3D Bioprinted Hybrid Hydrogel Scaffolds in Bone Tissue Engineering

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i. Project Summary

As the global organ transplant waiting list continues to grow, scientists are actively seeking alternative methods to address organ shortages. Tissue engineering, since its proposal in 1993, has emerged as a promising solution aimed at creating functional tissue replacements by combining principles from biology and engineering [1]. In this field, the advancement of 3D printing technology has revolutionized bone tissue engineering, particularly in the development of novel scaffold materials. The core of this proposal is to introduce our newly developed hybrid hydrogel scaffold material, fabricated using 3D bioprinting technology, for bone tissue engineering. These scaffolds are designed to mimic the complex microstructure of natural bones, aiming to provide ideal attachment points for bone cells while promoting bone tissue regeneration and repair [2].

Our research focuses on the unique properties of hybrid hydrogels, which combine the advantages of multiple biomaterials, including but not limited to the flexibility of polymers and the bioactivity of bioceramics. These characteristics enable hybrid hydrogel scaffolds to meet the requirements of bone conductivity, bone inductivity, and bone generativity while also providing necessary mechanical strength and biocompatibility. Although the prospects of 3D bioprinting technology in bone tissue engineering are promising, we also honestly discuss the current challenges faced in our proposal, including how to achieve effective vascularization of these scaffolds in host tissues, nutrient supply, and long-term mechanical stability. We explore the latest designs of biomaterials and how they help overcome these challenges, thus promoting the repair of bone defects.

In conclusion, we believe that through continuous research and technological innovation, 3D bioprinted hybrid hydrogel scaffolds will offer a novel approach for clinical treatment of bone defects, potentially significantly improving patient outcomes and quality of life.

ii. Intellectual Merit

The outcomes of this project will substantially enhance our understanding of biomaterial design in bone tissue engineering. Through a detailed analysis of cell interactions, biocompatibility, bioactivity, and the mechanisms of action in bone repair using mixed hydrogel scaffold materials, we will significantly contribute to the scientific knowledge base in this field. Furthermore, our research will provide valuable insights into the application of 3D bioprinting technology in tissue engineering, thereby advancing the development of personalized medical solutions.

iii. Broader Impacts

Our research addresses the critical societal need for improved treatment outcomes and quality of life for patients with bone defects and injuries, a demand that is growing due to an aging population and increased incidence of trauma. By advancing the application of 3D bioprinting technology in medicine, our project promotes personalized bone repair through the development of hybrid hydrogel scaffold materials that precisely mimic natural bone microstructure. This innovation not only enhances treatment efficacy but also drives economic growth by stimulating related industries such as biomaterials manufacturing, medical devices, and 3D printing technology, creating new job opportunities and contributing to overall economic development.

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1. Project Description

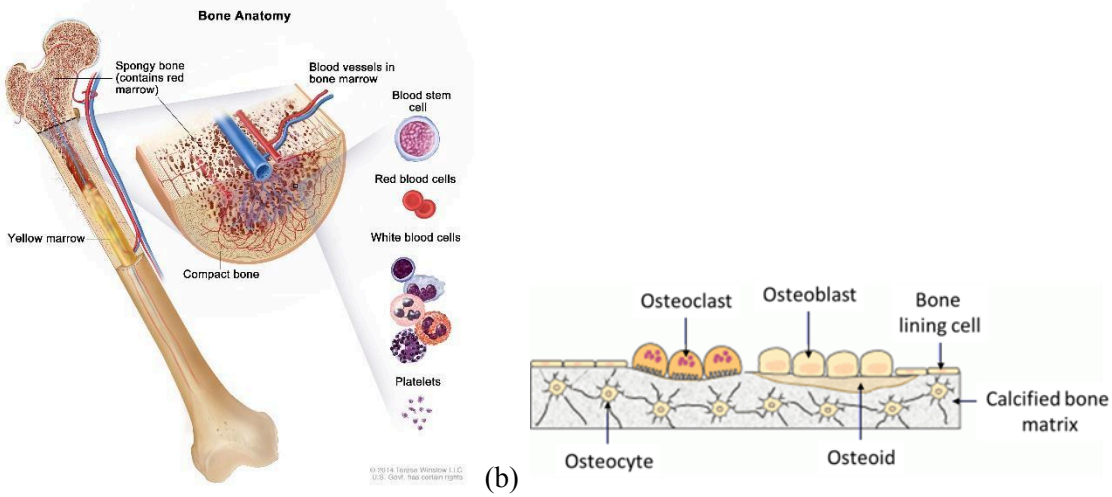
1.1 Background and Introduction

1.1.1 The Rise of Bone Tissue Engineering

The global shortage of organ donations is a growing problem. According to the U.S. Department of Health & Human Services, as of June 2017, there were about 120,000 patients in the United States who needed life-saving organ transplants, and only about 5,200 donors were available [3]. This supply-demand imbalance has led to long waiting lists and high death rates. Tissue engineering has been at the forefront of biomedical research since it was proposed in 1993 as a potential solution to the problem of organ shortage [1]. It involves combining engineering principles and life sciences to develop biocompatible materials that can replace or repair damaged tissues.

1.1.2 The Importance and Challenge of Bone Tissue Engineering

Bone tissue is composed of cancellous bone and compact bone, which have different structures and functions respectively as shown in Fig 1 (a). Cancellous bone has a porous structure, while compact bone is harder and denser. Both structures of bone participate in a dynamic remodeling process controlled by interactions between osteoblasts and osteoclasts where Fig 1 (b) states that osteoblasts are responsible for the formation of new bone, while osteoclasts are responsible for the absorption of old bone [4]. This bone remodeling process is essential for maintaining healthy bones. Although bone has a good ability to heal itself, large-scale bone injuries usually cannot be fully healed by the body itself [5].



(a) (b)

Fig 1. (a) Bone Anatomy: Compact bone makes up the outer layer of the bone. Cancellous bone is found mostly at the ends of bones and contains red marrow (taken with permission from [6]); (b) Different types of bone cells [7].

Traditional bone repair treatments, such as autograft and allograft, while effective in some cases, have problems with donor shortages, immune rejection, and the risk of disease transmission [8]. These limitations have prompted the medical community to seek alternatives, and bone tissue engineering offers a promising alternative. Bone tissue engineering restores, maintains, or improves function by synthesizing and/or regenerating bone tissue as shown in Fig 2, avoiding donor shortages, and reducing the risk of immune rejection. Compared to traditional surgery, this approach may reduce the risk of surgical complications, such as infection, bleeding, and anesthesia complications, and help restore bone function more fully. With the continuous progress of technology, bone tissue engineering has the potential to become a routine treatment for bone repair and regeneration, bringing better treatment results and quality of life to patients.

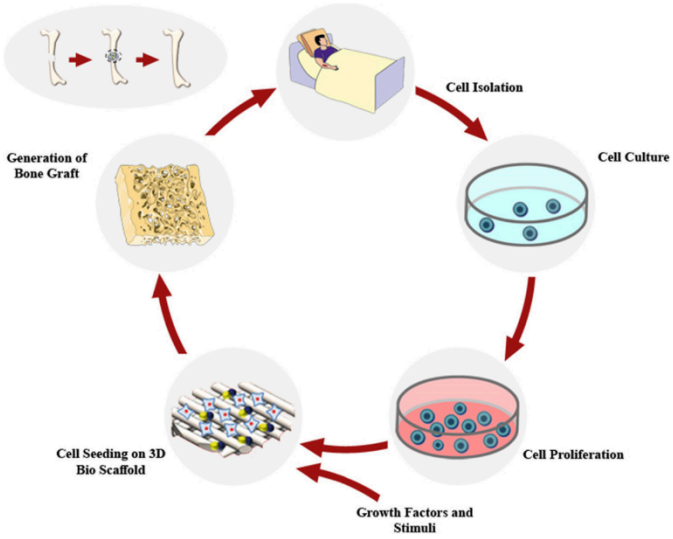


Fig 2. Bone tissue engineering: Derived stem cells from patients and seeding on bioscaffolds, cells then differentiate into osteoblast and produce bone grafts [9].

1.1.3 The Application of 3D Printing Technology in Bone Tissue Engineering

The introduction of 3D printing technology has brought revolutionary progress to the field of bone tissue engineering. Through precise regulation of the microstructure and physical and chemical properties of the scaffold, a complex and functional tissue engineering scaffold has been successfully created. These innovative scaffolds not only physically provide a stable support framework for new bone tissue, but also optimize the microenvironment for cell adhesion, proliferation and differentiation, significantly accelerating the process of bone regeneration and demonstrating unprecedented therapeutic potential [10].

Personalization is one of the highlights of 3D printing technology, which enables medical solutions to be tailored to each patient's unique anatomical characteristics and specific medical needs. This ability enables the 3D-printed bone scaffold to accurately match the patient's bone defect area, which not only improves the accuracy and success rate of treatment, but also greatly enhances the patient's comfort and rehabilitation experience, marking a major leap forward in personalized medical practice. The flexibility of 3D printing technology makes bionic design a reality, allowing engineers to replicate the fine structure of natural bone tissue, creating a growth environment that is highly similar to it. This design strategy not only enhances the structural and functional integration of the new bone with the original tissue, but also further catalyzes bone generation by simulating the mechanical properties and biological signals of natural bone.

1.1.4 Innovation and Potential of Hybrid Hydrogel Scaffold Materials

The choice of materials for 3D-printed scaffolds is critical to their biocompatibility and bioactivity. Researchers are developing and testing various biomaterials, including polymers, ceramics, and composites, to ensure that the scaffolds interact well with human tissues and support cellular activity and tissue growth [2]. Despite significant progress in the application of 3D printing technology in bone tissue engineering, existing scaffold materials still have limitations in simulating the structure and function of natural bone tissue. Traditional single-material scaffolds are often difficult to fully meet the requirements of biocompatibility, bioactivity, and complex structure [11]. To overcome these challenges, we propose a novel hybrid hydrogel material that cleverly combines natural and synthetic ingredients using alginate, gelatin, and cellulose nanocrystals (CNCs). This unique formulation not only ensures high biocompatibility, but also imparts excellent mechanical stability and regulated biodegradation properties

to the scaffold. Through careful design, the material can simulate the complex porous structure of natural bone, providing an ideal microenvironment to promote cell adhesion, migration, proliferation and differentiation, and accelerate the regeneration process of bone tissue.

Natural polymer hydrogels have garnered significant attention in the field of bioprinting, specifically due to their structural resemblance to the natural extracellular matrix (ECM). This similarity is crucial as it facilitates better support for cell adhesion, growth, and differentiation [12]. For example, cellulose, one of the most abundant biopolymers in nature, and its nanomaterials such as cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and bacterial nanocellulose (BNC), exhibit properties closely akin to the ECM and possess excellent rheological properties [13]. CNCs, derived from acid hydrolysis of cellulose, are particularly noteworthy for their high mechanical strength and low thermal expansion coefficient [12]. In addition to cellulose-based nanomaterials, alginate and gelatin—both of natural biological origin—are widely utilized in tissue regeneration. Their excellent biocompatibility and chemical structure make them ideal for supporting life processes [14]. However, the utility of pure alginate as a cell-supported printing material is limited unless it is combined with other biomaterials like gelatin, which can significantly enhance cell attachment and proliferation [15]. Furthermore, gelatin, a hydrolyzed product of collagen, is thermally reversible, adopting a solid structure at lower temperatures [15]. It contains natural cell-binding motifs that actively promote cell adhesion, proliferation, migration, and differentiation [16]. CNCs could promote gelation through hydrophobic and intramolecular hydrogen bond interactions which leads to CNC-based polymer composites exhibiting superior mechanical properties compared to pure polymers [17]. The crosslinking ability of CNCs is notably enhanced within the gelatin matrix, which is expected to improve both the mechanical and biological properties of bio-inks [15].

In particular, the application of 3D printing technology makes it possible to precisely customize the microstructure of the scaffold, mimicking the fine structure of natural bone, further optimizing the interaction between cells and materials, and promoting the full activation of cell functions. This precise structural control, combined with the biological activity of the material itself, creates a near-physiological growth platform for bone cells, significantly promoting the orderly construction and functional recovery of bone tissue. In addition, the adjustable degradation rate of the hybrid hydrogel scaffold is an important innovation, meaning that it can be broken down in time according to the specific schedule of bone regeneration, providing just the right support for the natural replacement of new bone tissue, which is particularly important for the repair of complex and large bone defects. This property, combined with the highly personalized capabilities of 3D printing technology, indicates that the material has broad application potential in the field of bone tissue engineering, especially in customized medical solutions.

In summary, the hybrid hydrogel scaffold material developed in this study overcomes the limitations of existing scaffold materials by its innovative material combination, fine structural design, enhanced biological activity and adjustable biodegradability, demonstrating great potential in promoting bone tissue regeneration, realizing precision medicine and solving complex bone defect repair. It lays a solid foundation for the future clinical application of bone tissue engineering.

1.2 Experimental methods and expected results

This project aims to develop an innovative hybrid hydrogel scaffold material for bone tissue engineering. Given the limitations of existing bone repair materials in terms of biocompatibility, bioactivity, and precise simulation of natural bone microstructure, our proposed new material is expected to provide significant performance improvements.

1.2.1 Methods

a. Preparation of Bioink

We plan to prepare our bioink using a combination of carefully selected materials, each with distinct properties that contribute to the overall performance of the bioink. Sodium alginate will be sourced from a supplier specializing in biomedical-grade materials, such as Sigma-Aldrich or Fisher Scientific, ensuring high purity and suitability for biomedical applications. Gelatin, derived from collagen, will be procured from reputable suppliers like Rousselot or Gelita, guaranteeing pharmaceutical or food-grade quality. To enhance the mechanical strength and stability of the bioink, we will incorporate CNCs obtained from specialized vendors such as CelluForce or American Process Inc. These vendors will provide CNCs of the appropriate size, shape, and purity for our application.

Here are the details of the materials and the process, where the chemical structure are shown in Fig 3:
Sodium Alginate: A natural polysaccharide known for its excellent biocompatibility and gelation properties. We will ensure that the alginate is of high purity and meets the requirements for biomedical applications.
Gelatin: Derived from collagen, gelatin will be procured to provide the necessary signals for cell adhesion and proliferation.
Cellulose Nanocrystals (CNCs): These will be obtained through acid hydrolysis of cellulose, a process that yields CNCs with very high mechanical strength and stability. We will collaborate with a vendor specializing in nanocellulose products to ensure that the CNCs are of the appropriate size, shape, and purity for our application.

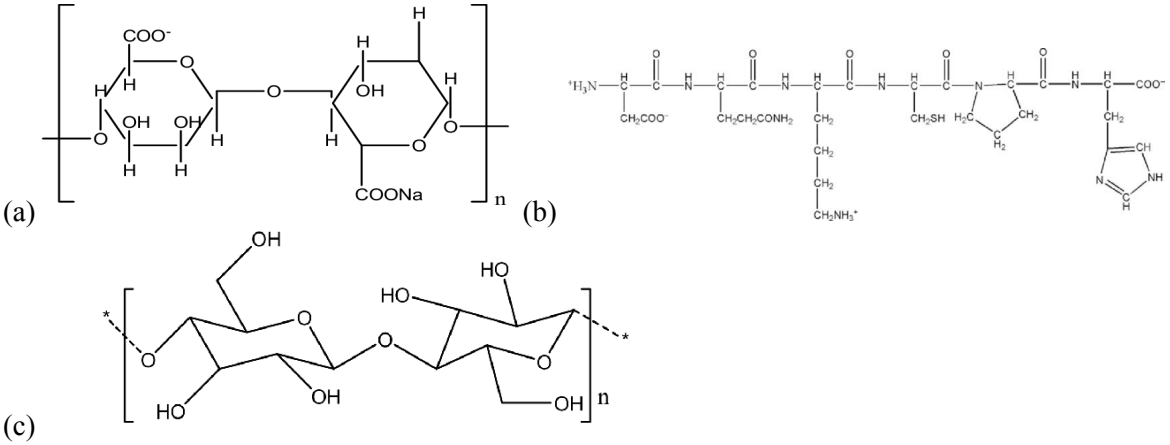


Fig 3. (a) Chemical structure of sodium alginate [18]; (b) Chemical structure of gelatin [19]; (c) Chemical structure of CNCs [20].

In our preparation process for a 50 mL solution, we will first dissolve the 3% (1.5g) sodium alginate and 4% (2g) gelatin in deionized water. This solution will then be gently heated to ensure complete dissolution and to promote the formation of a stable gel network. Following this, we will gradually add the 1% (0.5g) CNCs to the alginate-gelatin solution. We will continuously stir the mixture to ensure uniform dispersion of the CNCs throughout the solution. Throughout the mixing process, we will carefully control the temperature at 37 degrees Celsius, pH, and ionic strength to optimize the interaction between the alginate, gelatin, and CNCs. The mixture will be stirred continuously until a homogeneous bioink is obtained. This bioink will be designed to have the appropriate rheological properties, ensuring that it is suitable for extrusion-based 3D printing.

b. 3D Printing
The 3D bioprinter (CELLINK BIO-X) will be used for the printing experiments, which offers high precision and control over the printing process, essential for creating complex scaffold structures. The prepared bioink needs to be loaded into the bioprinter. Scaffold designs are going to be created using

computer-aided design (CAD) software SolidWorks, which provides models for printing. The bioprinter then follows these models to print the scaffolds layer by layer. Post-printing, the scaffolds are stabilized through physical and chemical crosslinking methods. Physical crosslinking was achieved by exposing the printed scaffolds to a calcium chloride solution, which crosslinked the alginate and chemical crosslinking involved using glutaraldehyde to crosslink gelatin, ensuring the scaffold maintained its shape and stability [11].

c. Evaluation of Physicochemical Properties

Scanning Electron Microscopy (SEM) is employed to observe the microstructure and pore architecture of the printed scaffolds. This analysis provides detailed images of the scaffold's surface morphology and internal structure, essential for understanding its mechanical properties and potential for cell infiltration.

- SEM can reveal the surface texture and topography of the scaffolds, which are important for cell attachment and growth. A rough surface with specific patterns can enhance cell adhesion and proliferation.
- The porosity of scaffolds is crucial for cell migration, nutrient diffusion, and waste removal. SEM can help visualize and measure the pore size and distribution, ensuring that the scaffold has an interconnected porous structure suitable for tissue ingrowth.
- SEM can provide images of how cells interact with the scaffold, such as the extent of cell spreading and the formation of ECM, which indirectly relate to the scaffold's mechanical integration with the tissue.

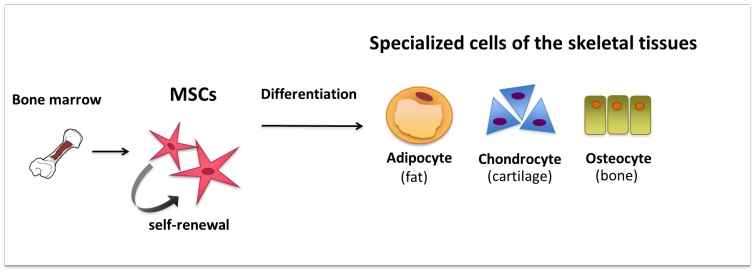
Fourier Transform Infrared Spectroscopy (FTIR) is used to analyze the molecular structure and chemical composition of the scaffolds. This technique confirms the presence of specific chemical bonds and functional groups, verifying the successful incorporation of all materials into the scaffold.

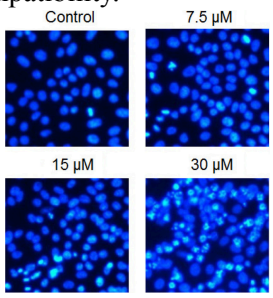
d. Biocompatibility and Cell Proliferation

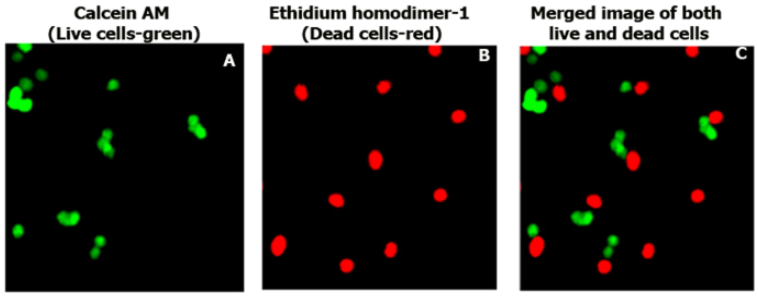
Human bone marrow mesenchymal stem cells (hBMSCs) which are shown in Fig 4 (a) are chosen as model cells due to their osteogenic potential and relevance in bone tissue engineering. hBMSCs are seeded onto the 3D-printed scaffolds and cultured under optimal conditions. The cell culture environment was maintained to promote cell attachment, proliferation, and differentiation.

Next, the 4',6-diamidino-2-phenylindole (DAPI) staining is performed to visualize cell nuclei, allowing for the assessment of cell distribution on the scaffolds, as shown in Fig 4 (b). This provided insight into the biocompatibility and cell-scaffold interactions.

In addition, a Live/Dead assay using calcein-AM and ethidium homodimer-1 (EthD-III) is conducted to evaluate cell viability that is stated in Fig 4 (c). This assay distinguished live cells (stained green) from dead cells (stained red), offering a direct assessment of scaffold biocompatibility.

(a) 

(b) 



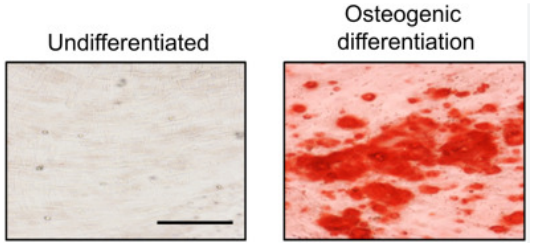
(c)

Fig 4. (a) hBMSCs [21]; (b) DAPI staining of cells [22]; (c) Live/Dead assay using calcein-AM and EthD-III [23]

e. Osteogenic Differentiation Potential

Alizarin Red-S (ARS) staining is used to detect calcium deposits, indicative of mineralization and osteogenic differentiation, which is shown in Fig 5 (a). The presence of red-stained mineralized nodules on the scaffold confirmed the scaffold's ability to support bone formation.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) shown in Fig 5 (b) will be utilized to quantify the expression of osteogenic markers such as Runx2, ALP, BMP-2, OCN, OPN, BSP, and COL1. This molecular analysis provides detailed information on the scaffold's capacity to induce osteogenic differentiation at the genetic level.



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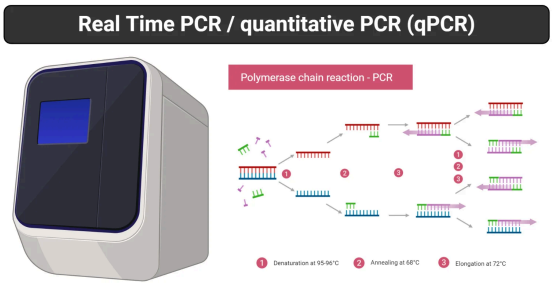

(b)

Fig 5. (a) ARS staining [24]; (b) qRT-PCR protocol [25]

1.2.2 Anticipated Outcomes

The aim is to develop Alginate/Gelatin bio-inks with CNCs added and to fabricate the intended scaffold structures using 3D printing technology. It is expected that the material combinations will be found feasible and suitable for 3D printing, with the scaffolds maintaining their shape and structure in a sterile environment.

We anticipate that the rheological properties of the bio-inks, to be assessed using an ARES-G2 rheometer, will be suitable for 3D printing. This will mean that the bio-inks will exhibit the appropriate viscosity and stability, which are crucial for preserving the shape and ensuring the precision of the printed structures.

SEM is expected to reveal a proper surface on the scaffold structures with a suitable porosity. This uniformity is anticipated to be conducive to cell adhesion and growth, which are essential for the scaffold's biocompatibility.

The scaffolds are expected to demonstrate excellent cell compatibility, supporting the survival and proliferation of hBMSCs. DAPI staining and live/dead cell assays are expected to confirm the non-toxicity of the scaffold material and its ability to promote cell proliferation. Furthermore,

fluorescence microscopy is expected to show that cells maintain a normal morphology and exhibit strong adhesion to the scaffold, suggesting a supportive environment for cell growth.

qRT-PCR is expected to reveal a significant upregulation of genes specific to osteoblast differentiation. This outcome would indicate that the scaffold materials are capable of effectively guiding stem cells toward osteogenic differentiation, a key goal of this research.

Overall, we anticipate that these outcomes will collectively validate the potential of the developed bio-inks and scaffolds for use in tissue engineering applications, particularly in bone tissue repair and regeneration.

1.3 Intellectual Merit

This research project shows its profound intellectual value and innovation driving force in multiple dimensions. First, with our groundbreaking design of a hybrid hydrogel scaffold material, we are pushing the boundaries of biomaterials science, especially in mimicking the complex structure and function of natural bone tissue with precision, bringing revolutionary material solutions to the field of bone tissue engineering. Secondly, the project deepens the exploration of 3D printing technology in biomedical applications, especially in the precision manufacturing of personalized bone tissue repair scaffolds, expanding the potential and application range of this technology.

By exploring in detail the mechanism of the interaction between the mixed hydrogel scaffold and bone marrow mesenchymal stem cells (hBMSCs), we plan to improve the basic understanding of how cells proliferate and differentiate on the artificial scaffold, but also provide a scientific basis for optimizing the cell-material interface interaction. In addition, in-depth research on the biocompatibility and bioactivity of new scaffolds provides scientific data support for the design of efficient and safe next-generation bone repair materials, which is the key to promoting the progress of bone regenerative medicine.

1.4 Broader Impacts

Social relevance and public health: Treatment of bone defects and injuries is essential to improve patients' quality of life. The new stent materials developed in this project will help reduce the number of surgeries, speed up the recovery process, and reduce the risk of long-term disability. This will have a direct impact on the physical health and psychological well-being of patients, while reducing the burden on the healthcare system.

Technological innovation and industry impact: Our research will promote the application of 3D bioprinting technology in the medical field and promote the development of personalized medicine. This will not only stimulate new business opportunities, but also drive innovation in related industries, including biomaterial manufacturing, medical devices and 3D printing technology.

Education and training: This program will provide valuable resources in the field of education. We will share our findings and experiences by publishing research papers, participating in academic conferences, conducting public lectures and educational programs. This will raise awareness among students and professionals about the importance of biomedical engineering and stimulate their interest in science and engineering.

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2. CV

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EDUCATION

University of California San Diego — Sep 2023 - Jun 2025 (Expected), La Jolla, CA

Master's degree in Mechanical Engineering

Xi'an Jiaotong-Liverpool University — Sep 2019 - Jun 2023, Suzhou, China

Bachelor in Intelligent Manufacturing Engineering (School of Intelligent Manufacturing Ecosystem)

WORK EXPERIENCE

Western Superconducting Technologies Co., LTD — Jun 2022 - Aug 2022, Xi'an, China

Electrical Design Intern

- Upgraded the program of the department's automatic marking project using Visual Studio (both in the user interface and C# code), which is used for identifying the side circle of cylindrical steel and automatically marking the specific production lot number.
- Produced an analysis report of how the program code communicated among different procedures, including PLC program, visual identification, marking operation, and database.

RESEARCH EXPERIENCE

5R-youBot Simulation Project — Feb 2024 - Mar 2024, San Diego, USA

- Wrote a matlab program which enables a 5R-youBot composed of a mobile chassis with four wheels and a 5R arm picking up, carrying, and placing a cube at designed locations in CoppeliaSim software.

Autonomous Robotic Platform for Inspection and Repairing Project — Sep 2022 - May 2023, Taicang, China

- Using OpenCV library and traditional vision algorithms to achieve real-time image acquisition, zooming in/out, making auxiliary lines, taking screenshots, video recording, and damage detection. Developing a graphical user interface.

Microchannel Heat Exchanger Project — Nov 2021 - May 2022, Suzhou, China

- Simulated the state change of dry/wet air after passing through the heat exchange channel using Ansys Fluent, so as to explore the influence of water vapor content in the air on the heat exchange efficiency.

Industrial Robotics Platform Programming Project (Haier) — Jul 2021 - Aug 2021, Nanjing, China

- Programmed a 6-axis manipulator with PLC (Simens TIA Portal) to automatically grasp the corresponding parts and assembled them into a complete Luban Lock. Designed the human-computer interface using the HMI module in Simens Portal.

Robot Modeling and Control Project — Jul 2020 - Aug 2020, Suzhou, China

- Designed the shell of a robot by Creo & completed the line patrol programming by Scratch.
- Improved the Scratch program using an ultrasonic wave sensor to detect the object so that the robot could identify and remove obstacles in its path.

EXTRACURRICULAR ACTIVITIES

XJTLU Student-Staff Liaison Committee — Sep 2021 - Sep 2022

Chair of Robotics & Intelligent Manufacturing Ecosystem

- Leading the meetings including setting the agenda. Representing the views of students in two schools. Solving issues that arise on behalf of the student body.

XJTLU InfoCo Club — Dec 2020 - Dec 2021

Deputy Director of Activity Department

- Proposed and organized activities related to the club. Kept up with the progress of activities and finished the follow-up work.

SKILLs

- **Languages:** Python, Matlab, C#
- **Functional:** Ansys Fluent, 3D modeling (Solidworks, Creo), Microsoft Office

3. Facilities and Budget

Facilities	Contents	Function	Location	Budget ($)
High-precision 3D bioprinters CELLINK BIO-X		printing complex scaffold structures	N/A	100,000
CAD software SolidWorks		design and simulate 3D printed stand models	EBU2 UCSD	N/A
Cell culture facilities	aseptic operating table, cell incubator, centrifuge, microscope	preparation of biological ink	Bioe Lab UCSD	N/A
Biological material preparation equipment	mixers, agitators, homogenizers		Bioe Lab UCSD	N/A
Material characterization equipment	scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR)	analyze the microstructure and chemical composition of the scaffold	Calt2 UCSD	N/A
Biological analysis tools	DAPI stain, Live/Dead cell activity kit, Alizarin Red-S stain	evaluate cytocompatibility and osteogenic differentiation potential	Bioe Lab UCSD	1,000
Real-time quantitative polymerase chain reaction (qRT-PCR) system		analyze the expression level of specific genes	N/A	10,000
Microcomputed tomography (μCT)		evaluate the internal structure and porosity of the scaffold	N/A	100,000
Chemical analysis equipment	pH meters and thermometers	measuring and adjusting the	Bioe Lab UCSD	N/A

		chemical properties of bio-inks		
Freeze-drying machine		freeze-drying of scaffolds to maintain their structural integrity	Bioe Lab UCSD	N/A
Bioreactor		simulate the in vivo environment for cell culture and tissue formation	Bioe Lab UCSD	N/A