancing Detection* **Ting-Yu Liu, Ming Chi University of Technology, Taiwan**
P1-23	*Low cost paper-based glucose sensor prepared by using commercial printer* **Binghuan Zhang, I-Shou University, Taiwan**
P1-24	*Preparation of novel membrane-based nucleic acid 3D printed biosensor platform and validating its target DNA detection* **Hsu-Hung Kuo, Yuan Ze University, Taiwan**
P1-25	*Biodegradable Adhesive Tissue-Mimicked Multichannel Microelectrode Arrays for Electrophysiological measurements applied to nerve, brain and cardiomyocytes* **Tzu-Ya Cheng, National Yang Ming Chiao Tung University, Taiwan**
P1-26	*Extracellular Matrix-Inspired All Hydrogel Biohybrid Neural Interfaces for Combined Microelectrode Array Technologies, Tissue Scaffolding, and Stem Cell Therapy* **Wan-Lou Lei, National Yang Ming Chiao Tung University, Taiwan**
P1-27	Photo-Responsive Nanozyme Sensor: Harnessing Emissive Oxidase-mimic Nanozyme for On-Site Antioxidant Detection in Human Saliva **Sanskruti Swain, National Central University, Taiwan**
P1-28	*Enhancing Peripheral Neural Cell Activity through the Combination of Conductive Hydrogels and Electrical Stimulation* **Jin-Xiu Yu, Feng Chia University, Taiwan**
P1-29	*A Magnetic-Responsive Injectable Photocrosslinkable Ink for the Applications of 4D Printed Bioactuators* **Tzu-Ching Chen, Feng Chia University, Taiwan**

P1-30	*Biodegradable Phosphocholine Cross-Linker With Ion-Pair Design for Tough Zwitterionic Hydrogel* **Yi-Yin Chen, National Central University, Taiwan**
P1-31	Complete zwitterionic double network hydrogels with great toughness and resistance against foreign body reaction and thrombus **Ying-Chieh Chuang, National Central University, Taiwan**
P1-32	*Development of methacrylic anhydride-modified carboxymethylcellulose hydrogels to deliver corticosteroids for corneal wound healing* **Ying-Qi Chen, National Taiwan University of Science and Technology, Taiwan**
P1-33	*Encouraging Enthesis Organ Development through Drug Release of Ligament, Bone, and Endochondral Ossification Cues using Mesoporous Silica Nanoparticles* **Qing-Xu Shi, National Taipei University of Technology, Taiwan**
P1-34	*Platelet-derived extracellular vesicles plus reduced graphene oxide co-laden polymer-coordinated hydrogel promotes diabetic wound restoration* **Ping-Chien Hao, Taipei Medical University, Taiwan**
P1-35	*Alginate-Tyramine Gel with Reinforcement by Plasma for Treatment of Arthritis* **Yu-Ming Chen, Taipei Medical University, Taiwan**
P1-36	*Development of modified hydroxypropyl methylcellulose hydrogel with drug loading as a vitreous substitute* **Chang-Lun Hsieh, Taipei Medical University, Taiwan**
P1-37	*Polypyrrole-polyethyleneimine nanopigments-woven medical products for restoring follicle* **Wei-Yung Huang, Taipei Medical University, Taiwan**
P1-38	*Harnessing Controlled-Degradable Microgel for Effective Chondrogenic and Osteogenic Differentiation of Human Mesenchymal Stem Cells* **Yu-Han Chen, National Tsing Hua University, Taiwan**
P1-39	*Effects of alginate hydrogel containing bacteriophage cocktail on macrophage-mediated inflammation and fibroblast migration* **Qiu-Yun Zheng, National Taipei University of Technology, Taiwan**
P1-40	*Novel Polypeptide Composite Fibrous Scaffold with Internal Chemical Boundary* **Chia-Hsien Lee, Ming-Chi University of Technology, Taiwan**
P1-41	*Development of Cancer Cell Membrane-Coated Indocyanine Green-Camptothecin-Loaded Perfluorinated Nanoparticles for Photodynamic Therapy in Triple-Negative Breast Cancer (MDA-MB 231): Material Fabrication and Functional Validation* **Ying-Ju Lin, National Central of University, Taiwan**
P1-42	*Extraction of ulvan and incorporation into thermosensitive electrospun fibers as a wound dressing* **Ping-Yu Chang, National Taiwan Ocean University, Taiwan**
P1-43	*Lanthanide Hydrogel: A Versatile Biomaterial with Stretchability and Self-Healing Abilities for Bioimaging* **Cheng-Zhen Yuan, National Kaohsiung University of Science and Technology, Taiwan**

P2-1	*Gelated cells as a robust cell-mimicking biomaterial system for cancer immunotherapy and tissue engineering* **Jung-Chen Lin, Academia Sinica, Taiwan**
P2-2	*Chitosan-based nerve guiding conduit for peripheral nerve repair* **Yi-Dan Chang, Asia University, Taiwan**
P2-3	*Photocrosslinkable Microbeads for Delivery of Dietary Supplement* **Huan-Jin Guo, Feng Chia University, Taiwan**
P2-4	*Gelatin-based Composite Microneedle patches for the Treatment of Keloid Scars* **Yong-Ji Chen, I-Shou university, Taiwan**
P2-5	*Development adhesive microneedles as an oral patch for recurrent aphthous stomatitis treatment* **Chi-Hsiang Wu, I-Shou university, Taiwan**
P2-6	*Development of Indocyanine Green and Camptothecin loaded Hyaluronic Acid Hydrogel for Photochemotherapy of Skin Cancer* **Chieh-Lin Chung, National Central University, Taiwan**
P2-7	*Smart Hydrogels for Trans-mucosal Drug Delivery: Evaluation the Efficacy of New Targeted Hyaluronic Acid-Drugs to Treatment Inflammatory Bowel Disease* **Pei-Xhan Wu, National United University, Taiwan**
P2-8	*Development of in situ dual-crosslinkable (dcHA) hyaluronic acid based injectable hydrogel as a dermal filler* **Bang Yu Wen, Yuanpei University of Medical Technology, Taiwan**
P2-9	*Development and evaluation of thermosensitive hydrogels for long-acting injection formulations* **Chih-Yu Hsieh, Taipei Medical University, Taiwan**
P2-10	Dual-crosslink of Self-assemble and Photocrosslinling Collagen Hydrogel Promote Vascular Tissue Engineering **Kuan-Ho Pan, National Tsing Hua University, Taiwan**
P2-11	*Pre-spheroidized by Chitosan Prompt Osteoblast-like MG63 Cells to Osteogenesis on NaOH Etching 3D printing Ti-alloy Scaffolds* **Ching-Wen Tsai,National Applied Research Laboratories, Taiwan**
P2-12	*Development of Perfluorinated Cancerous Exosomes for Enhanced Target Photochemotherapy in Triple-Negative Breast Cancer* **Zhi-Qiao Zuo, National Central University, Taiwan**
P2-13	Vitamin B12 Loaded Methylcellulose/Hyaluronic Acid Thermosensitive Hydrogel Ring for Ocular Drug Delivery **Yi-Xin Liu, National United University, Taiwan**
P2-14	Versatile photothermal nanozymes with glutathione depletion and thermal/acidity-triggered hydroxyl radical generation for combination cancer therapy **Wen-Hsuan Chiang, National Chung Hsing University, Taiwan**
P2-15	Mechanism of GNR@MIL-100(Fe) induced Macrophage Activation **Yen-Chang Chen, National Taiwan University, Taiwan**

P2-16	*The effect of metformin on carbonic anhydrase type 8 and glucose transporter type 2 in kidney and HK-2 cells* **Hsiang-Yu Liu, Tunghai University, Taiwan**
P2-17	*Hyaluronic acid surface-modified nanomedicine for the treatment of retinopathy in mice with blue light-induced damage* **Yen-Jen Lee, Taipei Medical University, Taiwan**
P2-18	*NIR-activated organic molecule-based nanocomposites with photothermal and photodynamic effects for cancer treatment* **Ming-Hsin Liu, National Taiwan University, Taiwan**
P2-19	*Nitrogen-doped polymer dots with tunable crosslinking density for nonconventional fluorescence and antibacterial behavior* **Jia-Qi Chen, China Medical University, Taiwan**
P2-20	*A three-in-one injectable hydrogel for reprogramming the immunosuppressive tumor* **Wu- Xun Chen, National Tsing Hua University, Taiwan**
P2-21	*IR820-loaded Fe(III)-rich nanozymes for glutathione-depletion/thermo enhanced chemodynamic/photothermal synergistic therapy* **Tzu-Chen Lin, National Chung Hsing University, Taiwan**
P2-22	*Development of AFP-targeting ICG-CPT-encapsulated PLGA nanoparticles emulsions for photochemotherapy of lung cancer* **Yen Po-Lung, National Central University, Taiwan**
P2-23	*Calcium-Zoledronic Acid Coordination Complex of Nanoparticles Combination with Thermal Effect for Treatment Breast Cancer Bone Metastasis* **Wong-Jin Chang, Taipei Medical University, Taiwan**
P2-24	*The feasibility of tea polyphenol nanoparticles as a drug for stimulating hair growth* **Chao-Yun Yen, Taipei Medical University, Taiwan**
P2-25	*Evaluation of eye drops contained small compound extracted from He Shou Wu for dry eye mice treatment* **Ting-Ying Huang, Taipei Medical University, Taiwan**
P2-26	*Investigating the Cardiac Effects of Drug Loaded Mesoporous Silica Nanoparticles on Heart Failure in Zebrafish* **Hong-Wei Wang, Taipei Medical University, Taiwan**
P2-27	*Stem cell–nanomedicine system as a theranostic bio-gadolinium agent for targeted neutron capture cancer therapy* **Yen-Ho Lai, China Medical University Hospital, Taiwan**
P2-28	*Development of functional polymers for drug delivery and bio-application* **Ruo-Yun Tao, Yuan Ze University, Taiwan**
P2-29	*Sorafenib-Loaded Superparamagnetic Nanoparticles Combined with External Electromagnetic Field for Precision Liver Cancer Treatment* **Xiao-Zhu Tang, National Taipei University of Technology, Taiwan**

P2-30	*Development of bacterial membrane coating-Indocyanine green and camptothecin co-loaded perfluorocarbon double nanoparticles for photochemoimmunotherapy of colorectal cancer* **Chin-Yu Tan, National Central University, Taiwan**
P2-31	*Development of controllable decorative tumor antigen-tethered spiked Virus-Like-PLGA-Nanoparticles (sVLPN) to induce potent immunogenicity against tumors in mice* **Yan-Ting Shen, Taipei Medical University, Taiwan**
P2-32	*Development of in situ thermosensitive poloxamer gels as ocular drug delivery systems of sodium phenylbutyrate for the treatment of open-angle glaucoma* **Chen-Hsuan Hsu, Taipei Medical University, Taiwan**
P2-33	*Bispecific T-cell engagers non-covalently decorated on PEGylated liposome fused with MHC molecular tumor antigen expressed exosomes derived from dendritic cells for colon cancer immunotherapy* **Ting-Wei Lin, Taipei Medical University, Taiwan**
P2-34	*Development of glycosylated PLGA nanoparticles as dendritic cell targeting delivery vehicles for therapeutic cancer vaccination* **Tung-Han Tsai, Taipei Medical University, Taiwan**
P2-35	*Treatment of skin cancer with dissolving ulvan microneedles containing curcumin in combination with X-ray radiation therapy* **Hsin-Yu Sun, National Taiwan Ocean University, Taiwan**
P2-36	*Developing a Local and Sequential Release System for Non-healing Diabetic Ulcer Therapy* **Tun-Hsiang Kao, National Taiwan University of Science and Technology, Taiwan.**
P2-37	*Anti-fibrotic microRNA/chitosan-based nanoparticles for the treatment of pulmonary fibrosis* **Yu-Chia Chang, Taipei Medical University, Taiwan**
P2-38	*Effect of a Topical Collagen Tripeptide on Antiaging and Barrier Dysfunction of Skin* **Kai-Wen Chang, Kaohsiung Medical University, Kaohsiung, Taiwan**
P2-39	*Using γ-PGA / MAO composite coatings for improvable biocompatibility and controllable drug release* **An-Nghia Nguyen, National Cheng Kung University, Taiwan**
P2-40	*Perioperative anesthetics combined hyperthermia on cancer precision medicine* **Wen-Hui Hsieh, Chun-Jen Huang, Jui-Aa Lin and Hsu-Wei Fang***
P2-41	*A simple green design of modification of doxorubicin onto GQD-nanogold composite for the inhibition of colon cancer* **Tseng-Yu Yeh, National Taipei University of Education, Taiwan**
P2-42	*Using the technology of DNA origami and the simulation of Magic DNA to develop various structures for drug delivery* **Hao-Wei Liu, Feng Chia University, Taiwan**

9/2 (Saturday)	
P3-1	*Development of silver-containing mesoporous bioactive glass combined Ebselen for wound dressing* **Chu-Yun Wei, Kaohsiung Medical University, Taiwan**
P3-2	*Degassing a decellularized scaffold enhances wound healing and reduces fibrosis during tracheal defect reconstruction: A preliminary animal study* **Nguyen-Kieu Viet-Nhi, Taipei Medical University, Taiwan**
P3-3	*Pd@VNU-2 and its Application in Radiation-Photothermal Combined Cancer Therapy* **Yu-Sheng Yu, National Taiwan University, Taiwan**
P3-4	*Development of Nanoparticle Loaded Microneedle Mediated Gene Delivery on the Application of Cancer Treatment* **Zi-Han Chen, National Tsing Hua University, Taiwan**
P3-5	*Study on Gelatin Methacryloyl / Hyaluronic Acid Methacryloyl Composite Hydrogel Cross-linked with Visible Light for Wound Dressings* **Wan-Rong Lin, National Taipei University of Technology, Taiwan**
P3-6	*Development of Antibacterial Composite Materials for the Bottom Layer of Dissolving Microneedles* **Zi-Hao Chen, National United University, Taiwan**
P3-7	*Development of magnetic temperature fiber materials for drug releasing* **Jou-Hsuan Huang, Chung Yuan Christian University, Taiwan**
P3-8	*Nanocomposite hydrogel-delivered GM-CSF and fucoidan-based nanoparticular vaccine for inhibition of tumor growth and metastasis* **Tsai-Hsuan Lin, Taipei Medical University, Taiwan**
P3-9	*In Situ Forming Transparent Quercetin/Hyaluronic Acid Hydrogel as A Vitreous Substitute* **Ching-Wen Chang, National United University, Taiwan**
P3-10	*Study on novel composite tissue-engineeringed tracheal grafts with bacteriostasis and photocurable 3D printing* **You-Cheng Tsai, Kaohsiung Medical University, Taiwan**
P3-11	*Development of a microfluidic device encapsulating mitochondria in liposomes for cell therapy* **Yen-Chin Hsu, Taipei Medical University, Taiwan**
P3-12	In vitro studies of kaempferol action on osteoblasts and osteoblasts **Pei-Ju Chang, China Medical University, Taiwan**
P3-13	*Development of a novel extraction wound dressing containing bimetallic nanoparticles bioactive glass for the prevention of medication-related osteonecrosis of the jaw* **Yi-Heng Dai, Kaohsiung Medical University, Taiwan**
P3-14	*3D Bioceramic burr hole scaffold fabricated by digital light processing for bone regeneration in rabbit calvarial defects* **Chih-Yun Lee, Kaohsiung Medical University, Taiwan**

P3-15	Plasma-Enabled Graphene Quantum Dot Hydrogel – Magnesium Composites as the Bioactive Scaffolds for In Vivo Bone Defect Repair **Pei-Chun Wong, Taipei Medical University, Taiwan**
P3-16	*Novel artificial tricalcium phosphate and magnesium composite graft facilitates angiogenesis in bone healing* **Yuan-Hsin Tsai, National Chung Hsing University, Taiwan**
P3-17	*Applying multiple surface treatments to enhance osseointegration of titanium dental implant through regulating bone remodeling* **Her-Hsiung Huang, National Yang Ming Chiao Tung University, Taiwan**
P3-18	*Chitosan wound dressing modified with bacteriophage promotes antibacterial activity against Staphylococcus aureus and bone cell growth* **Yu-Sheng Chen, National Taipei University of Technology, Taiwan**
P3-19	*Development of Biomimetic Gel with Osteoconductivity for Fixation of Permanent Bone Implants* **Chun-Yu Lin, National Central University, Taiwan**
P3-20	*Evaluation the effect of biomaterials on BMP-2 binding activity to BMPR-1A receptor* **Shu-Hui Wu, National Health Research Institutes, Taiwan**
P3-21	*Synergy of bone microenvironment stiffness and BMP-2 enhances breast tumor calcification by using a 3D model* **An-Lun Kuo, National Health Research Institutes, Taiwan**
P3-22	*Fucoidan-Decorated Epigallocatechin Gallate/Protamine Nanoparticles for Plaques-Targeted Delivery and Controlled Release of cargoes in Anti-Atherosclerosis Therapy* **Chi Lin, Taipei Medical University, Taiwan**
P3-23	*The Effect of heart and metabolites in PTZ induced heart abnormalities zebrafish* **Kuan-Lun Li, National Taiwan University, Taiwan**
P3-24	*Constructing Heart-specific Exosome Profile to Enable Research of Cardiovascular Disease* **Rosie Kao, National Taiwan University of Science and Technology, Taiwan**
P3-25	*Biomimetic Platelet Motor for Propelling Hirudin Peptide Delivery for Remotely Site-Specific Phototherapeutic Thrombolysis* **Yan-Ting Chen, Taipei Medical University, Taiwan**
P3-26	*Cold Atmospheric Plasma-Derived Nanoclusters for Lesion-Specific Multimodal Photo/Magnetic Thrombus Therapy* **Pei-Ru Jheng, Taipei Medical University, Taiwan**
P3-27	*Exosome/Secretome-derived from pcMSCs promoting endogenous progenitor stem cell and suppressing inflammatory condition in LPS-induced ARDS/ALI model* **Kajal Singh, Taipei Medical University, Taiwan**
P3-28	*In Vitro Treatment Effect of 3D cultured MSC-derived Exosomes on IL-1β Treated Chondrocyte : Exosomes Characterization and Anti-inflammation Evaluation* **Yu-Ping Chen, National United University, Taiwan**

P3-29	*Enabling Rapid Extracellular Vesicle Isolation from Cell Culture Media by Osmosis* **Casey Huang, National Taiwan University of Science and Technology, Taiwan**
P3-30	*Platelet-derived Extracellular vesicles (pEVs) based therapy for glaucoma-associated neuroinflammation and efficacy in ophthalmic drug delivery* **Huynh-Ngoc-Truc Nguyen, Taipei Medical University, Taiwan**
P3-31	*Mechanism of MIL-100(Fe) Induced Macrophage Activation & Its Application in Fibrosis Inhibition* **Po-Hsiu Cheng, National Taiwan University, Taiwan**
P3-32	*Fluorescent carbon nanodots via hydrothermal process from animal-derived materials for biolabeling and their antibacterial activity* **Shu-Ting Tung , China Medical University, Taiwan**
P3-33	*Aerosol delivery of Nintedanib in nanoformulation for pulmonary fibrosis treatment* **Hong-Ming Lin, Taipei Medical University, Taiwan**
P3-34	*MXene-Modified Injectable Microbeads for Gene Therapy in Neuron Regeneration for Traumatic Brain Injury Treatment* **Wan-Chi Pan, National Tsing Hua University, Taiwan**
P3-35	*Exploiting Erythrocyte-Mediated Delivery of PB-DLNP@RBC for Enhanced Drug Delivery and Gene Therapy* **Hao-Min Chang, National Tsing Hua University, Taiwan**
P3-36	*TiO2 Based Photodynamic and Photothermal Therapies for Glioblastoma Treatment* **Yu-Sheng Lee, National Tsing Hua University, Taiwan**
P3-37	*Development of magnetic triggered membrane for drug releasing application* **Jou-Hsuan Huang, Chung Yuan Christian University, Taiwan**
P3-38	*Single-Shot Nanosell Vaccine Eradicates MHC-low HPV-associated Tumor* **Chen-Hsueh Pai, Academia Sinica, Taiwan**
P3-39	*Evaluation of Eye Drops Containing Carbon Quantum Dots in Mice with Dry Eye Syndrome* **Po-Yu Lin, Taipei Medical University, Taiwan**
P3-40	*Contextual STING activation by 2'3'cGAMP-daunorubicin nanoparticle amplifies adaptive immunity in systemic cancer chemoimmunotherapy* **Kai-Chieh Yang, Academia Sinica, Taiwan**
P3-41	*Precision antigenic programming enhances anticancer vaccine efficacies* **Po-Cheng Tsai, Academia Sinica, Taiwan**
P3-42	*A Flexible and Fabric-Based Nanocomposite Design for Wound Healing Applications* **Po-En Hsu, National Central University, Taiwan**
P4-1	*Cytotoxicity, Transport, and Cellular uptake after exposure to polypropylene micro/nanoplastics produced by thermal oxidation reaction* **Suphatra Hiranphinyophat, Tohoku University, Japan**
P4-2	*Development of Kaempferol-Loaded Gelatin Nanoparticles for the Treatment of Choroidal Neovascularization* **Erh-Hsuan Hsieh, Taipei Medical University, Taiwan**

P4-3	*Temperature-Responsive Polymer-Antibody Conjugate for Biomarker Separation* **Maggie Shen, National Taiwan University of Science and Technology, Taiwan**
P4-4	*One-pot synthesis non-conventional fluorescent polymer dots with non-conjugated hyperbranched structure for drug delivery and bioimaging* **Siao-Cian Fan, China Medical University, Taiwan**
P4-5	*Associations between altered microbiota and functional brain images in fibromyalgia* **Nguyen Thanh Nhu, Taipei Medical University, Taiwan**
P4-6	*Additive manufacturing of Schwann cell-laden collagen nerve guidance conduits by freeform reversible embedding regulate neurogenesis towards peripheral nerve regeneration* **Su, Ching-Feng, China Medical University, Taiwan**
P4-7	*The abnormalities and metabolites of brain in pentylenetetrazol-induced seizures zebrafish* **Wen-Xu Wei, Chinese Culture University, Taiwan**
P4-8	*Neuron protective drug in an in-vitro model of glutamate-mediated neurotoxicity* **Chu-Chun Liang, National Tsing Hua University, Taiwan**
P4-9	*Application of decellularized small intestinal submucosa crosslinked with anthocyanin as an artificial nerve guiding conduits* **Xiang-Ting Huang, Asia University, Taiwan**
P4-10	*DEVELOP A TECHNOLOGY FOR MRI-MONITORING BREAST CANCER CELL SITUATION* **Yu-Jing Huang, National Health Research Institutes, Taiwan**
P4-11	*Effects of decellularization extracellular matrix on the behavior and differentiation of intestinal epithelial cells in both static and dynamic microfluidic environments.* **Aye Chan Phyu, National Taiwan University of Science and technology, Taiwan**
P4-12	*Preparation of Tumor Tissue Rich in Extracellular Matrix for Toxicity Test of Cancer Therapeutic Drugs* **Szu-Yi Ho, National Health Research Institutes, Taiwan**
P4-13	*The Process of Mouse Cochlear Hair Cell Differentiation Stimulated by Dynamically Regulating Ion Concentration* **Feng-Yuan Chien, National Central University, Taiwan**
P4-14	*A Tumor Accelerator Based on Multicomponent Bone Scaffolds and Cancer Cell Homing* **Chen-Ji Huang, National Health Research Institutes, Taiwan**
P4-15	*Biomimetic of a 3D breast cancer spheroid model using decellularized extracellular matrix for personalized therapeutics investigation* **Yen-Hong Lin, China Medical University, Taiwan**
P4-16	*Vessel on a chip with Hydrogel Based 3D Bioprinting Vessel-Like Construct* **Li-Ying Peng, National Tsing Hua University, Taiwan**
P4-17	*Development of bioinks for 3D bioprinting of breast cancer microenvironment modeling* **Ting-Wei Chang, National Tsing Hua University, Taiwan**

P4-18	*Effects of regenerative medicine in combination with physiotherapy for knee osteoarthritis: a network meta-analysis of randomized controlled trials* **Chun-De Liao, Taipei Medical University, Taiwan**
P4-19	*Therapeutic Potential of Chenopodium Formosanum Extracts for Early-Stage Osteoarthritis: Free radical scavenging activity and cell-compatibility* **Wan-Yi Xiao, National United University, Miaoli, Taiwan**
P4-20	*The prospect of applying 3D cultured osteogenic cell spheriods in surgical interventions for Empty Nose Syndrome* **Jing-Ke Chen, National Central University, Taiwan**
P4-21	*Promotion of Peripheral Nerve System Remyelination (and underline mechanism) by Cell Therapy* **Pei-Yi Ou Yang, National Cheng Kung University, Taiwan**
P4-22	*PLLA microparticle-loaded double-layered microneedle patches for effectively stimulating dermal collagen regeneration* **Chih-Chi Chang, National Cheng Kung University, Taiwan**
P4-23	*Advancement of Three-Dimensional Biomimetic Skin Substitutes for Burn Injury Skin Regeneration* **Ching-Yun Chen, National Central University, Taiwan**
P4-24	*Application of porcine-derived cartilage extracellular matrix to enhance the therapeutic efficacy of rheumatoid arthritis drug* **Sung-Han Jo, Pukyong National University, Korea**
P4-25	*Reduce cytotoxicity induced by fine particulate matter (PM2.5) via transporting mitochondria to human cardiomyocyte cells exposure to PM2.5* **Uyen Thi-Nhat Nguyen, Taipei Medical University, Taipei, Taiwan**
P4-26	*Mechanism of cutaneous wound repair in nude mice skin by picosecond laser-induced optical breakdown combined with polymer dots dressings* **Hoi-Man Iao, China medical university, Taiwan**
P4-27	*Improving cartilage reconstruction using the cell sheet engineering* **Yuan Tseng, National Taiwan University, Taiwan**
P4-28	*Polydopamine-dressed 3D stem cell spheroid-derived decellularized extracellular matrix scaffolds with for promoting tissue regeneration* **Pei-Ching Yang, National Tsing Hua University, Taiwan.**
P4-29	*3D bioprinting electrically conductive bioink on the application of Neural Tissue Engineering* **Yu-Chun Yeh, National Tsing Hua University, Taiwan.**
P4-30	*3D spheroids of bone marrow mesenchymal stem cells ameliorate traumatic brain injury by alleviating neuroinflammation and glutamate excitotoxicity* **Grace H. Chen, National Tsing Hua University, Taiwan.**
P4-31	*Development of nerve guidance conduit with spatial gradients of Schwann cells for repairing peripheral nervous system* **Chia-Hsin Ho, National Tsing Hua University, Taiwan.**

P4-32	*Transplantation of 3D spheroids of adipose-derived stem cells promotes rabbit Achilles tendon healing by enhancing tenocyte proliferation and suppressing M1 macrophages* **Shao-Wen Liu, National Tsing Hua University, Taiwan.**
P4-33	*Regulation of differentiation potential and sub-population by histone trimethylation and HDAC5 during spheroid formation of human adipose-derived stem cells* **Ming-Min Chang, National Cheng Kung University, Taiwan**
P4-34	*Enhanced β cell survival in subcutaneous space after co-transplantation of 3D stem cell spheroids with pro-angiogenic and anti-apoptotic potential* **Ying-Chi Kao, National Tsing Hua University, Taiwan.**
P4-35	*Cell Screening Approaches for Cochlear Progenitor Cells: Pre-Plate and Lgr5 Binding Protein as Antibody-Free Alternatives* **Sheng-Wen Chang, National Central University, Taiwan**
P4-36	*Effect of Near-infrared Laser Irradiance Photobiomodulation on Mitochondria Membrane Potential for Different Passages of Human Adipose-derived Stem Cell* **Wei-Chen Lin, Taipei Medical University, Taiwan**
P4-37	*Fabrication of 3D adipose tissue using engineered composite spheroids* **Jeongbok Lee, Hanyang University, Korea**
P4-38	*MicroRNAs-mediated cartilage regeneration using a lithium-containing calcium silicate bi-layered scaffold laden with exosome-based therapy* **Ting-You Kuo, China Medical University, Taiwan**
P4-39	*Dermal fibroblast-laden 3D-printed electroactive hydrogels for enhancing cutaneous wound healing through electrical stimulation* **Tai-Yi Hsu-Jiang, China Medical University, Taiwan**
P4-40	*Harnessing the multifunctional of ADSC-derived exosomes for accelerating healing of diabetic chronic wounds* **Min-Hua Yu, China Medical University, Taiwan**
P4-41	*Nano-Layered Magnetic Nanoparticles for Heat-Triggered Drug Release* **Nanami Fujisawa, National Institute for Materials Science, Japan**
P4-42	*OSMOTIC CONCENTRATION OF URINARY LIPOARABINOMANNAN FOR RAPID AND SENSITIVE DETECTION OF TUBERCULOSIS* **John J. Hill, National Taiwan University of Science and Technology, Taiwan**

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Enhancing Prediction of Cardiology Clinical Features of Coronary Heart Diseases Using Ensemble Learning Approaches

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Abstract:

As the population of Taiwan continues to age, there is a projected rise in the prevalence of Coronary Heart Disease (CHD) and other atherosclerotic diseases. This escalation is expected to impose a significant societal burden in the foreseeable future. Contemporary research has established that machine learning techniques can detect latent patterns from medical data, delivering valuable insights into diseases and facilitating precise disease prediction. CHD is an important research domain in healthcare. Despite significant strides in CHD prediction research in recent years, there remains a requirement to advance prediction accuracy. This study proposes a hybrid method called BOEL, combining Bayesian optimization (BO) with ensemble learning (EL) for CHD prediction. BO in BOEL aims to optimize the EL model's hyperparameters and improve CHD prediction accuracy. This paper presents an experimental framework designed for predicting CHD. The framework comprises four components: data preprocessing, feature selection, model training, and evaluation. The first stage involves the preprocessing of CHD data. Subsequently, relevant features are selected from the dataset, followed by k-fold cross-validation training of the machine learning model with the chosen data to avert overfitting. The final phase involves evaluating the trained model for accuracy. A flowchart of the BOEL methodology is depicted in Figure 1. We compare the performance of BOEL with five other forecasting algorithms, including CART (Classification and Regression Tree), support vector machine, k-nearest neighbors, AdaBoost, and artificial neural network. The experimental findings revealed that the BOEL model outperformed the alternatives for the accuracy and stability of CHD prediction. These results suggest the efficacy of the BOEL model in predicting coronary heart disease.

KEYWORDS: Coronary heart disease, ensemble learning, Bayesian optimization, support vector machine, artificial neural network

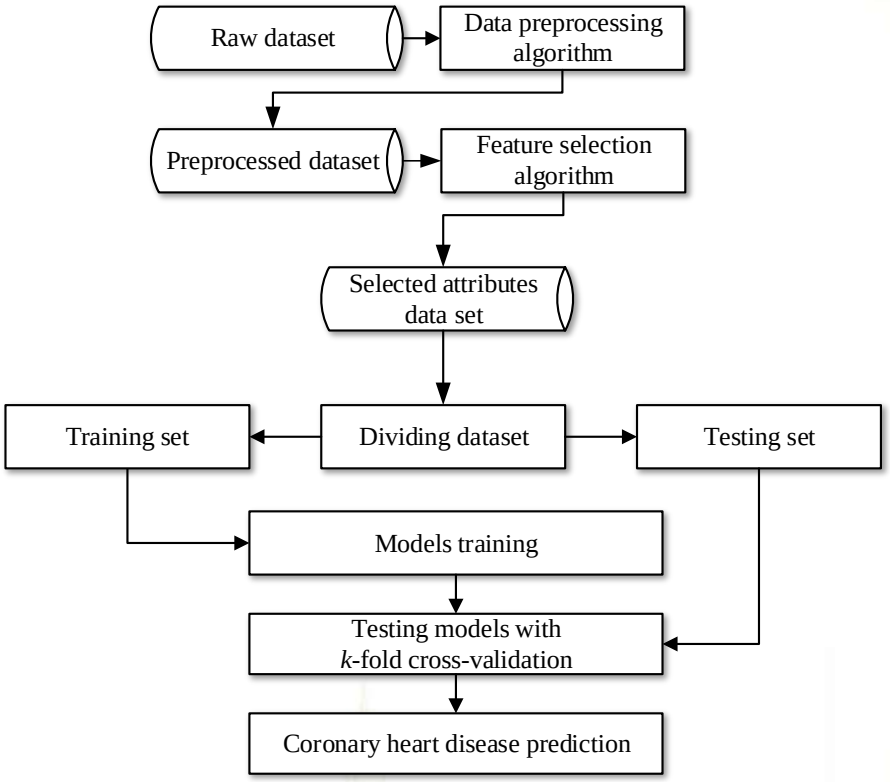

Figure. 1. BOEL flow chart for experimental framework

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Melanoma Lesion Detection Enhancement through Deep Hybrid Segmentation

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Abstract:

Early detection of melanoma is paramount for improved patient outcomes, as surgical intervention can lead to higher cure rates. Manual segmentation of suspected lesions is a commonly utilized method for early melanoma diagnosis. However, manual segmentation has several drawbacks, including misclassification and low efficiency. Therefore, developing an automatic image segmentation method is crucial to overcome these challenges. This study proposes an improved algorithm, HUSF (Hybrid U-Net and SegFomer), which combines the U-Net [2] and SegFormer [3]. Figure 1 represents the HUSF model involving a pre-trained SegFormer model that enhances the segmentation process, potentially improving the accuracy and reliability of skin cancer image segmentation. HUSF model offers two primary advantages over SegFormer: (1) it features a novel hierarchically structured Transformer encoder that generates multiscale features, eliminating the need for positional encoding and preventing performance degradation when testing resolution varies from training, and (2) it avoids the use of complex decoders. The performance of the proposed HUSF algorithm was evaluated by comparing it with other common models using two skin lesion datasets from the ISIC Challenges [1]. The experiment results indicate that the HUSF model exhibited superior performance compared to other models for three fundamental metrics: dice coefficient, intersection over union, and loss value. The overall conclusions drawn from the study imply that the HUSF model is adept at identifying skin lesions by enhancing composite coefficients and simplifying the network structure.

KEYWORDS: Melanoma, deep learning, sematic segmentation, U-Net, SegFormer

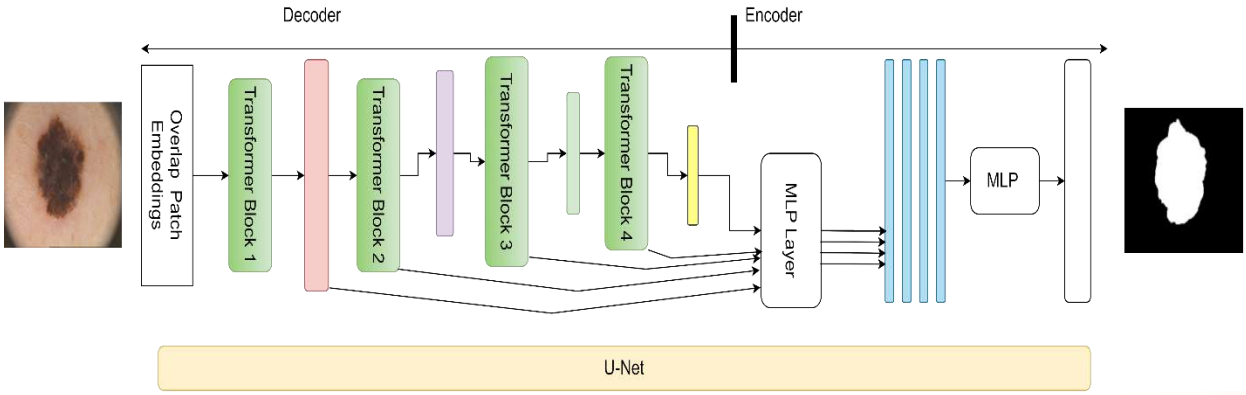

Figure. 1. HUSF architecture

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Design of biodegradable silk fibroin neural probe, for deep-brain chemical sensing and electrical stimulation

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Abstract:

Implantable neuro-recording microprobes have been implemented in different areas of the brain to provide diagnosis of patients with seizures and Alzheimer's. However, current needle-like intrusive nature of these devices face challenges of lack of sufficient probe flexibility, damage to brain tissue, and scar tissue formation, and secondary injuries caused by removal surgeries. Based on the aforementioned reasons, we dedicated to design biodegradable implants which reduced inflammatory reaction and avoiding removal surgeries. Here, we present a degradable microprobe for electrical signal stimulation, recording, and brain's released chemical sensing. We chose silk fibroin as the probe's substrate, because of its excellent physical and biomedical properties, such as transparency, mechanical strength and biocompatibility. Compared with the nondegradable device with substrate and passivation layer made of materials including SU-8 and parylene C, our degradable one can actively transform from a hard-brittle state to a flexible state after implantation, thus reducing tissue damage and enhancing its long-term recording stability. In addition to achieve transient neural signal recording and stimulation, the microprobes are also equipped with chemical signal detecting electrodes and an AgCl reference electrode. This allows the microprobe to detect chemicals (e.g., dopamine) released by the brain, which helps us manage the brain disorders more comprehensively.

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Application of the Unet model for cell segmentation and cell migration evaluation

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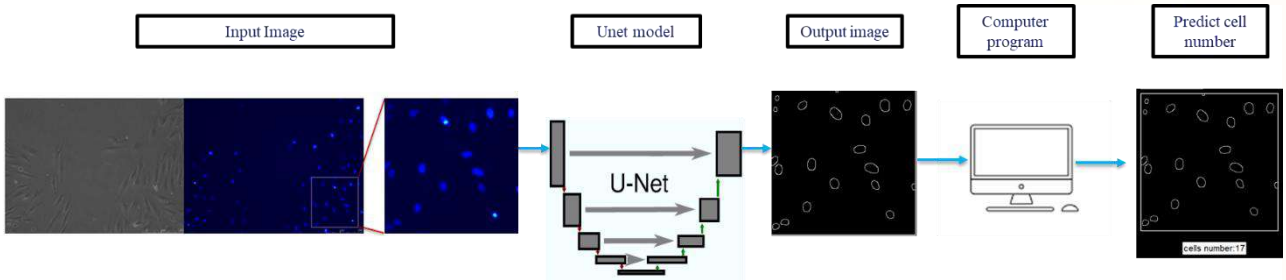
Abstract:

Cell migration serves as a crucial indicator in regenerative medicine. When tissue is damaged, such as from a wound or surgical incision, the surrounding cells begin to migrate as part of the repair and tissue regeneration process.

The cell scratch assay is the most commonly used method to evaluate cell migration because it simulates the *in vivo* wound healing process. However, the cell scratch assay, which generally uses the scratch area change to determine the migration ability of cells, can yield rapid results but is also prone to significant errors. Therefore, we propose an improved method that can reduce the error and get the result quickly in this experiment. We used Hoechst 33342 to stain the nuclei of adipose-derived stem cells (ADSCs) and take pictures. These images are then used to train the Unet model, a high-performing deep learning model in biomedical segmentation applications, to obtain the cell segmentation images [2]. Finally, we utilized a computer program to calculate the number of cells within the fixed area .

The principle of this method to evaluate cell migration ability is that if the number of cells increases significantly within a fixed area, it indicates a stronger cell migration capability. This approach allows for the rapid cell count and also mitigates errors introduced by human factors.

KEYWORDS: Cell migration, Cell counting, Unet, Cell segmentation, Deep learning



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Multilabel Object Detection To Predict Breast Cancer Lesions On Mammograms

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Abstract:

After lung cancer, breast cancer is the second most common cancer in the number of cases (1.7 million, accounting for 11.9% of all cancer cases) and the fifth leading cause of death (522,000 cases, accounting for 6.4% of all cancer deaths) in 2018. Among women, breast cancer is the most common cause of cancer in both developing and developed countries, with the number of cases increasing in all countries. Developing countries have a higher incidence of breast cancer than developed countries, with 883,000 cases compared to 794,000 cases, making it the number one disease among women in developing countries [1].

Early diagnosis and treatment of breast cancer can significantly improve disease progression and mortality [2–4], with mammography playing a vital role in the early detection of specific breast cancer lesions. A 2016 meta-analysis evaluating the effectiveness of mammogram screening in intermediate-risk women found a reduced risk of dying from breast cancer [5]: per 10,000 screenings per year, mammography reduced 2.9 deaths in women aged 39-49, 7.7 deaths in women aged 49-59, 21.3 deaths in women aged 59-69, and 12.5 deaths in women aged 70-74. Another European meta-study also found that mammogram screening reduced the risk of dying from breast cancer by 25-31% in the 50-69 age group [3]. Two observational studies in Sweden and Canada also found similar results, with a 26% and 44% reduction in the risk of dying from breast cancer [6, 7]. As a result, many countries have included mammography in their recommended screening programs for all women aged 40 to 50 years [8, 9]. In the US and UK, more than 42 million mammograms are performed yearly. Although mammography is widely accepted, assessing lesions on mammography remains challenging. The accuracy of mammography depends on radiologists' imaging technique, experience, and skill [10–12]. False positives can lead to patient anxiety and unnecessary invasive diagnostic tests [13]. Conversely, false negatives can lead to missed cancer, delayed diagnosis, and a complete inability to treat [14].

In this context, the emergence of supporting modelling - Artificial Intelligence (AI) - offers numerous possibilities for assisting in breast cancer screening and prognosis. A 2019 study evaluating the ability of AI to read screening mammograms, based on nearly 30,000 women from the UK and US, found that AI helped reduce false positive rates by 5.7% and 1.2% (in the UK and US, respectively), as well as reductions in false negative rates of 9.4% and 2.7% [15]. In an independent study involving six radiologists, the AI system also yielded outstanding results: the

area under the curves (AUC) read by the AI was higher than the average AUC of the readers by 11.5%. These developments have opened up numerous prospects for the widespread application of AI in breast cancer detection and for improving diagnostic quality. However, current AI models only detect the suspicious regions containing masses on mammograms without other types of lesions such as microcalcification, asymmetry, and architectural distortion. They also lack the interpretability of the Breast Imaging Reporting & Data System (BI-RADS) used by clinicians to evaluate the magnitude of malignancy for further management plans. Recently, VinDr-Mammo [16], a newly published dataset including the broad ground truth information of BI-RADS, and lesion types promises to foster the development of more comprehensive AI models in the future. Besides other object detection models, YOLO-V7 [17], short for "You Only Look Once version 7," is a state-of-the-art computer vision algorithm. It is a deep neural network that uses convolutional neural networks (CNNs) to identify and locate objects within images. YOLO-V7 is an improvement over previous versions of the YOLO algorithm, with enhanced accuracy and speed. This algorithm has many applications, including object detection in self-driving cars and security systems; however, its application in medical imaging is limited, regardless of the potential for detecting breast cancer lesions on mammograms.

In order to address the limitations of prior studies and leverage as much as the information provided, we herein propose a multilabel object detection approach using YOLO-V7. The contributions of our work include (1) A multilabel detector to learn and predict the suspicious mammary regions with BI-RADS levels and lesion types, which is practical for physicians in clinical settings; (2) An application for physicians and patients to try the model's predictive capabilities.

KEYWORDS: Artificial intelligence; Cancer detection; Precision medicine; Oncology; Medical imaging.

References:

Due to the two-page limit, please kindly refer to this link for the references.

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A deep learning algorithm to diagnose pediatric forearm fracture based on AO/OTA classification

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Abstract:

Background: Pediatric distal forearm fractures are one of the most common types of injuries among children; as a result, it is crucial to properly detect and classify these injuries so that the appropriate treatment can be planned and managed[1, 2] . Artificial intelligence and machine learning techniques have shown great potential in improving the accuracy and efficiency of fracture diagnosis and classification as a result of their application to fracture diagnosis and classification[3, 4]. According to this study, the findings could have significant implications for the diagnosis and treatment of pediatric distal forearm fractures, which would result in better patient outcomes and a reduction of healthcare costs.

Objectives: This study aims to investigate the functionality of a convolutional neural network (CNN) based AI algorithm to detect and classify pediatric distal forearm fractures, based on the AO/OTA classification system for pediatric fractures, in an attempt to identify errors in the detection and classification of pediatric fractures.

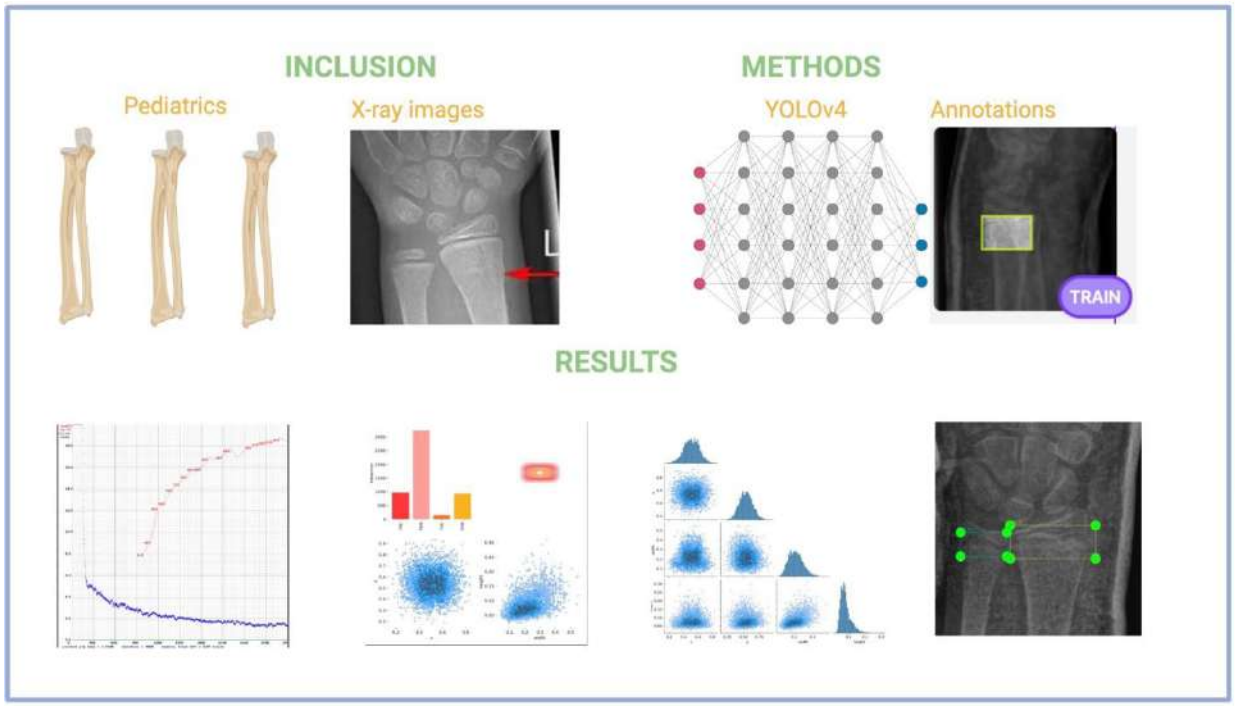
Methods: In this study, we obtained 20,327 images of wrist X-rays from GRAZPEDWRI-DX dataset published by the Department for Pediatric Surgery of the University Hospital Graz between 2008 and 2018. 6091 patients were included in this dataset. The images were labeled into 4 classes in fracture position divided into metaphysis and epiphysis in both radius and ulna depending on AO/ATO classification 2018. 2 pediatric radiologists and 2 orthopedists performed this task. We trained a CNN object detection model based on YOLOv4 with a training set of 6450 images, and a validation set of 1858 images. The model had 3000 batches with images scaled to 416x416 pixels.

Results: The results of our model indicate that the model was able to detect fractures with 0.917 precision, 0.93 recall, and an F1-score of 0.92. The average IoU is 70.75%. The mAP of 0.94 at an IoU threshold of 0.5, the most accurate precision is 0.99 with label combination FRE(radius and epiphysis), and with FRM , FUM, and FUE is 0.97, 0.80, 0.90, respectively.

Conclusion: The use of convolutional neural networks has been shown to provide clinicians with a highly accurate tool for detecting and classifying pediatric wrist fractures early in the disease process. A mobile app is also being developed as an additional application to help emergency clinicians obtain a quick guideline to treat these types of disorders by utilizing a mobile app.

KEYWORDS: deep learning, pediatric fracture, AO classification, YOLO.

Graphical abstract:



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Identification and validation of novel hypoxia-and immune-related gene signature for gastric cancer prognosis

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Abstract

Gastric cancer (GC) is ranked the fifth most common malignancy and the fourth leading cause of cancer-related death worldwide with a poor prognosis [1, 2] . Increasing evidence indicates that the presence of hypoxia and the immune status are clinically significant factors in the GC microenvironment [3, 4] . We aimed to generate a hypoxia-immune-related gene signature that may serve as an accurate prognostic tool for GC.

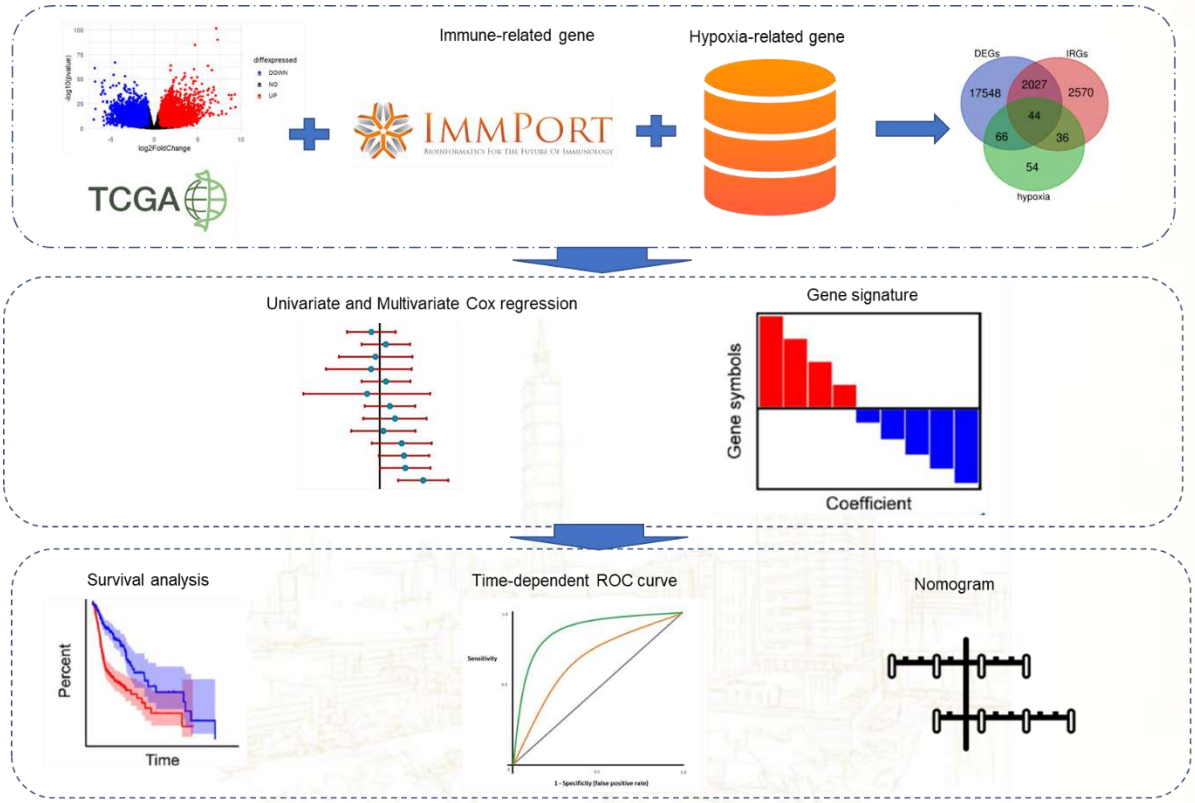
The Cancer Genome Atlas (TCGA) datasets were utilized to obtain transcriptomic and clinical data of GC samples. Next, immune-related genes from the ImmPort database and hypoxia-related genes from the Molecular Signatures database (MSigDB version 6.0) were acquired. Using univariate and multivariate Cox regression, a hypoxia- and immune-related gene signature was developed to predict the overall survival (OS) of GC patients. The signature's predictive ability was evaluated using Kaplan-Meier survival curves and time-dependent receiver operating characteristic (ROC) curves. Additionally, univariate and multivariate analyses of OS for both the risk score model and multiple clinicopathologic factors were conducted, followed by the construction of a nomogram to predict the prognosis. The results were validated using an independent validation cohort from the Gene Expression Omnibus.

The analysis yielded 44 DEGs that were identified as overlapping in both hypoxia and immune status. From this, five signature genes (INHA, TGFB3, SERPINE1, SRPX, GPC3) were selected for risk score calculated using univariate and multivariate Cox regression models. In both TCGA and GEO cohorts, the patients from the low-risk group had better OS than those in the high-risk group. Moreover, the risk score was significantly associated with T stage. Risk score is also an independent factor in predicting the prognosis GC compared to the clinical factors with

higher AUC. Ultimately, these findings suggest that the hypoxia- and immune-related gene signature holds significant prognostic potential for GC.

KEYWORDS: Gastric cancer, Immune-related genes, Hypoxia-related genes, Risk score, Bioinformatics

Graphic abstract



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Optimizing Embryo Selection in IVF through AI-Based Non-Invasive Ploidy Prediction:Integration of Time-Lapse and Clinical Data

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Abstract:

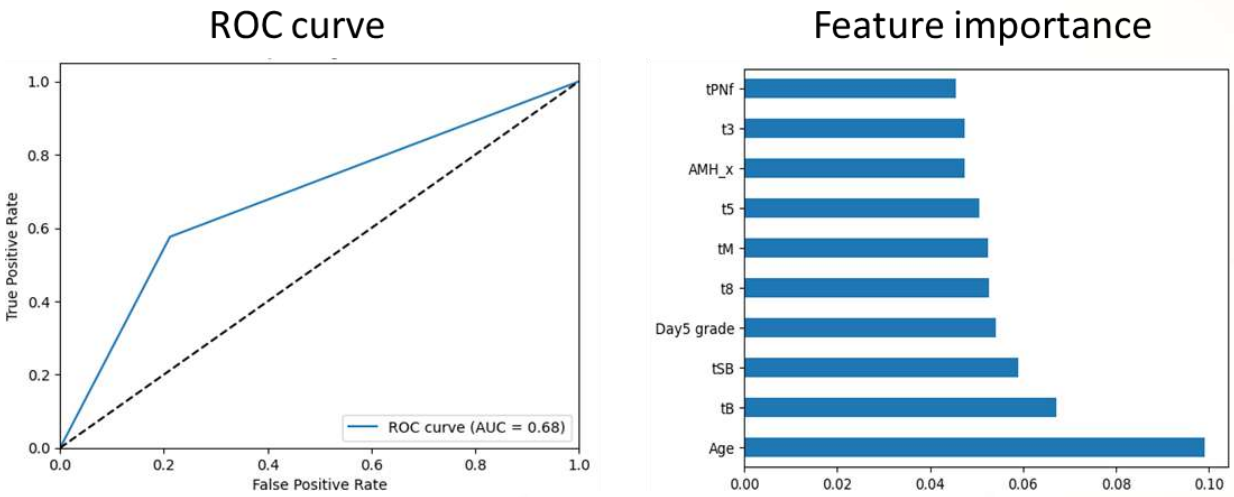
The embryo selection process presents itself as one of the chief challenges within IVF. There is a growing trend to consider the ploidy status when deciding which embryos to transfer. Both non-invasive and invasive methods are employed to select embryos with the highest potential for chromosomal normalcy, aiming to mitigate the risks associated with multiple embryo transfersand improve the chances of a successful pregnancy.

Non-invasive techniques, such as morphological and morphokinetic analysis, often require substantial time and can vary between embryologists, leading to inconsistencies. On the other hand, preimplantation genetic testing for aneuploidy (PGT-A) is associated with higher costs and potentially damaging the embryos [1]. However, artificial intelligence (AI) shows great promise as it offers a non-invasive and objective alternative. By leveraging AI, the accuracy and efficiency of predicting embryo ploidy can be optimized, thereby reducing the necessity for invasive procedures like PGT-A [2]. In this study, we developed machine learning models that integrated morphology, morphokinetic, and clinical data to predict the ploidy status of embryos at the blastocyst stage. Our dataset comprised 1,386 embryos with known ploidy results from Taipei Fertility Center in Taipei, Taiwan. Notably, all embryos underwent a biopsy irrespective of their grade or quality. The input variables for our models encompass morphokinetic parameters from tPNf to tB, morphology grade in D3 and D5, and 18 additional clinical variables. The random forest model emerged as the most effective through rigorous evaluation, exhibiting an accuracy of
0.71 and an area under the curve (AUC) of 0.68, outperforming conventional regression methods and alternative machine learning models. Feature importance analysis revealed that age and morphokinetic parameters such as tB, tSB, and D5 grade significantly impacted our predictive model. It corroborates existing knowledge regarding the influence of maternal age on embryo chromosomal integrity. Our findings indicate that an AI model incorporating embryonic morphological and morphokinetic characteristics, in conjunction with clinical data, holds promisefor accurately predicting embryo ploidy status.

Furthermore, integrating AI with time-lapse incubation presents an opportunity to revolutionize clinical workflows in assisted reproduction, backing embryologists to make informed decisions regarding embryo biopsy and reducing costs. Overall, our study highlights the potential of leveraging machine learning techniques to enhance the prediction of ploidy status, thusoptimizing embryo selection and ultimately improving outcomes in IVF.

KEYWORDS: artificial intelligence; embryo assessment; in vitro fertilization; ploidy prediction;time-lapse incubation

Graphic abstract



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Concentrates Urinary Biomarkers Via the Osmosis Processors

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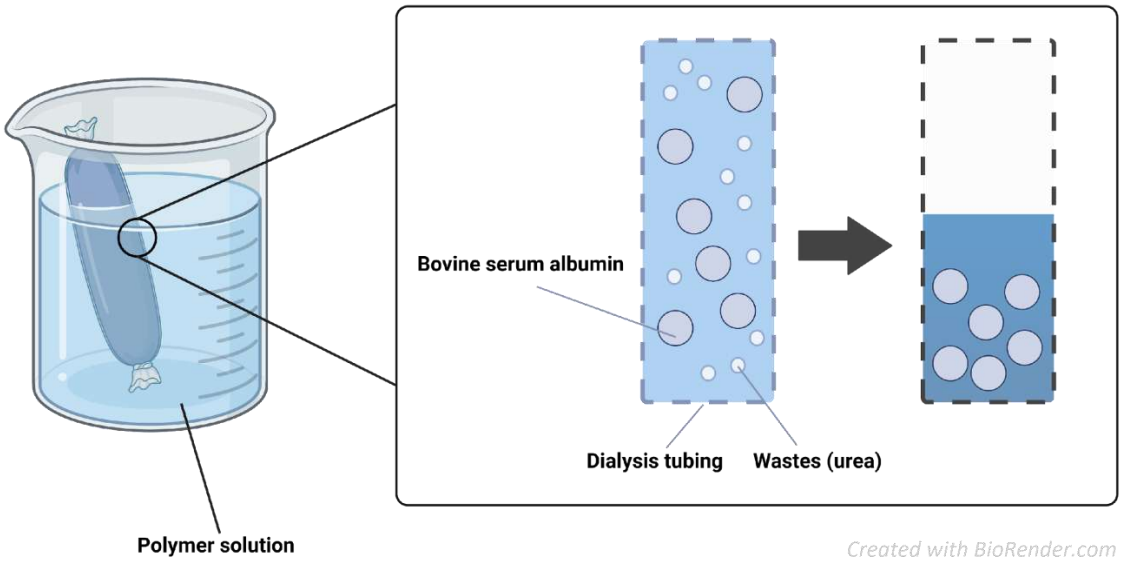
Abstract:

Urine is a type of biospecimen that is excreted from humans through the urinary system and urinary tract as a byproduct of metabolism. It contains metabolic waste products and other substances that can provide information about a person's overall health. Some research have been reported that there are various biomarker in the urine. For example, urothelial carcinoma (UC) is a cancer that begins in the urothelial cells, which is related to the urinary system. A couple of urine-based tests have been approved by Food and Drug Administration (FDA), but these tests suffer from low sensitivity and specificity [1]. Furthermore, urine composition has various substances, such as urea, protein, vitamins, electrolyte salts and ions which can be affected by different physiological variations, health conditions, as well as food consumption [2]. In addition, urine is a very diluted liquid which has low concentration of protein analytes. Therefore, it's necessary to purify and concentrate the specimen before using these biomarkers.

To address these issues, we develop an Osmosis Processor to concentrate urinary protein, purify the urine by removing the small molecules e.g., urea and reconditions the urine environment by spontaneous osmosis reaction. Urea exists in urine with relatively high concentration and is a chaotropic agent, which disrupts the solvating properties of water and potentially interfering with immunoassays. In this study, utilizing our device can easily remove 93% of the solution which original capacity is 15 mL, decrease urea concentration, adjust the suitable pH environment to enable immunoassay (e.g., ELISA) and enrich 10 times protein concentration. Polyethylene glycol was applied to drive osmotic pressure by using different molecular weight and mass concentration [3].



KEYWORDS: biomarker concentration, osmosis, polymers, biospecimen processing, urine

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Fucoidan microneedles with adjuvant effect for effectively enhancing antigen-specific immune responses

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Abstract:

The vaccine microneedle patch can deliver drug to the epidermis and dermis layer, which are rich in immune cells such as Langerhans cells and dermal DCs and induce immune response without difficult administration [1], avoid touching the nerves thereby reduce patient's pain and fear. In order to enhance the effect of vaccine, adjuvants are added as immunostimulatory agents. Fucoidan is a long chain sulfated polysaccharide extracted from various species of seaweeds, that has been found to have immunomodulatory effects by boosting innate and adaptive immunities. [2,3]

We use whole-profile fucoidan (WFU, molecular weight 10~2000k) as adjuvant and ovalbumin (OVA) as model antigen to produce dissolving vaccine microneedles (Fig. 1A), it can quickly dissolve after being pierced into the skin. The piercing depth is about 550 μm, indicates OVA-loaded WFU microneedle can successfully release antigens in the epidermis and dermis. Besides, the confocal images proved that antigen can retain in skin more than 3 days. The result of examination of major histocompatibility complex (MHC) expression on macrophages Raw 264.7 showed that WFU promoting the expression of MHC I and MHC II, activate helper T cells and thus enhance cell-mediated immunity and humoral immunity. To confirm whether immunization using WFU MNs improves the potency of the vaccine, Sprague-Dawley rats (SD rats) were immunized with subcutaneous injection (SC) saline or 130 μg OVA, microneedle (MN) containing OVA (130 μg) or OVA (130 μg) plus WFU (3000 μg). As shown in Fig. 1B, two weeks after boost vaccination, the antibody levels induced by OVA-loaded WFU MN treatment were significantly higher than those elicited by SC OVA ($p = 0.00096$) and MN OVA ($p = 0.00917$). Furthermore, compared with SC OVA, immunization using the OVA-loaded WFU MNs resulted in more robust immune responses ($p < 0.05$) after prime vaccination and lasted for eight weeks. The above results show that the WFU dissolving vaccine microneedles developed in this research, combined the advantages of vaccine microneedles and the adjuvant effect of the WFU, can effectively improve the performance of vaccine-specific antibodies and have the potential to serve as a new generation of vaccines.

Keywords: Microneedle, Vaccine, Adjuvant, Fucoidan, Transdermal drug delivery

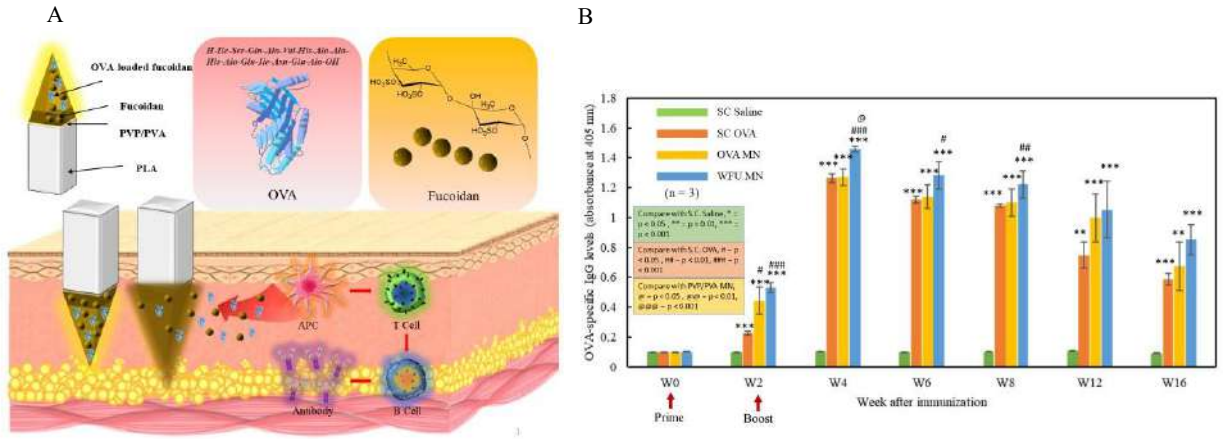

Fig. 1. A) The concept of using whole-profile fucoidan for transdermal immunization. B) OVA-specific IgG levels in serum, SC Saline: rats immunized by subcutaneous injection of 1 ml saline; SC OVA: rats immunized by subcutaneous injection of 130 μg OVA; OVA MN: rats immunized by application of microneedle patch containing 130 μg OVA; WFU MN: rats immunized by application of microneedle patch containing 130 μg OVA plus 3000 μg WFU.

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Investigating the effects of 830 nm low level lasers on ROS and melanin in melanocytes by single-cell analysis and evaluating their potential in the treatment of gray hair

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Abstract:

The main cause of senile hair graying is the aging of the melanocytes in the hair follicles, the reduction of its number, and the melanin can no longer be produced. At present, the protective mechanism of ultraviolet radiation (UVR) that causes melanocytes to produce melanin has been quite thorough. There have been researches exploring the relationship between visible light and melanocytes, but there are few related studies on the impact of near infrared red light on melanocytes. It has been confirmed that low-level laser therapy (LLLT) of red light and near-infrared light can promote tissue repair and regeneration. In recent years, low level near-infrared light therapy has been used to treat hair loss. The development potential of visible low level near-infrared light therapy is therefore obvious.

Based on the above factors, this study intends to explore the application of 830 nm near-infrared laser source to human epidermal melanocytes (Normal Human Primary Epidermal Melanocytes, HEM) with low-level laser light therapy and observe its photobiomodulation with a TE2000-U inverted microscope equipped with microchannels. Observation content includes followings:

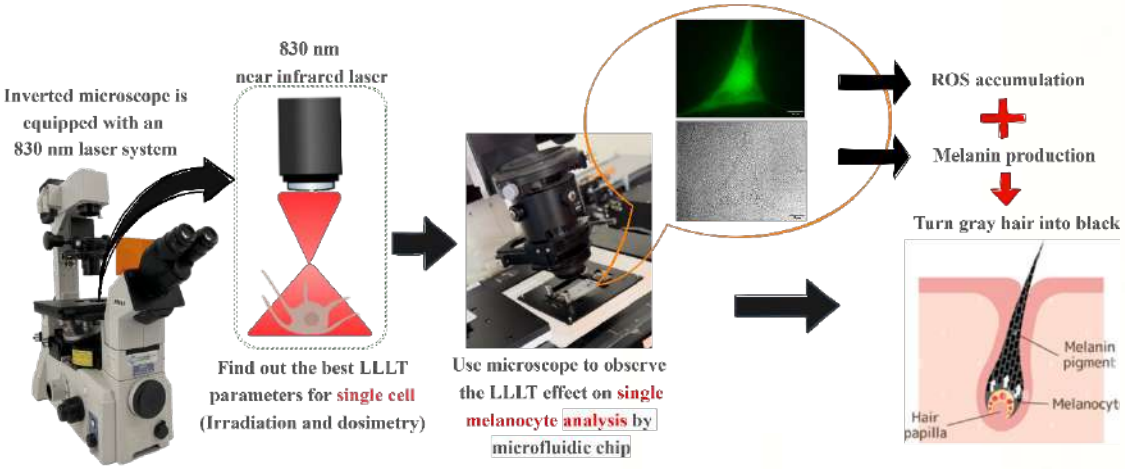
1. Cell viability after receiving low level near-infrared light therapy.
2. Real-time observation of changes in reactive oxygen species (ROS) in the mitochondria of melanocytes at the single cell level, marked with H_2DCFDA dye.
4. Discuss the relationship between ROS and changes in melanin, and evaluate the feasibility of using this relationship as a treatment for hair graying.

In this study, we calibrated the 830 nm laser system and calculated the actual output power of the laser passing through the objective lens, the lid of the 96-well cell culture dish, and PBS. We then used the photosensitive coupling device combined with

LabVIEW to calculate the laser irradiation area. We use 0 J/cm^2, 1 J/cm^2, 2 J/cm^2, 3 J/cm^2 dosage, and 25 mW/cm^2, 79 mW/cm^2 power density to treat melanocytes respectively with low-level laser, and finally use CCK-8 reagent to detect cell viability. The experimental results show that at the irradiance of 25 mW/cm^2 , there are no significant difference among the three groups of 0 J/cm^2, 1 J/cm^2, and 2 J/cm^2; The 1 J/cm^2 and 2 J/cm^2 groups will cause cell damage, where the cell viability decreased by 15% and 25% respectively compared to the 0 J/cm^2. However, the cell viability of the 3 J/cm^2 group slightly increased compared to the 0 J/cm^2.

This project also uses 25 mW /cm^2 irradiance and 1 J/cm^2 dosage to perform low-level laser treatment on single-cell biological level melanocytes in microfluidics. The average fluorescence intensity of ROS in most cells increased significantly after irradiation, proving that a low-level laser with a dosage of 1 J/cm^2 can indeed lead to an increase in the ROS generation, but according to our CCK-8 test, the same 1 J/cm^2 dosage could not increase the cell viability. This part still require more experimental data to determine the optimal light dose parameters to achieve the best balance between cell activity and ROS generation. In the bright field, we still cannot easily distinguish the obvious change of melanin secretion 30 mins after LLLT. In future research, it could be beneficial to use the secondary antibody immunofluorescence staining method to mark tyrosine (which is the precursor of melanin) related proteins, such as TRYP 1 or utilize live cell imaging, which will have a better understanding of the relationship between ROS and melanin in living single cell. This could further explore the feasibility of near-infrared light as a low level laser treatment for hair graying.

KEYWORDS: Grey hair, Low level laser therapy (LLLT), Melanocyte, Melanin, Near-infrared laser, Reactive oxygen species (ROS)



Schematic diagram of the study

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Aug. 31 – Sep. 3, 2023, Taipei, Taiwan

Machine learning for drug response prediction in lung cancer cell lines

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Abstract

Lung cancer is the most common type of cancer in both sex and leading cause of death globally. Despite efforts to improve early diagnosis and treatment approaches, lung cancer patients show the lowest survival rate among all cancer types. Personalized treatment strategies are currently being pursued, and one of the main challenges is predicting drug response for cancer patients. Although previous studies have attempted to develop machine learning (ML) models for drug response prediction, accurate prediction specifically for lung cancer patients and lung cancer cell lines remains limited [1, 2].

In this study, we aim to construct a model for predicting drug response in lung cancer cell lines by integrating comprehensive data including drug features and drug sensitivity. To achieve this aim, we first collected drug sensitivity data from Genomics of Drug Sensitivity in Cancer (GDSC) that included lung adenocarcinoma (LUAD) dataset including 62 cell lines interact to 288 drugs and squamous cell carcinoma (LUSC) dataset including 14 cell lines interact to 288 drugs. Then, we gathered the simplified molecular input line entry system (SMILES) from three sources including DrugBank, PubChem, and MedChemExpress [3, 4]. To construct our model, we first

constructed based model by using seven common ML classifiers (random forest, Adaboost, support vector machine, decision tree, k-nearest neighbors, naïve bayes, and logistic regression). Model performance was evaluated using 10 cross-validation and confusion matrix analysis. Then, to enhance model performance and identify important features, we employed three feature selection techniques, including feature importance analysis using coefficients and recursive feature elimination (RFE). Our optimal model indicates that the random forest classifier is the most effective algorithm for predicting drug response in lung cancer cell lines, achieving an accuracy of 0.79 on the LUAD dataset and 0.80 on the LUSC dataset. The coefficient technique identified STIP1 mutation as the most important feature, with a coefficient of 0.44, while RFE highlighted KRAS mutation and CDKN2A copy number loss as the most influential features.

This study demonstrates the potential of machine learning in advanced drug response prediction. Machine learning can contribute to improved personalized treatment strategies for lung cancer patients and further apply in others cancer types.

KEY WORDS: Drug response prediction, KRAS mutation, Machine learning, Personalized medicine, Precision medicine, Random forest.

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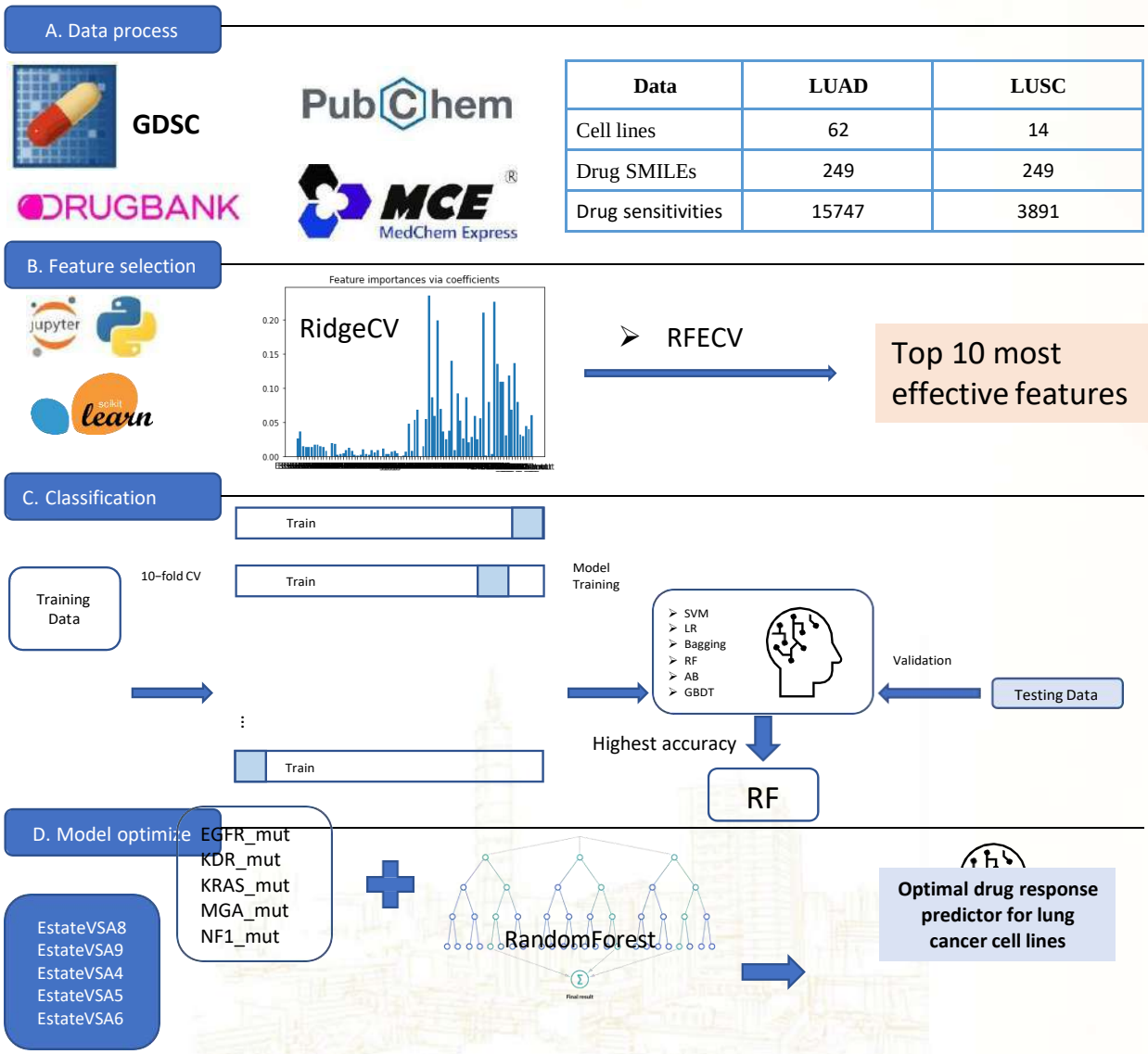
Graphical abstract/ Framework



Data	LUAD	LUSC
Cell lines	62	14
Drug SMILEs	249	249
Drug sensitivities	15747	3891

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ddPCR test of AI automated counting for COVID-19

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Abstract:

The COVID-19 [1] is an infectious disease caused by the most recently discovered coronavirus. The novel coronavirus outbreaks a public health emergency of international concern [2] for 4 years. Testing is important to help people reduce the spread of emerging variants of COVID-19 [3], and it is one of the pandemic prevention measures being taken by governments around the world. The accuracy and reliability of COVID-19 diagnostic tests is important to inform diagnosis and to ensure citizens can make the right decisions about their health. Polymerase chain reaction (PCR) [4] is a test to detect genetic material from a specific organism, such as the coronavirus. The PCR test can be used to diagnose people infected with COVID-19, and it is considered the most accurate available until now. ITRI researchers have engaged in the research of the PCR test owing to the global epidemic situation getting worse. There are different types of PCR, such as endpoint PCR, quantitative PCR (qPCR), and digital PCR (dPCR). They include the same reaction dynamics, but the ways the PCR products analyzed are different. Droplet dPCR (ddPCR) is a method for performing dPCR based on a sample that is fractionated into thousands two-phase liquid droplets, and PCR amplification will occur in each individual droplet.

Our experimental apparatus utilized the ddPCR technique is shown in the image above. It presents the research findings with Artificial Intelligence (AI) [3, 5] image recognition for integral characteristics of a process developing, when droplets are generated from the microfluidic device in a thermocycler. When a saliva (or paired nasopharyngeal) sample collected for a COVID-19 PCR test is sent into the experimental setting, the samples will be partitioned into many droplets. After repeated thermal cycling is performed for target amplification, we can detect the overall distributed fluorescence signals of droplets from a reaction to measure the amount of coronavirus DNA in a sample. An optical instrument that captures visual images is essential to detect the fluorescent light emitted from the droplets for ddPCR technique. Images captured by the camera built into the thermocycler can be analyzed with a powerful AI recognition system. Displaying location and counting number of each droplet within the images are intended to be done in the system.

Visit the URL link below. The video shows the process of identifying a few circular pieces of paper with a live stream. When we put the circular pieces of paper onto the table one by one, their images are captured by the camera of the top smartphone. A live video feed will be streamed from the smartphone to laptop over a wireless internet connection. Each circular piece of paper will be marked with bounding boxes for real-time AI inference, and the counted number will be displayed on another laptop's screen automatically. This concept of the experimental setup can be applied to the automated counting of droplets for the ddPCR platform. Therefore, an efficient detection of fluorescence signals of droplets may be accomplished due to the AI image recognition technology. With the specific technique, we can find how many fluorescent droplets and total droplets are in a sample, and it is very beneficial for DNA quantitative analysis of the ddPCR.

Keywords: Artificial intelligence, Image recognition, COVID-19, Polymerase chain reaction

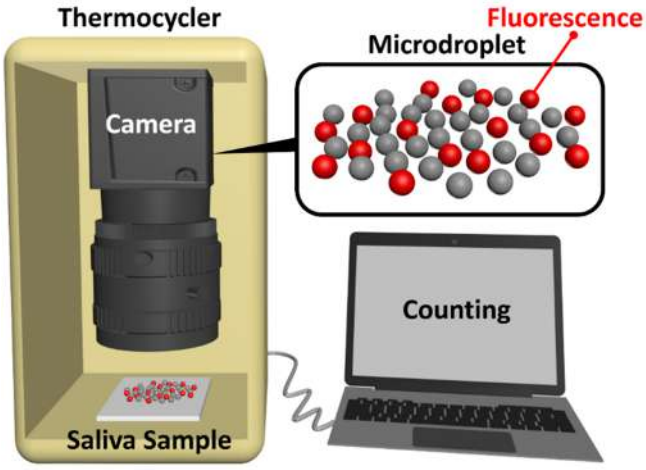
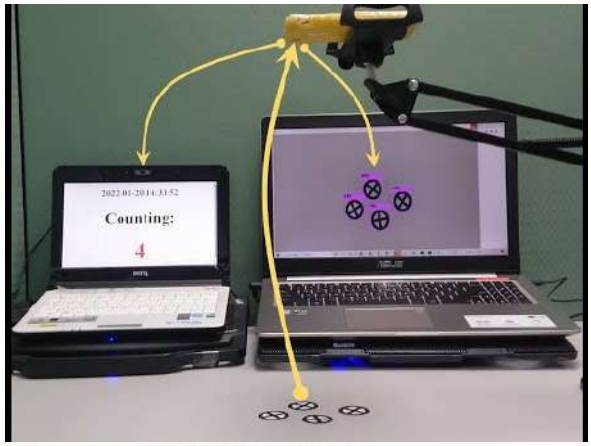


Fig.1. Sensitive ddPCR platform with image recognition for detecting COVID-19.



https://www.youtube.com/watch?v=EfpATr3KNfM

Fig.2. AI Counting Application enables the automated counting of droplets for the ddPCR test of COVID-19.

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Detection of Blood by Utilizing the Surface Enhanced Raman Spectroscopy Technique with The Help of Gold Nanorods And Silver Nanoparticles

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Abstract:

The detection and analysis of stains in forensic science labs and crime scene investigations are critical for determining variables related to a crime and those involved. Analyzing samples quickly and non-destrucitve, even when they are mixtures and small quantities, is essential for effective investigations. Non-destructive, fast, and cost-effective techniques are highly important in analyzing evidential materials, and spectroscopic methods have advanced significantly in recent years with the development of light detectors. This study focused on using Raman and Surface Enhanced Raman Spectroscopy (SERS) for detecting pure blood mixed stains. To reduce the fluorescence effect caused by the matrix components of the blood, Raman analyses were performed after dilution into four different concentrations. It was observed that reducing the concentration decreased the noise ratio and improved the detectability of the peaks. A simulated crime scene was created with using cherry juice to test the detectability of blood in a complex mixture of stains. Despite low peak intensity, the presence of blood was still detectable in the Raman spectrum.

The SERS spectrums of the blood samples were examined with gold nanorods and silver nanoparticles, and the latter provided greater signal enhancement due to their strong plasmonic properties. In conclusion, it has been determined that SERS is a potentially valuable tool for rapid and sensitive detection of blood.

KEYWORDS: Nanoparticles, Surface Enhanced Raman Spectroscopy, Forensic Sciences, Bionanotechnology, Identification of biological fluids.

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*: *This study bases on a part of doctoral/PhD thesis conducted at Hacettepe University, Institute of Science and Technology, Nanotechnology and Nanomedicine Division by Uğur Köroğlu.*

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Bacteriophage grafted superparamagnetic nanoparticle for detection of Shiga-toxin Escherichia coli (E.coli O157:H7)

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Abstract:

The number of bacteria in drinking water is one of the best biological indicators of water safety. Commercially available magnetic microbeads that are used to detect specific bacterial strains are grafted with bacterial antibodies, which bind with antigens to detect bacteria in the water or food. The production of antibodies is costly, and most of them function in narrow ranges of pH and temperature. The aim of this research was to produce a highly-specific strong-binding nanoprobe that could detect living bacteria from contaminated samples to avoid false positives. They were designed to be cost effective and could detect bacteria in samples in a wide range of samples.

We have successfully identified shiga-toxin producing bacteria (E coli O157:H7) and the bacteriophage (phage) that targeted this bacterial host from samples collected from sewage treatment plant. The phages were chemically grafted onto superparamagnetic iron oxide nanoparticles coated with chitosan to make E coli O157:H7 specific magnetic nanoprobes.

The following experiments on the phages were carried out to find its reduction of bacterial growth, morphology (transmission electron microscopy TEM), specificity, and lytic activity in various pH's. The experimental results show that the phage could survive in the environment of pH=4 to 10, had a latent period of 15 min, and belonged to the family of Siphoviridae with B1 morphotype.

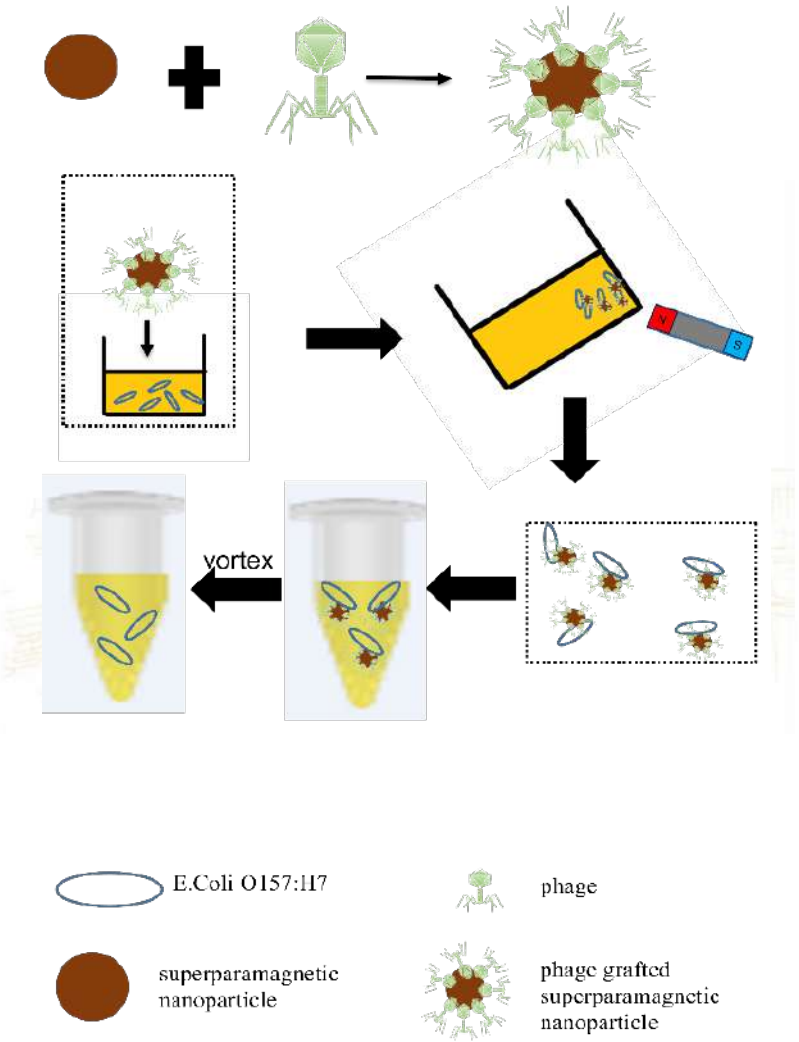
The superparamagnetic iron nanoparticles coated with chitosan were prepared by co-precipitation method. The chitosan on the nanoparticles was treated with MES and EDC/NHS before reacting with the phages and forming the nanoprobes. Fluorescent staining of the phages and TEM were used to confirm the presence of phages on the nanoprobes. The results showed that the phages were linked to the nanoprobes as the fluorescent phages moved with the nanoparticles toward a magnet under a microscope. Without the grafted phages the nanoparticles did not emit fluorescence.

The detection limit of the nanoprobe was tested by counting the numbers of bacterial cells extracted by 10 mg of the nanoprobes from bacterial solutions with concentrations ranging from 10^1 to 10^6 CFU/ml. The detection limit of 10 mg of nanoprobes was 10^2 CFU/mL. The increase

of the amount of probes from 10 mg to 50 mg lowered the detection limit to 10^1 CFU/mL.

The commercial magnetic microbeads cost tens of thousands of NT dollars. In this study, the phages were extracted from wastewater, and the synthesis of chitosan-coated nanoparticles required inexpensive and common chemicals. It is believed that if this probe can be further modified and improved in the future, it can become a powerful and economic option for detecting pathogenic bacteria in the environment.

KEYWORDS: Bacteriophage, chitosan, iron oxide nanoparticles, Escherichia coli (E.coli O157:H7), magnetic nanoprobes



Schematic Illustration : Escherichia coli O157:H7 Detection method via Bacteriophage grafted superparamagnetic nanoparticle separation

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

An ultra-thin soft electrode combined with portable multi-channel EEG acquisition system

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(Underline the name of the presenting author. The corresponding author(s) should be marked with an asterisk in the author list.)

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Abstract:

Electroencephalography (EEG) is a widely used technique for diagnosis of Epilepsy or other brain disease. Weak EEG signals due to artifact, environmental noise, patient discomfort limit the current application in clinics. The commonly used strategy is to incorporate EEG gel between skin and electrodes to stabilize signal transduction, while the viscous gel makes patient uncomfortable and has difficulty to be completely removed. In addition, the instrument used for EEG measurement in clinic is large in volume, which limits the life convenience of the patient in the treatment. Here, we proposed a new EEG recording system with high reliability and signal performance with combined design of an ultra-thin stretchable electrode arrays (Fig. 1) and a portable multi-channel EEG acquisition system (Fig. 2). The in-situ polymerization of dopamine (Dopa) in the synthesis of Pt-modified Ag NW based on Galvanic reaction resulted in the formation of inorganic/organic hybrid core-shell NW with robust conductivity, antioxidation ability and adhesive ability. Dopa with naturally occurring adhesive moiety imparts the adhesion between NW and PDMS, which benefits electrode manufacturing by transfer printing using an ultrathin PDMS with thickness < 150 μm to increase device-skin conformability. Combined with a new portable 8-channel EEG acquisition system with a small size of only 85*56*16.5 (mm*mm*mm) and the weight less than 40 grams, the state-of-the-art EEG recording system provides high signal quality with tiny value, lower cost and lower power consumption as compared with those derived from the clinically-used system.

KEYWORDS: Silver Nanowire (AgNW), Polydimethylsiloxane (PDMS), soft electrode, Electroencephalography (EEG)

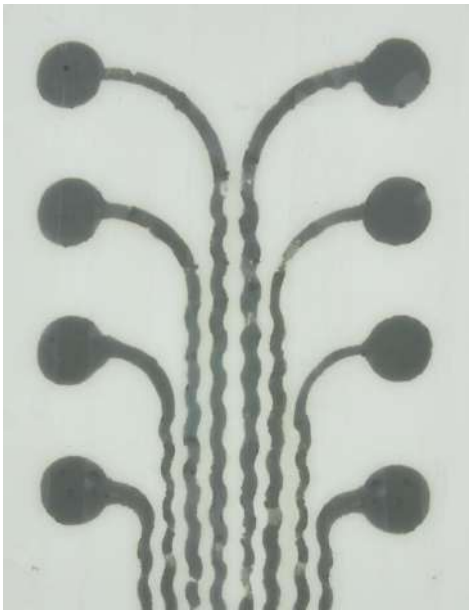

Figure 1. Ultra-thin stretchable electrode arrays

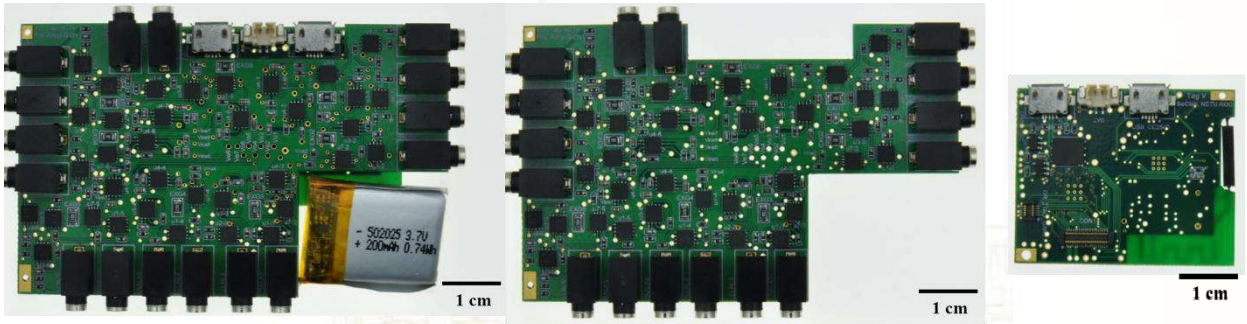

Figure 2. Portable multi-channel EEG acquisition system

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Using Multifunctional Hydrogel Conductivity to Detect Antibacterial Activity

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Abstract:

Hydrogels with high mechanical strength and good antibacterial ability are one of the focal points of biomedical materials. Here, we prepare hydrogel made of polyvinyl alcohol (PVA) cross-linked with borax and konjac glucomannan (KGM), which we named PKB3 hydrogel. Hydrogen bonds of polysaccharides from KGM and the borate bonds from borax might lead to the forming of hydrogel with high mechanical strength (96 kPa, 1041%) and good self-healing efficiency (74%). The PKB3 hydrogel was added with flower-shaped silver nanoparticles (F-AgNPs) to form PKB3/F-AgNPs hydrogel to endow it with good antibacterial ability and electrical conductivity, which can also increase the stress of the hydrogel (120 kPa). We further confirm its ability to be applied in antibacterial assessment by exploring its electrical conductivity before and after antibacterial experiments. Using electrical conductivity to detect antibacterial activity might reduce the complicated and high-cost antibacterial experiments using microbial culture. In order to increase the antibacterial ability of the hydrogel, we also discussed the antibacterial ability of the hydrogel before and after blue light irradiation. Because of the excellent softness and electrical conductivity of PKB3/F-AgNPs hydrogel, it can be perfectly attached to the human skin to detect skin epidermal movements such as skin deformation, facial expression, and throat vocalization. Therefore, besides its antibacterial detection ability, it also has the potential for a human body sensor.

KEYWORDS: hydrogel, self-healing, antibacterial, konjac glucomannan, body sensor

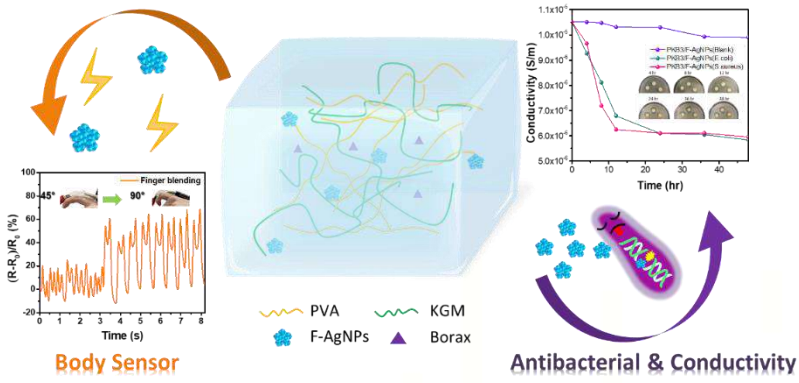

Figure 1. Schematic of the multifunctional PKB3/F-AgNPs hydrogel.

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Using a FET biosensor to measure the binding affinity between resveratrol and the serotonin 5-HT_{2A} receptor

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Abstract:

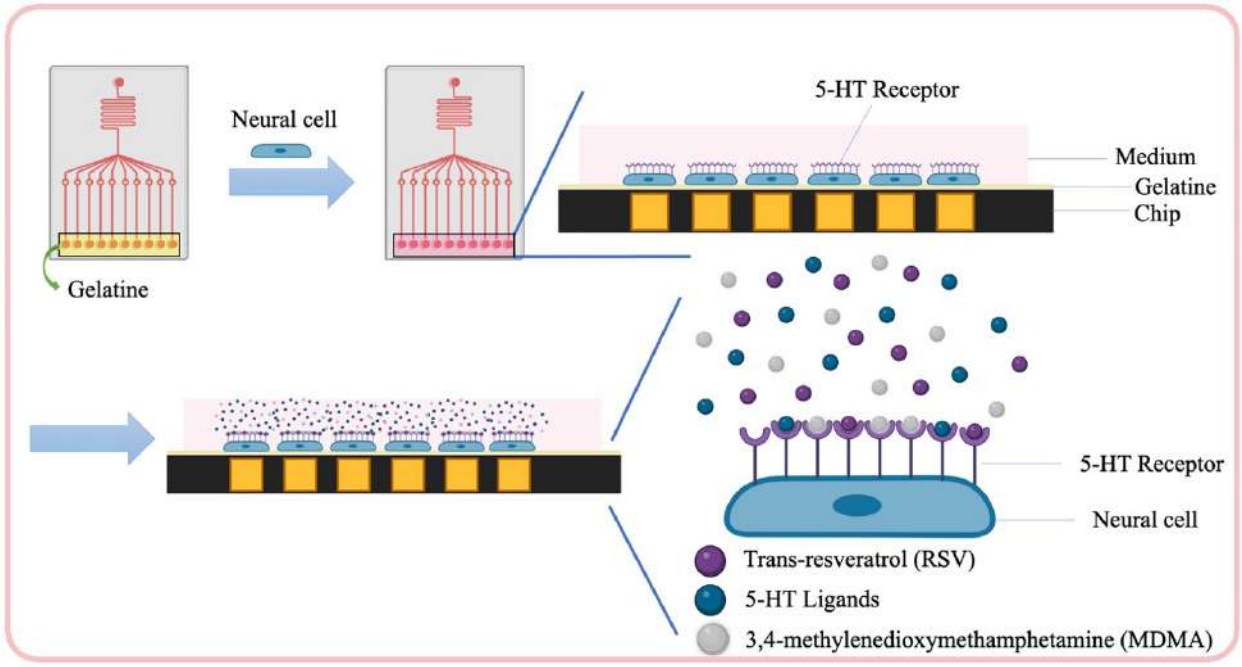
The 5-HT_{2A} receptor is a member of the serotonin (5-HT) receptor family. 5-HT_{2A} is a subtype of serotonin receptor that is primarily found in the central nervous system. It is a G protein-coupled receptor that binds to the neurotransmitter serotonin (5-HT) and plays a role in modulating various physiological and behavioral processes, including mood, cognition, perception, and hallucinations. The 5-HT_{2A} receptor has been implicated in the mechanism of 3,4-Methylenedioxymethamphetamine (MDMA)-induced toxicity [1].

Resveratrol, a natural polyphenolic phytoalexin found in plants, possesses functions such as antioxidant, anti-apoptotic, and anti-inflammatory activities. Cellular experiments have demonstrated that resveratrol can attenuate MDMA-induced neurotoxicity by mitigating neuronal apoptosis, thereby exerting neuroprotective effects. Animal studies indicate that resveratrol has the capability to competitively bind to serotonin (5-HT) binding sites, countering the effects of MDMA [2,3]. For continuous detection of drug effectiveness, the conventional techniques have several limitations when it comes to long-term monitoring. Therefore, a stretch-out electrical double layer (EDL)-gated field-effect transistor-based biosensor (FET biosensor) has been developed [4]. FET biosensor were utilized to amplify electrophysiological signals from the neuroblastoma cells.

In this study, we utilized FET biosensor to establish a drug screening platform and to in-vitro measure the binding affinity between 5-HT ligand of Neuro-2a cells and resveratrol, which has stronger binding affinity with in-vitro 5-HT ligand than MDMA did. We also proposed a model to represent the signal response of drugs on the cells. The gelatin was coated on the sensors as an adhesive layer for xxx cell attachment. FET biosensor successfully validated the binding affinity between RSV and the 5-HT receptor.

KEYWORDS: FET biosensor, Neuro-2a cells, Resveratrol, 5-HT_{2A} Receptor

Graphic abstract



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of an easy-to-use solid phase nucleic acid extraction device for next step isothermal amplification and CRISPR detection of target sequences

Yun-Hsien Chung (鍾運賢)[1,#], Chi-Ting Chen[2,#], Tao-Yun Yen[1], Peng-Wei Hsu[3,*], Hsien-Wen Yao[3], Chen-Han Huang[4], Chia-Chih Chang[2,*], Hsing-Ying Lin[1,*]

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Abstract

Nucleic acid, one of the important biomarkers in molecular diagnosis, can perform disease diagnosis that is more accurate than traditional methods. For instance, there is pseudo-progression, an increase in tumor size but a decrease in tumor border, may occur in glioblastoma, a type of malignant brain tumor by using traditional method to diagnose[1]. Although using nucleic acids for diagnosis can provide more accurate results, the detection process is limited by the equipment such as centrifuge. To solve the problem, a point-of-use (POU) diagnosis device is developed. The device can perform nucleic acid extraction, amplification, and detection from the specimen. In this study, a solid phase nucleic acid extraction method with syringe is reported. The method uses polymer bead functionalized with carboxyl group to capture nucleic acid. After nucleic acid purification, reverse transcription recombinase polymerase amplification (RT-RPA), an isothermal nucleic acid amplification method, will be performed. To report the result of nucleic acid amplification, CRISPR-associated enzyme will be used, which will hydrolyze nearby certain nucleic acid with reporter and releasing signal once nucleic acid with fluorescent dye[2]. Taking Cas12a (CRISPR associated protein 12a) as an example, when Cas12a-gRNA (guide RNA) complex binds to the target, it would cleave nearby single-stand DNA[3]. Our device for diagnosing at point of use may perform rapid purification and amplification process and provide accurate result.

KEYWORDS: Brain tumor, polymer bead, nucleic acid extraction, recombinase polymerase amplification, isothermal amplification, CRISPR

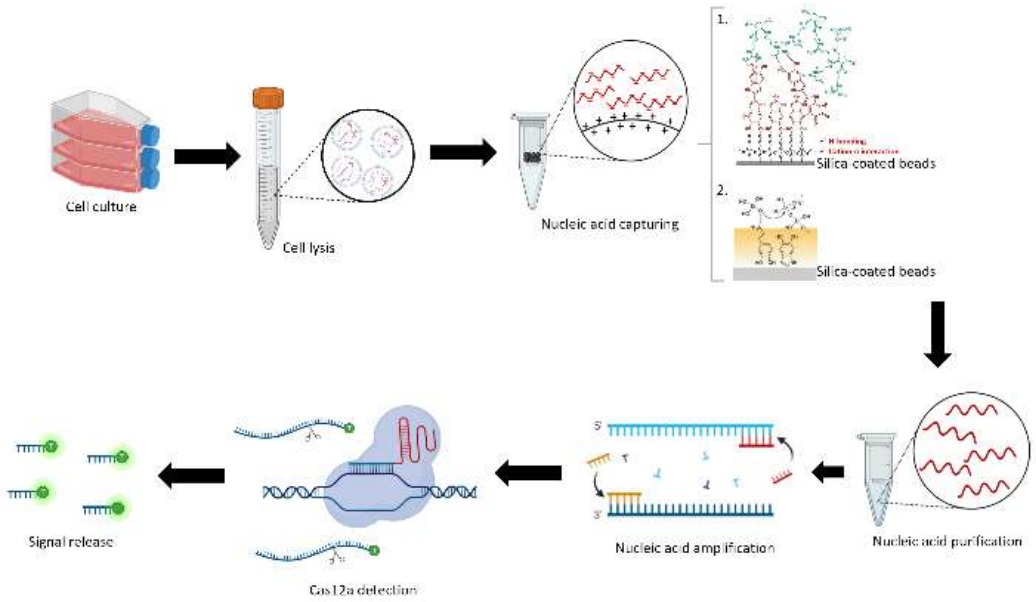

Fig. 1. The developed solid phase nucleic acid extraction device for the next step isothermal amplification and CRISPR-Cas12a detection of target sequences.

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Establish isothermal reverse transcriptase recombinase polymerase amplification (RT-RPA) for quick and highly sensitive nucleic acid analysis on a microfluid chip

Tao-Yun Yen[1](閻道蘊), Yu-He Liu[1], Li-Hsien Ho[1], I-Hsuan Chou[1], Zhi-Yang Wei[1], Yi-Chien Chen[1], Yun-Hsien Chung[1], Wei-Chun Lan[1], Hsien-Wen Yao[2], Peng-Wei Hsub[2,*], Chen-Han Huang[3], Hsing-Ying Lin[1,*]

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Abstract:

To overcome the requirements for complex equipment, researchers have developed technologies for a single-step, isothermal amplification of nucleic acids known as recombinase polymerase amplification (RPA) [1]. The currently commercialized ddPCR equipment is expensive, the operating technique is less popular, and fluorescence detection of images also takes a certain amount of time [2].

Glioblastoma (GBM) is an aggressive type of brain tumor that can be difficult to treat and diagnose due to the blood-brain barrier. Although monitoring and making a preliminary diagnosis of GBM is a major problem in clinical practice, liquid biopsy diagnosis is often not a clinical option. We attempted to approach glioblastoma (GBM) by analyzing exosomes from liquid biopsy. By using extracellular vesicles (EVs) as biomarkers, this study could provide noninvasive diagnosis with digital amplification through biosensors. This creates a platform with great potential for brain cancer diagnosis.

We have developed a) an isothermal real-time recombinase polymerase amplification (RT-RPA) system to amplify RNA samples; b) rapid analysis of amplified RNA combined with CRISPR Cas12a enzyme. And to accomplish the experiment as mentioned above, we also developed a droplet digital polymerase chain reaction (ddPCR) machine capable of detecting droplets with fluorescent signals generated through a miniature microfluidic chip.

Our results demonstrate that the data provide real-time analysis of fluorescence initiated through total 40 minutes of isothermal PCR cycles. Then, we can compare exosome nucleic acid and protein samples using a digital imaging sensor.

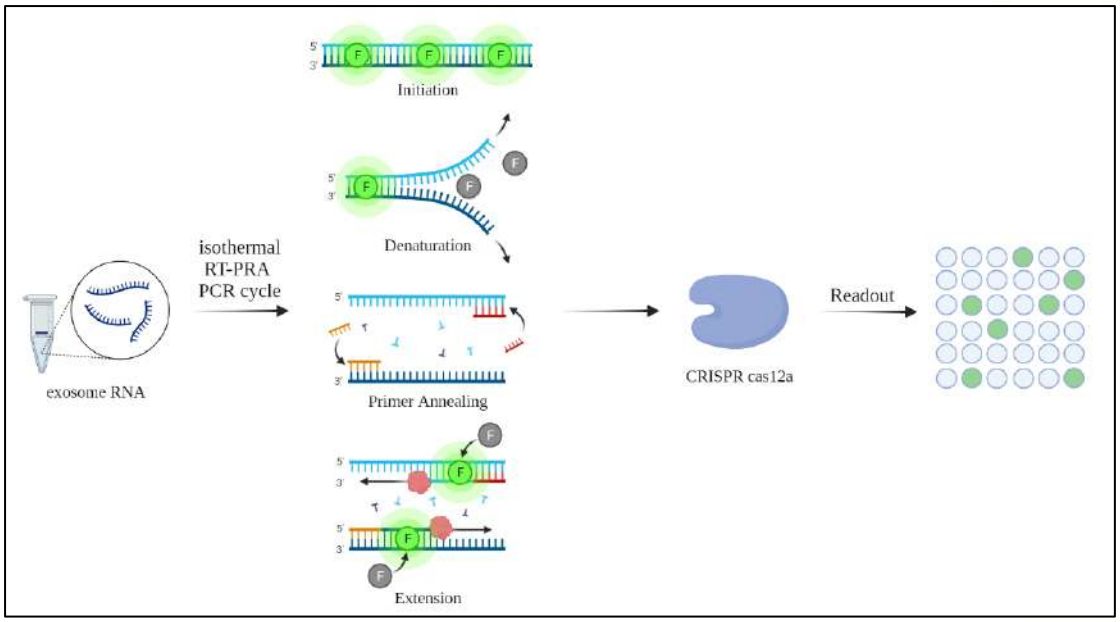

Fig 1. RNA sample through isothermal RPA step and CRISPR cas12a analysis.

KEYWORDS: Glioblastoma (GBM), recombinase polymerase amplification (RPA), ddPCR, CRISPR Cas12a

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Development of a microfluidic device for high-resolution liquid biopsy screening with an isothermal control

Wei-Chun Lan[1](藍偉峻), Yi-Chien Chen[1], Yu-He Liu[1], Zhi-Yang Wei[1], Li-Hsien Ho[1], Tao-Yun Yen [1], Yun-Hsien Chung[1], I-Hsuan Chou[1], Hsien-Wen Yao[2], Peng-Wei Hsu[2,*], Chen-Han Huang[3,*], Hsing-Ying Lin[1,*]

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Abstract:

Droplet digital polymerase chain reaction (ddPCR) is an extremely high-sensitivity technique for the absolute quantification of target nucleic acids. However, the commercial ddPCR equipment is too expensive and takes a certain amount of time to detect the fluorescence of images.

To solve the problem, we proposed a microfluidic droplet image detection system that can decode high-resolution images on microfluidic chips. It composes of a microfluidic cartridge, an image processing procedure, an embedded system, and a cloud database.

We have developed a microfluidic chip designed to be small and capable of detecting the concentration of relevant mRNAs in microscopic samples. Using photolithography with SU8 negative photoresist to retain the shape of the microfluidic channel on a silicon wafer. Then, using PDMS to obtain the channel by replicating them. Attaching the PDMS to a glass slide with oxygen plasma to form a microfluidic chip. Single and high-throughput chips are designed to accelerate droplet generation by adjusting the rates of oil and water phases. The system generates droplets on a microfluidic chip to separate samples into multiple independent units, allowing for absolute quantification of samples. Besides, using ESP32 controls the heating plate's temperature in the interval from 37°C to 42°C to do Recombinase Polymerase Amplification (RPA). TMP126 is used to be the temperature sensor to detect the temperature of the heating plate. With a friendly user interface (UI) on a touch panel and an app on a smartphone, the system allows for data collection via Bluetooth and cloud storage for real-time analysis. Results of the single channel microfluidic chip (10 mm × 25 mm) show that it can produce more than 25,000 droplets within 3 minutes. As a result, we estimate the high-throughput microfluidic chip can produce 32 times more than a single-channel chip. This portable microfluidic sensing platform features its inexpensive and low time cost, it can be used for early cancer diagnosis and treatment surveillance.

KEYWORDS: ddRPA, microfluidics, isothermal control, photolithography

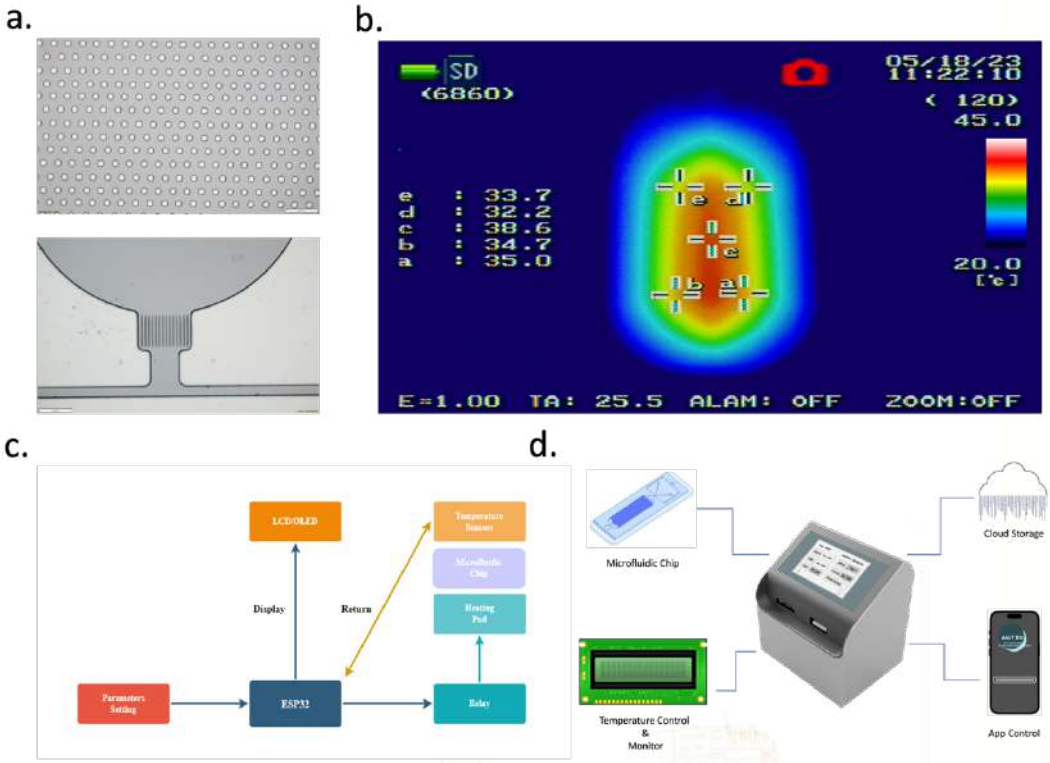

Fig. 1. (a) Image of fabricated wafer. (b) Heating pad of the thermal imager. (c) Flow chart of the isothermal control system. (d) A schematic diagram of the compact system.

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Silver Nano-island Arrays Deposited on Cicada Wings for Raman Enhancing Detection

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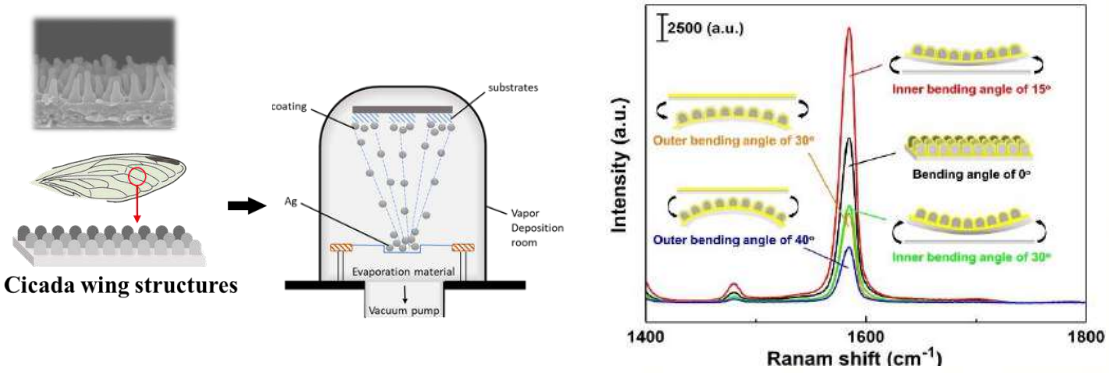
Abstract:

Surface enhanced Raman spectroscopy (SERS) detection is an analytical technology that has attracted much attention. This technology combines the "fingerprint" and rapid sensing technology of noble metal nanoparticle array and Raman spectrum. Recently, the flexible and wearable sensors are extensively developed in advanced coating materials, especially for label-free and culture-free rapid detection. This study focus on natural cicada wings (CW) are used as biomimetic templates for thermal evaporation treatment on cicadas. Cicada wings with 3D regular structure have excellent properties such as easy to obtain, regularly arranged nano arrays, low cost, super hydrophobic and environmental protection. We successfully fabricated the bionic 3D SERS substrates by depositing Ag nano-island arrays on the homogenous nano-columnar structures of CW substrate. The finite-difference time-domain (FDTD) simulation would be used to demonstrate the hot-spots effects of SERS detection. The bionic 3D SERS substrates exhibited the highest S/B ratio and SERS enhancement effect in the SERS detection due to the strong 3D hot spot effect. In addition, SERS intensity of CW-Ag-100nm would increase ~1.7 times while bending inner angle achieves 15°, displaying its flexible characterization for bio-sensing.

KEYWORDS: flexible and wearable sensors, cicada wings, SERS detection, Ag nano-islands, bio-environmental detection, FDTD simulation



- Ag nano-island arrays were deposited on bionic 3D SERS substrates (cicada wings, CW) by thermal evaporation for SERS detection.

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Low cost paper-based glucose sensor prepared by using commercial printer

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Abstract:

Diabetes is one of the most widespread chronic diseases, which affects hundreds of millions of people and is one of the main causes of death in the world[1]. Monitoring blood glucose level plays an important role in the treatment and management of diabetes. Due to the incurable nature of diabetes, patients need a large number of test strips for daily blood glucose monitoring. The traditional fingertip blood measurement method requires patients to endure frequent blood collection pain, and the measurement strip is also expensive. In the long run, this will bring huge discomfort and economic burden to patients. In addition, most of the materials used for glucose test strips are synthetic polymers[2] (PDMS, PET, PI), which are difficult to dispose of and cannot be recycled. Therefore, finding low-cost, degradable and green replacement materials is urgently. In recent years, paper has been widely used for its many advantages, in addition to its low cost: it is presented as a lightweight sheet, making it easy to transport and store; its foldability allows for multi-layer/folded paper designs; its porosity allows for bio/nano modifications as well as fluid transport; it also has good biodegradability and biocompatibility, which can be used for the development and application of flexible glucose sensing test strips[3].

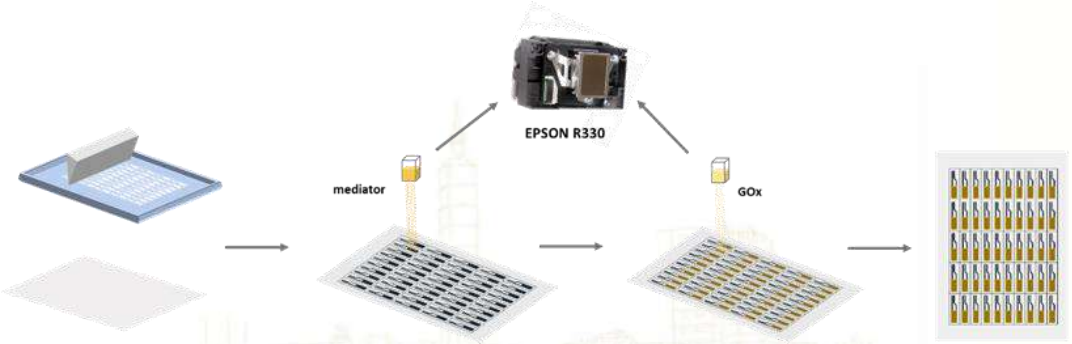
Currently, the preparation of glucose test strips is still limited to laboratories or factories. And there is no simple way can be used for producing glucose sensor test strips at home. Commercial printers, as essential tools for family life, have the advantages of simplicity, automation, and batch output. It is a very suitable equipment for batch and small-scale production of glucose sensing strips.

This study aims to provide a simple way to produce a green, low-cost paper-based glucose sensor by commercial printer. Here, we used paper as the electrode substrate, prepared carbon electrodes by screen printing, and used an Epson printer (R330) to immobilize the mediator ($K_3Fe(CN)_6$) and the Glucose Oxidase (GOx). In printer ink, add 20% glycerol to make sure the ink can be smoothly ejected from the nozzle. After that, Observation of morphology is using a microscope and electrochemical characterization is using an electrochemical workstation

(CHI660E). The experimental results show that the sensing electrode has clear edges, no pores, and the mediator and GOx distribution homogeneity. In the electrochemical characteristics, the △ Ep is 0.58mv, the electron transfer constant Ks is 1.2 * 10^{-3}, which indicated that the glucose sensing strips have good working voltage, good electron transfer constant, small inter batch difference, and good repeatability.

As above, this study using a commercial printer to prepare paper-based glucose sensing test strips was successful. Besides, the strip not only has good conductivity, but also low cost, degradability and good reproducibility. In the future, people can prepare their own glucose test strips at home using a commercial printer and bioink. This method has the possibility of being prepared anytime, anywhere, so it has great potential for development in point of care in the future.

KEYWORDS: glucose sensor, paper-based, EPSON, ink-jet printing



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Preparation of novel membrane-based nucleic acid 3D printed biosensor platform and validating its target DNA detection

Hsu-Hung Kuo[1], Yi-Nan Lin[1,2], Sung-Chieh Su[2], Jie-Ning Chuang[1], , Yu-Chen Chang[1], Li-Fen Huang[2], and *Yi-Ming Sun[1,2*]

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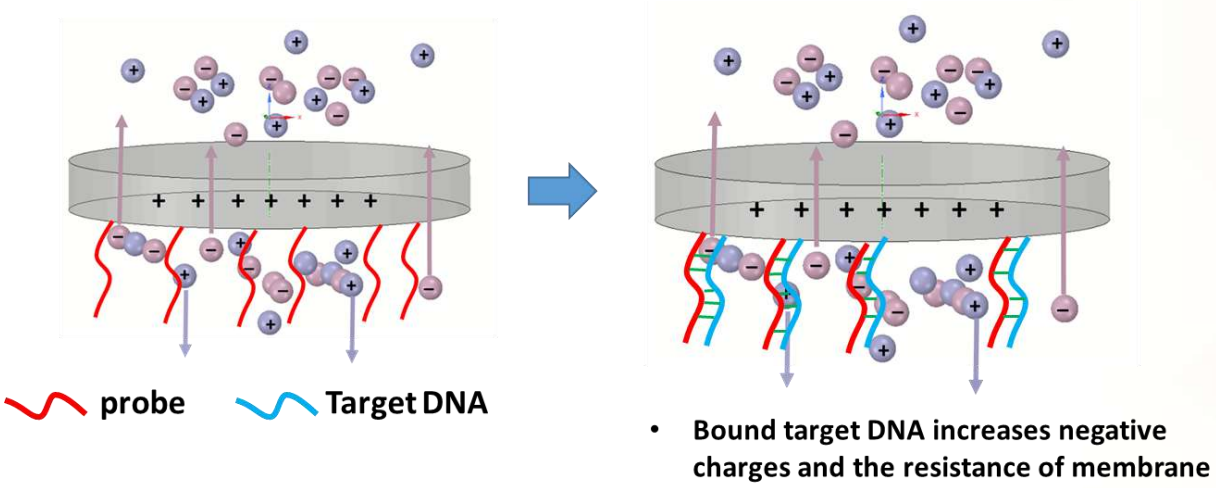
Abstract:

Nucleic acid biosensors are instrumental in the molecular diagnosis of diseases, and a membrane biosensor platform was developed by our collaborators using perm-selective membrane functionalized with gene-specific probes to detect target nucleic acids by monitoring changes in the current-voltage characteristics across the membrane. Originally, a heterogeneous commercial anion selective membrane has to be cut into tiny pieces and embedded to make the sensor heads. However, to facilitate the scale-up production of the biosensors, we have developed hydrogel-based homogenous anion selective membranes which can be synthesized *in situ* on the sensor heads to provide more consistent results and easier scalability.

Recently, we have made further improvements to the biosensor platform in several key areas. Firstly, we have improved the chemical composition of the anion selective membrane, resulting in a lower swelling ratio and better stability. Secondly, we have developed a method for membrane surface modification that allows for the functionalization of DNA probes with fewer steps. To validate the efficacy of the surface modification, we have conducted analyses using X-ray photoelectron spectroscopy. Thirdly, we have confirmed the specific binding of target nucleic acids to the DNA probes by measuring electrochemical and fluorescent signals. This validation process has ensured the accuracy and reliability of target DNA hybridization. Lastly, we have designed 3D-printed chips with membrane sensors that offer better functionality compared to the hand-made microfluidic chips used in the original studies. With these improvements, we hope to build a better biosensor system prototype to be used for food safety tests and future molecular diagnosis.

KEYWORDS: biosensor、DNA probes、3D-printed chips、current-voltage characteristics、membrane



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Biodegradable Adhesive Tissue-Mimicked Multichannel Microelectrode Arrays for Electrophysiological measurements applied to nerve, brain and cardiomyocytes

Tzu-Ya Cheng[1], Wan Lou Lei [1], Wei- Chen Huang[1, 2,*]

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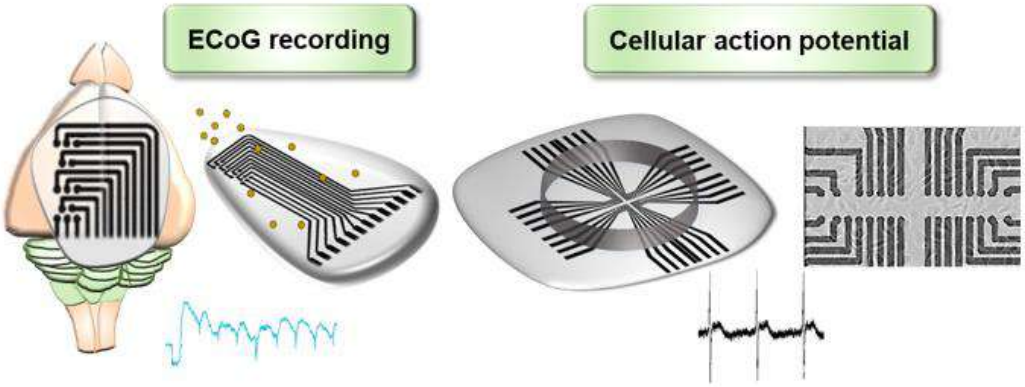
Abstract:

The microelectrode array (MEA) is used to study the electrophysiological signals in brain and cardiac cell networks and to treat brain disorders. However, most current MEA studies use silicon or rigid materials, which do not match the mechanical properties of brain, nerve and other tissues, leading to inflammation and functional loss. [1] [2] Therefore, we have developed a fully hydrogel MEA, whose electrodes can closely conform to tissues and accurately measure physiological signals. First, silk fibroin-dopamine/microbial transglutaminase (mTG)/Gelatin is developed as the substrate for MEA that can carry anti-inflammatory or disease drugs. In the second part, Gelatin Methacryloyl (GelMA) is grafted with graphene oxide (GO) and blended with the conductive polymer Poly (3,4-ethylenedioxythiophene) (PEDOT) as the hydrogel electrode conductor. In the third part, the biocompatible Polylactic acid (PLA) is used as the insulation layer. Finally, the conductive layer and the insulation layer are integrated onto the silk-mTG-Gelatin substrate by transfer printing. The electrochemical impedance at 1 kHz is lower than that reported in most literature. The soft MEA is not only used for in vitro cell recording of action potentials in cardiac cells HL-1, but also for real-time ECoG recording and epilepsy treatment, and is expected to improve the efficiency of treating various tissue diseases in the future.

KEYWORDS: Hydrogel electrode, Neural interfaces, Electrocorticography Grids, Brain implants, Electrical signals in cardiac cell networks, Microelectrode arrays.



Graphic abstract (not a mandatory requirement)

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Extracellular Matrix-Inspired All Hydrogel Biohybrid Neural Interfaces for Combined Microelectrode Array Technologies, Tissue Scaffolding, and Stem Cell Therapy

Wan-Lou Lei [1], Tzu-Ya Cheng[1], Chih-Wei Peng [2], Huai-En Lu[3], Wei-Chen Huang [4,*]

[1] Institute of Biomedical Engineering, National Yang Ming Chiao Tung University, Hsinchu, Taiwan

[2] Department of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan

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Abstract:

Implantable neural interfaces combined with cell transplantation inspire a new class of neuromodulation devices called biohybrid neural interfaces (BHNI). However, current BHNI were engineered by decorating cells on the surfaces of microelectrode arrays (MEAs) made of rigid, hydrophobic, and compactly structural materials, which will impose mechanical damage to the host tissues over the period of implantation. A key concept derived from nerve tissue engineering (NTE) is the use of nerve-based extracellular matrix (ECM) materials to promote neuronal residence and extended differentiation. Therefore, we propose here a new degradable, adhesive, and nerve tissue- mimicked MEA by leveraging the concepts derived from NTE and BHNI. The MEA tracks composed of doubly-crosslinked ECHs, called PDGO, with GelMA-doped PEDOT (PDGMA) as sparsely soft nanogels and GelMA-modified GO (GOGMA) as brittle skeletons to ensure high electrical/electrochemical conductivity. The device substrate is composed of gelatin/silk (GS) with adjustable gelation kinetics controlled by MTG. MTG encouraged covalently bonding interaction between GS and pre-fabricated PLA insulation layer and ECHs tracks, in turn permitting transfer printing to directly integrate the whole device in a batch without the use of any MEMS process or a sacrificial layer. The resultant hydrogel MEA enables site-specific electrical stimulation and neural signal detection with high performance in a time window of tissue repair and can provide an ideal stem cell niche for cell delivery. After implantation at the injury site in sciatic nerve, hydrogel MEA enables site-specific electrical stimulation and neural signal detection with high performance in a time window of tissue repair and can provide an ideal stem cell niche for cell delivery. The overall fusion of the biology and electricity systems overcome the applications of BHNI in host tissues, finally promoting the restoration of peripheral nerve injuries.

KEYWORDS: Neural interface, hydrogel, peripheral nerve injury, cell therapy, biohybrid neural interfaces (BHNI), microelectrode arrays (MEA)

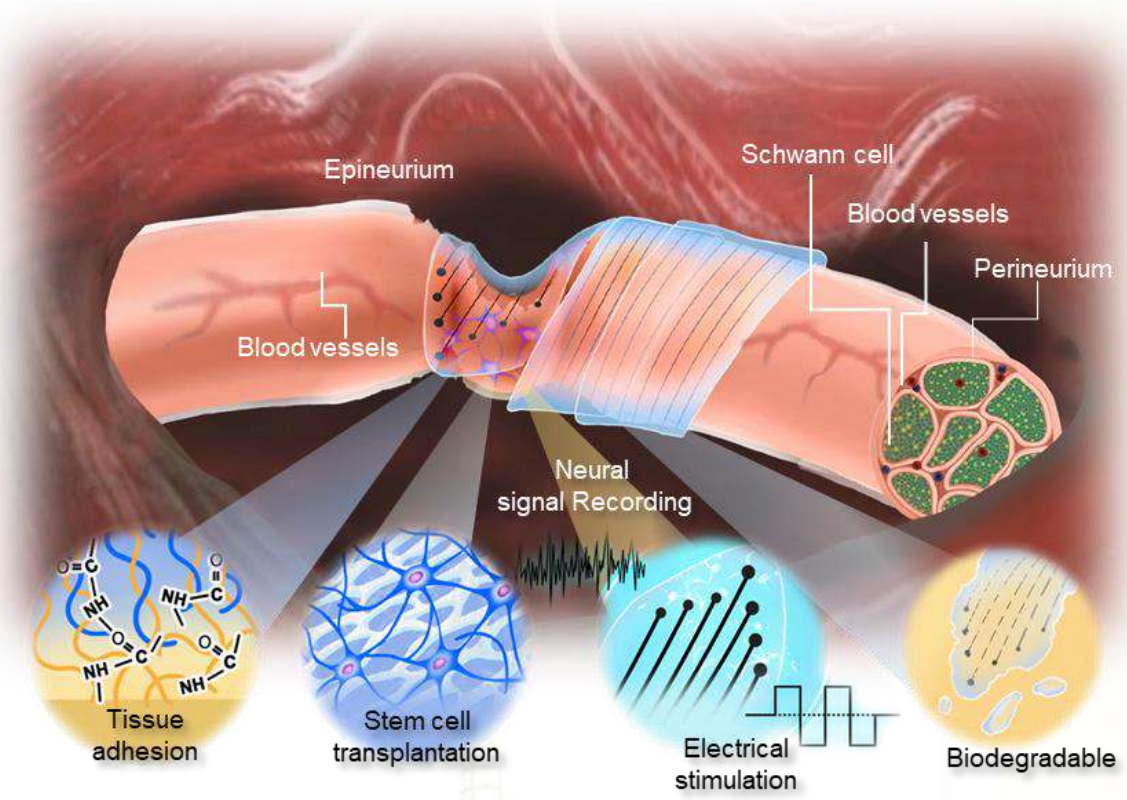

Figure 1. Biohybrid neural interfaces (BHNI) electrode arrays

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Photo-Responsive Nanozyme Sensor: Harnessing Emissive Oxidase-mimic Nanozyme for On-Site Antioxidant Detection in Human Saliva

Sanskruti Swain[1], Ting-Yi Lin[1], Hua-En Chueh[1], I-Hsuan Chou[2], Yun-Jung Ting[1], Hsing-Ying Lin[2], Chen-Han Huang[1]*

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Abstract:

Uncontrolled generation of free radicals in the human body due to lack of antioxidants leads to oxidative stress-related disorders like cancer, Alzheimer's disease, cardiovascular diseases etc. [1]. In this context, total antioxidant capacity (TAC) evaluation in body fluids represents an important bioanalytical parameter of oxidative stress. Nanozymes, nanomaterials with enzyme-like properties, are emerging as hotspots in research. While most possess peroxidase-mimic activity, oxidase-mimic nanozymes are superior due to their independence from toxic H_2O_2 for activation [2]. Utilizing light irradiation as an external stimulus to control nanozyme catalysis shows promise for enhancing efficiency with high spatiotemporal resolution and environmental friendliness [3].

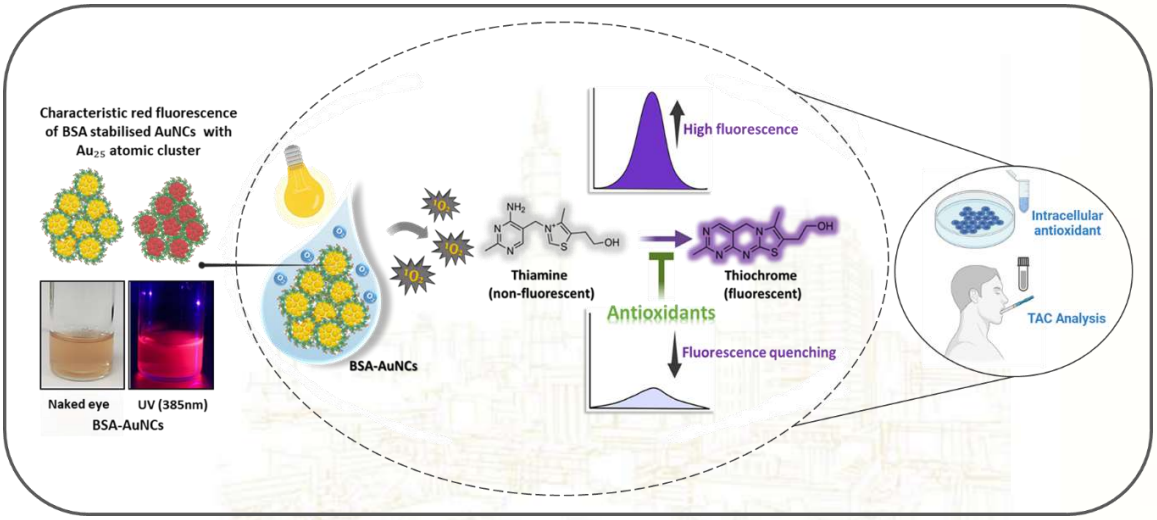
Herein, dithiothreitol-mediated red fluorescent gold nanoclusters (AuNCs) stabilized by bovine serum albumin (BSA) were synthesized, displaying maximum emission peak at 690-700nm when excited at 520nm with an average size of 2nm. The synthesized AuNCs were then subjected to investigation regarding their photocatalytic behavior, which demonstrated a photo-responsive oxidase-mimetic capability. Under visible light irradiation, BSA-AuNCs generated free radicals, with singlet oxygen being particularly prominent, leading to the conversion of non-fluorescent thiamine (TH) into fluorescent thiochrome. Notably, by alternately switching the light irradiation on and off, the oxidase-mimicking activity of the BSA-AuNCs displayed a staircase-like pattern, indicating the photo-controllable nature of the BSA-AuNCs' oxidase-like activity. This precise regulation enables the controlled oxidation of TH using the BSA-AuNCs. Additionally, the steady-state kinetic assay demonstrated that BSA-AuNCs exhibit a higher maximum reaction velocity (V_{max} = 3.79×10^{-3} M/sec), indicating their remarkable catalytic efficiency along with a lower Michaelis constant (K_m = 1.59×10^{-4} M), indicating a high affinity towards the substrate TH.

Due to the inherent free radical scavenging abilities of antioxidants, their effect on the catalytic activity of BSA-AuNCs was explored. It was observed that the presence of antioxidants resulted in a decrease in the fluorescence intensity of thiochrome (TC), indicating their inhibitory effect on the catalytic activity of BSA-AuNCs. This finding suggests the potential utility of BSA-

AuNCs as a biosensor for detecting and quantifying antioxidants by monitoring the changes in fluorescence intensity. The application of this detection strategy was employed to detect antioxidants within cancer cell lines. Extending the feasibility of this study, antioxidants were successfully detected and quantified in human saliva with enhanced selectivity and sensitivity. Significantly, the BSA-AuNCs enabled the non-invasive assessment of the total antioxidant capacity (TAC) of the body by utilizing human saliva.

Therefore, this study shows promise for on-site and real-time antioxidants evaluation, envisaging the possibility to utilize BSA-AuNCs as a biosensor for non-invasive routine screening and monitoring body TAC levels.

KEYWORDS: Antioxidants, Biosensor, BSA-AuNCs, Nanozymes, Photocatalytic behavior



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Enhancing Peripheral Neural Cell Activity through the Combination of Conductive Hydrogels and Electrical Stimulation

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Abstract:

Peripheral nerve injuries can cause temporary or permanent damage to the structure and physiological function of peripheral nerves. Currently, the standard therapeutic approach involves autologous transplantation. However, the availability of suitable tissue, either from the patient or donors, presents challenges and limits the applicability of autologous transplantation. Therefore, the use of artificial nerve transplantation represents a promising alternative treatment option. When repairing the neural system, it is crucial to select materials for reconstructing nerve conduits that possess high biocompatibility, degradability, excellent mechanical properties, and promote favorable conditions for neural growth to ensure successful tissue recovery. Additionally, electrical signal transmission and neuronal activity play vital roles in the nervous system. Consequently, electrical stimulation therapy is widely utilized in clinical treatments for various nervous system injuries. The electric fields generated by electrical stimulation can modulate ion channels, thereby influencing cell migration, proliferation, and growth direction. Furthermore, electrical stimulation can enhance the differentiation capacity of cells in the nervous system and promote nerve regeneration. In this study, we combined electrical stimulation therapy with gelatin methacryloyl (GelMA) hydrogels loaded with conductive metal carbide-soy phospholipid (MXene-SP). This combination demonstrated the potential of MXene-SP-loaded hydrogels carrying Schwann cells for tissue engineering, as it enhanced the activity of Schwann cells at specific frequencies and voltages through electrical stimulation. To further validate the potential of this material in constructing biomimetic neural tissue, we employed MXene-SP-loaded GelMA bioink for 3D bioprinting. The resulting constructs exhibited excellent biocompatibility, enabling the fabrication of multilayered 3D nerve conduits with high recognition rates and shape retention.

KEYWORDS: Conductive hydrogels, Electrical Stimulation, 3D nerve conduits, Peripheral nerve injuries.

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A Magnetic-Responsive Injectable Photocrosslinkable Ink for the Applications of 4D Printed Bioactuators

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Abstract:

Soft actuators have garnered significant attention in fields like micromanipulation and tissue engineering due to their potential applications in diverse areas, including clean energy, construction, industrial machinery, and aerospace. Unlike traditional rigid actuators, soft actuators employ flexible elastomers and polymers to create mechanical transformer structures, providing exceptional design flexibility and adaptability to varying environments. In this study, our focus centered on developing a bioink that incorporates magnetically responsive nanoparticles into photocrosslinkable biopolymers. This hybrid ink enabled us to manipulate the stiffness and modulate the actuation capability of the bioactuators. Additionally, we successfully employed the ink to fabricate biomimetic structures resembling those found in natural organisms. Furthermore, our findings indicated that the printed bioactuators exhibited reversible transformations, which can mimic the movements in natural organisms. This research underscores the potential of hybrid magnetically responsive photocrosslinkable inks for the rapid additive manufacturing of biomimetic structures, facilitating contactless magnetic actuation. Based on our design, the combination of 3D printing and stimuli-responsive materials opens up new possibilities for the 4D printing of advanced bioactuators.

KEYWORDS: 4D printing technique, magnetic-responsive materials, bioactuators.

2023 ISOMRM & BCRS
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Biodegradable Phosphocholine Cross-Linker With Ion-Pair Design for Tough Zwitterionic Hydrogel

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Abstract:

Hydrogels have been widely used in various biomedical applications based on their ability to provide 3D frames with tissue-like elasticity and high water content. However, the role of the cross-linking agents in the hydrogels was undervalued in terms of biocompatibility, mechanical properties, degradability, and hydration of gels. In this study, an innovative zwitterionic dimethacrylate 2-[2-{2-(Methacryloyloxy)ethyldimethylammonium}ethylphosphate]ethyl disulfide (MPCSS) for the development of biodegradable and biocompatible hydrogels with entirely bio-inspired PC structure is reported.

The MPCSS cross-linker includes the zwitterionic group providing nonfouling properties and a disulfide bond that can be degraded by reducing agents and enzymes. Moreover, MPCSS has an opposite arrangement of charged groups to that in the 2-methacryloyloxyethyl phosphorylcholine (MPC) monomer. The hydrogels developed from MPCSS and MPC allow the stronger mechanical properties upon electrostatic interaction between the oppositely charged groups and the higher water content than the MPC gels with the conventional cross-linker. The biocompatibility and fouling characteristics of MPC/MPCSS hydrogels are systematically investigated. Moreover, the degradation of MPCSS cross-linked hydrogels is evaluated through their weight loss and rheological data. Ultimately, MPC/MPCSS hydrogel is demonstrated to in situ encapsulate NIH-3T3 fibroblasts and provide an excellent 3D environment, facilitating cell remodeling and growth as a tissue scaffold.

KEYWORDS: biodegradable cross-linkers, cell encapsulation, hydrogel, ion-pair interaction, phosphocholine, zwitterionic

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Complete zwitterionic double network hydrogels with great toughness and resistance against foreign body reaction and thrombus

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Conventional tough hydrogels offer enhanced mechanical properties for load-bearing implants; however, their application is still hindered by a lack of biocompatibility. In this study, we demonstrate a new methodology for developing biocompatible double network (DN) hydrogels by using a responsive amphoteric polymer as a first framework.Tough DN hydrogels were formed by penetrating zwitterionic poly(sulfobetaine acrylamide) (PSBAA) into a swollen poly(lysine acrylamide) (PLysAA) network in an acidic or alkaline solution, and polymerizing under UV irradiation. The DN hydrogels were able to become zwitterionic entirely under physiological conditions, and possess excellent mechanical strength, comparable to conventional DN hydrogels with permanently charged polyelectrolyte frameworks.

Additionally, in vitro studies including biofouling, cytotoxicity and hemolysis were conducted to show the superior biocompatibility of the complete zwitterionic DN hydrogels. After the circulation of human blood in tubular DN hydrogels, the zwitterionic DN gels displayed negligible thrombus formation.

Furthermore, PLysAA/PSBAA hydrogels were implanted subcutaneously, showing excellent resistance against inflammatory response and long-term capsule formation. This work has presented a new strategy for synthesizing a biocompatible tough DN hydrogel to effectively mitigate the foreign body reaction to render great benefit for the development of biomedical implants.

KEYWORDS: Zwitterionic、Double network hydrogels

2023 ISOMRM & BCRS
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Development of methacrylic anhydride-modified carboxymethylcellulose hydrogels to deliver corticosteroids for corneal wound healing

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Abstract:

Corneal alkali burn is a severe ocular chemical injury that is associated with oxidative stress, inflammation and corneal neovascularization. Topical administration of corticosteroids is the first-line treatment for corneal alkali burns but have some limitations, including low ocular bioavailability, high dosage frequency and side effects. Carboxymethylcellulose (CMC) is a linear water-soluble polysaccharide and is a common composition of artificial tears. Modification of CMC with methacrylic anhydride (CMC-MA) can enhance mucoadhesion and prolong the precorneal retention time. Dexamethasone (DEX) with anti-inflammatory properties can suppress the inflammatory angiogenesis in cornea and even better than anti-vascular endothelial growth factor. In the study, a novel topical CMC-MA-based formulation was developed to release the DEX immediately under alkaline conditions and achieve prolonged therapeutic action at neutral pH that may improve the limitations of corticosteroids eye drops. CMC-MA was modified with succinic anhydride-grafted DEX (Suc-DEX). The characterization of developed hydrogels (MA-CMC-DEX) were analyzed by Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, rheometer, high performance liquid chromatography, scanning electron microscopy and in-vitro biocompatibility. The effects of optimized MA-CMC-DEX were demonstrated in corneal epithelial cells. The results revealed that MA-CMC-DEX hydrogels with mucoadhesive and pH-triggered drug release properties might have potentials for corneal wound healing applications.

KEYWORDS: corneal alkali burns, carboxymethylcellulose, dexamethasone, methacrylic anhydride.

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Encouraging Enthesis Organ Development through Drug Release of Ligament, Bone, and Endochondral Ossification Cues using Mesoporous Silica Nanoparticles

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Abstract:

Electrospun wavy PLLA (poly-L-lactic acid) fibers can be used as a scaffold for bone and ligament repair due to their biocompatibility and biodegradability. These fibers have a wavy morphology that mimics the natural architecture of bone and ligament tissue, which can enhance cell attachment and proliferation.

To further enhance the regenerative properties of the scaffold, we loading with a fibrin gel that contains Mesoporous Silica Nanoparticles (MSNs) loaded with TGF-β (transforming growth factor-beta) and dexamethasone. By combining these materials, it may be possible to create a scaffold for bone and ligament repair that can release TGF-β and dexamethasone in a controlled manner be created that provides mechanical support for tissues while also promoting their repair and regeneration.

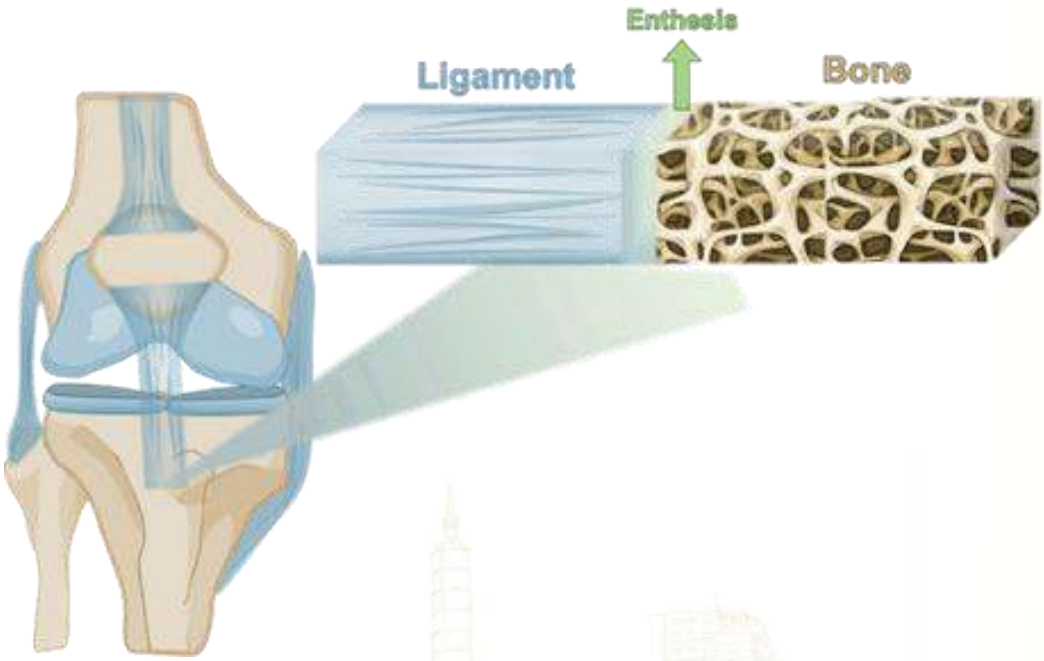
Mesoporous silica nanoparticles (MSNs) are a type of nanoparticle with a high surface area and pore volume. They can be loaded with various drugs or growth factors and can serve as a delivery vehicle to target specific tissues or cells. In this case, the MSN respectively loaded with transforming growth factor-beta (TGF-β) and dexamethasone.

TGF-β is a potent growth factor that plays a critical role in ligament repair. It can stimulate the proliferation and differentiation of mesenchymal stem cells (MSCs) into ligament-forming cells, while dexamethasone is a steroid that can reduce inflammation and enhance tissue regeneration. It has been shown to promote the differentiation of MSCs into osteoblasts, which can facilitate bone repair. MSN were able to release both agents over time, and that the released agents were able to promote the growth and differentiation of bone and ligament cells.

Overall, This composite scaffold can be used for bone and ligament repair applications, where there is a need for a strong and biocompatible material that can support tissue growth and healing.it show great potential in regenerative medicine as a promising strategy for bone and ligament repair, with potential clinical applications in the future. Further research is needed to

optimize their formulation and delivery for clinical use.

KEYWORDS: Mesoporous silica nanoparticles (MSN), Dexamethasone, transforming growth factor beta (TGF-β), regenerative medicine



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Platelet-derived extracellular vesicles plus reduced graphene oxide co-laden polymer-coordinated hydrogel promotes diabetic wound restoration

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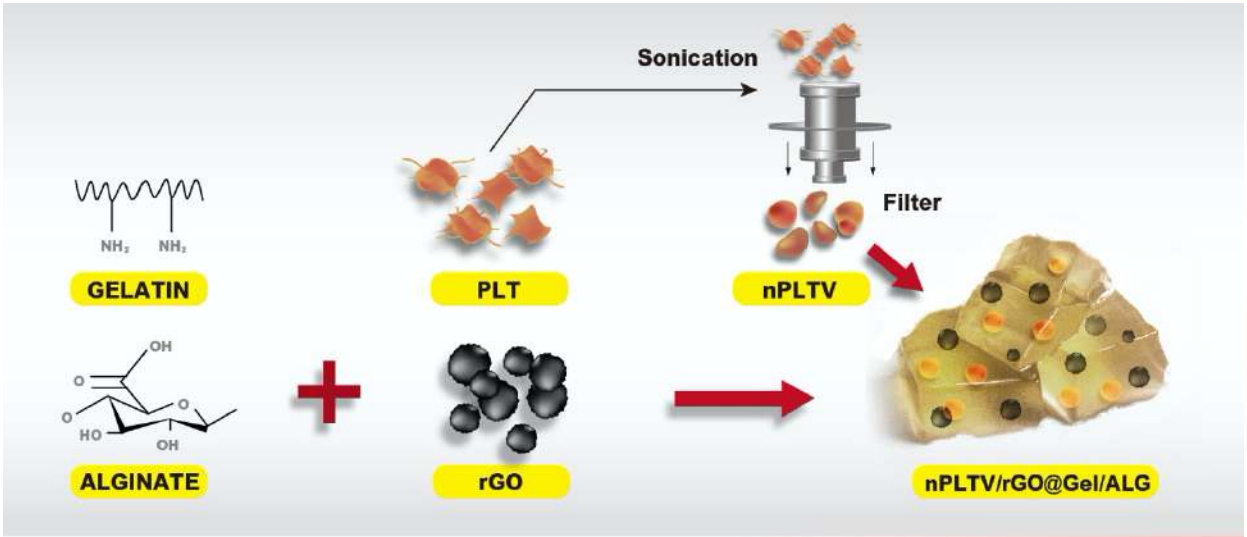
Abstract:

Diabetic wounds are hard to restore owing to invalid antibacterial effects, hindered heat shock protein (HSP) regulation, constrained angiogenesis, and persistent immunomodulation and inflammation. Photothermal-responsive reduced graphene oxide (rGO) has antibacterial and HSP-promoting activities. Nano-sized platelet-derived extracellular vesicles (nPLTVs) have abundant cytokines and growth factors, which facilitate cellular proliferation, angiogenesis, immunomodulation, and inflammation. Nevertheless, single medical therapy is constrained in its delivery efficiency and efficacy. Polymeric bioengineering can enhance the restrained performance of single medicines by integrating coordinated materials and medicines to obtain cooperative or complementary bioengineered medicines. Herein, rGO, gelatin (Gel), and alginate (ALG) were employed to fabricate an rGO-coordinated hydrogel (rGO@Gel/ALG) with satisfactory mechanical features, porous structure, biodegradability, and swelling ratio. The rGO@Gel/ALG was utilized as diabetic wound dressing for sustained release of the drug. nPLTVs were combined with the composite rGO@Gel/ALG hydrogel to form nPLTV/rGO@Gel/ALG. The nPLTV/rGO@Gel/ALG was biochemically and biophysically characterized and possessed good in vitro biocompatibility, inhibited inflammation, promoted wound healing, and regulated immune features. In a streptozotocin-induced diabetic rat wound model, the nPLTV/rGO@Gel/ALG hydrogel decreased expressions of inflammatory factors, regulated immunity, supported angiogenesis, and thus augmented diabetic wound healing. Interestingly, the nPLTV/rGO@Gel/ALG hydrogel elevated the expression of cellular protective signaling pathway-related HSP. The developed nPLTV/rGO@Gel/ALG hydrogel can be used as wound dressings to regulate immunity, inflammation, and angiogenesis of diabetic wounds and accelerate chronic wound healing.

KEYWORDS: hydrogel, redox graphene oxide (rGO), nano-platelet vesicles (nPLTV), photothermal effect, antibacterial, anti-inflammation, heat shock protein, immune regulation, angiogenesis, wound healing


Sonication
Filter
GELATIN
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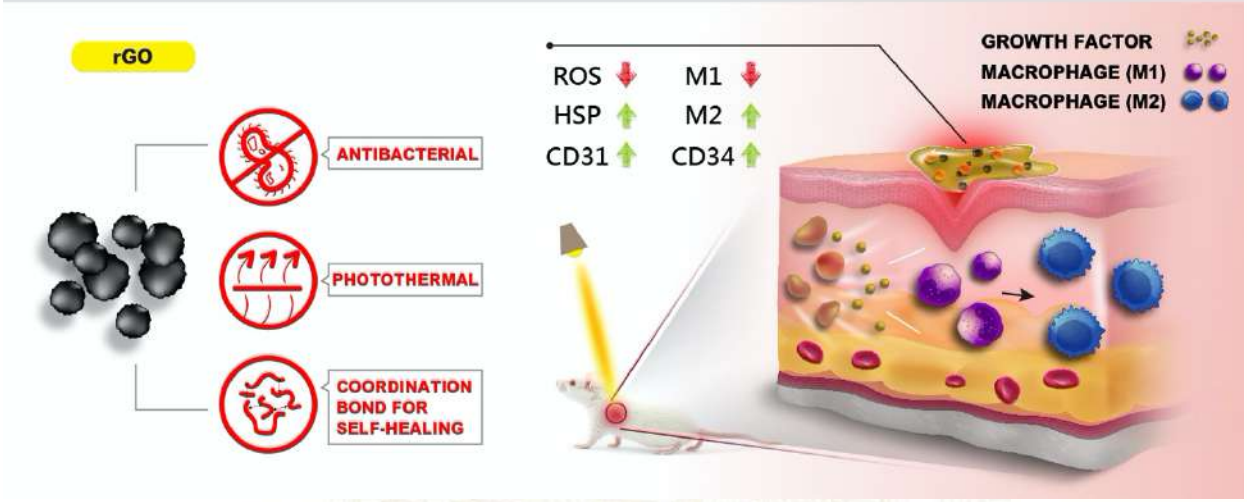
rGO
ANTIBACTERIAL
PHOTOTHERMAL
COORDINATION BOND FOR SELF-HEALING
ROS
HSP
CD31
M1
M2
CD34
GROWTH FACTOR
MACROPHAGE (M1)
MACROPHAGE (M2)

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Alginate-Tyramine Gel with Reinforcement by Plasma for Treatment of Arthritis

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(Underline the name of the presenting author. The corresponding author(s) should be marked with an asterisk in the author list.)

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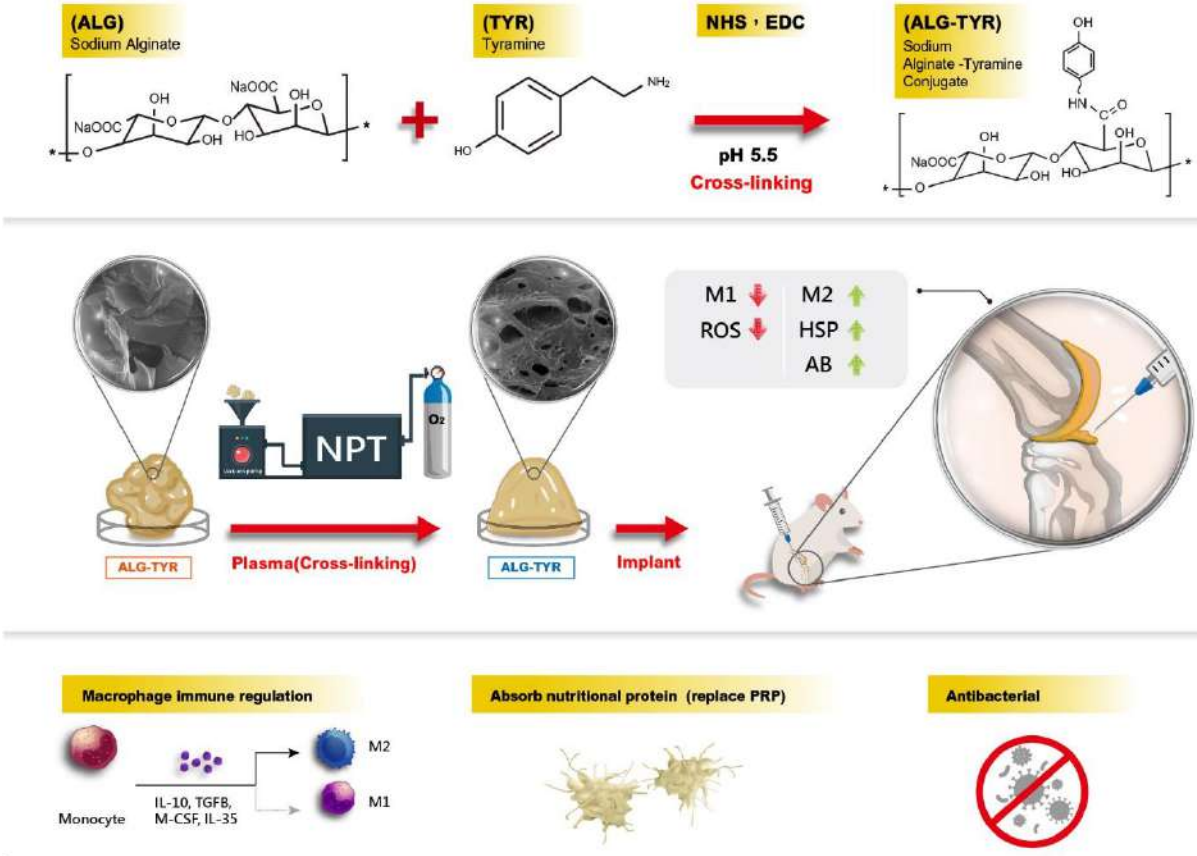
Abstract:

In this study, we conjugated tyramine (TYR) with alginate (ALG) for forming ALG-TYR and then treated it with 5 min oxygen plasma (ALG-TYR + P/5min). It was shown that ALG-TYR + P/5min revealed favorable viscoelastic, mechanical, self-concentrated, morphological, biocompatible, and cellular heat-shock protein amplification performances. Furthermore, ALG-TYR + P/5min not only effectively suppressed the inflammatory response in RA but also regulate the lesion immune. Once intraarticularly given the ALG-TYR + P/5min into the joints of zymosan-induced arthritis rats, we found that ALG-TYR + P/5min notably ameliorates syndromes of RA joint swelling and walk function, without initiating systematic unwanted side effects. This bioinspired and self-restorable ALG-TYR + P/5min thus can serve as an safe and efficient gel system, offering a prospective results to potentiate RA treatment.

Keywords: Alginate-Tyramine Gel, Immunomodulation, Treatment of Arthritis


(ALG)
Sodium Alginate
(TYR)
Tyramine
NHS，EDC
pH 5.5
Cross-linking
(ALG-TYR)
Sodium
Alginate -Tyramine
Conjugate
NPT
ALG-TYR
Plasma(Cross-linking)
ALG-TYR
Implant
M1
ROS
M2
HSP
AB
Macrophage immune regulation
Monocyte
IL-10, TGFB,
M-CSF, IL-35
M2
M1
Absorb nutritional protein (replace PRP)
Antibacterial

2023 ISOMRM & BCRS
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Development of modified hydroxypropyl methylcellulose hydrogel with drug loading as a vitreous substitutes

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Abstract:

[Introduction]

Vitreous and retinal-related diseases are often one of the culprits that cause people's vision loss, such as vitreous opacity and retinal detachment, which require vitrectomy for treatment. However, vitrectomy is often accompanied by side effects such as endophthalmitis, and although clinical vitreous substitutes are divided into many types, they may cause some serious side effects. Therefore, this study developed a new type of hydrogel grafted with hydroxypropylmethylcellulose (HPMC) methacrylic anhydride (MA), and added epigallocatechin gallate to the hydrogel EGCG gelatin nanoparticles, hydrogel mixed with nanoparticles, to study its application as a vitreous substitute, and to suppress possible postoperative endophthalmitis with EGCG.

[Materials and Methods]

In terms of material detection, use FTIR and NMR to confirm whether the synthesis and grafting are successful, and calculate the degree of crosslinking, and then analyze the transparency, refractive index, rheology, swelling, degradation rate and microstructure; and on the one hand, analyze the size of nanoparticles, zeta potential and TEM to confirm its microstructure; *In vitro* testing, use ARPE19 cells to conduct cytocompatibility analysis through two different methods: extraction method and direct contact method, to determine whether the hydrogel is toxic to cells, and use Live/dead to judge cell activity status; In terms of animal experiments, vitrectomy was performed on New Zealand white rabbits, and the hydrogel of this study was implanted, and the appearance, intraocular pressure, and corneal thickness of both eyes before and after the operation were compared to confirm the implantation. The transparency of the object and the condition of the retina were checked with an ophthalmoscope, and then the electroretinogram was used to objectively judge whether the function of the retina was affected. Finally, the structural integrity and inflammation of the eye were observed with tissue sections.

[Results]

In this experiment, a hydrogel with good rheological properties and easy injection was successfully prepared. The physicochemical properties of hydrogels are similar to those of natural vitreous. Two in vitro experiments, extraction method and direct contact method, confirmed that the hydrogel has good biocompatibility; in vivo experiments were based on 100 μM EGCG/gelatin nanoparticles, and three concentrations of low, medium and high concentrations were mobilized. Gelatin nanoparticles containing EGCG can exert anti-inflammatory effect after complete removal of vitreous humor. There was no significant change in corneal thickness after operation, and there was no significant difference in retinal potential a wave and b wave compared with the control group. There were no significant differences at several weeks postoperatively.

[Discussion]

In this experiment, we used HPMC grafted MA to solve the problem of premature degradation, and added EGCG contained gelatin nanoparticles to reduce the problem of endophthalmitis that may occur after surgery.

[Conclusions]

We have successfully prepared a hydrogel that is easy to inject and has good rheological properties. Its physicochemical properties are similar to natural vitreous, and it has been proved to have good biocompatibility in vitro and anti-inflammatory effect in vivo. This study demonstrates an injectable HPMC-grafted MA mixed with gelatin/EGCG nanoparticles as a vitreous substitute with appropriate physical properties and inflammation-inhibiting effects for vitrectomy.

KEYWORDS: **vitrectomy, postoperative inflammation, EGCG, HPMC, hydrogel**

2023 ISOMRM & BCRS
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Polypyrrole-polyethyleneimine nanopigments-woven medical products for restoring follicle

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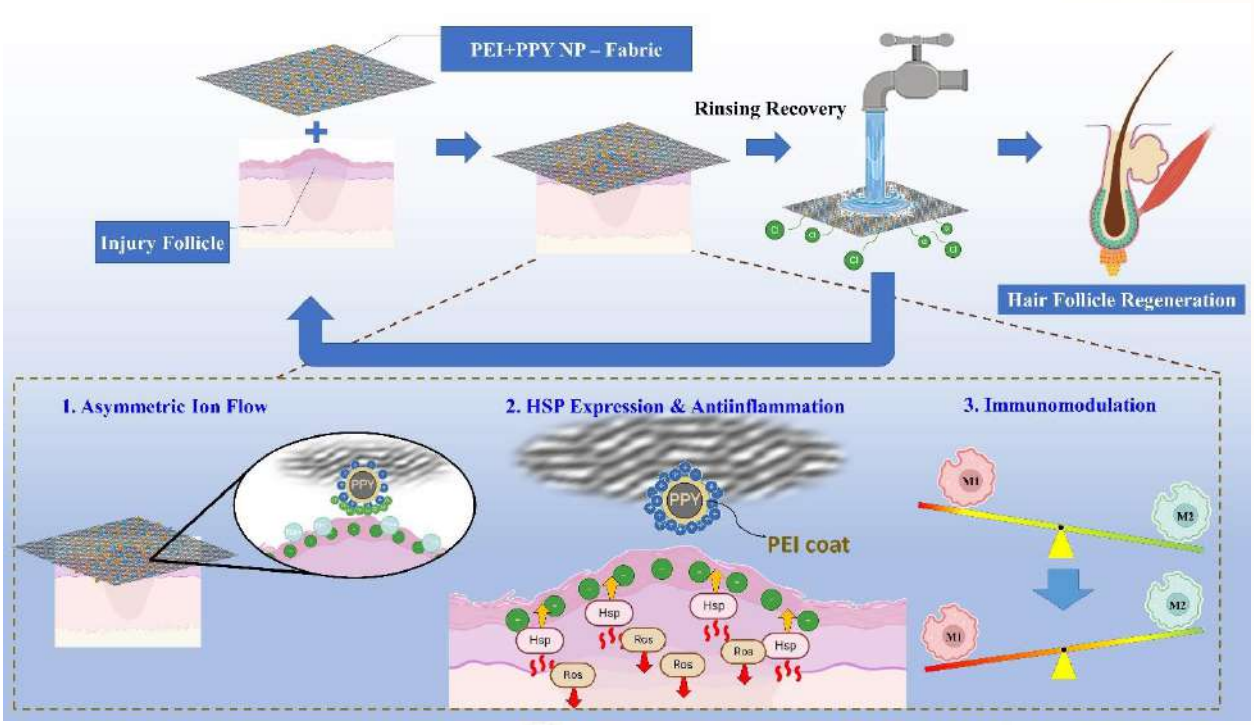
Abstract:
Hair loss is a popular but traumatizing illness in worldwide. No precise cause of hair loss has been recognized; nevertheless, it is well-known that numerous reasons, containing stress and heredity, could devastate hair shafts and follicles, which are a multifaceted mini-tissue of the skin[1, 2]. Numerous non-pharmacological and pharmacological therapies for hair loss are being developed; however, serious side effects or confines according to hair regeneration potential remain to happen[3, 4]. As recent substitutes, the method of nonpharmacological biophysical stimulation is being applied because it is a rather biocompatible and minimal-risk advanced therapy for the regeneration and regrowth of hair. It is principally categorized as electric stimulation as well as low-level laser therapy[5-8]. however, the consequential uneasiness and the necessity for outside components for instance electrical power supply is a problem in its common utilization. Herein, we introduce an innovative cationic fabric that utilizes a repeatable asymmetrical ionic flow in the physiological fluid to internally stimulate hair follicles and skin(Figure 1). The characterization, ultrasonic, biochemical, and histological experiments were conducted in a hair-less mouse model. The use of this development could significantly increase the heat shock protein expression and immunoregulation in the skin and the number of hair follicles in hair-less animals without any adverse risks.

KEYWORDS: polypyrrole-polyethyleneimine nanopigments-woven medical products, asymmetrical ionic convection, heat-shock protein, immunomodulation, follicle repair



(Figure 1)The schematic illustration depicts that the PE Fabric-PPy-PEI NPs can create repeatable asymmetric ion flow, HSP amplification, immunomodulation and antiinflammation in a drug- and power-free manner to biophysically stimulate follicle repair.

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2023 ISOMRM & BCRS
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Harnessing Controlled-Degradable Microgel for Effective Chondrogenic and Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Abstract:
The use of mesenchymal stem cells, either alone or in combination with scaffolds, has shown promise for tissue repair and regeneration. Recently, there has been growing interest in degradable microgel-based scaffolds for minimally-invasive cell delivery [1, 2]. These microgels can be designed to degrade gradually, creating space that enhances cell-cell interactions, cell migration, and tissue remodeling. An ideal degradable scaffold should degrade at a rate that matches the proliferation and differentiation of the encapsulated stem cells. In this study, we employed the microfluidic technique to create uniform-sized alginate dialdehyde/gelatin (ALG-GEL) microgels with specific material composition. By adjusting the relative flow rate of the oil and water phases, we could tune the size of the microgels. At an oil/water ratio of 800:1, the fabricated microgels had an average particle size of approximately 350 μm. The inter-connected pores in the lyophilized ALG-GEL microgels ranged from 5 to 30 μm, depending on the ALG to GEL ratio. We used fluorescence-labeled FITC-GEL/ADA to study hydrogel degradation and found that it could be flexibly controlled by altering the polymer composition. Upon encapsulation in ALG-GEL hydrogels, the stem cells exhibited focal adhesion and spreading initially, followed by continuous growth over a period of 4 weeks. The viability of the encapsulated cells remained high (>90%) even after injection through a 26 G needle, as confirmed by the Live/Dead assay. Additionally, we investigated the chondrogenic and osteogenic differentiation of the stem cells encapsulated in ADA/microgels. The stem cell-laden microgels were cultured in growth medium for one week and then exposed to chondrogenic or osteogenic differentiation medium for an additional three weeks. Real-time PCR analysis was performed to examine the expression of various markers related to stem cell differentiation. Among the different microgel formulations, human mesenchymal stem cells encapsulated in ADA/GEL (1.5/3.5) microgels exhibited enhanced chondrogenic differentiation. Osteogenic differentiation of the encapsulated stem cells in microgels is still ongoing. Based on our findings, we believe that ADA/GEL microgels hold great potential for miniaturized stem cell culture, promoting differentiation, and facilitating convenient injection. Further studies on this microgel system are warranted to explore its applications in cell therapy for bone and cartilage repair and regeneration.

KEYWORDS: chondrogenesis, stem cells, microgels, microfluidics, cell therapy

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Effects of alginate hydrogel containing bacteriophage cocktail on macrophage-mediated inflammation and fibroblast migration

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Abstract:

Antibiotic treatments were often used in the early stages of bacterial infections. The overuse of antibiotics leads to an increase in drug-resistant bacteria. Bacteriophage (phage) therapy was known to be able to fight drug-resistant bacterial infections.

Many studies have shown that phages are specific to bacteria and infect only their host bacteria without affecting other cells. However there is a lack of research on the impact of phages on the human immune system. Here we studied the effects of an alginate hydrogel film containing a mixture of phages (phage cocktail) on the phagocytosis and cytokine secretion of macrophage cells, and the migration of fibroblast cells. The results will be used to interpret the feasibility of using phages as biomaterials. The phage cocktail included three strains of phages targeting three bacteria commonly seen in skin infections: *E. coli*, *S. aureus*, and *P. aeru*.

Macrophage was chosen in this study as it is often seen in the first line of non-specific immune responses in the body. Macrophages, when come exposed to pathogens, such as lipopolysaccharide (LPS), a compound often found on Gram negative bacteria, will initiate phagocytosis and release active chemical species and cytokines to facilitate communication with other immune cells.

Fluorescently-stained phages were seen engulfed by macrophage cells under the microscope. The number of phages engulfed by macrophages was not affected by the addition of LPS to the culture media, and all three phages could induce macrophage phagocytosis.

The phage cocktail was embedded in or grafted on the surface of alginate hydrogel films. The number of phages grafted on the surface of alginate hydrogel film reached 4.17×10^5 PFU/film and the total number of the phages embedded in the hydrogel film reached 8.80×10^6 PFU/film. We found in this study that the presence of phages was pro-inflammatory, and stimulated macrophage cells to release NO and cytokines. When macrophages were first stimulated by LPS to release NO and cytokines, the presence of phages had an anti-inflammatory effect and reduced the secretions.

The cytokine measurement results show that different phages had different degrees of anti-inflammatory effects on the LPS-stimulated macrophage cells, and lowered its secretion of NO,

TNFα, and IL-1β compared to the group without phages ($P<0.05$). The phage cocktail grafted on the hydrogel film had a more significant effect in lowering the concentrations of NO and cytokines compared to the ones with phage embedded within.

The number of fibroblasts migrated across the membrane of the transwell decreased when phages were present in the well with LPS-stimulated macrophages. The reduction of pro-inflammatory cytokines by the phages seemed to prevent tissue fibrosis. The number of fibroblasts migrated across the membrane of the transwell increased when phages were present in the well with normal macrophages, phages aid in tissue repair by activating macrophages and attracting fibroblasts to migrate.

In conclusion, the presence of the phage cocktail in or on alginate films could regulate macrophage cell's responses, and induce the migration of fibroblasts to improve skin tissue repair.

KEYWORDS: Bacteriophage, Macrophage, Alginate film, Immune response, Lipopolysaccharide, cytokine

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Novel Polypeptide Composite Fibrous Scaffold with Internal Chemical Boundary

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(Underline the name of the presenting author. The corresponding author(s) should be marked with an asterisk in the author list.)

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Abstract:

Cell migration is a critical process in the development of mammalian tissue and various pathological conditions. In vitro, studies investigating the impact of chemical stimuli on cell behavior typically utilize 2D substrates, where cells respond to concentration gradients of different molecules. However, the development of polymeric scaffolds that can mimic cell migration in a 3D environment with chemical stimuli has been lacking. Here, a novel 3D composite scaffold with an internal chemical boundary is presented. This scaffold is fabricated via using electrospinning and mask-assisted electrospray techniques. By employing these methods, the deposition of PBG-N3 particles is confined to specific predetermined areas within the scaffold. The subsequent step involves selective surface modification of the particles through a click reaction, forming a chemical boundary. A fluorescent alkyne is used to visualize this boundary, enabling clear observation under a fluorescent microscope. This innovative biomaterial holds tremendous promise as a scaffold for investigating the influence of chemical stimuli on cell migration and growth in a 3D environment. It provides a platform that better reflects the physiological conditions for studying cell behavior, offering opportunities for advancing tissue engineering applications. By precisely controlling the distribution of chemical cues within the scaffold, researchers can gain valuable insights into the dynamics of cell migration and potentially develop strategies for tissue engineering, regeneration, and repair.

KEYWORDS: polypeptide, cell migration, 3D scaffolds, chemical boundary, surface modification

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Development of Cancer Cell Membrane-Coated Indocyanine Green-Camptothecin-Loaded Perfluorinated Nanoparticles for Photodynamic Therapy in Triple-Negative Breast Cancer (MDA-MB 231): Material Fabrication and Functional Validation

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Abstract:
Breast cancer represents the most prevalent malignancy among women. Combining chemotherapy with targeted therapy has proven effective in reducing the risk of recurrence. Nonetheless, the adverse effects associated with chemotherapy drugs can significantly impact patients' physiological well-being and potentially compromise treatment outcomes. In this research, we propose the utilization of Cancerous Membrane-Covered Indocyanine Green-Camptothecin-Loaded Perfluorinated Emulsions (CMICPEs) as a dual-function nanocarrier system for simultaneous photodynamic and chemotherapy treatment in triple-negative breast cancer (MDA-MB 231) under light irradiation. The expected outcomes aim to ameliorate severe chemotherapy-induced side effects, including nausea, vomiting, oral mucosal damage, diarrhea, hair loss, fatigue, and diminished appetite.

Preliminary analysis using a nanoparticle size and surface charge measurement instrument revealed that the particle size and surface potential of the ICPEs were determined to be 221.23 ± 3.49 nm and -28.28 ± 1.39 mV, respectively. The encapsulation efficiencies of ICG and CPT were found to be 97.7973% and 57.9367%, respectively. Moreover, the photothermal and photodynamic properties of the particles were assessed through near-infrared light irradiation. In vitro cell experiments were conducted to evaluate the specificity of the particles towards triple-negative breast cancer cells (MDA-MB 231), followed by cytotoxicity assays to compare the killing efficacy of the membrane-coated particles with that of free CPT solution.

The primary objective of this project is to develop a nanocarrier system that combines photodynamic therapy and targeted therapy to precisely and effectively eradicate the target cancer cells. By reducing the required dosage of chemotherapy drugs, this approach aims to minimize the adverse effects on patients and enhance their willingness to actively pursue treatment.

In conclusion, this study focuses on the development and validation of cancer cell membrane-coated indocyanine green-camptothecin-loaded perfluorinated nanoparticles for photodynamic

therapy in triple-negative breast cancer. The findings from this research hold the potential to significantly improve treatment outcomes by mitigating chemotherapy-related side effects and increasing patient adherence to therapy.

KEYWORDS: Breast cancer, Indocyanine green, Camptothecin, Perfluorocarbon, Nanoagent, Photochemotherapy

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Extraction of ulvan and incorporation into thermosensitive electrospun fibers as a wound dressing

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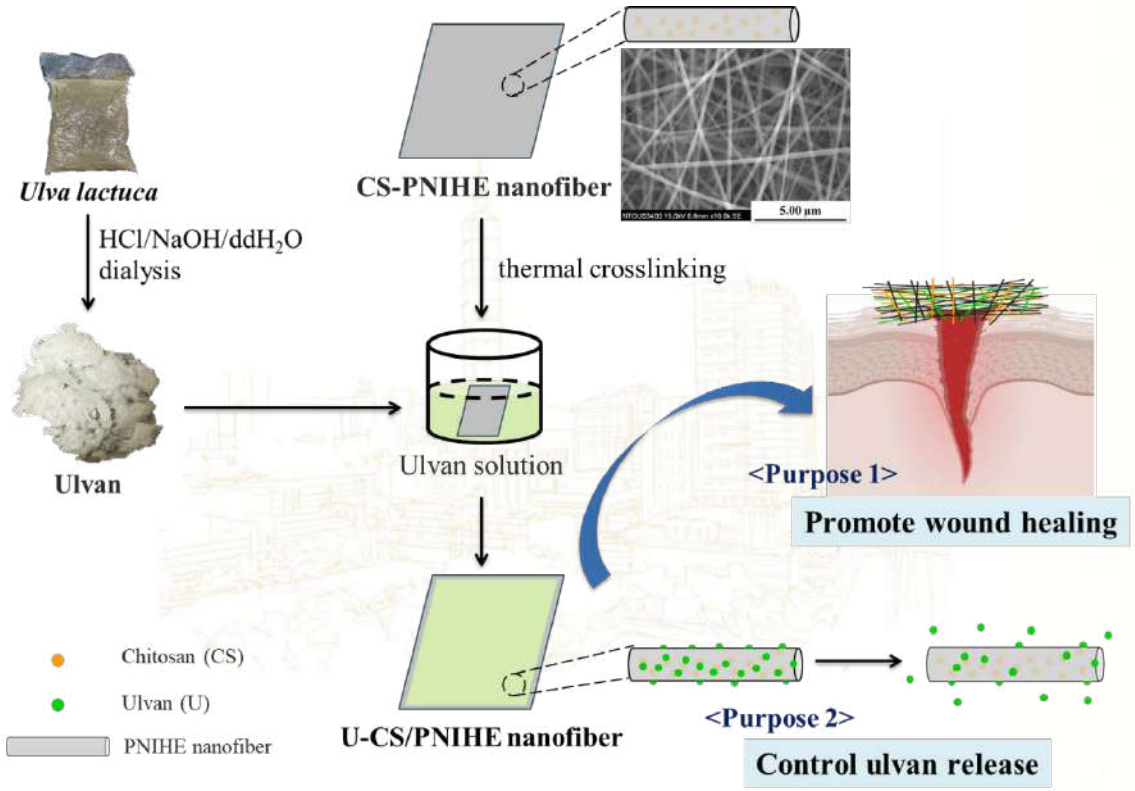
Abstract:

Electrospun nanofibers have small pore size, high porosity, large surface area, and exhibiting excellent performance as wound dressings. Ulvan, due to high biocompatibility and rich biological activities such as antibacterial and antioxidant properties, is widely used in the biomedical field. However, its biological activities are easily affected by extraction processes. Besides, its limited solubility in most solvents [1] and poor rheological properties [2] make it unfavorable for electrospinning. Therefore, this study aims to explore the effects of extraction solvents or dialysis on ulvan, followed by incorporating ulvan into thermosensitive chitosan/poly(*N*-isopropyl acrylamide-*co*-*N*-hydroxyethyl acrylamide) (CS/PNIHE) nanofibers. Additionally, the potential of ulvan for sustained delivery and promotion of wound healing will be evaluated. Ulvan was extracted by HCl, NaOH and ddH_2O, and purified by dialysis. All extracts had typical ulvan structures, with rhamnose as the main monosaccharide, and exhibited good stability up to 200 ℃. Acid extracted ulvan has low molecular weight, high rhamnose content, and good antioxidant activity ulvan. Although dialysis could enhance purity, taking into account yield and sulfate content, undialyzed ulvan extracted with acid would be added to the subsequently prepared nanofibers. To achieve continuous delivery of ulvan and enhance the stability of nanofibers in aqueous solution, PNIHE, possessing both thermosensitive and thermal crosslinking properties, was used as the electrospinning carrier material. The synthesized PNIHE was confirmed by ^{1}H NMR, indicated a chain ratio similar to the feed ratio. The cloud point temperature (T_{CP}) of PNIHE increased with an increase in the content of *N*-hydroxyethyl acrylamide. When the feed ratio of *N*-isopropyl acrylamide and *N*-hydroxyethyl acrylamide was 9:1 ($PNII_9HE_1$), the T_{CP} was close to body temperature. The PNI_9HE_1 solution prepared in H_2O/MeOH (30/70, v/v) solution was homogeneous and clear, ensureing its stability during electrospinning. White nanofibers were successfully electrospun using 5% (w/v) CS acetic acid

aqueous solution and 30% (w/v) P(NI_9HE_1) methanol solution at 3:7 (v/v) ratio. SEM images revealed that the nanofibers had a smooth and uniform structure with an average diameter of 172.7 ± 76 nm. In summary, CS/P(NI_9HE_1) nanofibers were successfully fabricated using the electrospinning technique. Furthermore, thermosensitive CS/P(NI_9HE_1) nanofibers loaded with acid-extracted undialyzed ulvan will be prepared, and their potential as wound dressings will be evaluated in the near future.

KEYWORDS: ulvan, extraction, electrospun nanofiber, thermosensitive, wound dressing



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Lanthanide Hydrogel: A Versatile Biomaterial with Stretchability and Self-Healing Abilities for Bioimaging

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Abstract:

This study presents a novel design for zwitterionic poly(sulfobetaine methacrylate) (PSBMA) hydrogels that possess exceptional properties of toughness and fluorescence, opening up vast possibilities for diverse applications. The synthesis of PSBMA hydrogels involved a careful balance of key components, including sodium dodecyl sulfate (SDS), lanthanide ions, stearyl methacrylate, and SBMA, resulting in a transparent solution. The precursor solution, initiated by APS/TEMED, led to the formation of physically crosslinked PSBMA hydrogels, which exhibit remarkable flexibility, self-healing capabilities, and strong adhesion to various materials. Additionally, the presence of lanthanide ions in the hydrogel imparts significant fluorescence upon exposure to ultraviolet light. By adjusting the ratio of Tb^{3+} to Er^{3+}, a range of fluorescent colors can be achieved, spanning from green to red, offering potential for imaging applications.

KEYWORDS: lanthanide-based hydrogel, flexibility, self-healing, adhesion, bioimaging

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Gelated cells as a robust cell-mimicking biomaterial system for cancer immunotherapy and tissue engineering

Jung-Chen Lin[1], Chen-Ying Chien[1], Chi-Long Lin[1], Chung-Yao Hsu[1], Jui-Yi Chen[1], Bing-Yu Yao[1], Yu-Han Liu[1], Zih-Syun Fang[1], Hui-Wen Chen[2], Che-Ming J. Hu[1]*

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Abstract:

The cell membrane serves as a crucial biological interface for cellular interactions with the external environment and other cells and relies on the support of the cytoskeleton to maintain its structure. In the recent years, more studies have been carried out for development of cell-mimicking biomaterials with retaining the characteristics of natural cell membranes. However, most existing biomaterials fail to mimic the intricate the dynamic surface chemistry of their live counterparts. Herein, we demonstrate a novel cellular fixation technique, intracellular hydrogelation, to create inanimate, cell-like biomaterials by facile transformation of live cell into stable, preservable constructs that retain the intricate membrane interface. By infusing PEG-diacrylate (PEG-DA) monomers to the intracellular domain using photo-activated radical polymerization to form intracellular hydrogel in cytosol, we show that the fluid cell membrane can be adequately preserved in natural cells, giving rise to intracellularly gelated cells (GCs) with robust stability. We validate retention of surface properties, membrane lipid fluidity, lipid order, and protein mobility on the GCs. Intracellular hydrogelation can be applied for the fixation of human primary dendritic cells (DCs) and xeno-free feeder layer.

For antigen-specific T cell expansion, we develop the G-DCs that can engage with antigen-specific T lymphocytes in a lifelike fashion and promote cell expansion in vitro and in vivo. In addition, the robust stability of the G-DCs further enabled their subsequent modification with cytokine-bearing carriers. G-DCs-expanded tumor antigen-specific T lymphocytes exhibit enhanced anti-tumor activity in vivo. In addition, intracellular hydrogelation applied to cellular monolayers leads to the creation of biomimetic devices suitable for tissue engineering applications. Intracellularly gelated feeder cell monolayers are stable for 180 days without cellular detachment, and they retain the protein presentation, membrane fluidity, and cellular topology of live cell monolayers. This biomimetic feeder layer is shown to sustain expansion of murine and human induced pluripotent stem cells.

Overall, intracellular hydrogelation ensures that cells maintain their membrane activity and function, and also provides a powerful research tool for cancer immunotherapy and regenerative medicine.

KEYWORDS: Intracellular hydrogelation, gelated cell, cell-mimicking biomaterial, biomimetic cell membrane, cancer immunotherapy, tissue engineering.

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Chitosan-based nerve guiding conduit for peripheral nerve repair

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Abstract:

Neurological damage poses significant challenges in therapeutic interventions, necessitating the exploration of effective repair strategies. Autologous nerve transplantation, the current standard approach, is limited by the scarcity and size constraints of available nerve grafts. [1][2] To address these limitations, the present study investigates the potential of chitosan-based nerve conduits as an alternative therapeutic option. Chitosan, known for its high biocompatibility, degradability, ease of manufacture, and cost-effectiveness, was selected as the primary material for fabricating the nerve conduits.

In this study, chitosan films were prepared using varying concentrations of sodium hydroxide (NaOH) (0.5M, 1.0M, 1.5M) to achieve smooth microstructures with minimal irregularities. Mechanical properties, such as tensile strength, were evaluated to assess the film's performance, resulting in values of 93.003±0.004. N, 192.254±10.257 N, and 184.303±1.556 N, respectively. The chitosan films were further crimped and fixed with collagen to create spacious conduit capable of accommodating nerve repair. The average inner diameters achieved were approximately 1.091±0.138mm, 1.322±0.0521mm, and 1.337±0.113mm for the respective NaOH concentrations. The corresponding average outer diameter sizes were measured as 1.632±0.152 mm, 2.0371±0.068 mm, and 1.700±0.147 mm respectively. The microstructure of the prepared nerve guiding conduit was observed using SEM, revealing a multilayer porous structure. The conduit not only provided mechanical support for fractured nerves but also demonstrated the required strength and flexibility to endure the stretching and contraction of surrounding muscles while maintaining an open inner channel. Bending tests revealed that the conduit with a NaOH concentration of 0.5M exhibited the best bending ability, with bending angles of 100.67±17.48°, 48.42±11.40°, and ±3.22° for the respective concentrations. Moreover, the degradation characteristics of the conduit were evaluated over a six-month period, demonstrating a gradual degradation while preserving structural integrity. The incorporation of collagen within the conduit facilitated nerve repair by promoting growth and filling the gap between the nerve and conduit. [1][3] The cytotoxicity of the conduit was assessed using PC12 cells through WST-1 and LDH assay. The results indicated that there was no significant cytotoxicity observed in any of the experimental groups.

In summary, this study provides valuable insights into the feasibility and usability of chitosan-based nerve guiding conduits for nerve repair. The findings regarding their mechanical properties, biocompatibility, degradation characteristics, and potential for nerve regeneration serve as essential references for future developments in neural conduit materials and technology.

KEYWORDS: Nerve guiding conduit (NGC), Nerve injuries, Chitosan

Graphic abstract

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Fig. 1. This is the production process of NGC.

2023 ISOMRM & BCRS
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Photocrosslinkable Microbeads for Delivery of Dietary Supplement

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Abstract:

Dietary supplements play a crucial role in meeting the nutritional requirements of the human body, as certain nutrients in food can be easily degraded by enzymes or rapidly metabolized. In this study, we aimed to develop a method for fabricating gelatin-based microbeads capable of encapsulating magnetic carriers to achieve sustained release of dietary supplements. To enhance the stability and compatibility of magnetic carriers with gelatin-based microbeads in aqueous environments, we employed two hydrophilic polymers, namely high molecular weight and low molecular weight polymers, to modify the magnetic carriers. Our results demonstrated that the modified magnetic carriers exhibited excellent dispersion in water. Moreover, we observed that the utilization of gelatin-based microbeads for encapsulating dietary supplement-loaded magnetic carrier microspheres enabled sustained release over a period of 7 days. These findings suggest that the integration of drug-loaded magnetic carriers with gelatin-based microbeads holds promising potential for applications in drug delivery.

KEYWORDS: Gelatin, Photocrosslinkable microbeads, Drug delivery

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Gelatin-based Composite Microneedle patches for the Treatment of Keloid Scars

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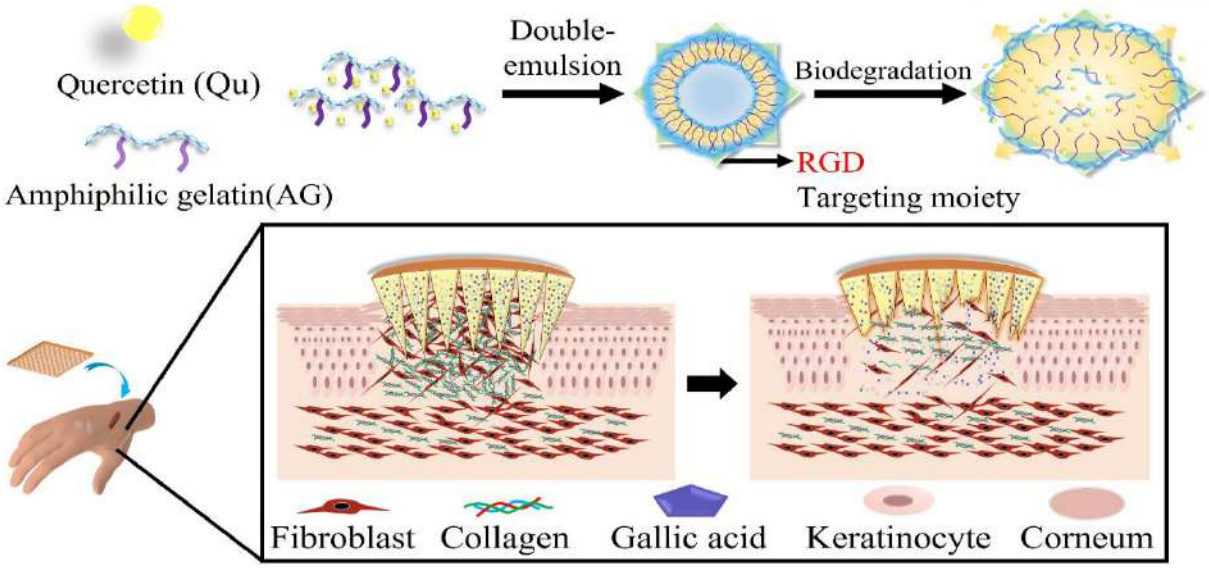
Abstract:

Keloid scars are abnormal tissue scars that occur during wound healing. During the proliferation stage of skin wound healing, some fibroblasts gradually differentiate into myofibroblasts to promote shrinkage of the wound edges. However, if fibroblasts are excessively activated during wound healing, excessive collagen can be secreted, resulting in an imbalance between the synthesis and degradation of the extracellular matrix (ECM), which leads to the formation of keloid scars. To date, various clinical therapeutic strategies have been developed for the treatment of keloid scars, including prophylactic treatment, surgical excision, laser therapy, and intralesional corticosteroid injection. Compared with other therapeutic strategies, prevention of pathological keloid scarring can help avoid unnecessary scarring; thus, prophylactic treatment is considered superior to other treatments. Specifically, amphiphilic gelatin (AG) was used to encapsulate hydrophobic quercetin (Qu) by forming a W/O/W drug nanocarrier via double emulsification, without additional surfactants. The RGD on the gelation can be used as cellular binding sites to enhance the affinity between cell and carrier. The hydrophilic surface of the Qu-loaded amphiphilic gelatin nanocarrier (QAGN) can be homogeneously dispersed in the gelatin of the MN which contains GA to enhance the efficiency of transdermal absorption. By controlling the release of GA and Qu, the composite MN with the nanocarrier can provide an additive effect between GA and Qu to achieve combinatory therapeutic efficacy and prevent keloid scar formation. As the dual drug released at different time, GA could be initially released for retarding of fibroblasts over growing, and quercetin was released as strong antioxidant to erase ROS generation for scar sustained invasion and has been the TGF-beta pathway inhibitor for regulating extracellular matrix production (collagen type I, III). As the cell viability, and ROS generation results shown that the GA indeed could inhibit fibroblasts proliferation. Furthermore, the data of qRT-PCR also indicated that the gene expressions of fibroblast (such as Col I, III) were downregulated in the dual drug synergy system. GA was added in the gelatin to fabricate the GA-loaded MNs for benefit direct delivering into the wound skin. The result has showed that GA was rapidly released at an earlier stage due to the dissolution of MN to inhibit fibroblast proliferation, as demonstrated in results. Cell viability was rapidly inhibited after GA was released in the short term (3 h) and displayed a significant downward trend after 24 h, particular in the GA and GA + QAGN groups. In contrast, the QAGN group did not present any obvious effect on the inhibition of fibroblast proliferation. Therefore, the contribution from the GA is very important in inhibiting the abnormal fibroblast proliferation. To further avoid excessive collagen deposition and prevent scar formation in the later stage, Qu was used because it is an onion extract that possesses multiple hydroxyl groups and can erase ROS. However, Qu is hydrophobic and not easily dispersed in an aqueous solution for cellular uptake, restricting its bioavailability and causing a major barrier to therapeutic uses. To improve the low bioavailability and poor absorption of Qu, AG nanoparticles was synthesized to encapsulate Qu. As expected, Qu in QAGN can be released slowly and

continuously as shown in the results. The ROS activity was significantly inhibited in the QAGN compared to GA groups after 24 h of culture, indicating the later-released Qu made a significant contribution to the inhibition of ROS. It is noted that the group of GA + QAGN can much enhance the efficacy, indicating that the combination of GA and Qu has the most significant inhibitory effect on fibroblast proliferation and ROS generation. We successfully designed a heterogeneous gelatin-structured composite MN system which modulated dual-drug release profile of GA and Qu to prevent keloid scar formation. The developed MN system can realize a combinatory effect by rapidly releasing the gallic acid due to the rapid dissolution of the MN after insertion, thereby effectively inhibiting the fibroblast proliferation and production of collagen. In addition, nanocarriers in the MNs can achieve a slow and sustained release of quercetin, thereby reducing the production of reactive oxygen species. In turn, the relative gene expression of Col I, III, and TGF-β1 was continuously downregulated to inhibit keloid scar formation for the MN with GA + QAGN group. Our preliminary results showed that the composite microneedle system shows great potential to realize the possibility of preventing keloid scar formation and in vivo experiments will be explored in the future.

KEYWORDS: keloid scar; gallic acid; quercetin; hydrogel microneedle; nanocarrier



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2023 ISOMRM & BCRS
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Development adhesive microneedles as an oral patch for recurrent aphthous stomatitis treatment

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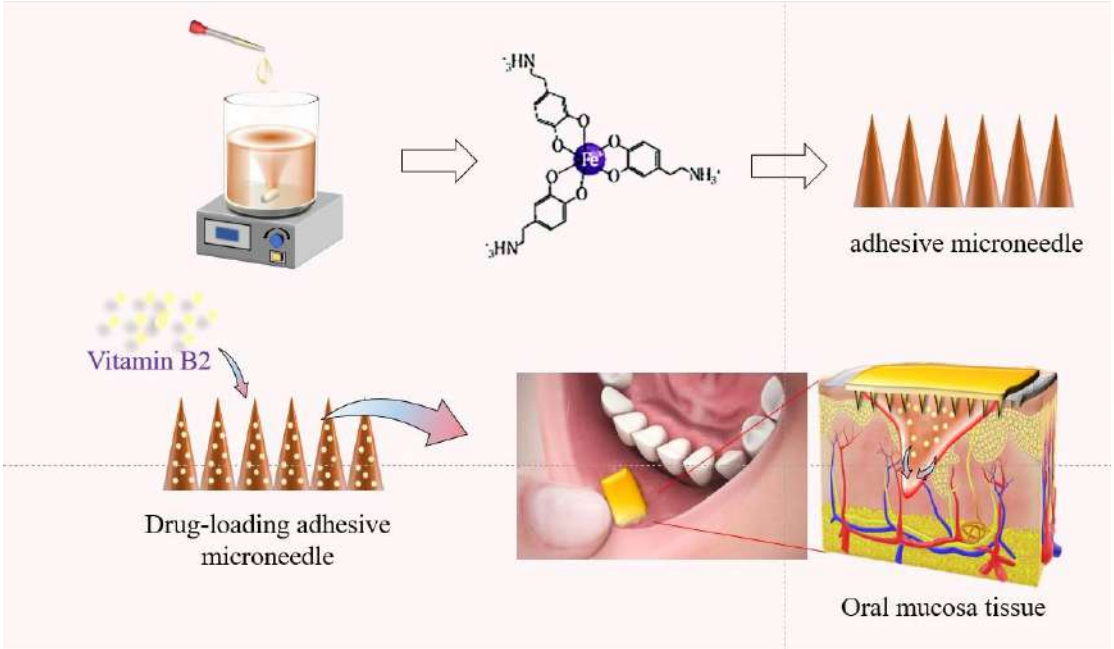
Abstract:
Recurrent aphthous stomatitis is a common oral mucosal ulcer disease, characterized by recurrent and painful ulcers of oral mucosa. The etiology of recurrent aphthous ulcers is still unknown, and possible pathogenic factors such as genetics, allergies, menstrual cycle, immune system dysfunction, and vitamin deficiency. Oral cream is often used as the method of administration. However, oral cream lacks mucous membrane adhesion and is easily washed away by food, liquid or even saliva, which could not achieve effective treatment. Currently, gel or patch products are used to form a protective film in the injure area and achieve anti-inflammatory and anti-allergic effects through the release of anti inflammatory drugs from the materials. However, the degradable patch made of natural or synthetic polymer has its limitation in use and composition, respectively. Moreover, the oral ulcer is covered by a yellow-gray pseudomembrane composed of fibrinous exudate and necrotic tissue. Therefore, the main purpose of this project is to develop an oral patch with adhesiveness and efficient drug delivery. In this study, dopamine will be used to modify hyaluronan to prepare sticky derivatives, which will be used as the material to develop adherent and degradable microneedle oral patch. Carbenoxolone and vitamin B2 will be added to the microneedle oral patch as anti-inflammatory drugs. Therefore, we will use the dopamine-modified hyaluronan with carbenoxolone extract and vitamin B2 to prepare a safe, durable, easy-to-use oral patch for recurrent aphthous ulcer treatment. From results indicate dopamine can be successfully grafted to hyaluronic acid via Schiff's base reaction, and the grafting rate could reach 33%. Moreover, hyaluronan sticky derivatives could be enhanced the mechanical properties with hydrogen bonds, π–π stacking, cation–π interactions, and metal coordination to form a stable molecular structure. Upper than 5% hyaluronan sticky derivatives that have excellent mechanical properties to penetrate the pseudomembrane. Vitamin B2 could be directly transported into the oral epithelial tissue. Furthermore, dopamine can effectively improve the biocompatibility of the patch without additional cytotoxicity, making the patch relatively safe to use. From the above results, this study has successfully developed a

oral microneedle patch with an adhesive effect, and this safe and easy-to-use composite structure patch can real-time release riboflavin within 30 min, inhibit wound inflammation. It can promote wound healing and has potential in the treatment of recurrent oral ulcers.

KEYWORDS: Recurrent aphthous stomatitis, hyaluronic acid, Catechol group, Oral patch.



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Development of Indocyanine Green and Camptothecin loaded Hyaluronic Acid Hydrogel for Photochemotherapy of Skin Cancer

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Abstract:

Skin cancer is one of the most common cancer. It mainly results from the UV radiation of the sun. Among the different types of skin cancer, melanoma is the most lethal and it accounts for about 10% of skin cancer. When melanocytes duplicate rapidly and mutate due to UV radiation, it becomes melanoma tumors. Although the occurrence of melanoma tumors is low, it has metastasis ability, which is hard to cure and fatal. Based on previous researches, melanoma tumors can be cured by immunotherapy and chemotherapy.

However, the severe side effect of the therapy causes bad physical and mental health of the patients. Camptothecin (CPT) is a plant extract from the bark and stem of Camptotheca acuminata (Happy tree). It is a topoisomerase inhibitor which have anticancer ability against breast, colon and stomach cancer.

Indocyanine Green (ICG) is a fluorescent dye used in medical diagnostics and it is a good photosensitizer. Also, it can absorb longer wavelength radiation to generate heat and ROS to kill tumors. It has been used to cure Pancreatic Cancer and Breast Cancer.

The ratio of HDI-PF127 and HA can also change the viscosity of the hydrogel. Hyaluronic Acid can be found in human body which is biodegradable and biocompatible. As there is overexpression of hyaluronic acid receptor CD44 in tumor cells, HA is a good drug carrier.The unique property of this hydrogel can allow the material to stay on skin and control the release of the drug, which can overcome the circumstance in skin surface drug delivery.

In order to reduce the side effect of the therapy, we have developed Indocyanine Green and Campytothecin are loaded Hyaluronic Acid Hydrogel (ICHHG) that provides both phototherapy and chemotherapy upon near-infrared light.

KEYWORDS: Skin cancer, Indocyanine green, Camptothecin, Hyaluronic Acid Hydrogel, Photochemotherapy

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Smart Hydrogels for Trans-mucosal Drug Delivery: Evaluation the Efficacy of New Targeted Hyaluronic Acid-Drugs to Treatment Inflammatory Bowel Disease.

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Abstract:

Inflammatory bowel disease (IBD) includes Ulcerative colitis (UC) and Crohn's disease (CD), which is a chronic autoimmune disease whose etiology is not yet fully understood persistent. It may increase the incidence of colorectal cancer because of the chronic inflammation. The current clinical first-line therapeutic drugs are the enemas with mesalamine, and the treated strategy is that increases the drug's concentration in the intestinal tissue. However, the UC favorite occur on the distal intestine, and the enema is easily excreted with the excrement due to physiological stimulation, which limits the therapeutic effect. In this study, we established a mucosal drug delivery system that combines the bioadhesive and biological targeting to increase the residence time of the drug in the inflamed tissues of the intestine. First at all, we developed a new enema formulation with a hydrogel grafted methylcellulose (MC), which is loaded an anti-inflammatory Hyaluronic acid-Mesalamine (HAMe) targeted drug. By adjusting the ratio of MC, HA and excipients to HAMe forming the smart MC-HAMe hydrogels with the thermosensitive property can control the time of sticking on the intestinal wall with body temperature. The MC-HAMe hydrogels can increase the concentration of the drug in the inflamed tissue of the intestine, and release the drug slowly through the degradation of hydrogel. The research results improve the problem of poor therapeutic effects of IBD treatment drugs, and expect to reduce the incidence of colorectal cancer.

KEYWORDS: inflammatory bowel disease, mucosal drug delivery system, bioadhesive, biological targeting, methylcellulose, hyaluronic acid-mesalamine

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Development of in situ dual-crosslinkable (dcHA) hyaluronic acid based injectable hydrogel as a dermal filler

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Abstract:

In recent years, injectable crosslinked hyaluronic acid (cHA) hydrogels have been widely used in biomedicine due to their high biocompatibility, biodegradability, and low toxicity. To improve the low mechanical strength and short half-life of cHA. In this study, cHA hydrogels composed of HA modified with three different degrees of substitution of maleimide ($HM_{10/20/30}$) and crosslinked with HA-SH (H_S) ($HM_{10}H_s/HM_{20}H_s/HM_{30}H_s$)[1], then HA-PEGDE ($H_P$) or HA-BDDE ($H_B$) was add into cHAs hydrogels to form double crosslinked HA (dcHA) hydrogels ($H_PHM_{10/20/30}H_S$/ $H_BHM_{10/20/30}H_S$).The rheological tests[2], such as strain amplitude sweep, strain frequency sweep, alternative-step test, and creep recovery test, will carry out on $HM_{10}Hs$, $HM_{20}Hs$, $HM_{30}Hs$, $H_PHM_{10}Hs$, $H_PHM_{20}Hs$, $H_PHM_{30}Hs$, $H_BHM_{10}Hs$, $H_BHM_{20}Hs$ and $H_BHM_{30}Hs$, and the commercially available Restylane® Lyft was used as the control group. In the cHA group, the mechanical strengths were G′=100~1000 Pa, it's similar with the Restylane®Lyft., therefore we hope the dcHAs group results will show higher hydrogel strength than commercially available products. Each group of dcHAs also show better linear viscoelastic regions (LVR) than commercially available products and show lower permanent strain in the creep recovery test. The morphology evaluation and preclinical trials were used to conform the properties of dcHAs.

In this study, we look forward to seeing the considerable potential of cHAs and dcHAs hydrogel as materials for medical applications, such as dermal fillers to smooth wrinkles or as biomaterial for bone regeneration.

KEYWORDS: double cross-linked HA, *in situ* injectable hydrogel, rheological study, biomedical materials.

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2023 ISOMRM & BCRS
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Development and evaluation of thermosensitive hydrogels for long-acting injection formulations

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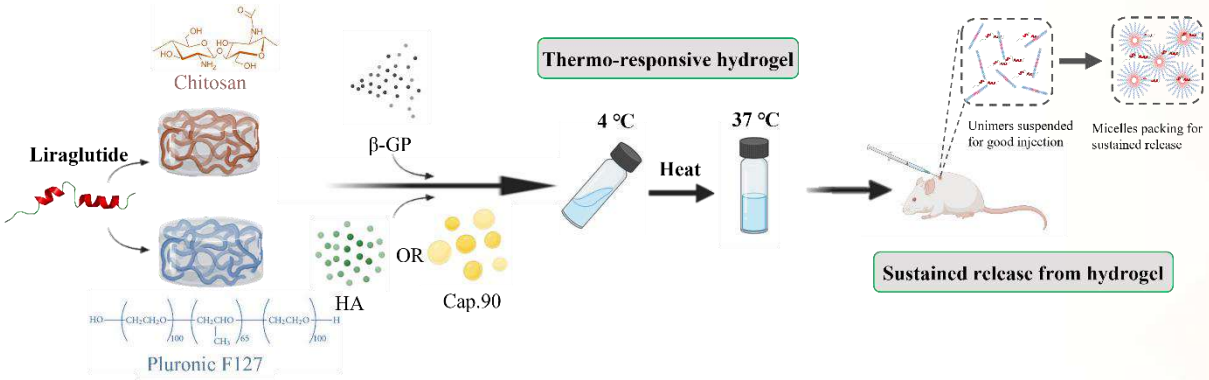
Abstract:

According to the report of the International Diabetes Federation（IDF）, the global prevalence of diabetes is estimated to reach 12.2% in 2045 and 90% of them belong to type 2 diabetes[1]. Liraglutide （Lira.） is the glucagon-like peptide-1 receptor agonist （GLP-1 RA ） which can activate pancreatic β cells making them proliferate and differentiate as well as inhibit their apoptosis, and also promote postprandial insulin secretion leading to lower risk of hypoglycemia. The half-life in the human body is 13 hours and daily subcutaneous injection is required in clinical application[2]. Therefore, the purpose of this study is to develop a long-acting injection using temperature-sensitive hydrogel prolonging the release time of liraglutide in vivo to achieve sustained and slow release effects, reducing drug injection rates and improving compliance[3].

In this study, the natural polymer chitosan（CS）and the synthetic polymer Pluronic F127 （PF127） were used as two kinds of hydrogel materials. The physicochemical properties were evaluated under different concentrations of polymers and auxiliary materials （Sodium hyaluronate, Capryol® 90, β-Glycerophosphate）.Results of gelation temperature showed that 15 and 16 % PF127 and 1%CS have gelation temperatures closer to physiological temperature. In the rheological evaluation,PF127 hydrogel had strong rigidity （larger G' value）, but was easily deformed by external force （small linear viscoelastic range）.Scanning electron microscope results indicated that $PF_{15}HA_{1.0}$ had well-arranged and uniformly sized pores. In the hydrogel degradation test, $LC_{1.0}$ β-GP_{15} and $MC_{1.0}$ β-GP_{15} still had 15-20% hydrogel residue on the 35th day. The in vitro dissolution test illustrated that the PF127 group had a slow release within 6 hours. The results of pharmacokinetics showed that $PF_{15}HA_{1.0}$, $PF_{16}Cap_{0.3}$ and $PF_{13}L_{0.15}Cap_{1.5}$ had slower drug release curves.

Based on the results of this study, we successfully developed the thermosensitive hydrogel for the evaluation of GLP-1 RA drug release. Lira. encapsulated by the hydrogel can prolong the time of maximum blood concentration （T_{max}） and the half-life of the drug （$T_{1/2}$ ） and increase the relative bioavailability.

KEYWORDS: hydrogel delivery system, Pluronic F127, Chitosan, Liraglutide, Long-acting injection.



(A schematic made by Biorender)

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Dual-crosslink of Self-assemble and Photocrosslinling Collagen Hydrogel Promote Vascular Tissue Engineering

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Abstract

In the wound-healing process, blood vessels play a crucial role in providing nutrients and oxygen in a timely manner to surrounding cells at injury sites to improve tissue repair and regeneration. Collagen is one of the main structural proteins in the extracellular matrix (ECM) of vertebrate cells. Due to its natural origin, excellent biocompatibility, low immunogenicity, biodegradability, and the ability to self-assemble into hydrogels, collagen hydrogel is widely used as a scaffold material for wound repair in clinical applications. Native collagen hydrogels formed through self-assembly collagen fibers can support cell migration, proliferation, and differentiation[1]; in addition, they also contribute to the viscoelasticity of hydrogels, enabling them to withstand mild strain or deformation[2]. In previous studies, collagen hydrogels have been used as carriers to encapsulate human mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs) to engineer functional vascular networks formed inside collagen gels through vasculogenesis, angiogenesis, and anastomose, which integrate with the host's vasculature and rapidly establish a vascular network to supply nutrients for tissue repair. However, collagen has inherent limitations, such as weak mechanical properties, significant gel contraction after cell encapsulation, and rapid degradation, which restrict its clinical use in large-sized defect repair. Previous attempts to enhance mechanical properties through various crosslinking methods have resulted in increased hardness that hindered the diffusion of nutrients, leading to cell necrosis[3].

Photocrosslinking is an alternative and widely used crosslinking method. After light exposure, photoinitiators release many free radicals, which react and quickly form covalent bonds between modified function groups on collagen molecules to form photocrosslinked collagen hydrogels. Patterned photocrosslinked collagen hydrogels created through the designed photomask provide some diffusion channels which allow oxygen and nutrient transportation throughout the hydrogel to increase cell viability and functionality. However, modifying photoactive functional groups on collagen

molecules is difficult due to pH sensitivity, tight triple-helical protein structure, and precipitation as increasing salt concentration to achieve optimal outcomes regarding yield, modification rate, and hydrogel stability. Additionally, the use of crosslinking agents can cause cell toxicity or instability of the hydrogel [4]. Therefore, new crosslinking methods need to be developed to address these issues.

In this study, a collagen-based hybrid hydrogel consisting of mixed native collagen and photocrosslinkable collagen is proposed. The photo-patterned region of the hydrogel crosslinked with covalent bonds can maintain volume and provide structure stability of the whole hydrogel, and other region self-assembly formed by hydrogen bonds between collagen fibers can provide a more suitable growth environment for cells. Moreover, results demonstrated that this dual-crosslinked collagen hydrogel can support the formation of vascular-like lumens during cell culture and further effectively generate microvessels integrated with the host vasculature in mice, which improves its capabilities in clinical use in tissue engineering and regeneration medicine.

KEYWORS: collagen-based hybrid hydrogel, photocrosslinking, angiogenesis, tissue engineering, wound healing

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Pre-spheroidized by Chitosan Prompt Osteoblast-like MG63 Cells to Osteogenesis on NaOH Etching 3D printing Ti-alloy Scaffolds

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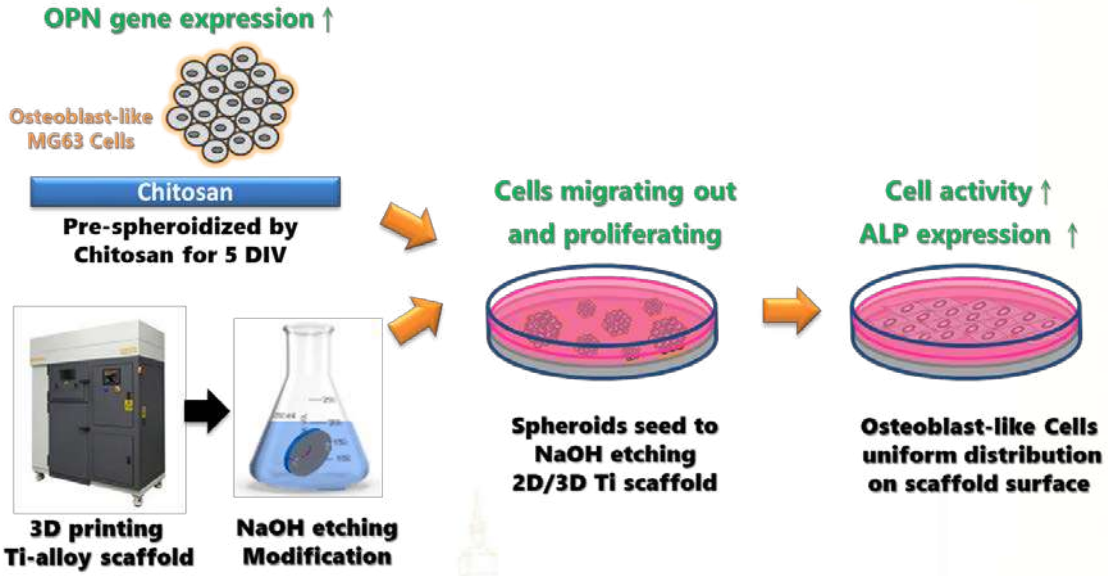
Abstract:

Titanium (Ti) alloy is one of the clinically better bone graft materials because it has lightweight, biocompatibility, and antibacterial properties. A thin oxide layer can form on its surface, which has excellent chemical stability and can help osteoblasts adhere to and induce bone cell growth [1]. Due to these advantages, this material has been widely used in 3D printing to produce bone grafts. 3D printing process can create scaffolds to provide a biologically similar microenvironment [2, 3] and biomechanical support. Before the implantation of bone grafts, sometimes the cell therapy technique is applied simultaneously to enhance the effectiveness of bone repair. However, it takes hours for cells to attach on the substrate during cell seeding. Before attaching, the cells sink to the bottom layers due to gravity, resulting in uneven distribution of cells throughout the scaffolds. Therefore, it is important to find a way to make the cells can attach and distribute well and precisely.

Although Ti alloy is a biocompatible material, this does not mean that osteoblasts can attach and grow well on untreated substrate, or even express proteins and behave normally. We need to find a post-treatment method for 3D printing Ti alloy scaffolds priority. Since grinding and polishing can't modify the internal part of 3D porous scaffolds, the chemical etching technology [4, 5] was only used in this study to process scaffolds. After chemical etching treatment (HCl, H_2SO_4, HCl+H_2SO_4, and NaOH solutions), the surfaces of these scaffolds were examined preliminarily. On the other hand, osteoblast-like cells were pretreated with chitosan before seeding onto 3D scaffolds. The osteopontin (OPN) gene expression of osteoblasts was upregulated after these cells naturally form suspended spheroids on chitosan for 5 DIV. After seeding onto chemical etching scaffolds, osteoblasts within the spheroids migrated out and proliferated better than non-prespheroidized osteoblasts. Osteoblast spheroids within the HCl+H_2SO_4 and NaOH groups expressed higher cell viability and ALP protein. Although these two groups had similar excellent effects, the mechanical strength of the scaffold in the HCl+H_2SO_4 group was weak, which would affect the therapeutic effect of bone grafting clinically. Therefore, NaOH would be a good choice for post-treatment of 3D printing Ti alloy scaffolds.

KEYWORDS: Titanium alloy, Chitosan, Ostoblasts, Cell spheroids, Chemical etching

Graphic abstract (not a mandatory requirement)



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Development of Perfluorinated Cancerous Exosomes for Enhanced Target Photochemotherapy in Triple-Negative Breast Cancer

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Abstract:
Breast cancer has emerged as one of the most prevalent forms of cancer in our modern era. In 2020, a significant milestone was reached as the number of individuals diagnosed with breast cancer surpassed those with lung cancer, establishing it as the leading cancer worldwide. Among various subtypes of breast cancer, triple-negative breast cancer stands out due to its heightened resistance to multiple drugs (MDR) and aggressive metastasis capabilities. To combat these challenges, scientists have turned to artificial nanocarrier technology for drug delivery and cancer treatment. Exosomes, small vesicles measuring approximately 100 nm, were initially discovered in 1983 on sheep reticulocytes. Initially disregarded as mere cellular waste, it wasn't until 2013 that the crucial role of exosomes in cell-to-cell communication and regulation became apparent, leading to increased research attention.

Taking inspiration from nature, we have devised a novel approach utilizing exosomes as carriers for anticancer drugs. This innovative concept can be likened to poisoning the food of cancer cells, enabling enhanced drug delivery and ultimately more effective cancer treatment. Our nanocarriers are composed of CPT, ICG, and PFCs, and exhibit dual functionality for both photothermal therapy and photodynamic therapy. Through dynamic light scattering (DLS) analysis, we have observed notable changes in the size and zeta potential of our drug-loaded nanocarriers. Encapsulation efficiency was determined using ultraviolet-visible spectroscopy (UV-Vis), while the impact of photothermal therapy/photodynamic therapy on our drug nanocarriers was evaluated using near-infrared (NIR) radiation at 808 nm and 6 W/cm².

The results obtained thus far highlight the tremendous potential of our developed nanocarriers in facilitating photothermal therapy/photodynamic therapy, preserving drug stability, and efficiently encapsulating drugs. These findings bring us closer to achieving breakthroughs in cancer treatment and pave the way for further advancements in nanomedicine.

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Vitamin B_{12} Loaded Methylcellulose/Hyaluronic Acid Thermosensitive Hydrogel Ring for Ocular Drug Delivery

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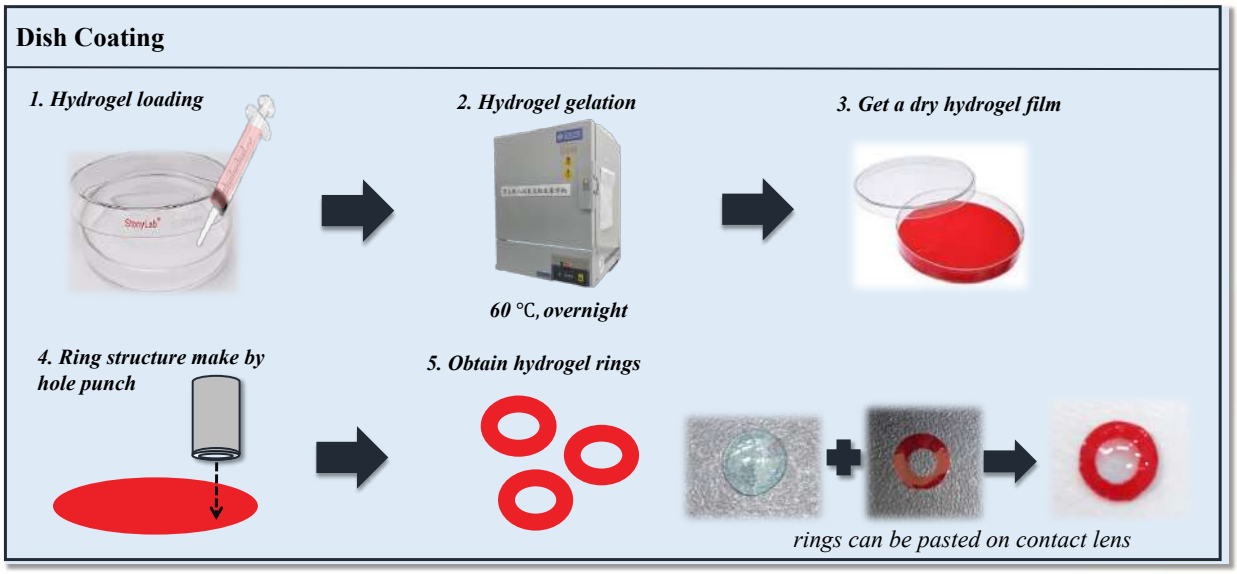
Abstract:

Nowadays, electronic devices have become a part of people's daily life, causing ocular diseases such as glaucoma, dry eye and visual fatigue to become more and more common. The global ophthalmic drugs market is expected to reach USD 42.7 billion by 2023. Topical instillation of eye drops is the most common treatment method for ocular-related-conditions. Although eye drops are the most direct and easiest way to treat the eyes, less than 5% of the active substance is absorbed by the eye due to first reflex blinking, drug-tear film mixing, nasolacrimal drainage and tear film turnover. Besides, topical eye drops require repeated administration at specific interval, patients may forget to use, and lead to therapeutic failure. Therefore, we would like to develop an easy-to-fabricate hydrogel ring as a drug carrier on contact lenses to increase the corneal surface residence time and improve bioavailability. We prepared drug-releasing rings with different concentrations of methylcellulose (MC) and hyaluronic acid (HA) and used rheometer to evaluate the gelation temperature. Then vitamin B_{12} was added to the MC/HA hydrogel to observe its drug release behavior. The degradation rate, light transmittance, and oxygen permeability of the drug release ring were also evaluated. Results showed that MC/HA/vitamin B_{12} hydrogel ring has well oxygen permeability, and it could sustain release vitamin B_{12} more than several hours. Importantly, the drug-releasing MC/HA/vitamin B_{12} hydrogel ring possess good cell compatibility when cultured with L929. According to above results, the MC/HA/vitamin B_{12} hydrogel ring could combined with contact lens and show its potential in ocular drug delivery vehicles for eye treatments in the future.

Keywords: *methylcellulose, hyaluronic acid, vitamin B_{12}, hydrogel ring, contact lenses*

This study was supported by National Science and Technology Council (Project number: MOST 110-2622-E-239-005).

Graphic Abstract



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Versatile photothermal nanozymes with glutathione depletion and thermal/acidity-triggered hydroxyl radical generation for combination cancer therapy

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Abstract:

To potently inhibit tumor growth and metastasis by chemodynamic/photothermal combination therapy, we designed versatile photothermal nanozymes capable of depleting glutathione (GSH) and generating hydroxyl radicals in the thermal/acidity-activated manner. Through chelation of Fe^{3+} ions with PEGylated chitosan (PEG-CS)-decorated polydopamine (PDA) nanoparticles, the attained Fe^{3+}@PEG-CS/PDA nanozymes were characterized to have a solid-like spherical shape of around 150 nm and exhibited outstanding colloidal stability. The Fe^{3+}@PEG-CS/PDA nanozymes displayed satisfied photothermal conversion efficiency (ca 43 %) and photothermal-stability after multiple irradiation of 808 nm near infrared (NIR) laser. Notably, Fe^{3+}@PEG-CS/PDA nanozymes under high temperature and acidic conditions effectively converted hydrogen peroxide (H_2O_2) into toxic hydroxy radical (·OH) via GSH-consumed and Fe^{2+}/Fe^{3+}-mediated Fenton reaction. The in vitro cellular uptake and cytotoxicity studies demonstrated that the endocytosed nanozymes effectively decomposed intracellular H_2O_2 to generate ·OH upon NIR-triggered hyperthermia, thereby prominently killing 4T1 mouse breast cancer cells and murine macrophages-like RAW 264.7 cells. The nanozymes considerably suppress 4T1 breast tumor growth in vivo and metastasis of cancer cells to major organs without significant systemic toxicity upon the chemodynamic/photothermal combination therapy. This work suggests the feasibility for applying such an advanced nanozyme in tumor treatment.

KEYWORDS: GSH depletion, nanozymes, polydopamine, Fenton reaction, chemodynamic/photothermal therapy

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Mechanism of GNR@MIL-100(Fe) induced Macrophage Activation

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Abstract:

When it comes to cancer therapy, there are many novel cancer therapies today, one of which is immunotherapy. Although traditional therapy is more mature and common, it has many disadvantages, such as surgical resection is more suitable for early stage, and once cancer cells start to metastasize, this method is not suitable. Chemotherapy can be used for cancer cells that have already metastasized, but it has many side effects, the concept of hurting one thousand and eight hundred. While radiotherapy has fewer side effects, the course of treatment takes a very long time. Immunotherapy activates the innate and adaptive immune systems to cure diseases with the important features of potency, specificity, and memory. By targeting the immune system instead of the tumor itself, cancer cells can be precisely recognized and destroyed in an antigen-specific manner. The most impressive characteristic of this therapy is that a long-term response can be realized through the memory of immune cells without collateral damage. Due to the low expression and mutation of tumor antigens, phagocytic APCs are usually unable to recognize tumor cells. By utilizing nanoparticles (NPs), the poor immunogenicity and low antigen sensitization of tumors can be overcome, consequently enhancing antitumor immunity. One such immunotherapy is to use the polarization of macrophages to cause the suppressive effect of cancer cells.

Macrophage cells are plastic and can initiate inflammatory (M1) or anti-inflammatory (M2) immune responses, the two major phenotypes of macrophages. In the innate immune system, the M1 phenotype could lead to antitumor immunity, offering new opportunities for tumor therapy. However, the polarization of TAMs to M2 phenotype is always associated with cancer growth, metastasis, and angiogenesis. Thus, depletion or reprograming of M2 macrophages into M1 phenotype will contribute significantly to combatting cancer [1].

Metal−organic frameworks (MOFs), with high porosity, surface modification ability, degradability, tunable pore size, and particle size, are excellent candidates as nanocarriers for imaging contrast agents and therapeutic cargoes. In the last experiment, when GNR@MIL-100(Fe) was used to conduct photothermal experiments in cells, it was found that this material would increase the index of macrophage polarization into M1 [2], so our rea wanted to explore which part of this material caused it. It may be the iron of MIL-100(Fe) itself or its different valence state, or the gold of GNR, so it is necessary to control variables and prepare materials: 1. Control the valence state of iron as a variable 2. Keep the Au content of GNR@MIL-100(Fe) the same as that of GNR 3. Keep the Fe content of GNR@MIL-100(Fe) the same as that of MIL-100(Fe)

KEYWORDS: Metal−organic frameworks (MOFs), Immunotherapy, macrophage polarization

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The effect of metformin on carbonic anhydrase type 8 and glucose transporter type 2 in kidney and HK-2 cells

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Abstract: In Taiwan, the cases of diabetes are increasing year by year, and the age of onset tends to be younger. Metformin (Metformin, Met) is a commonly used drug for the treatment of type 2 diabetes. Carbonic anhydrase 8 (CA8) is a novel protein with limited known biological functions. Previous research in the laboratory showed that the novel protein CA8 may be involved in the process of glucose metabolism, and the laboratory recently found that different concentrations of Met can cause two-way changes in the expression of CA8 in liver cells, and CA8 also interacts with glucose transporters that affect glucose transport. It is well known that Met and CA8 are similarly distributed in body tissues, including liver, kidney, and intestinal tract, indicating that Met may affect the expression and biological function of CA8 in these tissues. Because the kidney disease caused by type 2 diabetes will further affect the reabsorption function of the kidney, this function is related to the GLUT2 mentioned above, but we still don't know the role of CA8 in it, so in this project, I How different doses of Met affect the expression mechanism of the novel protein CA8 will be explored from two levels of human proximal tubular cells (HK-2) and mouse kidney. And further understand the interaction between CA8 and GLUT2 in kidney cells and whether it will be affected by Met. It is hoped that through this project, clearer guidelines and data can be provided on the dosage and timing of the use of Met in the future.

KEYWORDS:CA8,GLUT2,metformin,diabete

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Hyaluronic acid surface-modified nanomedicine for the treatment of retinopathy in mice with blue light-induced damage

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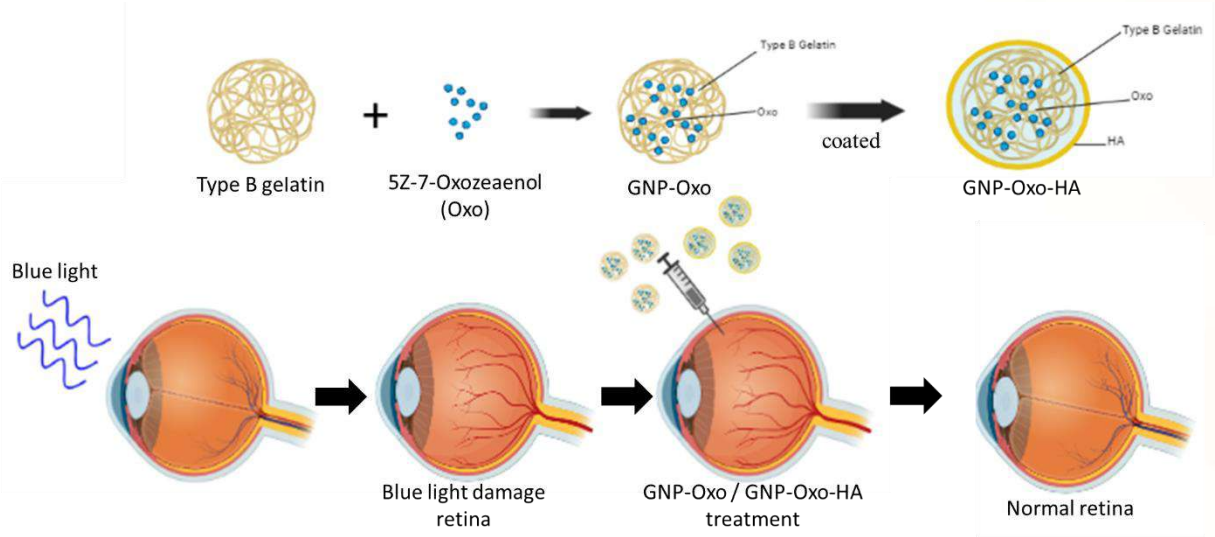
Abstract:

Blue light (BL) is high-energy visible light. Modern people have used mobile phones for a long time, BL cannot be filtered by the cornea and lens, and can directly reach the retina. we chose to use nanoparticles as drug carriers to treat eye diseases. Various biodegradable polymers, such as gelatin and hyaluronic acid are good nanoparticle materials. Among them, hyaluronic acid (HA) can smoothly pass through the vitreous body, combine with CD44 on retinal epithelial cells (RPE), and increase cell phagocytosis, which can improve the drug delivery efficiency. Therefore, in this study, we used gelatin nanoparticles (GNP) as a drug carrier, carried the anti-inflammatory drug 5Z-7-oxozeatenol (Oxo) as a nanodrug (GNP-Oxo) in the drug delivery system, and coated HA (GNP-Oxo) -HA) to investigate whether it is effective in treating retinas damaged by blue light. Materials testing uses dynamic light scattering (DLS), transmission electron microscopy (TEM), high performance liquid chromatography (HPLC) and other instruments. Retinal epithelial cells (RPE) cells were used for in vitro testing, and cell viability before and after blue light exposure was tested using the CCK-8 assay. Male mice (C57BL/6, 6 weeks old) were used for in vivo testing, and the state of the mice after exposure to blue light was observed using fundus fluorescein angiography (FFA) ,optical coherence tomography (OCT)and Electroretinogram(ERG).at the end of the experiment, sacrificed to observe histology by HE staining

KEYWORDS: Blue light damage, Retina , Gelatin nanoparticles (GNP) ,5Z-7-oxozeatenol (Oxo) , Hyaluronic acid (HA)


Type B gelatin
5Z-7-Oxozeaenol
(Oxo)
Type B Gelatin
Oxo
GNP-Oxo
coated
Type B Gelatin
Oxo
HA
GNP-Oxo-HA
Blue light
Blue light damage
retina
GNP-Oxo / GNP-Oxo-HA
treatment
Normal retina

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

NIR-activated organic molecule-based nanocomposites with photothermal and photodynamic effects for cancer treatment

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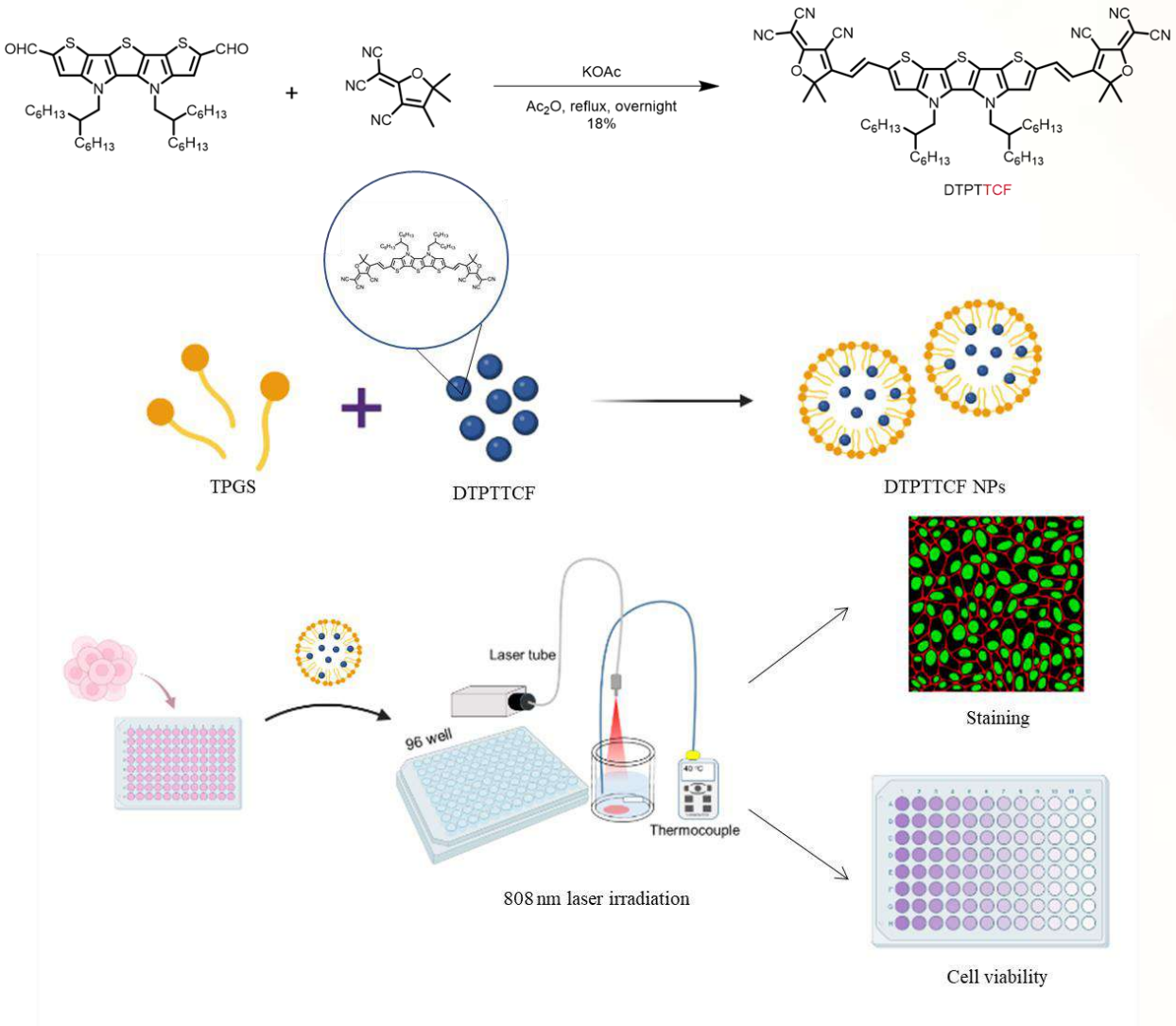
Abstract:

Nowadays, organic photosensitizers have become more popular and potential materials for medical application, especially in the tumor treatment study. Because of their tunability, we can adjust these conjugated organic molecules' energy level, planarity and solubility through changing or adding side chains and substituents on the backbone. Here, we show a stable organic photosensitizers nanoparticles based on DTPT-series photoactivated compounds with strong absorption in the near-infrared region (NIR). The DTPT nanoparticles are synthesized by self-assembling D-alpha-tocopherol polyethylene glycol 1000 succinate (TPGS) with DTPT-series molecules. TPGS-based micelle as the drug carrier can prevent organic molecules' aggregation and enable nanoparticles to be dispersed in the buffer due to the zeta potential. By designing the molecule structure, the absorption of the near-infrared region is strengthened, even when the molecules are encapsulated in the micelles. This increased potential for cancer therapy is due to the NIR light's deeper penetration depth in soft tissue, such as tumors. Additionally, these tuned molecules can be triggered by 808 nm and induce the photothermal effect and the photodynamic effect. For PTT, after DTPT nanoparticles are illuminated for few minutes, the environmental temperature can reach to over 45 °C, which can lead to cell death (43 to 49 °C) [1]. At the same time, reactive oxygen species (ROS) are produced due to PDT upon 808 nm laser excitation. The cell experiments confirm that ROS are generated in the cancer cell by DCFH-DA staining and prove its biosafety via comparing the cell viability if the DTPT nanoparticles are irradiated by NIR or not. This NIR-triggered nanoparticles system provides a promising and precise therapy against cancer cell.

KEYWORDS: photosensitizer, conjugated organic molecules, near-infrared region (NIR), photothermal effect, photodynamic effect, reactive oxygen species (ROS)



Graphic abstract

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2023 ISOMRM & BCRS
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Nitrogen-doped polymer dots with tunable crosslinking density for non-conventional fluorescence and antibacterial behavior

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Abstract:
This study first synthesized non-conventional fluorescent hyperbranched polymer dots through one-pot synthesis via A_2+B_3 approach. Subsequently, three type nitrogen-doped carbon dots with various crosslinking density were prepared via hydrothermal process. This synthesis process exhibits several advantages, including high yield, low cost, easy preparation, excellent photo-stability, good biocompatibility, and nanoscale particle. The results show that the quantum yield of resulted carbon dots increases with the increase in crosslinking density. Dynamic light scattering revealed that the size of resulted carbon dots dispersed in water was in the range of 5-10 nm. X-ray photoelectron spectroscopy confirmed a nitrogen content were approximately 8%. X-ray diffraction pattern showed a d-spacing of 4.6 Å, corresponding to the (002) plane of graphite. HRTEM image showed a lattice spacing of 2.2 Å, corresponding to the (100) plane of graphene. In addition, the safety assessment of human epidermal keratinocytes indicated low toxicity at 500 μg/ml. The nitrogen-containing carbon dots was suggested to behavior biological activity, and preliminary antibacterial tests were conducted using disk diffusion method. If antibacterial properties is confirmed, these dots could be used for the treatment of infectious wounds repair in the future.

KEYWORDS: Polymer dots, One-pot synthesis, Hydrothermal process, Antibacterial properties

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A three-in-one injectable hydrogel for reprogramming the immunosuppressive tumor microenvironment on triple-negative breast cancer therapy.

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Abstract:

This study proposes an innovative treatment approach for triple-negative breast cancer (TNBC), a subtype of breast cancer that lacks the receptors commonly found in breast cancer, with higher recurrence rates and a propensity of brain metastases. This study proposes a novel treatment approach aimed at addressing this predicament. An injectable and thermo-sensitive hydrogel system that integrates chemotherapy, photothermal therapy (PTT), and the regulation of the immunosuppressive tumor microenvironment was developed for treating TNBC. This hydrogel incorporates lactate oxidase (LOX), serving to repolarize tumor-associated macrophages (M2), from a tumor-supportive to a tumor-suppressive phenotype (M1) [1]. Subsequently, the 2D materials, MXene-SP, are encapsulated within the hydrogel, and upon exposure to an 808 nm near-infrared (NIR) laser to generate a mild increase in temperature within the tumor microenvironment. Consequently, tumor cells release damage-associated molecular patterns (DAMPs) thereby inducing dendritic cell (DC) maturation and fostering an immune-boosting effect [2]. Additionally, the thermo-sensitive hydrogel's internal microsphere, comprising methacrylate hyaluronan (HAMA) and methacrylate gelatin (GelMA), selectively target tumor cells and release doxorubicin (DOX) to enhance treatment efficacy [3]. By combining multiple therapeutic strategies, this hydrogel effectively regulates tumor microenvironments and may provide alternative for TNBC treatment in the future.

KEYWORDS: Triple-negative breast cancer (TNBC), injectable hydrogel, photothermal therapy, immunosuppressive tumor microenvironment, chemotherapy.

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IR820-loaded Fe(III)-rich nanozymes for glutathione-depletion/thermo enhanced chemodynamic/photothermal synergistic therapy

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Abstract:

Chemodynamic therapy (CDT) capable of converting intratumoral hydrogen peroxide (H_2O_2) into toxic ·OH by Fenton agents (such as Fe^{2+}/Fe^{3+}, Cu^{+}/Cu^{2+} or Mn^{2+}/Mn^{3+}) to kill cancer cells has attracted much attention due to its low side effect, high tumoral selectivity and without any external stimuli. However, high glutathione (GSH) level in the cancer cells would scavenge ·OH produced by Fenton agents, thereby hindering the performance of CDT [1,2].

To overcome the obstacle of CDT by combining the photothermal therapy, we fabricated unique hyaluronic acid (HA)-decorated Fe^{3+}-rich metal-organic frameworks (MOFs), NH_2-MIL-88B (MIL), as vehicles of IR820, a photothermal agent, for tumor-targeted chemodynamic/photothermal combined therapy. The obtained IR820@HA-MIL (IHM) nanozymes exhibited spindle-like shape, mono-dispersed size (about 174 nm) and outstanding stability in serum-containing milieu. Moreover, IHM nanozymes significantly promoted the photothermal conversion efficiency and stability of IR820. Notably, Fe^{3+} ions of IHM nanozymes oxidized GSH and further decomposed H_2O_2 into ·OH by GSH-depletion and Fe^{3+}/Fe^{2+}-mediated Fenton reaction. Furthermore, the hyperthermia/acidity-activated Fe^{3+}/Fe^{2+}-mediated Fenton reaction of IHM nanozymes effectively facilitated the production of ·OH.

KEYWORDS: Metal-organic frameworks, glutathione-depletion, Fenton reaction, chemodynamic/photothermal therapy

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Development of AFP-targeting ICG-CPT-encapsulated PLGA nanoparticles emulsions for photochemotherapy of lung cancer

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Abstract:

Cell membrane-encapsulated nanoparticle technology is becoming more and more frequently mentioned. This research developed a nano-particles AICEPNPs of poly-lactide-co-glycolide(PLGA) encapsulate not only with indocyanine(ICG) and camptothecin(CPT) by emulsion method , but also graft Alpha-fetoprotein (AFP) protein onto the surface of the particles.

AICEPNPs can be a carrier to carry both photosensitizer and an anti-cancer drugs for photodynamic and photothermal therapy .

Encapsulation brings effective protection , not to mention increasing the stability in the human body and the high biocompatibility of PLGA.

We encapsulate ICG,CPT into PLGA by using sonication, and the form of the particles will finally be nanoemulsions. The size and surface potential of nanoemulsions can be determined by Dynamic Light Scattering (DLS), and the difference between before and after grafting AFP protein can be compared.

The encapsulation and loading rates of CPT and ICG in PLGA nanoemulsions can also be measured by a UV-vis spectrophotometer. A drug release test and stability test were conducted for CPT and ICG respectively to know the trend of drug release and the stabilization effect of the photosensitizer.

In addition, due to the elevated temperature caused by AICEPNPs upon irradiation with 808 nm near-infrared light, it can cause damage to cancer cells. To enhance the effectiveness of ICG, we anticipate that this formulation can be combined with minimally invasive surgery, creating a small incision to deliver near-infrared light and allow the drug to exert its effects.

From the above experimental evaluation, we can initially confirm the development of

this technology and its potential as an emerging material for cancer treatment.

KEYWORDS: Lung cancer, Indocyanine green, Camptothecin, Poly-lactide-co-glycolide, Nanoagent, Photochemotherap

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Calcium-Zoledronic Acid Coordination Complex of Nanoparticles Combination withThermal Effect for Treatment Breast Cancer Bone Metastasis

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Abstract:

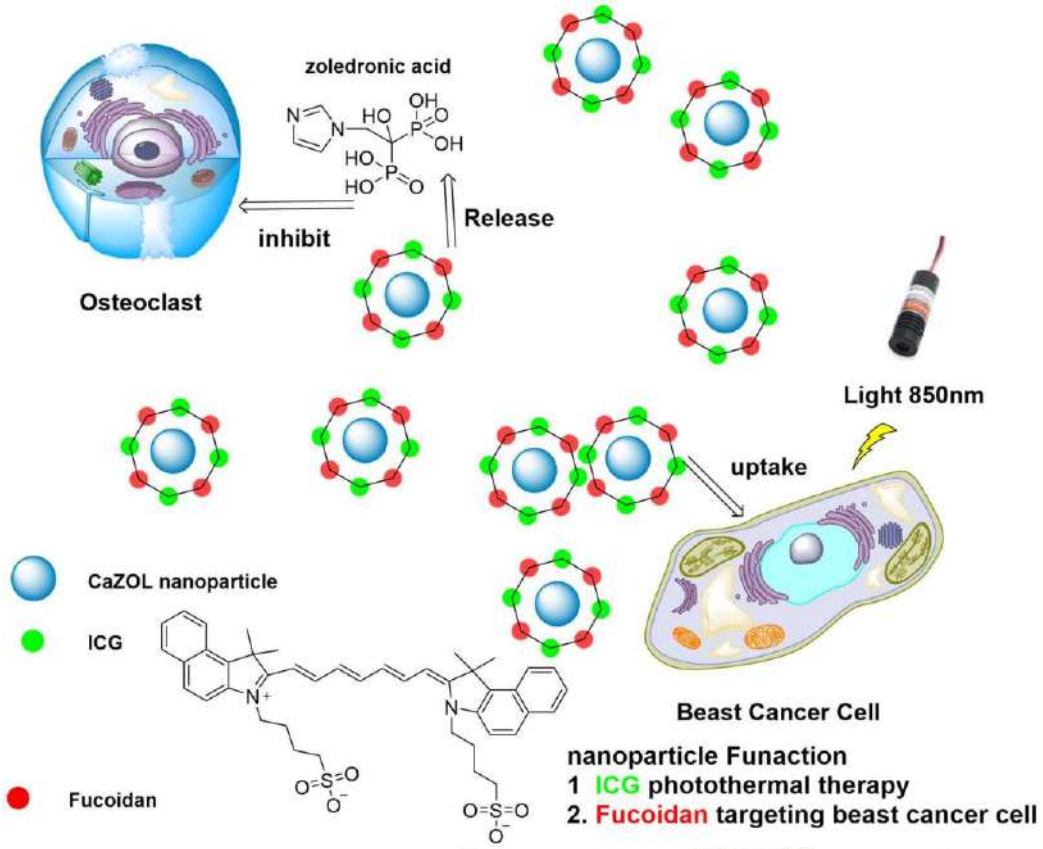
Zoledronic acid was the third-generation drug for osteoporosis therapy. Its chemical structure had bisphosphonate, which was unstable and readily reacted with metal or protein. Besides, BP (bisphosphonate) is a small molecule with a very short plasma half-life of only 1-2 hours in circulation [1]. However, high doses and repeated use are severe essential side effects that may occur, including affecting normal function bone mineralization and atrial fibrillation[2]. This shortcoming is significant disadvantage for the clinical applications of BP, especially in treating bone cancer.

In our approach, we can increase their drug bioavailability and reduce side effects via nanoparticles that solve those problems. Bisphosphonate structure was easy with Ca^{2+} ion coordination in the blood environment when intravenous injection of zoledronic acid. We have opted to use third-generation zoledronic acid to synthesize NPs directly with the bone material's calcium ions (CaZOL).

We used the calcium chloride and zoledronic acid mixture because nanoparticles were in HEPES buffer at room temperature. The reactor shows a white color solution for the calcium- zoledronic acid coordination complex of nanoparticle product [3]. Counties, adding fucoidan and ICG into reactor, and coating on the surface of CaZOL nanoparticles. The nanoparticle of Fu/ICG@CaZOL size was about 250nm by transmission electron microscope (TEM). XPS and TEM Mapping also demonstrate that Fucoidan was coated out of nanoparticles. In vitro, experiment has a promising anti-breast cancer (MDA-MB-231) and anti-osteoclast bioactivity that used Fu/ICG@CaZOL nanoparticles. Moreover, NIR 850nm irradiation decreases breast cancer (MDA-MB-231) and osteoclast cell cellular viability.

In this study, we designed and synthesized Fu/ICG@CaZOL nanoparticles that had fucoidan targeting breast cancer cells and ICG used NIR to induce photothermal effect in cells. In Vitro, breast cancer and osteoclast cells have significantly diminished of cellular viability under Fu/ICG@CaZOL nanoparticles treatment. There are high hopes for the Fu/ICG@CaZOL nanoparticles, which could potentially manage a variety of bone diseases and transport drugs or genes to bone tissue.

KEYWORDS: zoledronic acid, breast cancer bone metastasis, calcium-zoledronic acidnanoparticles, fucoidan, photothermal effect.



Graphic abstract

Reference :

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

The feasibility of tea polyphenol nanoparticles as a drug for stimulating hair growth

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Abstract:

Hair growth is controlled by a unique repetitive cycle comprised of anagen, catagen and telogen phases. [1] Dermal papilla cells (DPCs) helps to control hair growth not only in the normal hair cycle but also in the pathogenesis of certain conditions, for example in androgenetic alopecia. [2]. Therefore, factors affecting DPCs' functions in hair loss are important from the therapeutic viewpoint. Losing hair can have a significant impact on people's self-esteem and overall quality of life, as it may affect their appearance and confidence. Androgens such as testosterone (T) play a crucial role in the development and maintenance of male characteristics including the growth of facial and body hair. However, high levels of androgens, particularly a potent form called dihydrotestosterone (DHT), can also contribute to hair loss. [3]. Polyphenolic compounds found in green tea that has been shown to have anti-inflammatory, antioxidant, and anticancer properties. Some studies have suggested that the tea polyphenol (epigallocatechin-3-gallate, EGCG) may help hair growth [5]

Topical delivery of drugs or bioactive molecules usually exhibits an incomplete response. This phenomenon is mainly caused by the barrier features of the skin, which prompt the difficulty of drug permeation. Nanocarriers are considered an efficient strategy for catechin skin absorption because of their numerous advantages over conventional formulations, including improved storage stability, sustained release, targeted capability, and increased bioavailability.[6]

To explore this potential, we constructed nanoparticles (NPs) having a beneficial effect on hair growth. The NPs were made of gelatin-epigallocatechin gallate (abbreviated as GE NPs), its size is around142.1 nm, and zeta potential 23.2mV . And DHT-induced hair loss model in mice was created for exaim weather tea polyphenol, and free drug and nanodrug (GE NPs) were smeared on mice back once daily for to evaluate the feasibility of its role as a solution to clinical symptoms. After 9 day treatment, some new hair was observed, and after 15 day the damages area were almost recovered with new born hair at the high concentration of GE NPs treated group. The tea polyphenol nanoparticle can help to hair growth.

KEYWORDS: hair loss, dihydrotestosterone (DHT), tea polyphenol, gelatin, nanoparticles, skin

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Evaluation of eye drops contained small compound extracted from He Shou Wu for dry eye mice treatment

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Abstract:

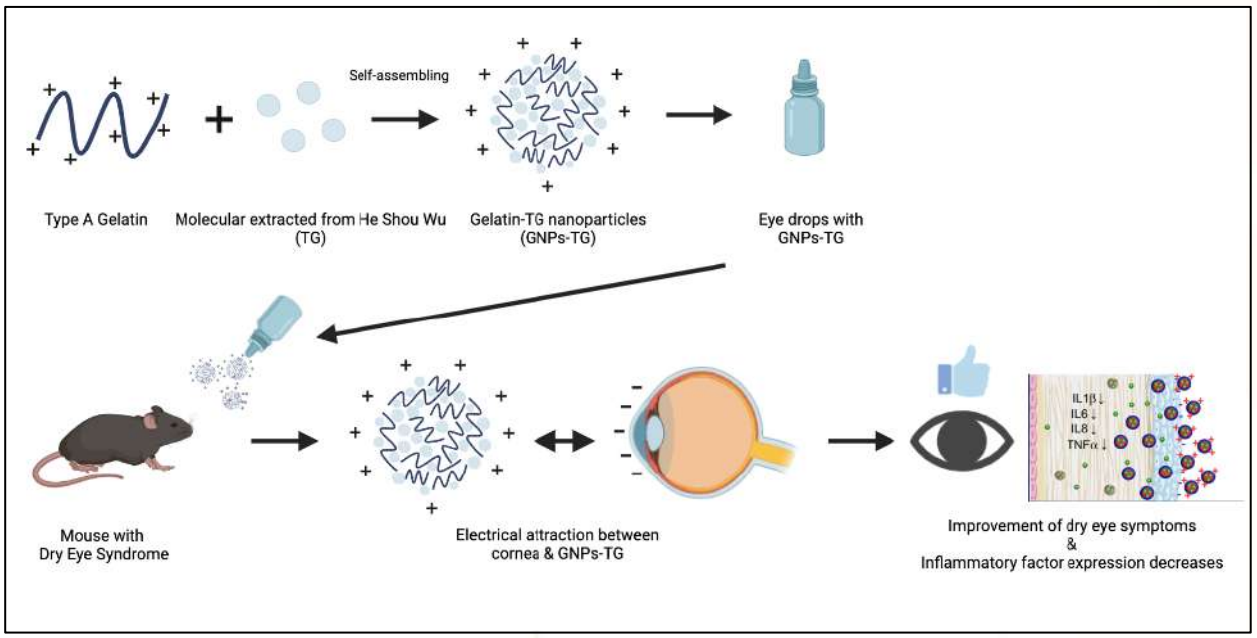
Dry-eye syndrome (DES) is usually accompanied by symptoms such as burning, stinging, and ocular surface inflammation [1]. However, the drugs on the market have some problems such as limited therapeutic effect, side effects, low drug utilization, and low therapeutic effect. Therefore, nanomedicine for ocular drug delivery can solved those problems. Due to the ocular barrier, the bioavailability of the drug on the ocular surface is very low, and it cannot be effectively treated, resulting in the need for repeated eye drops [2]. The polymer nano-drug carrier can make it easier to attach to the eye by adjusting the electrical properties of the carrier [2]. In addition, because the eye structure has collagen components, the eyes have low repellency to gelatin, and can metabolize it through the collagenase in the body without accumulating in the body.

In this study, gelatin nanoparticles (GNPs) were used as drug carrier for enhancing drug concentration on eyes. Nowadays, there are many active ingredients of Chinese herbal medicines, such as flavonoids, polyphenols, glucosides, etc., which have been proven to have anti-inflammatory functions, and small molecular extracted (TG) from He Shou Wu was encapsulated in GNP for treating dry eye via inhibiting the inflammation on ocular surface. TG loaded in GNPs were synthesized and prepared as eye drops for treat dry eye in mice.

A BAC induced dry eye model in mice was used, and GNPs-TG was dropped on mice twice daily. As a result, NPs can be formed by gelatin and TG mixture, and its particle size and zeta potential are 215 nm and 8.76 mV. DES mice after treated by GNPs-TG with some tear volume increase, and less cornea damaged was observed. It shows potential for GNP-TG to be a drug for treating DES, but a repeat test is needed to confirmed again.

KEYWORDS: Dry-eye syndrome (DES), Gelatin-nanoparticles (GNPs), Molecular extracted from He Shou Wu (TG), Self-assembly nanoparticles

Graphic abstract:



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Investigating the Cardiac Effects of Drug-Loaded Mesoporous Silica Nanoparticles on Heart Failure in Zebrafish

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Abstract:

Heart failure (HF) is attributed to abnormal heart function or structure, resulting in insufficient cardiac power output, which prevents the whole body from supplying the nutrient needed. Unfortunately, patients face a higher risk of mortality due to the loss of heart function caused by HF. Clinically, the effectiveness of HF drugs is often hindered by limited bioavailability, leading to the deterioration of cardiac function and subsequent mortality. Therefore, it is crucial to address these challenges and develop new therapeutic approaches, which have become an urgent clinical need.

Zebrafish is beneficial to the observation of the heart beating under a microscope. This study attempts to establish HF models and assessment techniques for cardiac function in zebrafish. By combining with nanotherapeutics, we demonstrate that the HF drug-loaded mesoporous silica nanoparticles (MSN) can effectively improve the limitations of drug toward the increased therapeutic efficacy. With the advantages of nanotechnology, the sustained release of HF drug can reduce the use of dosage, leading to the reduction of side effects.

KEYWORDS: heart failure (HF), mesoporous silica nanoparticles (MSN), zebrafish, drug delivery, cardiac function

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Stem cell–nanomedicine system as a theranostic bio-gadolinium agent for targeted neutron capture cancer therapy

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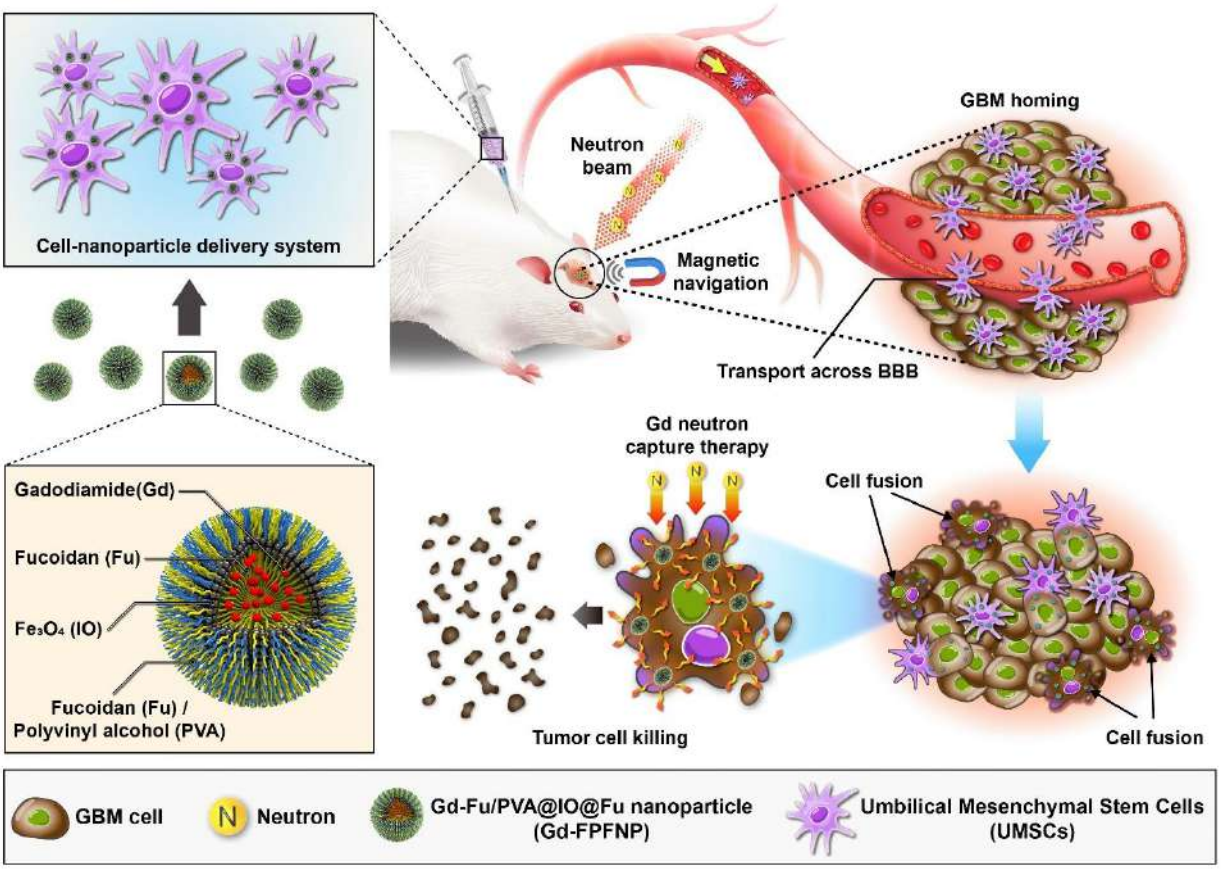
Abstract:

The potential clinical application of gadolinium-neutron capture therapy (Gd-NCT) for glioblastoma multiforme (GBM) treatment has been compromised by the fast clearance and nonspecific biodistribution of gadolinium-based agents. We have developed a stem cell–nanoparticle system (SNS) to actively target GBM for advanced Gd-NCT by magnetizing umbilical cord mesenchymal stem cells (UMSCs) using gadodiamide-concealed magnetic nanoparticles (Gd-FPFNP). Nanoformulated gadodiamide shielded by a dense surface composed of fucoidan and polyvinyl alcohol demonstrates enhanced cellular association and biocompatibility in UMSCs. The SNS preserves the ability of UMSCs to actively penetrate the blood brain barrier and home to GBM and, when magnetically navigates by an external magnetic field, an 8-fold increase in tumor-to-blood ratio is achieved compared with clinical data. In an orthotopic GBM-bearing rat model, using a single dose of irradiation and an ultra-low gadolinium dose (200 μg kg^{-1}), SNS significantly attenuates GBM progression without inducing safety issues, prolonging median survival 2.5-fold compared to free gadodiamide. The SNS is a cell-based delivery system that integrates the strengths of cell therapy and nanotechnology, which provides an alternative strategy for the treatment of brain diseases.

KEYWORDS: neutron capture therapy; GBM, polysaccharides nanoparticle , cellular fusion, cell delivery system, drug delivery system



Graphic abstract. Mechanism of action for stem cell–nanoparticle system (SNS) in gadolinium-neutron capture therapy (Gd-NCT). Gd-Fu@IO@PVA/Fu nanoparticle (Gd-FPFNP) associated with umbilical cord mesenchymal stem cells (UMSCs) as a bio-NCT agent can cross the blood brain barrier (BBB) and fuse with tumor cells under magnetic navigation for enhanced neuron capture therapy.

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of functional polymers for drug delivery and bio-application

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Abstract:

Nanoparticles have long been investigated for their potential in drug delivery applications [1]. Among them, lipid polymer hybrid nanoparticles (LPHNPs) have gained popularity as versatile carriers for drug delivery systems across different administration routes, including anticancer therapy, gene delivery, vaccine delivery, and bio-imaging [2]. In this study, we aimed to prepare LPHNPs using a nanoprecipitation method, incorporating a poly(β-butyrolactone) (PBL) core, a soybean lecithin monolayer, and a poly(ethylene glycol) (PEG) shell. Different mass ratios of lecithin/copolymer (10%, 15%, and 20%) were utilized to achieve optimal formulations. A PBL-PEG di-block copolymer was synthesized via ring-opening polymerization (ROP) with varying molar ratios of BL/EG (30:1, 40:1, and 50:1) to obtain different lengths of BL. The properties of the prepared nanoparticles, including particle size, polydispersity index (PDI), and particle morphology, were thoroughly investigated. The optimized formulation will subsequently be employed for encapsulating hydrophobic anticancer drugs, enabling an assessment of drug encapsulation efficiency and drug release profiles. The result shows that after the synthesis of the di-block copolymer, three different lengths of hydrophobic PBL were obtained. Subsequent formation of PBL-PEG micelles demonstrated that the particle size ranged from 100 to 186 nm with larger particle sizes observed at higher BL/EG ratios. The PDIs of these micelles ranged from 0.07 to 0.25. Notably, an increase in temperature resulted in a reduction in micelle size. Overall, these findings contribute to the understanding and development of LPHNPs as effective carriers for drug delivery applications.

KEYWORDS: Lipid polymer hybrid nanoparticle, Block-copolymer, Self-assembly, Drug delivery, Controlled drug release

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Sorafenib-Loaded Superparamagnetic Nanoparticles Combined with External Electromagnetic Field for Precision Liver Cancer Treatment

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Abstract:

Although the chemotherapy drug Sorafenib has been the first-line target drug for liver cancer approved by the US FDA for more than ten years, the effectiveness that Sorafenib can achieve is limited because of its various side effects and poor solubility. As the carrier of the drug delivery system, the superparamagnetic iron oxide nanoparticles can be directed to the site of leision in the presence of an external electromagnetic field to improve the precision and effectiveness the drug treatment.

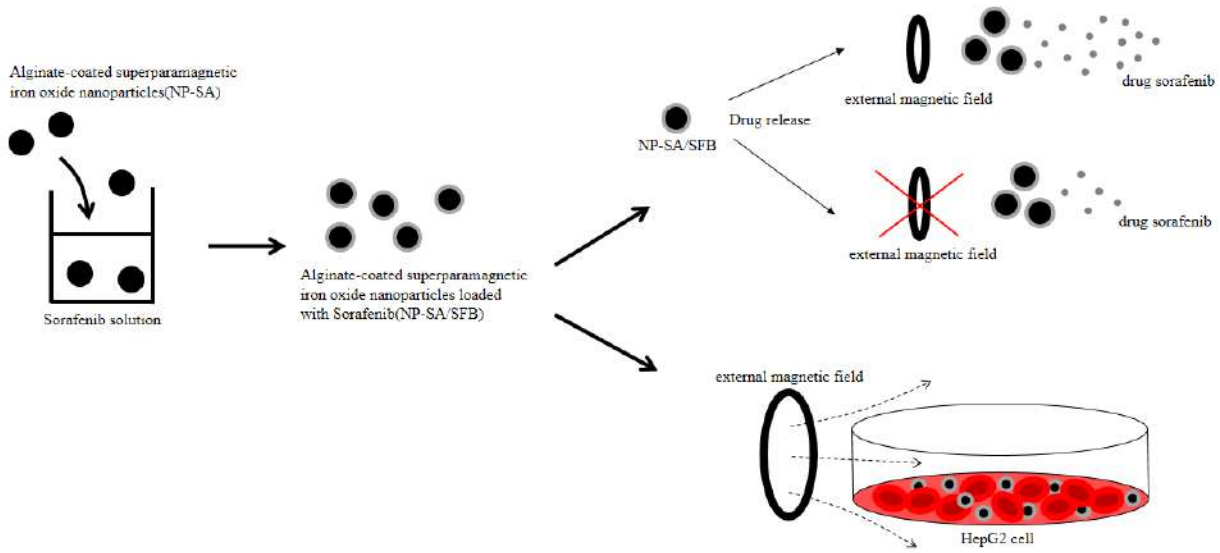
In this study, alginate-coated superparamagnetic iron oxide nanoparticles were prepared, loaded with the Sorafenib in the alginate gel coating, and exposed to an external magnetic field for 2 hours, to study the drug release of iron oxide nanoparticles and the drug's effect on humans liver cancer cell line HepG2.

Alginate-coated superparamagnetic iron oxide nanoparticles were prepared using a combination of a pre-gel method and co-precipitation method. [1] The drug release profiles from the iron oxide nanoparticles loaded with Sorafenib was measured with and without the presence of the external electromagnetic field (EMF). The results showed that the amount of drug release were 15 times higher ($p<0.05$) with external magnetic field than without external magnetic field.

Furthermore, in order to observe the system's effect on the growth for HepG2, we designed 7 experimental groups. To the HepG2 culture we added the following: 1. none (control group); 2. Sorafenib; 3. alginate-coated superparamagnetic iron oxide nanoparticles; 4. alginate-coated iron oxide nanoparticles loaded with Sorafenib; 5. None, with exposure to EMF for 2 h; 6. alginate-coated iron oxide nanoparticles with exposure to 2 h of EMF; 7. alginate-coated iron oxide nanoparticles loaded with Sorafenib with exposure to 2 h of EMF. We performed cell viability, Hoechst DNA staining and cell migration tests on the 7 groups. The results showed that EMF and iron oxide nanoparticles alone can slightly inhibit the growth of HepG2, and the iron oxide nanoparticles loaded with Sorafenib have a significant inhibitory effect on the growth

of HepG2, especially with the presence of external magnetic field. The results from the Hoechst dye staining showed that the iron oxide nanoparticles loaded with Sorafenib in the presence of EMF caused significant DNA damage in HepG2 cells. Based to the above results, the iron oxide nanoparticles loaded with Sorafenib could be used in combination with EMF and be a potential drug delivery system for the precision treatment of liver cancer.

KEYWORDS: Superparamagnetic iron oxide nanoparticles, Drug delivery, HepG2 cells, Sorafenib, electromagnetic field



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Development of bacterial membrane coating-Indocyanine green and camptothecin co-loaded perfluorocarbon double nanoparticles for photochemoimmunotherapy of colorectal cancer.

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Abstract:

Colorectal cancer is one of the most common types of cancer in the world. The current main treatment involves adjuvant radiotherapy and chemotherapy, followed by surgical removal of the tumor tissue. However, the treatment does not result in the complete eradication of the tumor cells, and the risk of recurrence after cancer treatment is high.

This study developed a bacterial membrane(BM) coated nanoparticle in which Indocyanine green(ICG) Camptothecin(CPT) encapsulated perfluorocarbon double nanoparticles(BM-ICPDNPs). BM-ICPDNPs perform photothermal therapy (PTT) and photodynamic therapy(PDT) by irradiating ICG with a near-infrared(NIR) laser and releasing CPT for chemotherapy. The bacterial membrane is abundant in pathogen - associated molecular patterns, which can effectively stimulate immune cell maturation and recruitment after photochemotherapy, to enhance the specificity of immune recognition and elimination of cancer cells.

Using DLS and PALS analysis, ICPDNPs have size of 214.47 ± 7.44 nm and negative surface charge(-11.65 ± 4.04 mV). After bacterial membrane coating, the size increases to 232.95 ± 5.7 nm, and the surface charge becomes -23.99 ± 8.13 mV. In vitro cell studies are expected to show that BM-ICPDNPs can stimulate the expression of NFkB and TLR2 in immune cells, demonstrating that BM-ICPDNPs can stimulate the immune system.

The goal of this study is to achieve the complete elimination of cancer cells by combining phototherapy, chemotherapy, and immunotherapy, to reduce the recurrence rate of cancer. This research can provide a new treatment method for cancer treatment and is expected to further develop a cancer treatment.

KEYWORDS: bacterial membrane, photochemoimmunotherapy, perfluorocarbon double nanoparticles, Indocyanine green, Camptothecin

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Development of controllable decorative tumor antigen-tethered spiked Virus-Like-PLGA-Nanoparticles (sVLPN) to induce potent immunogenicity against tumors in mice

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Abstract:

The vaccine trains the host immune system to produce antibodies and prevent the host from disease. The advantages of protein subunit vaccines are non-virus genome, non-infectious and simple conservation. There are a number of ways to formulate a protein-based (tumor antigen) cancer vaccine, including using free-floating protein, encapsulating protein in nanocarriers, or tethering protein to a nanoparticle. Among them, highly repetitive antigen protein fragments tethered to the surface of a nanoparticle mimicking the outer structure of spiked proteins on the virus, the ability of antigen-presenting cell phagocytosis can be improved to result in accumulating in lymph nodes and triggering subsequent immune responses [1, 2]. In this study, tumor antigen of ovalbumin (OVA, -SH) tethered to maleimide-PEG-PLGA nanoparticle via thio-maleimide crosslinking to form spiked virus-like-PLGA-nanoparticles (sVLPN), was developed as a protein subunit vaccine platform so that the vaccinated host can produce more potent and specific neutralizing antibodies. Ovalbumin-tethered sVLPN (O-sVLPN) was prepared by a single-emulsion solvent evaporation method with a yield ratio of 37.87±10.85%, particle size of 145.77±2.76nm and PDI of 0.19±0.03. At 4°C, the particle size distribution and uniformity remain stable for at least one month. The spherical structure and the peripheral lipid layer can be observed by TEM. *In vitro* study, the O-sVLPN co-cultured with the dendritic cell line (DC2.4) induces much higher CD80/CD86 expression in dendritic cells. The biodistribution of O-sVLPN *in vivo* image system (IVIS) shows the effective accumulation of lymphatic effect 6 h after I.M. administration. *In vivo* study, I.M. administration of O-sVLPN + adjuvant Quil-A (5 μg) in mice shows robust IgG and IgG1 effects. There were no obvious inflammatory, swollen regions, or depleted cells found in cardiac muscle fibers, hepatic cells, splenic pulp, pulmonary alveoli, or glomerular epithelial cells, indicating that these O-sVLPNs exhibited good biocompatibility in the major organs. Overall results show that the O-sVLPN can effectively enhance immune responses and have reliable safety as well. Thus, it showed great potential to be developed as a broadly applicable vaccine platform technology.

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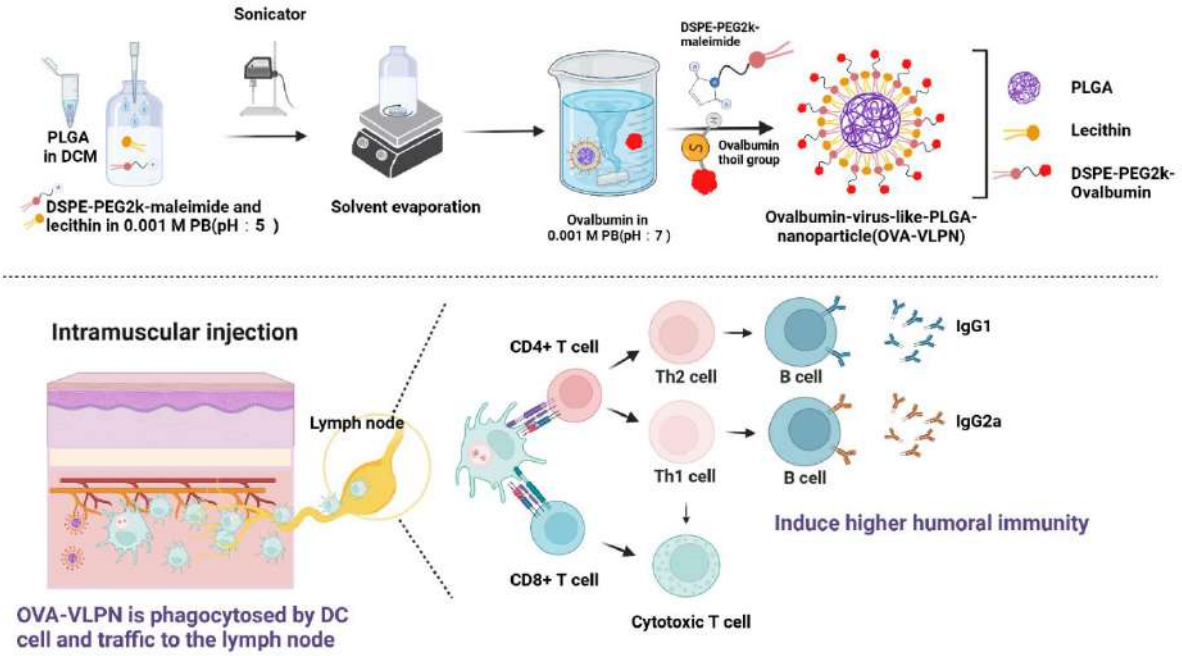
KEYWORDS: PLGA, cancer vaccine

Graphic abstract：



Schematic illustration of immunogenicity of OVA-virus-like-PLGA-nanoparticle(OVA-VLPN) to enhance dendritic cell uptake and traffic to the lymph node, induce higher specific humoral immunity.

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Development of *in situ* thermosensitive poloxamer gels as ocular drug delivery systems of sodium phenylbutyrate for the treatment of open-angle glaucoma

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Abstract:

Open-angle glaucoma (OAG) is a chronic disease that causes elevated intraocular pressure and optic nerve compression, potentially leading to permanent vision damage. At present, non-invasive eye drops are widely used for drug delivery, but their relatively low bioavailability necessitates frequent administration [1] .

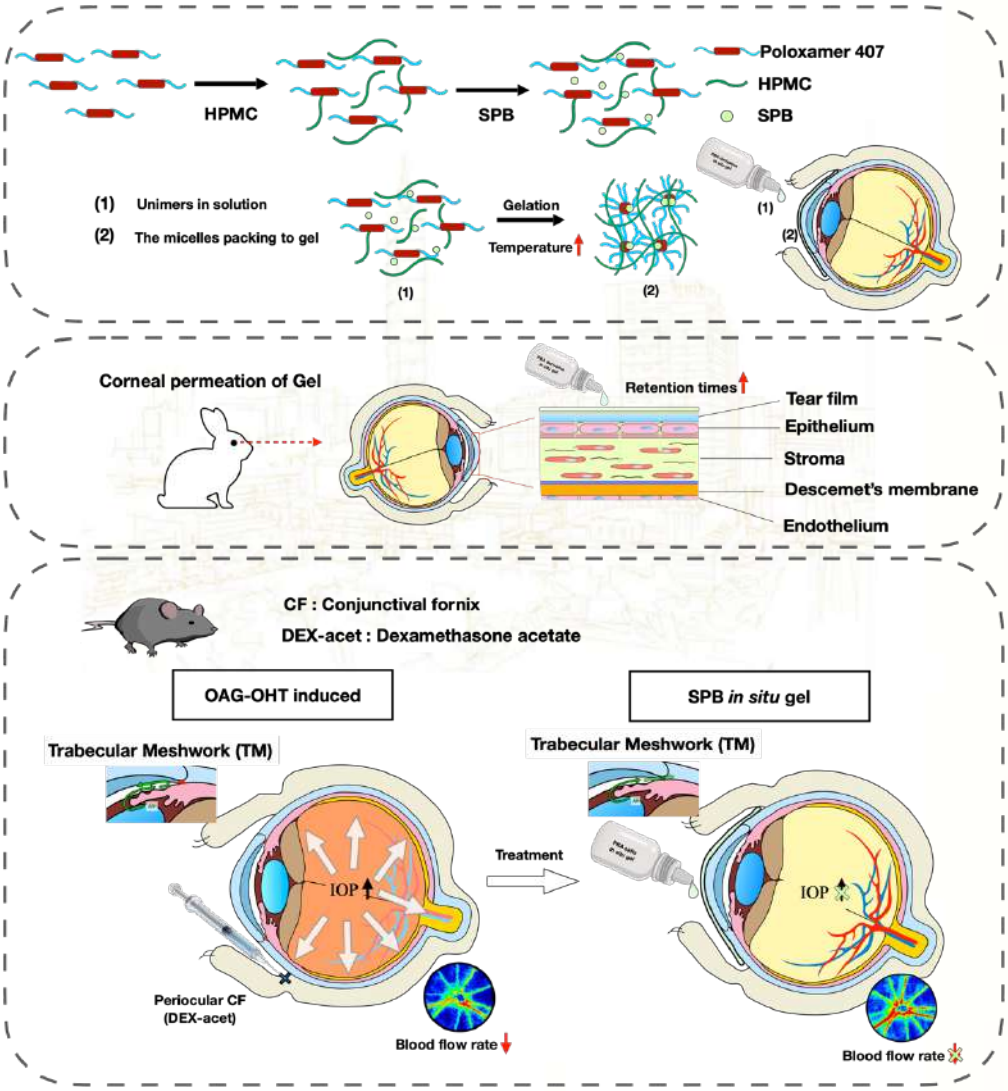
In this study, we used sodium phenylbutyrate (SPB) as model drugs for treating OAG. These drugs were loaded into thermosensitive *in situ* hydrogels prepared with poloxamer 407 (P407) and hydroxypropyl methylcellulose (HPMC). After adding SPB to the gel formulations, we selected the one with the best retention effects, $SPB_{1.13}P_{13}H_3$. The solution to gel transition temperatures for $SPB_{1.13}P_{13}H_3$ was 29.3 ± 0.8 °C with exhibiting shear-thinning characteristic demonstrating the suitability of $SPB_{1.13}P_{13}H_3$ for ophthalmic treatment. *Ex vivo* permeation tests showed that, compared with solution formulations, the gel systems ($0.33 \pm 0.09 \times 10^{-5}$ cm/sec) exhibited longer retention times and achieved drug release control compared to solution systems ($0.53 \pm 0.06 \times 10^{-5}$ cm/sec).

Long-term *in vivo* experiments on a dexamethasone acetate (DEX-acet)-induced glaucoma mouse model showed that treatment with DEX-acet alone significantly induced typical glaucoma symptoms in mice, such as elevated intraocular pressure, reduced optic nerve function, and decreased average blood flow rate. Furthermore, immunofluorescence staining of histopathological slices revealed a significant increase in Collagen I and Fibronectin accumulation in trabecular meshwork (TM) cells compared to the control group. However, concurrent treatment with SPB gels significantly reversed these DEX-acet induced effects in mice, including high intraocular pressure, optic nerve damage (oscillatory potentials, Ops), and decreased average blood flow rate. In addition, SPB gels significantly reduced DEX-acet induced Collagen I and Fibronectin accumulation. Based on these *in vivo* mouse experiment results, we found that SPB gels can effectively alleviate DEX-acet induced glaucoma symptoms. Furthermore, using fundus photography, fluorescein angiography, and optical coherence tomography (OCT) for retinal examination of all test groups, we found that neither DEX-acet nor drug treatment (SPB) caused structural damage to the retina. This further confirms that our DEX-acet induced high intraocular

pressure disease model only induced functional changes in the eyes of mice. In addition, it validated that the treatment drugs (SPB) do not cause toxic damage to the mouse retina under this treatment modality.

Our research results confirm that when SPB is combined with the gel system, an appropriate solution to gel transition temperature can be achieved, increasing drug retention time on the cornea and effectively alleviating glaucoma-induced high intraocular pressure, optic nerve damage, and extracellular matrix (ECM) accumulation. We hope this research can provide a new option for treating glaucoma.

Graphic abstract



KEYWORDS : Open-angle glaucoma, thermosensitive *in situ* gel, sodium phenylbutyrate.

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2023 ISOMRM & BCRS
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Bispecific T-cell engagers non-covalently decorated on PEGylated liposome fused with MHC molecular tumor antigen expressed exosomes derived from dendritic cells for colon cancer immunotherapy

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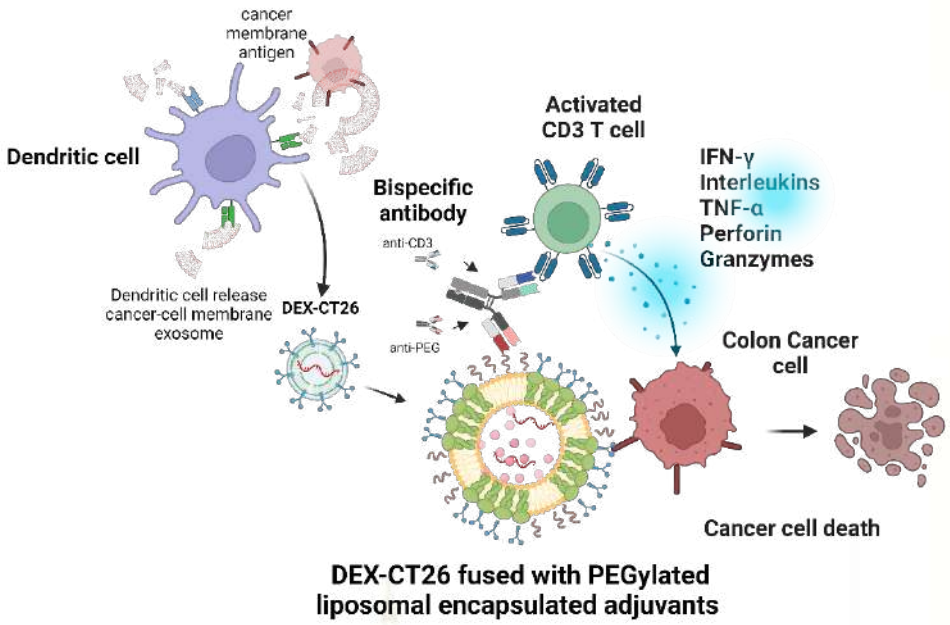
Abstract:

Colorectal cancer is one of the most prevalent cancers in the world. In recent years, the incidence of colorectal cancer in Taiwan was higher than in other cancers. Thus, how to develop more effective clinical treatment is an urgent need for colorectal cancer. In this study, the development of bispecific T-cell engagers non-covalently decorated on PEGylated liposome fused with MHC molecular tumor antigen expressed dendritic cells-derived exosomes for colon cancer immunotherapy was proposed [1]. Firstly, an anti-CD3/anti-PEG bispecific antibody (Anti-CD3/anti-PEG BsAb) was constructed and fabricated. In addition, PEGylated liposomal encapsulated adjuvant was successfully prepared with a particle size of 130-150 nm and good PDI quality (PDI<0.2). At the same time, dendritic cell-derived exosomes with MHC molecular antigen of CT-26 cancer cell (DEX-CT26) were successfully produced and collected with a size range of 30-150 nm. The Cryo-EM image of the DEX-CT26 showed the shell shape and particle range of DEX-CT26. The Immunoblot analysis of DEX-CT26 was assessed by CD9, CD63, and CD81 cell markers [2]. The yield rate for the DEX-26 was consistent per batch culture medium. The adjuvant release of CpG from PEGylated liposomal encapsulated adjuvant could be detected stably until 48 h. The DEX-CT26 could be fused with PEGylated liposomal encapsulated adjuvant and specifically bind with concentration-dependent anti-CD3/anti-PEG BsAb to target mouse spleen CD3 T cells by flow cytometry analysis. In vitro evaluation study, the PEGylated liposomal encapsulated adjuvant can be phagocytosed by dendritic cells through multiple endocytosis pathways and these deliveries can be phagocytosed by mouse spleen CD3 T cells as well. Moreover, the crucial level of multiple cytokines can be elevated by activated mouse spleen CD3 T cells. Overall, the results demonstrate the CD3-targeted immunoadjuvant-liposomal DEX-CT26 has the high potential to activate CD3 T cells to fight against colon cancer and the CT26 mouse cancer model will be well-established for further investigation in the near future.

KEYWORDS: colon cancer, targeted liposome, dendritic cell, exosome, cancer immunotherapy



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Development of glycosylated PLGA nanoparticles as dendritic cell targeting delivery vehicles for therapeutic cancer vaccination

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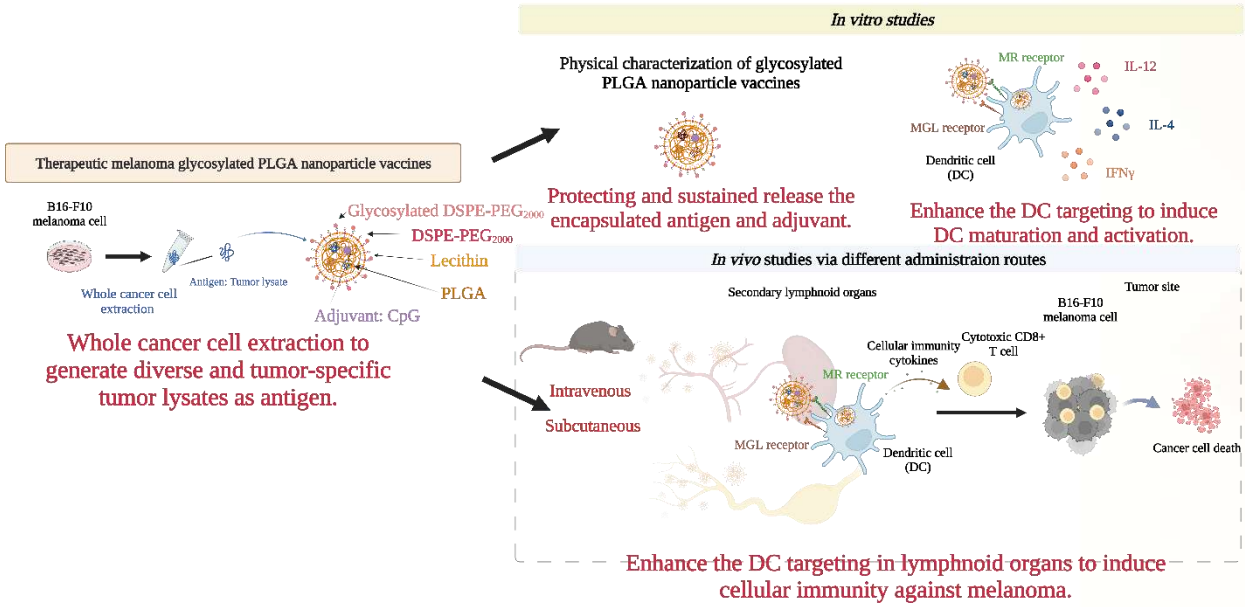
Abstract:

The development of cancer vaccines has recently garnered tremendous interest. However, the targeted delivery of antigens and adjuvants to dendritic cells (DCs) still remains challenging [1]. In this study, the glycosylated poly(lactic-co-glycolic acid) nanoparticles (NPs) we recently developed as effective DC-targeted delivery vehicles loaded with B16F10 melanoma cell-derived tumor lysate (TL) as the whole-tumor cell antigen and CpG as an adjuvant for therapeutic vaccination [2]. First, whole cancer cells are processed by developed subcellular fraction method to generate the diverse and antigen-rich tumor lysates. Surface modification of NPs with galactose (Gal) or mannose (Man) was carried out by a double-emulsion solvent evaporation method, and the products were respectively named fractionated tumor lysate (TLF)-CpG Gal-NPs and TLF-CpG Man-NPs. They exhibited a uniform particle size, high loading capacity, robust stability, and extended release. The TLF-CpG Gal-NPs were found to rapidly enhance antigen uptake and DC maturation. In the *in vivo* study, TLF-CpG Gal-NPs via intravenous (i.v.) route showed accumulate in the spleen more and rapidly (at 2 hours), via subcutanous (s.c.) route could rapidly (at 6 hours) distribute to the distal lymph node, increase the level of CD4+ T cells and IFN-γ in the spleen and IFN-γ in their serum, then promoted infiltration of cytotoxic CD8+ T lymphocytes in tumor tissues by immunohistochemistry staining of CD8b, which led to an efficient tumor-specific T cell immune response against B16-F10 tumor challenge and lung metastasis especially the group of TLF-CpG Gal-NPs. In conclusion, galactosylated NP vaccines loaded with whole-tumor cell antigen provided an effective platform to enhance the DC targeting for inducing cellular immunity and T-cell recruitment into tumor sites in vivo, thus showing great potential to be developed as a therapeutic cancer vaccine for immunotherapy.

KEYWORDS: therapeutic cancer vaccines, PLGA nanoparticles, tumor lysate, glycosylation, CpG ODN

Graphic abstract



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Treatment of skin cancer with dissolving ulvan microneedles containing curcumin in combination with X-ray radiation therapy

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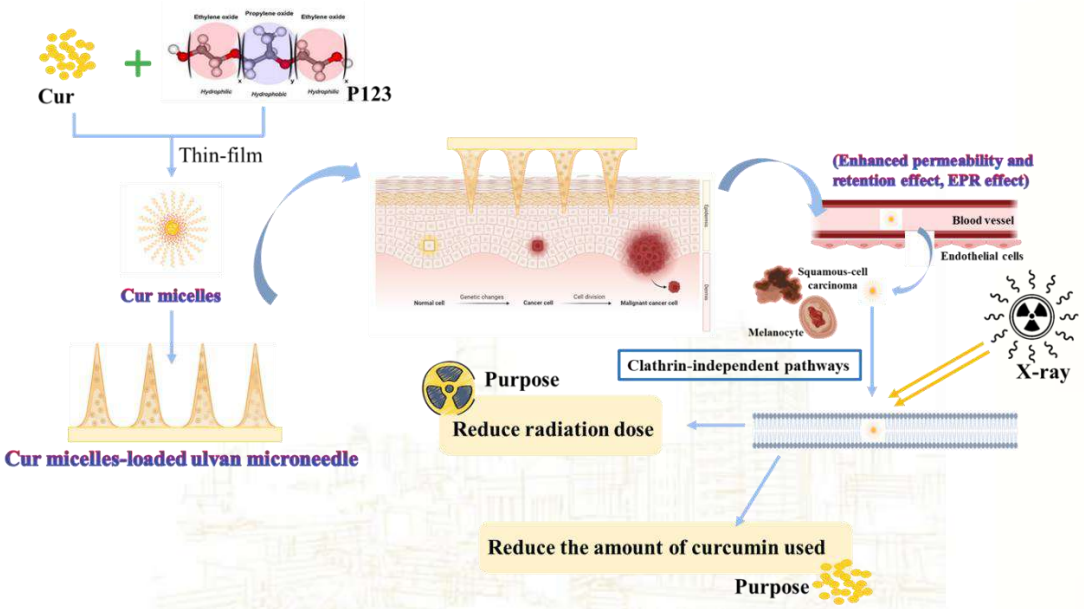
Abstract:

Skin cancer is one of the most common types of cancer worldwide, with malignant melanoma and human squamous cell carcinoma being highly invasive. The recurrence rate after surgical removal remains high. Therefore, this study aimed to develop dissolving ulvan microneedles (UMN) containing curcumin-micelles (Cur-m) to facilitate the penetration of the drug through the stratum corneum. Subsequently, the study utilized Cur as a radiosensitizer in combination with X-ray radiation therapy to synergistically treat skin cancer. First, The Cur-m was composed of Cur and the amphiphilic triblock copolymer Pluronic P123 at a weight ratio of 1:20. It was synthesized using thin film hydration method resulting in the formation of Cur-m with a particle size of 19.33 nm, an encapsulation efficiency of 98.27 ± 1.50%, and a drug loading of 4.68 ± 0.07%. Cur-m has a low critical micelle concentration of 0.0030% (w/v), indicating that it can maintain its integrity even after dilution and slowly release Cur over 72 hours. The half maximal inhibitory concentration (IC_{50}) of Cur-m on melanoma cancer cells (B16F10) and human squamous cell carcinoma cells (A431) was 9.79 μg/mL and 1.59 μg/mL, respectively. Because of its stable storage and good dispersibility in water, Cur-m was incorporated into 4% (w/v) ulvan microneedles using a two-layer casting method, forming curcumin-loaded dissolving ulvan microneedles (Cur-UMN). The prepared microneedles consist of 27×13 pyramid-shaped needle tips, with a height of about 626.00 μm and a width of about 249.90 μm, and an optimum aspect ratio of 2.51. The insertion ratio of the microneedles on porcine skin reached 99.56%, exhibiting good mechanical properties and enabling the drug to act locally on the skin or penetrate through the skin into the systemic circulation. The Cur content of a microneedle patch was about 5.05 ± 0.38 μg/cm^2. Upon observation with a stereomicroscope, Cur was almost concentrated at the needle tip and did not exhibit extensive diffusion towards the backing layer. When Cur-UMN was inserted into porcine skin with a force of 12 N, it dissolved rapidly by 43.02% after 30 seconds of

contact with the interstitial fluid, and experienced a reduction of nearly 79.02% in needle height after 2 minutes. In the franz cell drug release experiment, 80% of Cur rapidly penetrated the stratum corneum and reached the dermis within 4 hours. In summary, Cur-UMN has successfully fabricated and can be used in transdermal delivery systems to successfully deliver Cur. The potential of Cur-UMN as an adjuvant therapy for skin cancer will be evaluated in the near future.

KEYWORDS: skin cancer, transdermal drug delivery, microneedles, curcumin micelles, chemical synergistic radiotherapy



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Developing a Local and Sequential Release System for Non-healing Diabetic Ulcer Therapy

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ABSTRACT:

Chenopodium formosanum is a native pseudo-cereal crop in Taiwan. It is not only high in protein content but also rich in balanced amino acids and antioxidant components. The protein hydrolysates of Chenopodium formosanum have been found to possess potential antioxidant and anti-inflammatory properties. In our previous research, our team has successfully screened active peptides with anti-inflammatory effects from Chenopodium formosanum through solid-state fermentation. These peptides have a molecular weight of 10kDa.

The healing of wounds is a complex and dynamic physiological process, and the interactions between cells and tissues can affect wound healing. In particular, people with diabetes are prone to infections that will impair their ability to heal. Diabetic foot ulcers will lead to more complicated diseases and even death indirectly. Therefore, an effective treatment strategy is needed to address diabetic foot ulcers.

An ideal dressing for treating non-healing ulcers should be capable of the local and sequential release of drugs, ranging from hours to days. Hence, the central hypothesis of this study is to develop a drug delivery system with local and sequential release capabilities, which can enhance the effectiveness of anti-inflammatory drugs and improve wound healing in diabetic patients. In the sequential release aspect, we will use biodegradable polymeric materials to prepare micro- and nano-particles encapsulating anti-inflammatory peptides. In the local release aspect, we will employa biocompatible hydrogel as a carrier to encapsulate the nanoparticles, allowing for the sequential release of drugs at the wound site.

This experiment aims to develop a hydrogel-micro/nanoparticle composite system for the local and sequential release of anti-inflammatory peptides to enhance wound healing capabilities. In the experiment, we have achieved the following results:

A. Successful synthesis of the required PCADK polyketal polymer through step-growth polymerization, confirmed its functional groups through NMR, and determined its molecular weight (Mn=6400 Dalton, PDI=1.4) using GPC.

B. Completed the technique for preparing micro- and nanoparticles encapsulating anti-inflammatory peptides. The preparation of micro- and nanoparticles with different particle sizes was achieved by adjusting the homogenizer speed and ultrasonic disruptor power. The particle sizes were measured using DLS and SEM, with the diameter of blank microspheres being 2.19 ± 0.09 μm and the diameter of blank nanoparticles being 268 ± 35.4 nm.

C. Completed the quantitative analysis of the peptide (sequence: GGGGGKP) using gradient reverse-phase chromatography. The analysis method involved a 25 cm C18 column as the stationary phase, with mobile phase A: 0.1% TFA in 100% water and mobile phase B: 0.1% TFA in 100% acetonitrile. The gradient was set as 25-60% B (0-20 min) with a flow rate of 1.0 mL/min, and detection wavelength at 220 nm. This method will be used for future measurements of encapsulation efficiency and drug release.

Overall, the successful synthesis of the PCADK polymer, the technique for preparing micro- and nanoparticles, and the quantitative analysis method for the peptide lay a solid foundation for further research on the local and sequential release of anti-inflammatory peptides in wound healing applications. These findings contribute to the advancement of biomedical materials and hold potential for improving therapeutic strategies in wound healing.

KEYWORDS: Chenopodium formosanum, Peptide, Polyketal, Nanoparticles, Microparticles, Diabetic foot ulcers

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Anti-fibrotic microRNA/chitosan-based nanoparticles for the treatment of pulmonary fibrosis

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Abstract:

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and lethal fibrotic lung disease. Repetitive damages to the lung tissues that active transforming growth factor-beta (TGF-β) signaling pathway is involved in the pathological progress. In addition, dysregulation of microRNA (miR) expression is found in IPF patients. The disease-promoting changes in miRs cause a variety of fibrotic mechanisms such as TGF-β/Smads signaling and abnormal extracellular matrix (ECM) deposition. Despite miRs manipulation could be a promising therapeutic approach for fibrotic diseases, exogenous miRs can be degraded shortly *in vivo*. Accordingly, chitosan-based nanoparticles (NPs) were used for miR encapsulation and delivery in this study. Totally 18 anti-fibrotic miRs were lipotransfected to TGF-β-stimulated lung MRC-5 fibroblasts, and four miRs (miR-21 inhibitor, miR-92a mimic, miR-328 inhibitor, and miR-424 inhibitor) were selected. These miRs were further encapsulated in chitosan NPs by ionic gelation method and analyzed. For chitosan NPs loaded with scramble miR, the particle size was ranged 256.15±32.86 nm with the polydispersity index of 0.30±0.03 and a zeta potential of +20.52±1.26 mV. TGF-β-stimulated MRC-5 cells were then transfected with the anti-fibrotic miRs/chitosan NPs, and the results of qRT-PCR exhibited that the mRNA level of fibrotic and ECM genes was downregulated in treated cells. In addition, Western blotting analysis revealed that anti-fibrotic miRs/chitosan NPs transfection decreased pSmad2, α-SMA and vimentin protein productions. Furthermore, the wound closure and cell migration ability of transfected cells were inhibited. In conclusion, anti-fibrotic miRs/chitosan NPs could be a promising treatment for lung fibrosis.

KEYWORDS: pulmonary fibrosis, microRNA, chitosan, nanoparticle.

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Effect of a Topical Collagen Tripeptide on Antiaging and Barrier Dysfunction of Skin

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Abstract:

The process of skin aging is caused by DNA damage and the elevation of oxidative stress associated with the degradation of collagen. This may lead to skin barrier dysfunction, which can cause the formation of wrinkles, irregular pigmentation, skin dryness and an increase in the thickness of the epidermis and dermis. Collagen tripeptide (CTP) is a type of collagen that is hydrolyzed by enzymes. Compared to traditional collagen, it has better solubility, biocompatibility and lower allergenicity. In recent studies, CTP has been proven to improve skin barrier dysfunction and further provide anti-aging effects. To our best knowledge, collagen was shown poor permeability efficiency through the skin due to high molecular weight. Hence, this study aims to develop an optimized CIP formulation via various improvement strategies for skin permeability to maximize the skin transparency of CTP.

In this study, high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was employed to successfully analyze CTP using a pH 5.54 phosphate buffer and a mixture of MeOH 75:25 (v/v), under the conditions of a UV wavelength of 214 nm. Subsequently, a skin permeability study of the CTP-loaded formulations was conducted to confirm their effectiveness in penetrating the skin. Furthermore, a skin model based on cell lines was chosen to verify the efficiency of anti-aging and barrier dysfunction in the skin. In conclusion, the CTP-loaded formulation exhibited good permeability efficiency and demonstrated the potential to improve skin aging and barrier dysfunction.

KEYWORDS: skin aging, barrier dysfunction of skin, collagen, tripeptide

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2023 ISOMRM & BCRS
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Using γ-PGA / MAO composite coatings for improvable biocompatibility and controllabledrug release

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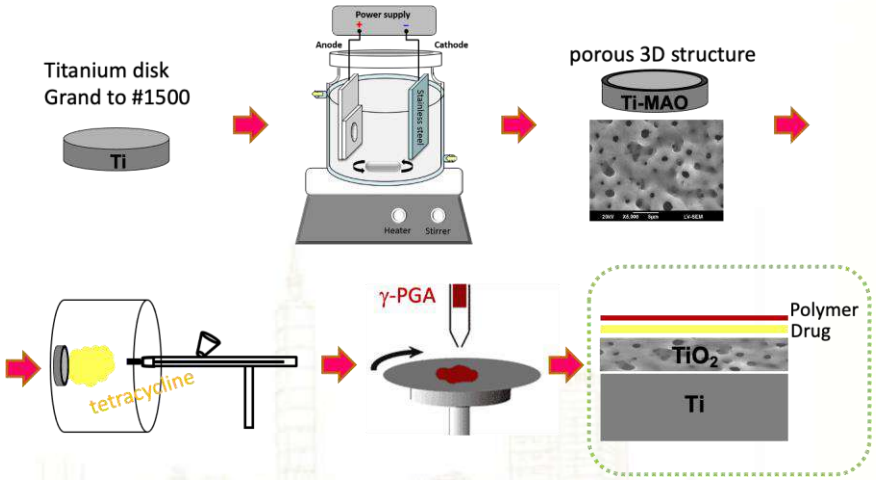
Abstract

Titanium and its alloys are commonly utilized in dental and orthopedic implants due to their favorable biocompatibility and reliable mechanical properties. However, bacterial surface biofilm formation and compromised immune response at the interface between the implant and surrounding tissue can result in persistent infections[1]. Inadequate osseointegration, extensive inflammation, and bacterial infection have been identified as key factors contributing to early failures of dental implants made from titanium[2].

In this particular research study, our primary focus was directed towards the advancement of a ceramic composite coated with a polymer, intended for effective delivery of antimicrobial drugs. To achieve this, we employed a method known as micro-arc oxidation (MAO), which enabled the creation of a porous structure comprised of calcium acetate hydrate ($Ca(CH_3COO)_2•H_2O$) and sodium phosphate monobasic monohydrate ($NaH_2PO_4•H_2O$). The purpose of this structure was to enhance the biocompatibility of titanium, a material commonly used in various orthopedic implants. A spray coating technique was utilized to load an aqueous drug onto the porous surface. Subsequently, we employed a layer-by-layer spin coating approach to apply gamma polyglutamic acid (γ-PGA), a widely recognized naturally occurring hydrophilic biodegradable polymer[3]. The coating process involved two distinct ratios of γ-PGA, characterized by their solubility properties: a water-soluble Na^+ type and a water-insoluble H^+ type. This application of γ-PGA acted as a protective barrier, effectively regulating the release of the drug. To assess the bactericidal impact of the antibiotic on the porous surface, we conducted evaluations using Escherichia coli (E. Coli) bacteria (ATCC® PTA- 10989TM). Furthermore, UV- Vis analysis of the obtained results indicated successful drug integration within the coatings, with subsequent release over time with antibiotic/γ-PGA solution could provide a high bactericidal effect against E.coli by the bactericidal effect of antibiotic.

The comprehensive findings derived from this study highlight the promising potential of employing a porous structure in conjunction with γ-PGA as an effective, economically viable formulation for localized, continuous and controled drug delivery. Notably, the antimicrobial efficacy of the antibiotic was not compromised upon its incorporation into the porous structure, as evidenced by the inhibition of E. coli growth. With the retained drug activity, this drug delivery system based on the porous structure holds significant promise for a wide range of applications in the fields of tissue engineering and pharmaceutical science.

KEYWORDS: Titanium, Gamma Polyglutamic Acid (γ-PGA), Micro-arc Oxidation (MAO),Drug release, Antibacterial



Graphic abstract: Schematic of experiment processes

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Perioperative anesthetics combined hyperthermia on cancer precision medicine

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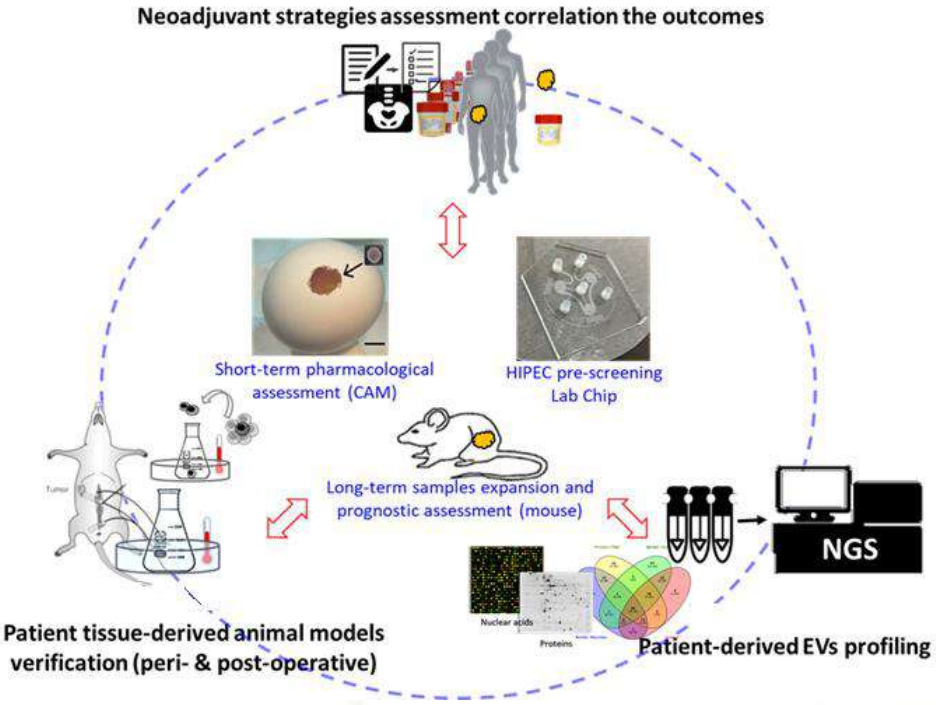
Abstract:

Choice of analgesic method and drug category has a substantial impact on cancer prognosis. We focus on lidocaine instead of others just because of its possibility of intravenous administration, which can facilitate patient recovery (Enhanced Recovery After Surgery; ERAS) after abdominal surgery by reducing morphine consumption, nausea, ileus and duration of admission. According to the ERAS protocol for use of intravenous lidocaine for colorectal surgery, lidocaine can benefit the patients both on postoperative recovery and potential of tumor suppression owing to a higher plasma concentration achieved than that from regional anesthesia/analgesia. Accordingly, researchers could optimize the effect of lidocaine on colon cancer behavior in a safe ERAS dose. Clinically relevant concentrations can be achieved differently in different models. As for tumors residing in human cavities (such as intraperitoneal metastasis intra-thoracic tumor or bladder cancer), tumor cells can be exposed directly to single injection with a sub-toxic dose of local anesthetic[1].

Several literatures reveal that lidocaine can strengthen the inhibitory effect of hyperthermia on cancerous skin cells[2]. We examined the effect of lidocaine at clinical relevant concentrations on non-small lung cancer and a preclinical animal model for hyperthermic intraperitoneal chemotherapy (HIPEC) has been setup (Figure 1), and validate the efficacy of lidocaine- enhanced hyperthermia by sub-toxic clinically applicable doses and aims for minimizing chemotherapeutic exposure during HIPEC.

In this study, we found that the clinically relevant concentrations of lidocaine have variable impacts on different kinds of tumors, and the results were opposite to some previous publications using high concentrations (much higher than clinically relevant ones) of lidocaine in tumor cells lines. Our results showed that, as for non-small cell lung cancer, clinically relevant concentration of lidocaine might reduce the inhibitory effect of 5-FU on cell migration; at clinically relevant concentration, lidocaine per se might even promote colon cancer cell behavior.

As a result, targeting precision medicine on an individual basis is of utmost importance in this field and clinically relevant concentration of lidocaine should be applied cautiously as a part of ERAS protocol.



KEYWORDS: Lidocaine. Administration, Intravenous, Hyperthermia, Chemotherapy, Colonic Neoplasms, Lung Neoplasms

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A simple green design of modification of doxorubicin onto GQD-nanogold composite for the inhibition of colon cancer

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Abstract:

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer mortality in the world [1], in clinical practice, chemotherapy is one of the common means to deal with colorectal cancer. Although chemotherapy drugs have a good tumor suppressing effect, there is no chemotherapy drug that can only destroy malignant cells without destroying normal tissues. In order to improve the side effects of drugs, it is necessary to make the drugs only act on cancer cells as much as possible. Therefore, the development of drug delivery systems is crucial to address these issues.

In drug delivery systems (DDS), various nanostructures, including liposomes, polymers, dendrimers, silicon or carbon materials, and magnetic nanoparticles, have been tested as effective carriers in drug delivery systems [2], coupled with the passive targeting effect of enhanced permeability and retention effect (EPR) , nano drug carriers can significantly increase the dosage of drugs in disease sites such as tumors, infections and inflammation sites, and reduce the toxicity and side effects of traditional drugs. Among these many types of nano particle carriers, carbon materials are an area of great interest. In recent years, scientists have successfully converted two-dimensional graphene into zero-dimensional graphene quantum dots, compared with previous carbon nanomaterials, graphene quantum dots have excellent fluorescence, smaller size, larger surface area and solubility, and lower cytotoxicity. These characteristics make graphene quantum dots have excellent functionality and application potential in drug carriers [3].

We developed a simple and environmentally friendly synthetic method to construct our drug delivery platform, these processes are consistent in the same solution and do not require the use of additional organic solvents (Figure 1). All the materials are evenly dispersed in the aqueous solution, and the functional groups on the graphene quantum dots are used to modify the biocompatible polymers, and then further connect the functional nanoparticles, loading drugs using the π-π stacking interaction properties between graphene and doxorubicin. The results showed that FTIR proved that the bonding of the carrier was successful, and the adsorption of the drug was confirmed by zeta potential and UV-Vis spectroscopy, and then TEM confirmed that the size of the carrier was uniform and suitable for biological tests. In cell experiments, the biological toxicity of the carrier itself is very low or even non-existent, and it has a high drug loading capacity,

which can quickly release drugs in an acidic environment, thereby corresponding to the acidic environment in cancer tissue, allowing the precise release of drugs to exert their poisonous effect on cancer cells.

KEYWORDS: Colorectal cancer (CRC), drug delivery systems (DDS), graphene quantum dots, nano drug carriers.

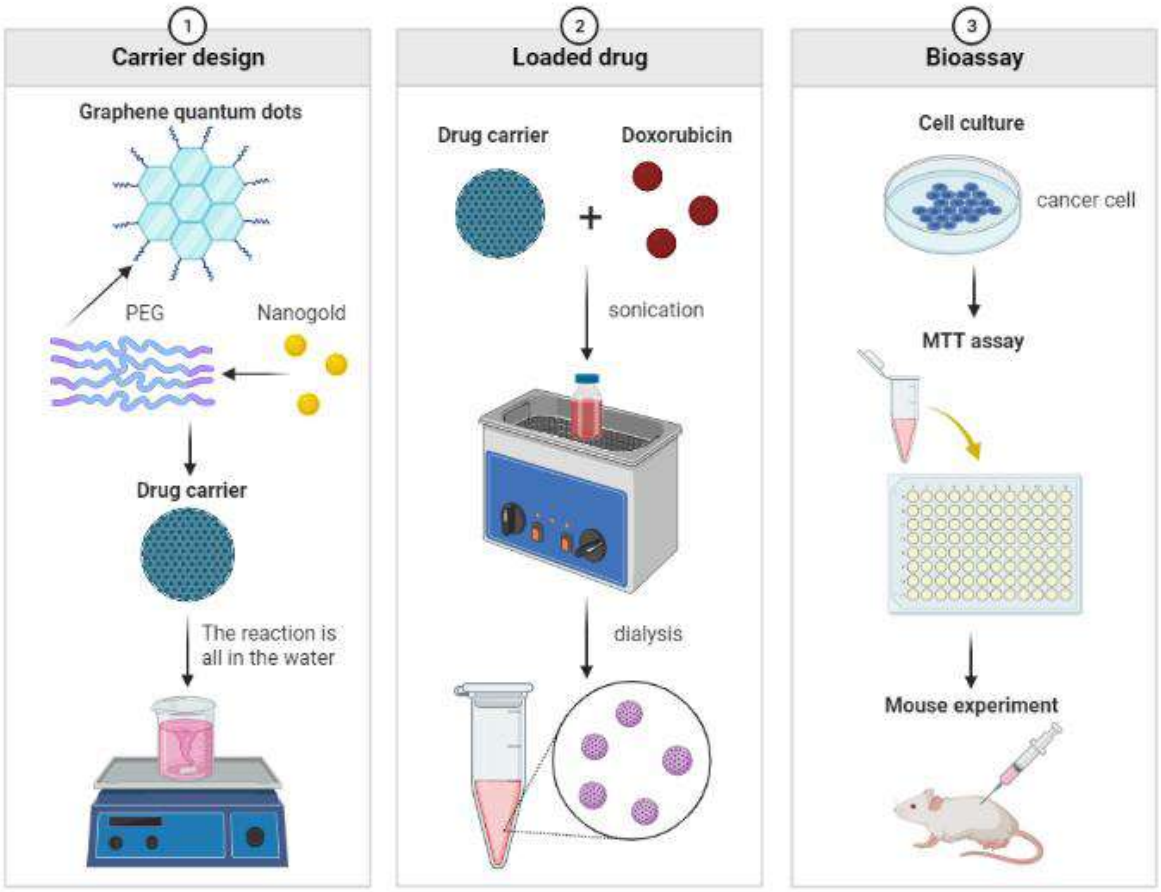

Figure 1. Graphic abstract

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Using the technology of DNA origami and the simulation of Magic DNA to develop various structures for drug delivery

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Abstract:

The aim of this project is to develop a precision medicine delivery system for the treatment of cancer with the DNA origami structure to form several distinguish physical structures. According to the principle of supramolecular self-assembly, physical structures such as micelles, vesicles, capsule, tetrahedral DNA nanostructure are produced by the simulation of Magic DNA for the model system of drug delivery. In order to equip this drug delivery system with targeting delivery function, for example, the four vertices of TDN are attached with the anticancer aptamer AS1411. Because AS1411 would specifically bound to nucleolin, the AS1411-modified TDN could accurately find out tumor sites, allowing the anticancer drugs loaded on it to be slowly released into cancer cells.

KEYWORDS: Self-assembly, DNA origami, Tetrahedral DNA nanostructure, Magic DNA, Aptamer

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of silver-containing mesoporous bioactive glass combined Ebselen for wound dressing

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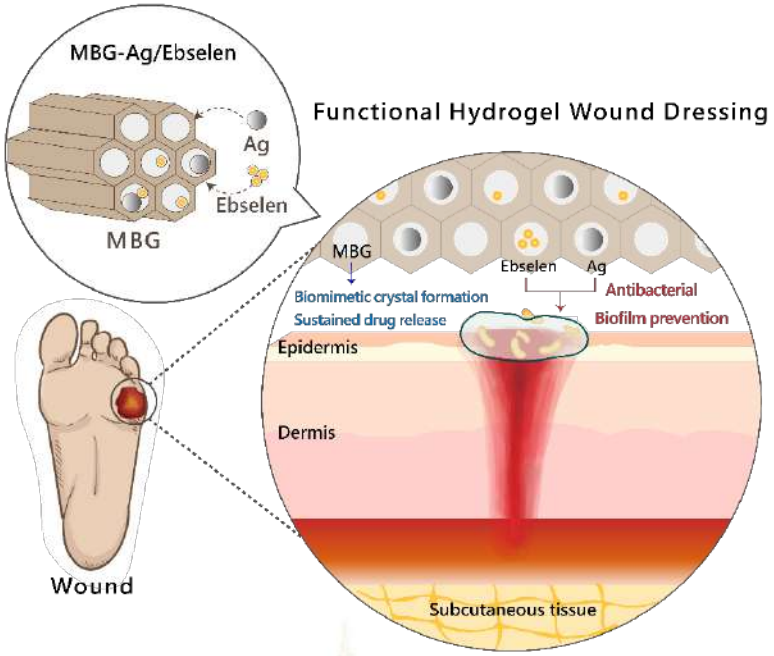
Abstract:

The increasing number of diabetes and bedridden individuals due to the aging population have significantly elevated the demand for wound care. The situation has attracted various industries, academia, and research institutions to invest in the development of innovative and high-value-added wound care products, highlighting the potential for future market growth. Antibiotic-resistant bacteria infections, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), was one of the most challenging issues in wound care. A recent study from Taiwan reported that MRSA accounts for a 74% of wound infections, limiting availability of antibiotics for clinical treatment. In the pursuit of novel solutions, ebselen, a selenium-containing small molecule drug, has shown promising antibacterial activities. Currently, ebselen, as investigational new drug, has undergone numerous phase II and III clinical trials for conditions such as hearing loss and neuroprotection. Recent studies have demonstrated that ebselen, in combination with silver nanoparticles/ions, exhibits synergistic antibacterial effects both *in vivo* and *in vitro*. However, the development and application of ebselen with silver co-loaded materials have not been extensively explored. Our laboratory has developed Ag/80S mesoporous bioactive glass, a technology that offers stable silver release capability, excellent antibacterial activity, and the ability to induce biomimetic crystal formation. In this study, we aim to assess the potential of incorporating ebselen and silver into mesoporous bioactive glass (MBG), resulting in MBG-Ag/Ebselen, for the treatment of wound infections. The combination of ebselen and silver could enhance the bactericidal ability synergistically, while the bioactive glass will facilitate wound support and expedite the healing process through its biomimetic crystal formation.

The characteristic absorption peaks of AgNPs and ebselen around 400 nm and 300 nm, respectively, were observed in ultraviolet-visible spectroscopy (UV-vis) analysis, indicating the successful incorporation of Ag and ebselen into the mesoporous bioactive glass. Furthermore, the adsorption-desorption (BET) analysis revealed that the surface area and pore volume of the material decreased after ebselen loading, suggesting successful loading of ebselen into MBG-Ag. The antibacterial efficacy of MBG-Ag/Ebselen against MRSA was evaluated by disk diffusion test and bacterial growth curve test. The experimental results demonstrated the synergistic antibacterial effect of MBG-Ag/Ebselen against MRSA. Further study will be conducted to evaluate the antibacterial efficacy against different strains of bacteria, to assess the *in vitro* bioactivity, and to examine the wound repair properties of MBG-Ag/Ebselen.

KEYWORDS: Drug-resistant bacteria, MBG-Ag, Ebselen, synergistic antibacterial, bioactive wound dressing



Graphic abstract (Figure. 1)

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3, 2023, Taipei, Taiwan

Degassing a decellularized scaffold enhances wound healing and reduces fibrosis during tracheal defect reconstruction: A preliminary animal study

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Abstract:

Few efforts have been made regarding the optimization of porcine small intestinal submucosa (SIS) to improve its biocompatibility. This study aims to evaluate the effect of SIS degassing on the promotion of cell attachment and wound healing. The degassed SIS was evaluated in vitro and in vivo, compared with the non-degassed SIS control. In the cell sheet reattachment model, the reattached cell sheet coverage was significantly higher in the degassed SIS group than in the non-degassed group. Cell sheet viability was also significantly higher in the SIS group than in the control group. In vivo studies showed that the tracheal defect repaired by the degassed SIS patch showed enhanced healing and reductions in fibrosis and luminal stenosis compared to the non-degassed SIS control group, with the thickness of the transplanted grafts in the degassed SIS group significantly lower than those in the control group (346.82 ± 28.02 μm vs. 771.29 ± 20.41 μm, $p < 0.05$).

Degassing the SIS mesh significantly promoted cell sheet attachment and wound healing by reducing luminal fibrosis and stenosis compared to the non-degassed control SIS. The results

suggest that the degassing processing might be a simple and effective way to improve the biocompatibility of SIS.

Keywords: tissue engineering; small intestinal submucosa; degas; tracheal patch model

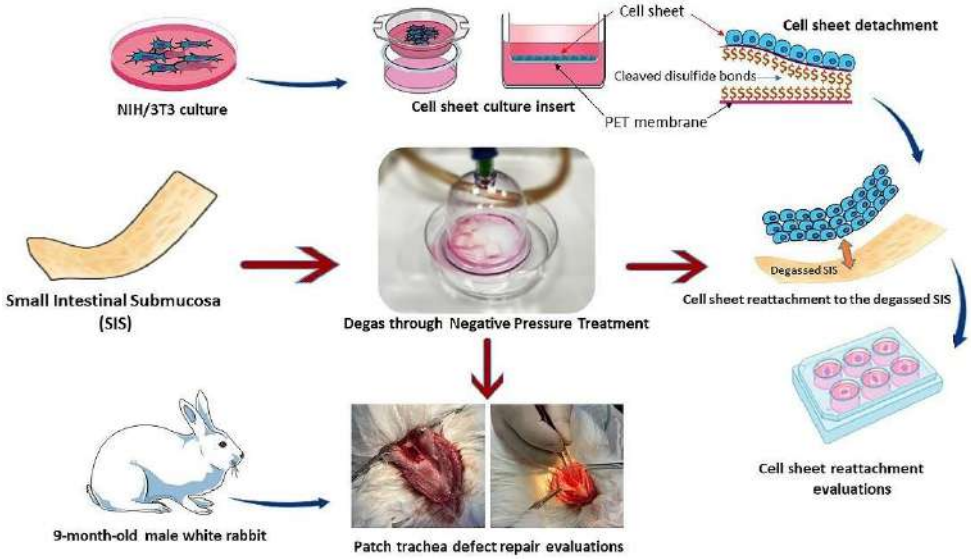

Figure 1. Flow diagram of the research design.

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Pd@VNU-2 and its Application in Radiation-Photothermal Combined Cancer Therapy

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Abstract:

Radiotherapy is often used in clinic ~~to kill malignant~~ tissues. However, different types of cancer might have different sensitivity to radiation. For example, Non-small Cell Lung Cancer and Skin Melanoma are considered radiation resistant, which means that higher dosage must be given during the treatment. Although higher doses of radiation can achieve curative effects, and it will also cause more damage to normal tissues near the tumor. In order to solve this problem, a novel method is to apply radiosensitizers. These sensitizers can be chemotherapy drugs which has synergistic effects with radiotherapy, such as cisplatin or nanomaterials containing high-Z elements such as Hafnium.

In this work, we successfully synthesized a Hf-based Metal-Organic Framework VNU-2 as radiosensitizer. Due to the high electron density of hafnium, when VNU-2 is irradiated by ionizing radiation, it can efficiently generate photoelectrons, secondary electrons and Auger electrons. Besides, its porous structure can avoid the self-quenching of photoelectrons and further improve the diffusion of reactive oxygen species (ROS) generated when electrons react with water molecules, make it more effective to cause direct and indirect damage to cancer cells.

On the other hand, we also found that the triple bond structure in the ligand can effectively adsorb noble metal ions. Therefore, we designed to first adsorb the palladium metal ions in the pores of VNU-2, and then reduce the palladium metal ions to Palladium nanoparticles. Since palladium nanoparticles have excellent absorption and photothermal efficiency in the range of UV-Vis and NIR, we use 808 nm NIR laser for photothermal therapy. The cell cycle analysis results show that after photothermal treatment, the proportion of the most radiation-resistant S phase decreases, which further improves the sensitivity of cancer cells to radiation therapy.

Until now, we have conducted in vitro experiments with the skin melanoma cell line B16F10 and the non-small cell lung cancer cell line A549 and obtained good results. Preliminary results of animal study based on skin melanoma model have also been obtained.

KEYWORDS:Ratiotherapy, radiosensitizer, photothermal therapy, metal-organic frameworks

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of Nanoparticle Loaded Microneedle Mediated Gene Delivery on the Application of Cancer Treatment

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Abstract:

Microneedles (MNs) are a promising method for drug delivery as they offer several advantages over traditional drug delivery methods. They can be used to deliver drugs to specific target tissues, reduce systemic side effects, and provide sustained release of drugs. Gelatin and silk fibroin (SF) are used in this study for fabricating MN patches due to their superior biocompatibility and biodegradability. The release rate of drugs can be regulated with the ratio of gelatin to silk fibroin (SF) in the MN patches. SEM imaging was performed, and the results showed the morphology of MN patches. Additionally, the drug release rate was tested for a duration of 7 days [1].

In this particular application, nanoparticles will be used to deliver the gene to cancer cells via the MN patch for the purpose of antiproliferative effect [2]. MTT and LDH analyses were used to evaluate the biocompatibility of MNs and nanoparticles. In addition, CCK8 and Live/Dead assays will be used to analyze biological activity. The sustained release provided by the patch can help to ensure that the nanoparticles remain in contact with the cancer cells for an extended period of time, increasing the likelihood of inducing apoptosis. The use of MN patches in both 2D and 3D environments is also noteworthy. Many drug delivery systems are designed for 2D cell cultures, which do not always reflect the complex nature of tumors in vivo. By testing the nanoparticle encapsulated in MN patches on 3D tumor spheroid, the researchers can gain a better understanding of their efficacy in a more realistic tumor model.

Overall, the ability to control the release rate of nanoparticles via the MN patch offers a significant advantage in drug delivery. By optimizing the ratio of materials in the patch, it is possible to achieve sustained release of drugs and increase their effectiveness in treating cancer.

KEYWORDS: microneedles (MNs), nanoparticle, gene delivery, cancer therapy

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Study on Gelatin Methacryloyl / Hyaluronic Acid Methacryloyl Composite Hydrogel Cross-linked with Visible Light for Wound Dressings

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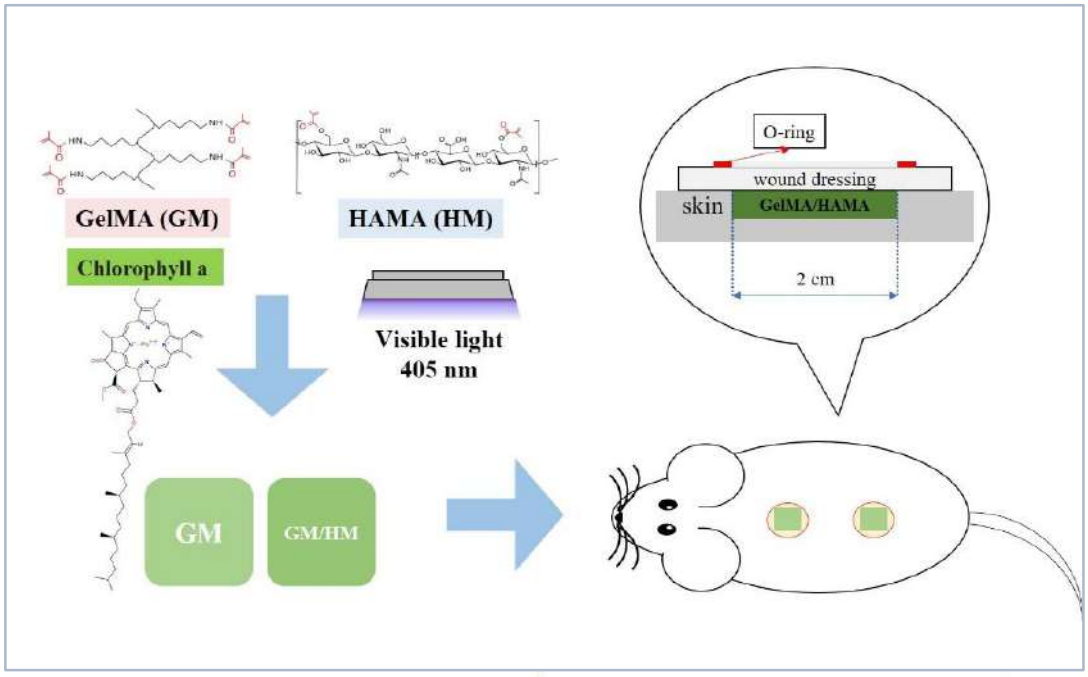
Abstract:

The skin is the outermost protective layer of the human body. It is in direct contact with the external environment and is extremely vulnerable to injury. When managed and treated in community care and hospitals, hospitalization and nursing expenses account for 80% to 85% of the total wound management costs for the treatment period. We can improve wound management efficiency by reducing healing time, optimizing the frequency of dressing changes, and preventing complications such as wound infections.

In this study, gelatin and hyaluronic acid were used, which were chemically modified into photosensitive materials, gelatin methacrylate (GelMA) and hyaluronic acid methacrylate (HAMA). The modified photosensitive material was added with chlorophyll as a photocrosslinking agent, and visible light was used as the crosslinking light source to produce a composite biological dressing. The biocompatibility of GelMA and GelMA/HAMA and their effects in promoting skin defect repair were evaluated with in vitro and in vivo tests.

The molecular structure and functional groups of the photosensitive material after chemical modification were confirmed by NMR and ATR/FT-IR. According to the compression performance test results, GelMA/HAMA had higher elastic properties than GelMA. After soaking in phosphate buffer to test its degradation properties, it was known that the degradation rate of GelMA/HAMA was significantly reduced. The CCK-8 result of tests showed that GelMA/HAMA led to well cell activity on mouse fibroblasts and human adipose-derived stem cell. The results of in vivo experiments in rats showed that the materials in this study have the potential to promote the regeneration of skin defects.

KEYWORDS: Methacrylic gelatin, Methacrylic hyaluronic acid, Visible light, Photo-cross-linking agents, Chlorophyll



Graphic abstract

2023 ISOMRM & Poster
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of Antibacterial Composite Materials for the Bottom Layer of Dissolving Microneedles

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Abstract:

Dissolving microneedle is a novel method of transcutaneous drug absorption, where drugs are delivered to the epidermis layer through painless punctures, enhancing the therapeutic effect of drug absorption. There are many potential applications of dissolving microneedles as a drug delivery system in various therapeutic areas, including cosmetic delivery, vaccine delivery, diagnosis and surveillance, cancer treatment, pain and inflammation management, diabetes, hair and scalp diseases, and inflammatory skin diseases. Dissolving microneedles (DMNs) are primarily composed of a soluble matrix that contains biocompatible polymers or polysaccharides. The tip of the microneedle dissolves upon contact with the interstitial liquid to facilitate the release of the active pharmaceutical ingredient. The release kinetics of the active pharmaceutical ingredients depend on the solubility of the constituent polymers, allowing for controlled drug delivery by adjusting the polymer composition or modifying the manufacturing process. DMNs offer advantages the high release rates and relatively low manufacturing costs. However, the production process for DMNs can be cumbersome that result in poor yields, which hinders the commercialization progress of microneedle products. Another drawback is the deposition of polymers in the skin after prolonged use, so that making them less suitable for long-term use.

The aim of our study is to find natural polymers for drug formulation to develop the preparation of DMNs and reduce the risk of infection at the site of skin puncture by incorporating antibacterial materials. In this experimental, the biodegradable thermoplastic modified starch are employed as the primary raw materials. The composite materials at the base of the needle were developed to possess sufficient mechanical strength for puncturing the stratum corneum, while ensuring the softness, flatness, flexibility, and antibacterial properties of the needle. In addition, it is expected that enhance both the yield and quality of the product by optimizing the process parameters.

KEYWORDS: Dissolving microneedles, drug delivery system, biodegradable, modified starch, stratum corneum, antibacterial material

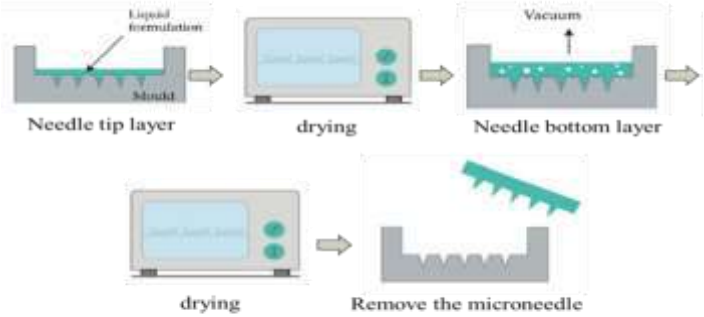

Fig.1 Microneedle manufacturing process

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of magnetic temperature fiber materials for drug releasing

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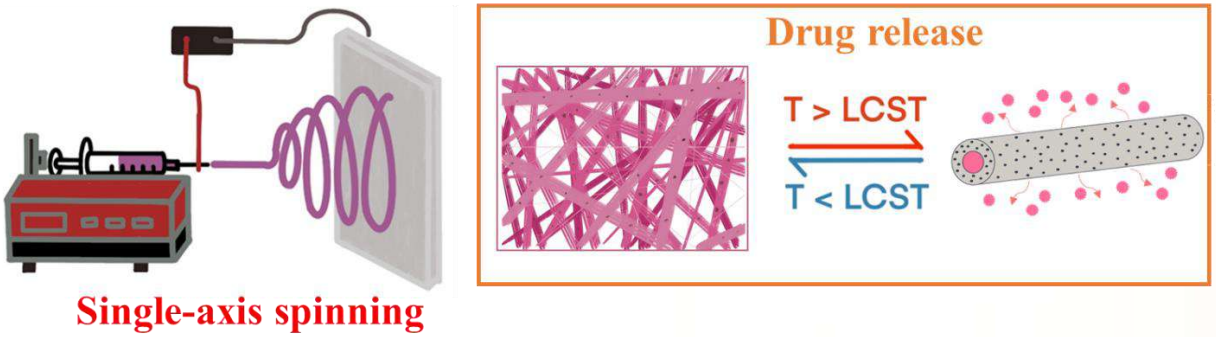
Abstract:

This study aims to develop a thin fiber with magnetic and temperature-responsive nanomaterials as a drug carrier for controlled drug release applications. The materials used consist of temperature-sensitive polymers. Which is poly(N-isopropyl acrylamide) (PNIPAAm), combined with polycaprolactone (PCL) and magnetic nanoparticles (MNPs). The thin fiber was produced using the electrospinning technique, resulting in highly porous structure with a large surface area. During the study, the ratio of magnetic nanoparticles was adjusted, and the patterns and structures of the fiber were observed using SEM and TEM. Rhodamine B was employed as the reagent to assess drug release. The effects of different concentrations of magnetic particles on the shrinkage and drug release of the spun-bond fiber were examined. The fiber underwent swelling triggered by heating the water temperature to study the drug release behavior. The experimental results revealed that higher concentrations of magnetic particles correlated with the increased heating temperature of the fiber, resulting in higher drug release rates. The development of these drug carriers in this study holds the potential to be utilized in numerous clinical applications due to their ability to carry various drugs and enable on-demand drug release.

KEYWORDS: Electrospinning, Magnetic nanoparticles, Temperature response polymer, Drug releasing



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Nanocomposite hydrogel-delivered GM-CSF and fucoidan-based nanoparticular vaccine for inhibition of tumor growth and metastasis

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Abstract:

Breast cancer is the most common cancer and the second leading cause of cancer-related deaths among women in the United States.[1] Triple-negative breast cancer (TNBC) lacks the common hormone receptors, making hormone therapy ineffective.[2] Although chemotherapy has shown good results in treating TNBC, it also has a high recurrence rate. The most common cause of death in TNBC is the invasion and metastasis to distant organs such as the lungs, liver, and bones.[3]

Due to the difficulty in treating metastatic TNBC, we plan to develop a vaccine to prevent both in situ and metastatic TNBC. In our experiment, we designed a hydrogel-delivered granulocyte-macrophage colony-stimulating factor (GM-CSF) and fucoidan-based nanoparticles as a cancer vaccine. The gelatin-hydroxyphenylpropionic acid hydrogel prolongs the release of GM-CSF, while the fucoidan-based nanoparticles combined with 4T1 cell lysate stimulate cellular immunity.[4-6] Nanoparticles (NPs) will be prepared by an ionic gelation method by complexation of positively charged polyetherimide (PEI) and negatively charged fucoidan, and will be used to encapsulate ovalbumin (OVA) or 4T1 tumor cell lysate. Fucoidan can enhance the activation of dendritic cells, while PEI can promote antigen cross-presentation by facilitating endosomal escape of antigen into cytosol. NPs and GM-CSF will further be encapsulated into hydroxyphenyl propionic acid-functionalized gelatin. Upon administration, the nanocomposite hydrogel will recruit and concentrate dendritic cells *in situ*, promoting their proliferation and activation. The recruited dendritic cells will then uptake NPs in the nanocomposite hydrogel, subsequently homing to the draining lymph node, and presenting antigen to T cells.

Our plan is to inject the vaccine subcutaneously in mice prior to subcutaneous injection of 4T1 cells and observe the tumor growth. This model represents the prevention of *in situ* TNBC. Through this animal models, we aim to confirm the effectiveness of our developed vaccine in inhibiting the occurrence of both *in situ* and metastatic TNBC.

KEYWORDS: Triple-negative breast cancer (TNBC), cancer vaccine, nanocomposite hydrogel

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

In Situ Forming Transparent Quercetin/Hyaluronic Acid Hydrogel as A Vitreous Substitute

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Abstract:

Vitreous is a clear, gel-like, water-rich substance that locates between the lens and the retina. It performs several vital roles in eye, including maintaining spherical eyeball shape, shock absorption and allowing light to penetrate to retina for vision. For people over the age of 50, the vitreous may degenerate, and patients may experience dark spots and floaters. In severe cases, vitreous would completely peel off from the retina, that may result in permanent loss of vision without further treatment. Clinically, colorless/inert gas, and silicone oil injection are the current treatments for vitreous replacement. However, they also have some limitations, such as patient having to keep inconvenient face-down position for several days when using colorless/inert gas injection treatment. Filling with silicone oil in vitreous has the risk of emulsification and may lead to cataract formation and intraocular inflammation. Since hyaluronic acid (HA) is one of the main components of the vitreous, we developed an *in situ* forming, colorless, transparent hydrogel with HA as a vitreous substitute in the study and incorporate with quercetin to form Quer-H hydrogel. Quercetin is a flavonoid extracted from natural vegetable and fruit plants with anti-inflammatory and antioxidant properties. The addition of quercetin in Quer-H hydrogel provides a better environment for vitreous due to its anti-inflammatory effect. Quer-H hydrogel can be prepared by simply mixed oxidated hyaluronic acid, adipic acid dihydrazide, and quercetin together. Fourier transform infrared spectroscopy (FTIR) and TNBS assay were used for HA oxidation confirmation. Quer-H hydrogel could be transformed from liquid to gel within 2 minutes. The refractive index of Quer-H hydrogel with 0 ~ 1000 μM quercetin was between 1.341 and 1.344, which is quite similar to human vitreous humor (1.336). Moreover, degradation time of Quer-H hydrogel was more than 2 weeks. Moreover, Quer-H showed well cell compatibility when cultivation retinal pigment epithelium with Quer-H hydrogel extraction medium together. In summary, Quer-H hydrogel has the advantages of similar refractive index, easy handling, degradable, natural and non-toxic. Based on above results, Quer-H hydrogel could be a promising vitreous substitute for retina supporting in the future.

keywords：*vitreous substitute, hyaluronic acid, quercetin, injectable hydrogel*

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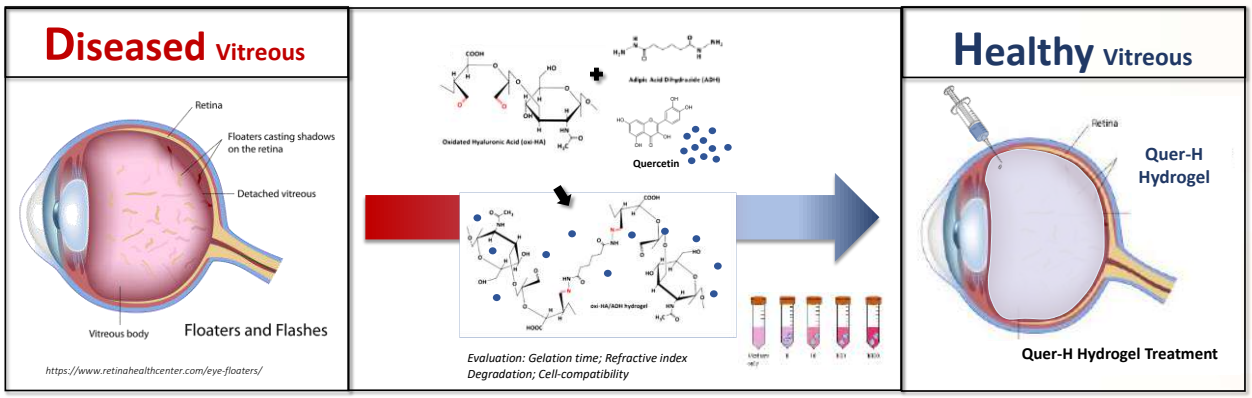
Graphic Abstract



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Study on novel composite tissue-engineeringed tracheal grafts with bacteriostasis and photocurable 3D printing

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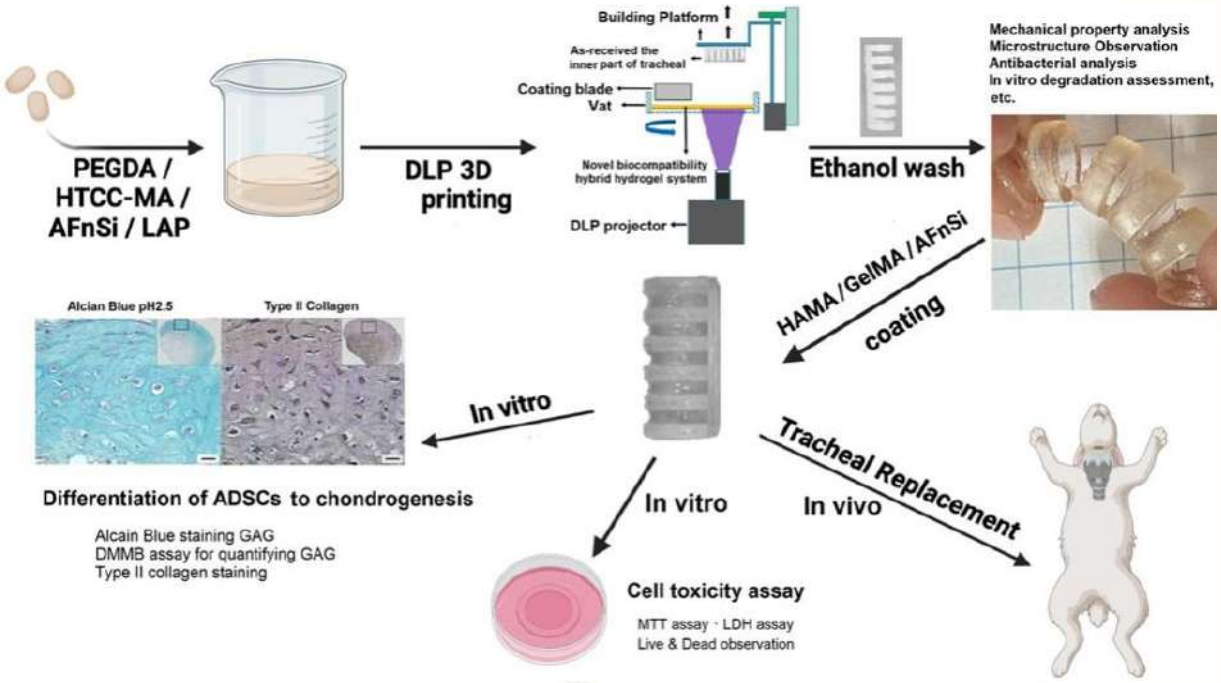
Abstract:

Tissue Engineering (TE) combined with hydrogel system and 3D printing has been quite active in the research field in recent years. By using 3D printing technology, bio-ink containing cells can be used to prepare tissue, organ models or customized medical materials. Help research drugs and find some potential treatments. Taking the artificial bronchus as an example, there are four types of auxiliary stents commonly used clinically: Bare metal stent, Covered metal stent, Dumon stent, and T-tube. Secondary injury during installation may indirectly lead to tissue inflammation and infection.

In our research, we design a strategy hope to combine the relevant shortcomings mentioned in the preface and use the hydrogel system combined with 3D printing for tissue engineering (TE). We developed the material with non-toxic, slow degradation, bacteriostatic, photocurable and printable which was preparing by the quaternary ammonium chitosan by reacting chitosan with Glycidyl trimethyl ammonium chloride (GTMAC) first then do the methacrylate reaction to obtain methacrylate quaternary ammonium chitosan (HTCC-MA). The structure of HTCC and HTCC-MA were confirmed by FT-IR, 1H NMR, 13C NMR and the mechanical properties and biological safety of the materials were also evaluated, and 3D printing of artificial bronchial stents with different ratios of HTCC-MA / PEGDA was done. In order to evaluate and discuss its relevant application in the future.

Keywords： 3D printing、Tissue Engineering、HTCC-MA、bacteriostasis、photocurable、 printable



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2023 ISOMRM & BCRS
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Development of a microfluidic device encapsulating mitochondria in liposomes for cell therapy

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Abstract:

Studies revealed that mitochondria can provide energy for cells, when the number of damaged mitochondria in cells reaches a certain level and cannot be repaired by themselves, it will cause cell damage, and then affect the normal operation of organs and tissues. However, mitochondria can only survive outside the cells for four hours. Herein, we fabricated a device that can encapsulate the mitochondria in the liposome. This device is a microfluidic channel that can force the mitochondria through the liposome to become a droplet. Because the liposome is a spherical vesicle having at least one lipid bilayer, and the cell membrane itself is also composed of a phospholipid, so the liposome can pass through the cell membrane directly. This characteristic helps us send the droplets back to the cell easily. We expect this device to prolong the mitochondria service time and be used in cell therapy.

Mitochondria have a double membrane structure and use aerobic respiration to generate adenosine triphosphate (ATP), which is used throughout the cell as a source of chemical energy. As we know, mitochondria can produce energy (A dominant role for the mitochondria is the production of ATP), cell death, storage of calcium ions, and heat production.[1] Relating to the mitochondria aging, reactive oxygen species are produced in mitochondria, as a byproduct of energy production. These highly charged particles damage DNA, fats, and proteins. Because of the damage caused by ROS, the functional parts of mitochondria are damaged.[2] More ROS are produced when the mitochondria can no longer function so well, worsening the damage further.[3]

A liposome is a spherical vesicle having at least one lipid bilayer. As the cell membrane itself is composed of phospholipids, liposomes can directly fuse with the membrane and release the cargo into the cytosol, so it has good biocompatibility. [4.5] First, it can encapsulate fat-soluble drugs and water-soluble drugs at the same time, reduce drug toxicity. Furthermore, a liposome can selectively distribute locally in certain tissues and organs, increasing the certainty of the drug substance on the lymphatic system.[6] In particular, anticancer drugs can target cancer cells without damaging normal tissues and cells. Moreover, after entering the body, the drug can be prevented from being degraded by the body's enzyme system and immune system due to the protection of the liposome membrane. In addition, using liposomes as a drug delivery system can effect-reduced diffusion in tissue, slowly releasing the drug into the blood.

The device comprises a microchannel and a glass slide. The microchannel is composed of

one big circle and a small circle. We need to cut a line for the outlet and the hole for the inlet. The droplet will be pushed through the microchannel, putting the liposome in the lower phase and adding the mitochondria from the inlet. (figure 1)

KEYWORDS: liposome, mitochondria, microfluidic device, cell therapy

(figure 1)

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

In vitro studies of kaempferol action on osteoblasts and osteoblasts

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Abstract:

Kaempferol is a natural flavonoid with various biological activities, including anti-inflammatory, antioxidant, and anti-allergic properties, found in numerous plants. Previous studies have indicated that the effects of kaempferol on osteoblasts vary depending on the concentration. While low concentrations promote osteoblast proliferation, activity, and calcification, high concentrations may decrease cell viability[1,2]. Additionally, kaempferol inhibits osteoclasts, leading to a reduction in bone resorption, particularly at an optimal concentration of 50 μM [3]. As a result, kaempferol has been investigated for its potential in treating osteoporosis. This study aimed to determine the optimal concentration of kaempferol for promoting osteoblasts and inhibiting osteoclasts to shorten bone repair time by incorporating the compound into orthopedic materials.

MG63 osteoblasts and RAW 264.7 cells were used to differentiate osteoclasts. Osteoblast viability and activity were analyzed using MTT and ALP assays, respectively, while mineralized nodules were assessed using Alizarin red staining. Osteoclast activity was measured using TRAP assay. Kaempferol solution was prepared by dissolving it in 100% anhydrous alcohol and diluting it into eight different concentrations using cell medium. The cells were then co-cultured with the drug at various time points in the experimental design. The incubation period was determined by the duration of osteoblast and osteoclast differentiation, and experimental analysis was performed after the incubation period ended.

The results indicated that low concentrations of kaempferol promoted osteoblast activity without affecting cell survival rates, while high concentrations led to decreased cell survival rates. Osteoclast inhibition was most pronounced at a concentration of 25 μM, with increasing concentrations leading to decreased cell survival rates. The optimal concentration of kaempferol was found to be 25 μM, as it simultaneously promoted osteoblasts and inhibited osteoclasts. This concentration could be incorporated into orthopedic materials to release the drug and treat bone defects caused by osteoporosis, thereby shortening bone repair time.

Our findings suggest that kaempferol has the potential to enhance bone repair and treat

osteoporosis. The optimal concentration of kaempferol, 25 μM, was found to promote osteoblast activity while inhibiting osteoclasts, which is critical in bone regeneration. These results suggest that kaempferol may be incorporated into orthopedic materials to enhance bone regeneration and reduce bone repair time. Further in vivo studies are warranted to confirm the potential of kaempferol in bone regeneration therapy.

KEYWORDS: osteoclast, osteoblast, Kaempferol

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Development of a novel extraction wound dressing containing bimetallic nanoparticles bioactive glass for the prevention of medication-related osteonecrosis of the jaw

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Abstract:

Studies have found that patients after receiving dental-related invasive treatment are prone to the side effects of Medication-Related Osteonecrosis of the Jaw (MRONJ) due to bacterial infection while taking medicine (some of anti-osteoporosis drugs and cancer treatment drugs) . *Actinomyces* are the main source of infection, and the representative bacteria is *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*). If infected, It is required to remove the necrotic bone by immediate surgical treatment. After the surgical treatment, it may lead to changes in the oral structure of the patient, resulting in masticatory dysfunction or appearance impact.

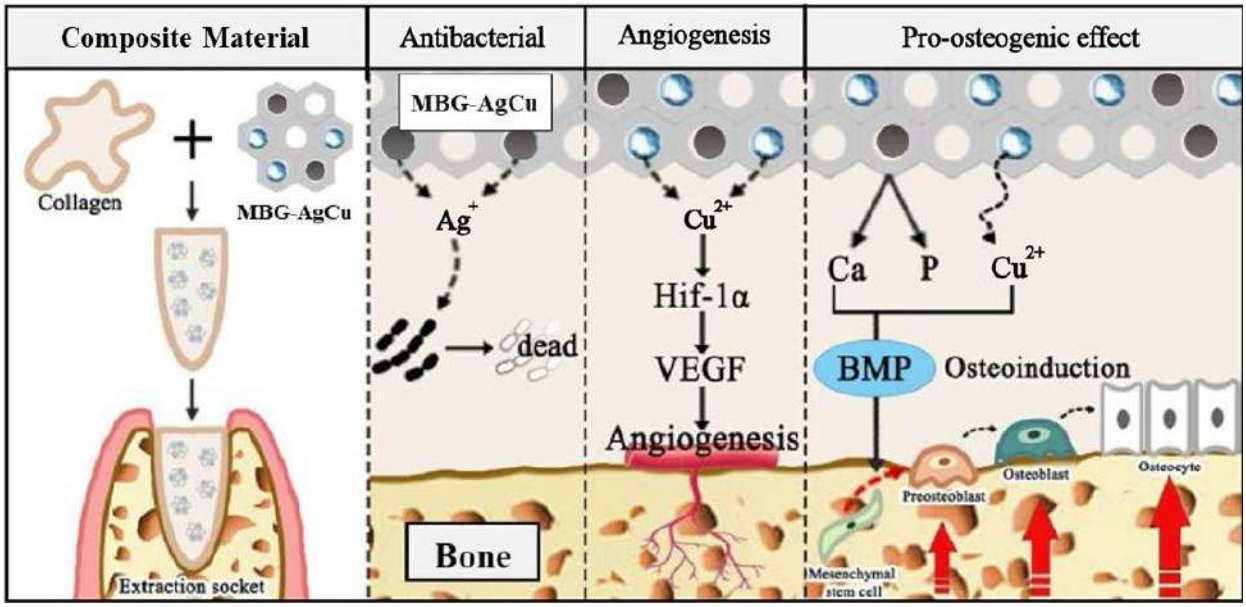
To prevent the occurrence of MRONJ, this study was synthesized containing silver-copper bimetallic nanoparticles mesoporous bioactive glass (MBG-Ag1Cu4) by sol-gel method and mixed with Type I Collagen to form a collagen matrix composite (CMC),which applied the high biocompatibility and osteoinductive properties of mesoporous bioactive glass, combined with the broad-spectrum antibacterial ability of silver ions, the angiogenesis-promoting properties of copper ions and the promote wound healing ability of collagen. CMC is used as a novel oral wound dressing to achieve the effects of preventing bacterial infection, alveolar bone repair and preservation. Analyze the crystal composition, molecular bonding and resonance absorption of AgNPs and CuNPs of CMC by X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and ultraviolet-visible spectroscopy (UV-vis). The results show that the (220) crystal plane of silver and the (111) crystal plane of copper appeared at $2\theta = 64.4$ and 43.3° respectively in the XRD pattern. In the FTIR spectrum, amide A, amide B and amide I in the collagen were detected at 3444cm^{-1}, 2933cm^{-1}, and 1653cm^{-1}, and Si-O-Si group of bioactive glass was detected at 1067cm^{-1} and 788cm^{-1}. The characteristic absorption peaks of AgNPs and CuNPs appeared around 400 nm and 700 nm, respectively, indicating that Ag and Cu were successfully doped on the mesoporous bioactive glass. The antibacterial efficacy of CMC against oral bacteria, *A. actinomycetemcomitans* and *E. faecalis*, was evaluated by disk diffusion test, bacterial growth curve test, and colony-forming test. The experimental results showed that CMC has antibacterial effect against *A. actinomycetemcomitans* and *E. faecalis*, while the minimum inhibitory concentration (MIC) was 10mg/mL and 20mg/mL, respectively, and the minimum bactericidal concentration (MBC) was 20mg/ml and greater than 20mg/mL, respectively.

In the future, *in vitro* bioactivity assays will be performed to evaluate the cell viability and

long-term pro-angiogenesis at different CMC concentrations, and Alkaline Phosphatase assay will be performed to evaluate the characteristics of CMC in accelerating wound repair and inducing bone regeneration.

Synthesis of the novel dressing and evaluation of *in vitro* bioactivity assays - antibacterial, angiogenesis, and pro-osteogenic effect (**Figure. 1**). Mixing bioactive glass with collagen utilizes the biodegradable properties of collagen to promote wound repair. Then the release of silver ions showed its antibacterial properties, the release of copper ions stimulated the expression of Hif-1 and VEGF to promote angiogenesis, and BMP expression was induced by copper ions, calcium and phosphorus in bioactive glass, resulting in bone cells proliferation and mineralization.

KEYWORDS: medication-related osteonecrosis of the jaw (MRONJ), bioactive glass, collagen, antibacterial, wound dressing



Graphic abstract (**Figure. 1**)

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

3D Bioceramic burr hole scaffold fabricated by digital light processing for bone regeneration in rabbit calvarial defects

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Abstract:

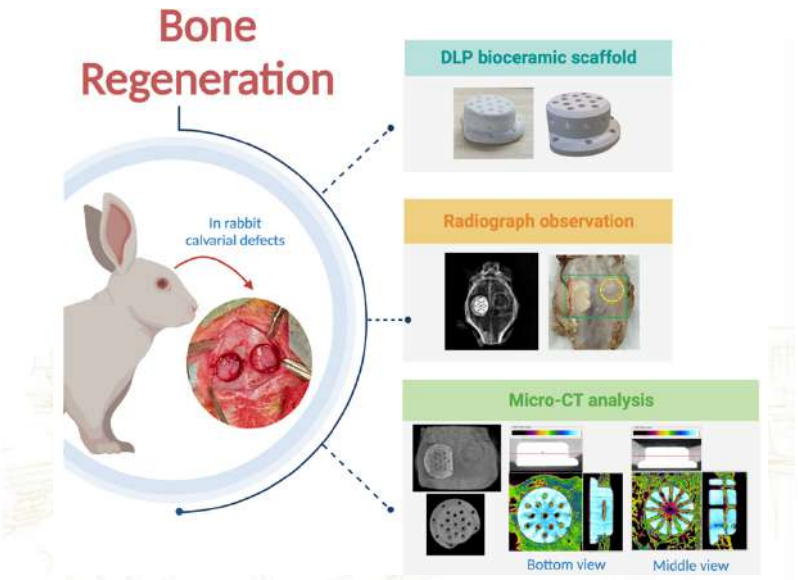
Bone defects resulting from trauma, infection, or disease represent a major clinical challenge in orthopedic surgery [1][2]. Autografts are considered the gold standard for bone regeneration. However, their use is limited due to donor site morbidity, availability, and donor tissue rejection [3][4]. Therefore, tissue engineering has emerged as a promising approach for bone regeneration. In our previous study, we evaluated the benefits of silicone oil sealing on the 3D printed bioceramics (3DP-BCs) green body, which was fabricated using robotic deposition, in terms of densification and structural stability during sintering. And demonstrated 3DP- BC scaffolds have better new bone regeneration efficiency in rabbit calvarial bone defect models than empty scaffolds and mold-forming bioceramic scaffolds (MF-BCs) [5].

In this study was to evaluate the use of a bioceramic scaffold fabricated using digital light processing (DLP) for bone regeneration in rabbit calvarial defects. The scaffold was composed of beta-tricalcium phosphate, which was known to promote bone growth and integration [6]. The scaffold had a porous structure with interconnected pores which allowed for cell infiltration, nutrient diffusion, and vascularization. We developed a critical sized calvarial defect model in rabbits in which the defect area was 8 mm in diameter, the height was 2 mm and there were defects in each rabbit, for a total of 16 defects in 8 rabbits. These defects were staggered in 3 groups, including the negative control (without a scaffold) group, infilled with competitive group, and the DLP bioceramic groups.

At three months post-surgery, the results showed that the bioceramic scaffold was able to

promote bone regeneration in the calvarial defects of rabbits. Micro-CT analysis revealed a significant increase in bone density in the experimental group compared to the control group. Histological analysis showed that the scaffold was well integrated with the surrounding tissue and supported the growth of new bone tissue. These findings suggest that the bioceramic scaffold fabricated using digital light processing may have potential for use in bone tissue engineering applications. The scaffold's porous structure and composition may enable it to support the growth of new bone tissue and promote bone regeneration. Future studies may further optimize the scaffold's design and composition to enhance its effectiveness for bone regeneration. Overall, this study represents a promising step towards the development of effective tissue engineering strategies for bone regeneration.

KEYWORDS: 3D printing, bioceramic, digital light processing, calvarial bone defect



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Plasma-Enabled Graphene Quantum Dot Hydrogel – Magnesium Composites as the Bioactive Scaffolds for In Vivo Bone Defect Repair

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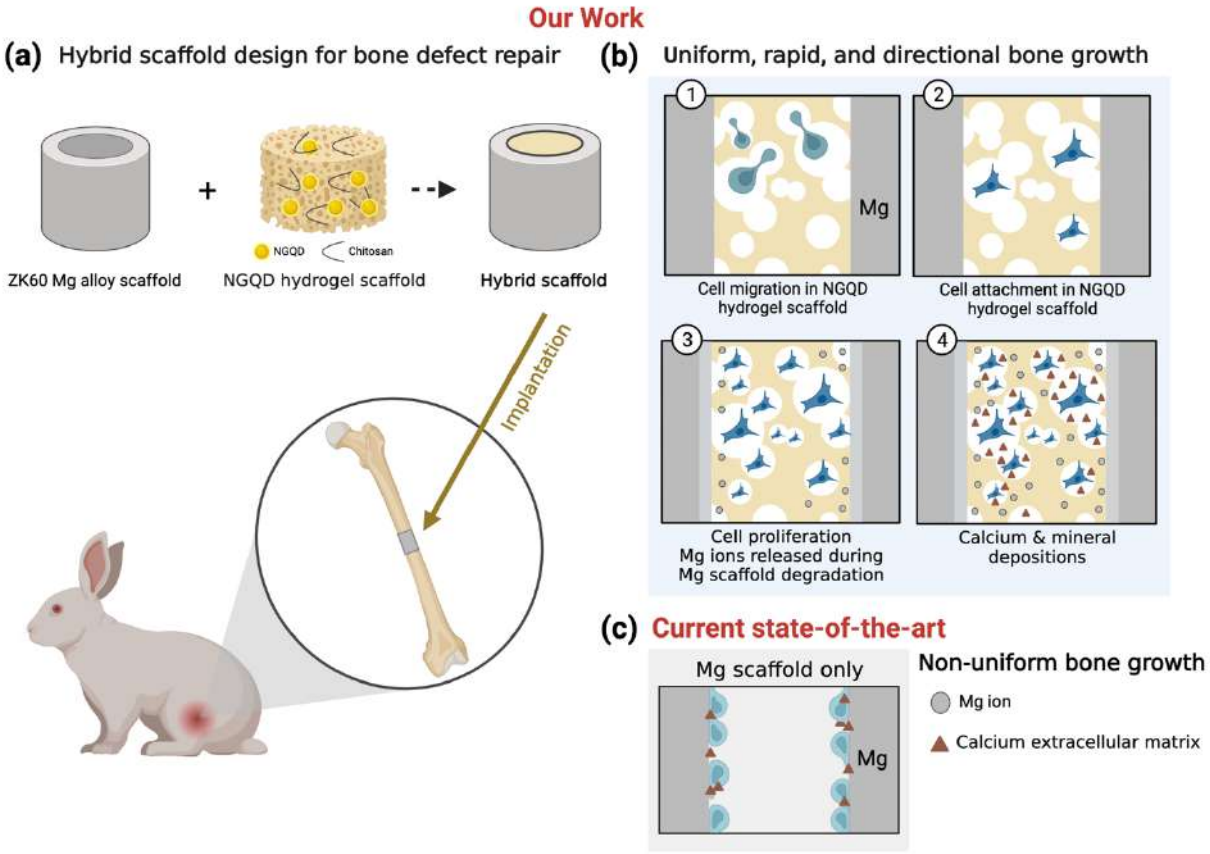
Abstract:

Bioactive and mechanically stable metal-based scaffolds have been widely utilized for bone defect repair. However, these conventional scaffolds often exhibit non-uniform cell growth, limiting the restoration of damaged tissue. To address this challenge, we have developed a novel composite scaffold by integrating a plasma nanotechnology-enhanced graphene quantum dot (GQD) hydrogel with magnesium (Mg) ZK60 alloy. The composite scaffold combines the strengths of its individual components to achieve enhanced functional bone defect repair. Firstly, it provides mechanical support akin to cortical bone and promotes calcium deposition through the release of Mg^{2+} during degradation. Secondly, the porous hydrogel facilitates improved uptake, migration, and distribution of osteoblasts. Finally, the NGQDs embedded in the hydrogel enhance osteoblast adhesion, proliferation, osteogenesis, and mineralization. Through in vivo studies, our hybrid scaffold demonstrates significant osteogenic ability enhancement, resulting in accelerated, uniform, and directed bone growth across the hydrogel channel compared with a control Mg-based scaffold. This work not only provides valuable insights into the design of multifunctional hybrid scaffolds for bone defect repair, but also opens up possibilities for their application in diverse areas beyond the scope of this study.

KEYWORDS: bone defect repair, composite scaffold, graphene quantum dot, magnesium alloy, plasma nanotechnology



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2023 ISOMRM & BCRS
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Novel artificial tricalcium phosphate and magnesium composite graft facilitates angiogenesis in bone healing

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Abstract:

Background: Massive bone defects posed a significant challenge for orthopedic surgeons. Autologous/allograft bone grafting cannot achieve an excellent bony union. The most commonly used clinical methods to achieve bone reconstruction are the Induced Membrane Technique (Masquelet technique) and Distraction Osteogenesis (Distraction Osteogenesis), but both are time-consuming processes to use these two techniques to achieve bone union. The current use of growth factor (bone morphogenetic protein 2, BMP-2) is a potent protein for bone healing, but it is expensive. Studies have confirmed that magnesium deficiency will induce inflammation and hinder the process of angiogenesis. The addition of magnesium ions will be one of the keys to promoting angiogenesis.

Methods: This study aims to develop biomedical materials that can promote bone repair and be applied to large-volume bone defects. It is mainly composed of tetracalcium phosphate (TTCP) and calcium dihydrogen phosphate monohydrate (MCPM) powder, magnesium powder, and collagen.

Results: The data showed that the sub-micron CPC powder, composed of tetracalcium phosphate/ monocalcium phosphate monohydrate (TTCP/MCPM; 3.5:1 ratio) with a setting time shorter than

15 min and the compressive strength was 4.39±0.96MPa, revealing that the sub-micron CPC powder had an adequate setting time and mechanical strength. The particle size, composition, and microstructure were also investigated as well.

Conclusion: We found the biocompatibility of the sub-micron CPC sponge, which contained magnesium, resulted in better proliferation and osteogenic induction effects without cytotoxicity. A CPC sponge containing magnesium also promoted angiogenesis. In summary, we introduced a novel CPC sponge, which could serve as a promising material used in bone regeneration for massive bone defects.

KEYWORDS: Massive bone defect, Magnesium, Tricalcium Phosphate, Bone graft

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Applying multiple surface treatments to enhance osseointegration of titanium dental implant through regulating bone remodeling

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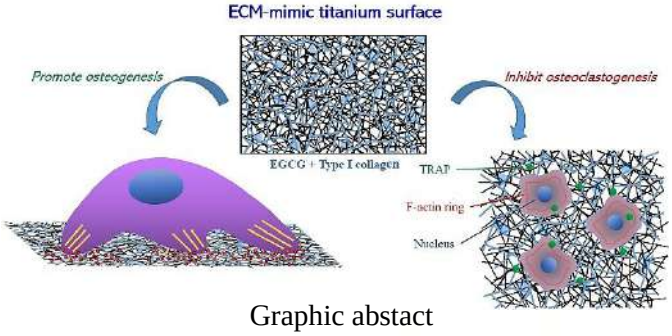
Abstract:

Although titanium is widely used for dental implants, there are still patients with low bone quality who require longer bone healing and osseointegration after implantation. The aim of this study was to modulate bone remodeling [1] by changing the surface of titanium implants, *i.e.*, to shorten the required bone healing time in patients with low bone quality, thus achieving rapid and good osseointegration. *In vitro* experiments confirmed that the titanium surface treated with a mixture of sandblasting/acid etching/alkaline produced a three-dimensional mesh-like pore structure that mimicked the appearance of extracellular matrix (ECM). Type I collagen was then cross-linked with the natural cross-linking agent epigallocatechin-3-gallate (EGCG) and immobilized on the titanium surface. The collagen-immobilized ECM-like titanium surface simultaneously promoted osteogenesis and inhibited osteoclast activity *in vitro*. A surface treatment process consistent with the above has now been successfully applied to commercial screw-type titanium dental implants. Then, *in vivo* studies using a rat model are now underway. It is anticipated that *in vivo* experiments will demonstrate that this multiple surface treatment process for titanium dental implants can promote osseointegration in patients with low bone quality by reducing bone healing time.

KEYWORDS: titanium, surface treatment, type I collagen, epigallocatechin-3-gallate, bone remodeling, osseointegration.



Graphic abstact

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Chitosan wound dressing modified with bacteriophage promotes antibacterial activity against Staphylococcus aureus and bone cell growth

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Osteomyelitis, a disease caused by *Staphylococcus aureus* (*S. aureus*), is considered to be one of the most infectious bone diseases that is difficult to resolve with antibiotic therapy. Bacteriophage (phage) therapy is one of the emerging tools that has the potential to replace antibiotics and fight drug resistant bacteria.

In this study, we isolated a strain of phage specifically targets *S aureus* phage from sewage water. The phages were chemically linked to the surface of chitosan films. The film samples were tested for the antibacterial activity and biocompatibility with osteoblast cells in order to evaluate the feasibility of using phages as an antibacterial biomaterial.

The phage isolated belongs to the family Myoviridae and exhibits good activity in a wide range of temperatures and pHs. Through fluorescent staining, we showed that the phages were successfully immobilized on the surface of the films. The film with phages encapsulated slowly released the phages over time. Finally, the in vitro antibacterial tests show that the films made by this methods had good antibacterial activity, and the antibacterial rate reached 99% within 24 hours . The initial tests also showed that osteoblasts cultured with *S. aureus* with the presence of phages were able to proliferate in three days, and the group without the phages failed to survive.

These results showed that the chitosan films modified with phages had good antibacterial effects against *S. aureus*, which lead to the survival of bone cells in the presence of *S aureus*. The study had demonstrated potential use of the phage-modified films as wound dressings to treat osteomyelitis infected with *S. aureus*.

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of Biomimetic Gel with Osteoconductivity for Fixation of Permanent BoneImplants

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Abstract:

Osteoporosis is an age-related disease characterized by an imbalance between osteoblasts and osteoclasts. When fractures occur, bone screws and plates are commonly used in combination. However, bone screws can lead to loosening. Currently, the clinical solution is to use bone cement in conjunction with bone screws.After clinical evaluation by physicians, some patients may have implants that remain permanently in their bodies. However, the application of bone cement in osteoporotic patients can cause stress shielding and ineffective osteointegration with the bone tissue. This study aims to develop a biocompatibility gel and construct a biomimetic structure that can be used in conjunction with bone screws. The application of the gel is expected to enhance osteointegration and osteoconduction of the implant with the surrounding bone tissue. This promotes the migration of cells from the surrounding bone tissue into the gel, leading to mineralization and ultimately achieving permanent fixation of the bone screws at the implant site. In the study, a quantitative mixture of gelatin and sodium polyacrylate was used, and the experimental groups were divided into those with and without the addition of β-tricalcium phosphate. The gel was then validated for its biocompatibility, physical properties, chemical characteristics, and functionality. Based on the current experimental results, the gel experimental groups were Gel and Gel-6B, showed no cytotoxicity. XRD and FTIR analysis revealed the components in both experimental groups. No high temperature was generated during polymerization. pH remained neutral within 28 days. When the gel groups were co-cultured with single osteoblasts or primary bone tissue and observed under confocal microscopy, it was found that single osteoblasts experiment showed proliferation and mineralization within the gel, and the gel facilitated the migration and growth of osteoblasts from primary bone tissue into the gels. CT imaging confirmed the filling capacity of the gel, and its ability to assist in bone defect healing has been verified in animal experiments.

KEYWORDS: Osteointegration, osteoconductivity, osteoporosis, biomimetic, implant

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Evaluation the effect of biomaterials on BMP-2 binding activity to BMPR-1A receptor

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Abstract:

It is currently known that BMP type I receptors (BMPR-1A/BMPR-1B) can bind to bone morphogenetic protein 2 (BMP-2) which promoting mesenchymal stem cells (MSCs) differentiation into osteoblasts and cartilage cells, and muscle cells differentiate into bone cells. Studies shown that BMP-2 activity must be based on bio-materials, however, whether the characteristics of bio-materials themselves affect the function of BMP-2, or even whether the combination of BMPR1A and BMPR1B affect the role of bone formation has not yet been clear.

Therefore, this study used Surface Plasmon Resonance (SPR) for analyzing the differences in affinity between BMP-2 and BMP receptors when BMP-2 was immobilized on different bio-materials. Results showed that most of the bio-materials had better affinity to BMPR1A, while gelatin had the best effect on BMPR1B. And then, C2C12 was used to evaluate the effect of bio-materials on bone differentiation of cells. The results indicated that most of the bio-materials showed obvious bone cell differentiation marker, enzyme-alkaline phosphatase (Alkaline phosphatase, ALP) activity, however, the expression on gelatin was poor. Our results hypothesize that most bio-materials allow cells to bind BMPR1A via BMP-2 promoting bone differentiation. In the future, the three-dimensional scaffolds will be used to verify the cell mineralization caused by different bio-materials combined with BMP-2 and BMPR1, so as to evaluate the most suitable bio-materials for bone repair.

KEYWORDS: Bio-materials; bone morphogenetic protein 2 (BMP-2); BMPR-1A receptor; bone differentiation

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Synergy of bone microenvironment stiffness and BMP-2 enhances breast tumor calcification by using a 3D model

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Abstract:

Breast microcalcifications are associated with lower survival rates in patients with breast cancer [1]. The size and quantity of these microcalcifications increase with the malignant tumor, indicating a correlation between the degree of calcification and cancer progression [2]. Breast cancer cells most commonly metastasize to the bone, where the microenvironment following metastasis exhibits higher concentrations of bone morphogenetic protein-2 (BMP-2), a member of the transforming growth factor beta (TGF-β) superfamily. BMP-2 induces calcification in breast cancer cells has been confirmed [3]. Due to the synergistic effect of growth factors and mechanical signals on cellular functions and the activation of various signaling pathways, BMP-2 has shown a synergistic effect with a stiff matrix on myoblasts, leading to osteogenic differentiation [4]. Therefore, this study aims to explore the synergistic effect of BMP-2 and matrix stiffness in the bone microenvironment on accelerating breast cancer cell calcification. As microcalcification is primarily composed of hydroxyapatite (HAP), which accounts for 70% of the bone's composition, increased calcification enhances the stiffness of the microenvironment, resulting in feedback that further accelerates calcification and promotes breast cancer progression. Experimental approaches involve using bone-like scaffolds of different stiffness cultured in BMP-2-containing medium, combined with 3D dynamic cell culture systems that enables long-term culture and simulates a realistic physiological environment by promoting cell-cell and cell-extracellular matrix (ECM) interactions, this setup aims to validate the research objective by observing the degree of calcification.

KEYWORDS: bone morphogenetic protein-2 (BMP-2), breast cancer, calcification, stiffness, 3D cell culture

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Fucoidan-Decorated Epigallocatechin Gallate/Protamine Nanoparticles for Plaques-Targeted Delivery and Controlled Release of cargoes in Anti-Atherosclerosis Therapy

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Abstract:

Lacking an efficient delivery system is an obstacle to drug development in atherosclerosis therapy. Inflammatory endothelial cells, foamy macrophages, and a high amount of protease engage by atherosclerosis plaque formation and growth. Among the participated cells, both highly expressed markers, P-selectins and scavenger receptor A (SR-A), are the antigen of fucoidan (Fu), which makes Fu could serve as a suitable material for targeting atherosclerosis plaques. (-)-Epigallocatechin-3-gallate (EGCG), a natural phytochemical, present a brilliant anti-inflammatory and anti-atherogenic effect. However, the human trials of EGCG were defeated with low stability and non-targeting effects. Thus, we aim to develop a delivery system to overcome it. In this study, EGCG/protamine nanoparticle has been assembled, then further decorated with fucoidan to construct plaque-targeted and anti-atherogenic nanocarriers ((EGCG/Prot)@Fu NPs). The particles were well-fabricated and identified by dynamic light scattering, transmission electron microscope, circular dichroism spectroscopy, and Fourier-transform infrared spectroscopy. The stability and release profile of particles is correlated with pH value, and the release of EGCG and Fu can be further triggered by trypsin. In vitro studies demonstrated that this fucoidan-decorated particle has a high binding affinity with inflammatory endothelial cells and foamy macrophages through P-selectins and SR-A. In addition, the particles reduce the monocyte's (THP-1) adhesion by 17.3-fold by decreasing the ICAM-1, VCAM-1, and E-selectin expression on inflammatory endothelial cells (HUVECs). Moreover, the ROS level, NO release, and ox-LDL uptake of foamy macrophages are also significantly reduced by the (EGCG/Prot)@Fu NPs. In vivo studies were established by introducing a 12-week high-fat diet to ApoE-/- mice. The results demonstrated that particles prefer to accumulate at the atherosclerotic aorta by 5.9-fold. Besides, the particles significantly decreased lipid accumulation, plaque size, and the expression of inflammatory cytokines (TNF-α and IL-6) compared with Fu/EGCG

mixture after 8-week treatment with 2.4 mg NPs/2-3 days (i.v. injection). In this study, we establish a novel nanoparticle assembled by EGCG and protamine, which can protect the EGCG and response release by atherosclerotic ectopic trypsin. On the other hand, we provide a strategy of using fucoidan to target delivering drugs to atherosclerosis plaques. This novel delivery system with three natural bioactive components could become a potential anti-atherogenic agent.

KEYWORDS: Fucoidan, Atherosclerosis, Epigallocatechin gallate, Targeting, drug delivery

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

The Effect of heart and metabolites in PTZ-induced heart abnormalities zebrafish

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Abstract:

As we know, seizure may cause sudden harsh of electrical activity in brain, temporary changes in behavior and movement. Besides, it may also have some effects on cardiovascular injury. But we still do not understand the underlying mechanism clearly.

4-6 month-old adult AB type zebrafishes were used as animal model. After exposed to 10mM PTZ for 20 min, we used Western blot and RT-PCR to explore the abnormal phenomenon in their hearts. We also used general oxidative stress indicator, CM-H2DCFDA (100μl for 15 min), to detect ROS level in heart tissue. Then we used UPLC-MS to figure out the metabolites differences in hearts between normal and PTZ-treated zebrafish.

Our results showed that treating PTZ may make ROS level increase in zebrafish heart. We also found metabolites which may represent as biomarkers to investigate its underlying mechanism. Furthermore, we established PTZ-induced seizure zebrafish model that might serve as the good platform for therapeutic natural compound candidate research in epilepsy.

KEYWORDS: metabolites, pentylenetetrazol (PTZ), heart, zebrafish

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2023 ISOMRM & BCRS
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Constructing Heart-specific Exosome Profile to Enable Research of Cardiovascular Disease

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Abstract:

Exosomes, a specific type of extracellular vesicles (EVs), play a significant role in both disease progression and diagnosis. They achieve this by facilitating the transfer of exosomal cargoes from one cell to another [1]. An illustrative example involves the contribution of miR-320, which is found in exosomes derived from cardiomyocytes, to the increased risk of atherosclerosis in diabetic patients. The presence of miR-320 inhibits the regeneration of myocardial endothelial cells, rendering the blood vessels susceptible to damage caused by elevated glucose levels in the bloodstream [3].

Identifying exosomes for specific diseases and/or tissues is very challenging. For example, exosomes from cardiomyocyte cell culture media and/or plasma have been utilized to study cardiovascular disease [4]. However, the exosomes from one single cell culture cannot represent the heart tissue complexity. On the other hand, exosomes in plasma originate from various tissues, which lead to convoluted analysis [5, 6]. To address the challenges, explant models, such as placental perfusion, have been employed to generate tissue-specific exosomes. Explants offer the advantage of preserving tissue integrity and functionality, making them suitable for investigating transport mechanisms and related studies [7].

We proposed that the explant method, specifically heart perfusion, could be employed to generate heart-specific exosomes with a more comprehensive profile, thus supporting studies related to cardiovascular disease. For our research, we utilized mice as the animal model. After the collection, the heart perfusate is concentrated through osmosis. And then, exosomes were isolated using ExoQuick-TC. BCA assay showed that there was 50-150 μg protein per milliliter sample; the particle size was around 120 nm (CI: 92 - 146 nm), measured by DLS. In addition, western blot demonstrated CD9 detection in the particles collected from the perfusate, and RT-PCR was employed to characterize the miRNA cargoes. The exosomes will be utilized to treat hear-related cells to investigate the functional difference by comparing to other sources, including cell culture media and plasma.

KEYWORDS: Extracellular vesicles, exosomes, cardiovascular disease, organ explant

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2023 ISOMRM & BCRS
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Biomimetic Platelet Motor for Propelling Hirudin Peptide Delivery for Remotely Site-Specific Phototherapeutic Thrombolysis

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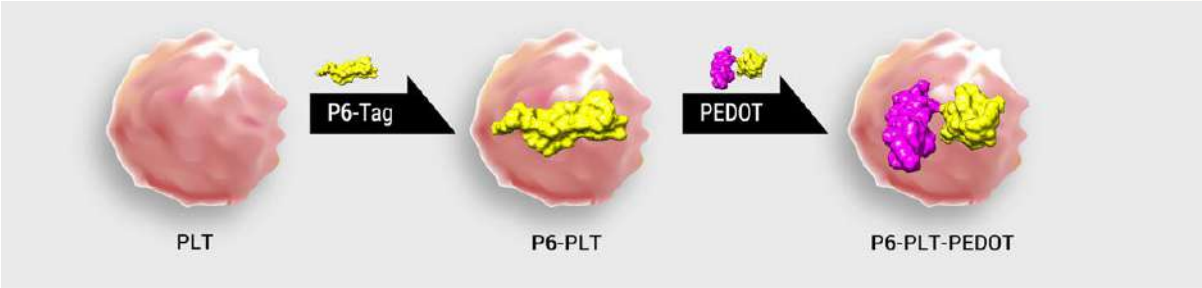
Abstract:

A cotton-ball shaped platelet (PLT)-mimetic self-assembly framework-bioengineered phototherapeutic poly(3,4-ethylenedioxythiophene) PEDOT carrier system capable of target delivering hirudin peptide (P6) toward thrombus lesions, which generates P6@PEDOT@PLT motor, for remote site-specific thrombolysis and powerful anticoagulation, is proposed. Regulated with the special proteins on PLT, the P6@PEDOT@PLT motor accumulates at the thrombus lesion and next ruptures under near-infrared (NIR) irradiation, thus attaining sequential drug release desirable. In the meantime, the movement ability of P6@PEDOT@PLT motor upon NIR irradiation is cable of effectively propelling them to infiltrate deeply into thrombus lesions for enhanced bioavailability. The described biomimetic (P6@PEDOT@PLT motor) proposes a promising possibility in enhancing the effectiveness of antithrombotic treatment in thrombus-related disorders.

KEYWORDS: Phototherapeutic Thrombolysis, Hirudin Peptide, Restoration of Vascular, Platelet Motor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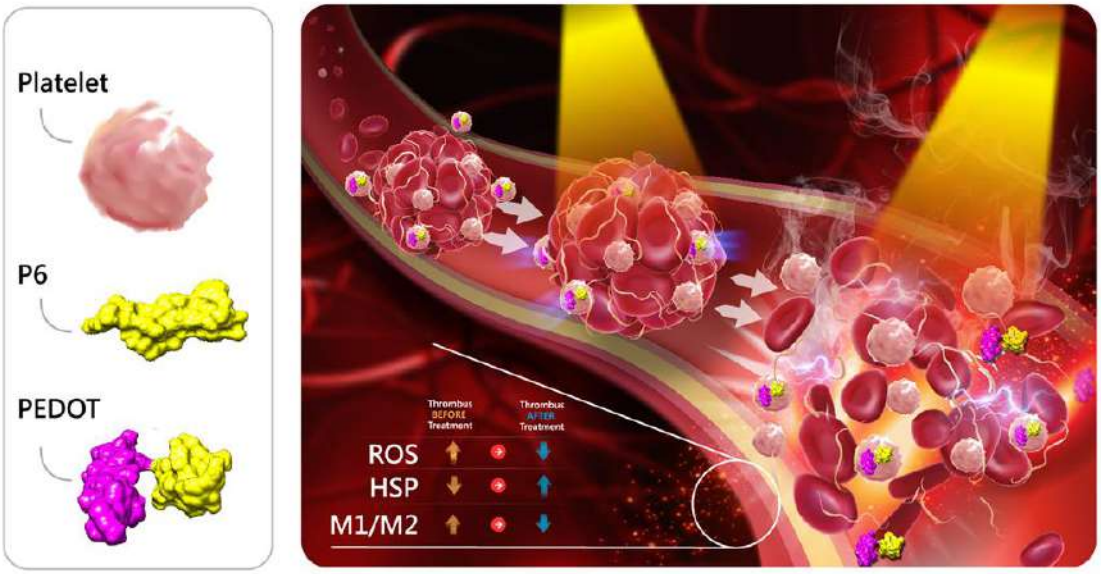
Platelet
P6
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ROS
HSP
M1/M2

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Cold Atmospheric Plasma-Derived Nanoclusters for Lesion-Specific Multimodal Photo/Magnetic Thrombus Therapy

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(Underline the name of the presenting author. The corresponding author(s) should be marked with an asterisk in the author list.)

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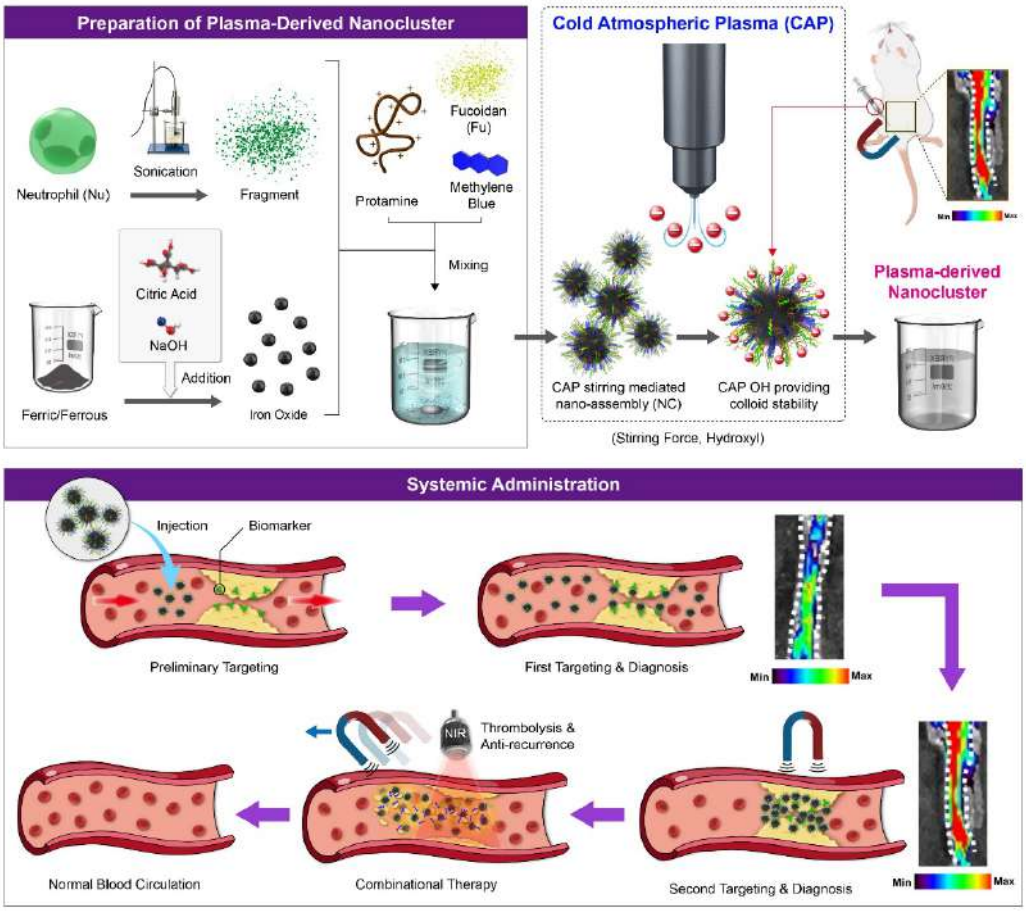
Abstract:

A biocompatible, magnetic, fluorescent, well-characterized, multimodal targeting theranostic platform was engineered for formulating photothermal and photodynamic agents. This multimodal theranostic technique is capable of being remotely visualized and magnetically navigated toward thrombi, remotely irradiated by near-infrared (NIR) phototherapies, and non-invasively activated by actuated magnets for further mechanical therapy. Magnetic guidance is capable of also improving the penetration of nanomedicines to thrombi. In a mouse thrombus model, thrombosis clot residues were reduced by *ca.* 80% and with no risk of adverse effects or of rethrombosis. This strategy not only allows the progression of thrombolysis but also hastens the lysis rate, thus promoting its prospective usage in time-critical thrombolytic management.

Keywords: multimodal theranostic, photo/magnetic thrombolysis, cold atmospheric plasma, biomarker, nanocluster



Graphic abstract (not a mandatory requirement)

By exploiting elevated BM levels of CXCL12 and P-selectin, thrombus-targeted imaging and antithrombotic CAP-derived MB-Nu/Fu-IO nanocarriers (NCs) were designed to specifically target thrombus clots to image thrombi (for preliminary targeting), be attracted by a magnet (for second targeting and deep thrombus penetration), be irradiated by NIR (for PDT/PTT phototherapies), and be actuated by an alternative magnetic field (for mechanical thrombolysis, MT), resulting in the prevention of thrombus formation in injured vasculature.

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2023 ISOMRM & BCRS

Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Exosome/Secretome-derived from pcMSCs promoting endogenous progenitor stem cell and suppressing inflammatory condition in LPS-induced ARDS/ALI model

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Abstract

SARS-CoV- 19 infections spread worldwide unabated and leading to ARDS respiratory disease, which has resulted in world health crises. ARDS (Acute respiratory distress syndrome) due to covid-19 and other etiologies results from lung injury to the epithelial cells barrier resulting in pulmonary edema which causes acute respiratory failure; recovery requires epithelial cell regenerations [1]. During ARDS, AT2 cells (alveolar type 2 epithelial stem cells) damage or proliferate for repair, exit the cell cycle, and enter into the transition state, but due to chronic inflammation not able to differentiate into AT1 (alveolar type 1epithelial cells), and persistence of transition state cause ARDS [2]. However, achieving protection from the virus from the whole respiratory tract, avoiding epithelial regeneration, and suppressing inflammatory cytokine storm remains a major challenge. In our study, we investigated the therapeutic effect of exosomes derived from of pcMSCs (placental mesenchymal stem cells) for the activation of progenitor stem cells torepair the lungs in ARDS.

Acute respiratory distress syndrome (ARDS)/Acute lung injury (ALI) is a serious clinical illness with a high mortality rate. Currently, mechanical ventilation and fluid management are the main symptomatic therapy for ARDS/ALI. Most ARDS/ALI patients face a poor prognosis, due to a lack of effective treatment. Even recently, the SARS-CoV-2-induced ARDS/ALI pandemic spread worldwide unabated [3]. However, achieving protection from lung damage, and progenitor stem cell death and calming the subsequent cytokine storm remains a major challenge. Here, we hypothesized that an inhaled/intratracheal administration of pcMSC-derived exosome or secretome will regenerate the endogenous progenitor cells and ameliorate the inflammatory condition to protect the lung injury and has been a promising prospect for the treatment of ARDS/ALI. We found pcMSC-derived exosomes/secretomes can reduce inflammation, inhibit apoptosis, and promote cell renewal. The inhaled exosomes or secretome significantly reduce ARDS/ALI lung injury effectiveness over the whole course of the respiratory system *in vitro* and *in vivo*. Moreover, we also demonstrated that the inhaled/intratracheal administration pcMSC- derived exosomes efficiently neutralize proinflammatory cytokines, cause an alternative landscape of lung-infiltrated immune cells, and alleviate hyperinflammation of lymph nodes. In summary, an ARDS/ALI mouse model, the inhaled/intratracheal pcMSC-derived exosome or secretome show significant therapeutic efficacy by regulation of the multisystem inflammatory syndrome reduce acute mortality, and AT2 cells activation for lung regeneration suggesting a powerful synergic strategy for the treatment of patients with severe ARDS/ALI via non-invasive administration.

KEYWORDS: ARDS, exosome, secretome, AT2 (alveolar type 2 epithelial stem cells), progenitor stem cell, lung regeneration.

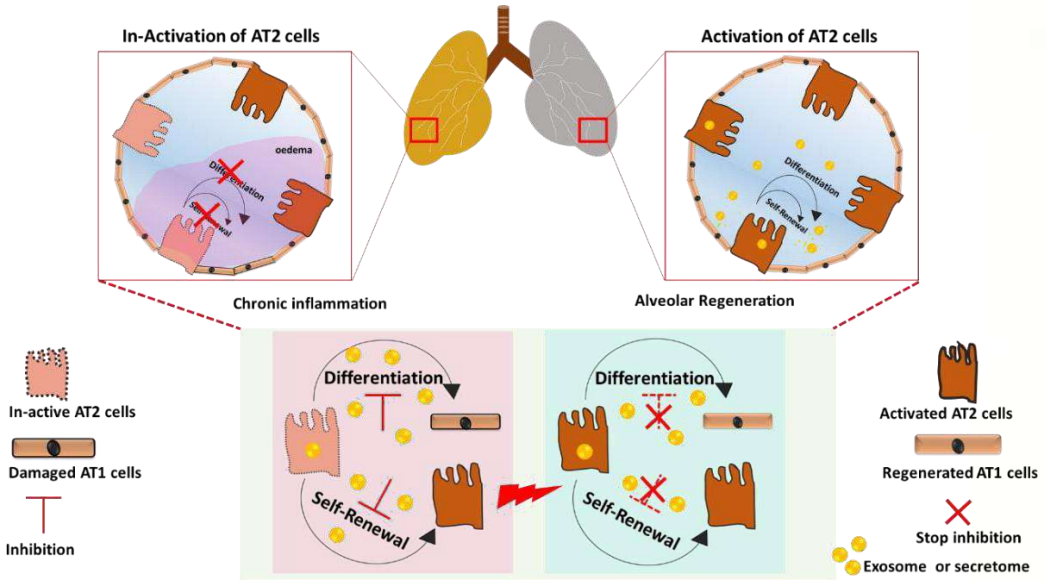

Fig: Graphical abstract

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In Vitro Treatment Effect of 3D cultured MSC-derived Exosomes on IL-1β Treated Chondrocyte: Exosomes Characterization and Anti-inflammation Evaluation

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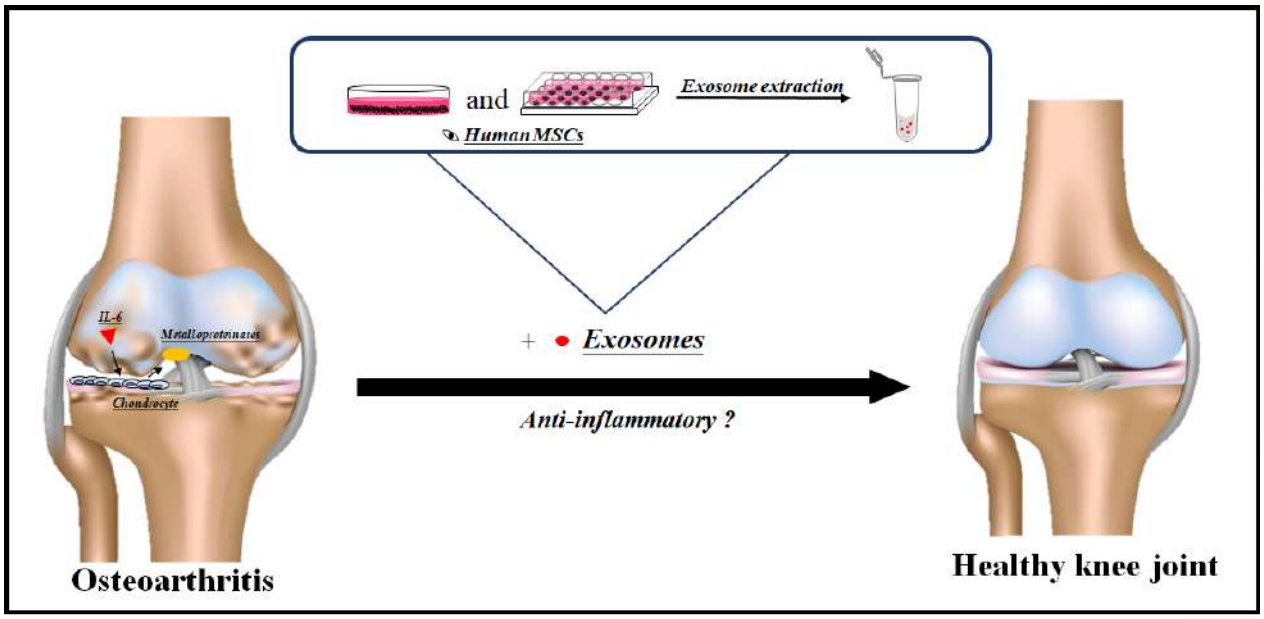
Abstract:

Osteoarthritis (OA) is one of the common joint diseases. The prevalence of this chronic articular cartilage degenerative disease gradually increases with age, and half of the world's elderly population over the age of 65 suffers from OA. The main symptoms of OA include joint bone sclerosis, synovial inflammation and cartilage degeneration. Multiple factors, such as pro-inflammatory cytokines and oxidative stress, are thought to contribute to the destruction and damage of OA cartilage. Current clinical treatments of OA include pain-killer taking, steroids and hyaluronic acid injection. However, these methods can only relieve pain and cannot restore cartilage function. Mesenchymal stem cells (MSCs) injection are another choice for OA therapy because of their multi-potent differentiation and self-renewal ability. But, the processing of MSC has some limitations, such as long proliferation time and liquid nitrogen transportation. Thus, in this study, we would like to examine whether MSC-derived exosome can be used for cartilage repair. We investigated the treatment effect of 2D and 3D cultured MSC-derived exosomes on IL-1β treated chondrocyte. According to Nanoparticle Tracking Analysis (NTA) and BCA protein concentration data, we found the mean size of exosomes in each group ranged from 113 to 132 nm, and protein concentration was between 500 to 700 μg/ml in each group. Following 10 ng/ml IL-1β stimulation, 10^6 and 10^7 exosomes were added to each well for cell viability evaluation. The results indicated that the cell viability was higher in the 10^7 exosomes group compared to the 10^6 exosomes group. Additionally, gene expression analysis using real-time PCR was performed on chondrocytes cultured with 10^7 particles/ml. Results showed that 3D-cultured MSC-derived exosomes could promote chondrocyte survival in the presence of IL-1β inflammation cytokine, inhibit IL-6 mRNA synthesis, and slightly assist the synthesis of cartilage matrix components. Current study showed the particle size, protein content, cell-compatibility and anti-IL-6 mRNA synthesis ability of 3D-cultured MSC-derived exosomes. However, lots of studies such as ELISA and GAG/DNA assay should be done in the future to evaluate the treatment effect on protein level.

keywords：*osteoarthritis, mesenchymal stem cell, exosome, gene expression*

This study was supported by National Science and Technology Council (Project number: MOST 109-2314-B-239-001-MY3).

Graphic Abstract



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Enabling Rapid Extracellular Vesicle Isolation from Cell Culture Media by Osmosis

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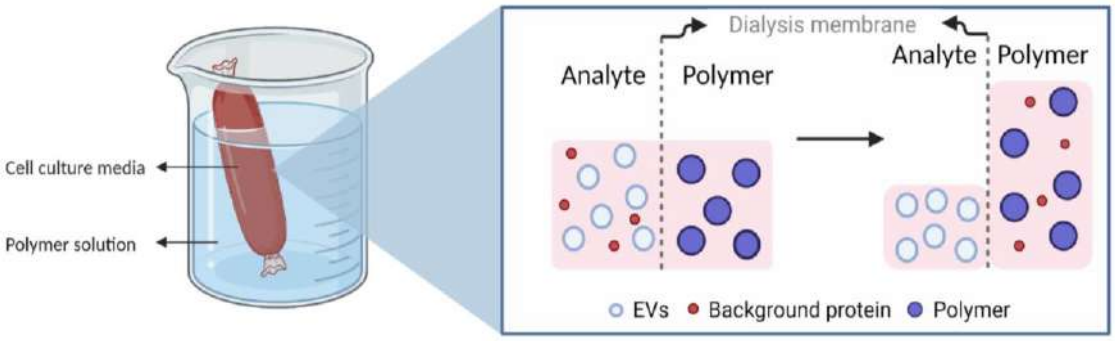
Abstract:

In order to realize the potential of exosomes in biotechnology and clinical fields, there is a need for isolation approaches that can purify exosomes from various solutions, including culture medium and human body fluids (plasma, serum, urine) [1]. For therapeutic and life science research, extracellular vesicles (EVs) are isolated from conditioned cell culture medium due to relatively higher consistency and purity. The commonly used methods to isolate EVs are precipitation, ultracentrifugation, ultrafiltration, and size exclusion chromatography (SEC). However, these have several disadvantages such as time-consuming, protein containmiation, low purity etc. For example, ultracentrifugation, the gold standard for EV isolation, takes 140-600 mins to process and results can vary between operators [2]. Ultracentrifugation may lead to vesicle damage due to the extremely strong centrifuge force and repetitive steps. Therefore, there's a need to develop a novel method to address the challenges.

We have previously demonstrated a simple device to improve biomarker detection limits nearly 100-fold *via* osmosis [3]. Here we hypothesized that osmosis can be utilized as an efficient method for EV isolation from cell culture supernatant, requiring less time and less user steps than ultracentrifugation. The osmosis can also be scaled up for a larger specimen volume. To achieve this, cardiac-derived cells were cultured in EVs-depleted medium for 3 days and the supernatant was harvested for EVs isolation. The osmosis utilized the cellulose ester (CE) membrane with 1000 kDa molecular weight cutoff as a semi-permeable layer to retain EVs while removing excessive soluble proteins and substances.

After a 2-hour osmosis, the sample volume was reduced ~50-fold, and protein concentration increased ~10-fold. The average particle sizes are 150 ± 22, and 131.3 ± 9.2 nm for osmosis and ultracentrifugation respectively. Furthermore, the protein and particle recovery efficiencies for osmosis were 20.45% and 23.72%, while for ultracentrifugation, they were 0.8 % and 0.6 %, respectively. The results clearly indicate that the osmosis resulted in significantly higher EV recovery. Additionally, osmosis is a gentle process, which reduces the possibility of exosome damage compared to ultracentrifugation's strong centrifugal force. Therefore, the process can potentially be utilized for manufacturing EV to facilitate life science research.

KEYWORDS: Exosome, isolation, osmosis



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Platelet-derived Extracellular vesicles (pEVs) based therapy for glaucoma-associated neuroinflammation and efficacy in ophthalmic drug delivery

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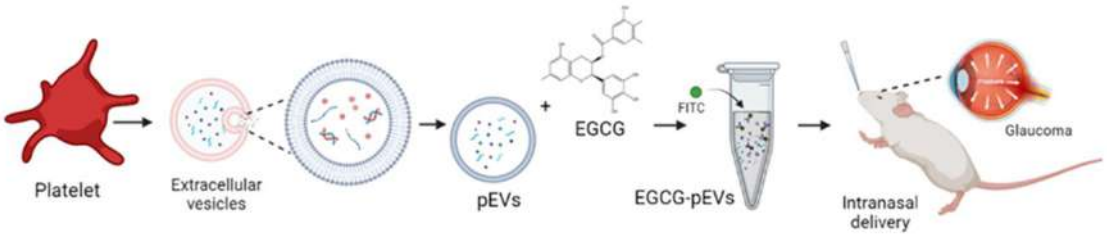
Abstract:

Glaucoma is a condition that causes irreversible blindness in most people everywhere in the globe. Neurodegeneration and related neuroinflammation cause visual impairment in glaucoma. Because of several biological barriers, intraocular distribution of medications is a continuing difficulty. These barriers prevent pharmaceuticals that are systemically administered from reaching the intraocular region. As a direct consequence, a novel therapy method is urgently needed. Platelet-derived extracellular vesicles (pEVs) contain P-selectin ligands, and it is hypothesized that these ligands will target P-selectin overexpression in inflammatory retinal ganglion cells to facilitate greater drug delivery to the eye. EGCG-pEVs are pEVs that have been loaded with epigallocatechin gallate (EGCG), and these EGCG-pEVs have features that make them neuroprotective, anti-inflammatory, and immunomodulatory. This research investigates the possibility of developing a novel treatment strategy using the intranasal delivery (IND). It is hypothesized that the supplied EGCG-pEVs would overcome the ocular morphological and physiological barriers to the retinal lesion, increasing EGCG bioavailability and therapeutic efficacy against glaucoma. This will be accomplished via intranasal to retinal administration with P-selectin bio-interactions. The material characteristics of pEVs and EGCG-pEVs with diameters ranging from 130 to 200 nm and negative charge were measured using DLS/Zeta potential in this work. The morphology and density distribution of the materials were determined using NTA, SEM, and TEM methods. Furthermore, FTIR and NMR methods are utilized to identify the bindings, formulations, chemical structures, and components in composites. The EGCG-loaded pEVs had a loading capacity more than 50%, and the drug release rate was effective at pH=5 and after 12 hours of testing. *In-vitro* investigations were conducted using retinal ganglion cell lines (RGC), and MTT and live/dead test methodologies were studied with varied doses/concentration of EGCG-pEVs. Furthermore, a glaucoma animal model was utilized *in-vivo*, and mice treated with FITC-EGCG-pEVs through the IND route for over a month demonstrated superior outcomes

compared to those treated via IP injection or eyedrops. It is hypothesized that P-selectin-mediated drug transport to the eye lesions would augment therapeutic EGCG administration, distribution, and accumulation near the inflammatory eye lesions, resulting in an increased inhibitory effect against glaucoma. The newly developed EGCG-pEVs, with their one-of-a-kind mode of drug administration, have significant potential for clinical ophthalmology.

KEYWORDS: Epigallocatechin Gallate (EGCG), Platelet-derived Extracellular vesicles (pEVs), P-selectin, Glaucoma, intranasal to retinal drug delivery, anti-inflammation.

Graphic abstract



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2023 ISOMRM & BCRS
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Mechanism of MIL-100(Fe) Induced Macrophage Activation & Its Application in Fibrosis Inhibition

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Abstract:

Immunotherapy is a relatively new way among many cancer treatment methods, mainly using the immune system to treat chronic infectious diseases and cancer treatment. Among them, the tumor-associated macrophages (TAM) polarization mechanism is one of the immunotherapy strategies. It is expected to polarize TAM from the M2 phenotype to the M1 phenotype to attack cancer cells. As a keloid scar with a mechanism similar to cancer cells, the excessive proliferation of fibroblasts will produce excess collagen, causing the scar tissue to expand beyond the original injured area. However, it is currently difficult to eradicate keloid scars in many treatments, so our strategy is to use an iron-based porous metal-organic framework (MIL-100(Fe)) to treat keloid scars. The preliminary research results pointed out that MIL-100(Fe) has the function of polarizing macrophages into the M1 phenotype. In wound repair, M2 phenotype macrophages are a part of the repair mechanism, so we want to use MIL -100(Fe) injected into keloid scars. It is expected to reduce the expression of M2 phenotype macrophages and, at the same time, achieve the range of reducing keloid scars. The experimental results indicate that MIL-100(Fe) can reduce the expression of collagen I and smooth muscle actin in keloid scars, which means that MIL-100(Fe) can reduce the hyperplasia of keloid scars. However, the mechanism of MIL-100(Fe) in the treatment of keloids has not yet been clarified, but preliminary results have shown that MIL-100(Fe) has the potential to treat keloid scars.

KEYWORDS: keloid treatment, immunotherapy, metal-organic framework, MIL -100(Fe)

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Fluorescent carbon nanodots via hydrothermal process from animal-derived materials for biolabeling and their antibacterial activity

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Abstract:

Carbon nanodots (CNDs) are carbon nanomaterials with a particle size less than 10 nanometers, with good biocompatibility, low cytotoxicity, a variety of fluorescent properties, high water solubility and simple synthesis [1] [2]. It can be applied to many different fields and research, such as nanocarriers, biological imaging, photocatalysis and solar cells [3]. Biomass extracted from natural resources are very attractive due to their eco-friendliness, usability and cost-effectiveness [4] [5].

In this study, synthetic CNDs from animal-derived materials (AM) were prepared using hydrothermal process for 0 h, 3 h and 6 h treatment. The results showed that the recovery yields of CND-AM-0, CND-AM-3 and CND-AM-6 were 81.97%, 67.27% and 64.92%, respectively, while the quantum yields were 20.68%, 14.05% and 7.63%, respectively. CND-AM-0 has an excitation wavelength of 330 nm, while CND-AM-3 and CND-AM-6 have an excitation wavelength of 320 nm. The emission wavelengths of CND-AM-0, CND-AM-3 and CND-AM-6 are 414 nm, 423 nm and 421 nm, respectively. CND-AM has a particle size of about 2-11 nm, while the zeta potential is negatively charged. In this study, CNDs were prepared by cost-effective methods and simple green synthesis. It is expected that the synthesized nanostructures can be applied to biolabeling, making them novel tracing nanocarriers with antibacterial activity simultaneously.

KEYWORDS: Carbon nanodots (CNDs), animal-derived materials (AM), hydrothermal process, fluorescence, biolabeling, antibacterial activity

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2023 ISOMRM & BCRS
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Aerosol delivery of Nintedanib in nanoformulation for pulmonary fibrosis treatment

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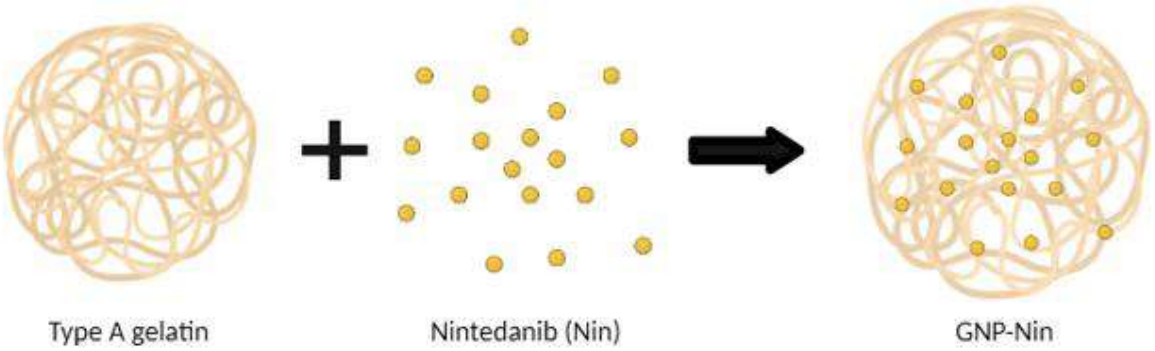
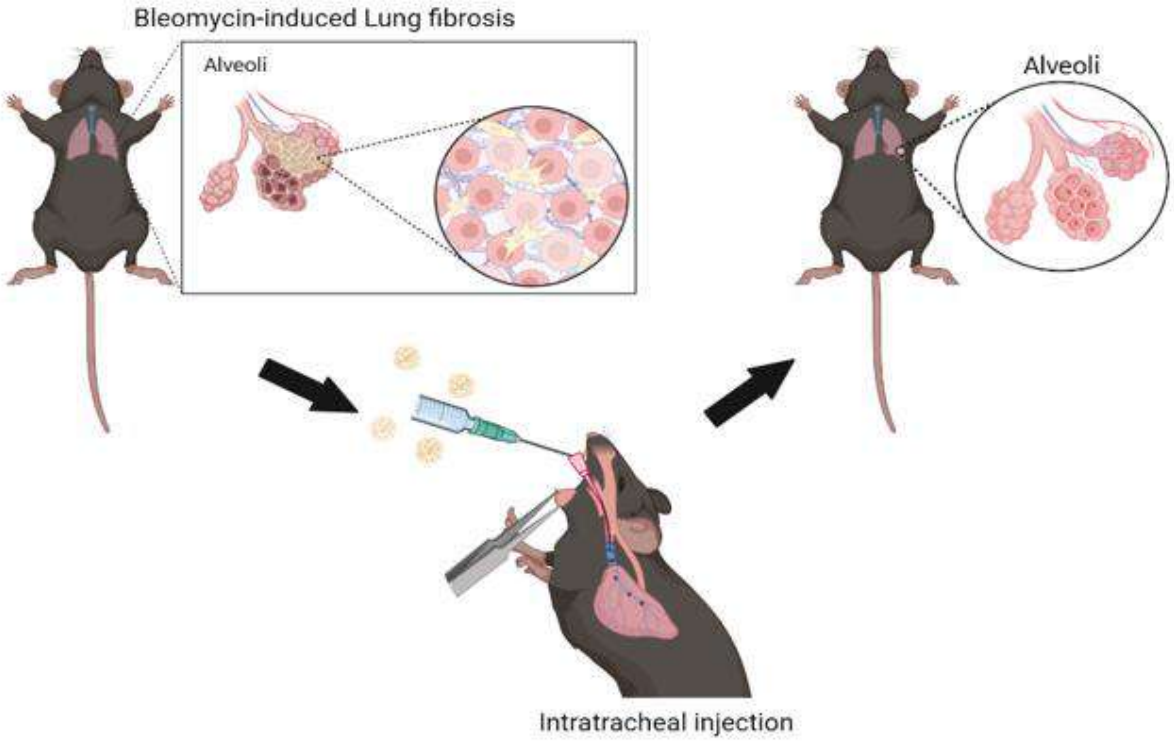
Abstract:

Pulmonary fibrosis is a very serious lung disease that can permanently damage the lung structure of patients, leading to loss of lung function and even death. Idiopathic pulmonary fibrosis is another common form of chronic progressive disease of unknown etiology. The disease process is the proliferation and activation of fibroblasts in the alveolar periphery and excessive production of extracellular matrix. Nintedanib (Nin) is an FDA-approved anti-fibrosis drug that inhibits RTK families such as FGFR, PDGFR and FGFR. Oral treatment can delay the progression of fibrosis, but long-term use of high-dose drugs can cause serious side effects. Gelatin nanoparticles (GNP) are a widely used drug delivery system with high stability and biocompatibility. Nanocarriers can optimize the pharmacokinetics, biodistribution, and slow-release characteristics of drugs. The goal of our research is to optimize the properties of Nintedanib through gelatin nanoparticles and treat the lungs through respiratory delivery. Materials test the particle size and zeta potential with dynamic light scattering (DLS), the morphology with transmission electron microscopy (TEM), and high performance liquid chromatography (HPLC) test drug encapsulation and drug release rate. We choose fibroblast to do in vitro tests to check the safety and inhibit the ability of drug-loaded nanoparticles (GNP-Nin). Male mice (C57BL/6, 6-8 weeks old) were used for in vivo testing, Bleomycin (BLM) induces lung fibrosis to build a disease model, we first lung function before and after inducement, then test nanoparticles deposition in vivo after treatment, the lung tissue was collected and stained to observe the tissue before and after treatment of pulmonary fibrosis after the sacrifice.

KEYWORDS: Idiopathic pulmonary fibrosis, Nintedanib, Receptor tyrosine kinesis, Gelatin nanoparticles


Type A gelatin
Nintedanib (Nin)
GNP-Nin


Bleomycin-induced Lung fibrosis
Alveoli
Alveoli
Intratracheal injection

2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

MXene-Modified Injectable Microbeads for Gene Therapy in Neuron Regeneration for Traumatic Brain Injury Treatment

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Abstract:

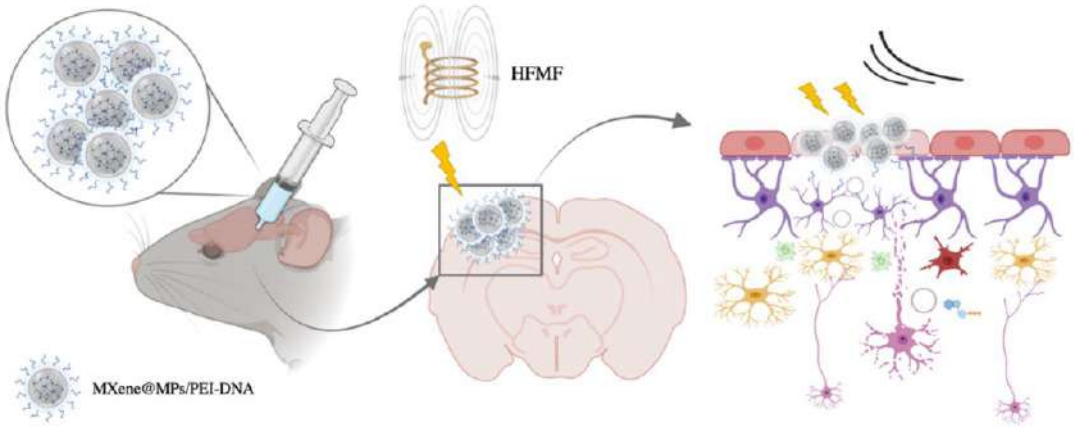
Traumatic brain injury (TBI) is a significant global public health concern, affecting millions of individuals annually and leading to substantial injury-related fatalities and long-term disabilities, with profound implications for physical, cognitive, and behavioral functions [1]. Injectable hydrogel-based microbeads have emerged as promising cell healing scaffolds, effectively filling the traumatic site and mitigating brain atrophy and neuron degeneration [2]. However, traditional hydrogel implants may impede cell infiltration, migration, and trigger immune responses.

In this study, we introduce a novel formulation of GelMA microbeads (MBs) incorporated with MXene (MX), a versatile two-dimensional nanomaterial, and polyethylenimine (PEI), to enhance cell infiltration, neuron regeneration, and gene transfection for neuron growth [3]. The PEI-MX@GelMA microbeads (PEI-MX@MBs) demonstrate exceptional gene delivery capability, hydrophilicity, and biocompatibility. Moreover, the porous structure of the PEI-MX@MBs enables superior cell infiltration and nanomaterial loading potential, which further enhances the recovery of traumatic area. Subsequent application of a high-frequency magnetic field (HFMF) promotes the efficient endosomal escape of DNA and generates electricity through the magnetoelectric effect by the MX coating. This magnetoelectric effect stimulates neuron regeneration, further enhanced by the incorporation of microRNA sponge plasmid [4].

These multifunctional microbeads offer a promising platform for wound filling and gene therapy in TBI patients, effectively addressing critical requirements in the field.

KEYWORDS: Traumatic brain injury, Microbeads, MXene, gene therapy, high-frequency magnetic field

Graphic abstract:



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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Exploiting Erythrocyte-Mediated Delivery of PB-DLNP@RBC for Enhanced Drug Delivery and Gene Therapy

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Abstract:

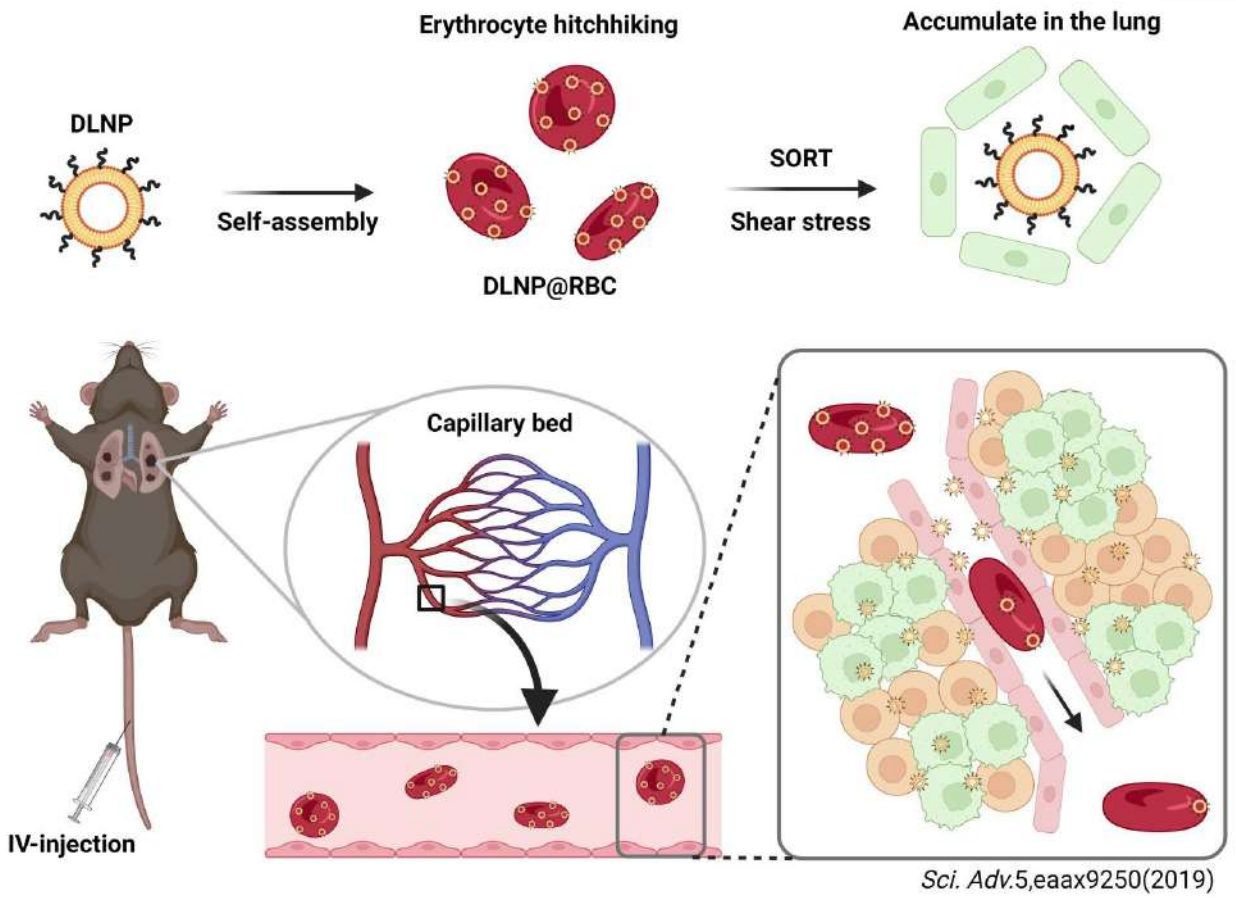
Lung cancer, ranked second in terms of incidence and first in terms of mortality by the American Cancer Society's statistics, represents a formidable malignancy with a dismal prognosis [1]. Unfortunately, current treatment modalities lack efficacy specifically targeting lung cancer, resulting in suboptimal therapeutic outcomes and an unmet medical need.

In our study, a lung-specific delivery system was developed by loading cationic liposomes (DLNP) carrying PDL-1 siRNA and prussian blue nanocubes (PB) on erythrocyte (PB-DLNP@RBC), aiming to enhance gene therapy targeting lung cancer. Liposomes have gained widespread usage in clinical applications due to their excellent biocompatibility and low toxicity . However, conventional liposomes suffer from a short circulation half-life, which prompted us to incorporate mPEG into DLNP to address this issue. The erythrocytes as carriers has successfully prolonged the circulation time of DLNP, enabling evasion of immune clearance and leveraging the shear stress within the pulmonary microvasculature to enhance targeting effects [2]. Additionally, DLNP@RBC demonstrates the ability to reach and accumulate within the lung's capillary network following intravenous administration, leading to increased DLNP accumulation [3]. The following surface modification of liposomes with selective organ targeting (SORT) molecules enhances their lung-targeting capabilities [4]. Moreover, in our system, PB is encapsulated within the cationic liposomes (PB-DLNP). This configuration allows for the electrostatic adsorption of nucleic acid drugs and facilitates endosomal escape through the proton sponge effect [5]. The Fenton-like reaction of PB induces cell membrane destabilization, thereby enhancing siRNA transfection efficiency.

This multifunctional drug and gene delivery platform demonstrates enhanced lung-specific delivery in biodistribution, offering a novel approach for lung cancer treatment.

KEYWORDS: Lung cancer, Erythrocytes based delivery, Cationic liposomes, mPEG, Drug/Gene delivery

Graphic abstract:



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TiO_2 Based Photodynamic and Photothermal Therapies for Glioblastoma Treatment

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Abstract:

Glioblastoma, an aggressive tumor of the central nervous system, is typically managed with surgical resection followed by radiation therapy. However, complete tumor removal is challenging, and recurrence is common[1].

In this study, we synthesized titanium dioxide nanosheets (TNSs) and modified them with TAPB-DMTP-COF (covalent organic framework) and ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) to function as a photothermal therapy agent. Previous reports have demonstrated that TNSs possess broad absorption in the near-infrared (NIR-II) wavelength range. When exposed to 1064 nm light, TNS, acting as a photosensitizer, can generate heat and reactive oxygen species (ROS) to eradicate tumor cells.

Glutathione (GSH), an endogenous antioxidant, plays a crucial role in maintaining the cellular redox balance and scavenging free radicals to protect cells from damage[2]. In our study, $ABTS^{\bullet+}$ is capable of scavenging GSH, thereby augmenting the efficacy of photodynamic therapy and achieving a synergistic effect when combined with photothermal therapy.

KEYWORDS: Glioblastoma, Convection-enhanced delivery, Titanium dioxide nanosheets, COF, ABTS, Photothermal therapy, Photodynamic therapy

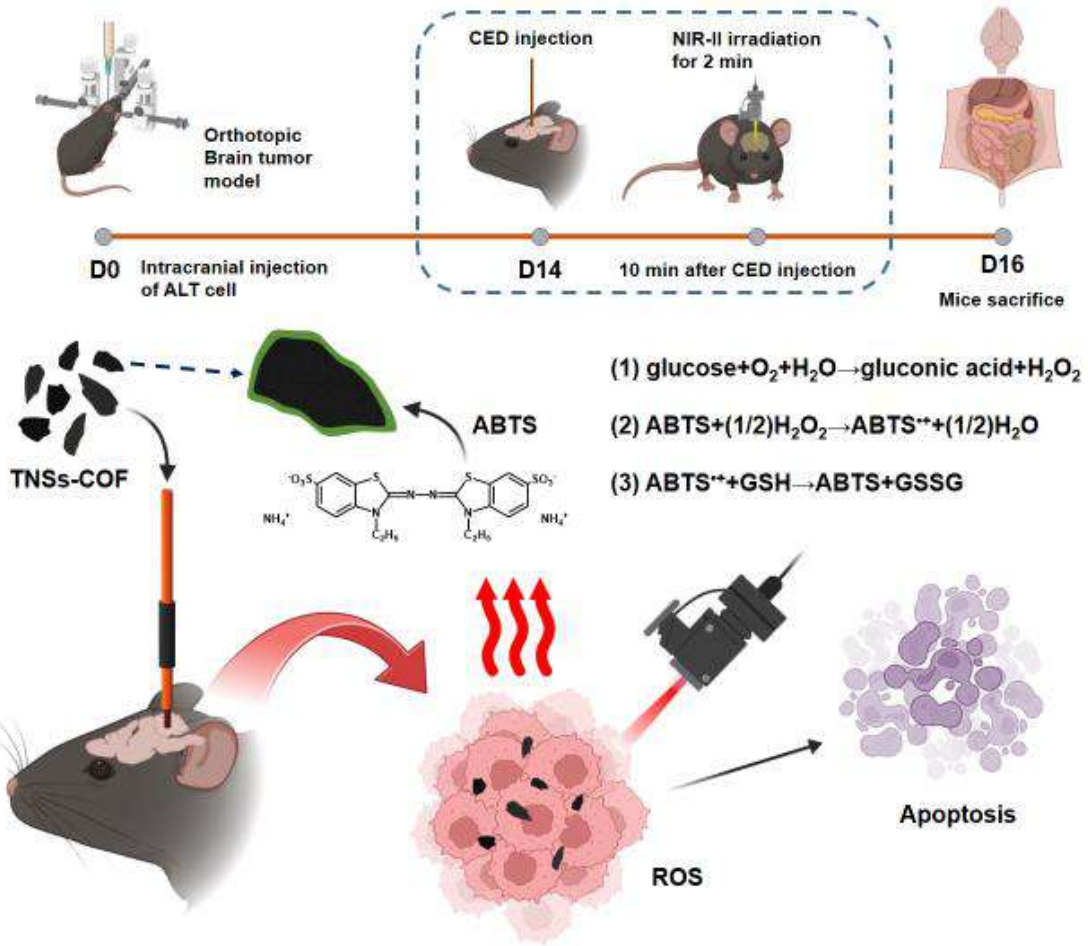

Figure 1: Schematic representation of TiO_2 nanosheets (TNSs) modified with COF for NIR-II photodynamic and photothermal therapies in glioblastoma treatment.

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Development of magnetic triggered membrane for drug releasing application

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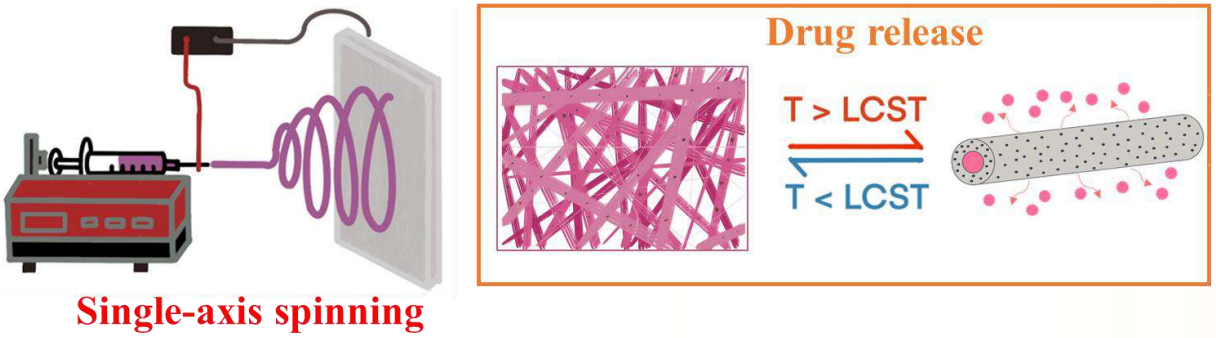
Abstract:

This study aims to develop a thin fiber with magnetic and temperature-responsive nanomaterials as a drug carrier for controlled drug release applications. The materials used consist of temperature-sensitive polymers. Which is poly(N-isopropyl acrylamide) (PNIPAAm), combined with polycaprolactone (PCL) and magnetic nanoparticles (MNPs). The thin fiber was produced using the electrospinning technique, resulting in highly porous structure with a large surface area. During the study, the ratio of magnetic nanoparticles was adjusted, and the patterns and structures of the fiber were observed using SEM and TEM. Rhodamine B was employed as the reagent to assess drug release. The effects of different concentrations of magnetic particles on the shrinkage and drug release of the spun-bond fiber were examined. The fiber underwent swelling triggered by heating the water temperature to study the drug release behavior. The experimental results revealed that higher concentrations of magnetic particles correlated with the increased heating temperature of the fiber, resulting in higher drug release rates. The development of these drug carriers in this study holds the potential to be utilized in numerous clinical applications due to their ability to carry various drugs and enable on-demand drug release.

KEYWORDS: Electrospinning, Magnetic nanoparticles, Temperature response polymer, Drug releasing



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2023 ISOMRM & BCRS
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Single shot Nanosell vaccine eradicates MHC^{low} HPV associated tumor

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Abstract:

Human papillomavirus (HPV) infections pose a significant global health burden due to their association with various cancers, including cervical, anal, and oropharyngeal cancers. Therapeutic interventions for established infections and associated malignancies remain limited. MHC-I downregulation is a strategy employed by HPV to evade immune recognition and elimination. By suppressing MHC-I expression, infected cells become less susceptible to cytotoxic T lymphocyte (CTL) surveillance. In recent years, several therapeutic vaccines being developed in clinical trials, including protein, peptide, nucleic acid, and cell-based vaccines but shown limited anti-cancer effects[1]. Previous clinical studies also found MHC-I downregulation after therapeutic vaccine treatment[2]. Therefore, reversing MHC-I downregulation is crucial for enabling effective immune responses against HPV-infected cells.

Combining our PLGA hollow nanoparticle and peptide liberation design, we encapsulate HPV antigen and adjuvant in a programmable nanoshell vaccine. We demonstrate single dose HPV/NS vaccine can induce high magnitude antigen-specific CD8 T cells and eliminate the large HPV-associate tumor even in the MHC-I downregulation clone but not in other vaccine platforms. In human-relevant models, we also show induce HLA-A allele-specific T-cell response and promising anti-tumor effect by NS vaccination. In conclusion, our HPV therapeutic nanoshell vaccine targeting MHC-I downregulation in cancer cells represents a potential breakthrough in cancer immunotherapy. We also demonstrate the potential to translate to clinical and found the epitopes for humans in the human-relevant model. By circumventing the immune evasion tactics employed by HPV-infected cells, this innovative vaccine holds promise for enhancing anti-tumor immune responses and improving clinical outcomes in patients with HPV-associated cancers.

KEYWORDS: Human papillomavirus (HPV), PLGA hollow nanoparticles, Therapeutic vaccine

Reference

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2023 ISOMRM & BCRS
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Evaluation of Eye Drops Containing Carbon Quantum Dots in Mice with Dry Eye Syndrome

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Abstract:

Dry eye syndrome is a common eye disease (DES), variant therapeutic agents for effectively DES treatment is demanded. Carbon quantum dots (CQD) can not only prevent the fibrillation of type I collagen fibers but also be used to treat floaters by destroying collagen fibers in eyes, it's shown the potential of CQDs applied in ophthalmology. CQD also revealed anti-bacterial and anti-inflammatory effect; therefore, it shows the potential for using CQD for relief the ocular inflammation of DES.

The CQD was prepared from Prof Sabine's lab, and it's adjusted to variant concentration as eye drops for used in animal test. A benzalkonium chloride (BAC) induced DES mouse model was created, CQD contained eye drops was drop on mice eye once daily. There were 4 tested groups performed :(1) Normal group (without any induction and treatment) (2) PBS group, (3) CQD-1 group (CQD: 25 μg/mL), and(4) CQD-2 group (CQD : 250 μg/mL). The treatment was lasted for 21 days, and the clinical symptom (tear volume, fluorescent stain of cornea, intraocular pressure) of DES were analyzed during the stationary period. And histological examination of whole mice eyeball was also proceeded.

In conclusion, the intraocular pressure in all eye drop groups had no significant change. The CQD-2 treated eyes showed the best therapeutic effect of all groups with marked increase in tear production, less damaged cornea. The thickness of the cornea is observed to be very similar to the normal group. Overall, the use of CQD contained eyedrops with a concentration of 250 μg/mL shows the potential as a therapeutic agent for treating DES.

KEYWORDS: Carbon Quantum Dots (CQD), Nanoparticle, Dry Eye Syndrome (DES)

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Contextual STING activation by 2'3'cGAMP-daunorubicin nanoparticle amplifies adaptive immunity in systemic cancer chemoimmunotherapy

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Abstract:

Cancer immunotherapy has seen significant advancements in exploring the cGAS-STING pathway, a vital immune defense mechanism against malignancies. STING, activated by the cyclic GMP-AMP (cGAMP) binding, triggers a cascade of immune responses pivotal for antiviral defense and the stimulation of anticancer immunity. However, the delivery of natural STING agonists, like cGAMP, has been challenging due to their poor efficiency and susceptibility to serum degradation. Synthetic STING agonists have been designed to overcome these hurdles but raise concerns regarding off-target effects and the long-term implications of continuous STING activation. Recent clinical trials have questioned the effectiveness of STING agonist monotherapy as it has been linked with tumor resistance and immune evasion, highlighting the need for a more nuanced approach. These findings have illuminated the importance of contextual STING activation, a strategy that considers the specific cellular and immunological environment, thereby necessitating sophisticated treatment designs. Building upon this necessity, we hypothesized a novel approach using our proprietary nanoshell platform. This platform delivers a combination of Daunorubicin, a broad-spectrum anticancer drug, and a STING agonist, synchronizing cytotoxic killing and immune stimulation. This temporal and spatial synchronization facilitates what we term 'contextual STING activation,' modulating a multitude of favorable anticancer attributes. It effectively manipulates STING agonist effects on cancer cells, modulates immunogenicity of cell debris, and remodels the tumor microenvironment, leading to enhanced tumor suppression and heightened anticancer immunity. The essence of our approach lies in leveraging the context-dependent role of Type-I IFNs. Our platform facilitates the sequential delivery of cytotoxic treatment, inducing CDN-loaded cancer cell debris. This debris concurrently delivers tumor antigens and CDNs to antigen-presenting cells (APCs), enabling simultaneous antigen presentation followed by Type-I IFN induction for optimal antigen-specific T-cell activation. Our proprietary nanoshell platform exemplifies the importance of contextual STING activation,

accentuating the difference between STING monotherapy and combination therapy. Our approach marks a significant milestone in STING-mediated anticancer therapies, opening avenues for innovative nanomedicines and enriching the field of combinatorial immunotherapy.

KEYWORDS: Contextual STING Activation, Combinatorial Systemic Delivery, PLGA Hollow Nanoshell, TME Re-Programming, STING Agonist.

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Precision antigenic programming enhances anticancer vaccine efficacies

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Abstract:

Cancer vaccine has been widely investigated in clinical research but exhibit limited therapeutic efficacy. One major hurdle that has been overlooked in current cancer vaccine design is the intracellular barriers in antigen processing. T-cell priming requires the recognition of precise epitope(short peptide between 8 and 12 amino acids in length) presented by Major Histocompatibility Complex(MHC) on the surface of antigen presenting cells(APCs). Antigens taken up by APCs have to overcome a series of intracellular barriers for successful T cell recognition, including cytosolic mobility, proteasomal processing, cytosolic peptidase degradation, TAP transport and MHC binding. On top of this, antigen formulations can greatly influence their presentation along with immunogenicity, however, the complex correlation between antigen formulations, antigen processing and the corresponding T-cell response remains difficult to discover due to antigens' diverse intrinsic traits and variable delivery efficiency.

Herein, we introduce a nanoshell cancer vaccine with epitope liberation peptide tailoring that enhance antigen immunogenicity and anticancer activity. Via asymmetric ionic stabilization, polymeric nanoshells co-encapsulating tumor epitopes and immune adjuvants are prepared, we are able to uniformly deliver equal molar of peptides in different formulations to APCs dwelling in lymph nodes. The peptide modifier constituted with 4 aspartate and 3 glycine is added to the N-terminus of the peptide antigen for hydrophilicity enhancement and liberating epitopes from intracellular barriers. We showed that the epitope liberation tailoring extends antigens' intracellular half-life and propagates sustained antigen presentation in *in vitro* evaluation, exhibiting superior immunogenicity *in vivo*, moreover, the peptide modifier is universally adaptable to a variety of tumor epitopes. We further validated our nanoshell vaccine's antitumor activity in different murine tumor models, compared with clinically-used long peptide vaccine design, our vaccine platform exhibited significantly improved tumor growth control and survival prolongation.

KEYWORDS: Nanoshell

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A Flexible and Fabric-Based Nanocomposite Design for Wound Healing Applications

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Abstract:

Technological advancements in device miniaturization towards wearable electronics have evoked significant interests in recent years. Triboelectric nanogenerators (TENGs), which transfers ambient mechanical energy into electricity has been widely investigated for their power sustainability, material accessibility, fabric compatibility, and diverse applicability to wearable electronics [1]. Specifically, the combination of Electrical stimulation (ES) and TENG technology has been treated as a promising approach in wound healing applications [2, 3].

In this study, we designed a facile process to produce a fabric-based nanocomposite as a multi-functional triboelectric sensors with excellent durability and sustainability. More importantly, we also found that the electrical stimulation from the designed device can be applied to promote wound healing process. We believe that this device can pave the way for not only future sustainable energy, healthcare sensing, and medical monitoring, but also shed light on new opportunities of future wearable therapeutic system.

KEYWORDS: Flexible device, wearable technology, healthcare, wound healing,

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Cytotoxicity, Transport, and Cellular uptake after exposure to polypropylene micro/nanoplastics produced by thermal oxidation reaction

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Abstract:

With the ever-increasing plastic pollution, nanoplastics (NPs), defined as small plastic debris with a diameter below 1 μm, are widespread in the environment and can be ingested through food, water, and air, posing a threat to human health. However, there are a knowledge gap in how exposure to NPs changes cellular function and affects animal and human organism.[1,2] Among plastics, polypropylene (PP) is widely used for plastic packaging as well as medical applications. However, risk assessment for PP nano-plastics is still limited due to a lack of reference materials and validated detection methods.[3] Recently, thermal oxidation processes have been applied in the PP recycling process in the aqueous environment, utilizing diverse reactive oxygen species. The use of oxygen can significantly reduce the temperature of polymer degradation and change the composition of the resulting products with advantages of high performance, simplicity, and nontoxicity.[4] In this study, we proposed the thermal oxidation process under high pressure to fabricate PP into the nanoscale (OPPNPs). Briefly, PP pellets (300 mg) were placed in a 50 mL high pressure vessel together with a given volume (5 mL) of the hydrogen peroxide (H_2O_2) as a solvent. The vessel was sealed and then transferred to the preheated oven at different temperatures (125, 150, and 175°C) for 24 hours. The OPPNPs formed were fractioned by centrifugation at 50000 rpm for 15 minutes. The physical properties, size distribution and morphological properties of OPPNPs were analyzed by FT-IR, DLS and SEM, respectively. For cytotoxicity test of the OPPNPs, intestinal human cell line (Caco-2) was pre-cultured in 24-well plates for two days, followed by exposing the OPPNPs for 24 hours at different concentrations (1-1000 μg mL^{-1}). The cellular endocytosis was also investigated using methyl-β-cyclodextrin, Chlorpromazine HCl, Ethyl-isopropyl amiloride, and Cytochalasin D as inhibitors.

The production of OPPNPs was performed by a thermal oxidation process using hydrogen peroxide (H_2O_2) as a solvent. The effects of reaction temperature (125, 150, and 175ºC) on the PP decomposition were investigated. The result showed no significant degradation of PP pallets after treatment at 125ºC, while the treated PP appeared significantly different in the elevated temperature to 150 and 175ºC. FTIR spectrum shows the appearance of oxygen-containing oxidation products of a complex structure, as evidenced by a wide band in the region of 3350-3500 cm^{-1} and an intense band at 1714 cm^{-1}, which corresponded to the stretching vibration of hydroxyl group and C=O group, respectively. Additional testing using XPS also confirmed an increase in oxygen content within the OPPNPs. The hydrodynamic diameter distribution of OPPNPs decreases with increasing the reaction temperature. The obtained OPPNP at 175 ºC were sphere-like particles with a size distribution of 100-250 nm (Fig 1A). LDH assay results obtained after 24 h of incubation in concentration-dependent of OPPNPs are shown in Fig 1B. Cell viability of Caco-2 cells demonstrated that the OPPNPs has relatively low cytotoxicity (500 µg mL^{-1}) but slightly higher than that of bare PP. This phenomenon might be due to the smaller size of oxidized PP particles. Endocytic pathways showed that transportation of OPPNPs into the cell decreased with inhibition of caveolae-mediated endocytosis (MβCD), clathrin-mediated endocytosis (Chl) and micropinocytosis (EIPA). Thus, OPPNPs model might be a promising nanoplastic PP model for future studies to assess downstream biological responses.

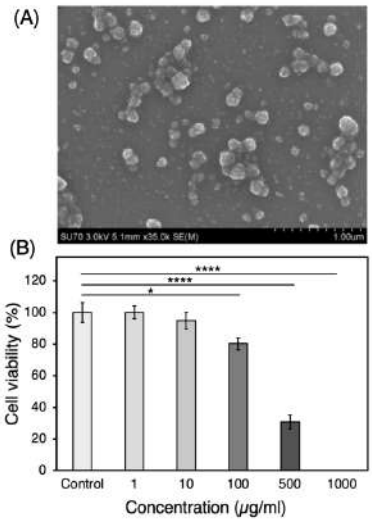

Fig 1. (A) SEM image of OPPNP97 at 175°C for 24h. (B) Cytotoxicity of OPPNPs tested by LDH assay. The graph shows mean ± standard deviation.

KEYWORDS: polypropylene, nanoplastics, thermal oxidation, human cells, cytotoxicity, cell uptake

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Development of Kaempferol-Loaded Gelatin Nanoparticles for the Treatment of Choroidal Neovascularization

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Abstract:

Choroidal neovascularization (CNV) is a major cause of visual loss, which happens when blood vessels from the choroid break through the Bruch membrane, and grow into the subretinal space which cause vision dysfunction. The major treatment in clinics is to inject anti-VEGF agent to the eyes for vessels inhibition. The existing therapies for CNV have their own drawbacks. Kaempferol is a natural flavonol which can be found in a variety of plants. Kaempferol possess not only antiangiogenic effect but also anti-inflammation, and anti-oxidation. With this multifunction, we chose kaempferol as candidate drug for inhibiting vessels formation in CNV.

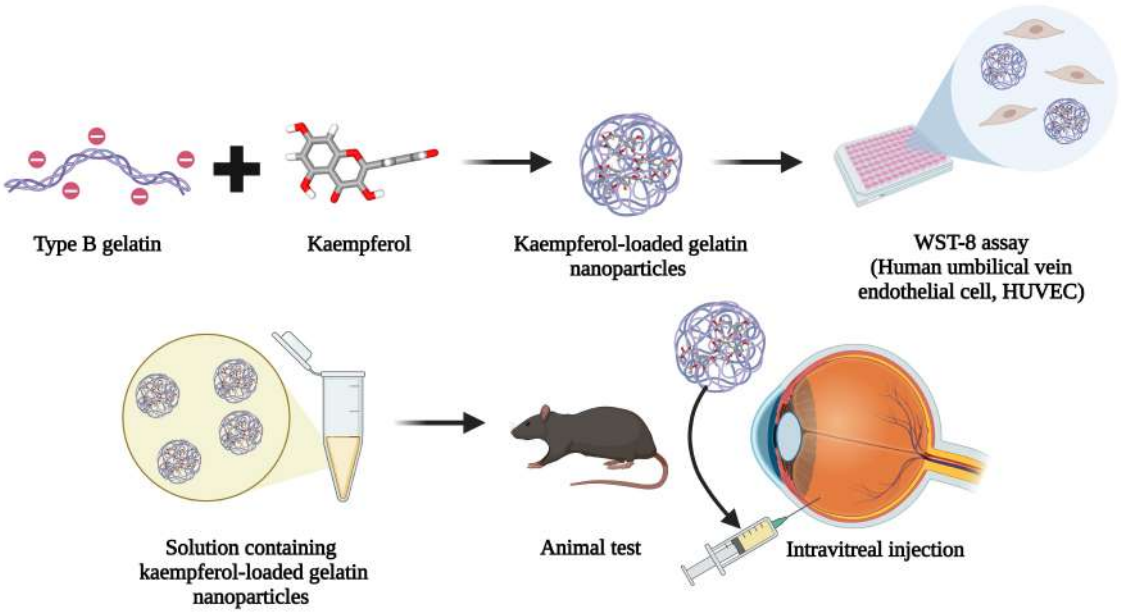
Nanometric scale technology has been widely used in ocular medicine with its advantages, which includes overcoming eye barriers, increasing contact time with its target tissue, and reaching a specific site without disturbing normal cells. This improves the poor bioavailability which traditional administrating routes used to have. We chose gelatin as our nanocarrier due to its good biocompatibility and biodegradability. This study is to seek another option for dealing with CNV by giving kaempferol-loaded gelatin nanoparticles (GNP-KA) through intravitreal injection, and evaluate its therapeutic effect in a mice laser induced CNV model

The size, zeta potential and polydispersity (PDI) of the GNP-KA were measured by DLS (Dynamic Light Scattering). The morphology was observed under TEM (Transmission Electron Microscopy). Drug encapsulation rate was calculated with HPLC (High Performance Liquid Chromatography). In vitro study, Human umbilical vein endothelial cell (HUVEC) was cultured with GNP- KA to test the cell viability by WST-8 assay. In vivo study, Brown Norway Rats with CNV which were induced by

laser are treated via intravitreal injection to evaluate the ability to inhibit the Choroidal neovascularization via images of FFA (Fundus Fluorescein Angiography).

The results showed that kaempferol loaded with pH 10 Type B gelatin nanoparticles had its smallest size and the most stable condition. The size was about 218 nm, zeta potential around -39.32±1.89 mV, and had a drug encapsulation rate of 68 %. Data from TEM showed round and well-distributed nanoparticles. From the in vitro experiment, WST-8 assay showed that GNP-KA had the better ability to suppress the angiogenesis ability of HUVEC after 3 days when compared to the KA group. It evidently proved that the GNP-KA in nano-scale can efficiently uptake by cells. After intravitreal injection with GNP-KA in mice eyes, the vessels images in posterior eyes were acquired via fundus fluorescein angiography (FFA). After the images of vessels leakage condition acquired by FFA were analyzed by the software, ImageJ. Quantification data of vessels revealed that GNP-KA could inhibiting choroidal neovascularization as the best therapeutic effect compared to free KM groups.

KEYWORDS: Choroidal neovascularization (CNV)、Kaempferol-loaded gelatin nanoparticles (GNP-KA)、Intravitreal Injection (IVT Injection)



Schematic diagram of the study

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Temperature-Responsive Polymer-Antibody Conjugate for Biomarker Separation

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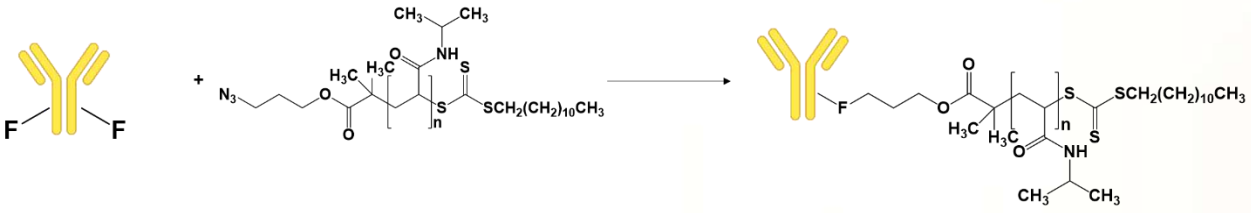
Abstract:

Detecting analytes in the biofluids has been utilized in various biomedical applications. For the existing technology, magnetic beads separation has proven to be convenient tool for selective separation [1]. Magnetic beads with surface immobilized antibodies, the mainstream technology, have been utilized for analyte isolation to enable biomarker detection.

Magnetic beads offer significant advantages in bioseparation due to their large particle diameters, typically around 10 micrometers. However, the use of micrometer-sized particles introduces poor diffusion characteristics. To overcome this limitation, our approach involved the utilization of nanoscale polymer materials. By employing these nanoscale polymers, we aimed to enhance the diffusion properties and improve the overall performance of the bioseparation process.

Here, we developed to conjugate temperature-responsive polymer with antibody, and the difference between magnetic beads and temperature-responsive polymer is particle dimension. To address the challenge, we developed temperature-responsive polymer-antibody conjugates.

To conjugate polymer with antibody, we use poly(n-isopropylacrylamide) which is temperature responsive polymer and is azide terminated to do click chemistry with linker that is DBCO-TFP ester to binding with antibody. The polymer-antibody conjugation utilized click chemistry. Specifically, the antibody was modified with DBCO-dPEG$_4$-TFP ester, and then grafted with azido-poly(N-isopropylacrylamide).



However, the dimension of polymer particle is around nanometer, so we believed that our polymer in solution would be higher efficient to diffusion. The conjugates' hydrodynamic radius are 100-time smaller than the magnetic beads. Therefore, the conjugates can rapidly diffusion to drive real-time analyte recognition.

KEYWORDS: temperature-responsive polymer, biomarker separation, click chemistry

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One-pot synthesis non-conventional fluorescent polymer dots with non-conjugated hyperbranched structure for drug delivery and bioimaging

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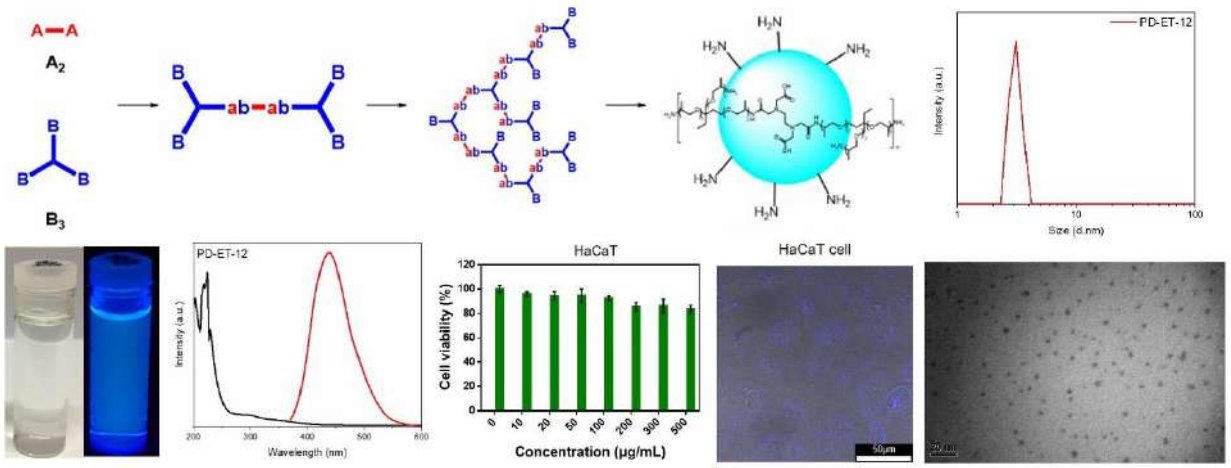
Abstract:

Nonconventional fluorescent polymer dots (PDs) had non-conjugated hyperbranched poly(amic acid) structure, property of anti-photobleaching and anti-fluorescence extinction. We adopted A_2+B_3 polymerization to one-pot synthesize polymer dots, employing dianhydrides as the A_2 components and triamine as the B_3 components. In aqueous solutions, PDs exhibited strong fluorescence with emission at 438 nm, the quantum yield of 14.0%. Observing through dynamic light scattering and transmission electron microscope displayed the sizes of the PDs nanoparticles were 3−5 nm. PDs were low-cytotoxic toward human keratinocyte HaCaT cells at concentrations of 500 μgmL^{-1}. Confocal microscopic bioimaging revealed that the PDs were located within the cells after treatment for 6 hours.[1] PDs had high water dispersibility, non-conventional fluorescence, nanoscale particles and a large number of peripheral functional groups for further modification. PDs appear to have potential of drug carriers and bioimaging, and can be extended to drug delivery systems to improve the interaction of traditional drug, water solubility, biodistribution, targeting and biodegradability.

Folic acid (FA) is one of the biologic molecules which has been targeted overexpressed-folic acid receptor (FR) on the surface of cancer cells. The physiological FA is transported using the cell membrane-associated proteins or folic acid receptor via receptor-mediated endocytosis. FR has overexpression in various human carcinomas including breast, ovary, kidney, lung.[2] Therefore, we would like to conjugate FA to PDs for enhancing the FR-mediated targeting delivery of therapeutic agents.

KEYWORDS: Nonconventional fluorescent polymer dots, drug delivery, bioimaging, folic acid, folic acid receptor-mediated targeting delivery



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Additive manufacturing of Schwann cell-laden collagen nerve guidance conduits by freeform reversible embedding regulate neurogenesis towards peripheral nerve regeneration

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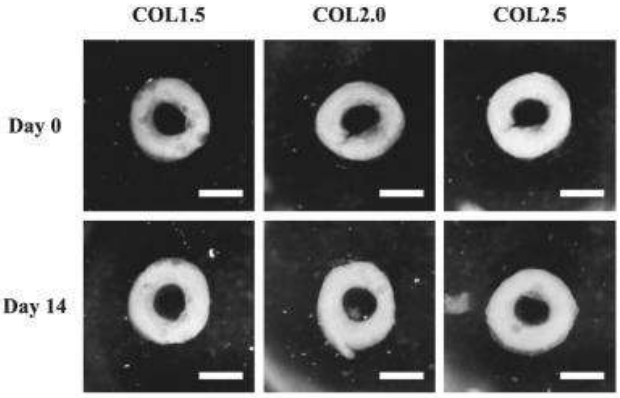
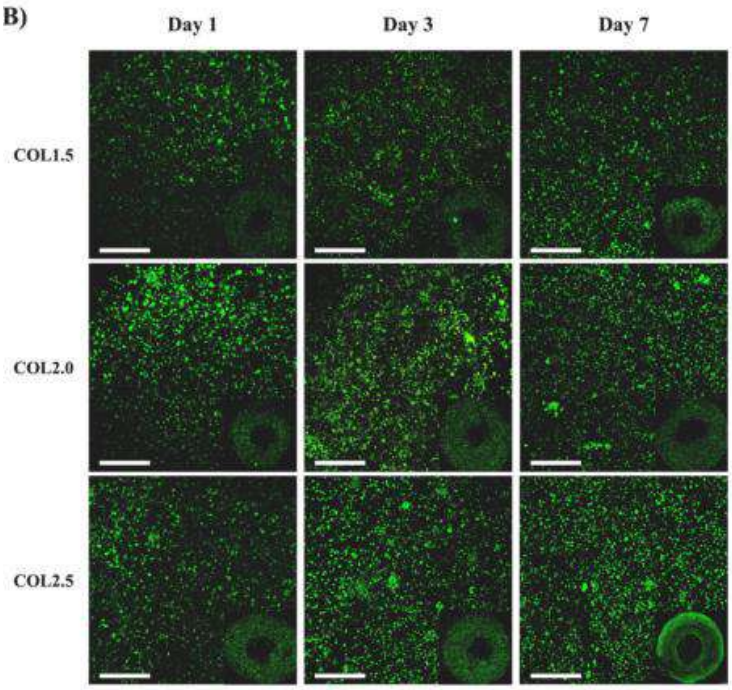
Abstract:

Peripheral nerve injury is a common clinical problem that could be debilitating to one's quality of life.[1] The complex nerve guidance conduits (NGCs) with cells in order to improve nerve regeneration.[2] Therefore, we used freeform reversible embedding of suspended hydrogels to fabricate Schwann cells (SCs)-laden collagen/alginate (Col/Alg) NGCs.[3] First, we evaluated Col influence on the characteristics of NGCs. After which, Wharton's jelly mesenchymal stem cells (WJMSC) are seeded onto the inner channel of NGCs and evaluated neural regeneration behaviors. Results indicated the SCs-laden NGCs with 2.5 % Col found the highest proliferation and secretion of neurotrophic protein. Furthermore, co-culture of SCs promoted differentiation of WJMSC as seen from the increased neurogenic-related protein in NGCs. To determine the molecular mechanism between SCs and WJMSC, we demonstrated the neurotrophic factors secreted by SCs act on tropomyosin receptor kinase A (TrkA) receptors of WJMSC to promote nerve regeneration. In addition, our study demonstrated SCs-derived exosomes had a critical role in regulating neural differentiation of WJMSC. Taken together, this study demonstrates the fabrication of SCs-laden Col/Alg NGCs for nerve regeneration and understanding regarding the synergistic regenerative mechanisms of different cells could bring us a step closer for clinical treatment of large nerve defects.

KEYWORDS: Additive manufacturing , Nerve guidance conduits, Schwann cell



Morphology of different COL group NGC. The scale bar is 1.5 mm



Live/dead assay of SC encapsulated in NGC with different COL contents at culture periods of 1, 3 and 7 days.

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2023 ISOMRM & BCRS
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The abnormalities and metabolites of brain in pentylenetetrazol-induced seizures zebrafish

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Abstract:

Epilepsy is a central nervous system (CNS) disorder that causes frequent seizures. A sudden harsh of electrical activity is occurred in the brain due to a seizure that causes temporary changes in behavior, movement or feelings. However, the underlying mechanism of seizure is still unclear.

4-6 month-old adult AB type zebrafishes were exposed to 10mM PTZ for 20 min. Then, we used Western blot and RT-PCR to explore the abnormal phenomenon in brains of PTZ-induced epilepsy zebrafish. We also treated brain tissue with 100μl general oxidative stress indicator (CM-H2DCFDA) for 15 min, to detect ROS level via ELISA reader. Besides, we used UPLC-MS to investigate the metabolites differences between normal and PTZ-treated zebrafish.

We found that PTZ-induced seizure may increase ROS level in zebrafish brain. And we also found metabolites which may represent as biomarkers to investigate its underlying mechanism. We established PTZ-induced seizure zebrafish model that might serve as the good platform for therapeutic natural compound candidate research in epilepsy.

KEYWORDS: pentylenetetrazol (PTZ), metabolites, brain, zebrafish

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Neuron protective drug in an in-vitro model of glutamate-mediated neurotoxicity

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Excitotoxicity induced by glutamate can manifest in various neurological disorders, including stroke, traumatic brain injury, epilepsy, Alzheimer's disease, and Parkinson's disease. Previous research has demonstrated that estradiol possesses neuroprotective properties, effectively reducing cell death resulting from excitotoxicity. In this study, we employed a novel approach to differentiate neural stem cells (NSCs) on a polyelectrolyte multilayer films composed of PLL-PLGA, generating intricate neural networks. Subsequently, a model of nerve injury was established by subjecting the neural networks to high concentrations of glutamate. The concentration of glutamate was assessed using immunofluorescence staining, followed by evaluating the neuroprotective effects exerted by HA-modified estrogen (HAE2). HAE2, a water-soluble derivative of estrogen, exhibited superior protective effects compared to unmodified E2. To ascertain the reduction of cytotoxicity and oxidative stress, the PI/annexin V apoptosis assay was used and reactive oxygen species (ROS) levels was measured. Furthermore, the activation of glial cells, quantification of glutamate transporter recovery, and assessment of Ca2+ ion channel functionality were determined through western blot analysis of GFAP, GLT-1, PTX3, and the utilization of fluo-4. Notably, our observations on the brain on chip designed in our previous study indicated enhanced synaptic growth, with astrocytes playing a pivotal role in the excitotoxicity protection model, surpassing the significance of neurons.

Keywords: excitotoxicity, neuroprotective, estradiol, astrocyte, neural networks

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Application of decellularized small intestinal submucosa crosslinked with anthocyanin as an artificial nerve guiding conduit

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Abstract:

Clinical applications of nerve guiding conduit (NGC) span a wide range of peripheral nerve injuries (PNI), including traumatic nerve damage, nerve compression, and surgical repairs. In this study, decellularized small intestinal submucosa (SIS) was prepared and coiled to form the nerve guiding conduit. To prolong degradation time and meet the mechanical properties required for clinical use, a natural and low-toxicity oligomeric proanthocyanidins (OPC) was used as a crosslinking agent. OPCs are natural compounds found in various plants, known for their antioxidant and anti-inflammatory properties, and have been extensively studied for their potential in neuroprotection and regeneration. The prepared NGC with an inner diameter of Ø 1.98 mm, an outer diameter of Ø 2.5 ± 0.15 mm, and a length of 1.5 cm were prepared by crosslinking with different concentrations (5wt%, 7.5wt%, 10wt%) of oligomeric proanthocyanidins (OPC). The properties of NGC, including microstructure, degradation behavior, swelling index, and kinking resistance, were assessed and evaluated. SEM observation revealed that the NGC exhibited a multilayered porous structure. As the concentration of the crosslinking agent increased, the porous structure became more compact. The kinking resistance test aimed to determine the flexibility and resistance of the NGC to kinking under various conditions. The results of kinking resistance demonstrated that higher concentrations of OPC decreased the bending angle. The cytotoxicity of NGC was evaluated using PC12 cell line in accordance with ISO 10993-5. WST-1 and LDH analyses revealed no significant cytotoxicity in all groups. The results of cell culture demonstrated that OPCs crosslinked nerve guiding conduits provide a conducive environment for PC12 cells to extend axons. OPC crosslinked SIS nerve guiding conduits represent a potential biomaterial for nerve regeneration.

KEYWORDS: Nerve Guidance Conduit (NGC), Oligomeric proanthocyanidins (OPC), Small intestinal submucosa(SIS), decellularization

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2023 ISOMRM & BCRS
Aug. 31 – Sep. 3 2023, Taipei, Taiwan

DEVELOP A TECHNOLOGY FOR MRI-MONITORING BREAST CANCER CELL SITUATION

Yu-Jing Huang, Hung-Yuan Tsai, Gin-Shin Chen, Li-Wei Kuo, Guo-Chung Dong (董國忠)

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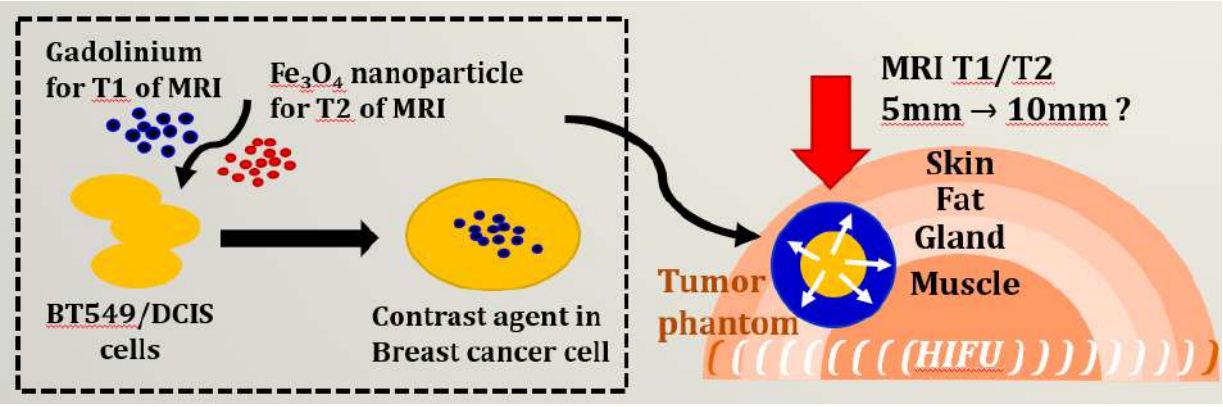
Abstract:

On current HIFU-MR therapy via MR images to make sure HIFU burning position and area, but it is different to understand the treated situation. In this study, the bioactive phantom was applied on HIFU-MR therapy that measured the HIFU burning situation on cell level. In previous studies, the structures of breast phantoms with tumor have been standardized, and this year developed a technique for evaluate cancer cells situation after treatment. Heating on cancer cells to investigate the extent damage by temperature to induce cancer cells release uptake MRI contrast agent. Measured the T1 value on different cell situations by hyperthermia to monitor breast cancer cell situated level. From the results, the higher temperature, more easily release MRI contrast agent from cell. At 45°C, only a few cells died, about 40% were damaged, and 50% were still alive. This technology can provide biological information for ultrasound therapy development and to monitor biological status using MR image.

KEYWORDS: gelatin hydroxyapatite glutaraldehyde (GHG) scaffolds; MR images; ultrasound therapy; breast phantoms



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Effects of decellularization extracellular matrix on the behavior and differentiation of intestinal epithelial cells in both static and dynamic microfluidic environments.

Phenpitcha Charoensaensuk [1, †], Chi-Tien Chen[2, †], Tuan Ngoc Anh Vo[3], Aye Chan Phyu[1], Chun-Min Lo[2], Pin-Chuan Chen[3], Chen-Yu Kao [1,4,*]

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† These authors contributed equally to this work.

Abstract:

Caco-2 cells monolayer is an accepted in vitro intestinal model for predicting drug permeability and absorption in pharmaceutical companies and regulatory agencies [1, 2]. However, achieving complete differentiation of Caco-2 monolayer cells requires a minimum of 21 days [3], making it a time-consuming and expensive process with limited throughput. To address this challenge, ECM (extracellular matrix) was applied to promote Caco-2 cell migration and differentiation. In common practice, gels made from rat tail type I collagen are used as a scaffold in cell culture. These collagen gels incorporate the hypothesized inductive properties of the ECM component and promote cell polarization [4]. Collagen type I is highly valued for its excellent biocompatibility, structural support, and ability to enhance cell adhesion and proliferation. However, commercial rat tail collagen type I is quite expensive. Therefore, in our laboratory [5-7], we have developed B-ECM (porcine bladder extracellular matrix) as a replacement for commercial collagen type I. B-ECM, with its main component being collagen type I, may effectively mimic the natural characteristics of the intestinal extracellular matrix (ECM).

The B-ECM coated substrate model demonstrated a desirable phenotype and functional similarity to the collagen type I coated substrate Caco-2 model in terms of ECIS (Electric cell-substrate sensing) measurement, cell attachment, and spreading. The B-ECM-coated substrate showed a shorter waiting time for cell spreading (13.17 nF/h) compared to the COL-I-coated substrate (8.7 nF/h). Additionally, the migration of Caco-2 cells was observed through the halfway resistance value of the plateau (T50) after turning off the Electric Fence (EF). The results indicated enhanced migration on both B-ECM-coated electrodes (0.079 h) and COL-I- coated electrodes (0.079 h). Furthermore, the trans-epithelial electrical resistance (TEER) measurement reached 300 $\Omega.cm^2$ after 3 days of culture, indicating the development of tight

junctions between the cells. The phenol red permeability (Papp) was found to be less than 2×10^{-6} cm/s after 7 days of culture, indicating a low level of paracellular transport. Overall, these findings demonstrate that the B-ECM-coated substrate model effectively supports Caco-2 cell behavior and functions, comparable to the collagen type I-coated substrate model, as evidenced by ECIS measurements, cell spreading, migration, TEER, and phenol red permeability. However, the use of a dynamic system to mimic the in vivo intestinal environment using a microfluidic device with a PDMS substrate bonded with PET has not been successful in cell culture. Unfortunately, the system breaks down after only 3 days of culture.

KEYWORDS: Extracellular Matrix, Decellularization, Caco-2, cell adhesion, cell migration, ECIS; electric fence (EF), wound healing assays, microfluidic chip, APTES bonding, PDMS, PET porous membrane

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Preparation of Tumor Tissue Rich in Extracellular Matrix for Toxicity Test of Cancer Therapeutic Drugs

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Abstract:

The tumor tissue formed by cancer cells has always been a major obstacle in the clinical treatment of cancer. Many literatures have pointed out that the matrix tightly wrapped around some cancer tumor tissues may be one of the reasons for the decrease in the penetration ability of therapeutic drugs. However, in the current drug treatment efficacy and preclinical testing of new drugs, most of the animal experiments or two-dimensional cell culture are used as the reference basis for dosage and efficacy development. The predicament that the drug dosage and cell type performance are quite different from the results of animal experiments[1].

Therefore, in order to shorten the dose and effect difference between the two-dimensionally cultured monolayer cells and the direct effect on tissues and organs in animal experiments, and provide a clinical efficacy evaluation method for combined treatment before administration as the goal for tumor treatment; To this end, the laboratory has established a set of techniques to allow cancer cells to produce extracellular matrix in vitro. Under the stimulation of inducing extracellular matrix secretion factors, this technique can secrete different levels of extracellular matrix around the cells, and over time a cell cluster formed by cell aggregation under regulation; finally, after optimizing the above conditions, a tumor tissue rich in extracellular matrix was obtained, followed by a drug toxicity test on the tumor tissue in vitro with commonly used chemotherapeutic drugs, and we found in the results that culturing tumor tissue rich in extracellular matrix are similar to the results of animal experiments, and the effect of drug resistance was also observed during treatment. However, in the tumor tissue without extracellular matrix, it can still be clearly observed that the drug is poisoned and dissolved. Therefore, we verified that the extracellular matrix is not only one of the indispensable elements of tumor tissue, but also plays an important role in the drug tolerance of tumor tissue, and also established a set of in vitro regulated extracellular matrix culture tumor tissue methods.

KEYWORDS: Extracellular Matrix (ECM), Tumor Tissue, Toxicity Test, ECM-rich, In-vitro Three-dimensional Culture

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

The Process of Mouse Cochlear Hair Cell Differentiation Stimulated by Dynamically Regulating Ion Concentration

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Abstract:

Sensorineural hearing loss (SNHL) is the most common form of hearing loss, encompassing damage to the cochlea, auditory nerve, or central nervous system. It can be caused by various factors such as noise, aging, congenital conditions, ototoxic agents, diseases, head injuries, and systemic conditions. In mammals, the hair cells in the inner cochlea are responsible for detecting sound, and their damage leads to irreversible hearing loss. Consequently, the regeneration of hair cells to restore hearing has been a significant challenge. During mammalian development, the composition of the cochlear endolymph changes from a high-sodium/low-potassium solution to a low-sodium/high-potassium solution in adulthood. However, conventional hair cell culture mediums typically use high-sodium formulations, which do not mimic the in vivo environment accurately. In this study, we have developed an ion exchange method using alginate replacement colloid to dynamically adjust the sodium/potassium concentration in the cell culture process. This approach allows for the growth and differentiation of primary cochlear cells in a sodium/potassium concentration closer to the developing mouse embryo environment. We successfully generated inner ear organoids that are adapted to survive in a high-potassium environment. Staining results have indicated the

presence of hair cell markers (Atoh1, Myo7, and F-actin) in the cochlear organoid, suggesting the presence of mature hair cells. Further studies will focus on conducting physiological function tests on these hair cells. In summary, we have established an ion exchange method to modify the sodium/potassium concentration during the cell culture process, enabling the growth and differentiation of hair cells in an environment that closely resembles the natural inner ear. This ion exchange method has the potential for broader applications in other in vitro models that require precise ion adjustments. We believe that the method described in this study could provide valuable insights for research on sensorineural hearing loss.

KEYWORDS: Cochlea, Hair Cell, Ion Exchange

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A Tumor Accelerator Based on Multicomponent Bone Scaffolds and Cancer Cell Homing

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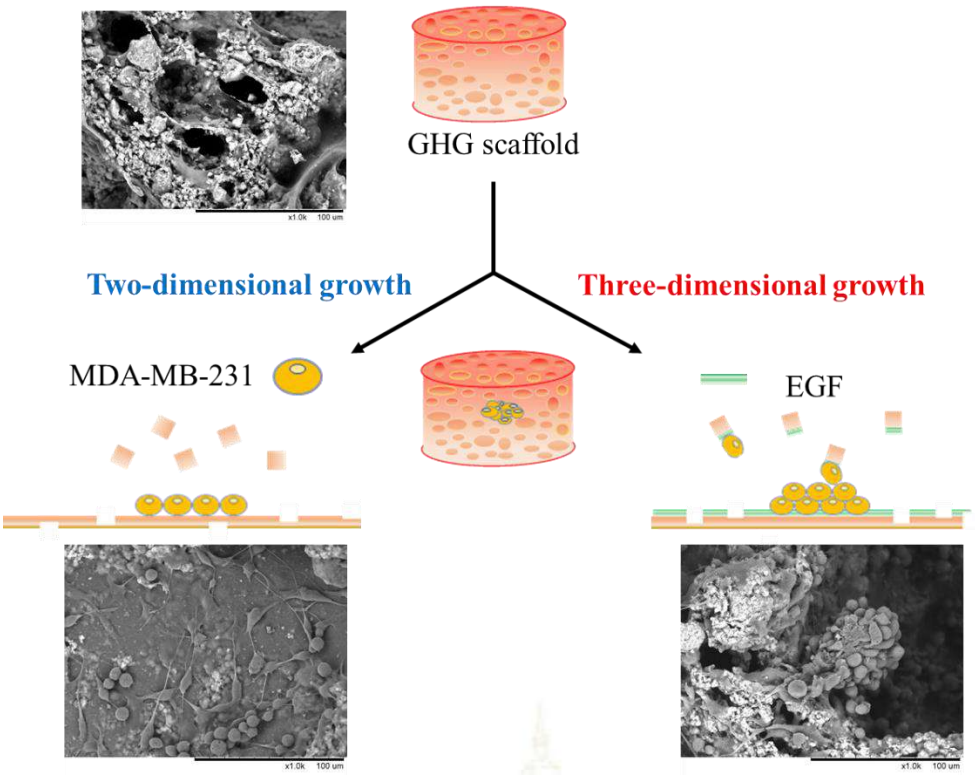
Abstract:

Bone tissue attracts cancer cell homing biologically, mechanically, or chemically. It is difficult and time consuming to identify their complex cross-talk using existed methods. In this study, a multi-component bone matrix was fabricated using gelatin, hydroxyapatite (HAp), and epidermal growth factor (EGF) as raw materials to investigate how "acellular" bone matrix affects cancer cell homing in bone. Then, EGF-responsive cancer cells were cultured with the scaffold in a dynamical bioreactor. For different culture periods, the effects of HAp, gelatin, and EGF on the cell adhesion, proliferation, 3D growth, and migration of cancer were evaluated. The results indicated that a small amount of calcium ion released from the scaffolds accelerated cancer MDA-MB-231 adhesion on the surface of inner pores. Moreover, degradable gelatin key caused cancer cell growth on the scaffold surface to turn into a 3D aggregation. Despite this, the formation of cancer spheroids was slow, and required 14 days of dynamic culture. Thankfully, EGF promoted cancer cell adhesion, proliferation, and migration, and cancer spheroids were observed only after 3-day culture. We concluded that the combination of the multiple components in this scaffold allows cancer cells to meet multiple requirements of cancer dynamic progression.

KEYWORDS: epidermal growth factor (EGF); MDA-MB-231; gelatin hydroxyapatite glutaraldehyde (GHG) scaffolds; microenvironment



Graphic abstract

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Biomimetic of a 3D breast cancer spheroid model using decellularized extracellular matrix for personalized therapeutics investigation

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Abstract:

Breast cancer remains a significant global health challenge, contributing to high cancer-related mortality rates. Its complex and diverse characteristics, including clonality and heterogeneity, pose significant hurdles in effective treatment strategies. Despite advancements in therapeutic options and genomic analyses, the emergence of recurrent mutations and subsequent drug resistance continues to hinder treatment efficacy, particularly in late-stage patients. Consequently, there is a pressing need to develop innovative approaches that bridge the gap between genomic profiling and personalized therapeutic responses [1]. The tumor microenvironment is a complex network of vasculature, tissue-specific extracellular matrix (ECM), immune cells, fibroblasts, and tumor cells, which collectively interact to influence cellular behavior and drug delivery. To address the need for patient-specific cancer models that faithfully mimic the tumor microenvironment, we propose a novel approach. Specifically, we present an *ex vivo* breast cancer model utilizing decellularized ECM (dECM) derived from breast tissues to create a biomimetic tumor microenvironment [2]. Our results illustrated that the fabrication process of this *ex vivo* breast cancer spheroid model, where breast cancer cells are encapsulated within adipose extracellular matrix (AdECM) to form cancer spheroids in the presence of vascular cells, thus emulating the microenvironment. A co-clinical trial involving various breast cancer patients was conducted, and their treatment responses were recorded and compared with the outcomes obtained from the ex vivo patient-specific cancer spheroids. In addition, we demonstrate that the developed breast cancer spheroid model accurately reproduces patient responses to chemotherapeutic agents and targeted therapies, achieving high accuracy, sensitivity, and specificity. This novel *ex vivo* predictive model holds promise for personalized breast cancer therapy and has the potential to enhance the quality of clinical care.

KEYWORDS: breast cancer, spheroid, extracellular matrix, drug screening, precision therapeutics

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Vessel on a chip with Hydrogel Based 3D Bioprinting Vessel-Like Construct

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Abstract:

The vascular system is a critical component of Vertebrate Animals, playing essential role in the development and functioning of all organs and tissues. Therefore, *in vitro* models of blood vessels can significantly contribute to drug development and medical research. However, recapitulating the complex construction of cell-laden vascular constructs is still challenging.

Herein, we developed a novel method to prepare bioink by double-tube mixing the alginate, EDTA, crosslinking agent, and silk methacrylate (SilMA). This method produces a highly homogeneous bioink with excellent biocompatibility, superior structural stability, and also improved the mechanical properties. Rheological measurements demonstrate that bioink has highly shear thinning which provides a supportive environment for the printed cells.

Human endothelial cells (ECs) have a natural ability to self-assemble and form blood vessel-like structures, known as capillary-like networks, when provided with the appropriate conditions. The bioink-embedded human endothelial cells (ECs) is printed into custom-manufactured fluidic chips that integrates fluid flow and three-dimensional (3D) co-cultures so promotes spontaneously organizing and forming micro-vessel. To verify the identity of ECs on the chip, immunofluorescence staining will be used to confirm the expression of ECs markers such as CD31 (PECAM-1) and VE-cadherin (CD144).

The circulatory system facilitates the delivery of immune cells, growth factors, and nutrients to promote tissue development and repair. It is considered that the vessel-on-a-chip model may provide a valuable tool for studying diseases and therapies in a controlled environment, enabling researchers to investigate precision medicine approaches and drug screening.

KEYWORDS: Double-Tube bioink prepartion, alginate, silk methacrylate (SilMA), vessel-on-a-chip model, micro-vessel

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Development of bioinks for 3D bioprinting of breast cancer microenvironment modeling

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Abstract:

Breast cancer ranks first among female cancers. Currently, many unknown mechanisms exist, which makes it difficult to make accurate predictions or treatments for breast cancer. Therefore, it is important to develop *in vitro* models that mimic the microenvironment of breast cancer and explore the underlying mechanisms.

The two most basic elements of a cancer model are cancer cells and the extracellular matrix (ECM). The ECM can provide cancer cell scaffolds, construct a 3D microenvironment, and simulate cell-ECM interactions. The cell-ECM interactions will lead to tumor heterogeneity. It is revealed that the hardness of breast tumors is mainly affected by the ECM, with the inner part being the softest and the outer part becoming harder and harder [1]. Previous literature also revealed that breast cancer cells demonstrated better proliferation ability in softer ECM and higher invasive abilities in the harder ECM [2].

Therefore, to develop a microenvironment that closely mimics the hardness of real tumors, collagen and hyaluronic acid with modification were used to synthesize photocurable bioinks (ColMA and HAMA) to simulate tumor ECM. Collagen is the most important protein in the breast cancer microenvironment, and it can provide cell binding sites and promote cell adhesion, attachment, proliferation, and signal transduction. It also has a strong impact on tumor invasion. HA is pro-inflammatory and activates signaling pathways that promote survival, migration, and invasion within both tumor and host cells through binding to HA receptors such as CD44 [3]. Series of different composition HAMA/ColMA hydrogels were subjected to mechanical testing, biocompatibility testing, and SEM analysis of materials. For breast cancer cells, HER2 MCF-7 and triple-negative MBA-MB-231 cells were used in this study and tumor spheroid were formed and embedded in different composition of bioinks through bioprinting for printing and photocuring. The proliferation ability and invasive ability of two kinds of breast tumor spheroid in the hydrogels with different composition and stiffness will be analyzed.

A breast cancer model by spheroid laden 3D bioprinting and microfluidic chip will be fabricated. Fluorescent immunostaining will be performed to observe the tumor spheroid morphology, proliferation, and expression of Epithelial-mesenchymal transition (EMT) markers

to verify whether the breast cancer model could simulate real tumor behavior.

KEYWORDS: Breast cancer microenvironment, tumor model, tumor spheroid, collagen-hyaluronic hydrogel, 3D bioprinting

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Effects of regenerative medicine in combination with physiotherapy for knee osteoarthritis: a network meta-analysis of randomized controlled trials

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Abstract

Background: Knee osteoarthritis (KOA) are associated with high risk of sarcopenia and disability. Physical therapy (PT) as well as regenerative medicine using intra-articular injections (IAI) are effective treatments on pain relief and physical mobility recovery in middle-aged and older people with KOA [1, 2]. The relative effects among treatment regimens of various regenerative IAIs and its combination with PT for preventing sarcopenia KOA remain unclear.

Purpose: The purpose of this network meta-analysis (NMA) was to investigate the relative effects among monotherapy of regenerative IAIs and PT, and the combined treatment regimens on muscle mass and strength gain for KOA.

Methods: The present study was conducted according to the guidelines recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis [3]. The protocol of this systematic review was registered in the PROSPERO registry (CRD42022336304). Seven electronic databases (PubMed, EMBASE, CINAHL, the Cochrane Library, PEDro, the China Knowledge Resource Integrated Database, and Google Scholar) were systematically searched from inception until June 2022. Randomized controlled trials (RCTs) that reported the effects of regenerative IAIs combined with PT for KOA were identified. The regenerative IAIs included ozone, dextrose prolotherapy, platelet-rich plasma, and mesenchymal stem cell. No limitation was imposed on the publication year or language. Main outcome of interest included muscle mass and leg strength measures. The included RCTs were analyzed through NMA and risk-of-bias assessment. Ranking probabilities of effect estimation among treatments per outcome were expressed by the surface-under-the-cumulative-ranking (SUCRA) score [4]. Network meta-regression was performed to explore the moderators of treatment efficacy on each main outcome [5].

Results: We included 48 RCTs along with 94 treatment arms in the NMA. A total of 3136 patients

who had mean age of 61.2 (range, 46.2–80.9) years and mean body mass index of 30.8 (range, 23.1–35.4) kg/m^2 were recruited. In summary, dextrose prolotherapy plus PT was the most effective treatment for muscle mass gain [standard mean difference (SMD) = 2.42; SUCRA = 0.95] among all treatment arms. In addition, platelet-rich plasma plus PT yielded the highest rank of treatment efficacy for strength gain (SMD = 1.16; SUCRA = 0.96) compared with other treatment options. Disease severity (i.e., Kellgren and Lawrence grading system for classification of KOA) influenced the treatment outcome. Trials enrolled a greater number of patients who were classified as moderate to severe disease severity may obtain greater treatment effects on muscle mass (β = 1.34; 95% credible interval = 1.18, 2.59) and strength (β = 0.56; 95% credible interval = 0.09, 1.07).

Conclusion: Combined treatment regenerative IAI plus PT exerts additional benefits on preventing sarcopenia for middle-aged and older adults with KOA, compared to either IAI alone or PT alone. Such treatment efficacy may be moderated by disease severity.

Implications: Regenerative IAI in combination with PT, especially the dextrose prolotherapy and platelet-rich plasma, exert extra benefits in addition to the monotherapy for KOA.

Keywords. osteoarthritis, sarcopenia, regenerative medicine, injection, physiotherapy

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Therapeutic Potential of *Chenopodium Formosanum* Extracts for Early-Stage Osteoarthritis: Free radical scavenging activity and cell-compatibility

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Abstract

Human cartilage may produce abnormal free radicals in the joints due to aging, working environment or genetic disorder, which can trigger inflammation and cause mild to severe joint degeneration, such as osteoarthritis (OA). When articular cartilage is attacked by free radicals, due to its natural avascular structure, the self-renewal rate of the matrix become quite slow. Thus, it is difficult to rely on the patient's own cells or tissue repair the degenerated cartilage. Age, gender, menopause, genetics, nutrition and bone density often lead to increased susceptibility to OA. In Taiwan, according to the Ministry of Health and Welfare statistics, the prevalence of knee joint related degeneration in the population is 15%. One in five people over the age of 60 suffers from joint degeneration. More than 70% of the population over the age of 70 suffers from osteoarthritis. Clinically, hyaluronic acid injections are often used in the treatment of early OA, but this treatment can only passively relieve short-term pain, and cannot improve joint inflammation and promote cartilage regeneration. Chenopodium formosanum (Djulis) is a cereal crop in Taiwan, it contains starch, dietary fiber, proteins and essential amino acids, antioxidant compounds, such as betacyanins, betaxanthins, flavonoids. We also found in the literature that Djulis has anti-oxidant and collagen synthesis enhancement ability in skin treatment. Therefore, we would like to extend the application of Djulis extract to early-stage OA treatment in the study. First, we used ABTS assay and DPPH assay to evaluate it's free radical scavenging activity, and then total phenolic content, and total flavonoid content were also measured by Folin-Ciocalteu's phenol reagent and sodium nitrite/ aluminum chloride reaction. Then, we evaluated chondrocyte cell viability under the stimulation of IL-1β and Djulis extract. Results showed that total phenolic content was 0.04605 g gallic acid/g extract, and total flavonoid content was 0.16681 g Quercetin/g extract. The IC50 of DPPH radical was 69.35185 μg/ml and a radical was 69.35185 μg/ml. Moreover, the cell viability was similar among Djulis extract groups (0.1 ~ 100 μg/ml) and control group, which means the Djulis extract has good cytocompatibility. Further studies, such as gene expression analysis and ELISA protein evaluation will be done. According above results, we suppose Djulis extract could be a promising therapy, and it can be mixed with hyaluronic acid injections for early-stage OA treatment in the future.

Keywords *: osteoarthritis, Chenopodium Formosanum(djulis), antioxidant, chondrocyte*

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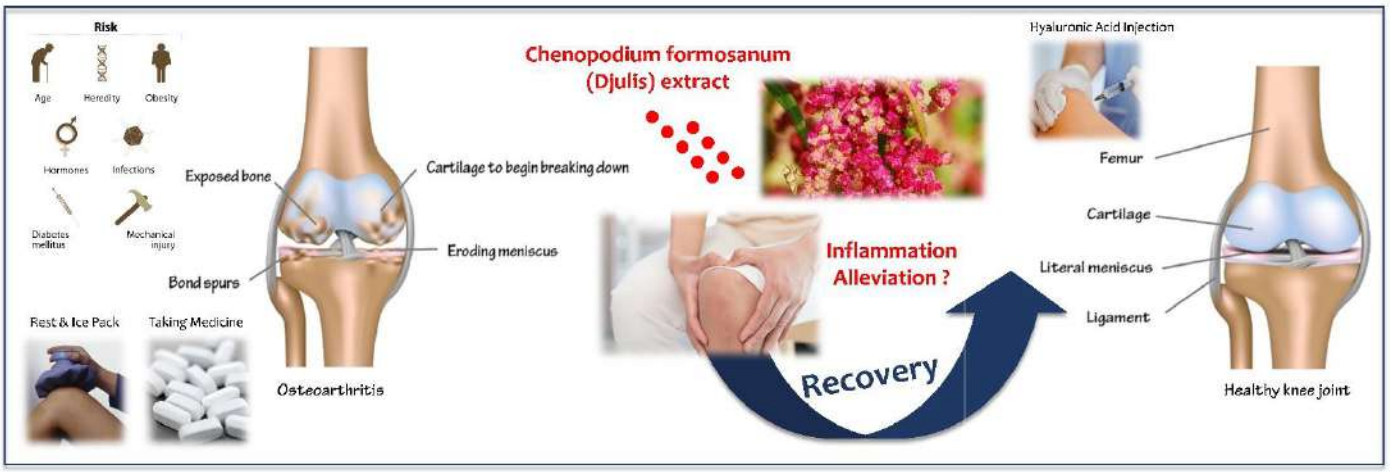
Graphic Abstract



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The prospect of applying 3D cultured osteogenic cell spheriods in surgical interventions for Empty Nose Syndrome

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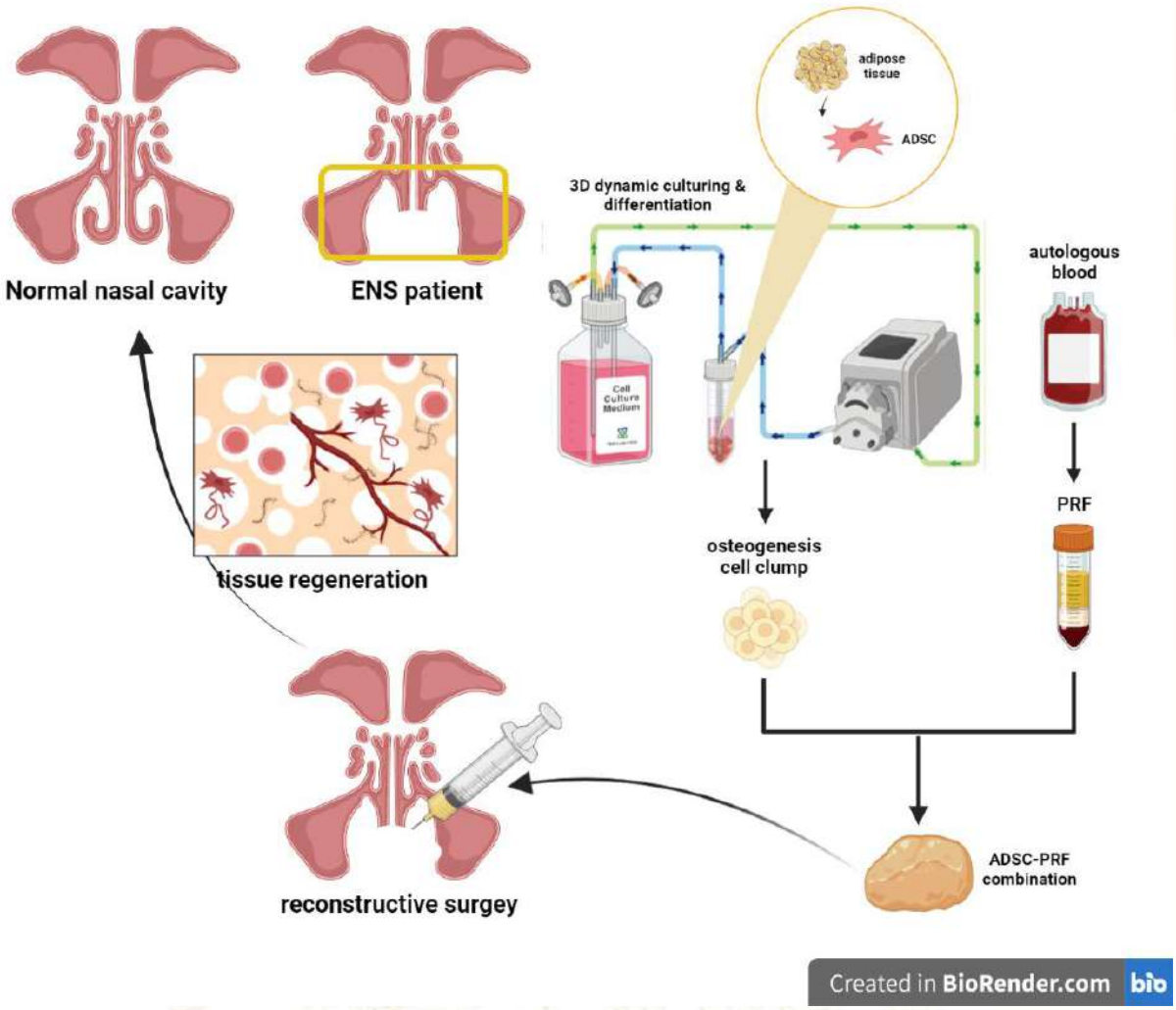
Abstract:

Empty Nose Syndrome (ENS) is a clinical entity related to the overly removal of nasal turbinate structure and is therefore associated with turbinate reduction surgery. The pathogenesis of ENS varies from changes in laminar physiological airflow, induction of mucosa functions and deficient neural sensation. As a result, ENS patients suffer from numerous symptoms such as dyspnea, nasal dryness, nasal burning, paradoxical nasal obstruction, fatigue and sleep disorder. In addition to physiological illness, patients also experienced psychological problems due to cerebral hypoxia and sleep deprivation. In some severe cases, ENS even drives depression and suicide thoughts among its victims. There is still no specific effective treatment for ENS nowadays. Many studies have been exploring the possibility of cell-based treatment on ENS, believing that the new developing cell technologies could overcome the existing limitation of current treatment and lead to the significant and more stable improvement in quality of life of ENS patients.With the same point of view, this study aims to develop a bone-like structure with the combination of 3D dynamic culturing meschymal stem cell (MSC) and platelet rich fibrin (PRF) as a surgical implant to reconstruct the damaged nasal turbinate in ENS. In the 3D dynamic culture of MSC, we apply the co-culture of neuron stem cell (NSC) and endothelia cell (EC). According to several studies, the growth factors secreted by NSC show a positive effect on MSC in osteogenesis. By introducing this co-culture system into the 3D dynamic MSC culturing, we expect that the bone regeneration efficiency for the damaged turbinate tissue would be increase after the implant surgery. The three types of cells will first be seeded inside the scaffolds seperately and apply to the bioreacter system in our lab. After 21 days of culturing, the MSC spheriods will be seperated from the alginate scaffold and analystic studies will be carried out. Combination of cell spheroids with PRF will be done in the future work for clinical use.

KEYWORDS: Empty Nose Syndrome (ENS), meschymal stem cell (MSC), platelet rich fibrin

(PRF), three dimensional dynamic culturing



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Promotion of PNS remyelination (and underline mechanism) by cell therapy

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Abstract:

Peripheral nerve injury (PNI) is a common type of nervous system damage that leads to dysfunction or damage. PNI is classified into three levels: neurapraxia, axonotmesis, and neurotmesis. Neurotmesis is one of the most severe types of nerve damage, caused by complete fracture of nerve bundles or trunks. During neurotmesis, myelin degenerates and Schwann cells (SCs) clean up the myelin debris to promote axonal regrowth. Cell therapy is a technique that utilizes the physiological functions of cells to treat or repair damaged or diseased tissues. It involves the injection of specific cells into a living body to act at the site of injury and provide a therapeutic effect. The current surgical method for cell therapy on nerve, which involves using silicone conduits, is an emerging treatment that promotes tissue repair and regeneration by transplanting cells into damaged tissues through silicone channels. However, the use of silicon conduits may be limited by their stability and biocompatibility, as well as the challenge of controlling cell transport and localization. Based on the above, we are going to test that therapies of different cell types could promote nerve remyelination in greater repair efficiency.

In this work, we first tested the feasibility of using different cells such as adipose stem cell (ASC), ASC-derived SCs, rat Schwann cell (RT4), mouse myoblast (C2C12), and C2C12-derived myotube, then double-confirmed our findings using western blot or PCR. We created animal models of sciatic nerve injury and utilized transmission electron microscope (TEM) and focused ion beam (FIB) techniques to analyze the structures of both healthy and injured nerves. We also stained the tissue with several neuron markers, including MPZ, S100β, and p75NTR, to evaluate nerve regeneration. Finally, we co-cultured RT4 cell with rat dorsal root ganglion (rDRG) or C2C12-derived myotube with mouse dorsal root ganglion (mDRG) to investigate the role of different types of cells in remyelination.

We are going to demonstrate the feasibility of using specific cells for cell therapy by forming cells into cell sheet, as evidenced by the results of Western blot and PCR analyses. In animal models, tissue sectioning, IHC staining, and transmission electron microscopy (TEM) will be performed to observe the ultrafine structure of the myelin sheath in both healthy and injured nerves. Next, axonal regeneration is affected by co-culturing rDRG with RT4 cells or mDRG with C2C12-derived myotube, *ex vivo*. We will validate the applicability of this cell therapy to peripheral nerves in animals and the ability to control the nerve remyelination.

KEYWORDS: peripheral nerve injury, neurotmesis, regeneration, remyelination, cell therapy

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PLLA microparticle-loaded double-layered microneedle patches for effectively stimulating dermal collagen regeneration

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Abstract:

Skin aging is an inevitable physiological process. Both increasing chronological age and cumulative exposure to external factors such as ultraviolet radiation, cause wrinkles and reducing elasticity of the dermis. Collagen atrophy is a major factor in skin ageing, the production of collagen decreases and its degradation increases, which leads to a reduction in collagen total amount [1]. Dermal filler is a nonsurgical treatment for the relaxation of facial wrinkles and for the augmentation of the aging face. Collagen and hyaluronic acid (HA) are temporary fillers, can quickly correct the volume loss when injected into the skin [2]. Poly-L-lactic acid microparticles (PLLA MPs) are semipermanent injectable fillers stimulate foreign body inflammatory response in the host, which in turn elicits macrophage adhesion and attack, foreign body giant cell formation, and neocollagenesis. Within 3 weeks, the gradual deposition of collagen around the provides a natural-looking outcome and potentially last up to 25 months. Over the course of 9 months, the PLLA MPs are degraded and metabolized as lactic acid. PLLA MPs should be injected into the correct depth of the skin with professional injection technique, otherwise it may cause papules or nodules [3-4].

In this study, we fabricated a double-layered PLLA MPs loaded HA with PLA supporting microneedles (MN). With the MN applicator, we can control the same insertion depth of the skin every time. Also, we sought to explore the potential of tiny wounds in the dermis created by MN for collagen regeneration [5]. Each MN patch was loaded with PLLA MPs 931 ± 27 μg (n = 5). When inserted into the skin, the HA layer can be dissolved within 20 minutes and release PLLA MPs into the dermis with an approximate insertion depth of 769 ± 122 μm (n = 5). We evaluated the degradation of the PLLA MPs for 0, 4, 12 weeks in dorsal skin model of SD rat. The quantitative analysis from 3D confocal reconstruction images showed PLLA MPs degraded over time. And about 60.1 ± 5.8 % and 55.7 ± 10.4 % of PLLA MPs were existed at 4 and 12 weeks, respectively. We will conduct in vivo animal test to explore the availability of PLLA MPs-loaded HA/PLA MNs as a potential dermal filler.

KEYWORDS: skin aging, microneedles, poly-L-lactic acid (PLLA), foreign body reaction, neocollagenesis

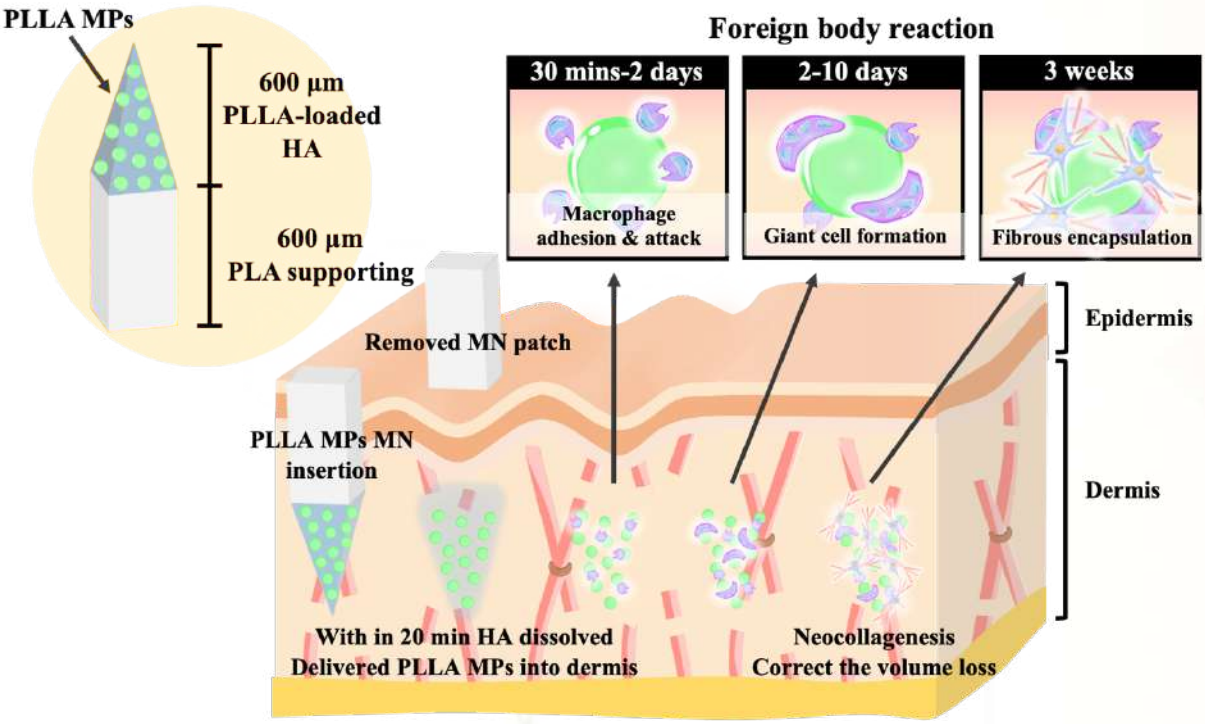

Fig. 1 The concept of this study

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Aug. 31 – Sep. 3 2023, Taipei, Taiwan

Advancement of Three-Dimensional Biomimetic Skin Substitutes for Burn Injury Skin Regeneration

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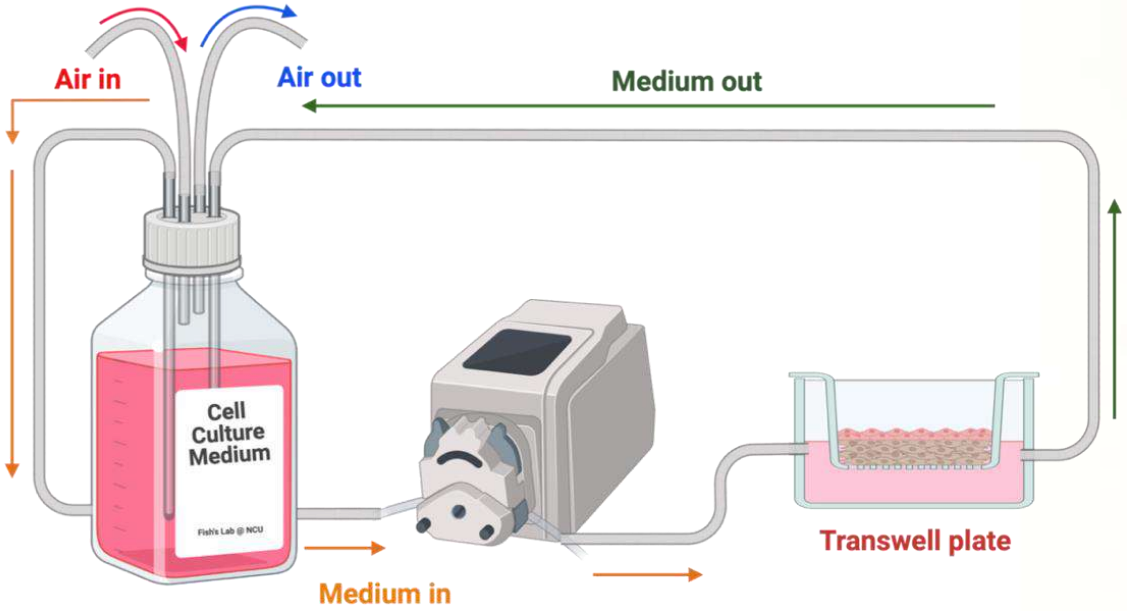
Abstract:

Burns are the most common type of skin injury, and severe burn patients often require life-saving skin graft surgeries. The gold standard treatment for these patients is autologous split-thickness skin graft (STSG). However, in cases where patients have extensive wound areas, there is often a shortage of healthy autologous skin available for grafting. This has led to the emergence of various skin substitutes, which can be categorized as epidermal, dermal, or bilayered substitutes. These substitutes are considered when patients with severe burns and large wound areas undergo skin graft surgeries. However, monolayered substitutes have limited effectiveness in promoting wound healing, while bilayered substitutes are not widely used due to their high cost and time-consuming nature. As a result, our project aims to address these limitations by utilizing a self-designed bioreactor and 3D cell culture techniques to culture autologous skin cells (fibroblasts and keratinocytes) from patients. The bioreactor creates a biomimetic skin tissue with a continuous flow system that mimics capillary flow from the hypodermis, providing nourishment to the biomimetic skin tissue, promoting tissue growth, and enhancing the barrier properties of the epidermis. The objective of this project is to accelerate the culture period and reduce the cost of developing biomimetic skin substitutes.

KEYWORDS: Fibroblasts, Keratinocytes, Collagen, Tissue Engineering, Bioreactor System



Biomimetic Skin Substitutes with Self-Designed Bioreactor System

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Application of porcine-derived cartilage extracellular matrix to enhance the therapeutic efficacy of rheumatoid arthritis drug

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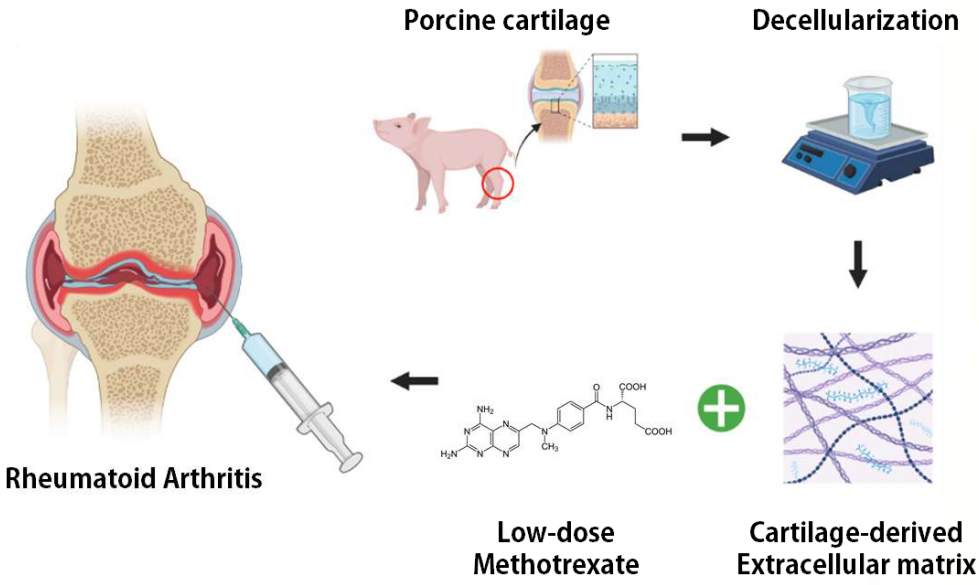
Abstract:

Rheumatoid arthritis (RA) is a chronic inflammatory disorder affecting joints and other organs. The global rheumatoid arthritis market is expected to grow in the coming years due to the increasing prevalence of rheumatoid arthritis, the rising geriatric population, and increasing research and development activities. Methotrexate (MTX), a disease-modifying anti-rheumatic drug (DMARD), is often used for RA treatment and can be combined with other biological DMARDs for increased effectiveness. However, prolonged use of DMARDs may result in adverse effects and limited therapeutic outcomes.

This study focused on decreasing MTX's dose and side effects. Cartilage extracellular matrix (CECM) has anti-inflammatory and anti-vascular properties and stimulates stem cell migration, adhesion, and differentiation into cartilage cells. When extracted from porcine cartilage and combined with MTX, CECM demonstrated no effect on synovial cells (SW 982), but efficiently suppressed macrophage cell (RAW 264.7) activity. Furthermore, low-dose MTX mixed with CECM led to enhanced anti-inflammatory effects. In a mouse model of collagen-induced arthritis, low-dose MTX mixed with CECM resulted in a significant reduction in RA-related cytokines in the blood and provided the most favorable cartilage preservation outcomes compared to other groups. In summary, using CECM as an enhancer for RA treatment can potentially improve therapeutic outcomes, decrease drug side effects, and encourage joint tissue regeneration.

KEYWORDS: Rheumatoid arthritis, inflammation, extracellular matrix

Graphic abstract



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Reduce cytotoxicity induced by fine particulate matter (PM2.5) via transporting mitochondria to human cardiomyocyte cells exposure to PM2.5

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Abstract:

Fine particulate matter (PM 2.5) is deemed a carcinogenic factor and one of the most dangerous health causes with persistent high ambient concentrations. Researchers are continuing to explore the toxicity mechanism and also solutions to regulate the effect of harmful components of PM2.5.

Our goal was to reveal mitochondria as a therapy to prevent and destroy fine particle exposure. In this study, we used human myocardial cells (AC16) exposed to PM2.5 as a positive control and evaluated the effects on proliferation, apoptosis, cellular aging, and reactive oxygen species (ROS) production.

Firstly, we found that PM2.5 have affected to viability at concentrations 46.64ug/ml, also significantly different gene expression profiles of cells exposed to PM2.5 and obtained related to metabolic pathways by microfluidic dynamics culture system. The results showed that PM2.5 may be relevant oxidative stress in cell mitochondria.

Then, mitochondria were isolated from healthy donors and transported without needing an additional source with only one step within 5 minutes at a normal centrifugal rate (2000xg) into PM2.5 administrated AC16 cells. To discovery our goals, the confocal images and flow cytometry results showed an efficiency of replacing and removing damaged mitochondria that were affected by PM2.5. On the other, ATP production-related OCR was investigated for metabolic activity of recipient cells that recovered by transferred mitochondria. Through the transfer directly healthy mitochondria from donors into PM2.5-exposured AC16 cells by general centrifugation in the laboratory, we established a simple and efficient approach to the transfer and treatment that may offer a platform for mitochondria therapy in the future

KEYWORDS: Mitochondria, AC16, PM2.-exposure cells, delivery, centrifuge.

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Mechanism of cutaneous wound repair in nude mice skin by picosecond laser-inducedoptical breakdown combined with polymer dots dressings

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MOST 110-2113-M-039-007

Abstract:

The human skin is the outer covering of the body in protecting the body against stimulation from the environment and temperature regulation. Skin damage is a relativelycommon clinical manifestation. Skin wounds not only damage the barrier function of the skin but also affect the ability of the skin to sense temperature, pain, and touch. Most cutaneous wound can repair naturally, however, in patients with chronic diseases or the elderly, wounds ofdifferent etiologies sometimes stagnate a state of pathologic inflammation due to a lack of cellfunction, and low levels of growth factors and result in chronic wounds. In thisstudy, weuse a picosecond laser combined with polymer dots (PDs) in wound healing therapy. Picosecondlaser can produce laser-induced optical breakdown (LIOB) on the skin, excites carriers throughthermal energy, and then oscillates at the junction of the skin epidermis and dermis(photomechanical effect) to induce microscopic vacuole production, which induces TGF-β smadpathway stimulates collagen proliferation and tissue repair.[1] Furthermore, PDs has shown great potential in various biomedicine applications and its sizes were below 5 nm with several distinct merits, such as fluorescence properties, low cytotoxicity, anti-inflammation, and bacteriostasis.[2] PDs can induce the epithelial-mesenchymal transition (EMT) process via promote the activation of the TGF-β non-samd pathway. Increasing the migration of epithelialcells and further promoting cutaneous wound healing. Both activate the TGF-β smad/non-smad pathway to promote wound healing, respectively. Therefore, to verify whether the wound repair triggered by the two different mechanisms can produce a synergistic effect, this study established a nude mouse wound healing experimental model and histological examination to evaluate the wound healing effect of picosecond laser and PDs in nude mice. It has been proved that the combination of picosecond laser and PDs can promote EMT, accelerate epithelial cell migration, rapid re-epithelialization to form the epidermal barrier, reduce inflammation and collagen reorganization and regeneration, and achieve wound healing with less scarring.

KEYWORDS: Laser-induced optical breakdown (LIOB), polymer dots (PDs), wound healing, epithelial-mesenchymal transition (EMT)

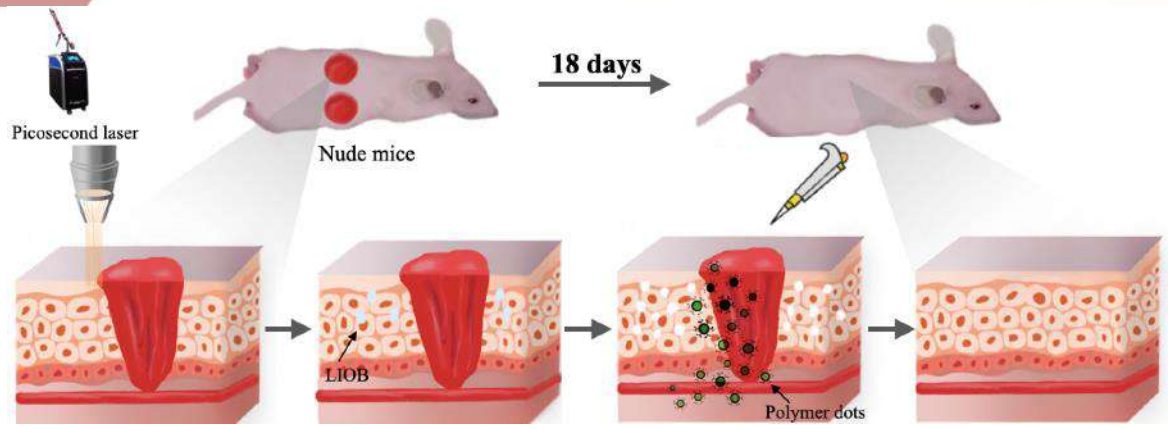

Figure 1. Picosecond laser combined with polymer dots (PDs) in wound healing therapy.

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Improving cartilage reconstruction using the cell sheet engineering

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Abstract:

Osteoarthritis is the most prevalent cause of human disability nowadays, with a current global population of 240 million. Nevertheless, aside from adjuvant therapy for the initial symptoms, the only clinical option is the total knee replacement. Furthermore, present study remains restricted by technical constraints, resulting in it challenging to achieve autologous regeneration merely through stimulation. Consequently, in recent decades, cell therapy, which artificially provides cells, has emerged as one of the main academic avenues for cartilage regeneration.

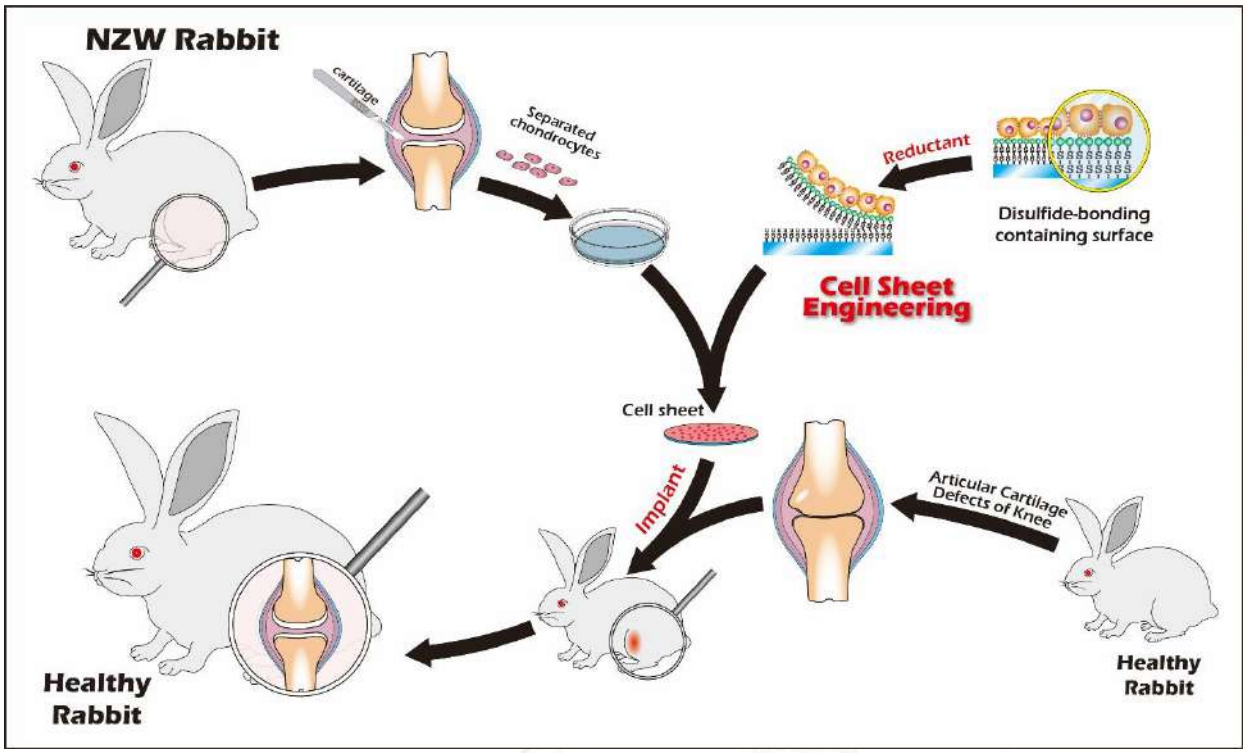
To improve the inefficiency of injecting cell suspensions and the adhesion rate and cell viability of transplanted cells, cell sheet engineering has arisen as an emerging development avenue of chondrocyte treatment. Nowadays, compared to the chondrocytes employed in this study, stem cells are riskier as they possess a larger capacity for regeneration. Based on the aforementioned factors, this study assumes that functional cells from the differentiated terminal have superior therapeutic utility, and as a result, it explores the possibility of utilizing a chondrocyte sheet to restore function to damaged cartilage tissue.

In our study, we develop an experimental cultured insert with favorable cell growth and a detach function to culture cell sheet. Initially, the functional insert for culturing the chondrocyte sheet needs to undergo a low-pressure carbon dioxide plasma reaction, and then polyglutamic acid is grafted. The results demonstrate that the surface stiffness of the functional culture insert is diminished from 878.6 MPa to 1.5 MPa, a finding that is supported by other approaches such as the Atomic Force Microscope, Surface Electron Spectroscopy for Chemical Analysis, and water contact angle measurement.

Finally, primary chondrocytes are cultured into cell sheets and successfully detached in this study. The cells are still viable after detachment, and the survival period after detachment is observed to be up to three weeks. In the future, we are going to investigate the curative effect of lamellar chondrocytes on knee articular cartilage repair in rabbit model animals, as well as attempt to utilize artificial intelligence navigation for minimally invasive surgery, so that the transplantation can be accurately positioned on the affected part to improve effectiveness and reduce side effects of surgery. This method is expected to aid in cartilage reconstruction.

KEYWORDS: Cartilage Reconstruction, Regenerative Medicine, Cell Therapy, Cell Sheet

Engineering, Bone Navigation Surgery



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Polydopamine-dressed 3D stem cell spheroid-derived decellularized extracellular matrix scaffolds with for promoting tissue regeneration

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Abstract:

Scaffolds for tissue engineering aim to mimic the native extracellular matrix (ECM), providing both physical support and biochemical signals to modulate multiple cell behaviors [1]. However, synthetic materials cannot precisely replicate the intricate composition and architecture of the native matrix, which contains essential physiological and biochemical factors [2]. To address this limitation, we developed three-dimensional decellularized ECM (dECM) scaffolds derived from mesenchymal stem cell (MSC) spheroids, offering an *in vivo*-like microenvironment with crucial biochemical signals and physical support for cells. To enhance the bioactivity of dECM scaffolds, polydopamine (PDA), a versatile and biocompatible material with antioxidant and anti-inflammatory potentials [3-6], was utilized as a surface modifier for dECM scaffolds. Additionally, the strong adhesive characteristics of PDA can act as a binder to promote the assembly of multiple dECM scaffolds into a tissue engineering scaffold. Consequently, dECM-PDA scaffolds hold tremendous potential to revolutionize scaffold-based tissue engineering applications, enabling the development of complex and functional tissue constructs with enhanced bioactivity.

KEYWORDS: stem cell spheroids, cell-derived matrix, polydopamine, surface modification, mesenchymal stem cells, tissue engineering scaffolds.

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3D bioprinting electrically conductive bioink on the application of Neural Tissue Engineering

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Abstract

Neurological damage is a severe injury, for which there is currently no ideal treatment. Several kinds of scaffolds have been developed to mimic the shape and structure of the natural nerve tissue. However, how to improve the biological function of neural cells remains a major challenge.

In recent years, 3D bioprinting scaffold have gained significant popularity as a method for *in vitro* models that can effectively replicate the neural growth environment. This study endeavors develop a bioink suitable for 3D printing by incorporating a conductive material mimic the 3D nerve environment. We aim to investigate the neuronal differentiation following 3D printing and electrical stimulation.

In this work, we modified silk fiber using glycidyl methacrylate (GMA) to prepare photocrosslinkable methacrylate silk fibroin (SilMA)[1]. Subsequently, we integrated pectin extracted from fruit peels and MXene-soybean phosphate, a conductive material modified with soybean phosphate, to fabricate a series of hybrid bioinks. Sil-MA is a renowned for its remarkable biocompatibility and superior mechanical properties. By addition with pectin, the printability of the bioink can be improved. Moreover, Modification of MXene with soybean phosphate improves its dispersibility in water, thereby increasing the concentration of MXene in the hydrogel and enhanced dispersion.

Following Fourier-transform infrared spectroscopy (FT-IR) and Nuclear Magnetic Resonance(H-NMR) were used to identify the GMA modification on silk fibroin, which demonstrated the successful modification of GMA. Additionally, the exceptional dispersion of the modified MXene-SP was confirmed through Transmission electron microscopy(TEM), and the microstructure of the hydrogel was observed via Scanning Electron Microscopy(SEM). Furthermore, the mechanical properties, rheological behavior, and biocompatibility of the hydrogel were evaluated. Finally, the neural stem cell spheroid laden 3D bioprinting was used to fabricate the construct and the neuronal cell differentiation was observed, and the effect of electrical stimulation were also determined.

It is suggested that hydrogels loaded with conductive materials may exhibit a higher degree of neuronal differentiation compared to hydrogels without conductive materials. Moreover, external electrical stimulation may further enhance the neurite outgrowth and functionality[2]. In the future, it holds the potential to develop more complex 3D *in vitro* models and apply them practically in neural tissue repair.

Keyword: methacrylate silk fibroin (SilMA), Pectin, conductivity, neural stem cells, differentiation

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3D spheroids of bone marrow mesenchymal stem cells ameliorate traumatic brain injury by alleviating neuroinflammation and glutamate excitotoxicity

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Abstract:

Traumatic brain injury (TBI) is a leading causes to brain damage that results in serious neurological complications. TBI triggers multiple complex pathological reactions that cause primary and secondary injuries, leading to glutamate excitotoxicity, oxidative stress, and neuroinflammation.[1–3] Although recent studies have shown that stem cells hold promise for alleviating glutamate excitotoxicity post-TBI,[4–6] the low efficacy of cell transplantation remains unsatisfactory. Recent reports confirm that aggregation of MSCs into three-dimensional (3D) spheroids results in enhanced paracrine secretion, anti-inflammatory, and neuroprotective potentials.[7–9] Herein, we investigated the capability of 3D spheroids of mouse bone marrow mesenchymal stem cells (BMSC) in protecting central nervous system against glutamate excitotoxicity by *in vitro* studies and an *in vivo* mouse TBI model. Our results showed that 3D BMSC spheroids effectively rescued cortical neurons from exogenous glutamate-induced excitotoxicity *in vitro*. Moreover, co-culturing astrocytes, cortical neurons, and BMSCs under glutamate treatment showed alleviated excitotoxicity on cortical neurons, which could be attributed to the increased expression level of glutamate transporter in astrocytes after receiving the 3D BMSC spheroid-derived secretome. In the *in vivo* study, the transplantation of 3D BMSC spheroids in a mouse TBI model effectively alleviated neuron excitotoxicity, demonstrating a superior neuroprotective potential. In summary, our findings demonstrate the therapeutic potential of 3D BMSC spheroids, which can modulate the uptake of glutamate by astrocyte and thus effectively alleviate post-TBI cortical neuron excitotoxicity.

KEYWORDS:

Stem cell spheroids, bone marrow mesenchymal stem cells, glutamate excitotoxicity, traumatic brin injury

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Development of nerve guidance conduit with spatial gradients of Schwann cells for repairing peripheral nervous system

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Abstract:

The injury of peripheral nerves leads to physical, psychological, and economic burdens on patients and their family [1]. Recently, nerve guide conduits that incorporate topographical, chemical, and biological cues have been developed to provide a favorable environment for nerve regeneration. For example, conduits with growth factor gradients have been developed to direct cell migration and neurite extension [2]. However, the incorporation of a single or even a few types of neurotrophic factors in conduits achieves limited therapeutic outcomes. In injured peripheral nerves, Schwann cells are known to support nerve regeneration by secreting a broad spectrum of factors that enhance neuronal survival, promote axonal regeneration and modulate macrophages [3]. In this study, we presented a method for generating gradients in Schwann cell density on a membrane composed of aligned fiber, which was then rolled up to create conduit. To evaluate their potential to promote neurite sprouting, rat dorsal root ganglions (DRGs) were isolated and seeded into the fabricated nerve conduits. Compared to the group with a uniform Schwann cell density, the conduit with a spatially-graded distribution of Schwann cells significantly promoted neurite extension of DRG toward the end with higher cell density. In summary, by engineering spatial gradients of Schwann cells in nerve conduits, the efficiency of neurite extension can be significantly improved, making it a promising strategy for accelerating peripheral nerve regeneration.

KEYWORDS: nerve guide conduit, spatial gradient, Schwann cells, peripheral nerve regeneration

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Transplantation of 3D spheroids of adipose-derived stem cells promotes rabbit Achilles tendon healing by enhancing tenocyte proliferation and suppressing M1 macrophages

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Abstract:

Tendons possess limited regenerative potential, which necessitates a prolonged healing period, and inevitably results in fibrotic scarring, leading to suboptimal prognosis. Stem cell-based therapies have been considered as a promising approach to accelerate tendon repair [1]. However, direct administration of stem cells to the site of injury results in limited therapeutic outcomes due to significant cell loss from target tissue [2]. To improve the post-engraftment survival rate and cellular functionality, the present study aimed to explore the feasibility of using three-dimensional (3D) spheroids of adipose-derived stem cells (ADSCs) to promote tendon repair.

Primary ADSCs isolated from rabbit fat pads were seeded into 12% methylcellulose (MC) hydrogel-coated plates and incubated for 24 h for spheroid formation. The harvested ADSC spheroids were intratendinously transplanted into the sutured stumps of a rabbit model of Achilles tendon rupture. After four weeks, we observed that transplantation ADSCs in a 3D spheroid configuration was more effective in promoting the healing of sutured stumps than delivery of conventional single-cell suspensions. Next, a series of *in vitro* experiments were conducted to explore the potential mechanisms underlying the observed *in vivo* therapeutic effects. We found that ADSC spheroid-derived conditioned medium (CM) effectively promoted tenocyte proliferation and migration through activating the ERK signaling. Moreover, the ADSC spheroid-derived CM significantly suppressed the expression and secretion of pro-inflammatory cytokines in lipopolysaccharide-stimulated mouse bone marrow-derived macrophages *via* the COX-2/PGE2/EP4 signaling axis. In summary, the results of this study demonstrate the effectiveness of using ADSCs in a 3D spheroid configuration to enhance tendon regeneration. These findings thereby lay a foundation for future clinical applications of stem cell spheroid-based therapy for the management of tendon injury.

Keywords: tendon regeneration, tenocytes, adipose-derived stem cells, cell therapy, cell spheroids, immunomodulation

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Regulation of differentiation potential and sub-population by histone trimethylation and HDAC5 during spheroid formation of human adipose-derived stem cells

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Abstract:

Adipose-derived stem cells (ASCs), known as multipotent mesenchymal stromal cells, are widely used in *regenerative medicine* and clinical application in recent years. Our previous work have demonstrated that chitosan induced spheroid formation and differentiation potential of human ASCs (hASCs) into a mixed population of neural lineage-like cells which possess therapeutic efficacy [1]. However, the underline mechanisms of neuronal differentiation potential of hASCs is still unknown. The development and repair of the nervous system requires the coordinated expression of several specific genes. Epigenetic factors, such as histone modifications, are known to play a pivotal role in controlling adult stem cell self-renewal and differentiation [2] (1). Here, we aim to explore the epigenetic regulation and differential gene expression profiles of neurospheroids induced from hASCs.

During spheroid formation of hASCs, cell cycle arrest and epigenetic marks (histone trimethylations) were induced. Reduced histone acetyltransferase activities, increased histone deacetylase activities and nuclear translocation of histone deacetylase 5 (HDAC5) in spheroids were also observed. Inhibition of these epigenetic marks and HDAC5 resulted in a reduction in size of spheroids and neuronal marker expressions in spheroids. These findings indicated that these epigenetic marks and HDAC5 play a crucial role in spheroid formation and differentiation potential of hASCs. In addition, the scRNA-seq and cell fate trajectory analyses identified an endpoint state and a transient state of subgroups differentiated from hASCs. The predicted network from IPA analysis and the results of inhibition experiments indicated that histone

trimethylation were major players in regulating gene expression while HDAC5 had a lesser impact on the differentiation potential in the specific transient subgroup. These findings indicate that epigenetic regulators, such as histone methylation, may have a more prominent role in driving the spheroid formation and differentiation potential of hASCs. The identification of key regulatory genes and pathways involved in spheroid formation and neural differentiation of hASC can inform the development of novel strategies for regenerative medicine. By targeting and manipulating these regulatory mechanisms, it may be possible to enhance the therapeutic potential of hASC for tissue repair and regeneration.

Keywords Histone trimethylation, HDAC5, differentiation potential, sphere formation, adipose-derived stem cells, regenerative medicine.

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Enhanced β cell survival in subcutaneous space after co-transplantation of 3D stem cell spheroids with pro-angiogenic and anti-apoptotic potential

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Abstract:

Type I diabetes is an autoimmune disease characterized by the impairment of insulin-secreting β cells by patient's own immune system [1]. While exogenous insulin injection is a common therapy, it may not precisely mimic the endogenous insulin secretion profile [2]. Islet transplantation, which involves the infusion of exogenous islets into the portal vasculature of liver, has been reported to make patients insulin independent [3]. However, the loss of infused cells over time leads to a gradual loss insulin independency, making the portal vasculature a less optimal site for maintain long term viability of engrafted cells [4]. Alternative sites for cell transplantation have been studied, with the subcutaneous space emerging as a superior site for islet transplantation [5]. However, slow neovascularization at subcutaneous sites results in limited oxygen and nutrition supply, leading to the death of transplanted islets [6]. In the present study, three-dimensional (3D) cell spheroids of mesenchymal stem cells and vascular endothelial cells were fabricated and employed for co-transplantation with β cells into subcutaneous space. Our *in vitro* results demonstrated that the prepared 3D stem cell spheroids secreted multiple proangiogenic and prosurvival factors, promoting angiogenesis and preventing β cell death. Co-transplantation of 3D stem cell spheroids with β cells effectively promoted local neovascularization, thereby enhancing the survival of engrafted β cells. These results emphasize the effectiveness of 3D stem cell spheroids in promoting the survival and function of subcutaneously transplanted β cells.

KEYWORDS: Type I diabetes mellitus, umbilical cord blood mesenchymal stem cell, human umbilical vein endothelial cells, 3D cell spheroid

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Cell Screening Approaches for Cochlear Progenitor Cells: Pre-Plate and Lgr5 Binding Protein as Antibody-Free Alternatives

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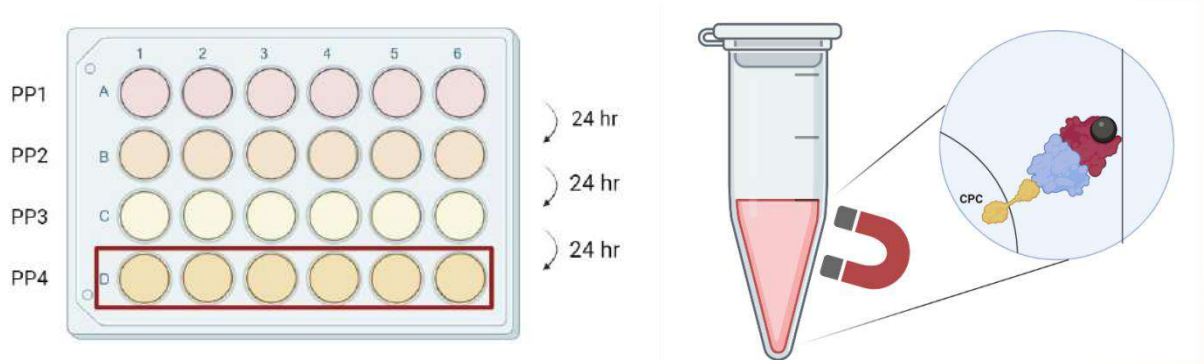
Abstract:

Sensorineural hearing loss is predominantly attributed to the irreversible damage incurred by auditory hair cells, making them a focal point of investigation in hearing loss research. However, a significant constraint in studying auditory hair cells stems from their limited regenerative capacity in mature mammalian organisms. This constraint imposes limitations on the availability of research-ready cells for comprehensive studies in this field. [1, 2] In pursuit of expanding research opportunities in the field of auditory hair cells, considerable efforts have been dedicated to the isolation of cochlear progenitor cells (CPCs). These specialized cells hold the remarkable capability to replicate and undergo differentiation into functional auditory hair cells, even in the postnatal stage. [3] The objective behind these endeavors is to establish methodologies for in vitro production and cultivation of hair cells, enabling further exploration and advancement in auditory research.

Numerous studies have elucidated the significant role of Lgr5, a pivotal protein expressed on cochlear progenitor cells (CPCs), in the process of hair cell differentiation. Conventionally, the identification of Lgr5 protein is achieved through antibody-based affinity sorting methods. However, the challenge lies in the subsequent detachment of antibodies from isolated CPCs without compromising their viability. In light of this challenge, we propose two alternative approaches: the Pre-plate method and the utilization of Lgr5 binding protein (LBP) as a targeting agent. Both methods have been devised to provide gentler yet effective strategies for CPC isolation, maintaining cell viability and preserving their regenerative potential.

KEYWORDS: cochlear progenitor, pre-plate, Lgr5, binding protein, cell screening

A B



Graphic Abstract: Strategies for Cochlear Progenitor Cell Isolation. (A) Pre-plate method: A physical cell screening approach harnessing the differential adhesion characteristics of distinct cell types. Cochlear progenitor cells have been observed to form non-adherent spheroids, enabling their isolation by allowing non-target cells to adhere to a 24-well plate. (B) Lgr5 binding protein coupled with magnetic beads: The specific binding of Lgr5 binding protein to cochlear progenitor cells facilitates their isolation using a magnet.

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Acknowledgements

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Effect of Near-infrared Laser Irradiance Photobiomodulation on Mitochondria Membrane Potential for Different Passages of Human Adipose-derived Stem Cell

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Abstract:

Wounds are usually healed within a few months for healthy people. However, for those who have diseases such as diabetes or larger wound areas, the healing process can be slow or even non-existent. To enhance chronic wound healing process, low-level laser therapy (LLLT), now commonly referred to as photobiomodulation (PBM), is a potential solution. LLLT utilizes a specific wavelength of light as a signal for cells to create a cellular environment prone to cell proliferation and migration, thereby accelerating wound healing.

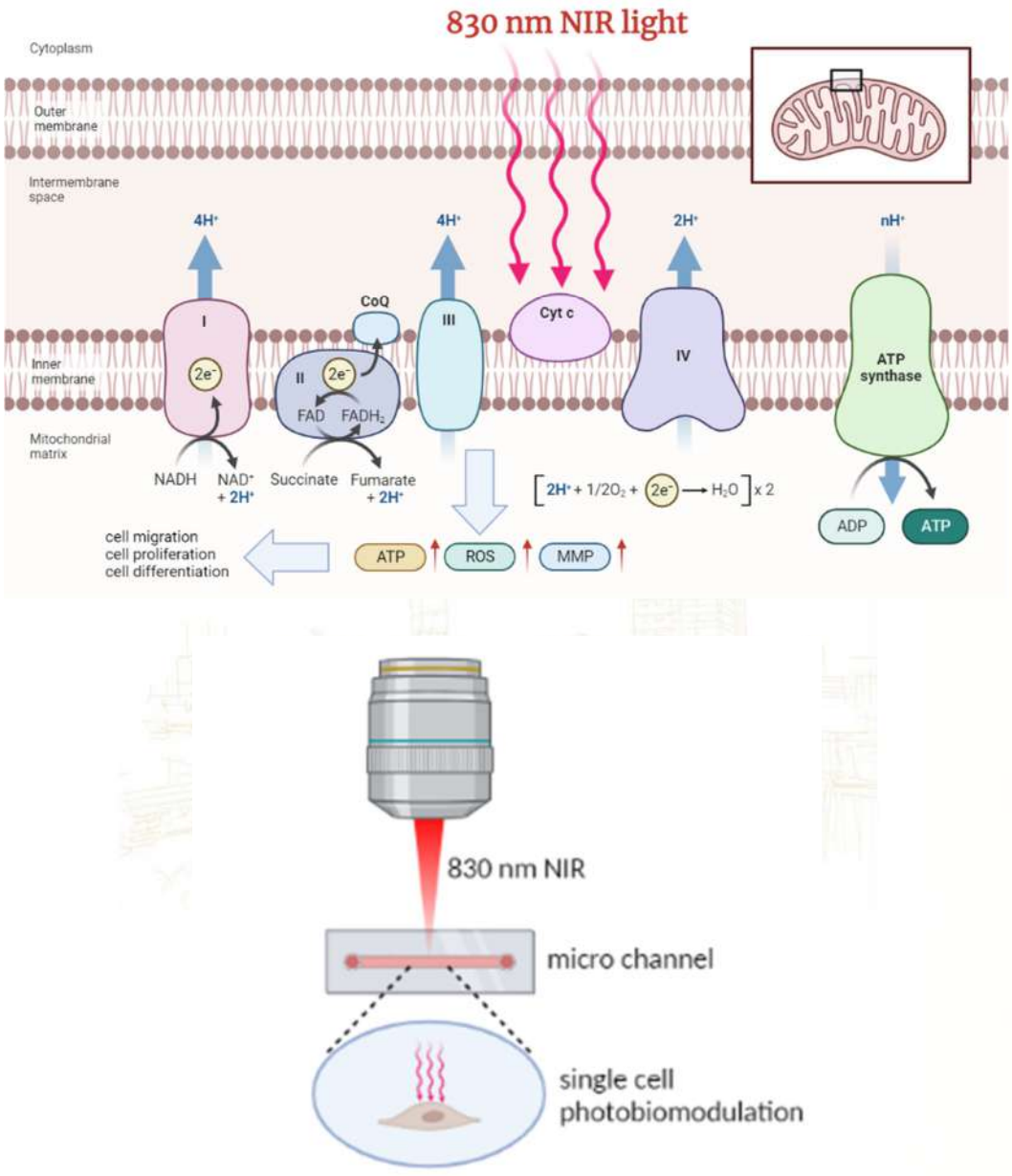
Stem cells are commonly used in tissue engineering to promote wound healing. Human adipose-derived stem cells (hADSCs) are more easily to obtain large quantities and have less invasive extraction procedures compared to bone marrow-derived mesenchymal stem cells (BMSCs). Therefore, hADSCs held great potential in tissue engineering. As the mechanism of action is not yet fully understood, ongoing research aims to shed more light on this topic.

In this study, we used an 830 nm laser to investigate the changes in mitochondria membrane potential (MMP) of hADSCs before and after light exposure. We employed different irradiances (W/cm^2) and exposure times (s) under the same dosage (J/cm^2), as well as different cell passages. One objective was to determine whether altering the irradiance had a significant effect on the changes in mitochondrial membrane potential.

First, we made micro-channels and seeded cells inside for single cell photobiomodulation. We used three types of modulation modes to irradiate specific passages of hADSCs. After capturing fluorescent images stained by Rhodamine 123 (Rh 123), we analyzed the results by comparing the mean intensity ratio of mitochondrial membrane potential before and after irradiation at the same single living cells. We found out there were significant differences between the control group and the irradiation group, suggesting these methods could serve as precision photobiomodulation modes for human adipose-derived stem cells in the future.

KEYWORDS: Photobiomodulation (PBM), Low-level laser therapy (LLLT), Human adipose-derived stem cells (hADSCs), Near-infrared (NIR) laser light, Irradiance, Mitochondria membrane potential (MMP)

Graphic abstract:



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Fabrication of 3D adipose tissue using engineered composite spheroids

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Abstract:

The use of 3D cell spheroids incorporating biomaterials have become increasingly popular due to the ability to control cell functionality by delivering signals directly to the spheroid. Recently, researchers have focused on the development of 3D artificial adipose tissue for reconstruction. However, the fabrication of 3D adipose tissue has been challenging due to the low viability of mature adipocytes and the complex components required for adipogenic differentiation (such as IBMX, indomethacin, dexamethasone, and insulin). Furthermore, high vascularization mimicking natural adipose tissue has been difficult because differentiation medium prevent endothelial cells from sprouting for vascularization.

In this study, we fabricated 3D vascularized fat tissue using adipo-inductive nanofibers incorporated co-culture spheroid of human adipose tissue-derived stem cells (hADSCs) and human umbilical vein endothelial cells (HUVECs) (3:1) in Gel-MA hydrogel. Adipogenic nanofibers (AF) were created by immobilizing insulin onto indomethacin-incorporated nanofibers (IF) using polydopamine. AF was then incorporated into co-culture spheroids with hADSCs and HUVECs. The cells encapsulated spheroids not only proliferated and sprouted actively in Gel-MA hydrogel, but also differentiated into mature adipocytes with homogeneously many lipid droplets and high vascularization in the hydrogel. These 3D vascularized adipose tissue construct will be applied as a 3D artificial adipose tissue model for treatment and reconstruction.

KEYWORDS : 3D adipose tissue, hADSCs, HUVECs, Spheroid, Gel-MA hydrogel

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MicroRNAs-mediated cartilage regeneration using a lithium-containing calcium silicate bi-layered scaffold laden with exosome-based therapy

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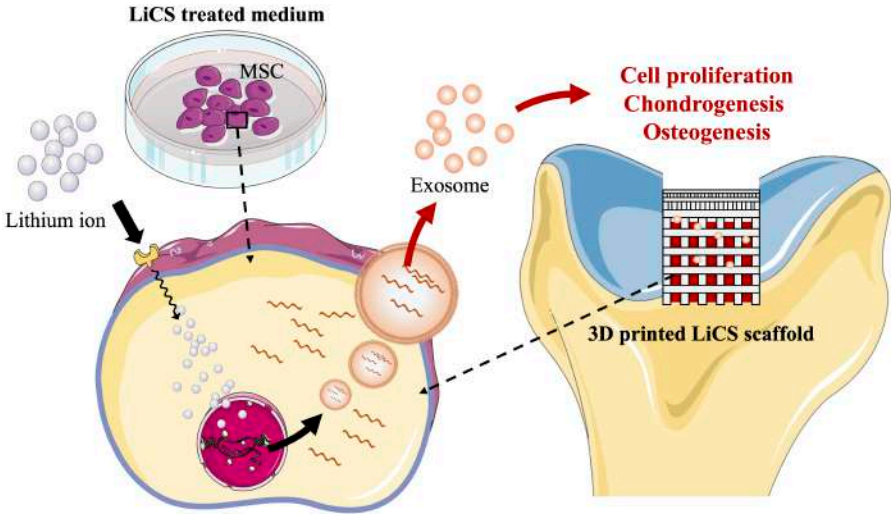
Abstract:

Cartilage tissue structure may be impacted or pulled by external forces; osteonecrosis of joints caused by bone infarction or ischemia; severe degenerative arthritis [1]. Especially in arthritis, which is the most common geriatric disease, the morbidity rate increases significantly with age. The existing repair surgery is not effective for the regeneration of newly formed tissue. Studies have confirmed that the regeneration part is mostly fibrous tissue and fibrocartilage, so the subchondral bone cannot be effectively reconstructed, and it tends to deteriorate in long-term follow-up results. At present, the international trend of osteochondral transplantation is to use double-layer scaffold osteochondral transplantation. The subchondral bone supporting structure can provide blood flow, and the material has the function of providing osteoinduction [2]. Therefore, in this study, a lithium-containing calcium silicate ceramic support (LiCS) scaffold was used as the main material for the lower layer of osteochondral regeneration, and the decellularized cartilage matrix bioink was used to carry cellular exosomes as the upper cartilage layer for repair. This technology uses 3D printing technology to prepare a two-layer biological scaffold porous scaffold, which can accurately control the wire diameter and porosity of the scaffold. In addition to establishing the pore connectivity of LiCS, it can also construct dual materials on a single scaffold to achieve two Robust mechanical integration between the layers. The in vitro soaked microstructure of LiCS has deposited apatite-like spheres, indicating that the scaffold has excellent biological activity. In addition, it has suitable mechanical strength and degradation properties. The identification of cartilage differentiation-related proteins and the specific staining of cartilage extracellular matrix were used to determine that lithium-containing calcium silicate scaffolds were beneficial to cartilage differentiation. The exosome size can be calculated to be approximately 92.5 nm from the nanoparticle tracking analyzer. From the analysis of the miRNA heat map carried by exosomes, it was found after quantitative analysis that a total of 3150 genes were up-regulated, and the expression of cartilage-related genes and immune regulation-related genes were all related. It is hoped that this technology can accelerate cartilage regeneration.

KEYWORDS: calcium silicate, lithium, exosomes, bilayer scaffold, 3D printing

Graphic abstract



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Dermal fibroblast-laden 3D-printed electroactive hydrogels for enhancing cutaneous wound healing through electrical stimulation

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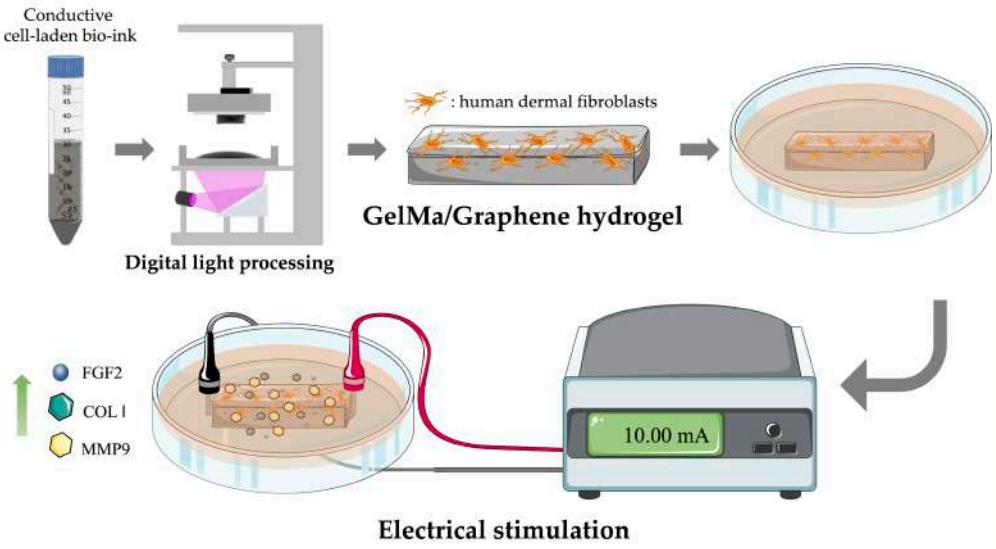
Abstract:

The skin is the body's largest organ and acts as a protective barrier against various threats. Cutaneous injuries require a complex healing process, and interruptions in this process can significantly hinder wound closure, especially in cases of large wounds or patients with underlying conditions like diabetes. Consequently, there is a pressing need for effective therapies to address cutaneous injuries [1]. Hydrogels have emerged as a promising approach for cutaneous wound healing due to their simplicity in fabrication and their ability to incorporate biological molecules. These hydrogels not only serve as temporary skin replacements but also have the potential to enhance tissue regeneration. Moreover, they create an ideal healing environment conducive to the regeneration of damaged skin [2]. Recent research has highlighted the potential benefits of incorporating electroactivity into hydrogels to further enhance cellular activities. Conductive hydrogels, in particular, have shown promising results in promoting wound healing. The introduction of electrical stimulation has been found to guide cellular behavior and improve the overall healing process [3]. In this study, GelMA combined with graphene was used to fabricate conductive hydrogels using a digital light processing (DLP) three-dimensional printer. The hydrogel encapsulates human dermal fibroblasts, which are subsequently subjected to electrical stimulation. Physical properties, biocompatibility, and biodegradability assessments were performed to evaluate the effectiveness of the composite scaffolds. By harnessing the advantages of hydrogels and integrating electroactivity, novel therapeutic approaches may be developed to effectively address cutaneous injuries and improve patient outcomes. This strategy holds promise for future applications in wound healing and skin tissue regeneration.

Graphic abstract

KEYWORDS: Gelatin-methacrylate, graphene, electrical stimulation, wound healing

Graphic abstract



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Harnessing the multifunctional of ADSC-derived exosomes for accelerating healing of diabetic chronic wounds

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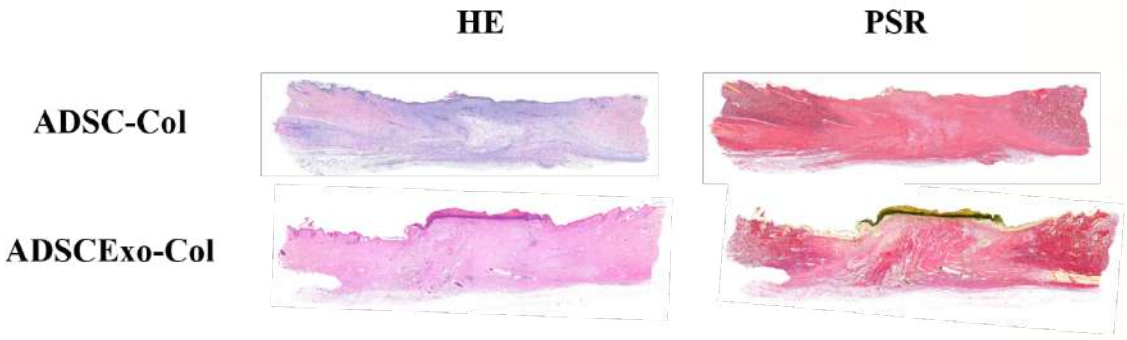
Abstract:

Chronic wounds have emerged as a significant focus in contemporary healthcare, encompassing conditions such as diabetic foot ulcers, peripheral artery ulcers, autoimmune disorders, varicose veins, pressure ulcers, burns, radiation injuries, and paralysis. These chronic diseases often progress to chronic wounds, which are associated with high rates of complications and mortality, frequently leading to substantial healthcare costs and a burden on caregiving resources [1]. In recent years, with advancements in biotechnology, extensive research has been conducted on stem cell therapy and exosome applications for chronic wound care. Additionally, studies on modulating M1/M2 macrophages to promote wound healing and incorporating exosomes have significant implications for future trends in chronic wound treatment [2]. Therefore, the objective of this study is to develop a biofabricated skin matrix capable of regulating exosome release behavior. This skin substitute aims to temporarily replace the protective function of natural skin, preventing moisture loss, providing insulation, and safeguarding against external injuries, including physical trauma and bacterial invasion. Adipose-derived stem cells (ADSC) were used as the source of exosomes, as they are commonly utilized in clinical practice for treating challenging wounds and can modulate wound healing at different stages. The bioprinted skin matrix was engineered to enable controlled and sustained exosome release, offering diverse functionalities to promote wound healing. Our result showed that the histological evaluation of animal skin sections stained with HE and PSR revealed distinct results for ADSC-Col (ADSC-laden matrix) and ADSCExo-Col (ADSC-derived exosome laden in matrix) groups. In the ADSCExo-Col group, HE staining exhibited enhanced collagen deposition and organization compared to the control group, indicating the beneficial effects of exosome presence within the collagen matrix. Additionally, the PSR staining in the ADSCExo-Col group demonstrated increased collagen content, as indicated by the intense red coloration, further confirming the positive impact of exosome incorporation. All in all, this study explored the use of

exosomes as a vehicle for drug delivery and assessed their impact on wound healing, presenting a novel avenue for advancing wound healing through pharmaceutical interventions.

KEYWORDS: Chronic wounds, diabetic foot, ADSC, exosomes, wound healing



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Nano-Layered Magnetic Nanoparticles for Heat-Triggered Drug Release

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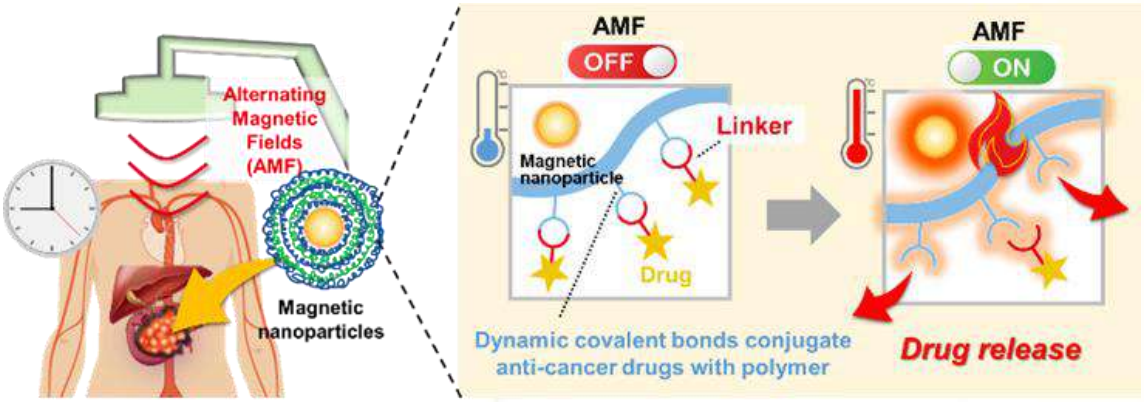
Abstract:

In recent years, diseases such as cancer, heart disease, and stroke, which are caused by a complex interplay of lifestyle, genetic factors, and intrinsic factors of each patient, have become the leading causes of death. Against this background, drug therapy and its precise spatiotemporal control in accordance with patient homeostasis and drug mechanism of action have become important [1]. Our laboratory has been developing smart drug delivery platforms which enable flexible drug release in response to external stimuli from outside the body. In this study, we focused on the Diels-Alder (DA) reaction, a temperature-responsive dynamic covalent bonding. Drugs were conjugated to the side chains of polymers via DA. The polymers were then coated on the magnetic nanoparticles by layer-by-layer (LbL) method. The system enables on-demand drug release by irradiation of an alternating magnetic field (AMF) [2].

To achieve this, poly(styrene sulfonate-*co*-furfuryl methacrylate)(poly(NaSS-*co*-FMA)) was synthesized which has polyanionic segment containing functional groups capable of DA to electrostatically coat the polymer on the surface of magnetic nanoparticles by LbL. The composition of the polymer was calculated by ^{1}H NMR measurement and contained up to 19% furan groups. The rate of linker incorporation via the DA into the furan group of the side chain was calculated from the increase in the integrals at 3.70 ppm (endo) and 3.38 ppm (exo), which are newly exhibited with the progress of the reaction. The retro DA was confirmed above 80°C, and the reaction at 90°C took approximately 10 min to complete. Subsequent verification of the thin film formation of poly(NaSS-*co*-FMA)/poly allylamine using quartz crystal microbalance confirmed that the crystal frequency decreased with each step, indicating that the thin film was formed by alternating layers. Furthermore, the stacking of polymers on the surface of magnetic nanoparticles by LbL successfully formed a thin film on the surface of magnetic nanoparticles by confirming an increase in particle size in addition to the inversion of surface charge by each polymer. Irradiation of the polymer-coated magnetic nanoparticles with an AMF suppressed the temperature increase of the aqueous dispersion of smart magnetic nanoparticles, suggesting that the temperature increase *in vivo* is suppressed. However, accurate temperature measurement of the magnetic nanoparticle surface still needs to be investigated. Therefore, we will confirm the usefulness of this system by controlling and measuring the local heat generation of magnetic

nanoparticles, evaluating the linkage of drug release, and evaluating the activity of the released drug.

KEYWORDS: Magnetic Nanoparticles, Layer-by-Layer, Heat-Triggered Controlled Release, Diels-Alder Reaction



Graphic abstract

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OSMOTIC CONCENTRATION OF URINARY LIPOARABINOMANNAN FOR RAPID AND SENSITIVE DETECTION OF TUBERCULOSIS

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Abstract:

Tuberculosis (TB) is a communicable disease caused by Mycobacterium tuberculosis (Mtb) infection. Conventional clinical and laboratory tests used to diagnose active TB, such as bacterial culture, sputum smear microscopy, and rapid molecular tests, suffer from limitations in sensitivity, accessibility, and speed[1]. Additionally, these tests require sputum samples, which pose challenges in collection procedures and safety concerns. The World Health Organization (WHO) has endorsed the lipoarabinomannan (LAM) antigen as an alternative biomarker for point-of-care TB diagnosis[1]. However, current lateral flow assays (LFAs) designed to detect urinary LAM exhibit sensitivity levels well below WHO standards, mainly due to the low concentration of LAM present in urine[2, 3]. To address this issue and enable sensitive and rapid urinary biomarker detection via LFAs, we introduce the osmotic processor—a device that employs osmosis to concentrate analytes[4]. The process efficiently removes water molecules from the urine specimen while retaining the target analyte.

In this study, we designed the osmotic processor to concentrate urine samples spiked with LAM at concentrations of 1280, 640, 160, 40, and 10 pg/mL. We then applied the samples, both before and after concentration, onto LFAs. Prior to osmotic concentration, the LFA was unable to detect LAM below 640 pg/mL in urine. However, after a 15-minute osmotic concentration, the LFA successfully detected LAM levels as low as 10 pg/mL in the original sample. Furthermore, we evaluated the osmotic processor using urine specimens from TB-positive patients. Prior to osmotic concentration, samples with lower LAM concentrations displayed faint LFA test bands, but after osmotic concentration, the test bands became significantly stronger. Similarly, samples with higher LAM concentrations exhibited increased test band intensity after osmotic concentration. These results indicate a substantial enrichment of urinary LAM through osmotic concentration, highlighting the viability of this method for processing clinical specimens. Beyond its application in TB diagnosis, the osmotic processor holds great potential for concentrating other types of biomarkers, such as DNA, proteins, and exosomes, for diagnostic, disease monitoring, and therapeutic purposes. Overall, our study demonstrates the efficacy of the osmotic

concentration technique and its broader implications in improving the accuracy and efficiency of urinary biomarker detection in various medical applications.

KEYWORDS: bioprocessing, osmosis, lateral flow assay, point-of-care diagnostics

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