

ADVANCED 3D BIOPRINTED CONDUCTIVE TISSUE FOR CARDIAC REGENERATION

by Wafa Al Shamery

Thesis submitted in fulfilment of the requirements for
the degree of

Doctor of Philosophy

**under the supervision of Assoc/Prof Carmine Gentile, Prof.
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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Wafa Al Shamery declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy in the School of Biomedical Engineering, Faculty of Engineering, and Information Technology at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Disclaimer

This PhD project was significantly impacted by the COVID-19 pandemic and the lockdown restrictions imposed by the Australian Government from June to October 2020. As a result, I was unable to access the laboratory at UTS to begin my project and conduct experiments. During this period, I focused on my literature review for six months.

In 2021, I completed two online subjects for my PhD: Technology Research Preparation, which provided a foundation in research design and methodology, and Technology Research Method, which focused on advanced research techniques. Another lockdown was imposed in NSW due to the Delta variant, further limiting my laboratory access from June to October 2021.

In 2022, I completed my CA2 on December 20th. I generated preliminary results for my specific aims 1 and 2 between 2020 and 2023. However, essential media for cell culture expired and had to be reordered, delaying my experiments for an additional eight months (March 2023 to October 2023). I finished my training to work with animals on August 18, 2023. Therefore, I was able to start my final experiments between November 1, 2023, and January 15, 2024. This intense two-month period led to significant stress in completing all the required experiments.

Throughout my PhD, I faced numerous challenges, including pandemic-related delays, lockdown restrictions, and the personal loss of a family member. Despite these obstacles, I persevered and successfully completed my research.

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Abbreviation

Alg-Gel	4g Alginate+8g Gelatine
Alg	Alginate
Au	Gold
BM	Brillouin microscopy
BFS	Brillouin Frequency Shift
BMSCs	Bone Marrow-Derived Mesenchymal Stem Cells
BSA	Bovine Serum Albumin
CaCl ₂	Calcium Chloride
CM	Cardiomyocyte
CNTs	Carbon Nanoparticles
CNTs	Carbon Nanotubes
CP	Conductive Polymers
CS-g-PPY	Chitosan-Graft-Polypyrrole
CS-g-PPY	Chitosan-Graft-Polypyrrole
CSs	Cardiac Spheroid
CVD	Cardiovascular Disease
DHR-3	Dynamic Hybrid Rheometer
DMEM	Dulbecco's Modified Eagle's Medium-High Glucose
ECM	Extracellular Matrix
LVEF%	Left Ventricular Ejection Fraction %
FS%	Fractional Shorting
G	Graphene
Gel	Gelatine
GelMA	Gelatine Methacryloyl
GNP	Gold Nanoparticles
GO	Graphene Oxide
H&E	Hematoxylin And Eosin
HACEC	Human Coronary Artery Endothelial Cell
HCF	Human Fibroblast
hiPSC	Human Induced Pluripotent Stem Cell
hMSC	Mesenchymal Stem Cell
hUC-MSC	Human Umbilical Cord Mesenchymal Stem Cell
iPSC-CM	Induced Pluripotent Stem Cell-Derived Cardiomyocyte
LAD	Left Anterior Descending Artery
LV	Left Ventricular
LVADs	Left Ventricular Assist Devices
MI	Myocardial Infarction
MTE	Myocardial Tissue Engineering
PANI	Polyaniline
PANINTs	Polyaniline Nanotubes
PCL	Poly(E-Caprolactone)
PET	Polyethylene Terephthalate
PGLDs	Polyglycerol Dendrimers

PGS	Poly (Glycerol Sebacate)
PPY	Polypyrrole
rGO	Reduced Graphene Oxide
SEM	Scanning Electron Microscope
SF	Silk Fibroin
SS	Silk Sericin
TDS	Total Dissolved Solid
TEVG	Tissue Engineered
VSC	Vascularised Cardiac Spheroid
VSMC	Vascular Smooth Muscle Cell
XRD	X-Ray Diffraction

List of Manuscript Contributions

This thesis does not contain materials that have been published before. However, I have contributed to two published articles during my PhD studies. My contribution in terms of SEM analysis and hydrogel preparation was predominantly technical. These include the following articles:

- Tarsitano, M., Ming, C. L. C., Bennar, L., Mahmodi, H., Wyllie, K., Idais, D., Al Shamery, W., Paolino, D., Cox, T. R., & Kabakova, I. (2024). Chlorella-enriched hydrogels protect against myocardial damage and reactive oxygen species production in an *in vitro* ischemia/reperfusion model using cardiac spheroids. *Biofabrication*, 17(1), 015006. (<https://iopscience.iop.org/article/10.1088/1758-5090/ad8266/meta>)

My role involved preparing the samples for a resubmission of the manuscript to conduct scanning electron microscopy analyses of the hydrogels and to interpret the results. This work was crucial for understanding the microstructural characteristics of the hydrogels, which played a significant role in their potential applications in cardiac tissue engineering.

- Polonchuk, L., Suriya, L., Lee, M. H., Sharma, P., Ming, C. L. C., Richter, F., Ben-Sefer, E., Rad, M. A., Sarmast, H. M. S., Al Shamery, W., Tran A, H., Vettori, L., Haeusermann, F., C Filipe, E., Rnjak-Kovacina, J., Cox, T., Tipper, J., Kabakova, I., & Gentile, C. (2021), **Toward engineering heart tissue from bioprinted Cardiac spheroids**. *Biofabrication*, 13. (4), 045009. (<https://iopscience.iop.org/article/10.1088/17585090/ac14ca?elqTrackId=facff08dee304afb6c36b6e87dc4a2a6>).

My role involved preparing the samples for a resubmission of the manuscript.

Conferences Presentations

5th Australian Bioprinting Workshop – November 20–21, 2023, University of Technology Sydney (UTS), Sydney, Australia

Oral presentation: *Advanced 3D Bioprinted Conductive Tissues for Cardiac Regeneration*.

Engagement with General Public

The image on this page depicts the artwork “Heart”, featured in the museum exhibition ‘You Only Get to Die Once (2030)’, created in collaboration between artists Dr Paul Brown, Ms Linda Dement, and the Cardiovascular Regeneration Team (Assoc/Prof Carmine Gentile). In collaboration with capstone student Sruthy Gnanachelvan from Assoc/Prof Gentile’s team at the UTS School of Biomedical Engineering, I prepared the hydrogel from Assoc/Prof Gentile’s lab and 3D printed the heart patch. Assoc/Prof Carmine Gentile reviewed this work. The 3D heart model was printed by ProtoSpace at UTS.



Thesis Abstract

This thesis explores the use of conductive polymers (CPs) and hydrogels in cardiac tissue engineering, focusing on their potential to repair and regenerate myocardial tissue following myocardial infarction (MI). Cardiac repair aims to provide structural support for damaged heart tissue with the formation of a scar, while cardiac regeneration aims to replace damaged tissue with new, functional tissue. The role of CPs in enhancing the electrical conductivity and mechanical properties of engineered cardiac tissues is central to this research.

Previous studies in our team developed alginate-gelatin (Alg-Gel) hydrogels for cardiac tissue engineering applications. In this study, I aimed to improve the conductivity of Alg-Gel hydrogels by incorporating and comparing conductive materials, such as graphene, graphene oxide, gold nanoparticles, polypyrrole, and polyaniline. First, these hydrogels were evaluated for their effects on conductivity, pore size, printability and durability of Alg-Gel hydrogels. Then, they were tested with freely dispersed cardiac cells, as well as in the presence of cardiac spheroids, a three-dimensional *in vitro* culture model of the heart, for their effects on cell viability and toxicity, and contractile activity. Finally, these hydrogels were tested in an *in vivo* mouse model of MI using echocardiography and histological analysis to evaluate functional and structural recovery.

The conductive hydrogels demonstrated good biocompatibility, supporting high cell viability and promoting the formation of functional cardiac tissue. The hydrogels showed enhanced contractile activity, with spheroid integration leading to improved tissue functionality. The patches exhibited superior printability and durability, maintaining their structure and function under physiological conditions. *In vivo* results demonstrated that conductive hydrogel-based patches containing G and GO significantly improved myocardial function, as evidenced by increased ejection fraction and reduced fibrosis by day 28 post-implantation.

These findings suggest that conductive hydrogels offer significant promise for cardiac tissue engineering, providing a potential alternative to a heart transplant. The study contributes to advancing regenerative therapies for MI, with potential applications in both

clinical and research settings, highlighting the importance of optimising biomaterials for cardiac repair.

ADVANCED 3D BIOPRINTED CONDUCTIVE TISSUE FOR CARDIAC REGENERATION

Thesis with publication graphical overview

PART 1 INTRODUCTION

1.1 Thesis introduction, hypothesis and aims



1.2 Literature Review



1.3 closing Remarks to part 1

PART 2 CHARACTERISATION OF CONDUCTIVE HYDROGEL

2.1 Introduction and relevant to Part 2



2.2 Development and Characterization of Conductive Hydrogel for Cardiac (*in vitro*)



2.3 Closing Remarks to part 2

PART 3 SPHEROID RESPONSE TO CONDUCTIVE HYDROGELS

3.1 Introduction and relevant to Part 3



3.2 Spheroid Response to Conductive Hydrogels (*in vitro*)



3.3 Closing Remarks to part 3

PART 4 CONDUCTIVE HYDROGEL ENHANCES HEART FUNCTION IN MICE

4.1 Introduction and relevant to Part 4



4.2 conductive patch transplantation (*in vivo*)



4.3 Closing Remarks to part 4

PART 5 DISCUSSION, CONCLUSION AND

5.1 Introduction and relevant to Part 5



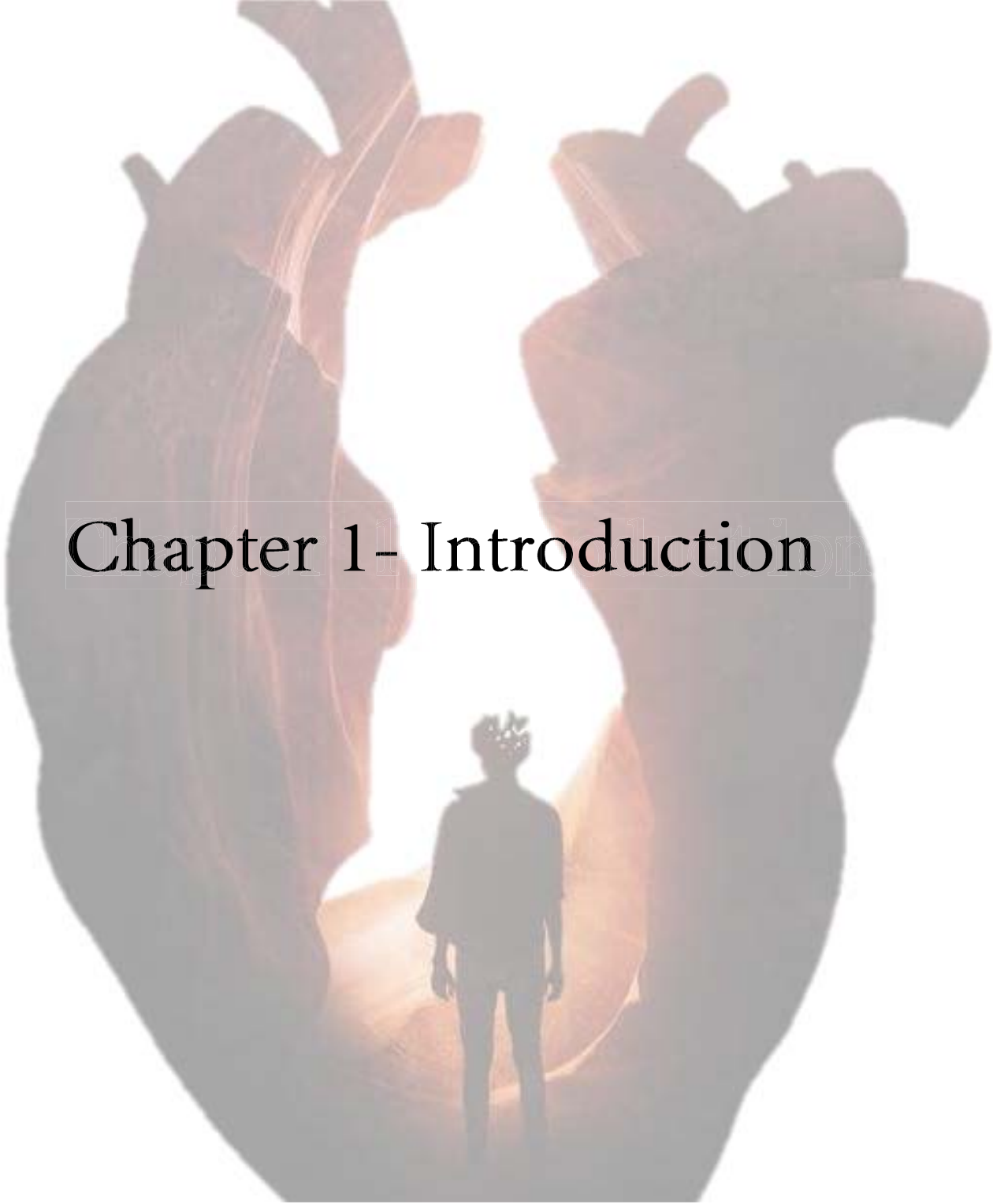
5.2 Thesis Discussion



5.3 Thesis Future
direction



5.4 Thesis Conclusion

A conceptual image featuring a large, dark heart shape. Inside the heart, a small silhouette of a person stands with their back to the viewer, looking out towards a bright, glowing light source. The heart's interior is filled with a warm, orange-red glow, suggesting a path or a journey. The overall composition is centered and evokes themes of love, hope, and personal growth.

Chapter 1- Introduction

Chapter 1- Abstract

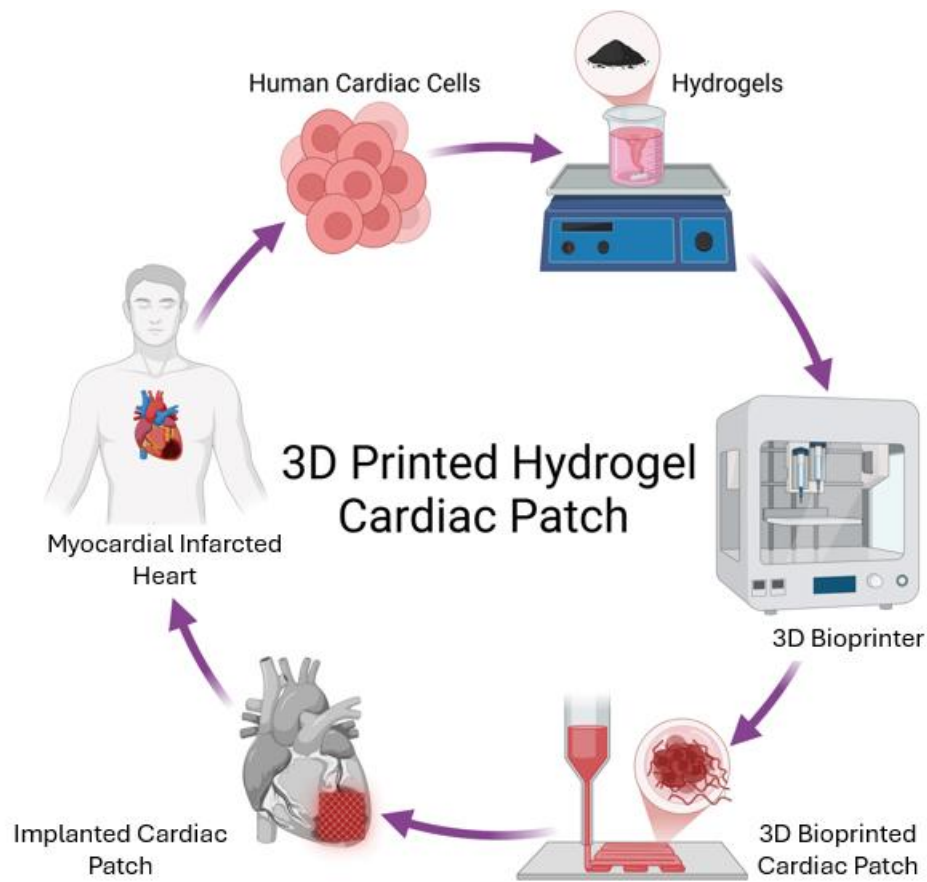
This chapter explores the role of conductive polymers (CPs) in cardiac regeneration. Cardiac repair refers to the healing process in which damaged heart tissue is replaced by scar tissue rather than native cardiac muscle, providing structural reinforcement without functional recovery. In contrast, cardiac regeneration involves the formation of new, functional cardiomyocytes, restoring both the structure and function of the heart.

CPs play a crucial role in enhancing cardiac regeneration by providing electrical conductivity and mechanical properties that mimic the native myocardium. These polymers facilitate electrical signalling between cardiomyocytes, improving functionality and promoting synchronised contraction. Additionally, CPs have been shown to enhance cardiomyocyte differentiation, support cellular adhesion, and improve integration with native heart tissue. By bridging damaged areas and providing bioelectrical cues, CPs create a more favourable microenvironment for cell survival, maturation, and tissue remodelling, contributing to functional recovery.

Hydrogels are biocompatible, three-dimensional polymer networks widely used in tissue engineering due to their high-water content, tunable mechanical properties, and ability to support cell adhesion and differentiation. While they have broad applications across various tissues, this research focuses on optimising hydrogel composition and conductive polymer (CP) concentration to enhance electrical conductivity and cardiomyocyte integration—critical factors for restoring heart function after myocardial infarction (MI).

The major objective of Chapter 1 is to establish whether CPs could facilitate cardiac regeneration based on the existing literature. This Chapter examines conductive biomaterials used in cardiac tissue engineering, evaluating their potential to enhance contractile activity and electrical conductivity within cardiac scaffolds. Additionally, it explores advancements in hydrogel formulation and how these materials influence mechanical, electrical, and biological properties, ultimately assessing their role in promoting cardiac cell function and tissue regeneration. This chapter has been published as a review article in iScience.

Graphical Abstract



(created with BioRender.com)

1. Introduction

Cardiovascular diseases, including myocardial infarction (MI), remain a leading cause of mortality worldwide (Nowbar et al., 2019). Current treatments, such as pharmacological therapy, implantable devices, and heart transplants, can alleviate symptoms and improve survival, but do not fully restore lost cardiac function. Tissue engineering has emerged as a promising alternative, aiming to develop biomaterials that integrate with native heart tissue and promote functional recovery.

This chapter provides context for my research and presents a detailed background on conductive polymers (CPs) and biomaterials in cardiac bioengineering. CPs possess electrical conductivity, biocompatibility, and mechanical flexibility, making

them promising candidates for cardiac tissue engineering applications. Their ability to enhance electrical signalling, support cardiomyocyte function, and facilitate tissue integration has positioned CPs as a potential solution to overcome the limitations of traditional therapies. This chapter examines how CPs have been modified to improve their conductivity, stability, and biocompatibility, as well as their impact on cardiac regeneration (Mihic et al., 2015).

Additionally, this chapter explores the current challenges and advancements in hydrogel-based CP formulations, focusing on their role in mimicking the myocardium's structural and electrical properties. The potential applications of CPs extend beyond cardiac regeneration to include advanced *in vitro* models, which can be used to study cardiac function and test novel therapeutic strategies.

This chapter establishes the scientific framework for this research, defining its aim, scope, and research questions. The thesis objectives and hypotheses are outlined at the end of the chapter.

1.1. Background

Cardiovascular disease (CVD), including myocardial infarction (MI), is a leading cause of death worldwide, claiming approximately 18.6 million lives annually (Roth et al., 2020). MI occurs when the heart is deprived of oxygen due to blocked blood flow, resulting in heart muscle damage (Wu et al., 2021). This condition presents a significant threat to individuals' lives and health, often leading to pathological structural abnormalities within the heart ventricles. Immediate medical attention is crucial to reduce further damage and improve outcomes, especially given the limited availability of treatment options such as heart transplants (Heusch & Gersh, 2017). Moreover, complications like heart failure can arise due to the heart's inability to self-repair. Regenerative medicine research aims to develop therapies for repairing damaged heart tissue, offering hope for enhancing outcomes and improving the

quality of life for those affected by CVD (Fernández-Avilés et al., 2017). Currently, available treatment options such as heart transplants, face challenges due to the scarcity of donor organs, underscoring the urgent need for alternative approaches, such as cardiac tissue engineering using conductive biomaterials (Sáez et al., 2014).

Conductive polymers have emerged as essential materials in supporting and stimulating cells for heart repair. Their engineered properties, along with electrical and electromechanical stimulation, provide various benefits in aiding stem cell function (Petty et al., 2020). Optimising the interaction between cardiac progenitor cells and conductive polymer surfaces is crucial for advancing our understanding of biomaterial interactions with cardiac cells and optimising materials for cardiac tissue regeneration (Gelmi et al., 2014). Furthermore, conductive biomaterials play a pivotal role in post-myocardial infarction recovery by restoring electrical functions and repairing damaged myocardium. These biomaterials effectively lower scar tissue resistivity, increase field potential, and improve conduction velocity, thereby enhancing heart function (He et al., 2020). In the field of cardiac tissue engineering, conductive substrates are designed to mimic the electrical properties of the myocardium, thereby improving blood-pumping ability and reducing scar size. These substrates not only enhance cardiomyocyte maturation and integration with the native myocardium but also contribute to the restoration of heart function. Moreover, conductive scaffolds facilitate stem cell differentiation into cardiomyocytes, enhancing the expression of cardiac proteins and promoting electrical coupling and contractility of cells, ultimately leading to improving cardiac function post-MI (Yin et al., 2023). This chapter explores the application of various biomaterials and conductive polymers in cardiac tissue engineering.

1.1. Medical problem: Heart failure, Current Treatments, and Challenges in Cardiac Regeneration

MI is commonly known as a heart attack, is caused by atherosclerosis, which occurs when plaque builds up with fatty material and other substances in the arteries. These plaques narrow the arteries, leading to either decreased or completely halting blood flow, which prevents the heart from functioning properly and can lead to total heart failure (Jawad et al., 2007a; Jawad et al., 2007b; Kadiri, 2017).

Treatment options for MI focus on preventing the progression to heart failure rather than offering a true cure. These treatments include antiplatelet agents like aspirin and clopidogrel to prevent blood clots, thrombolytics such as alteplase to dissolve existing clots, and anticoagulants like heparin to reduce the risk of further clot formation. While these therapies can help manage the immediate consequences of MI, long-term care and interventions are required to address heart function and prevent complications.(Collen et al., 1988). Beta-blockers and ACE inhibitors also help manage heart workload and blood pressure, while statins lower cholesterol levels. However, if these treatments are insufficient, a heart transplant may be considered for patients with end-stage heart failure (Strauss et al., 2023). Heart failure is a condition where the heart is unable to pump blood effectively to meet the body's demands, leading to symptoms such as shortness of breath, fatigue, and fluid retention. Clinical measurements to diagnose heart failure include the assessment of ejection fraction (EF), which is measured via echocardiography to evaluate the heart's pumping efficiency. Additionally, physical examination findings such as edema and jugular venous distention, symptom evaluation (e.g., dyspnea, orthopnea), and elevated biomarkers like B-type natriuretic peptide (BNP) are important for diagnosis.

However, the severe shortage of donor organs significantly restricts its availability, causing many patients on the waiting list to die each year (Gelmi et al., 2014).

Current studies have explored various approaches to address heart failure, ranging from pharmacological treatments to surgical interventions. Traditional therapies focus on managing symptoms and preventing disease progression, including the use of beta-blockers, ACE inhibitors, and devices like left ventricular assist devices (LVADs) (Khazanie et al., 2016). Additionally, heart transplantation remains a critical option for patients with end-stage heart failure. However, emerging approaches, including the use of regenerative therapies, are gaining attention. One such innovation is the use of conductive polymers in tissue engineering. Conductive polymers offer promising properties for creating scaffolds that mimic the natural electrical conductivity of cardiac tissue, potentially facilitating the regeneration of heart muscle and restoring normal electrical function in damaged tissues (Ul Haq et al., 2023)

Advancements in biomaterial-based therapies and 3D bioprinting, which involve the layer-by-layer printing of living cells and biomaterials to create complex tissue structures, have the potential to revolutionise cardiac regeneration and significantly improve outcomes for

heart failure patients. By providing a personalised treatment approach that overcomes the limitations of traditional heart transplants, these innovations offer hope for a brighter future in cardiovascular medicine (Matthews et al., 2022).

Biomaterials, whether synthetic or natural, play a crucial role in cardiovascular treatment by facilitating tissue repair and regeneration (Polonchuk et al., 2021). Injectable hydrogels for cardiac tissue engineering were investigated by Navaei et al. (2016) to close the gap between MI and the shortage of donor heart transplants. This article presents the development of an injectable biohybrid hydrogel for cardiac tissue engineering, combining PNIPAAm (synthetic polymer) with gelatin (natural polymer) to create a dual-cross-linkable matrix with enhanced properties. Key features include excellent bioactivity, high mechanical strength, a 90% cell survival rate, and proper cardiac cell spreading. The hydrogel outperforms mono-culture conditions in co-culturing cardiomyocytes with fibroblasts, leading to enhanced synchronous tissue contractions. *In-vitro* testing shows promising results, overcoming limitations of previous injectable biomaterials, with potential applications in cardiac tissue engineering and cell delivery.

As the myocardium is an electrically active tissue and cardiac cells have synchronised contractile activity through the propagation of electrical signals, the bioengineered tissue should be electrically conductive. The absence of electrical conductance in cardiac patches could hinder cell engraftment and prevent the synchronised contractile activity between transplanted cells and host tissue. A wide range of electroconductive biomaterials has already been investigated as potential candidates to improve electrical conductivity in the heart (Hasan et al., 2015; Solazzo et al., 2019).

1.2. Cardiac Bioengineering

A key component in this field is the use of conductive biomaterials, which enhance the electrical conductivity of bioengineered tissues, improving their integration and function. Ensuring the safety of these materials is crucial, as they must be biocompatible, non-toxic, and capable of maintaining their structural and functional stability in the body to avoid adverse reactions. Rigorous testing and quality control measures are essential to ensure that these materials not only support tissue regeneration but also minimise risks related to immune response or long-term degradation (Vunjak-Novakovic et al., 2010).

Successful clinical applications of bioengineered tissues depend on maturation protocols, vascularisation, and engraftment with host tissue. Conductive biomaterials support these processes by facilitating electrical communication between cells, which is essential for the proper function of cardiac tissue. In addition to cell sources, bioengineering techniques such as cell reprogramming, gene editing, and the delivery of protein factors through engineered particles enhance the properties of cells (Tomov et al., 2019).

Innovations in technologies, such as protein decoration and the use of cardiac patches embedded with conductive materials, are essential for advancing cardiac regenerative medicine. These approaches help overcome the limitations of live cell therapy, which include challenges like poor cell survival, limited integration with host tissue, and inefficient electrical coupling. By enhancing the engraftment and functionality of engineered cardiac tissue, these technologies improve the prospects for successful integration in clinical applications, offering a promising avenue for repairing heart tissue and restoring cardiac function.(Chingale et al., 2021).

1.3. Biomaterials for Heart Tissue Engineering

Biomaterials are essential in supporting cells and guiding their development into functional tissue (Kim et al., 2000). Each type of biomaterial has unique characteristics, but they all function as supportive structures that promote the growth and specialization of targeted tissues, as outlined in Table 1. These biomaterials are typically either synthetically engineered or derived from essential natural substances, with subsequent processes used to tailor their morphology and properties for specific applications(Nguyen et al., 2019) 3D bioprinting has emerged as a transformative tool in the field of tissue engineering. When combined with conductive biomaterials and hydrogels, 3D bioprinting enables the fabrication of engineered cardiac tissues that mimic the electrical and mechanical properties of the native heart, enhancing their functionality and integration in regenerative therapies (Wang et al., 2021). This section will explore the various biomaterials used in cardiac tissue engineering, as well as their broader applications in other areas of tissue engineering.

1.3.1. Alginate

Alginate, a natural compound derived from seaweed, is known for its biocompatibility, biodegradability, non-toxicity, and non-immunogenicity. These advantageous properties make it highly suitable for several medical applications, such as drug delivery, wound healing, and tissue engineering (Mi et al., 2002; Reis et al., 2016).

In cardiac tissue engineering, alginate has been used as an injectable cell delivery system because it can easily gel when exposed to ions such as calcium. These properties make it effective for delivering cells to the heart tissue during regenerative treatment (Koh & Atala, 2004; Wang et al., 2010). The gelation process results from the interaction between the alginate molecules and calcium ions present in the surrounding environment. When alginate comes into contact with calcium ions, it undergoes a cross-linking reaction, forming a gel-like structure. This gel can then encapsulate cells, protecting them during delivery and facilitating their release at the target site within the heart tissue (Kaviani et al., 2024).

A study by Leor et al. (2005) explored the potential of alginate in cardiac engineering by developing specialised scaffolds to support implanted heart cells in a rat myocardial infarction model. These alginate-based scaffolds provided a controlled release of therapeutic factors to create an optimal environment for heart cell survival and integration. Additionally, the study examined the effects of specific cytokines, including granulocyte-colony stimulating factor (G-CSF), stromal-derived factor-1 (SDF-1), leukemia inhibitory factor (LIF), liver growth factor-1 (LGF-1), and erythropoietin (EPO), on promoting tissue healing and reducing scar formation after a heart attack. The results indicated that these cytokine-loaded scaffolds significantly increased cell retention, reduced infarcted area, and improved left ventricular (LV) function, offering promising approaches for heart tissue repair and regeneration.

In 2008, Landa et al. (2008) investigated the potential of alginate's properties by developing absorbable biomaterials using a calcium-crosslinked alginate solution, which transforms into a hydrogel upon injection into an infarcted area. The research focused on alginate's impact on cardiac remodelling and function in the infarcted area. The study used a rat myocardial infarction (MI) model to examine how this alginate hydrogel influenced cardiac tissue recovery and function. The research aimed to understand how the alginate hydrogel specifically affected cardiac remodelling in infarcted tissue.

Echocardiographic and histological analyses showed that the alginate-treated rats had significantly reduced left ventricular (LV) dilation compared to the untreated control group in infarcted animals. The control group would have consisted of rats with myocardial infarction that were not treated with the hydrogel. The results showed comparable or superior benefits to neonatal cardiomyocyte transplantation in rats treated with the alginate hydrogel, improving cardiac function and reducing infarct expansion. Alginate also reshaped the left ventricle, preventing further enlargement and promoting increased arteriogenesis. The hydrogel further promoted neovascularisation, with an observed increase in vascular density within the treated infarct area. Compared to fibrin, alginate consistently enhanced left ventricular functional shortening and reduced infarct size, with both materials boosting arteriogenesis (Landa et al., 2008; Wang et al., 2010).

In 2009, a study conducted by Yu et al. (2009) investigated the long-term effects of alginate on the heart using a mouse model that mimicked chronic heart disease caused by tissue damage. The study compared modified and unmodified alginate formulations, incorporating RGD peptides and calcium chloride, to evaluate their effects on cardiac tissue when administered directly to the damaged area of the left ventricle (LV). Results revealed that the modified alginate combined with RGD promoted angiogenesis and blood vessel formation and that both alginate forms improved LV function. Additionally, Yu developed a method to deliver mesenchymal stem cells (hMSCs) by encapsulating them in microbeads made from RGD alginate and injecting them into the affected region, which increased stem cell retention, slowed tissue remodelling, and further facilitated angiogenesis. Interestingly, no significant difference was observed *in vivo* between using RGD alginate alone and in combination with cells. This study suggests that RGD alginate is a cell-free approach to stimulate angiogenesis and support functional recovery in cardiac applications (Majid et al., 2020a, 2020b; Yu et al., 2009).

1.3.2. Chitosan

Chitosan is a natural, biocompatible, and non-toxic material derived from chitin, commonly used in controlled drug delivery systems. Its ability to form nanoparticles through ionic interactions with tripolyphosphate (TPP) makes it ideal for targeted drug delivery and

tissue engineering applications. Chitosan nanoparticles (CNP) are known for their prolonged circulation times in the bloodstream and excellent biocompatibility (Rajam et al., 2011).

In 2017, a study by Ye and Qiu (2017) focused on using chitosan combined with conductive biomaterials, such as carbon-based nanofibers and nanotubes, to enhance cardiac repair and function following myocardial infarction. These conductive scaffolds were designed to mimic the heart's electrical environment, thereby supporting improved electrical signal transmission across cardiac cells. The materials were combined to create scaffolds that enhance heart function post-myocardial infarction by promoting cell adhesion, proliferation, and elongation, key factors in the integration and survival of transplanted cells. These studies did not involve bioprinted tissues; rather, they focused on creating scaffolds that supported cell-based therapies. The scaffolds were intended to facilitate the adhesion and function of transplanted cells (not bioprinted tissues), enhancing the expression of cardiomyocyte-specific markers and leading to improved cardiomyocyte (CM) functionality and synchronous contraction, critical for restoring coordinated heartbeats and overall heart performance (Ye & Qiu, 2017). Cui et al. (2018) further expanded on these findings, exploring how these scaffolds could improve the functionality of cardiomyocytes and enhance heart tissue regeneration, promoting better outcomes after myocardial injury. The study by Cui et al. (2018) also did not involve bioprinted tissues, as it focused on the use of scaffolds supporting cells.

A study conducted by Mihic et al. examined the effects of chitosan-graft-polypyrrole (CS-g-PPY) hydrogel compositions as implants for treating damaged cardiac tissues and enhancing heart function post-myocardial infarction.(Mihic et al., 2015). The CS-g-PPY hydrogel was developed to improve electrical conductivity and mimic the natural electrical properties of cardiac tissue. CS-g-PPY demonstrated improved electrical conduction and calcium signal propagation in *ex vivo* tests, enhancing the communication between cardiomyocytes necessary for synchronised contractions. *In vivo* experiments on rats showed that CS-g-PPY treatment led to echocardiogram readings similar to pre-infarction levels after one week, with faster transverse conduction velocities and elongated rather than rounded scar tissue, indicating better tissue integration and repair. CS and CS-g-PPY reduced scar size and improved various cardiac function parameters compared to saline injections, suggesting CS-g-PPY's potential to restore cardiac electrical and structural integrity after myocardial infarction (Hackett et al., 2017), as seen in Figure 1.

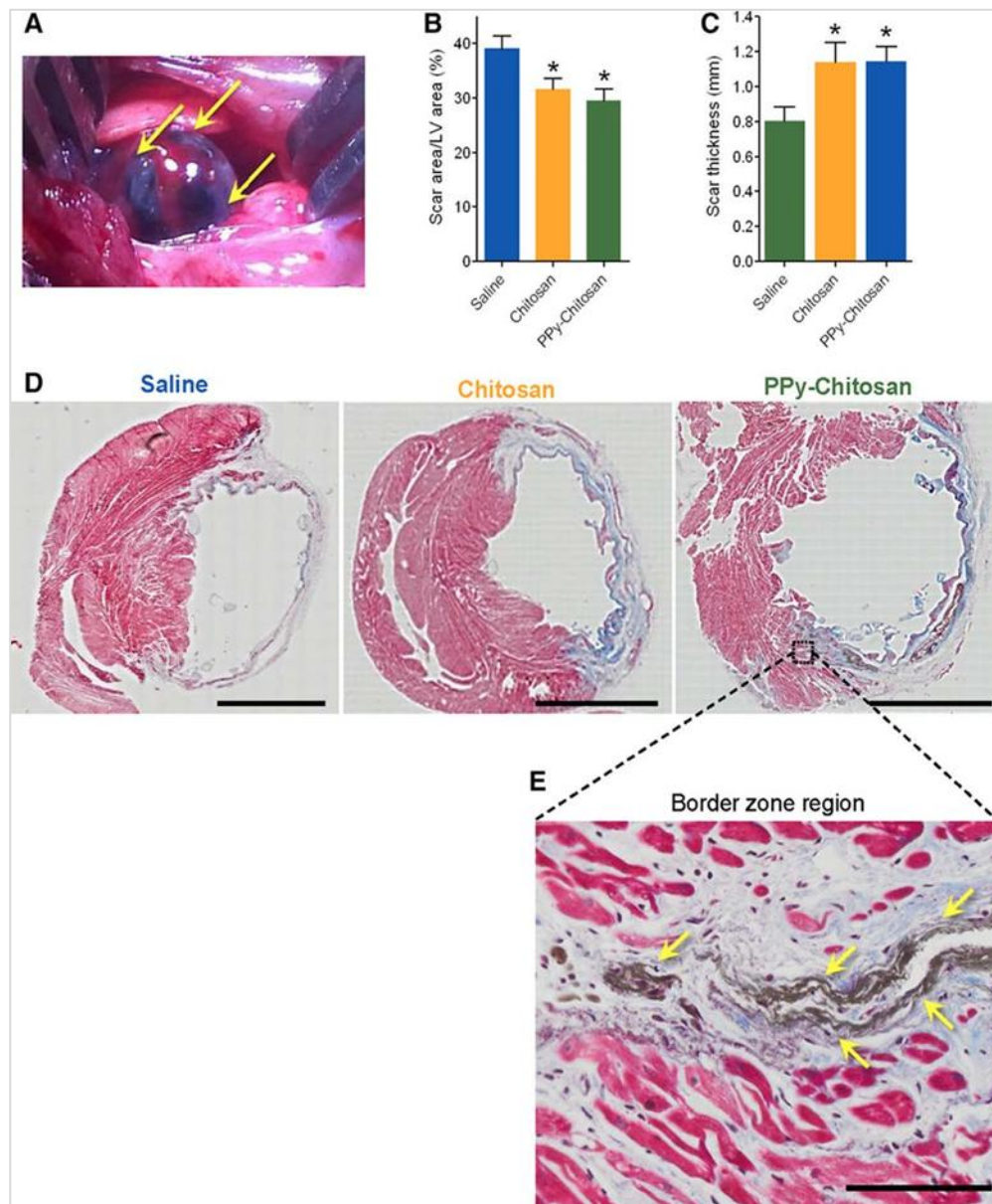


Figure 1 . Chitosan-graft-polypyrrole (CS-g-PPY) hydrogels reduce scare size in post-MI rat hearts. **A.** Intraoperative photograph displaying three darker areas for CS-g-PPY was injected into the border area of the rat heart one-week post-MI. **B** and **C.** Eight weeks post-injection, scar area and thickness were measured in a heart treated with saline (n=6), chitosan (n=8), and CS-PPY (n=8). CS-PPY and chitosan significantly reduced scar compared to saline ($P < 0.05$). **D.** Representative heart sections from each group, taken from a similar transverse section below the papillary muscles. **E.** Masson trichrome staining reveals PPY-chitosan

particles in the scar and border zone of the heart eight weeks post-injection (arrows). Scale bar:100 μ m (Mihic et al., 2015).

1.3.3. Gelatine

Gelatine is a useful biomaterial widely used in medical applications such as wound healing and tissue engineering (Schrieber & Gareis, 2007). Due to its biocompatibility and biodegradability, gelatine provides a supportive environment for various cell types, including cardiac, bone, and cartilage cells, making it highly versatile in tissue engineering.

Gelatine is derived from collagen, typically from animal connective tissues such as skin, bones, and tendons. It is composed of amino acids like glycine, proline, and hydroxyproline, forming a triple-helix structure. Gelatine is bioactive and demonstrates its ability to support cell recruitment, attachment, and growth, facilitating natural healing processes. Its composition and structure enable it to mimic the extracellular matrix (ECM), which is essential for cellular functions and tissue integration.

Gelatine has successfully been used to transport corneal endothelial cells for transplantation, enhancing cell growth and alignment. Moreover, gelatine-based scaffolds show efficacy in regenerating bone and cartilage tissue, offering properties similar to physiological tissues, which is particularly beneficial for bone regeneration (Mihic et al., 2015).

In cardiac applications, gelatine can be modified or combined with other materials to improve its mechanical strength and stability, enabling its use in cardiac patches and as a supportive matrix for cell delivery (Hackett et al., 2017), as seen in Figure 2.

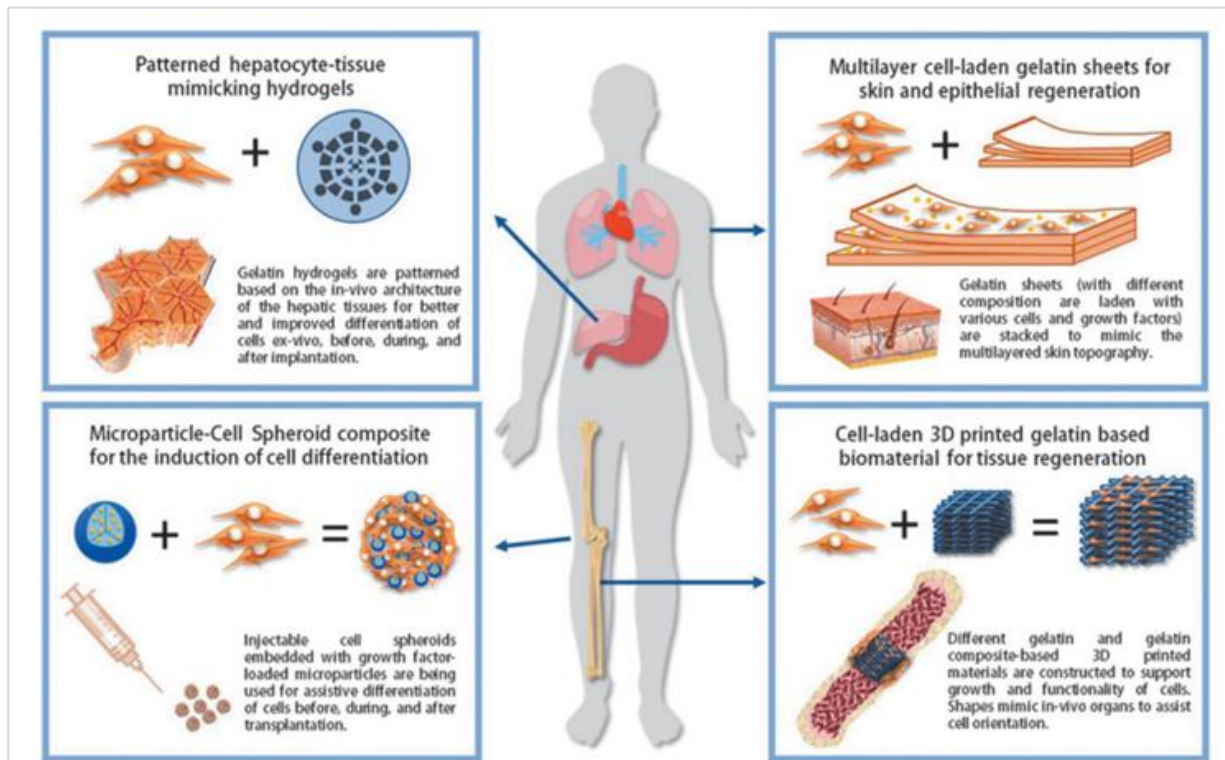


Figure 2. Medical Application of Gelatine Biomaterials. This figure highlights the advancements in gelatin biomaterials, showcasing their use of multi-layered cell-laden sheets for skin and corneal regeneration, patterned hydrogels that mimic natural microenvironments for targeted cell differentiation, injectable spheroids for cell transplantation, and 3D-printed scaffolds for repairing bone and cartilage. (Bello et al., 2020).

A study conducted by Polonchuk et al. (2021) showed that the combination of gelatine with alginate produced hydrogels that supported cardiac cell viability, showing promise for regenerative medicine. The study highlighted that the alginate-gelatine combination created an ECM-like environment, which enhanced cell survival. Another study utilised gelatine in 3D-printed Alginate/gelatine (AlgGel) patches containing cardiac spheroids, which were transplanted onto the outer surface of the heart in a mouse myocardial infarction model. Gelatine played a crucial role in formulating the bioink for printing the patches, contributing to their structural integrity and biocompatibility. The results showed that transplanting these gelatine-incorporated patches led to enhanced heart function, likely by upregulating cardiac-specific gene expression and reducing inflammation in the host tissue, which contributed to improved contractile function and decreased adverse remodelling (Roche et al., 2023).

1.3.4. Gelatine Methacryloyl (GelMA)

GelMA is commonly used in heart tissue engineering due to its excellent cell compatibility and tunable mechanical properties, especially with UV cross-linking (Sharifi et al., 2021; Yue et al., 2015). These hydrogels mimic the natural extracellular matrix (ECM), promoting cell growth and tissue formation. GelMA's mechanical properties can be finely adjusted with light irradiation in the presence of photoinitiators, allowing it to be microfabricated into controlled structures, such as microchannels or patterned configurations, which support vascularisation and tissue integration. Its flexibility to replicate specific tissue stiffness and elasticity makes it particularly suitable for cardiac applications with vital mechanical properties. GelMA has proven effective in bone, cartilage, cardiac, and vascular tissue engineering, as well as drug delivery and biosensing (Kurian et al., 2022). However, there are some limitations. UV cross-linking, although crucial, can negatively impact cell viability (Chansoria et al., 2021). Additionally, GelMA has limited long-term stability in physiological conditions unless further modified (Xiao et al., 2019), which could hinder its sustained effectiveness in tissue regeneration applications.

A study conducted by Nikkhah et al. (2012) investigated the formation of endothelial cord structures using micropatterned GelMA hydrogels. Through precise micropatterning techniques, the researchers created GelMA constructs with varying shapes and heights to examine their effects on endothelial cell behaviour and organisation. They discovered that the shape of the GelMA constructs was crucial in guiding the development of well-organised and durable cord structures by endothelial cells. Specifically, constructs with optimised heights and geometries provided the most favourable microenvironment, enhancing cell alignment, elongation, and connectivity, which are essential for forming stable vascular networks. Their study underscores the potential of GelMA hydrogels in controlling the morphology of endothelial cells, which is vital for applications in tissue engineering, such as creating vascularised tissue constructs for cardiac and other organ regeneration.

In a study conducted by Lee et al. (2019), the researchers explored the use of GelMA for cardiac tissue engineering combined with carbon nanotube (CNTs) and reduced graphene oxide (rGO), demonstrating improvements in electrophysiological properties, electrical conductivity, mechanical stiffness, and the maturation of cardiac myocytes (CMs). The integration of these

conductive materials aimed to replicate the electrical environment of native cardiac tissue, essential for synchronised cell contractions. Additionally, adding fibronectin to GelMA hydrogels enhanced CM survival and spreading, providing an extracellular matrix component facilitating cell adhesion and proliferation. The study findings indicated that different functionalizations led to distinct forms of tissue maturation: Graphene oxide (GO) resulted in atrial-like tissue, carbon nanotubes (CNTs) in ventricular-like tissue, and reduced graphene oxide (rGO)-GelMA in a mixed phenotype resembling cardiac muscle cells. This differentiation suggests that tailored functionalisation of GelMA with specific conductive materials can guide the formation of specific cardiac tissue types, supporting the development of biomimetic heart models for research and therapeutic applications (Elkhoury et al., 2021), as seen in Figure 3.

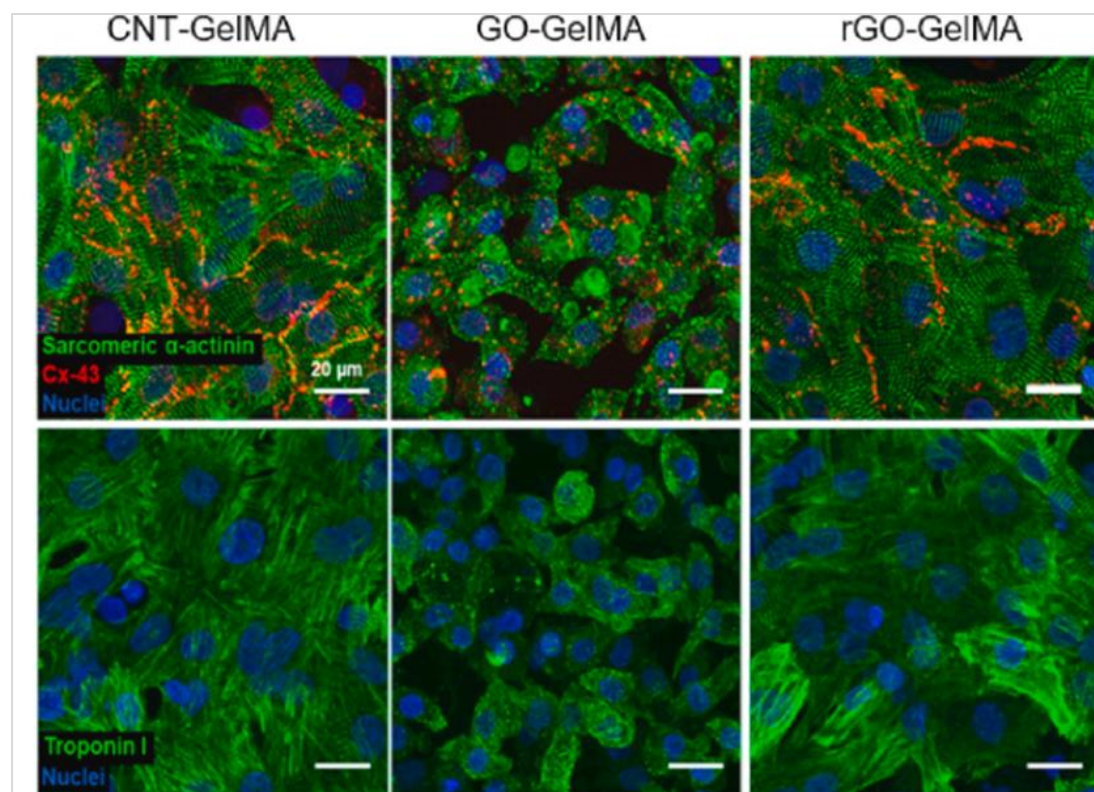


Figure 3. Effects of carbon nanoparticles on GelMA constructs. This image shows GelMA constructs modified with carbon nanotubes, highlighting increased expression of connexin 43 (Cx-43) and troponin I, as well as enhanced cellular alignment. **A.** Immunostaining for sarcomeric α -actinin (green), connexin 43 (Cx-43, red), and nuclei (blue), showing distinct phenotypes of cardiac tissues after 5 days of culture. Cross-sectional views are shown at the

bottom. **B.** Immunofluorescence images of cardiomyocytes on scaffolds stained for troponin I (green) and nuclei (blue), demonstrating cardiac differentiation (Lee et al., 2019).

In another study, cardiomyocytes in GelMA-coated microchannels displayed organised growth guided by fluid flow, resulting in tissue similar to cardiac fibres with inherent beating capabilities (Yue et al., 2015).

Annabi et al. (2013) developed a heart-on-chip platform using photocrosslinked GelMA hydrogels to coat microfluidic devices for drug screening in biomedical engineering. They demonstrated that the GelMA solution could be injected into microchannels and UV-crosslinked to form a coating of controllable thickness. This system allows cell incorporation within the hydrogel, creating a tissue-mimicking microenvironment with physiological conditions, such as oxygen/nutrient supply and shear stress. This microfluidic GelMA platform shows potential for studying cell-biomaterial interactions, biosensing, and drug screening by closely replicating native tissue environments (Yue et al., 2015).

A study by M. B. Chen et al. (2013) focuses on using GelMA to develop a microfluidic platform with a bilayer membrane device to simulate physiological cardiovascular cell-cell interactions. While the study does not specifically address conductive polymers or materials, the platform's use of GelMA hydrogel could potentially be combined with conductive materials in future research to enhance the electrical properties of the system. Incorporating conductive polymers into such platforms could help mimic the electrical conductivity of native tissues, which is crucial for studying cardiac and vascular cell behaviour under electrical stimulation or flow-induced shear stress. This approach may provide new insights into the role of electrical signals in vascular and valvular biology.

1.3.5. Collagen

Collagen is a structural protein that is primarily derived from the extracellular matrix (ECM) and is found abundantly in connective tissues throughout the body. It plays a critical role in providing structural support to the heart, facilitating stem cell attachment, and transporting essential molecules necessary for heart repair and rejuvenation (Golpanian et al., 2016). Collagen is typically crosslinked through enzymatic or chemical processes, which help to stabilize its structure and enhance its mechanical properties. These crosslinks are crucial for

maintaining the integrity of the heart, distributing internal forces, and providing the required strength and flexibility for optimal heart function. Its importance in cardiac function cannot be overstated (Tanzer, 1973).

Collagen-based biomaterials for tissue engineering were investigated by Wang et al. (2023), examining their diverse applications, potential avenues for future development, and challenges in the field. The study's emphasis on tissue regeneration underlines the versatility of collagen, particularly its ability to combine with other substances and serve as an extracellular matrix (ECM), providing structural support and biochemical cues that promote cell adhesion, proliferation, and differentiation. Collagen's adaptability makes it suitable for various tissue engineering applications, from cardiac patches and skin grafts to bone scaffolds, due to its biocompatibility and ability to mimic natural tissue environments.

Regarding the types of collagens, the study likely refers to type I collagen, which is predominant in bone, skin, and tendon, and type II collagen, which is found in cartilage, as these types are commonly used in tissue engineering applications. Additionally, type III collagen, which plays a role in skin and blood vessels, and type IV collagen, found in the basement membrane, are also important in some tissue engineering contexts, especially when mimicking the ECM.

The study also highlighted the potential for enhancing collagen's mechanical and biological properties through cross-linking or incorporation with synthetic polymers, aiming to improve its stability and functionality in dynamic environments, such as the heart. This versatility contributes to collagen's significance in biomaterial development, making it a promising candidate for future advancements in regenerative medicine.

Curley et al. (2019). investigated myocardial tissue engineering as a strategy to treat heart failure resulting from myocardial infarction (MI). They aimed to repair damaged left ventricles using minimally invasive procedures involving injectable biopolymers combined with cells. These biopolymers included alginate, fibrin, collagen, and chitosan, which served as scaffolds to promote tissue regeneration and improve cardiac function. The study developed biocompatible heart muscle patches that closely resembled the natural myocardium and supported tissue repair in the infarcted area. The injectable biopolymers provided a scaffold that facilitated cell delivery and retention in the damaged tissue, enhancing localised

regeneration potential. Collagen emerged as a crucial component in this approach, providing structural support, stiffness, and resistance to deformation, which is critical for maintaining the heart muscle's integrity during contractions. The myocardial collagen matrix, primarily composed of type I and III collagens, is key to preserving heart muscle structure and function. Each collagen type contributes differently to the myocardium's mechanical strength and elasticity, essential for promoting stem cell differentiation into cardiomyocytes and supporting cardiac tissue regeneration and functional recovery post-MI (Omenetto & Kaplan, 2010; Tao et al., 2012)

1.3.6. Silk

Silk is a natural biomaterial used in cardiac tissue engineering due to its remarkable biocompatibility, mechanical properties, and versatility in forming films, scaffolds, and hydrogels. It comprises silk fibroin (SF) and silk sericin (SS), with SF offering strong mechanical properties that can be tailored through structural modifications, while SS enhances cell adhesion and proliferation and controls antioxidant properties beneficial for cardiac tissue repair. The extraction methods employed significantly impact the properties and control antioxidant applications of SF and SS in tissue engineering. Additionally, silk-based materials are cost-effective, durable, and can be utilised for *in vivo* tracking due to their photoluminescent properties (Gupta & Mandal, 2021; Omenetto & Kaplan, 2010).

The impact of SF on alginate-gelatine (Alg-Gel) hydrogels for cardiac bioink formulations was explored by Vettori et al. (2024). The study aimed to improve Alg-Gel hydrogels' structural and functional properties for cardiac tissue engineering. Adding 1% SF enhanced the hydrogel's elasticity and printability without compromising cell viability, allowing the bioink to be extruded smoothly and form stable, well-defined structures suitable for 3D bioprinting. This addition also improved the contractile function of cardiac spheroids, supporting synchronised beating and mimicking native heart tissue functionality. However, a higher SF concentration (2%) negatively affected pore size and cell viability, reducing nutrient and oxygen diffusion, which hindered cell growth and function. The study concluded that 1% SF in Alg-Gel hydrogels optimise mechanical properties and cardiac cell function, showing promise for 3D bioprinted cardiac tissue applications and advancing the development of functional cardiac constructs.

Cetin et al. (2021) explore the use of 3D silk fibroin scaffolds for cardiac tissue engineering, focusing on the potential of human adipose-derived mesenchymal stem cells (hAD-MSCs) for cardiac regeneration. The research investigates the properties of SF scaffolds, such as their morphology, swelling behaviour, and biodegradability. The results show that SF scaffolds can support the proliferation and cardiomyogenic differentiation of hAD-MSCs. The study further demonstrates that SF scaffolds promote the expression of key cardiac biomarkers, such as troponin I, connexin 43, and myosin heavy chain, which are crucial for cardiac tissue function. These findings suggest that SF scaffolds could be a promising material for cardiac tissue engineering, with potential applications in repairing heart tissue damaged by conditions like myocardial infarction. Integrating conductive materials, like conductive polymers or nanoparticles, into the scaffolds could improve their electrical conductivity, enhance cell-to-cell communication and promoting better tissue integration and functionality in cardiac applications.

1.3.7. Fibrin

Fibrin is a natural biopolymer found primarily in the blood, where it plays a crucial role in the blood clotting process. It is produced when fibrinogen, a soluble protein in the blood, is converted into insoluble fibrin strands during coagulation. Fibrin is notable for its biocompatibility, ability to integrate cells and growth factors, and biodegradability. It is essential in cardiovascular repair as a scaffold for cardiac tissue engineering. Its flexibility allows for the tailored mimicry of the ECM, making it ideal for crafting heart valves, cardiac patches, and small-diameter vessels. Moreover, fibrin-based injectable cardiac tissue engineering aims to bolster cell therapy outcomes in cardiovascular repairs, leveraging fibrin's capacity to enhance cell therapy efficacy by facilitating specific homing and in-situ cell retention (Barsotti et al., 2011).

A study conducted by Chang et al. (2022) developed a fibrin-based cardiac patch containing neuregulin-1 (NRG-1) for treating myocardial infarction (MI). Male C57BL/6 mice (6–8 weeks old) were divided into four groups: Sham, MI, Patch, and Patch+NRG-1, which were used to assess the effectiveness of the treatment in improving MI heart function. The patch leverages the biocompatibility and ECM-like structure of fibrin, combined with the regenerative capabilities of NRG-1 via the ErbB signalling pathway, to promote cardiomyocyte

proliferation, reduce cell apoptosis, encourage angiogenesis, and inhibit fibrosis. However, a major limitation of fibrin is that it can restrict the exchange of oxygen and nutrients with the outer environment, which may affect the long-term viability of the cells within the patch.

Histology results (Masson's Trichrome staining) revealed reduced myocardial fibrosis and infarct size on Day 28 after MI. Immunohistochemistry results for TUNEL staining indicated reduced cell apoptosis (7.06% in the Patch+NRG-1 group vs. 33.02% in the MI group). Also, CD31 staining demonstrated increased blood vessel formation in the infarct border zone, and Ki67/cTnT/DAPI staining revealed increased cardiomyocyte proliferation in the Patch+NRG-1 group. Echocardiography results for M-mode imaging showed improved left ventricular function, with significantly higher left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) values in the Patch+NRG-1 group compared to the MI group (36.78% vs 21.88%, respectively).

In clinical practice, LVFS values in heart failure patients often fall below 30%, and values above 30% indicate improved cardiac function, suggesting that the patch may have clinical relevance in improving heart function and patient survival. Higher LVFS values are associated with better survival rates, so the observed improvement in LVFS suggests potential benefits for patients with myocardial infarction and heart failure.

Collectively, all results for the Patch+NRG-1 group showed significant benefits, including reduced cell apoptosis, improved left ventricular contraction, enhanced cardiac function, reduced myocardial fibrosis, and smaller infarcted scar size. These outcomes target different aspects of MI recovery: reducing apoptosis protects cardiomyocytes, improving contraction and cardiac function enhances heart performance, while reducing fibrosis and scar size prevents further heart damage. Together, these findings highlight the patch's promising potential for MI treatment.

Shaik et al. (2023) investigated the development and testing of fibrin-enriched cardiac extracellular matrix (Fn-cECM) hydrogels to promote angiogenesis. The cardiac extracellular matrix (cECM) is the natural matrix found in cardiac tissue, providing structural and biochemical support to heart cells. It is derived from the heart tissue itself and is crucial for maintaining tissue integrity and function (Kafili et al., 2023). The research demonstrated the characteristics of hydrogels by comparing storage moduli, complex moduli, swelling ratios, and degradation rates between cECM and Fn-cECM. Fn-cECM hydrogels exhibited faster gelation times, improved mechanical stiffness, and lower swelling ratios compared to cECM, making them suitable for pregel solution mixing and catheter delivery. The study demonstrated that Fn-cECM hydrogels promoted robust vascular network formation in human umbilical vein endothelial cells (HUVECs) and enhanced angiogenesis by increasing tube formation and VEGF secretion, as seen in Figure 4. Furthermore, the Fn-cECM hydrogels induced more robust tube network formation and VEGF secretion in human mesenchymal stem cells (hMSCs) and promoted angiogenic sprouting in hMSC spheroids. The findings suggest that blending fibrin with cECM hydrogels accelerates gelation and enhances proangiogenic properties, making Fn-cECM a promising material for cardiac tissue engineering. However, rapid degradation observed in Fn-cECM hydrogels may require further modification for improved stability, and the study recommends future optimisation and evaluation in myocardial infarction models.

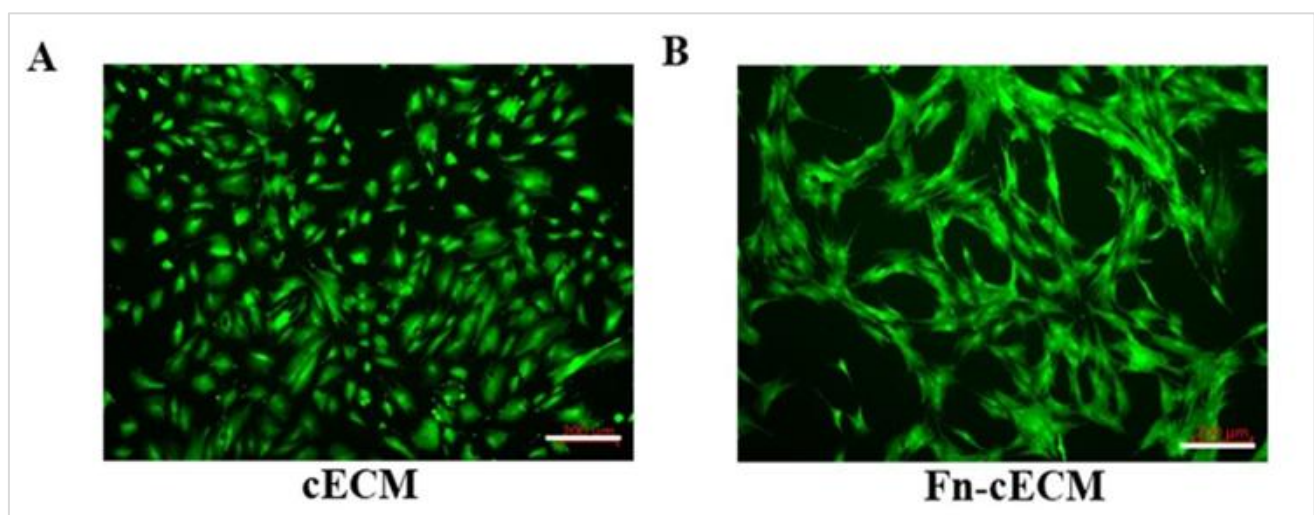


Figure 4. Vascular network formation and VEGF secretion by hMSCs seeded on hydrogels. **A.** Representative images of Calcein AM-stained hMSCs seeded on cECM. **B.** Representative images of Calcein AM-stained hMSCs seeded on Fn-cECM hydrogels, demonstrating differences in vascular network formation. Scale bar: 200 μm (Shaik et al., 2023)

Table 1. Strengths and Limitations of Biomaterials in Cardiac Tissue Engineering.

Biomaterial	Strengths	Limitations
Alginate	- Biocompatible, biodegradable, non-toxic, and non-immunogenic (Ahmad et al., 2021; Martău et al., 2019). - Forms gels with calcium ions, enabling effective cell delivery and encapsulation (Orive et al., 2006). - Promotes vascularisation, reduces infarct expansion, and prevents LV enlargement (Xu & Lam, 2018).	- Low mechanical strength when used alone (LeRoux et al., 1999). - Requires modification or combination with other materials, such as conductive polymers (e.g., Polypyrrole, Polyaniline), graphene, or carbon nanotubes, for improved structural support and conductivity (Draget et al., 1997).
Chitosan	- Supports cardiac repair by promoting cell adhesion (Chen et al., 2018). - Conductive when combined with materials like polypyrrole (Y. Chen et al., 2013). - Enables controlled scar tissue formation and improves signal conduction (He et al., 2020).	- Conductivity needs enhancement through additional materials like polypyrrole (Zarei et al., 2021). - Variable degradation rate that impacts its stability for prolonged cardiac use (Domengé et al., 2021; Kean & Thanou, 2010).
Gelatine	- Supports cell recruitment, attachment, and growth (Moscato et al., 2008). - Effective in enhancing heart function when used in cardiac patches (Cristallini et al., 2019). - Biodegradable and mimics physiological properties for bone and tissue engineering (C Echave et al., 2017; Gorgieva & Kokol, 2011).	- Rapid degradation and limited mechanical stability on its own (Stevens, 2009) - Conductivity may require enhancement through combination with other material such as onductivity may require enhancement through combination with other materials, such as conductive polymers (e.g., polyaniline, polypyrrole) or carbon-based nanomaterials (e.g., graphene) (Gorgieva & Kokol, 2011).
GelMA (Gelatin Methacryloyl)	- Excellent cell compatibility and tunable mechanical properties with UV cross-linking (Sharifi et al., 2021; Yue et al., 2015). - Mimics the ECM and supports cell alignment and tissue structure formation (Xiao et al., 2019).	- UV cross-linking is required, which may affect cell viability (Chansoria et al., 2021; Lim et al., 2019). - Limited long-term stability in physiological conditions without modification (Xiao et al., 2019). - Modifications (e.g., incorporation of conductive polymers such as

	- Effective for cardiac, vascular, and bone tissue engineering (Kurian et al., 2022; Xiao et al., 2019).	polypyrrole or polyaniline, carbon nanomaterials like graphene oxide or carbon nanotubes, or gold nanoparticles) are required for improved stability and conductivity (Kaviani et al., 2024)
Collagen	- Naturally supportive of cell attachment and cardiac tissue structure (Terracio et al., 1991). - Provides flexibility and is a key ECM component aiding stem cell differentiation (Lee et al., 2011). - Useful in injectable hydrogels and cardiac patches (Wu et al., 2019).	- It requires conductive materials (e.g., polypyrrole, polyaniline, graphene oxide, or gold nanoparticles) to enhance electrical conductivity in cardiac applications Q. Li et al. (2022) - Rapid degradation can impact durability in cardiac tissue engineering (de Souza, 2002). - sources Sourcing from animal origins may impact immune compatibility and quality; however, other commonly used biomaterials, such as gelatine, Matrigel, fibrin, and chitosan, are also derived from animal sources (Rezvani Ghomi et al., 2021).
Silk	- Excellent biocompatibility, customizable mechanical strength (C. Wang et al., 2019). - Supports cell adhesion and proliferation (Mandal & Kundu, 2009). - Enhances mechanical properties when added to other biomaterials (Vepari & Kaplan, 2007).	- Higher concentrations may reduce cell viability and affect structural porosity (L. Huang et al., 2019). - It requires complex fabrication steps, such as electrospinning or chemical modification, to improve specific properties like mechanical strength and biocompatibility (Li et al., 2015; Q. Wang et al., 2019). - Biodegradable and can be combined with conductive materials, such as polypyrrole, polyaniline, graphene oxide, or carbon nanotubes, to enhance electrical conductivity and support cardiac tissue function (Vasconcelos et al., 2008)

Fibrin	- High biocompatibility and flexibility in mimicking ECM, not conducive by itself (Nazari et al., 2020). - Enhances cell integration, cardiac patches, and cell retention. - Effective in 3D bioprinting applications for cardiac constructs (Chang et al., 2022).	- Low mechanical strength, degrades relatively quickly (Collet et al., 2003), and lacks intrinsic electrical conductivity, often requiring blending with conductive materials (e.g., polypyrrole, graphene oxide) for cardiac applications (Mehdikhani & Ghaziof, 2018) - Limited cell survival in the middle of the material due to limited diffusion of nutrients and oxygen in thicker fibrin layers (Nazari et al., 2020). - Quick gelation reduces working time in certain applications (Janmey et al., 2009).
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1.4. Conductive Polymers for Cardiac Bioengineering

Recent research has focused on exploring the potential of conductive polymers in regenerative medicine, particularly in cardiac tissue engineering. Conductive hydrogel formulations, for instance, have positively impacted cardiac cells by promoting electrical conduction in non-conductive areas. This property is particularly valuable for mimicking native tissue and preserving the electrical connection between myocardial cells in an infarcted heart. Understanding the critical role of electrical signalling in the native myocardium and cell proliferation during disease compared to healthy tissue is paramount. Such insights help identify similarities and differences between the two cell types, crucial for correcting specific signalling pathways in disease models to restore them to a healthy state (Ribeiro et al., 2017; Saravanan et al., 2018).

In addition to their role in regenerative medicine, conductive polymers also play an essential role in heart function and the electrical coupling of heart cells within engineered scaffolds, which allows heart cells to communicate and coordinate contraction, restoring heart function in damaged tissue (Ketabat et al., 2018). A variety of conductive polymers, such as carbon nanotubes, gold nanostructures, graphene, polypyrrole, polyaniline, and Maxene, have been investigated for their potential to improve electrical conductivity in the heart (Prajapati

& Kandasubramanian, 2019). This section will describe some of the conductive polymers used in biomedical engineering, including cardiac applications.

1.4.1. Carbon nanotube

Carbon nanotubes (CNTs) are derived from carbon atoms and can be produced through methods such as chemical vapor deposition (CVD), arc discharge, or laser ablation. They hold promise for regenerating cardiac tissue due to their strong nature and unique mechanical and electrical conductive properties (Moorthi et al., 2017). CNTs are easily chemically modified, making them excellent candidates for creating engineered cardiac structures. Combining CNTs with polymeric scaffolds enhances cardiac regeneration by improving cardiomyocyte behaviour, such as synchronized contractility (Shin et al., 2015). The elongated shape of CNTs is advantageous for replicating the anisotropic structure of heart muscle, enabling the creation of finely detailed scaffolds to accurately mimic cardiac tissue (Barrejón et al., 2021).

A study by Barrejón et al. (2021) investigated the use of carbon nanotubes (CNTs) to develop scaffolds for regenerating damaged cardiac tissue. The study highlights that incorporating CNTs significantly enhances cardiomyocyte synchronous contractility. The elongated shape of CNTs effectively mimics the structural features of the myocardium, enabling the creation of micropatterned scaffolds with nanoscale precision to better replicate cardiac tissue architecture. Micropatterning was achieved by using techniques such as nanoimprinting, laser etching, or electrospinning, which allow for the precise control of scaffold structure at the micro and nanoscale. This study underscores the potential of engineered CNT constructs as innovative platforms for improving cardiac tissue functionality.

1.4.2. Gold nanoparticles

Gold nanoparticles are small gold particles typically ranging in size from 1 to 100 nanometers in diameter. Their shape can vary, with common forms including spherical, rod-like, and cylindrical shapes, which contribute to their unique optical, electronic, and chemical properties (Abdelhalim et al., 2012). These nanoparticles can be customized with different compounds for various medical uses. They are well-tolerated by the body and can be precisely targeted, showing promise for innovative therapeutic applications (Sztandera et al., 2018).

1.4.3. Graphene

Graphene is a highly promising material for tissue engineering due to its exceptional ability to interact with a wide range of biomolecules, including DNA, proteins, enzymes, lipids, and carbohydrates. These interactions make graphene valuable for applications in regenerative medicine, as it can facilitate cell attachment, enhance signalling pathways, and support tissue growth (Shin et al., 2018). The ability of graphene to interact with DNA is particularly beneficial as it can aid in the delivery of genetic material and improve gene therapy applications. By embedding graphene into scaffolds, its stability and efficiency in gene delivery are enhanced, allowing for more precise control over cellular behaviour, such as promoting cell differentiation, proliferation, and tissue repair (Shin et al., 2016).

Furthermore, graphene has become an essential material in biomedical applications due to its unique properties, including its ability to influence cell growth and control the release of bioactive compounds, making it particularly valuable in regenerative medicine. Its exceptional conductivity, mechanical strength, and chemical stability, derived from its honeycomb lattice structure, allow for a wide range of potential application (Shin et al., 2016; Shin et al., 2018). Graphene can also be engineered into different forms, such as single-layer graphene and graphene oxide, which increases its flexibility and adaptability for specific uses (Geim, 2009). Since its discovery in 2004, graphene has garnered significant interest and found widespread use across many scientific disciplines (Novoselov et al., 2004).

Graphene has gained widespread attention across various fields of biomedical engineering due to its unique properties, aiding in extensive cytotoxicity analyses to ensure safe clinical applications. Several studies have assessed the biological toxicity of graphene on different cell types, including fibroblasts, epithelial cells, and neurons (Ikram et al., 2022; Mousavi et al., 2021; Yang et al., 2013). A study by Dreanca et al. (2020) focused on dental materials, demonstrating graphene's biocompatibility through *in vitro* and *in vivo* tests; the implications extend beyond dentistry. The results revealed that the materials were non-toxic to dental cells and did not cause acute toxicity, inflammation, or systemic organ toxicity in animal models, confirming the material's potential for broad biomedical applications, including tissue engineering and regenerative medicine. In cardiac applications, graphene's biocompatibility holds promise for enhancing heart tissue engineering, where it can support cardiac regeneration and facilitate better integration with heart cells.

A study by Fakhrali et al. (2021) focused on creating a composite nanofibrous scaffold that incorporates graphene using electrospinning. These scaffolds were made by the cross-linking of water-soluble polymers, and cells were loaded into them for further testing. The researchers conducted various analyses, including imaging, X-ray diffraction (XRD), water contact angle measurement, tensile testing, and biodegradation assessments. The MTT assay was used to assess sample cytotoxicity, and the behavior of human cardiac monocytes on the scaffold was studied. Results showed that adding PGS (poly(glycerol sebacate)) to PCL increased degradation rates, while graphene helped maintain structural integrity. PGS is a biodegradable elastomer often used in cardiac and vascular tissue engineering due to its flexibility and biocompatibility. PCL increased degradation rates, while graphene helped maintain structural integrity. Cell attachment, growth, proliferation, and migration on the scaffolds were observed over 1, 3, and 7 days, indicating promising cell behavior on the hybrid nanofibers.

1.4.4. Graphene oxide

Graphene oxide (GO), a functional derivative of graphene, has garnered significant attention for its potential in biomedical applications due to its remarkable versatility and biocompatibility (Nanda et al., 2015). Over time, researchers have increasingly explored its use in a variety of fields, including drug delivery, tissue engineering, biosensors, and medical imaging, owing to its unique properties that enable effective interaction with biological systems and enhancement of therapeutic outcomes (Pinto et al., 2013).

A study conducted by Choe et al. (2019) focused on graphene oxide, aiming to create microgel systems for delivering MSCs with strong antioxidant properties to protect them after a heart attack. The research found that MSCs enclosed in reduced GO/alginate microgels demonstrated higher survival rates under oxidative stress compared to MSCs not enclosed in the microgels, which resulted in better outcomes in heart attack models by reducing damage and improving heart function

Research conducted by J. Li et al. (2022) investigated the incorporation of graphene oxide (GO) into bio-ink for tissue engineering applications. Bio-ink refers to a specialized material used in 3D bioprinting, composed of a blend of biomaterials (such as alginate, gelatine, or other polymers) that can support cell encapsulation and enable tissue regeneration. By

combining GO with alginate (Alg) and gelatine (Gel), 3D printability for cell support was significantly enhanced. Compared to traditional bio-inks, the addition of GO improved the structural integrity and bioactivity of the printed scaffolds.

The concentration of GO used in the bio-ink formulations ranged from 0.05% to 0.5% (w/w). Specifically, the researchers found that 0.05%-0.1% (w/w) GO in an Alg/Gel hydrogel improved 3D printability and printing resolution. A concentration of 0.05% (w/w) GO was used to evaluate the effect on bio-ink preparation and performance. The highest achievable GO concentration was 0.5% (w/w), as higher concentrations resulted in undispersed GO content that could cause nozzle clogging during printing. When GO content exceeded 0.2%, the morphology deteriorated due to decreased storage modulus and GO flakes potentially hindered the crosslinking of Alg after printing.

The results demonstrated that adding GO to the bio-ink improved cell affinity and viability, making it a promising candidate for novel bio-ink formulations. This study underscores the potential of GO for advanced tissue engineering applications.

1.4.5. MXene

MXene is a two-dimensional material with metallic conductivity, optical and mechanical properties, hydrophilicity, chemical stability, and a large surface area (Salim et al., 2019). It is produced by etching the “A” metal layer in the MAX phase, creating 2D nano-layers of MXenes with surface functional groups. MXene is being explored for applications in cardiac repair (Lee et al., 2022; Zhang et al., 2019). Additionally, MXene reduces bacterial attachment and proliferation, enhancing its biocompatibility (Rasool et al., 2016). When paired with electrical field stimulation, these biohybrid platforms, which may include MXene combined with hydrogels, polymeric scaffolds, or other bioactive materials, greatly improve cell growth and maturation, especially in induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) (Asaro et al., 2023). Compared to traditional scaffolds or materials that do not utilize electrical stimulation, these biohybrid systems show significantly enhanced cell differentiation, proliferation, and functional maturation.

In 2023, Asaro et al. (2023) developed a conductive biohybrid platform using collagen type I with MXene (Ti₃C₂Tx). This platform exhibited enhanced electrical properties and mechanical strength while inhibiting bacterial attachment. It facilitated iPSC-derived

cardiomyocytes' attachment, elongation, and maturation under external electrical stimulation. The study highlights the potential of this biohybrid platform for a range of biological applications that require electrical conductivity, demonstrating promising outcomes for use in tissue engineering and regenerative medicine.

Another study on MXene-based materials for cardiac repair was conducted by Ye et al. (2020). This study focused on the potential in biomedical applications, particularly tissue engineering. MXene-based hydrogels exhibit excellent electrical, chemical, and mechanical properties, supporting cardiac tissue regeneration. Studies have shown that MXene-based hydrogels can enhance the expression of cardiac-related genes, improve the synchronous beating of cardiomyocytes, and have no cytotoxicity, aiding cardiac repair after myocardial infarction. Additionally, MXene quantum dots in hydrogels offer anti-inflammatory properties and promote cellular activity, making them promising for tissue repair and treatment of inflammatory and degenerative diseases, including cardiac applications (Rafieerad et al., 2019).

1.4.6. Polypyrrole

Polypyrrole (PPy) is a conductive polymer widely studied in tissue engineering. It is valued for its biocompatibility, conductivity, and ease of synthesis (Liang & Goh, 2020). Several cell types, including neurons, fibroblasts, and endothelial cells, have been shown to adhere and grow on PPy substrates in the lab, suggesting potential applications in cardiac tissue regeneration and electrical signal transmission. Research indicates that PPy-containing nanofibers can enhance cell attachment, proliferation, and the expression of cardiac-specific proteins (Guo & Ma, 2018).

Guo and Ma (2018) utilized electrically conducting polymers, including PPy, to fabricate conductive biomaterials for tissue regeneration, covering formats such as films, nanofibers, hydrogels, and scaffolds. Their research demonstrated the capacity of conductive polymers to stimulate cells with electrical signals, showcasing the development of degradable conductive composite films and copolymers. These innovations have been shown to enhance cell behaviour and promote tissue regeneration in tissue engineering applications.

In a study by Kai et al. (2011), PPy was utilised in cardiac tissue engineering to create bioactive scaffolds for cardiac regeneration. These scaffolds were generated by incorporating various concentrations of PPy into poly(ϵ -caprolactone) (PCL)/gelatine nanofibrous scaffolds.

Increasing the concentration of PPy (ranging from 0% to 30%) resulted in thinner fibres and higher tensile strength. The PPG15 scaffold, containing 15% PPy in PCL, exhibited an optimal balance of conductivity, mechanical strength, and biodegradability, which enhanced cell attachment, growth, and the expression of cardiac-specific proteins compared to the PPG30 scaffold. The study underscored the critical role of conductivity, mechanical properties, and biodegradability in designing scaffolds for cardiac regeneration.

The study by Zanzanizadeh Ezazi et al. (2020) aimed to develop a biodegradable cardiac patch with conductivity and elasticity for heart tissue engineering. They combined poly (glycerol sebacate) (PGS), collagen, and PPy using the evaporation method, which involves dissolving the polymer blend in a solvent, followed by solvent evaporation to create thin, solid films or coatings. This approach was used to treat myocardial infarction. Results revealed that the composite containing 5% PPy exhibited increased blood wettability compared to pure PGS, indicating its potential for attracting proteins due to PPy's hydrophobic nature. Furthermore, the 0.5-5% PPy patch demonstrated improved conductivity, reaching 0.06 S compared to lower PPy concentrations. The study also assessed the patches' mechanical properties, degradation rate, protein adsorption, drug release, and cell viability, highlighting their potential in cardiac repair applications.

1.4.7. Polyaniline

Polyaniline (PANI) is a conductive polymer commonly used in cardiac tissue engineering due to its biocompatibility and ability to support cell development and growth (Ul Haq et al., 2021). Conductive polymers, like PANI, are extensively researched for their conductivity and biocompatibility with cardiac tissue regeneration, presenting potential treatment options for MI (Cui et al., 2016; Ul Haq et al., 2021). Polyaniline nanotubes (PANINTs), particularly when dendronized (as PGLD-PANINTs), have been investigated for their potential as scaffolds in cardiac tissue engineering. The study conducted by Moura and de Queiroz (2011) focuses on assessing the biocompatibility and promoting cardiomyocyte proliferation using PGLD-modified PANINTs.

The research indicates that PGLD-coated PANINTs are non-cytotoxic to Chinese hamster ovary (CHO) cells, and microcurrent stimulation supports the differentiation of cardiac cells cultured on these scaffolds. PGLD-PANINTs have shown the potential to serve as a

biocompatible, electroactive three-dimensional matrix for cardiac tissue engineering. These findings were confirmed through XPS analysis, which showed the presence of PGLD on the surface of the PANINTs. The study also demonstrated that PGLD-PANINTs electrospun fibres, produced from a 10% wt/vol dispersion of PGLD-PANINTs in a 50:50 mixture of dichloromethane (DCM) and dimethylformamide (DMF), promoted better proliferation and differentiation of myocardial cells compared to casting PGLD-PANINTs films. The electrospinning process was performed at an applied voltage of 15–20 kV, and the resulting scaffolds showed excellent biocompatibility with no toxicity in CHO cells, indicating high cell viability. These electroactive and biocompatible characteristics suggest the potential use of PGLD-PANINTs in heart tissue engineering, highlighting the material's promising role in advancing cardiac regeneration and offering new avenues for treatment in myocardial infarction.

Ghovvati et al. (2022) explored the incorporation of PANI into electroconductive biomaterials for cardiac tissue engineering. They discovered that integrating PANI into scaffolds made of polymeric materials enhanced cell stimulation, facilitated the controlled release of therapeutic agents, and improved cardiac function in rat models of myocardial infarction. The study demonstrated significant improvements in cardiomyocytes' maturation, contraction, and electrical signalling, underscoring the potential of PANI-based biomaterials for promoting cardiac tissue regeneration and enhancing heart function.

A study by Liu et al. (2020) investigated the use of PANI in cardiac tissue engineering applications. They discovered that PANI significantly improves cardiomyocyte adhesion, proliferation, and responses to electrical stimulation, thereby beneficially altering cell electrophysiology and promoting tissue formation (Rocha et al., 2021). Additionally, PANI was found to form a conductive complex with polymeric materials, resulting in excellent electrical and mechanical properties that support cardiomyocyte growth. This research highlights the critical role of PANI in the repair of myocardial infarction, specifically by enhancing cardiomyocyte growth, improving electrical conductivity, and fostering tissue development through better cell communication and alignment.

Table 2. Conductive Polymer Properties and Applications

Conductive polymers	Properties	Applications
Carbon Nanotube (CNT)	Strong mechanical and electrical conductance makes them suitable for improving cardiac regeneration and drug delivery (Jalilinejad et al., 2023; Shar et al., 2023).	Cardiac regeneration, drug delivery (crosses the blood-brain barrier), biosensors, tissue engineering (cell tracking, microenvironment sensing, stem cell growth), bioimaging (contrast agents in ultrasound/photoacoustic imaging), cancer therapy, infectious disease and CNS disorder diagnostics and treatment (Gao et al., 2024; Murjani et al., 2022; Parvin et al., 2024).
Gold Nanoparticles (GNPs)	Biocompatibility and size-dependent toxicity influence their use in drug delivery and bio-imaging (Karnwal et al., 2024; Xia et al., 2016).	Drug delivery (targeted delivery of drugs/DNA), cancer treatment (photothermal therapy), bio-imaging (CT and photothermal microscopy), bone tissue engineering, and biosensors (Chen & Wu, 2024; Huang et al., 2023; Vines et al., 2019)
Graphene	Exceptional conductivity and mechanical strength support cell growth and bioactive molecule release, essential for drug delivery and biosensors (Bellet et al., 2021).	Drug delivery, biosensors (sensitive electromechanical sensors), tissue engineering (conductive scaffolds), antimicrobials (implant coatings), deep brain implants, neural interfaces (improved brain signal detection), blood glucose sensors (H. Huang et al., 2019)
Graphene Oxide (GO)	Semi-conductivity and antimicrobial properties make it suitable for tissue engineering and biosensors (Anegbe et al., 2024; Fares & Radaydeh, 2024).	Tissue engineering, biosensors, cardiac regeneration, antimicrobial coatings for medical devices and packaging, drug delivery, clinical diagnostics, and DNA sequence detection (Harish et al., 2022; Nguyen et al., 2020).
MXene	High conductivity and mechanical properties help in tissue engineering and biohybrid platforms to support cardiomyocyte maturation (Iravani & Varma, 2021; Seidi et al., 2023).	Tissue engineering, regenerative medicine, and biohybrid platforms to enhance cardiomyocyte maturation and attachment (Asaro et al., 2023; Kang et al., 2023).
Polypyrrole (PPy)	Conductivity and high biocompatibility make it suitable for cardiac tissue engineering and wound healing (Fonner et al., 2008; Huang et al., 2014)	Cardiac tissue engineering, enhancing electrical signalling in cardiac scaffolds, biodegradable patches for myocardial infarction repair, neural regeneration, drug delivery, biosensors, and wound healing (Liang & Goh, 2020; Shokrollahi et al., 2023).

Polyaniline (PANI)	Highly conductive and biocompatible, ideal for cardiac regeneration and conductive scaffolds to restore electrical function (Beygisangchin et al., 2024; G. Wang et al., 2017; Zare et al., 2019).	Cardiac regeneration, conductive scaffolds for restoring electrical function in injured hearts, promote cardiomyocyte maturation and electrical signalling (Arteshi et al., 2018; Jia et al., 2024).
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1.5. Conclusion

In conclusion, cardiac tissue engineering is essential for myocardial tissue regeneration. This approach involves creating engineered myocardial tissue with specific functional properties, initially *in vitro*, to replace damaged myocardium. The tissue is then transplanted into the heart *in vivo* to restore cardiac function. Recent advancements have led to the development of engineered cardiac patches using both natural and synthetic biomaterials.

This chapter identified the significant roles of electrically CPs in cardiac tissue engineering. These conducting polymers offer numerous advantages, including biocompatibility, adjustable conductivity, ease of synthesis, and flexibility, making them ideal for creating bioactive scaffolds. These scaffolds not only support cell electrical signalling but also enhance cardiac function post-implantation.

Conductive polymers differ from hydrogels, which are typically water-based materials that retain high water content and are used for their soft, flexible nature and ability to mimic natural tissues. While hydrogels are effective for tissue engineering due to their hydration and cell-supporting properties, they generally lack the electrical conductivity needed for efficient cell-to-cell communication. In contrast, conductive polymers provide the necessary electrical conductivity, which is crucial for integrating implanted materials with native myocardial tissue and enhancing the regenerative potential of the engineered tissue.

Despite promising *in vitro* and *in vivo* studies, substantial challenges remain in transitioning these materials from lab-based modes to clinical applications. Various fabrication techniques have been developed to produce conductive biomaterials, such as composite hydrogels, enhancing their potential for tissue regeneration. However, further research is crucial to fully explore these materials' potential in cardiac regeneration and to ensure their properties align

with the dynamic mechanical environment of the beating heart. This can be achieved by improving the mechanical strength, elasticity, and conductivity of these materials to better mimic the heart's natural tissue properties. Future work must refine this material and its applications to develop safe, effective, and reliable cardiovascular disease therapies.

The literature highlights the role of conductive materials in cardiac tissue engineering, particularly for enhancing electrical conductivity and promoting cellular integration and regeneration. However, while previous studies have shown promising results in improving cellular behaviour and tissue function with conductive polymers, the specific impact of combining these polymers with Alg- Gel hydrogels have not been fully explored in the context of myocardial regeneration. This is important because optimising the conductive elements in these hydrogels could directly contribute to more effective cardiac repair strategies. By enhancing the contractile activity in cardiac spheroids and improving heart function *in vivo*, this research addresses critical gaps in current treatments, offering potential solutions for heart failure and cardiac tissue regeneration. Addressing these gaps will help develop more efficient therapies for myocardial infarction and other heart conditions, which could ultimately lead to better patient outcomes. Although prior studies have shown that adding conductive polymers enhances electrical conductivity in hydrogels, the specific effect on the contractile function of bioprinted cardiac spheroids and myocardial function remains underexplored. Therefore, this thesis directly tests this by incorporating conductive elements into a 4% Alginate and 8% Gelatine hydrogel to evaluate improvements *in vitro* and a mouse MI model. Accordingly, the following hypothesis was developed and is presented on the next page.

1.6 Hypothesis

The addition of conductive elements to 4%alginate/8%gelatine hydrogels will improve the contractile activity in *in vitro* cardiac spheroids and improve cardiac function in an *in vivo* mouse model of heart failure.

1.6.1 Aims and Objectives

This project will test the hypothesis through the following aims and objectives:

Aim 1: To generate and characterise 4% alginate/8% gelatine hydrogels containing conductive elements.

This aim focuses on optimising the concentration and formulation of each selected conductive component and conducting comprehensive material characterisation to validate their suitability for cardiac tissue engineering. Key experiments include scanning electron microscopy (SEM) to examine pore structure, Brillouin microscopy and compression testing to assess mechanical properties, and the evaluation of printability, durability, and electrical conductivity. All experiments will be performed without cells to establish the fundamental properties of each hydrogel before proceeding to cell-based studies.

Aim 2: To evaluate if the addition of conductive elements to 4% alginate/8% gelatine hydrogels improves contractile activity in *in vitro* cardiac spheroids.

This aim will determine whether conductive elements enhance cardiac spheroid viability and contractile function *in vitro*. It will include detailed analyses of cell viability, spheroid viability, contractile activity (including measurement of contraction frequency and fractional shortening percentage), immunostaining to assess structural and cellular markers, and optical mapping to visualise and quantify electrical activity within the spheroids. These combined analyses will provide comprehensive evidence of the functional benefits of conductive hydrogels and inform the selection of optimal formulations for *in vivo* testing.

Aim 3: To evaluate if the addition of conductive elements to 4% alginate/8% gelatine hydrogels improves cardiac function in an *in vivo* mouse model of heart failure.

This aim will assess whether the conductive hydrogels developed in Aims 1 and 2 improve cardiac function when implanted in a mouse myocardial infarction model. Cardiac

function will be measured after 28 days using echocardiography to assess left ventricular ejection fraction (LVEF%). Following functional assessment, hearts will be collected for histological analyses, including H&E staining and fibrosis quantification using Masson's Trichrome and Picrosirius Red, to evaluate tissue structure, fibrosis, and patch engraftment.

Review

Toward strategies to advance the conductive properties of hydrogel formulations for cardiac tissue engineering

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SUMMARY

This review article aims to elucidate the role of conductive polymers (CPs) in cardiac bioengineering, focusing on scaffolds and biomaterials required for cardiac tissue engineering. It explores the unique features of CPs that make them well-suited for biomedical applications and explores how these polymers have been optimized to exhibit these properties. Moreover, the review provides insight into the importance of conductive biomaterials and their applications for cardiac repair and regeneration. Furthermore, the review seeks to inform and guide readers in this evolving field, highlighting the significant potential of conductive biomaterials in advancing cardiac tissue engineering. By understanding the capabilities and optimization strategies of CPs, researchers can effectively utilize these materials to develop innovative therapies for myocardial repair and regeneration, ultimately contributing to improved outcomes for patients suffering from heart attacks.

INTRODUCTION

Cardiovascular disease (CVD), including myocardial infarction (MI), is a leading cause of death worldwide, claiming approximately 18.6 million lives annually.¹ MI occurs when the heart is deprived of oxygen due to blocked blood flow, resulting in heart muscle damage. This condition presents a significant threat to individuals' lives and health, often leading to pathological and structural abnormalities within the heart ventricles. Immediate medical attention is crucial to reduce further damage and improve outcomes, especially given the limited availability of treatment options such as heart transplants.² Moreover, complications like heart failure can arise due to the heart's inability to self-repair. Regenerative medicine research aims to develop therapies for repairing damaged heart tissues, offering hope for enhancing outcomes and improving the quality of life for those affected by CVD.³

Currently, available treatment options, such as heart transplants, face challenges due to the scarcity of donor organs, underscoring the urgent need for alternative approaches, such as cardiac tissue engineering using conductive biomaterials.⁴ Conductive polymers have emerged as essential materials in supporting and stimulating cells for heart repair. Their engineered properties, along with electrical and electromechanical stimulation, provide various benefits in aiding stem cell function.⁵ Optimizing the interaction between cardiac progenitor cells and conductive polymer surfaces is crucial for advancing our understanding of biomaterial interactions with cardiac cells and improving materials for cardiac tissue regeneration.⁶

Furthermore, conductive biomaterials play a pivotal role in post-MI recovery by restoring electrical functions and repairing

damaged myocardium. These biomaterials effectively lower scar tissue resistivity, increase field potential, and improve conduction velocity, thereby enhancing heart function.⁷ In the field of cardiac tissue engineering, conductive substrates are designed to mimic the electrical properties of the myocardium, thereby improving blood-pumping ability and reducing scar size in heart. These substrates not only enhance cardiomyocyte (CM) maturation and integration with the native myocardium but also contribute to the restoration of heart function. Moreover, conductive scaffolds facilitate stem cell differentiation into CMs, enhancing the expression of cardiac proteins and promoting electrical coupling and contractility of cells, ultimately improving cardiac function post-MI.⁸ This review explores the application of various biomaterials and conductive polymers in cardiac tissue engineering.

MEDICAL PROBLEM: HEART FAILURE, CURRENT TREATMENTS, AND CHALLENGES IN CARDIAC REGENERATION

MI, commonly known as a heart attack, is caused by atherosclerosis, a condition in which plaque composed of fatty materials and other substances builds up in the arteries. This accumulation narrows the arterial lumen, reducing or completely obstructing blood flow. As a result, the heart is deprived of oxygen and nutrients, impairing its function and potentially leading to total heart failure,^{3,9} as shown in Figure 1. Common medications for treating MI include antiplatelet agents such as aspirin and clopidogrel to prevent blood clots, thrombolytics such as alteplase to dissolve existing clots, and anticoagulants such as heparin.¹¹



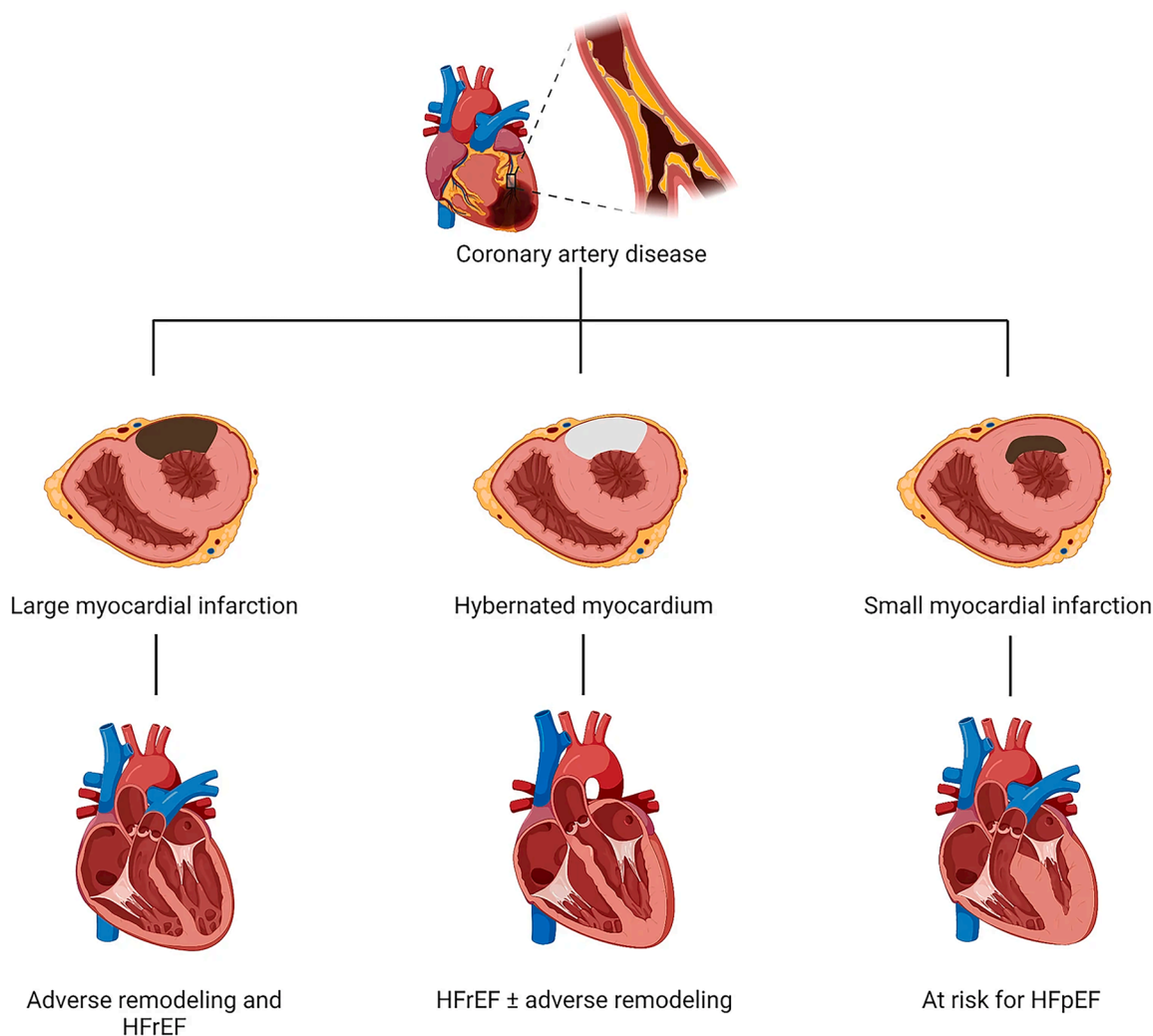


Figure 1. Myocardial infarction in the human heart

The build-up of plaque, composed of fatty materials and other substances, over time in the arteries is the primary cause of myocardial infarction in the human heart, leading to heart failure in patients. Reproduced with permission from Del Buono et al.¹⁰

Beta-blockers and angiotensin-converting-enzyme inhibitors also help to manage heart workload and blood pressure, while statins lower cholesterol levels. However, if these treatments are insufficient, a heart transplant may be considered for an end-stage heart failure.¹²

Currently, a heart transplant is a crucial treatment option for end-stage heart failures. However, this is associated with risks and limitations. The severe shortage of donor organs significantly restricts its availability, resulting in many patients on the waiting list dying each year.⁵ Currently, approximately 3,700 heart transplants are performed globally each year, while approximately 30,000 patients await transplantation. Immunosuppression strategies in heart transplantation adhere to several key principles.¹³ First, the risk of graft rejection is at its highest immediately after the surgery, prompting regimens to start with high levels of immunosuppression that are gradually reduced over the first year to the lowest effective maintenance levels.¹⁴

Second, using low doses of multiple drugs is favored over high doses of fewer drugs to avoid overlapping toxicities. Third, excessive immunosuppression is avoided due to its association with increased risks of infection and malignancy. Achieving the right balance of immunosuppression requires a carefully controlled approach by maintaining the right balance between the toxicity and optimum immune response. Immunosuppressive regimens are typically categorized into induction, maintenance, and rejection therapy, with induction providing intense early suppression and maintenance continuing throughout the patient's life to prevent rejection.¹⁵

An LVAD (left ventricular assist device) is an artificial heart pump used for patients, including pediatric patients, with severe heart failure, particularly those waiting for a heart transplant. It helps the weakened heart pump blood into the aorta, restoring normal blood flow and supporting patients who may not survive until a donor's heart becomes available.¹⁶ In addition, ECMO

(extracorporeal membrane oxygenation) is a life support system for individuals with severe heart or lung dysfunction. It maintains blood circulation and helps regulate oxygen and carbon dioxide levels in the blood.¹⁷ However, LVADs lack in providing the biological support a patient needs.

Current studies have explored innovative approaches to address heart failure, such as using conductive polymers in tissue engineering. Conductive polymers offer promising properties in creating scaffolds that mimic the natural electrical conductivity of cardiac tissues. This supports the integration of implant materials with the host tissue and facilitates electrical signaling essential for proper heart function.¹⁸ Over the last few years, the use of stem cells for cardiac regeneration has provided promising results in preventing ventricular dilation and cardiac dysfunction in preclinical studies.¹⁹ However, there are still some hurdles to overcome, such as challenges in selecting the appropriate cell type for transplantation,²⁰ mode of cell delivery,²¹ cell survival,²² paracrine signaling, and homing.²³ Furthermore, the absence of efficient engraftment of donor cells with host tissue prevents optimal electrical communication.²⁴

Injectable hydrogels for cardiac tissue engineering were investigated by Hasan et al.²⁵ to close the gap between MI and the shortage of donor heart transplants. These hydrogels can repair damaged cardiac tissues and include natural and synthetic biopolymers, such as collagen, fibrin, alginate, hyaluronic acid, and PEG-based materials. Animal studies have shown increased wall thickness, improved cardiac function, and enhanced angiogenesis. However, further research is needed to optimize hydrogel properties and translate these therapies to human clinical applications. More recently, tissue engineering approaches involving an interactive environment between cells, scaffolds, and bioactive molecules have been explored to generate cardiac patches that improve cell engraftment by providing an optimal growing environment.^{25,26}

As the myocardium is an electrically active tissue and cardiac cells have synchronized contractile activity through the propagation of the electrical signals, the bioengineered tissue should be electrically conductive. The absence of electrical conductance in cardiac patches could hinder cell engraftment and prevent the synchronized contractile activity between transplanted cells and host tissues. A wide range of electroconductive biomaterials has already been investigated as potential candidates to improve electrical conductivity in the heart.^{25,27}

Biomaterials, whether synthetic or natural, play a crucial role in cardiovascular treatment by facilitating tissue repair and regeneration.⁴ Synthetic materials such as polymers and metals offer strength and durability, while natural materials deliver excellent functionality.²⁸ Combining stem cell therapy with biomaterials shows promise in improving cardiovascular treatment outcomes by enhancing cell engraftment and survival after transplantation.

Advancements in biomaterial-based therapies and 3D bioprinting have the potential to revolutionize cardiac regeneration and significantly improve outcomes for heart failure patients. By providing personalized treatment that overcomes the challenges of traditional heart transplants, these innovations offer hope for a brighter future in cardiovascular medicine.^{2,29}

CARDIAC BIOENGINEERING

Cardiac bioengineering aims to create functional cardiac tissues to repair or replace damaged heart tissues.³⁰ A key component in this field is the use of conductive biomaterials, which enhance the electrical conductivity of engineered tissues and improve their integration and functionality.³¹ These biomaterials are critical for ensuring synchronized contractions and effective electrical signaling in engineered cardiac tissues.³²

Various cell sources, including mesenchymal stem cells (MSCs), cardiosphere-derived progenitor cells, human embryonic stem cells, and human-induced pluripotent stem cells (hiPSCs), are used to generate CMs. hiPSCs are particularly advantageous due to their ethical acceptability and reduced immunogenicity, allowing the production of unlimited autologous CMs.³³

Successful clinical applications depend on maturation protocols, vascularization, and integration with the host tissue. Conductive biomaterials support these processes by facilitating electrical communication between cells, which is essential for the proper function of cardiac tissue. In addition to cell sources, bioengineering techniques such as cell reprogramming, gene editing, and the delivery of protein factors through engineered particles enhance the properties of both cells and biomaterials.³⁴

Innovations in technologies such as protein decoration and the use of cardiac patches embedded with conductive materials are essential for advancing cardiac regenerative medicine. These approaches might help overcome the limitations of live cell therapy and improve the prospects for successful integration and functionality of engineered cardiac tissue in clinical applications.³⁵

BIOMATERIALS FOR HEART TISSUE ENGINEERING

Biomaterials are essential in supporting cells and guiding their development into functional tissues.³⁶ While each type of biomaterial has unique characteristics in tissue engineering, they all function as supportive structures that promote the growth and specialization of targeted tissues, as shown in Table 1.¹⁰⁸ These biomaterials are typically either synthetically engineered or derived from essential natural substances, with subsequent processes used to tailor their morphology and properties for specific applications.¹⁰⁹ This section will explore the various biomaterials used in cardiac tissue engineering.

Alginate

Alginate, a natural compound derived from seaweed, is known for its biocompatibility, biodegradability, non-toxicity, and non-immunogenicity. These advantageous properties make it highly suitable for several medical applications, such as drug delivery, wound healing, and tissue engineering.^{110,111}

In cardiac tissue engineering, alginate has been used as an injectable cell delivery system because it can easily form gel when exposed to ions such as calcium. These properties make it effective for delivering cells to the heart tissue during regenerative treatments.^{112,113} The gelation process results from the interaction between the alginate molecules and calcium ions present in the surrounding environment. When alginate comes

Table 1. Strengths and limitations of biomaterials used for cardiac tissue engineering

Biomaterial	Strengths	Limitations
Alginate	- biocompatible, biodegradable, non-toxic, and non-immunogenic.^{37,38} - forms gels with calcium ions, enabling effective cell delivery and encapsulation.³⁹ - promotes vascularisation, reduces infarct expansion, and prevents LV enlargement.⁴⁰	- low mechanical strength when used alone.⁴¹ - requires modification or combination with other materials, such as conductive polymers (e.g., polypyrrole and polyaniline), graphene, or carbon nanotubes, for improved structural support and conductivity.⁴²
Chitosan	- supports cardiac repair by promoting cell adhesion.⁴³ - conductive when combined with materials like polypyrrole.⁴⁴ - enables controlled scar tissue formation and improves signal conduction.⁴⁵	- conductivity needs enhancement through additional materials like polypyrrole.⁴⁶ - variable degradation rate that impacts its stability for prolonged cardiac use.^{47,48}
Gelatin	- supports cell recruitment, attachment, and growth.⁴⁹ - effective in enhancing heart function when used in cardiac patches.⁵⁰ - biodegradable and mimics physiological properties for bone and tissue engineering.^{51,52}	- rapid degradation and limited mechanical stability on its own⁵³ - conductivity may require enhancement through combination with other material such as conductivity may require enhancement through combination with other materials, such as conductive polymers (e.g., polyaniline and polypyrrole) or carbon-based nanomaterials (e.g., graphene).⁵²
GelMA (Gelatin Methacryloyl)	- excellent cell compatibility and tunable mechanical properties with UV crosslinking.^{54,55} - mimics the ECM and supports cell alignment and tissue structure formation.⁵⁶ - effective for cardiac, vascular, and bone tissue engineering.^{56,57}	- UV crosslinking is required, which may affect cell viability.^{58,59} - limited long-term stability in physiological conditions without modification.⁵⁶ - modifications (e.g., incorporation of conductive polymers such as polypyrrole or polyaniline, carbon nanomaterials like graphene oxide or carbon nanotubes, or gold nanoparticles) are required for improved stability and conductivity⁶⁰
Collagen	- naturally supportive of cell attachment and cardiac tissue structure.⁶¹ - provides flexibility and is a key ECM component aiding stem cell differentiation.⁶² - useful in injectable hydrogels and cardiac patches.⁶³	- it requires conductive materials (e.g., polypyrrole, polyaniline, graphene oxide, or gold nanoparticles) to enhance electrical conductivity in cardiac applications Li et al.⁶⁴ - rapid degradation can impact durability in cardiac tissue engineering.⁶⁵ - sourcing from animal origins may impact immune compatibility and quality; however, other commonly used biomaterials, such as gelatin, matrigel, fibrin, and chitosan, are also derived from animal sources.⁶⁶
Silk	- excellent biocompatibility, customizable mechanical strength.⁶⁷ - supports cell adhesion and proliferation.⁶⁸ - enhances mechanical properties when added to other biomaterials.⁶⁹	- higher concentrations may reduce cell viability and affect structural porosity.⁷⁰ - it requires complex fabrication steps, such as electrospinning or chemical modification, to improve specific properties like mechanical strength and biocompatibility.^{71,72} - biodegradable and can be combined with conductive materials, such as polypyrrole, polyaniline, graphene oxide, or carbon nanotubes, to enhance electrical conductivity and support cardiac tissue function⁷³
Fibrin	- high biocompatibility and flexibility in mimicking ECM, not conductive by itself.⁷⁴ - enhances cell integration, cardiac patches, and cell retention. - effective in 3D bioprinting applications for cardiac constructs.⁷⁵	- low mechanical strength, degrades relatively quickly,⁷⁶ and lacks intrinsic electrical conductivity, often requiring blending with conductive materials (e.g., polypyrrole and graphene oxide) for cardiac applications.⁷⁷ - limited cell survival in the middle of the material due to limited diffusion of nutrients and oxygen in thicker fibrin layers.⁷⁴ - quick gelation reduces working time in certain applications.⁷⁸

into contact with calcium ions, it undergoes a cross-linking reaction, forming a gel-like structure. This gel can then encapsulate cells, protecting them during delivery and facilitating their release at the target site within the heart tissues.⁶⁰

A study by Leor et al.¹¹⁴ explored the potential of alginate in cardiac engineering by developing specialized scaffolds to support implanted heart cells in a rat MI model. These alginate-based scaffolds provided a controlled release of therapeutic factors to create an optimal environment for heart cell survival and integration. Additionally, the study examined the effects of specific cytokines, including G-CSF, SDF-1, LIF, LGF-1, and EPO, on promoting tissue healing and reducing scar formation after a heart attack. The results indicated that these cytokine-scaffolds significantly increased cell retention, reduced infarcted area, and improved left ventricle (LV) function, offering promising approaches for heart tissue repair and regeneration.

Landa et al.¹¹⁵ investigated the potential of alginate's properties by developing absorbable biomaterials using a calcium-crosslinked alginate solution, which transforms into a hydrogel upon injection into an infarcted area. The research focused on alginate's impact on cardiac remodeling and function in the infarcted area. The study used a rat MI model to examine how this alginate hydrogel influenced cardiac tissue recovery and function. The research aimed to understand how the alginate hydrogel specifically affected cardiac remodeling in infarcted tissues.

Echocardiographic and histological analyses showed that the alginate-treated rats had significantly reduced LV dilation compared with the untreated control group in infarcted animals. The control group would have consisted of rats with MI that were not treated with the hydrogel. The results showed comparable or superior benefits to neonatal CM transplantation in rats treated with the alginate hydrogel, improving cardiac function and reducing infarct expansion. Alginate also reshaped the LV, preventing further enlargement and promoting increased arteriogenesis. The hydrogel further promoted neovascularization, with an observed increase in vascular density within the treated infarct area. Compared to fibrin, alginate consistently enhanced LV functional shortening and reduced infarct size, with both materials boosting arteriogenesis.^{113,115}

A study conducted by Yu et al.¹¹⁶ investigated the long-term effects of alginate on the heart using a mouse model that mimicked chronic heart disease caused by tissue damage. The study compared modified and unmodified alginate formulations, incorporating arginylglycylaspartic acid (RGD) peptides and calcium chloride, to evaluate their effects on cardiac tissues when administered directly to the damaged area of the LV. Results revealed that the modified alginate combined with RGD promoted angiogenesis and blood vessel formation and that both alginate forms improved LV function. Additionally, Yu developed a method to deliver human MSCs (hMSCs) by encapsulating them in microbeads made from RGD alginate and injecting them into the affected region, which increased stem cell retention, slowed tissue remodeling, and further facilitated angiogenesis. Interestingly, no significant difference was observed *in vivo* between using RGD alginate alone and in combination with cells. This study suggests that RGD alginate is a cell-free approach to

stimulate angiogenesis and support functional recovery in cardiac applications.^{28,116}

Chitosan

Chitosan is a natural, biocompatible, and non-toxic material derived from chitin, commonly used in controlled drug delivery systems. Its ability to form nanoparticles through ionic interactions with tripolyphosphate makes it ideal for targeted drug delivery and tissue engineering applications. Chitosan nanoparticles are known for their prolonged circulation times in the bloodstream and excellent biocompatibility.¹¹⁷

A study by Ye and Qiu¹¹⁸ focused on using chitosan combined with conductive biomaterials, such as carbon-based nanofibers and nanotubes, to enhance cardiac repair and function following MI. These conductive scaffolds were designed to mimic the heart's electrical environment, thereby supporting improved electrical signal transmission across cardiac cells. The materials were combined to create scaffolds that enhance heart function post-MI by promoting cell adhesion, proliferation, and elongation, key factors in the integration and survival of transplanted cells. These studies did not involve bioprinted tissues; rather, they focused on creating scaffolds that supported cell-based therapies. The scaffolds were intended to facilitate the adhesion and function of transplanted cells (not bioprinted tissues), enhancing the expression of CM-specific markers and leading to improved CM functionality and synchronous contraction, critical for restoring coordinated heartbeats and overall heart performance.¹¹⁸ Cui et al.¹¹⁹ further expanded on these findings, exploring how these scaffolds could improve the functionality of CMs and enhance heart tissue regeneration, promoting better outcomes after myocardial injury. The study by Cui et al.¹¹⁹ also did not involve bioprinted tissues, as it focused on the use of scaffolds as supporting cells.

A study conducted by Mihic et al.¹²⁰ examined the effects of chitosan-graft-polypyrrole (CS-g-PPY) hydrogel compositions as implants for treating damaged cardiac tissues and enhancing heart function post-MI. The CS-g-PPY hydrogel was developed to improve electrical conductivity and mimic the natural electrical properties of cardiac tissue. CS-g-PPY demonstrated improved electrical conduction and calcium signal propagation in *ex vivo* tests, enhancing the communication between CMs necessary for synchronized contractions. *In vivo* experiments on rats showed that CS-g-PPY treatment led to echocardiogram readings similar to pre-infarction levels after one week, with faster transverse conduction velocities and elongated rather than rounded scar tissue, indicating better tissue integration and repair. CS and CS-g-PPY reduced scar size and improved various cardiac function parameters compared to saline injections, suggesting CS-g-PPY's potential to restore cardiac electrical and structural integrity after MI,¹²¹ as seen in Figure 2.

Gelatin

Gelatin is a useful biomaterial widely used in medical applications such as wound healing and tissue engineering.¹²² Due to its biocompatibility and biodegradability, gelatin provides a supportive environment for various cell types, including cardiac, bone, and cartilage cells, making it highly versatile in tissue engineering.

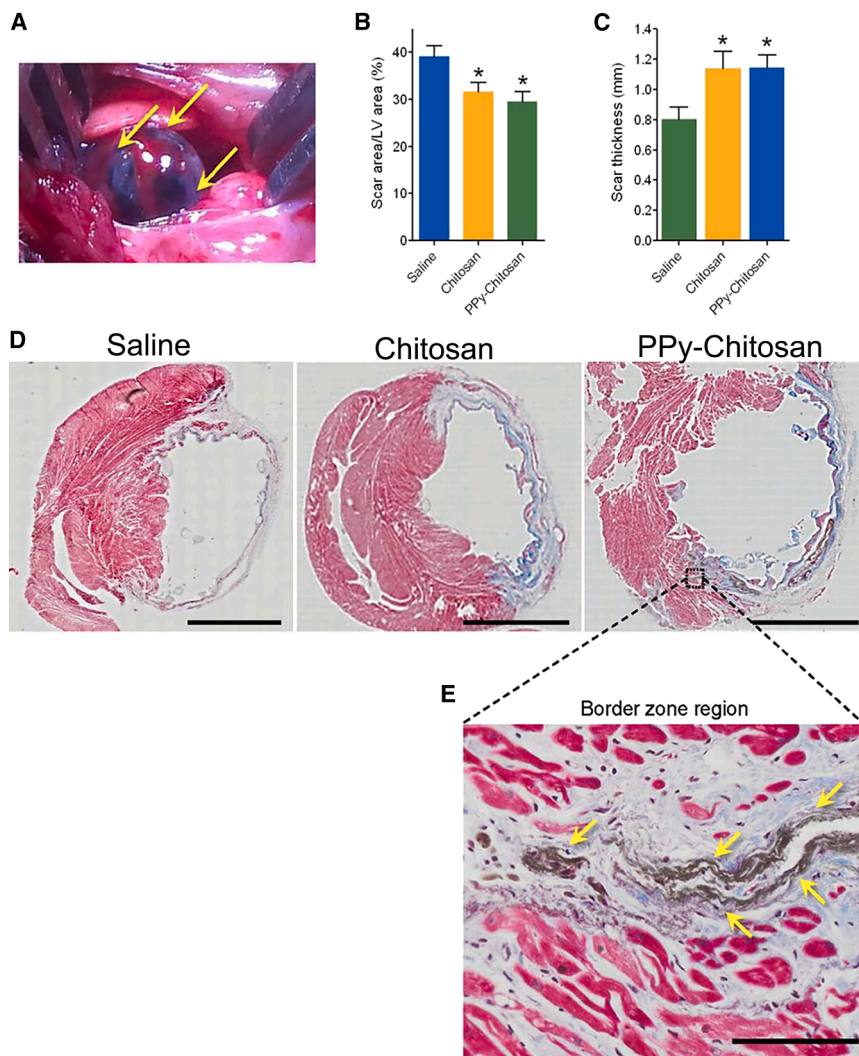


Figure 2. Chitosan-graft-polypyrrole hydrogels reduce scar size in post-MI rat hearts

(A) Intraoperative photograph displaying three darker areas for CS-g-PPY was injected into the border area of the rat heart one week post-MI. (B and C) Eight weeks post-injection, scar area and thickness were measured in a heart treated with saline ($n = 6$), chitosan ($n = 8$), and CS-PPY ($n = 8$). CS-PPY and chitosan significantly reduced scar compared to saline ($p < 0.05$). (D) Representative heart sections from each group, taken from a similar transverse section below the papillary muscles. (E) Masson trichrome staining reveals PPY-chitosan particles in the scar and border zone of the heart eight weeks post-injection (arrows). Scale bars: 100 μm . Reproduced with permission from Mihic et al.¹²⁰

medicine. The study highlighted that the alginate-gelatin (Alg-Gel) combination created an ECM-like environment, which enhanced cell survival. Another study utilized gelatin in 3D-printed Alg-Gel patches containing cardiac spheroids, which were transplanted onto the outer surface of the heart in a mouse MI model. Gelatin played a crucial role in formulating the bioink for printing the cardiac tissues in another study as well, contributing to their structural integrity and biocompatibility. The results showed that transplanting these gelatin-incorporated patches led to enhanced heart function, likely by upregulating cardiac-specific gene expression and reducing inflammation in the host tissue, which

contributed to an improved contractile function and decreased adverse remodeling.¹²⁴

Gelatin methacryloyl

Gelatin methacryloyl (GelMA) is commonly used in heart tissue engineering due to its excellent cell compatibility and tunable mechanical properties, especially with UV crosslinking.^{54,55} These hydrogels mimic the natural ECM, promoting cell growth and tissue formation. GelMA's mechanical properties can be finely adjusted with light irradiation in the presence of photoinitiators, allowing it to be microfabricated into controlled structures, such as microchannels or patterned configurations, which support vascularization and tissue integration. Its flexibility to replicate specific tissue stiffness and elasticity makes it particularly suitable for cardiac applications with vital mechanical properties. GelMA has been proven to be effective in bone, cartilage, cardiac, and vascular tissue engineering, as well as drug delivery and biosensing.⁵⁷ However, there are some limitations. UV crosslinking, although crucial, can negatively impact cell viability.⁵⁸ Additionally, GelMA has limited long-term stability

Gelatin is derived from collagen, typically from animal connective tissues such as skin, bones, and tendons. It is composed of amino acids like glycine, proline, and hydroxyproline, forming a triple-helix structure. Gelatin is bioactive and demonstrates its ability to support cell recruitment, attachment, and growth, facilitating natural healing processes. Its composition and structure enable it to mimic the extracellular matrix (ECM), which is essential for cellular functions and tissue integration.

Gelatin has successfully been used to transport corneal endothelial cells for transplantation, enhancing cell growth and alignment. Moreover, gelatin-based scaffolds show efficacy in regenerating bone and cartilage tissue, offering properties similar to physiological tissues, which is particularly beneficial for bone regeneration.¹²⁰ In cardiac applications, gelatin can be modified or combined with other materials to improve its mechanical strength and stability, enabling its use in cardiac patches and as a supportive matrix for cell delivery,¹²¹ as seen in Figure 3.

A study conducted by Polonchuk et al.⁴ showed that the combination of gelatin with alginate produced hydrogels that supported cardiac cell viability, showing promise as a regenerative

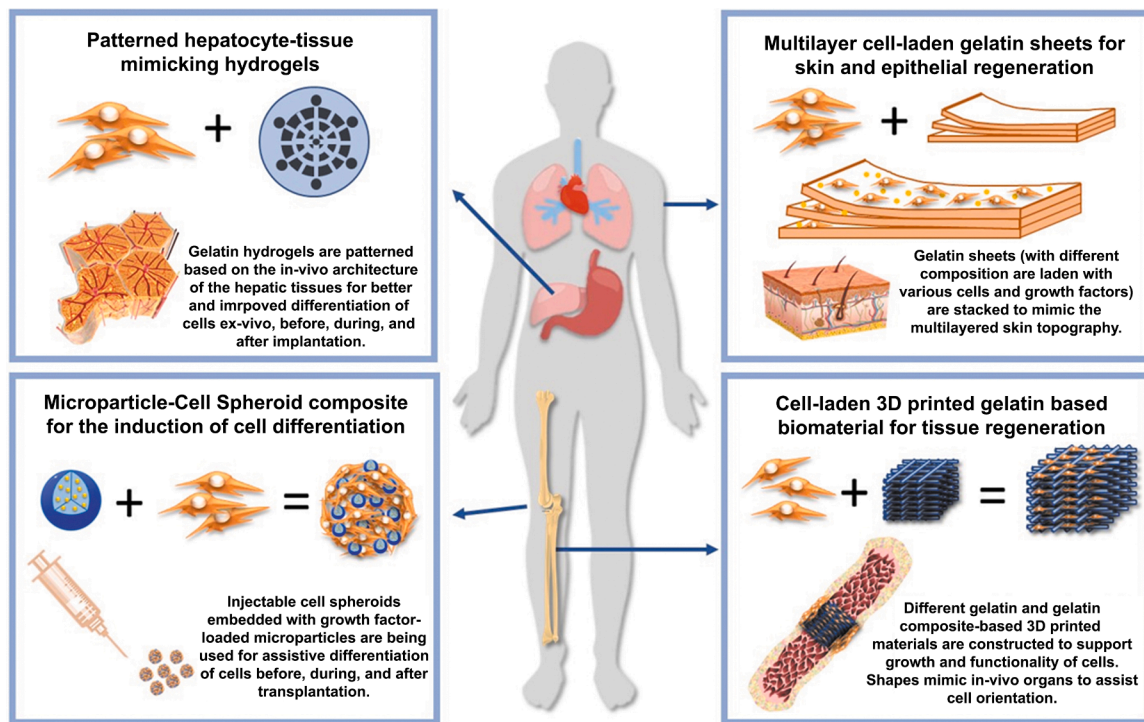


Figure 3. Medical applications of gelatin-based biomaterials

This figure highlights the advancement in gelatin biomaterials, showcasing their use of multi-layered cell-laden sheets for skin and corneal regeneration, patterned hydrogels that mimic natural microenvironments for targeted cell differentiation, injectable spheroids for cell transplantation, and 3D printed scaffolds for repairing bone and cartilage. Reproduced with permission from Bello et al.¹²³

in physiological conditions unless further modified,⁵⁶ which could hinder its sustained effectiveness in tissue regeneration applications.

A study conducted by Nikkhah et al.¹²⁵ investigated the formation of endothelial cord structures using micropatterned GelMA hydrogels. Through precise micropatterning techniques, the researchers created GelMA constructs with varying shapes and heights to examine their effects on endothelial cell behavior and organization. They discovered that the shape of the GelMA constructs was crucial in guiding the development of well-organized and durable cord structures by endothelial cells. Specifically, constructs with optimized heights and geometries provided the most favorable microenvironment, enhancing cell alignment, elongation, and connectivity, which are essential for forming stable vascular networks. Their study underscores the potential of GelMA hydrogels in controlling the morphology of endothelial cells, which is vital for applications in tissue engineering, such as creating vascularized tissue constructs for cardiac and other organ regeneration.

In a study conducted by Lee et al.,¹²⁶ the group explored the use of GelMA for cardiac tissue engineering combined with carbon nanotubes (CNTs) and reduced graphene oxides (rGOs), demonstrating improvements in electrophysiological properties, electrical conductivity, mechanical stiffness, and the maturation of CMs. The integration of these conductive materials aimed to replicate the electrical environment of native cardiac tissues, essential for synchronized cell contractions. Additionally, adding

fibronectin to GelMA hydrogels enhanced CM survival and spreading, providing an ECM component facilitating cell adhesion and proliferation. The study findings indicated that different functionalizations led to distinct forms of tissue maturation: GO resulted in atrial-like tissues, CNTs in ventricular-like tissues, and rGO-GelMA in a mixed phenotype resembling cardiac muscle cells. This differentiation suggests that tailored functionalization of GelMA with specific conductive materials can guide the formation of specific cardiac tissue types, supporting the development of biomimetic heart models for research and therapeutic applications,¹²⁷ as seen in Figure 4.

In another study, CMs in GelMA-coated microchannels displayed organized growth guided by fluid flow, resulting in tissues similar to cardiac fibers with inherent beating capabilities.⁵⁵

Annabi et al.¹²⁸ developed a heart-on-chip platform using photo-crosslinked GelMA hydrogels to coat microfluidic devices for drug screening in biomedical engineering. They demonstrated that the GelMA solution could be injected into microchannels and UV-crosslinked to form a coating of controllable thickness. This system allows cell incorporation within the hydrogel, creating a tissue-mimicking microenvironment with physiological conditions, such as oxygen/nutrient supply and shear stress. This microfluidic GelMA platform shows potential for studying cell-biomaterial interactions, biosensing, and drug screening by closely replicating the native tissue environment.

Chen et al.¹²⁹ developed a GelMA-based microfluidic platform with a bilayer membrane device to simulate physiological

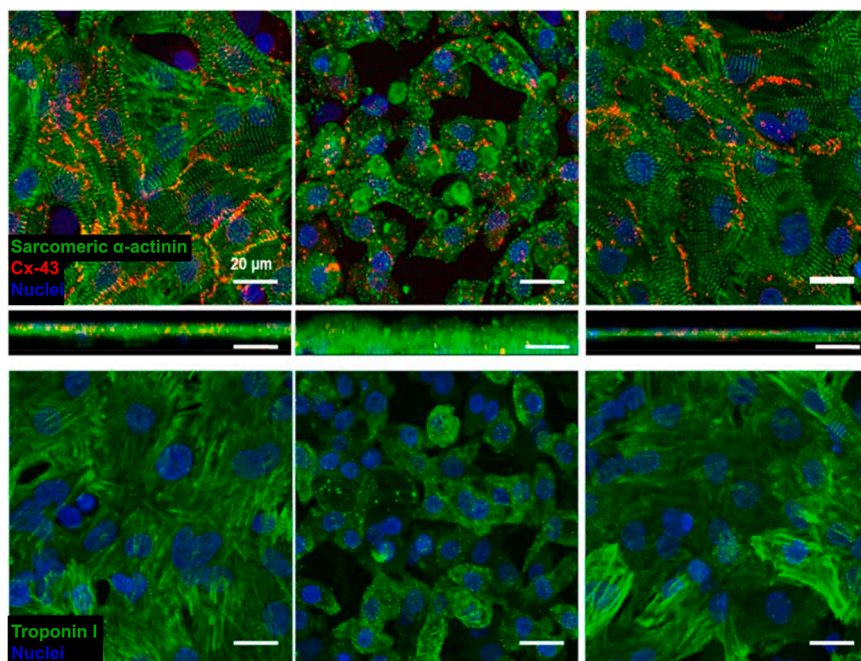


Figure 4. Effects of carbon nanoparticles on GelMA constructs

This image shows GelMA constructs modified with carbon nanotubes, demonstrating increased levels of Cx43 and troponin I expression and improved cellular alignment. Reproduced with permission from Nikkhah et al.¹²⁶

cardiovascular cell-cell interactions. While the study does not specifically address conductive polymers or materials, the platform's use of GelMA hydrogel could potentially be combined with conductive materials in future research to enhance the electrical properties of the system. Incorporating conductive polymers into such platforms could help mimic the electrical conductivity of native tissues, which is crucial for studying cardiac and vascular cell behavior under electrical stimulation or flow-induced shear stress. This approach may provide new insights into the role of electrical signals in vascular and valvular biology.

Collagen

Collagen is a structural protein that is primarily derived from the ECM and is found abundantly in connective tissues throughout the body. It plays a critical role in providing structural support to the heart, facilitating stem cell attachment, and transporting essential molecules necessary for heart repair and regeneration.¹³⁰ Collagen is typically crosslinked through enzymatic or chemical processes, which help to stabilize its structure and enhance its mechanical properties. These crosslinking methods are crucial for maintaining the integrity of the heart, distributing internal forces, and providing the required strength and flexibility for optimal heart function. Its importance in cardiac function cannot be overstated.¹³¹

Collagen-based biomaterials for tissue engineering were investigated by Wang et al.,¹³² examining their diverse applications, potential avenues for future development, and challenges in the field. The study's emphasis on tissue regeneration underlines the versatility of collagen, particularly its ability to combine with other substances and serve as an ECM, providing structural support and biochemical cues that promote cell adhesion, proliferation, and differentiation. Collagen's adaptability makes it suitable for various tissue engineering applications, from cardiac

patches and skin grafts to bone scaffolds, due to its biocompatibility and ability to mimic natural tissue environments.

Regarding the types of collagens, the study likely refers to type I collagen, which is predominant in bone, skin, and tendon, and type II collagen, which is found in cartilage, as these types are commonly used in tissue engineering applications. Additionally, type III collagen, which plays a role in skin and blood vessels, and type IV collagen, found in the basement membrane, are also important in some tissue engineering contexts, especially when mimicking the ECM.

The study also highlighted the potential for enhancing collagen's mechanical and biological properties through crosslinking or incorporation with synthetic polymers, aiming to improve its stability and functionality in dynamic environments, such as the heart. This versatility contributes to collagen's significance in biomaterial development, making it a promising candidate for future advancements in regenerative medicine.

Curley et al.¹³³ investigated myocardial tissue engineering as a strategy to treat heart failure resulting from MI. They aimed to repair damaged LVs using minimally invasive procedures involving injectable biopolymers combined with cells. These biopolymers included alginate, fibrin, collagen, and chitosan, which served as scaffolds to promote tissue regeneration and improve cardiac function. The study developed biocompatible heart muscle patches that closely resembled the natural myocardium and supported tissue repair in the infarcted area. The injectable biopolymers provided a scaffold that facilitated cell delivery and retention in the damaged tissue, enhancing localized regeneration potential. Collagen emerged as a crucial component in this approach, providing structural support, stiffness, and resistance to deformation, which is critical for maintaining the heart muscle's integrity during contractions. The myocardial collagen matrix, primarily composed of type I and III collagens, is key to preserving heart muscle structure and function. Each collagen type contributes differently to the myocardium's mechanical strength and elasticity, essential for promoting stem cell differentiation into CMs and supporting cardiac tissue regeneration and functional recovery post-MI.^{134,135}

Silk

Silk is a natural biomaterial used in cardiac tissue engineering due to its remarkable biocompatibility, mechanical properties, and versatility in forming films, scaffolds, and hydrogels. It comprises silk fibroin (SF) and silk sericin (SS), with SF offering strong

mechanical properties that can be tailored through structural modifications, while the SS enhances cell adhesion and proliferation and controls antioxidant properties beneficial for cardiac tissue repair. The extraction methods employed significantly impact the properties and control antioxidant applications of SF and SS in tissue engineering. Additionally, silk-based materials are cost-effective, durable, and can be utilized for *in vivo* tracking due to their photoluminescent properties.^{135,136}

The impact of SF on Alg-Gel hydrogels for cardiac bioink formulations was explored by Vettori et al.¹³⁷ The study aimed to improve Alg-Gel hydrogel's structural and functional properties for cardiac tissue engineering. Adding 1% SF enhanced the hydrogel's elasticity and printability without compromising cell viability, allowing the bioink to be extruded smoothly and form stable, well-defined structures suitable for 3D bioprinting. This addition also improved the contractile function of cardiac spheroids, supporting synchronized beating and mimicking native heart tissue functionality. However, a higher SF concentration (2%) negatively affected pore size and cell viability, reducing nutrient and oxygen diffusion, which hindered cell growth and function. The study concluded that 1% SF in Alg-Gel hydrogels optimize mechanical properties and cardiac cell function, showing promise for 3D bioprinted cardiac tissue applications and advancing the development of functional cardiac constructs.

Cetin et al.¹³⁸ explored the use of 3D SF scaffolds for cardiac tissue engineering, focusing on the potential of human adipose-derived MSCs (hAD-MSCs) for cardiac regeneration. The research investigates the properties of SF scaffolds, such as their morphology, swelling behavior, and biodegradability. The results show that SF scaffolds can support the proliferation and cardiomyogenic differentiation of hAD-MSCs. The study further demonstrates that SF scaffolds promote the expression of key cardiac biomarkers, such as troponin I, connexin 43, and myosin heavy chain, which are crucial for cardiac tissue function. These findings suggest that SF scaffolds could be a promising material for cardiac tissue engineering, with potential applications in repairing heart tissue damaged by conditions like MI. Integrating conductive materials, such as conductive polymers or nanoparticles, into the scaffolds could improve their electrical conductivity, enhancing cell-to-cell communication and promoting better tissue integration and functionality in cardiac applications.

Fibrin

Fibrin is a natural biopolymer found primarily in the blood, where it plays a crucial role in the blood clotting process. It is produced when fibrinogen, a soluble protein in the blood, is converted into insoluble fibrin strands during coagulation. Fibrin is notable for its biocompatibility, ability to integrate cells and growth factors, and biodegradability. It is essential in the cardiovascular repair mechanism as a scaffold for cardiac tissue engineering. Its flexibility allows for the tailored mimicry of the ECM, making it ideal for crafting heart valves, cardiac patches, and small-diameter vessels. Moreover, fibrin-based injectable cardiac tissue engineering aims to bolster cell therapy outcomes in cardiovascular repairs, leveraging fibrin's capacity to enhance cell therapy efficacy by facilitating specific homing and *in situ* cell retention.¹³⁹

Chang et al.⁷⁵ developed a fibrin-based cardiac patch containing neuregulin-1 (NRG-1) for treating MI. Male C57BL/6 mice (6–8 weeks old) were divided into four groups: Sham, MI, Patch, and Patch+NRG-1, which were used to assess the effectiveness of the treatment in improving MI heart function. The patch leverages the biocompatibility and ECM-like structure of fibrin, combined with the regenerative capabilities of NRG-1 via the ErbB signaling pathway, to promote CM proliferation, reduce cell apoptosis, encourage angiogenesis, and inhibit fibrosis. However, a major limitation of fibrin is that it can restrict the exchange of oxygen and nutrients with the outer environment, which may affect the long-term viability of the cells within the patch.

Histology results (Masson's trichrome staining) revealed reduced myocardial fibrosis and infarct size on day 28 after MI. Immunohistochemistry results for TUNEL staining indicated reduced cell apoptosis (7.06% in the Patch+NRG-1 group vs. 33.02% in the MI group). Also, CD31 staining demonstrated increased blood vessel formation in the infarct border zone, and Ki67/cTnT/DAPI staining revealed increased CM proliferation in the Patch+NRG-1 group. Echocardiography results for M-mode imaging showed improved LV function, with significantly higher LV ejection fraction and LV fractional shortening (LVFS) values in the Patch+NRG-1 group compared with the MI group (36.78% vs. 21.88%, respectively). In clinical practice, LVFS values in heart failure patients often fall below 30%, and values above 30% indicate improved cardiac function, suggesting that the patch may have clinical relevance in improving heart function and patient survival. Higher LVFS values are associated with better survival rates, so the observed improvement in LVFS suggests potential benefits for patients with MI and heart failure. Collectively, all results for the Patch+NRG-1 group showed significant benefits, including reduced cell apoptosis, improved LV contraction, enhanced cardiac function, reduced myocardial fibrosis, and smaller infarcted scar size. These outcomes target different aspects of MI recovery: reducing apoptosis protects CMs, improving contraction and cardiac function enhances heart performance, while reducing fibrosis and scar size prevents further heart damage. Together, these findings highlight the patch's promising potential for MI treatment.

Shaik et al.¹⁴⁰ investigated the development and testing of fibrin-enriched cardiac ECM (Fn-cECM) hydrogels to promote angiogenesis. The cardiac ECM (cECM) is the natural matrix found in cardiac tissues, providing structural and biochemical support to heart cells. It is derived from the heart tissue itself and is crucial for maintaining tissue integrity and functions.¹⁴¹ The research demonstrated the characteristics of hydrogels by comparing storage moduli, complex moduli, swelling ratios, and degradation rates between cECM and Fn-cECM. Fn-cECM hydrogels exhibited faster gelation times, improved mechanical stiffness, and lower swelling ratios compared to cECM, making them suitable for pre-gel solution mixing and catheter delivery. The study demonstrated that Fn-cECM hydrogels promoted robust vascular network formation in human umbilical vein endothelial cells and enhanced angiogenesis by increasing tube formation and vascular endothelial growth factor (VEGF) secretion. Furthermore, the Fn-cECM hydrogels induced more robust tube network formation and VEGF secretion in hMSCs and promoted angiogenic sprouting in hMSC spheroids.

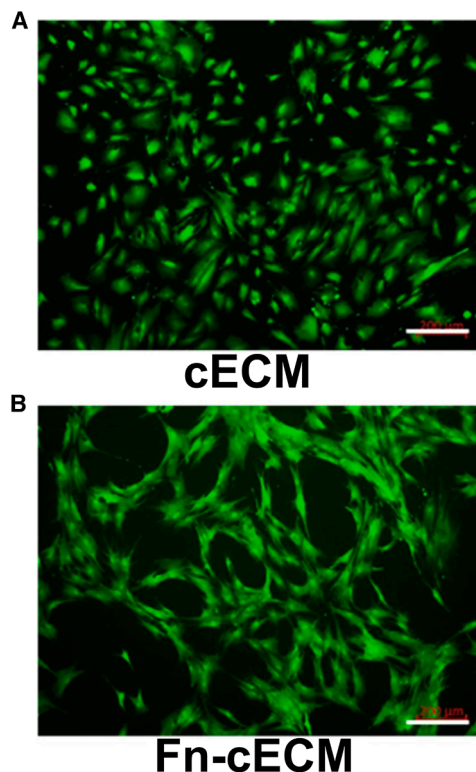


Figure 5. Vascular network formation and VEGF secretion by hMSCs seeded on hydrogels

(A) Representative images of Calcein AM-stained hMSCs seeded on cECM. (B) Representative images of Calcein AM-stained hMSCs seeded on Fn-cECM hydrogels, demonstrating differences in vascular network formation. Scale bars: 200 μ m. Reproduced with permission from Shaik et al.¹⁴⁰

The findings suggest that blending fibrin with cECM hydrogels accelerates gelation and enhances proangiogenic properties, making Fn-cECM a promising material for cardiac tissue engineering. However, rapid degradation observed in Fn-cECM hydrogels may require further modification for improved stability, and the study recommends future optimization and evaluation in MI models as seen in Figure 5.¹⁴⁰

CONDUCTIVE POLYMERS FOR CARDIAC BIOENGINEERING

Recent research has focused on exploring the potential of conductive polymers in regenerative medicine, particularly in cardiac tissue engineering (Table 2). Conductive hydrogel formulations, for instance, have positively impacted cardiac cells by promoting electrical conduction in non-conductive areas. This property is particularly valuable for mimicking native tissue and preserving the electrical connection between myocardial cells in an infarcted heart. Understanding the critical role of electrical signaling in the native myocardium and cell proliferation during disease, compared to healthy tissues, is paramount. Such insights help to identify similarities and differences between the two cell types, crucial for correcting specific signaling pathways in disease models to restore them to a healthy state.^{142,143}

In addition to their role in regenerative medicine, conductive polymers also play an essential role in heart function and the electrical coupling of heart cells within engineered scaffolds, which allows heart cells to communicate and coordinate contraction, restoring heart function in damaged tissues.¹⁴⁴ A variety of conductive polymers, such as carbon nanotubes, gold nanostructures, graphene, polypyrrole, polyaniline, and MXene, have been investigated for their potential to improve electrical conductivity in the heart.¹⁴⁵ This section will describe some of the conductive polymers used in biomedical engineering, including cardiac applications.

Carbon nanotubes

CNTs are derived from carbon atoms and can be produced through methods such as chemical vapor deposition, arc discharge, or laser ablation. They hold promise for regenerating cardiac tissue due to their strong nature and unique mechanical and electrical conductive properties.¹⁴⁶ CNTs are easily chemically modified, making them excellent candidates for creating engineered cardiac structures. Combining CNTs with polymeric scaffolds enhances cardiac regeneration by improving CM behavior, such as synchronized contractility.¹⁴⁷ The elongated shape of CNTs is advantageous for replicating the anisotropic structure of heart muscles, enabling the creation of finely detailed scaffolds to accurately mimic cardiac tissues.¹⁴⁸

A study by Barrejón et al.¹⁴⁸ investigated the use of CNTs to develop scaffolds to regenerate damaged cardiac tissues. The study highlights that incorporating CNTs significantly enhances the CM synchronous contractility. The elongated shape of CNTs effectively mimics the structural features of the myocardium, enabling the creation of micropatterned scaffolds with nanoscale precision to better replicate cardiac tissue architecture. Micropatterning was achieved by using techniques such as nanoimprinting, laser etching, or electrospinning, which allow for the precise control of scaffold structure at the micro- and nano-scale. This study underscores the potential of engineered CNT constructs as innovative platforms for improving cardiac tissue functionality.

Gold nanoparticles

Gold nanoparticles are small gold particles typically ranging in size from 1 to 100 nm in diameter. Their shape can vary, with common forms including spherical, rod-like, and cylindrical shapes, which contributes to their unique optical, electronic, and chemical properties.¹⁴⁹ These nanoparticles can be customized with different compounds for various medical uses. They are well-tolerated by the body and can be precisely targeted, showing promise for innovative therapeutic applications.¹⁵⁰

Graphene

Graphene is a highly promising material for tissue engineering due to its exceptional ability to interact with a wide range of biomolecules, including DNA, proteins, enzymes, lipids, and carbohydrates. These interactions make graphene valuable for applications in regenerative medicine, as it can facilitate cell attachment, enhance signaling pathways, and support tissue growth.¹⁵¹ The ability of graphene to interact with DNA is particularly beneficial as it can aid in the delivery of genetic material

Table 2. Properties and applications of conductive biomaterials

Conductive polymers	Properties	Applications
Carbon nanotube (CNT)	strong mechanical and electrical conductance makes them suitable for improving cardiac regeneration and drug delivery. ^{79,80}	cardiac regeneration, drug delivery (crosses the blood-brain barrier), biosensors, tissue engineering (cell tracking, microenvironment sensing, stem cell growth), bioimaging (contrast agents in ultrasound/photoacoustic imaging), cancer therapy, infectious disease, and CNS disorder diagnostics and treatment. ^{81–83}
Gold nanoparticles (GNPs)	biocompatibility and size-dependent toxicity influence their use in drug delivery and bio-imaging. ^{84,85}	drug delivery (targeted delivery of drugs/DNA), cancer treatment (photothermal therapy), bio-imaging (CT and photothermal microscopy), bone tissue engineering, and biosensors ^{86–88}
Graphene	exceptional conductivity and mechanical strength support cell growth and bioactive molecule release, essential for drug delivery and biosensors. ⁸⁹	drug delivery, biosensors (sensitive electromechanical sensors), tissue engineering (conductive scaffolds), antimicrobials (implant coatings), deep brain implants, neural interfaces (improved brain signal detection), blood glucose sensors ⁹⁰
Graphene oxide (GO)	semi-conductivity and antimicrobial properties make it suitable for tissue engineering and biosensors. ^{91,92}	tissue engineering, biosensors, cardiac regeneration, antimicrobial coatings for medical devices and packaging, drug delivery, clinical diagnostics, and DNA sequence detection. ^{93,94}
MXene	high conductivity and mechanical properties help in tissue engineering and biohybrid platforms to support cardiomyocyte maturation. ^{95,96}	tissue engineering, regenerative medicine, and biohybrid platforms to enhance cardiomyocyte maturation and attachment. ^{97,98}
Polypyrrole (PPy)	conductivity and high biocompatibility make it suitable for cardiac tissue engineering and wound healing ^{99,100}	cardiac tissue engineering, enhancing electrical signaling in cardiac scaffolds, biodegradable patches for myocardial infarction repair, neural regeneration, drug delivery, biosensors, and wound healing. ^{101,102}
Polyaniline (PANI)	highly conductive and biocompatible, ideal for cardiac regeneration and conductive scaffolds to restore electrical function. ^{103–105}	cardiac regeneration, conductive scaffolds for restoring electrical function in injured hearts, promote cardiomyocyte maturation and electrical signaling. ^{106,107}

and improve gene therapy applications. By embedding graphene into scaffolds, its stability and efficiency in gene delivery are enhanced, allowing for more precise control over cellular behavior, such as promoting cell differentiation, proliferation, and tissue repair.¹⁵²

Furthermore, graphene has become an essential material in biomedical applications due to its unique properties, including its ability to influence cell growth and control the release of bioactive compounds, making it particularly valuable in regenerative medicine. Its exceptional conductivity, mechanical strength, and chemical stability, derived from its honeycomb lattice structure, allow for a wide range of potential applications.^{151,152} Graphene can also be engineered into different forms, such as single-layer graphene and GO, which increases its flexibility and adaptability for specific uses.¹⁵³ Since its discovery, graphene has garnered significant interest and found widespread use across many scientific disciplines.¹⁵⁴

Graphene has gained widespread attention across various fields of biomedical engineering due to its unique properties, aiding in extensive cytotoxicity analyses to ensure safe clinical applications. Several studies have assessed the biological toxicity of graphene on different cell types, including fibroblasts, epithelial cells, and neurons.^{155–157} A study by Dreanca et al.¹⁵⁸ focused on dental materials, demonstrating graphene's biocom-

patibility through *in vitro* and *in vivo* tests; the implications extend beyond dentistry. The results revealed that the materials were non-toxic to dental cells and did not cause acute toxicity, inflammation, or systemic organ toxicity in animal models, confirming the material's potential for broad biomedical applications, including tissue engineering and regenerative medicine. In cardiac applications, graphene's biocompatibility holds promise for enhancing heart tissue engineering, where it can support cardiac regeneration and facilitate better integration with heart cells.

A study by Fakhrali et al.¹⁵⁹ focused on creating a composite nanofibrous scaffold that incorporates graphene using electrospinning. These scaffolds were made by the crosslinking of water-soluble polymers, and cells were loaded into them for further testing. The researchers conducted various analyses, including imaging, X-ray diffraction, water contact angle measurement, tensile testing, and biodegradation assessments. The MTT assay was used to assess sample cytotoxicity, and the behavior of human CMs on the scaffold was studied. Results showed that adding PGS (poly[glycerol sebacate]) to poly(ϵ -caprolactone) (PCL) increased degradation rates, while graphene helped maintain the structural integrity. PGS is a biodegradable elastomer often used in cardiac and vascular tissue engineering due to its flexibility and biocompatibility. PCL increased degradation rates, while graphene helped to maintain structural integrity. Cell

attachment, growth, proliferation, and migration on the scaffolds were observed over 1, 3, and 7 days, indicating promising cell behavior on the hybrid nanofibers.

Graphene oxide

GO, a functional derivative of graphene, has garnered significant attention for its potential in biomedical applications due to its remarkable versatility and biocompatibility.¹⁶⁰ Over time, researchers have increasingly explored its use in a variety of fields, including drug delivery, tissue engineering, biosensors, and medical imaging, owing to its unique properties that enable effective interaction with biological systems and enhancement of therapeutic outcomes.¹⁶¹

A study conducted by Choe et al.¹⁶² focused on GO, aiming to create microgel systems for delivering MSCs with strong antioxidant properties to protect them after a heart attack. The research found that MSCs enclosed in rGO/alginate microgels demonstrated higher survival rates under oxidative stress compared to MSCs not enclosed in the microgel, which resulted in better outcomes in heart attack models by reducing damage and improving heart function.

In another study by Li et al.,¹⁶³ GO was used to generate bio-inks for tissue engineering applications. By combining GO with alginate and gelatin, 3D printability for cell support was significantly enhanced. The concentration of GO used in the bio-ink formulations ranged from 0.05% to 0.5% (w/w). Compared to traditional bio-inks, the addition of GO improved the structural integrity and bioactivity of the printed scaffolds. Specifically, the researchers found that 0.05%–0.1% (w/w) GO in an Alg-Gel hydrogel improved 3D printability and printing resolution. A concentration of 0.05% (w/w) GO was used to evaluate the effect on bio-ink preparation and performance. The highest achievable GO concentration was 0.5% (w/w), as higher concentrations resulted in undispersed GO content that could cause nozzle clogging during printing. When GO content exceeded 0.2%, the morphology deteriorated due to decreased storage modulus and GO flakes potentially hindered the crosslinking of alginate after printing. These results support that adding GO to the bio-ink can improve cell affinity and viability, making it a promising candidate for novel bio-ink formulations.

MXene

MXene is a 2D material with metallic conductivity, optical and mechanical properties, hydrophilicity, chemical stability, and a large surface area.¹⁶⁴ It is produced by etching the “A” metal layer in the MAX phase, creating 2D nano-layers of MXenes with surface functional groups. MXene is being explored for applications in cardiac repair.^{165,166} Additionally, MXene reduces bacterial attachment and proliferation, enhancing its biocompatibility.¹⁶⁷ When paired with electrical field stimulation, these biohybrid platforms, which may include MXene combined with hydrogels, polymeric scaffolds, or other bioactive materials, greatly improve cell growth and maturation, especially in induced pluripotent stem cell-derived CMs (iPSC-CMs).⁹⁷ Compared to traditional scaffolds or materials that do not utilize electrical stimulation, these biohybrid systems show significantly enhanced cell differentiation, proliferation, and functional maturation.

Asaro et al.⁹⁷ developed a conductive biohybrid platform using collagen type I with MXene (Ti3C2Tx). This platform exhibited enhanced electrical properties and mechanical strength while inhibiting bacterial attachment. It facilitated iPSC-derived CMs’ attachment, elongation, and maturation under external electrical stimulation. The study highlights the potential of this biohybrid platform for a range of biological applications that require electrical conductivity, demonstrating promising outcomes for use in tissue engineering and regenerative medicine.

Another study on MXene-based materials for cardiac repair was conducted by Ye et al.¹⁶⁸ This study focused on the potential in biomedical applications, particularly tissue engineering. MXene-based hydrogels exhibit excellent electrical, chemical, and mechanical properties, supporting cardiac tissue regeneration. Studies have shown that MXene-based hydrogels can enhance the expression of cardiac-related genes, improve the synchronous beating of CMs, and have no cytotoxicity, aiding cardiac repair after MI. Additionally, MXene quantum dots in hydrogels offer anti-inflammatory properties and promote cellular activity, making them promising candidate for tissue repair and treatment of inflammatory and degenerative diseases, including cardiac applications.¹⁶⁹

Polypyrrole

Polypyrrole (PPy) is a conductive polymer widely studied in tissue engineering. It is valued for its biocompatibility, conductivity, and ease of synthesis.¹⁰¹ Several cell types, including neurons, fibroblasts, and endothelial cells, have been shown to adhere and grow on PPy substrates in the laboratory, suggesting potential applications in cardiac tissue regeneration and electrical signal transmission. A study indicates that PPy-containing nanofibers can enhance cell attachment, proliferation, and the expression of cardiac-specific proteins.¹⁷⁰

Guo and Ma¹⁷⁰ utilized electrically conducting polymers, including PPy, to fabricate conductive biomaterials for tissue regeneration, covering formats such as films, nanofibers, hydrogels, and scaffolds. Their research demonstrated the capacity of conductive polymers to stimulate cells with electrical signals, showcasing the development of degradable conductive composite films and copolymers. These innovations have been shown to enhance cell behavior and promote tissue regeneration in tissue engineering applications.

In a study by Kai et al.,¹⁷¹ PPy was utilized in cardiac tissue engineering to create bioactive scaffolds for cardiac regeneration. These scaffolds were generated by incorporating various concentrations of PPy into PCL/gelatin nanofibrous scaffolds. Increasing the concentration of PPy (ranging from 0% to 30%) resulted in thinner fibers and higher tensile strength. The PPG15 scaffold, containing 15% PPy in PCL, exhibited an optimal balance of conductivity, mechanical strength, and biodegradability, which enhanced cell attachment, growth, and the expression of cardiac-specific proteins compared to the PPG30 scaffold. The study underscored the critical role of conductivity, mechanical properties, and biodegradability in designing scaffolds for cardiac regeneration.

The study by Ezazi et al.¹⁷² aimed to develop a biodegradable cardiac patch with conductivity and elasticity for heart tissue engineering. They combined PGS, collagen, and PPy using the

evaporation method, which involves dissolving the polymer blend in a solvent, followed by solvent evaporation to create thin, solid films or coatings. This approach was used to treat MI. Results revealed that the composite containing 5% PPY exhibited increased blood wettability compared to pure PGS, indicating its potential for attracting proteins due to PPY's hydrophobic nature. Furthermore, the 0.5%–5% PPY patch demonstrated improved conductivity, reaching 0.06 S compared to lower PPY concentrations. The study also assessed the patches' mechanical properties, degradation rate, protein adsorption, drug release, and cell viability, highlighting their potential in cardiac repair applications.

Polyaniline

Polyaniline (PANI) is a conductive polymer commonly used in cardiac tissue engineering due to its biocompatibility and ability to support cell development and growth.⁷ Conductive polymers, such as PANI, are extensively researched for their conductivity and biocompatibility with cardiac tissue regeneration, presenting potential treatment options for MI.^{6,7} PANI nanotubes (PANINTs), particularly when dendronized (as PGLD-PANINTs), have been investigated for their potential as scaffolds in cardiac tissue engineering. A study conducted by Moura and de Queiroz¹⁷³ showed that PGLD-modified PANINTs could promote cell growth. The study showed that PGLD-coated PANINTs are non-cytotoxic to Chinese hamster ovary (CHO) cells, and microcurrent stimulation supports the differentiation of cardiac cells cultured on these scaffolds. PGLD-PANINTs have shown the potential to serve as a biocompatible, electroactive 3D matrix for cardiac tissue engineering. These findings were confirmed through XPS analysis, which showed the presence of PGLD on the surface of the PANINTs. The study also demonstrated that PGLD-PANINTs electrospun fibers, produced from a 10% w/v dispersion of PGLD-PANINTs in a 50:50 mixture of dichloromethane and dimethylformamide, promoted better proliferation and differentiation of myocardial cells compared to casting PGLD-PANINTs films. The electrospinning process was performed at an applied voltage of 15–20 kV, and the resulting scaffolds showed excellent biocompatibility with no toxicity in CHO cells, indicating high cell viability. These electroactive and biocompatible characteristics suggest the potential use of PGLD-PANINTs in heart tissue engineering, highlighting the material's promising role in advancing cardiac regeneration and offering new avenues for treatment in MI.

Ghovvati et al.¹⁷⁴ explored the incorporation of PANI into electroconductive biomaterials for cardiac tissue engineering. They discovered that integrating PANI into scaffolds made of polymeric materials enhanced cell stimulation, facilitated the controlled release of therapeutic agents, and improved cardiac functions in rat models of MI. The study demonstrated significant improvements in CMs' maturation, contraction, and electrical signaling, underscoring the potential of PANI-based biomaterials for promoting cardiac tissue regeneration and enhancing heart function.

A study by Liu et al.¹⁷⁵ investigated the use of PANI in cardiac tissue engineering applications. They discovered that PANI significantly improves CM adhesion, proliferation, and responses to electrical stimulation, thereby beneficially altering

cell electrophysiology and promoting tissue formation.¹⁷⁶ Additionally, PANI was found to form a conductive complex with polymeric materials, resulting in excellent electrical and mechanical properties that support CM growth. This research highlights the critical role of PANI in the repair of MI, specifically by enhancing CM growth, improving electrical conductivity, and fostering tissue development through better cell communication and alignment.

CONCLUSIONS AND FUTURE DIRECTIONS

This review highlighted the significant role of electrically conductive polymers in cardiac tissue engineering. These conducting polymers have numerous advantages, including biocompatibility, adjustable conductivity, ease of synthesis, and flexibility, making them ideal for creating bioactive scaffolds. These scaffolds support cell electrical signaling and enhance cardiac function post-implantation. Unlike hydrogels, conductive polymers provide the necessary electrical conductivity for effective cell communication, which is crucial for integrating implanted materials with native myocardial tissues.

Despite promising results in both *in vitro* and *in vivo* studies, substantial challenges remain in transitioning these materials from laboratory-based modes to clinical applications. Several fabrication techniques have been developed to produce conductive biomaterials, such as composite hydrogels, enhancing their potential for tissue regeneration. However, further research is crucial to fully explore these materials' potential in cardiac regeneration and to ensure their properties align with the dynamic mechanical environment of the beating heart. Future work must refine this material and its applications to develop safe, effective, and reliable CVD therapies.

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AUTHOR CONTRIBUTIONS

W.A.S. and C.G. jointly decided to focus on the topic at the center of the review article; W.A.S. performed the literature search and drafted the original manuscript; C.G. critically revised the work.

DECLARATION OF INTERESTS

The authors claim no competing interests.

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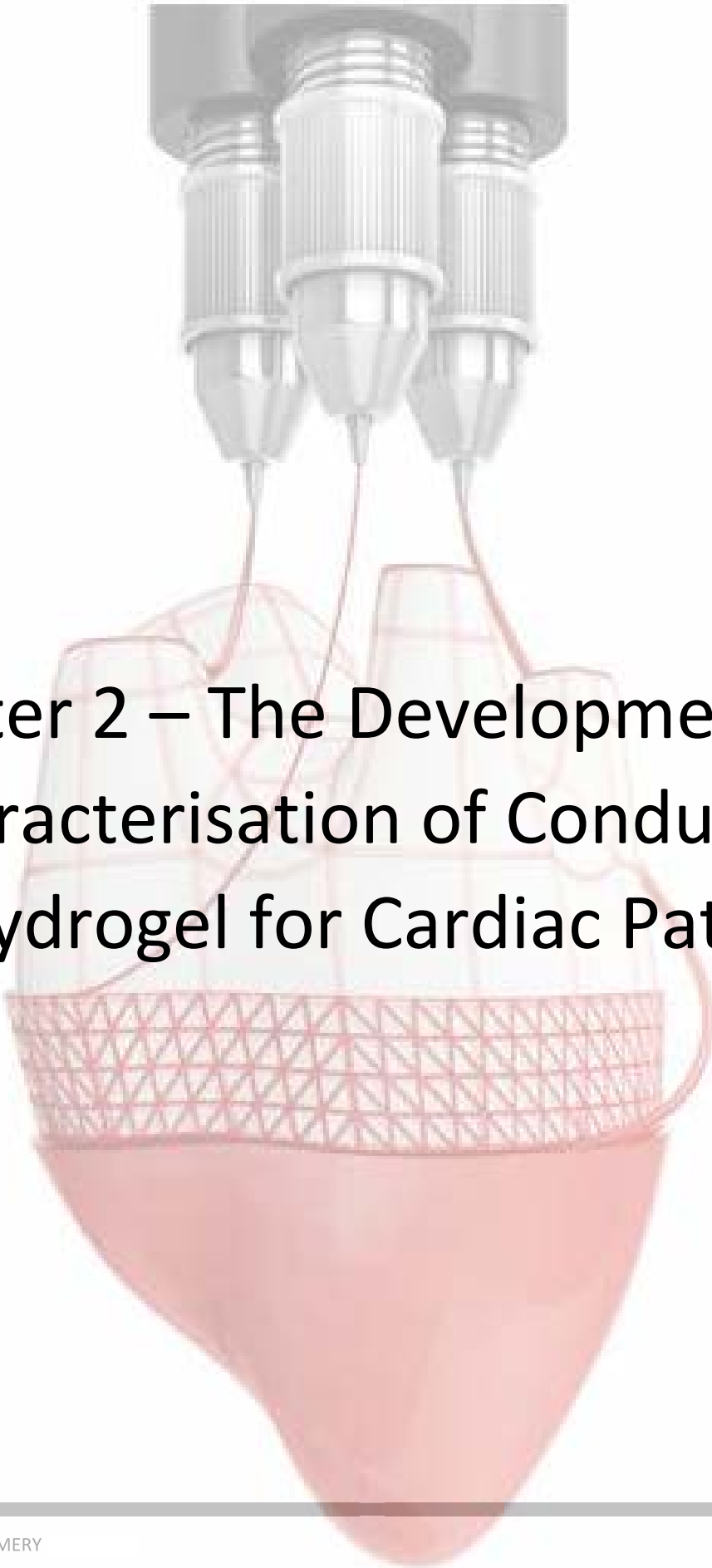
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Chapter 2 – The Development and Characterisation of Conductive Hydrogel for Cardiac Patch

Chapter 2: The development and characterisation of conductive hydrogel for cardiac patches

Abstract

This Chapter focusses on the development and characterisation of conductive hydrogels for the cardiac tissue regeneration following myocardial infarction (MI). It examines how conductive materials improve electrical conductivity and cell signalling in cardiac tissue. Based on the literature reviewed in Chapter 1, conductive polymers such as graphene, graphene oxide, gold nanoparticles, polypyrrole, and polyaniline were selected and tested for biocompatibility, toxicity, and their ability to support cardiomyocyte, endothelial, and fibroblast viability, as well as nutrient exchange. These polymers were incorporated into hydrogels to mimic the native heart extracellular environment. The hydrogels were prepared by combining 4% alginate and 8% gelatine with the selected conductive polymers in a culture medium. Mechanical properties were assessed using Brillouin microscopy and compression testing to ensure the hydrogels matched the stiffness of native cardiac tissue. Porosity, important for oxygen and nutrient diffusion, was evaluated using scanning electron microscopy (SEM). The hydrogels' printability for 3D bioprinting applications was also evaluated, ensuring accuracy and high cell viability over time. The conductive hydrogels were further examined through conductivity measurements, stretching, and durability evaluations to assess their suitability for cardiac tissue engineering. These results indicate that the developed conductive hydrogels have potential as materials for cardiac patches, capable of enhancing tissue regeneration and electrical connectivity in infarcted cardiac tissue. This chapter provides a comprehensive strategy for optimising hydrogels for cardiac repair following MI.

2 Introduction

This chapter builds on the background presented in Chapter 1 by focusing on the development and evaluation of conductive hydrogels for myocardial infarction (MI) repair. Conductive polymers, including graphene, graphene oxide, gold nanoparticles, polypyrrole, and polyaniline, were selected for their ability to enhance electrical conductivity and cell-to-cell signalling, which are essential for supporting cardiac tissue regeneration. These materials

were tested for their biocompatibility and ability to support endothelial and fibroblast cell viability.

To assess their suitability for cardiac applications, the polymers were incorporated into 4% alginate/8% gelatine hydrogels and tested using several characterisation techniques. Brillouin microscopy was used to measure the stiffness and compare it to that of native heart tissue. Compression testing was used to check the mechanical strength and how well the hydrogels could handle physical stress. Scanning electron microscopy (SEM) was used to look at pore structure, which affects how well oxygen and nutrients can move through the material. 3D bioprinting was used to create structured hydrogel patches, and printability tests were done to make sure the materials could be printed accurately and consistently. Durability testing was also used to see if the hydrogels could stay stable over time, especially under conditions similar to those in the heart. Stretch testing was also conducted to mimic the natural movement of heart tissue and evaluate the hydrogel's response to repeated mechanical strain. Together, these tests gave a full picture of how suitable the conductive hydrogels are for use in cardiac tissue engineering.

2.1 Materials and Methods

2.1.1 Hydrogel Preparation and Crosslinking

Hydrogels were prepared based on an optimised protocol adapted from previous reports (Polonchuk et al., 2021). Alginic acid sodium salt (4% w/v) and gelatine (8% w/v) powders were sterilised under UV light for 30 minutes. A medium was prepared by adding 1% (v/v) penicillin–streptomycin and 1% (v/v) L-glutamine to 100 mL of DMEM and warming it to 65 °C. The alginate, gelatine, and the specified amount of conductive polymer (Table 3) were then gradually added to the warmed medium and mixed continuously using a magnetic stirrer until fully dissolved and homogeneous.

The selected conductive polymer concentrations were based on preliminary optimisation and previous studies demonstrating suitable conductivity and biocompatibility (Ketabat et al., 2018; Saravanan et al., 2018). Once prepared, the hydrogels were transferred to sterile tubes and stored at 4 °C until use (Figure 5). A sterile 2% (w/v) CaCl₂ solution was applied to the cold hydrogels for ionic crosslinking during experiments.

Table 3. Composition and preparation conditions for conductive hydrogels used in cardiac regeneration.

Hydrogel composition	Conductive Material	DMEM (mL)	Temperature (°C)	Mixing Time (min)
4 g Alginate + 8 g Gelatine	None	100	65	30
4 g Alginate + 8 g Gelatine + 2 g Gold Nanostructures	Gold Nanostructures (Au)	100	65	30
4 g Alginate + 8 g Gelatine + 0.01 g Graphene	Graphene (G)	100	65	30
4 g Alginate + 8 g Gelatine + 0.01 g Graphene Oxide	Graphene Oxide (GO)	100	65	30
4 g Alginate + 8 g Gelatine + 0.025 g Polypyrrole	Polypyrrole (PPY)	100	65	30
4 g Alginate + 8 g Gelatine + 0.025 g Polyaniline	Polyaniline (PANI)	100	65	30

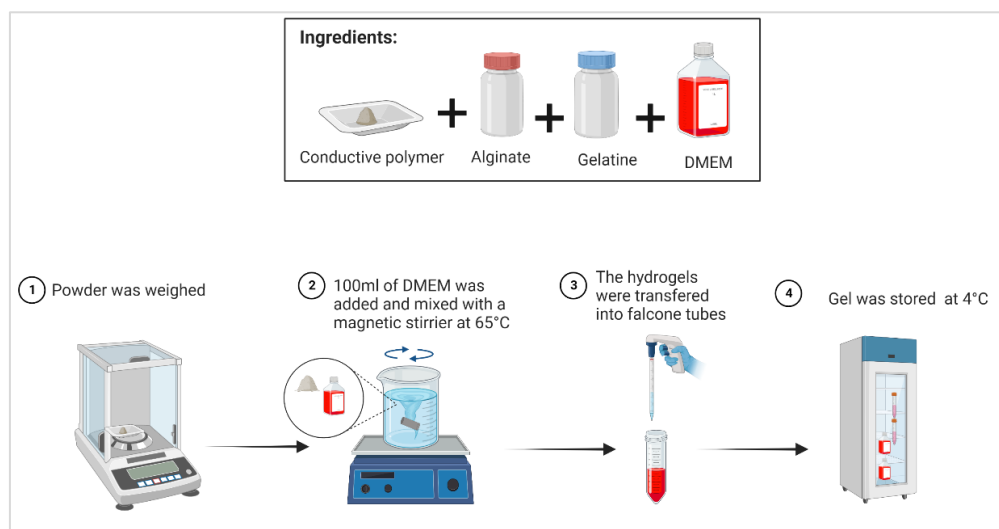


Figure 5. Schematic diagram showing the preparation of conductive hydrogels.

2.1.2 Scanning Electron Microscope (SEM) Analysis

Pore size measurements of the Alg–Gel hydrogels, with and without conductive polymers, were performed using scanning electron microscopy (SEM), as previously described (Baptista et al., 2020; Polonchuk et al., 2021), to assess the effect of incorporating conductive materials into Alg-Gel hydrogels. Conductive hydrogels were crosslinked using a 2% (w/v) CaCl_2 solution in Dulbecco's phosphate-buffered saline (DPBS), prepared by dissolving 2 g of CaCl_2 in 100 mL of DPBS, and crosslinking was performed on ice for 30 minutes to stabilise the hydrogel structure. Each sample was mounted on aluminium stubs with double-sided carbon adhesive tabs and dried in a vacuum oven (Gallenkamp OVA031.XX1.5, Manchester, UK) at 120°C for 12 hours. Dried samples were coated with a 10 nm layer of gold–platinum using a K575X sputter coater (Emitech Quorum Technologies Ltd, UK). SEM imaging was performed using a Supra-SEM (Zeiss, Macquarie Park, NSW, Australia) equipped with a thermionic tungsten electron gun at an acceleration voltage of 10 kV and a working distance of 10.5 mm. For each conductive hydrogel, eight random fields of view were captured using the Zeiss SmartSEM operating system. Pore area (μm^2) was quantified from SEM images using FIJI (ImageJ) software by thresholding and measuring pore regions, as shown in Figure 6.

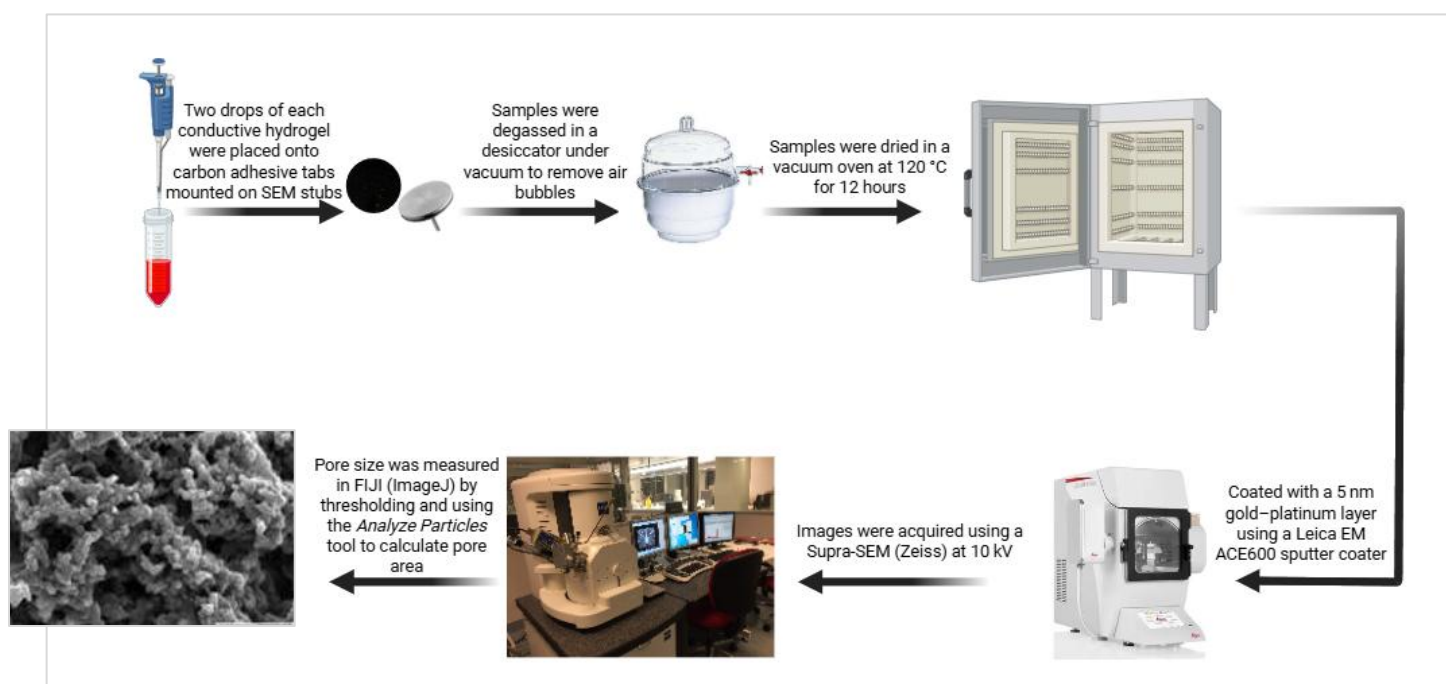


Figure 6. Workflow for preparing and imaging conductive hydrogel samples for scanning electron microscopy (SEM) and pore size analysis. Two drops of each hydrogel were placed on carbon adhesive tabs mounted on SEM stubs, degassed in a desiccator, and dried in a vacuum oven at 120 °C for 12 hours. Samples were coated with a 5 nm gold–platinum layer using a Leica EM ACE600 sputter coater. Images were acquired at 10 kV with a Supra-SEM (Zeiss) and pore size was quantified in FIJI (ImageJ) using the Analyze Particles tool.

2.1.3 Mechanical Testing of Conductive Hydrogel

2.1.3.1 Brillouin Microscopy

Brillouin microspectroscopy was used to determine the viscoelastic properties of the conductive hydrogels listed in Table 1, following the protocol described by Polonchuk et al. (2021). For each hydrogel composition, measurements were performed under three conditions: without crosslinking, after crosslinking for 30 minutes, and after crosslinking in culture medium following incubation at 37 °C for 1 hour in a 48-well plate. Hydrogels were crosslinked using a 2% (w/v) calcium chloride (CaCl_2) solution (2 g CaCl_2 dissolved in 100 mL DPBS) on ice for 30 minutes to stabilise the gel structure. Each condition was tested in triplicate ($n = 3$). Raw Brillouin spectra were collected and fitted using the damped harmonic oscillator model. The Brillouin frequency shift (BFS) was calculated by averaging the Stokes and anti-Stokes peaks to minimise laser frequency drift and measurement variation. Data analysis was performed using Ghost 7.00 software (JRS Instruments, Switzerland), as shown in Figure 7.

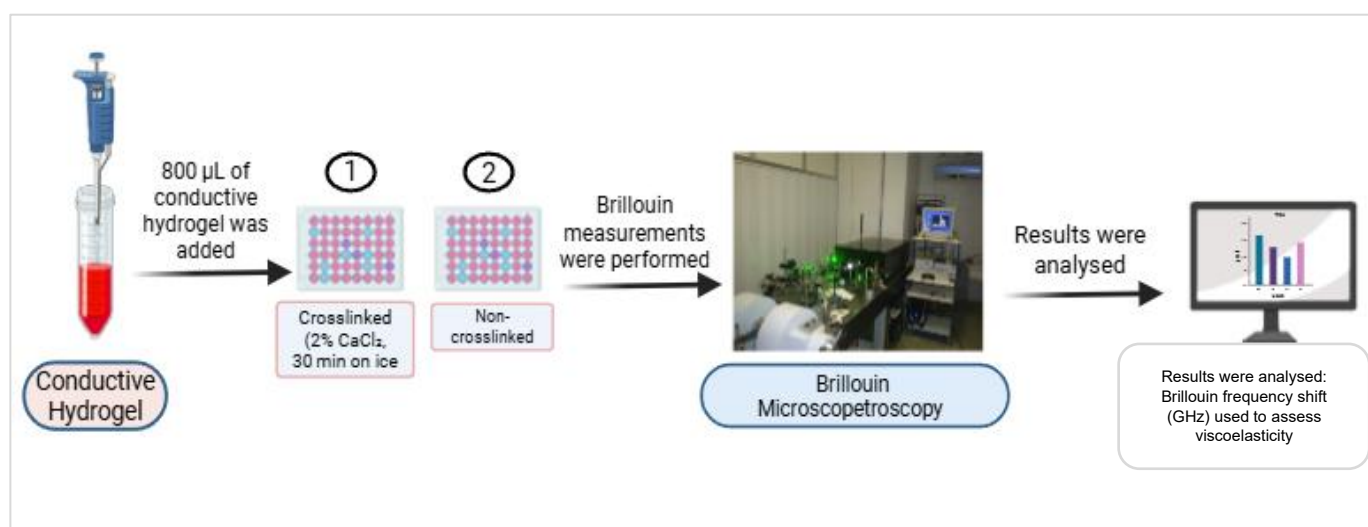


Figure 7. Schematic diagram showing the preparation and measurement workflow for Brillouin microscopy analysis of conductive hydrogels.

2.1.3.2 Compression and Rheology Testing of Conductive Hydrogels

The Young's modulus of each conductive hydrogel sample ($n = 4$) was measured by performing unconfined compression tests using a Dynamic Hybrid Rheometer (DHR-3, TA Instruments). Alginate–gelatine hydrogels containing conductive polymers were crosslinked in a 2% (w/v) calcium chloride (CaCl_2) solution (2 g in 100 mL DPBS) for 1 hour to ensure structural stability. For each formulation, an 8 mm diameter biopsy punch was used to prepare hydrogel discs, which were placed between the rheometer's parallel plate system. Compression tests were carried out at 25 °C with a compression rate of 10 mm min⁻¹. During measurements, the axial force was gradually increased from 0 N to 0.2 N while recording axial force (N) and gap distance (mm). Stress–strain curves were constructed for each replicate, and Young's modulus (kPa) was determined from the linear region of the curve, following the method described by Papanicolaou et al. (2022) (Figure 8). All tests were performed on the same day to maintain experimental consistency.

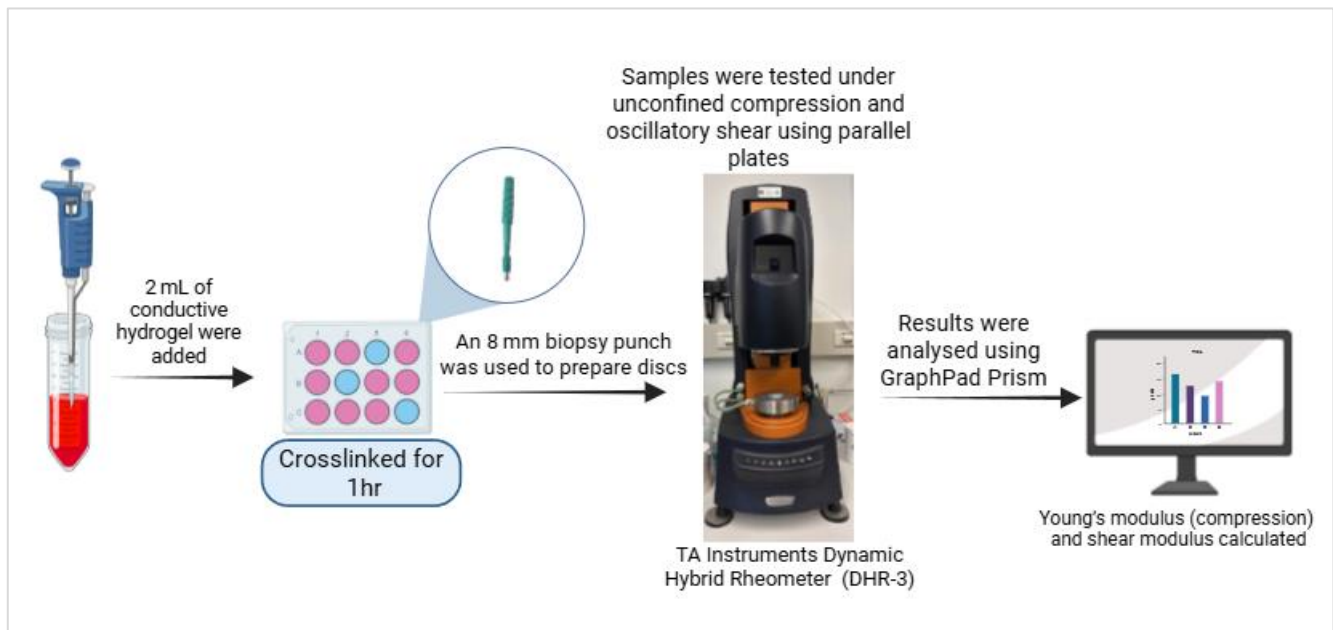


Figure 8. Schematic showing preparation and mechanical testing of conductive hydrogels for determination of compressive and shear moduli using a dynamic hybrid rheometer (DHR-3).

2.1.4 Hydrogel Conductivity Measurement

The conductivity chip was designed in this study and used to measure the conductivity of the conductive hydrogels. For each hydrogel, measurements were performed on the control medium (DMEM) and crosslinked hydrogels after 1 hour of incubation at 37 °C on day 0 and day 7. Each condition was tested in triplicate ($n = 3$). The procedure for conductivity measurement is illustrated in Figure 9. Data were analysed using GraphPad Prism to compare changes before and after crosslinking.

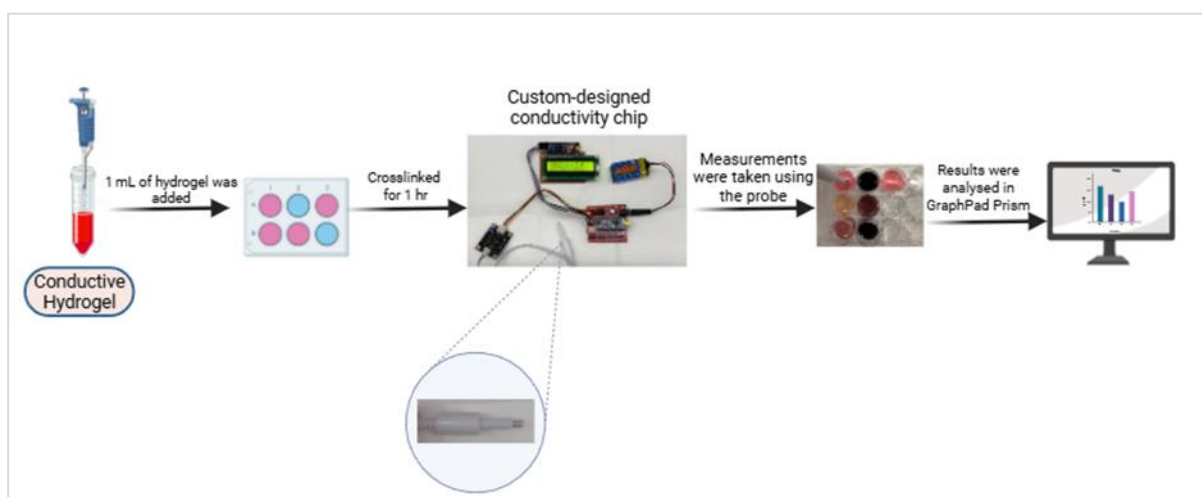


Figure 9. Schematic diagram showing the measurement of hydrogel conductivity using a custom-designed conductivity chip and probe. Results were analysed in GraphPad Prism.

2.1.5 Optimising Conductive Hydrogel Composition for Printability and Durability

Printability was assessed to evaluate the accuracy and structural integrity of the conductive hydrogel patches over 28 days. The aim was to ensure the hydrogels could be printed at low pressure settings suitable for maintaining shape fidelity. Conductive hydrogels were prewarmed to 37 °C in a water bath and transferred into sterile 3 mL cartridges (CELLINK, Gothenburg, Sweden) using Luer lock syringes and connectors to avoid air bubbles. All pre-

printing steps were performed inside a biosafety cabinet, and the BIO X6 bioprinter chamber (CELLINK) was sterilised with UV light before use. Extrusion-based printing was performed using the BIO X6 printer fitted with a temperature-controlled printhead and a 22G nozzle (inner diameter 0.41 mm). Six replicates ($n = 6$) were printed for each hydrogel formulation. The printhead and printbed temperatures were maintained at 28 °C and 10 °C, respectively, to ensure consistent extrusion and rapid gelation. The printed constructs measured 20 mm × 20 mm × 0.5 mm.

After printing, conductive hydrogels were crosslinked in a 2% (w/v) CaCl_2 solution for 1 hour, as previously described. Residual crosslinking solution was removed, and the patches were imaged using an EVOS M7000 Imaging System (Thermo Fisher Scientific, Scoresby, VIC, Australia). Dulbecco's Modified Eagle's Medium (DMEM) was added to each well, and samples were incubated for 24 hours at 37 °C and 5% CO_2 before imaging and strand measurement analysis with ImageJ software (NIH FIJI). The culture medium was refreshed every 2 days. Imaging was performed on day 0 to assess print fidelity and on days 1, 3, 7, 14, and 28 to monitor durability, following the method described by Roche et al. (2021), as seen in Figure 10.

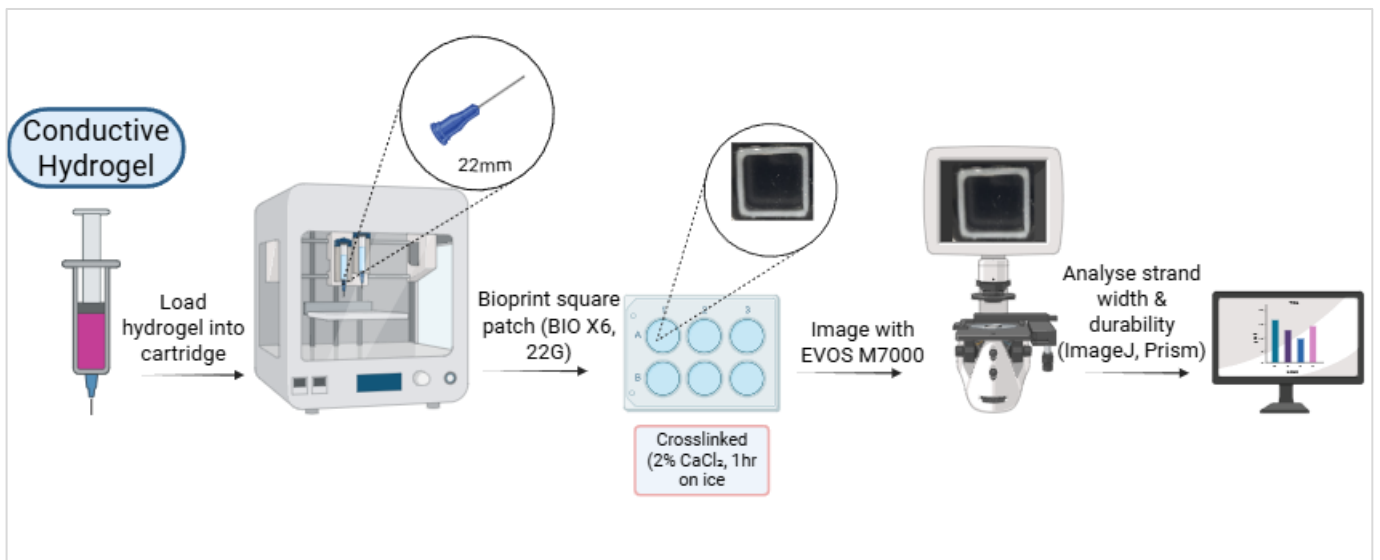


Figure 10. Schematic workflow for the printability and durability assessment of conductive hydrogels. Conductive hydrogel formulations were loaded into a sterile 3 mL cartridge and bioprinted as square patches using a BIO X6 printer with a 22G nozzle. Printed constructs

were crosslinked in 2% (w/v) CaCl_2 on ice for 1 hour, then imaged using an EVOS M7000 Imaging System (Thermo Fisher Scientific). Strand width and structural durability were analysed in ImageJ (NIH FIJI) and results processed in GraphPad Prism.

2.1.6 Stretching Testing for Conductive Hydrogels

Conductive hydrogels were prewarmed at 37°C and loaded into cartridges to be printed as a 3-layer patch using a BIO X6 extrusion-based printer with a 22-gauge size nozzle. Three replicate bioprints were printed ($n=3$). The linear infill percentage was 14%. The patch was crosslinked with calcium chloride (CaCl_2) and placed in the incubator for 1hr. The patch was placed in the Taihoya ATMS Dynamic Culture machine, and the screw was tightened. The Taihoya machine was set to 12 volts, which equals 60 beats per minute for 24 hrs as seen in Figure 11.

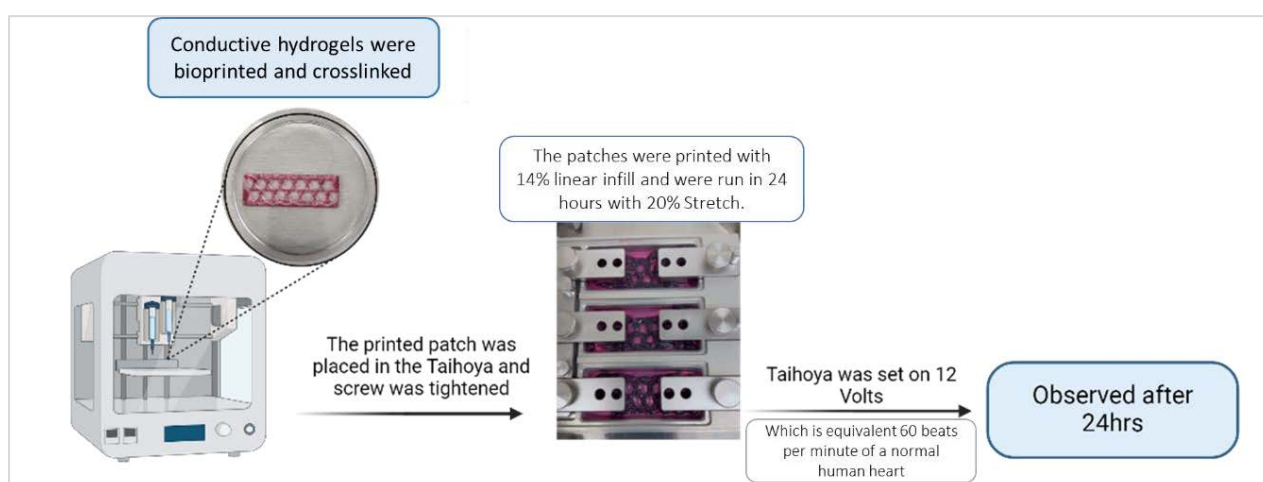


Figure 11. Schematic representation of the Taihoa ATMS Dynamic Culture System used to assess the stretchability and mechanical behaviour of conductive hydrogel patches under dynamic conditions.

2.1.7 Statistical Analyses

Statistical analyses were performed using GraphPad Prism 9.4 (GraphPad Software, CA, USA). Printability and durability were performed in technical replicates ($n=6$), Brillouin microspectroscopy and compression test were performed in technical replicates ($n=4$), and

conductivity and Taihoya were performed in technical replicates (n=3). Significance was determined using One-Way ANOVA (with Turkey's post hoc analysis) or Two-Way ANOVA (with Sidak's post hoc analysis). All values are presented as mean $\pm$ SEM (standard error of the mean). P values less than 0.05 were considered statistically significant.

2.3 Results

2.3.1 SEM Analysis of Pores Size Effects from Adding Conductive Polymers to Alg-Gel

To evaluate the effect of adding conductive polymers to Alg-Gel hydrogels, scanning electron microscopy (SEM) was used to analyse changes in pore structure. These structural features are essential for oxygen and nutrient diffusion and influence hydrogel conductivity, cell adhesion, and synchronised contractile activity. SEM images clearly showed distinct pore architectures for each conductive formulation, including differences in pore diameter, pore density, and overall network organisation.

In contrast, the graphene oxide (GO) hydrogels showed a significant reduction in pore size relative to Alg-Gel ($P < 0.01$), indicating a denser structure that may restrict oxygen and nutrient flow. SEM micrographs of GO hydrogels revealed tightly packed pores with narrow interconnections, forming a compact network that limits diffusion pathways. Gold nanoparticle (Au) and polyaniline (PANI) hydrogels also displayed smaller, more compact pores than Alg-Gel ($P < 0.05$; Figure 12B), potentially limiting their conductivity enhancement. Both Au and PANI hydrogels exhibited smoother internal surfaces and fewer open channels, reflecting reduced porosity compared to the control.

Graphene (G) hydrogels did not show a significant difference in pore size compared to Alg-Gel. SEM analysis confirmed that G maintained a porous and interconnected network similar to the control, with consistent pore diameters and uniform distribution. Overall, the results demonstrate that the type of conductive polymer has a measurable impact on pore structure

In summary, PPY hydrogels exhibited larger and more numerous pores, supporting enhanced electrical conductivity. SEM revealed an open, highly porous network in PPY hydrogels, suggesting greater surface area for cell attachment and improved ionic movement.

Conversely, GO, Au, and PANI hydrogels showed smaller or fewer pores, which may reduce conductivity. G hydrogels remained structurally similar to the control. These findings underscore the significance of pore structure in influencing the material performance of conductive hydrogels for cardiac tissue regeneration.

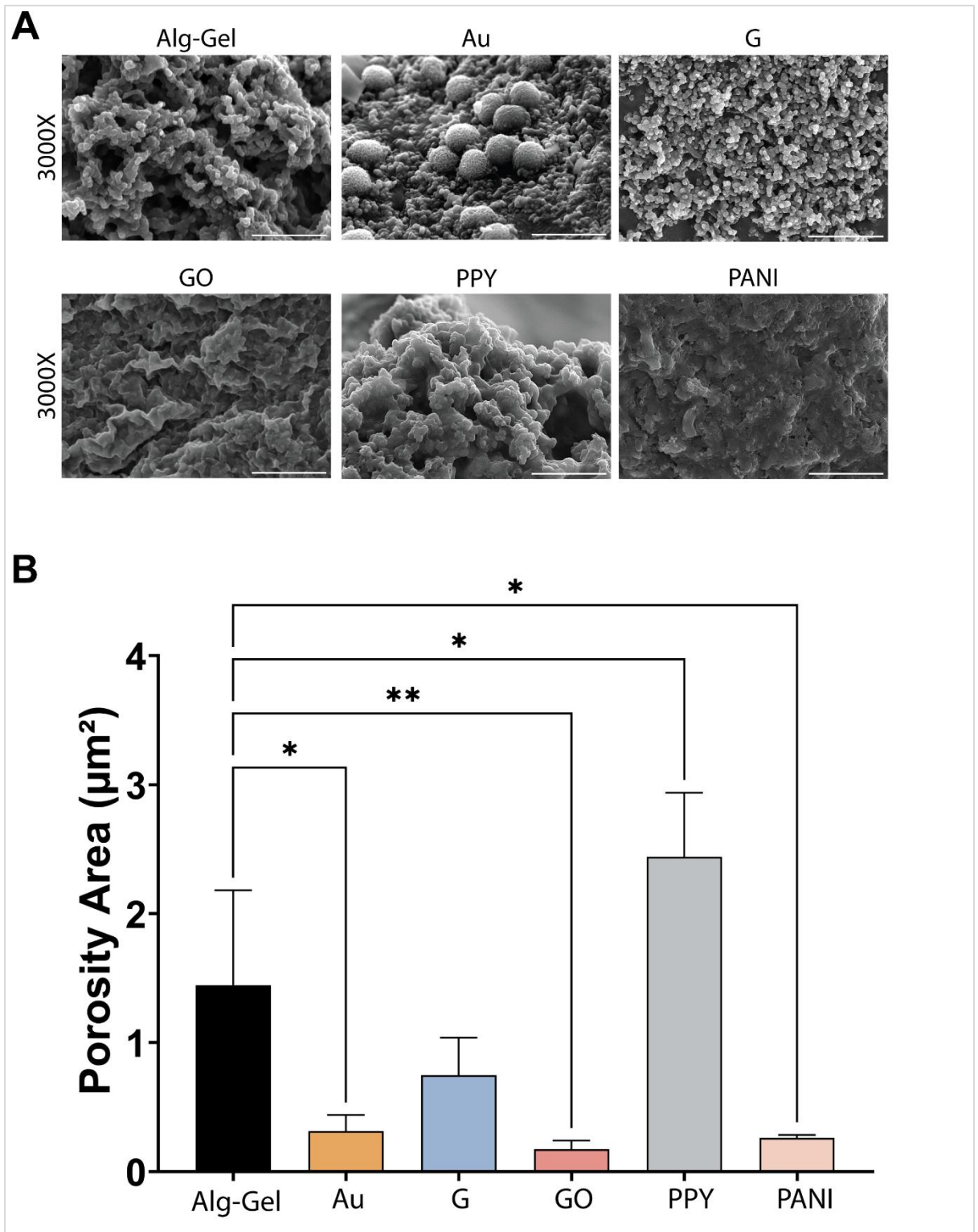


Figure 12. SEM analysis of pore morphology and pore area in conductive hydrogels. (A)

Scanning electron microscope (SEM) images were taken after drying each hydrogel sample at 120 °C for 12 hours and coating it with gold-palladium. The following formulations were examined: control (4 g alginate + 8 g gelatine, Alg-Gel), Alg-Gel + 4 g gold (Au), Alg-Gel + 0.01 g graphene (G), Alg-Gel + 0.01 g graphene oxide (GO), Alg-Gel + 0.25 g polypyrrole (PPY), and Alg-Gel + 0.025 g polyaniline (PANI). All SEM images were taken at low magnification (1000×; scale bar = 10 µm) and high magnification (3000×; scale bar = 2 µm). **(B)**

Quantification of pore area (µm²) from SEM images shows differences in porosity among the hydrogels containing different conductive polymers. Porosity in the Au, G, GO, PPY, and PANI groups was compared to the control Alg-Gel hydrogel. Data are presented as mean ± SEM.

Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test.

* p < 0.05, ** p < 0.01.

2.3.2 Adding Conductive Polymers to Alg-Gel Formulations Changes Mechanical Properties

Brillouin microspectroscopy was used on the different conductive hydrogels to determine their elasticity and viscosity. Furthermore, classic rheology was used to validate and complement the data obtained from Brillouin microspectroscopy. This combined approach enables better control over the mechanical properties of hydrogel bioinks used in 3D bioprinting for cardiac tissue engineering.

Brillouin measurements were collected by analysing the frequency shift (GHz) before and after crosslinking. Before crosslinking, GO hydrogels showed significantly higher frequency shift values than Alg Gel (control) (Figure 13A; P < 0.05), indicating increased stiffness in the uncrosslinked state. After calcium crosslinking, the frequency shift values became comparable, and no significant differences were detected among any of the hydrogel groups (Figure 13B), demonstrating that crosslinking equalised stiffness across the formulations.

Compression testing of Young's modulus (Figure 13C) showed no significant differences in elastic modulus among the conductive hydrogels and Alg Gel, with all samples falling within a similar range. However, shear modulus analysis (Figure 13D) showed a significant reduction in PANI hydrogels compared to Alg Gel (P < 0.05), reflecting lower viscosity and greater susceptibility to deformation under shear.

Overall, these findings show that while some conductive polymers, such as GO, influence stiffness before crosslinking, the final mechanical characteristics after crosslinking remain broadly similar. PANI is the only formulation that demonstrates a noticeable reduction in shear modulus, which may affect its printability and performance under physiological loading.

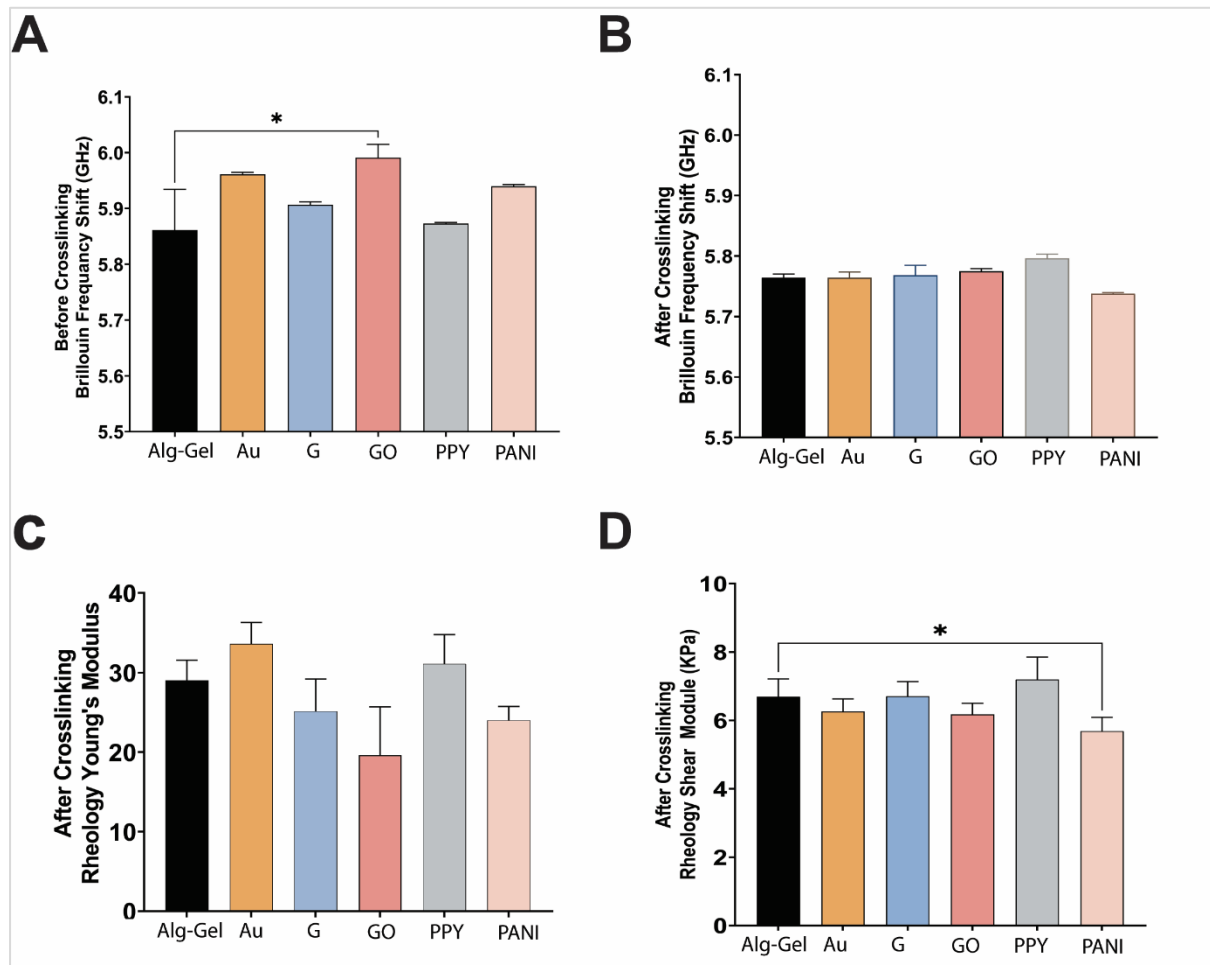


Figure 13. Brillouin microspectroscopy and rheological characterisation of conductive hydrogels. **A.** Brillouin frequency shift measurements of conductive hydrogels before crosslinking. Frequency shift (GHz) values are shown for hydrogels composed of 4% alginate and 8% gelatine mixed with either 4 g gold (Au), 0.1 g graphene (G), 0.1 g graphene oxide (GO), 0.01 g polypyrrole (PPY), or 0.01 g polyaniline (PANI), compared to the control (Alg-Gel: 4 g alginate + 8 g gelatine). **B.** Brillouin microspectroscopy measurements of the same

hydrogels after crosslinking. **C.** Compressive modulus calculated from the frequency shift, where a higher shift indicates increased stiffness and reduced compressibility. **D.** Shear modulus of the hydrogels, where lower values indicate reduced viscosity. Data are presented as mean $\pm$ SEM ($n = 4$ independent replicates). Statistical significance was determined using one-way ANOVA with Tukey's post hoc test. $*p < 0.05$.

2.3.3 Hydrogels Conductivity

To assess the stability and conductivity performance of the conductive hydrogels over time, a total dissolved solids (TDS) sensor module with a probe was used to measure the conductivity of the hydrogels at two time points: Day 0 and Day 7. TDS refers to the total concentration of dissolved ions, salts, and organic matter in a liquid. The TDS sensor estimates electrical conductivity based on ion mobility in the medium, making it suitable for evaluating the conductive properties of hydrogels. This test aimed to determine whether the addition of conductive polymers enhanced or maintained the electrical conductivity of the hydrogels during incubation.

At Day 0, there were no statistically significant differences in conductivity between the polymer-containing hydrogels and the control Alg-Gel (Figure 14A). However, by Day 7, hydrogels containing gold (Au), graphene (G), and graphene oxide (GO) exhibited significantly higher conductivity compared to Alg-Gel ($P < 0.0001$; one-way ANOVA with Tukey's post hoc analysis). PPY hydrogels showed no significant difference from the control, while PANI hydrogels demonstrated a significant increase in conductivity compared to Alg-Gel ($P < 0.05$), as shown in Figure 14B.

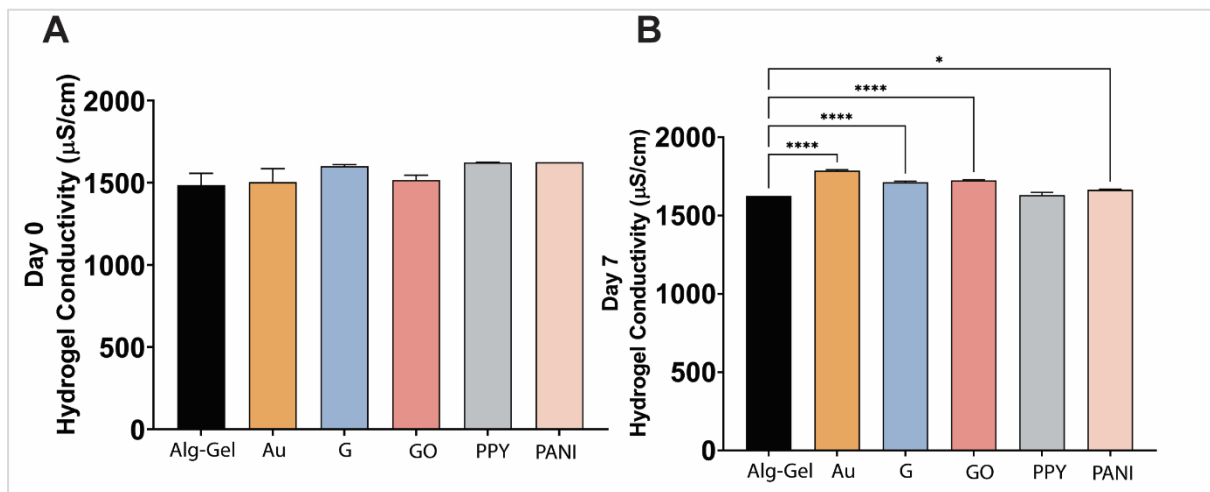


Figure 14. Conductivity measurement of hydrogels using a TDS sensor module. A.

Conductivity measurements at Day 0 for hydrogels composed of 4% alginate and 8% gelatine mixed with 4 g gold (Au), 0.1 g graphene (G), 0.1 g graphene oxide (GO), 0.01 g polypyrrole (PPY), or 0.01 g polyaniline (PANI), compared to the control (Alg-Gel). No significant differences were observed. **B.** Conductivity at Day 7, showing significant increases in Au, G, GO, and PANI groups compared to Alg-Gel. Data are presented as mean $\pm$ SEM; n = 4 independent replicates. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. *p < 0.05, ****p < 0.0001.

2.3.4 Addition of Conductive Polymers Improves the Printability and Durability of Alg-Gel

Printability refers to the ability of a hydrogel to be successfully extruded and patterned into a 3D structure using the BIO X6 bioprinter (CELLINK Life Sciences). It was assessed by measuring the width of the extruded hydrogel filament and comparing it to the nozzle diameter, providing a metric for shape fidelity. This evaluation is essential for ensuring that bioprinted cardiac patches are fabricated with precision and reproducibility.

Durability describes the hydrogel's capacity to retain its shape and structural integrity over time when incubated in medium at 37 °C. Durability was assessed by visually monitoring printed patches over 28 days and quantifying the proportion of samples that remained structurally intact. The printability analysis showed that G exhibited reduced print fidelity compared to Alg-Gel (control: 4% alginate + 8% gelatine), with significantly wider printed lines ($P < 0.05$; one-way ANOVA with Tukey's post hoc test; Figure 15A–B). GO also exhibited reduced printability, as indicated by a significantly increased line width compared to the control ($P < 0.01$). Au, PPY, and PANI did not show significant differences in print fidelity relative to Alg-Gel. All hydrogels were printed using a 22-gauge nozzle (inner diameter 410 μ m), with six replicates per group.

To assess structural durability, each hydrogel group was printed into six replicate grid-shaped patches and incubated in DMEM at 37 °C. Visual assessment over 28 days indicated that most hydrogels retained their structure without obvious degradation. However, due to the limited sample size and lack of quantification, additional analysis is needed to confirm these findings (Figure 15C).

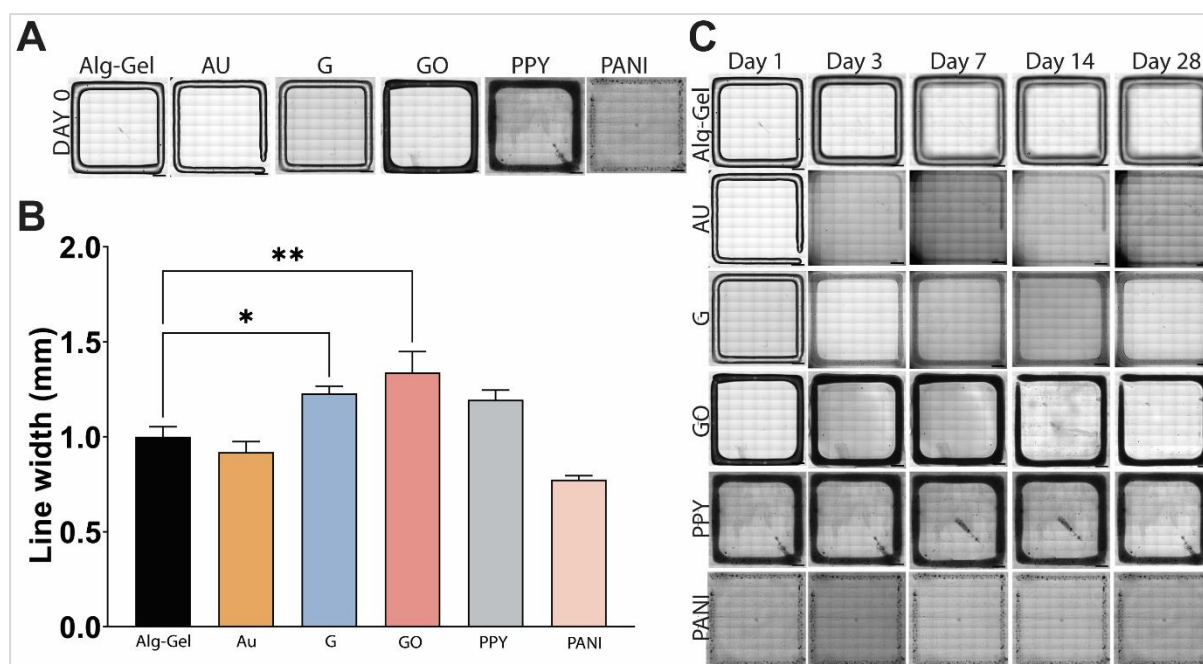


Figure 15 Printability and structural stability of conductive hydrogel patches. A.

Representative images of bioprinted hydrogel lines using a BIO X6 extrusion printer with a 22-gauge nozzle. Line fidelity was evaluated immediately after printing. **B.** Quantification of printed line width (mm) showed that hydrogels containing Au and GO had significantly higher line width, indicating reduced printability compared to the control Alg-Gel ($P < 0.05$ and $P < 0.01$, respectively; one-way ANOVA with Tukey's post hoc test). **C.** Visual assessment of patch integrity over 28 days. Images were taken on days 0, 7, 14, and 28. At each time point, patches were visually evaluated for shape retention and dissolution. Most conductive hydrogel patches retained their shape across all time points. The data shown are representative of 3 independent prints per group.

2.3.5 Stretchability Test for Conductive Hydrogels.

The stretchability test was conducted using a dynamic culture system (ATMS Boxer QQA, manufactured by TAIHOYA, Taiwan). The hydrogels were 3D printed with 14% linear infill and subjected to cyclic stretching for 24 hours at 12 volts, equivalent to 60 beats per minute ($n=3$ technical replicates), simulating the average human heart rate. No significant differences were observed in physical damage or structural integrity after 24 hours of cyclic

stretching among the conductive hydrogels (G, GO, and PPY) compared to the control Alg-Gel.

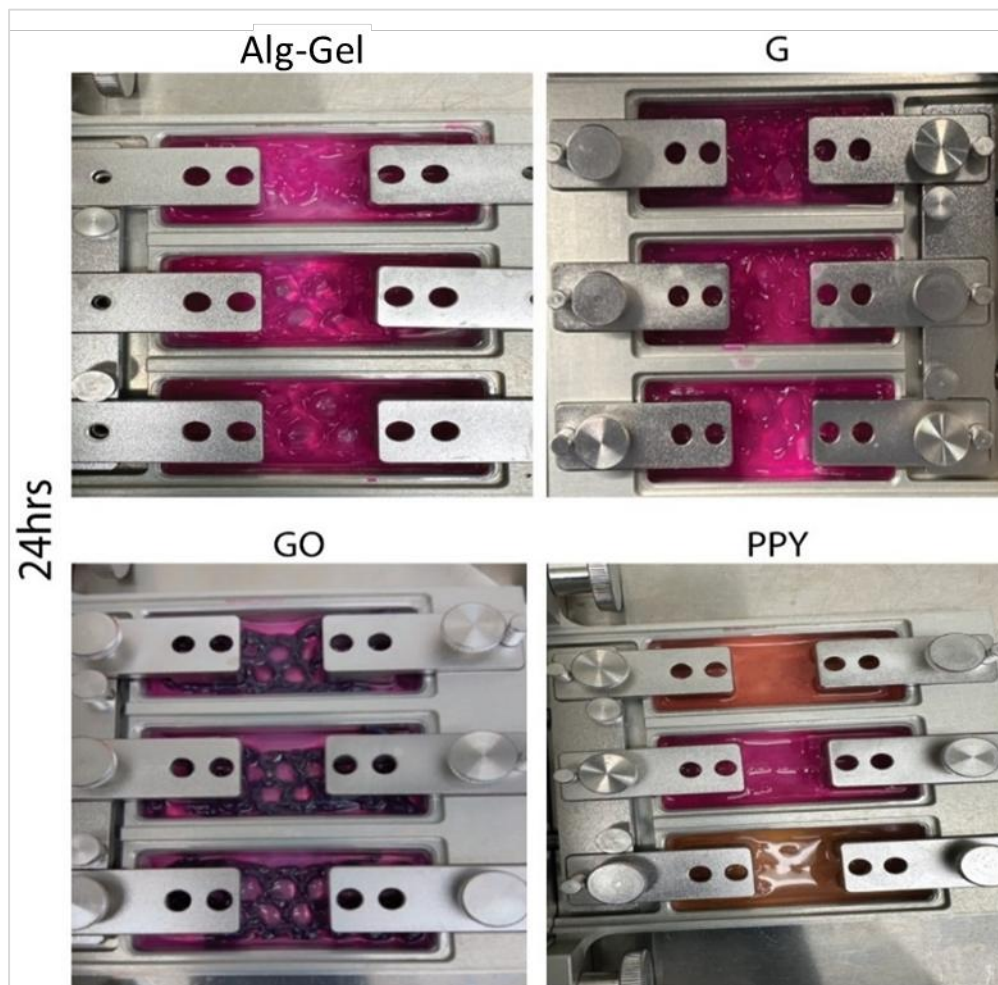


Figure 16. Stretchability assessment of 3D bioprinted conductive hydrogel patches. Images show the structural integrity of patches made with different formulations: (A) Alg-Gel: 4% alginate + 8% gelatine (control), (B) G: Alg-Gel + 0.1 g graphene, (C) GO: Alg-Gel + 0.1 g graphene oxide, and (D) PPY: Alg-Gel + 0.01 g polypyrrole after 24 hours of cyclic stretching in the Taihoya dynamic culture machine. All patches were printed with 14% linear infill and exposed to 60 bpm at 12 volts. No visible tears, degradation, or detachment were observed in any group, indicating good mechanical stability under cyclic load.

2.3.6 Statistical Analyses

The data was analysed using GraphPad Prism 9.5 software (GraphPad Software, CA, USA). Each experiment was performed in 6 technical replicates (n=6). Significance was determined using one-way ANOVA with Tukey's post hoc test. The results are presented as

mean $\pm$ SEM (standard error of the mean). P-values less than 0.05 were considered statistically significant.

2.4 Discussion

In this chapter, different conductive polymers were mixed with 4% alginate and 8% gelatine hydrogels and evaluated for their mechanical properties, printability, durability, conductivity, and elasticity, in comparison to the non-conductive hydrogel control group (AlgGel). Alg-Gel hydrogels have previously been shown to support cardiac cell viability and contractility in both *in vitro* and *in vivo* (Polonchuk et al., 2021; Roche et al., 2023; Roche et al., 2021). However, Alg-Gel hydrogels exhibit limitations, such as poor durability under physiological conditions (Roche et al., 2021; Vettori et al., 2024).

SEM analysis was used to evaluate the porosity level in different conductive hydrogels compared to the control, the non-conductive hydrogel Alg-Gel. A previous study conducted by Fedorovich et al. (2011) demonstrated that higher porosity can enhance nutrient transport, waste removal, and oxygen diffusion, which are crucial for cell growth. In this study, PPY hydrogels showed significantly higher porosity than Alg-Gel ($P < 0.05$; Figure 12B), suggesting improved capacity for oxygen and nutrient diffusion. In contrast, GO, Au and PANI hydrogels exhibited smaller and more compact pores, reflecting a denser internal structure. These differences in pore size likely result from how each conductive polymer interacts with the Alg-Gel matrix: PPY particles disperse more loosely, creating larger and more open pores, whereas GO promote tighter packing and reduced pore openings. Since porosity affects both nutrient diffusion and hydrogel density, these structural variations also influence the mechanical behaviour of each formulation.

Before crosslinking, GO hydrogels showed a significantly higher Brillouin frequency shift than Alg-Gel ($P < 0.05$; Figure 13A), indicating increased stiffness. After crosslinking, however, no significant differences were observed (Figure 13B), and compressive modulus values remained comparable (Figure 13C). The measured stiffness (19.63–33.59 kPa) falls within the physiological range for cardiac tissue (~ 50 kPa) (Querceto et al., 2022), suggesting that these hydrogels may provide adequate mechanical support. Notably, PANI hydrogels showed slightly lower mechanical stress responses, indicating unique behaviour under dynamic loading that

may require further investigation. While crosslinking helped standardise stiffness, some differences persisted, particularly for PANI, highlighting the importance of further optimisation for cardiac applications.

Although GO appeared stiffer in the Brillouin measurements, this trend was not observed in the compression results. This discrepancy is directly linked to differences in porosity and measurement scale. Brillouin microspectroscopy probes stiffness at the microscale, where it is highly sensitive to polymer packing, pore compaction, and local water content. Because GO hydrogels exhibit smaller and denser pores, the polymer network is more tightly organised, making the material appear stiffer when assessed at the microscale before crosslinking. In contrast, compression testing assesses bulk mechanical behaviour. After calcium crosslinking, the overall network becomes more uniform across all formulations, reducing the influence of GO's tighter pore structure and resulting in similar bulk stiffness among the groups. Therefore, the discrepancy reflects the fact that Brillouin detects microscale stiffness influenced by porosity, whereas compression testing measures bulk stiffness after structural homogenisation. This means the two techniques are complementary rather than contradictory.

Conductivity was measured *in vitro* using a total dissolved solids (TDS) sensor. On day 0, there were no significant differences between the hydrogels and the control. By day 7, however, PANI, Au, G, and GO showed significantly higher conductivity than Alg-Gel (Figure 14B). The electrical conductivity of native myocardium is approximately 0.16 S/m (Raghavan et al., 2009); the Alg-Gel control was measured at 0.1625 S/m, while the conductive hydrogels ranged from 0.172–0.178 S/m, indicating enhanced conductivity relative to the control. Although these values slightly exceed the native myocardium, they could potentially enhance signal propagation in infarcted tissue, where conductivity is typically reduced. However, since TDS measurements do not fully replicate *in vivo* electrophysiology, further validation is needed.

Printability and durability were assessed to determine the viscosity and printability of the conductive hydrogels for 3D bioprinting. The Alg-Gel control structure remained intact after 28 days, consistent with previous reports (Roche et al., 2021). No significant differences in printed line width were found for Au, PPY, and PANI, whereas G and GO had significantly wider lines, indicating lower viscosity or reduced filament integrity. Nonetheless, all constructs

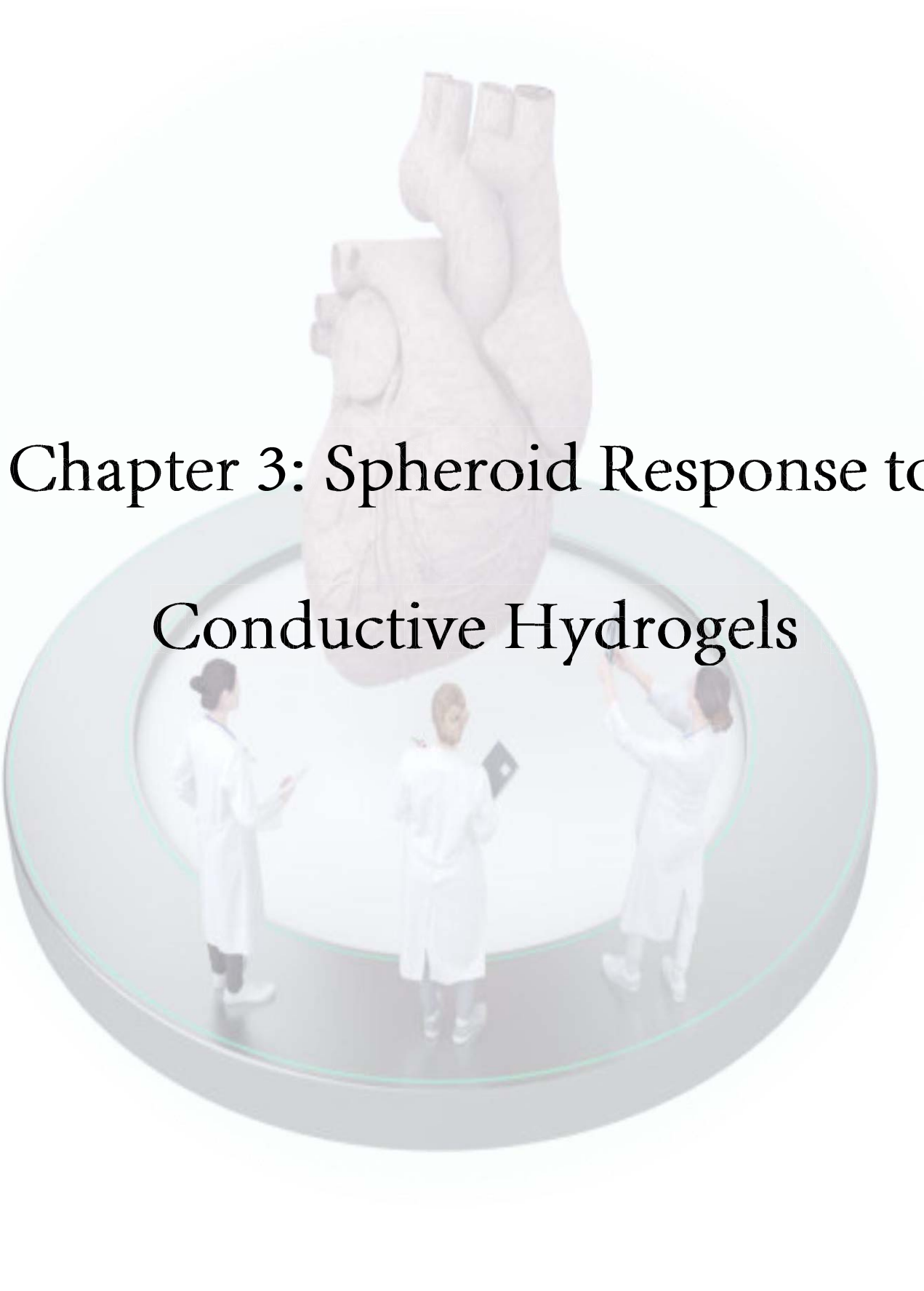
maintained their shape throughout 28 days, confirming good structural durability (Figure 15A–B).

Stretchability was tested using a dynamic stretch culture platform (ATMS Boxer QQA, TAIHOYA, Taiwan), applying a 20% stretch at 12 volts (60 beats per minute) for 24 hours to mimic the human heart rate. No significant differences in stretchability were observed between the conductive hydrogels and Alg-Gel, indicating comparable mechanical stability under cyclic loading.

In summary, this study demonstrated that incorporating conductive polymers into Alg-Gel hydrogels can modulate porosity, stiffness, conductivity, printability, and stretchability. PPY improved porosity, GO increased pre-crosslinking stiffness, and Au, G, and GO enhanced conductivity. All hydrogels showed good printability and durability, with no significant stretchability differences. These findings underscore the potential of conductive hydrogels for cardiac tissue engineering and highlight the need for further long-term *in vivo* studies.

2.5 Conclusion

This study compared conductive hydrogels with a non-conductive control (Alg-Gel) to assess their suitability for cardiac tissue engineering. GO, G, Au, and PANI showed improved conductivity, while GO also enhanced printability. All hydrogels maintained their structure over 28 days, and no major differences were found in stretchability or post-crosslinking stiffness. However, PANI showed unusual mechanical behaviour, highlighting the need for further optimisation. Overall, selecting suitable hydrogels requires assessing electrical, mechanical, and printing properties, with future work needed to validate long-term performance.



Chapter 3: Spheroid Response to Conductive Hydrogels

Chapter 3: The effects of conductive hydrogels on cardiac cells *in vitro*

Abstract

Chapter 3 focuses on evaluating the effects of conductive hydrogels on cardiac cells, both as single dispersed cells and within spheroid cultures. Conductive hydrogels have been used to facilitate electrical signalling, which is essential for synchronising the contraction of cardiac cells. In this study, human coronary artery endothelial cells (HCAECs), human cardiac fibroblasts (HCFs), and human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were co-cultured and incorporated into spheroids to mimic the natural cellular environment of the heart. These spheroids were then tested with the hydrogels generated in Chapter 2, and their response was assessed for cell viability, cytotoxicity, contractile activity, and conduction velocity. The viability and toxicity of cells within the hydrogels were analysed using live/dead assays, while contractile activity was evaluated by measuring contraction frequency and fractional shortening % over 28 days. Additionally, spheroid immunostaining was performed to measure changes in cell populations over time. Results showed that G and GO hydrogels demonstrated the lowest toxicity ratios across timepoints, particularly at Day 14, indicating enhanced biocompatibility compared to the control and PPY groups. Overall, the findings demonstrate that conductive hydrogels, especially G and GO, can effectively support spheroid viability and functional performance, highlighting their promise for bioengineered cardiac tissue applications.

3 Introduction

Cardiac tissue engineering has emerged as a promising approach for treating myocardial infarction. A key aspect of this research is developing biocompatible and conductive hydrogels that can support the growth and function of cardiac cells, mimicking the natural environment of heart tissue. Conductive hydrogels are especially useful for this application because they can facilitate the electrical signalling required for the synchronised contraction of cardiac cells (Gil-Cabrerizo et al., 2023).

In this study, our team developed functional cardiac tissues as cardiac spheroids (CSs) that better mimic human cardiac pathophysiology compared to conventional 2D cultures.

These CSs reproduce the complexity of native cardiac tissue by combining human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), human cardiac fibroblasts (HCFs), and human coronary artery endothelial cells (HCAECs) at physiologically relevant ratios. This multicellular configuration enables robust cell–cell and cell–matrix interactions, supporting enhanced viability, electrical coupling, and tissue formation. By using CSs, this study aimed to create a more physiologically relevant in vitro model and to improve the therapeutic efficacy of bioprinted cardiac patches for myocardial infarction (Polonchuk et al., 2017; Polonchuk et al., 2021; Sharma & Gentile, 2021b)

This study aims to identify the most suitable conductive hydrogel formulations for cardiac patches to treat myocardial infarction. We will evaluate the spheroid interaction with different conductive hydrogels, assessing 2D cell cultures (endothelial and fibroblast cells), 3D spheroids (cardiac cells, endothelial cells, and fibroblast cells), contractile activity, and conduction velocity using optical mapping. The aim is to optimise the hydrogel composition to support the spheroid's cell viability, minimise cytotoxicity and functionality.

Overall, this project investigates the spheroid response to different conductive hydrogels, focusing on their potential application in creating an effective cardiac patch. The results of this study will provide valuable insights into the design and development of biomaterials for cardiac tissue engineering, with the aim of improving outcomes for patients with myocardial infarction.

3.1 Materials and Methods

3.1.1 Cell Viability

The co-cultured human coronary artery endothelial cells (HCAECs, Cell Applications, Inc., San Diego, CA, USA) and human fibroblasts (HCF, Cell Applications, Inc., San Diego, CA, USA) were cultured separately in T-flasks using a 1:1 ratio of MesoEndo Cell Growth Medium (Sigma Aldrich) and CF Growth Medium (Sigma Aldrich) and incubated at 37°C in 5% (v/v) CO₂. The media were changed every second day. After the cells reached 80% confluence, they were seeded in 96-well plates at a density of 3×10^4 cells/mL and incubated overnight. The following day, 50 µL of conductive hydrogel was added on top of the seeded cells, followed by 50 µL of calcium chloride solution (2%, prepared by dissolving 2 g of calcium chloride in 100 mL of PBS). Cross-linking was performed for 30 minutes on ice. All samples were then incubated for 7 days

at 37°C. To analyse cell viability within each of the different conductive hydrogel samples, the cells were stained with calcein-AM (live cells, Live/Dead Assay, ThermoFisher), ethidium homodimer-1 (dead cells, Live/Dead Assay, ThermoFisher), and Hoechst stain (nuclear stain) on day 7 for cell viability analysis, as shown in Figure 17.

3.1.2 Fluorescence Microscopy

The stained endothelial and fibroblast cells were imaged using an EVOS M7000 microscope with the 2x objective lens (0.06 NA / 5.62 WD) for cell viability analysis. Cells were imaged with GFP LED illumination for green/live cells, RFP LED illumination for red/dead cells, and DAPI LED illumination to highlight the cell nuclei. The whole surface area of each well was imaged for each sample via the automated image stitching technique provided in EVOS M7000. The images were analysed in FIJI software, as shown in Figure 17.

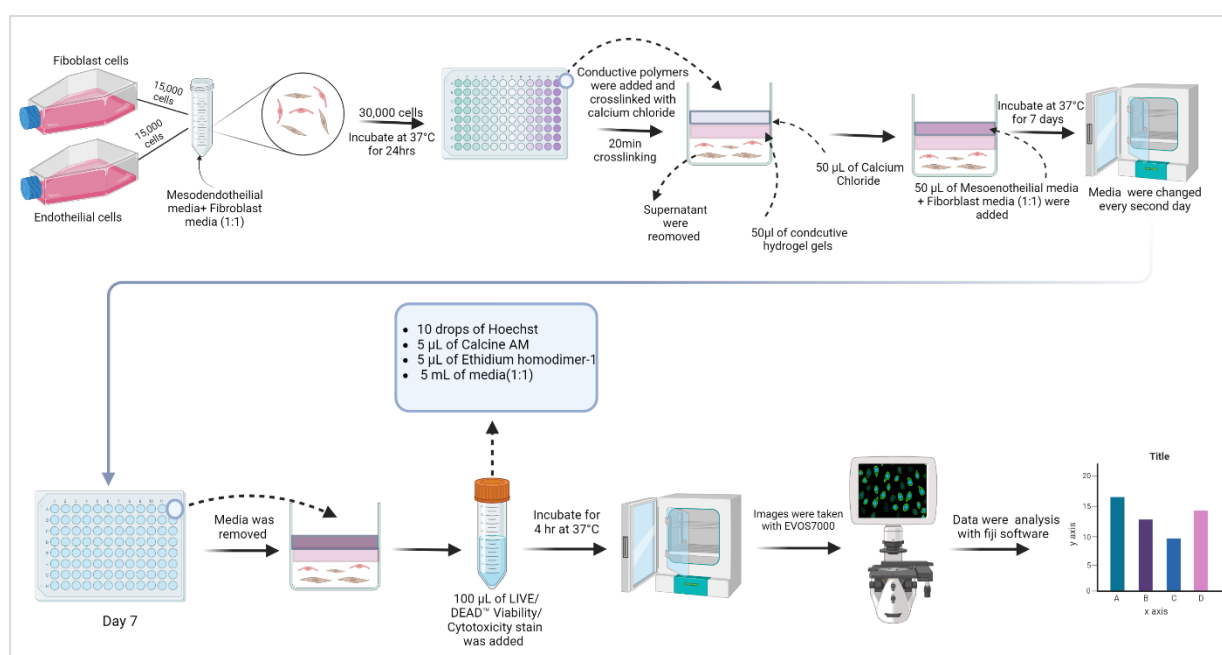


Figure 17. The schematic diagram for the co-culture of endothelial and fibroblast cells with the addition of conductive polymers, which were incubated for 7 days. Calcein-AM, Ethidium homodimer-1 and Hoechst stains were used for live/dead cell viability analysis.

3.1.3 Development of Human Cardiac Spheroid

Human CSs were prepared as previously described (Tarsitano et al., 2024). Human cardiac fibroblasts (HCFs) and human coronary artery endothelial cells (HCAECs) were purchased from Cell Applications (San Diego, CA, USA) and cultured separately in HCF Medium and MesoEndo Growth Medium, respectively. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs; iCell® Cardiomyocytes) were obtained from FujiFilm Cellular Dynamics (Madison, WI, USA) and maintained according to the manufacturer's instructions. Briefly, iCMs were plated in iCell® Plating Medium for one day and then cultured in iCell® Maintenance Medium for two additional days.

To generate cardiac spheroids, HCFs, HCAECs, and iCMs were mixed at a 1:1:2 ratio and resuspended in CS medium, which consisted of HCF Medium, MesoEndo Growth Medium, and iCell® Maintenance Medium combined at a 1:1:2 ratio. The cell suspension was seeded into non-adherent 2% (w/v) agarose 3D Petri Dish® moulds (Microtissues Inc., Providence, RI, USA), prepared according to the manufacturer's instructions, and incubated at 37 °C with 5% CO₂. The CS medium was replaced every two days until spheroids were formed. Six replicates were prepared for each conductive hydrogel in each experiment.

3.1.4 Cell Viability of Spheroids in Conductive Hydrogels

Ten spheroids were bioprinted into 1 mL of each conductive hydrogel to form patches measuring 10 × 10 mm, which were placed in 6-well plates (n = 6 replicates per hydrogel type) and cross-linked with 1 mL of calcium chloride. Each well contained 10 mL of culture medium, and all samples were incubated for 7, 14, and 28 days at 37°C. To analyse the live and dead cells within each sample, the cells were stained with calcein-AM (1 µL/mL), ethidium homodimer-1 (1 µL/mL), and Hoechst (NucBlue™ Live ReadyProbes™, Thermo Fisher) on days 7, 14, and 28. Staining was performed by adding the dyes directly to the 10 mL medium, and the samples were incubated at 37°C in 5% CO₂ for 3 hours. After incubation, the samples were washed three times with warm DPBS and then imaged using a STELLARIS 8 CM1 (Leica Microsystems Pty Ltd, Macquarie Park, NSW, Australia). Cell viability was analysed by fluorescence, and the toxicity ratio was calculated using the following formula:

$Toxicity\ ratio = \frac{fluorescence\ Ethidium\ Homodimer-1}{fluorescence\ Calcein\ AM}$ and was normalised to the toxicity ratio of the Alg-Gel control group, as shown in Figure 18.

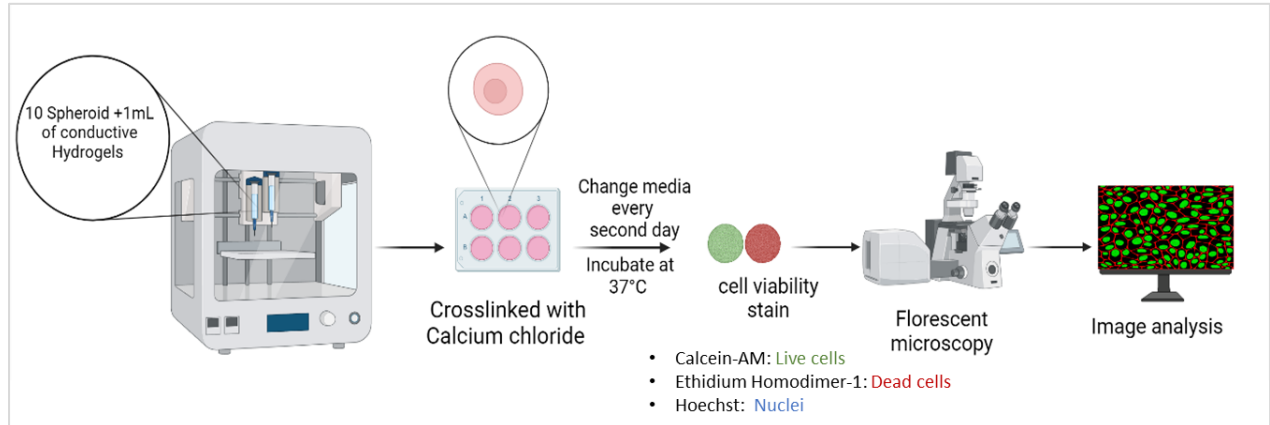


Figure 18. Schematic overview of the experimental workflow for spheroid cell viability analysis in conductive hydrogels. Ten cardiac spheroids were bioprinted into each 1 mL hydrogel patch (10 × 10 mm), cultured for 7, 14, and 28 days, and stained with calcein-AM (live), ethidium homodimer-1 (dead), and Hoechst (nuclear). Fluorescence imaging was performed to calculate the toxicity ratio, normalised to the Alg-Gel control group.

3.1.5 Contractile Activity of CSs Bioprinted in Conductive Hydrogels

Ten CSs were bioprinted into 1 mL of each conductive hydrogel using the BIO X6 (CELLINK Life Sciences, Boston, MA, USA) to form individual patches, as previously described (Tarsitano et al., 2024; Vettori et al., 2024). The bioprinted patches were incubated at 37°C in 5% (v/v) CO₂, and the culture medium was replaced every two days with fresh medium for up to 28 days. On the 28th day, 20-second videos were recorded for each sample using the EVOS M7000 Cell Imaging System (ThermoFisher Scientific, Waltham, MA, USA) (Figure 19). The contractile properties of the CSs were analysed by measuring the contraction frequency (number of contractions per second) and the fractional shortening percentage (FS%). Fractional shortening percentage was calculated using the formula:

$$FS\% = \frac{(Dc - Dr)}{Dc}$$

where D_c is the diameter of the contracted spheroid and D_r is the diameter of the relaxed spheroid. Video analysis was performed using ImageJ software (NIH, Bethesda, MD, USA). These measurements were used to assess spheroid viability and functional performance within the conductive hydrogel patches.

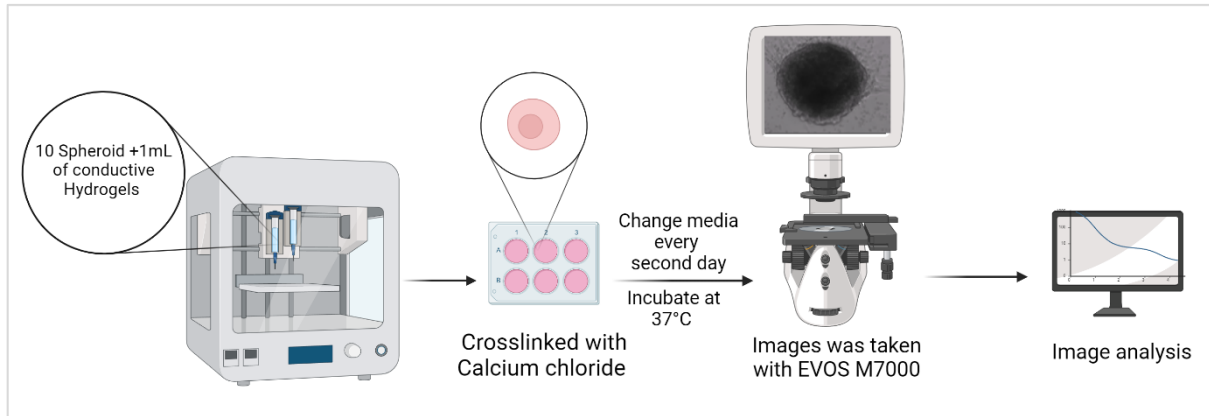


Figure 19. The schematic diagram of spheroid contractile activity measurement within conductive hydrogels using the EVOS M7000 Cell Imaging System on days 7, 14, and 28.

3.1.6 Spheroid Immunostaining in Conductive Hydrogels

Conductive hydrogels containing CSs were prepared as shown in Figure 18. Six replicates were fixed with 100 μ L of 4% paraformaldehyde (Sigma Aldrich) for 3 hours at room temperature (RT). All samples were washed three times with 0.01% (v/v) PBSA (DPBS containing 0.01% sodium azide). Samples were permeabilised using 100 μ L of 0.02% (v/v) Triton X-100 in PBSA for 1 hour and blocked with 3% (w/v) bovine serum albumin (BSA) in 0.01% PBSA for each well. Plates were sealed with Parafilm and incubated at 4°C overnight.

For immunostaining, samples were incubated with primary and secondary antibodies at 4°C overnight, as previously described (Tarsitano et al., 2024). Primary antibodies included mouse anti-human CD31 (6.25 μ g/mL, Abcam, Cambridge, UK) for labelling HCAECs, Troponin T-C Antibody (CT3) Alexa Fluor 546-conjugated (10 μ g/mL, Abcam) for cardiomyocytes, and Alexa Fluor 488 anti-vimentin antibody (2 μ g/mL, Abcam) for fibroblasts. The secondary antibody was Donkey anti-mouse Alexa Fluor 790 (21 μ g/mL, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). NucBlue™ Live ReadyProbes™ (2 drops/mL, Thermo Fisher Scientific, Waltham, MA, USA) was used to stain nuclei. Specifically, for final staining,

25 μL of CTNT (10 $\mu\text{g}/\text{mL}$), 2 μL of vimentin (2 $\mu\text{g}/\text{mL}$), and 2 drops of Hoechst dye were mixed and diluted in 50 μL of 3% (w/v) BSA and 0.01% (v/v) PBSA per well. Samples were parafiled, covered with aluminium foil, placed on a rocker at room temperature for 1 hour, then incubated at 4°C overnight. After staining, samples were washed three times with PBSA. Images were collected using LAS X software with an LSCM Leica Stellaris DMI-8 confocal microscope (Leica Microsystems) and analysed with ImageJ software (NIH Fiji, Bethesda, MD, USA). Each staining was measured individually, and data were normalised against CSs embedded in Alg-Gel hydrogels as seen in Figure 20.

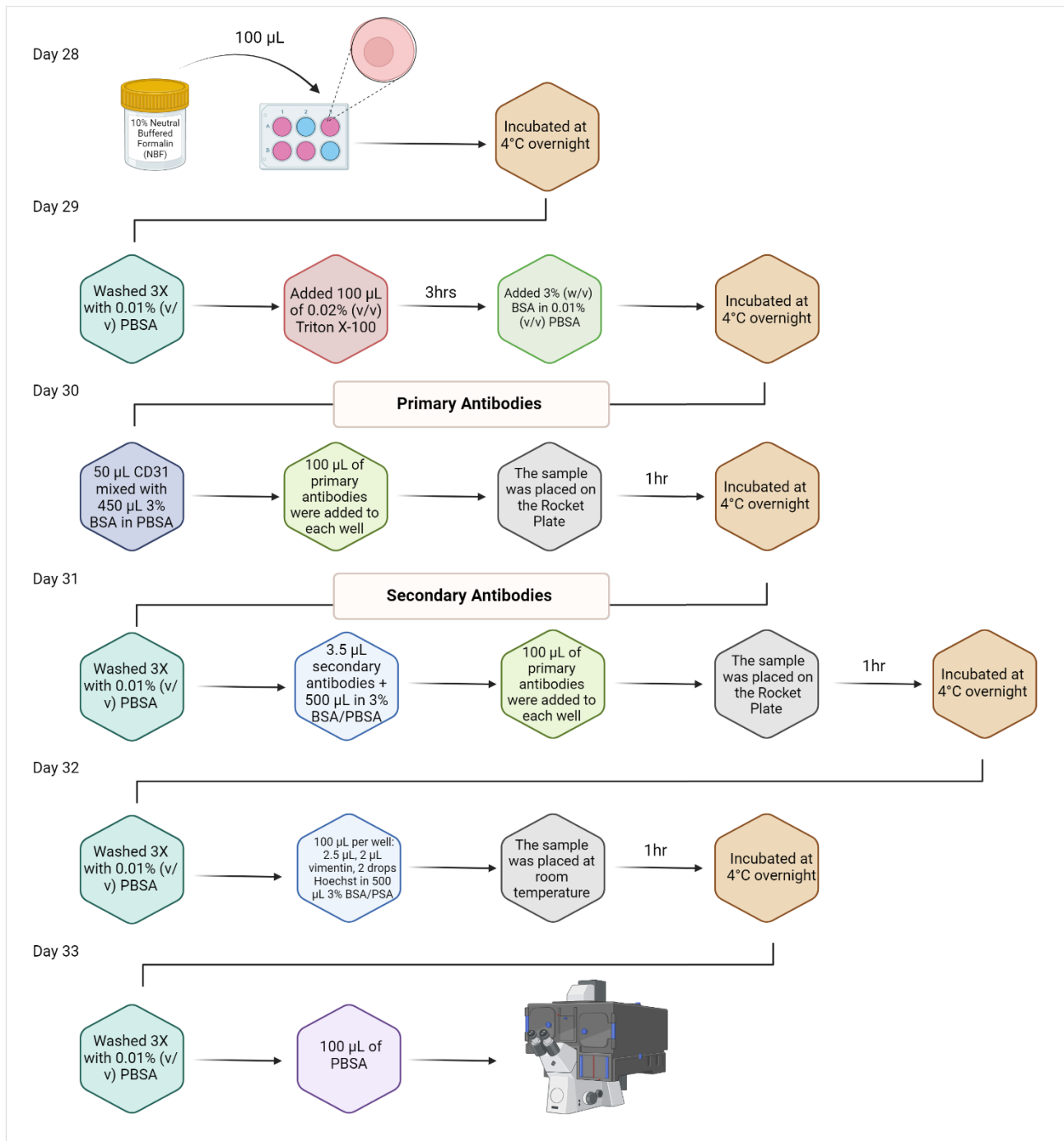


Figure 20. Spheroid immunostaining in conductive hydrogels. Six replicates were fixed with 10% neutral buffered formalin and incubated at 4°C overnight. Samples were washed with 0.01% PBSA, permeabilised with 0.02% Triton X-100, and blocked with 3% BSA in PBSA. Immunostaining was performed using a primary antibody targeting CD31 to label HCAECs, followed by Alexa Fluor 647-conjugated secondary antibodies. Cardiac myocytes, fibroblasts, and nuclei were stained with CTNT, vimentin, and Hoechst, respectively. Samples were imaged using a Leica Stellaris DMI-8 confocal microscope and analysed with ImageJ software.

3.1.7 Optical Mapping

Optical mapping measures cardiac tissue's electrical activity and calcium signalling to assess conduction velocity (CV), as seen in Figure 21. To perform optical mapping of the conductive hydrogels, a dye solution was prepared consisting of 2 mL of culture medium containing a final concentration of 1.77 μM Rhod-2 AM (fluorescent Ca^{2+} indicator, Abcam, Melbourne, VIC, Australia), 0.02% Pluronic™ F-127 (Thermo Fisher Scientific, Scoresby, VIC, Australia), and 50 μM RH237 (Santa Cruz Biotechnology Inc., Heidelberg, Germany). Samples were placed in a 6-well plate with 3 mL of medium containing the dye solution and incubated at 37 °C for 1 hour. Samples were washed three times with 1× PBS and incubated for 40 minutes at 37 °C in the MappingLab Optical Mapping System. Each TTL system cable was connected appropriately: D05 for LED lights, D06 for stimulation (D07 kept off) for ECG, and D08 for cameras. The acquisition time was set uniformly across channels (e.g., 30–30-second recording: D05 for 3000 ms, D06 for no stimulation, D07 off, and D08 for 29,500 ms with an initial 500 ms pause). Images were collected using the optical mapping software OMapRecord 4.0 and processed using OMapScope 5.6.8 (Mapping Lab Ltd, Manchester, UK).

Conduction velocity was calculated from the activation time maps generated from the first derivative of the optical signals using custom MATLAB code, as previously described in our lab (Tarsitano et al., 2024). Processing filters were applied to generate waveforms with low noise-to-signal ratios, including a Gaussian convolution of 9×9 , a zero-phase filter window of 30, and theta smoothing intensity of 1.5. Waveform graphs were selected based on a region of interest to display the lowest signal-to-noise ratio. A single peak representing a single contraction was used for calculating CV values for both action potential and calcium signals, with temporal sequence recordings (slowed to 1/20th of the original speed) to display signal propagation across different regions of the CSs.

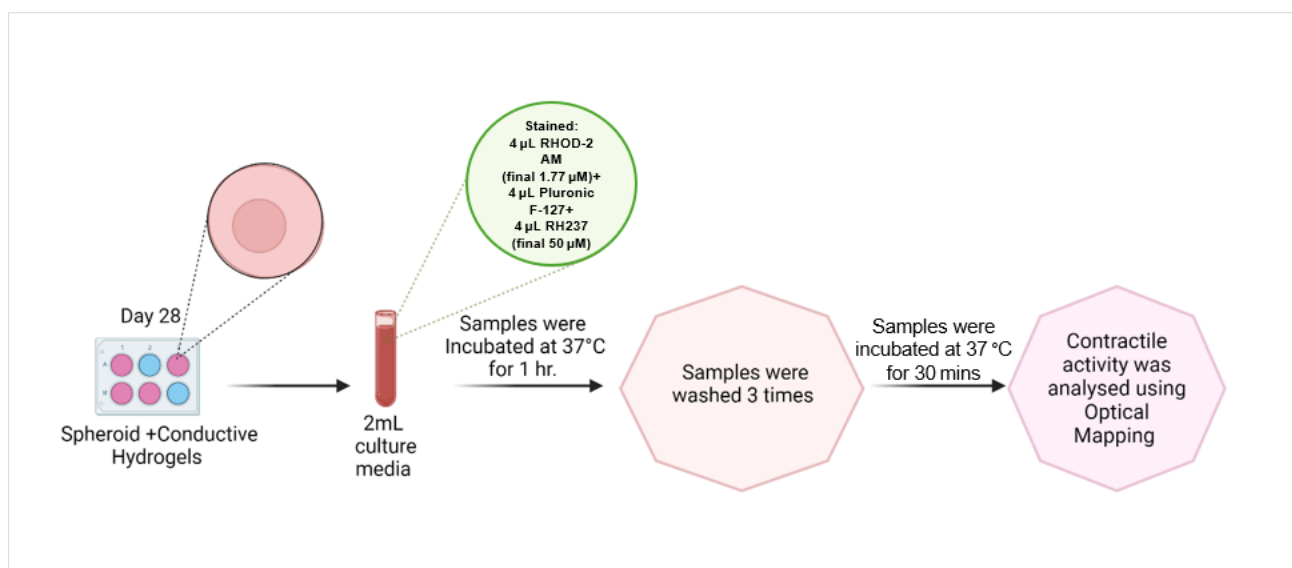


Figure 21. Schematic workflow of bioprinted spheroids within conductive hydrogels analysed for contractile activity using optical mapping on day 28. Spheroids were bioprinted into conductive hydrogels, stained with Rhod-2 AM (1.77 μ M final), Pluronic™ F-127 (0.02%), and RH237 (50 μ M final), incubated, washed, and imaged using the MappingLab Optical Mapping System.

3.1.8 Statistical Analyses

The data was analysed using GraphPad Prism 9.5 software (GraphPad Software, CA, USA). Each experiment was performed in 6 technical replicates ($n=6$). For cell viability, the images were taken from the whole well, and the total number of cells within the field of view was accounted for in the statistical analysis. Significance was determined using one-way ANOVA with Tukey's post hoc test. The results are presented as mean $\pm$ SEM (standard error of the mean). P-values less than 0.05 were considered statistically significant.

3.2 Result

3.2.1 Cell Viability Embedded in Conductive Hydrogels

Cell viability is an important indicator of the health and functionality of cells within engineered constructs. For cardiac tissue engineering, it is critical to develop biomaterials that maintain high cell viability and support robust cell integration and growth. Cytotoxicity testing is therefore essential to ensure that conductive hydrogels are

biocompatible and safe for cardiac applications. In this preliminary experiment, cardiac spheroids were embedded in Alg-Gel hydrogels containing different conductive polymers (GO, PPY, Au, PANI) and cultured for 7 days. Cell viability was assessed using live/dead staining with Calcein-AM (live cells) and Ethidium homodimer-1 (dead cells), followed by fluorescence imaging and quantification. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test.

The results (Figure 22 A–B) show that PPY, Au, and PANI hydrogels exhibited higher cytotoxicity compared to the control Alg-Gel hydrogel ($p < 0.05$). In contrast, GO hydrogels maintained high cell viability similar to the control. These findings highlight the need to optimise the formulation of PPY, Au, and PANI hydrogels to reduce cytotoxicity and improve biocompatibility for future cardiac applications.

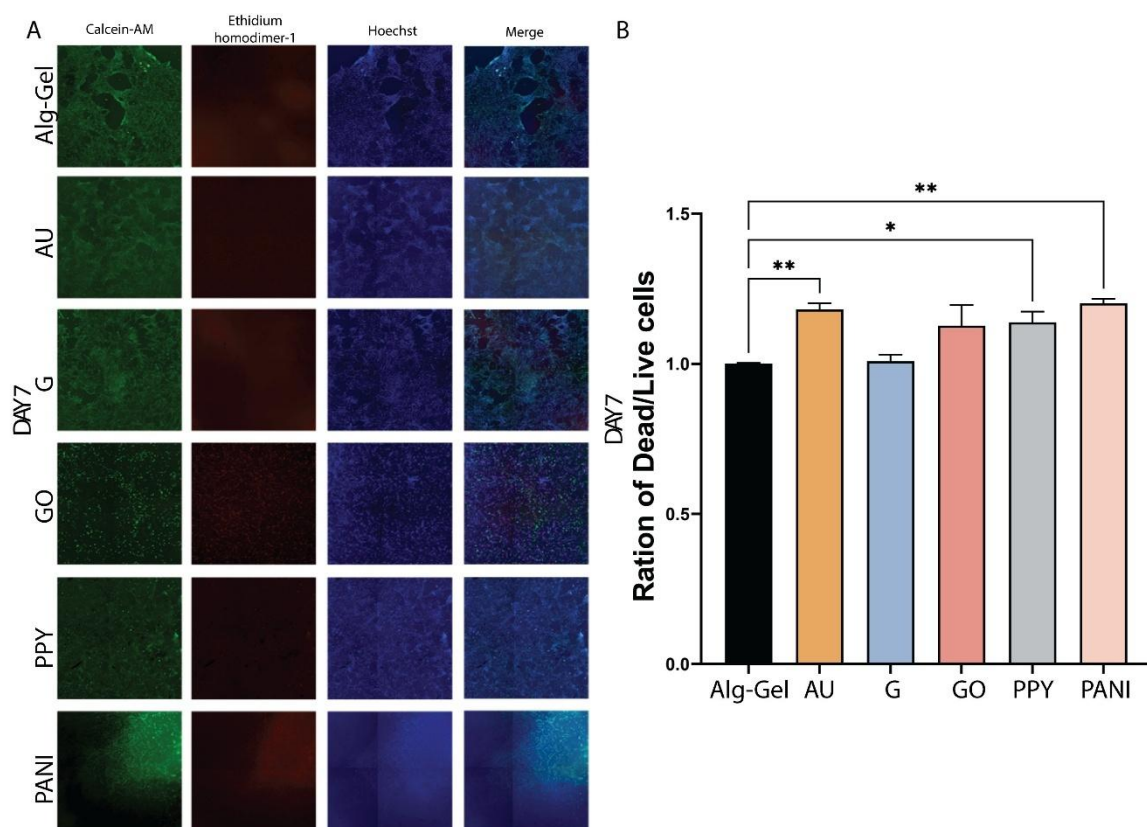


Figure 22.Dead and live cell ratio of endothelial cells and fibroblast cells, which were in co-culture conditions within the mentioned concentrations of the different conductive polymers. **B.** Data is presented as mean $\pm$ SEM. Statistical significance was determined using Two-Way ANOVA (Tukey's post hoc analysis). * $p < 0.05$, ** $p < 0.01$.

3.2.2 Cell Viability of CSs Bioprinted in Conductive Hydrogels

Our statistical analysis of bioprinted spheroids stained with live (calcein-AM) and dead (ethidium homodimer) dyes showed no significant differences ($p > 0.05$) in cytotoxicity for the conductive hydrogels G, GO, and PPY compared to the Alg-Gel control at days 7, 14, and 28 (Figure 23B–F). Across all formulations, the viability remained high, with more than 85–90% of cells staining positive for calcein-AM, while dead cells represented only a small fraction of the total population. This consistent viability profile across three time points confirms that the addition of conductive polymers did not introduce any detectable cytotoxic effects.

Qualitative assessment of the confocal images further supported these findings. Spheroids maintained a compact, rounded morphology with uniform green fluorescence, indicating stable metabolic activity and intact structural organisation (Figure 23 A, C, E). No signs of spheroid fragmentation, irregular shapes, or increases in peripheral cell death were observed, and the distribution of live and dead cells appeared comparable across all conductive hydrogels and the control group.

Together, the statistical and visual data confirm that G, GO, and PPY hydrogels provide a biocompatible environment capable of supporting long-term cardiac spheroid survival and structural integrity over 28 days. These results demonstrate that incorporating conductive materials into Alg-Gel does not compromise cell viability, making these formulations suitable for downstream functional analyses.

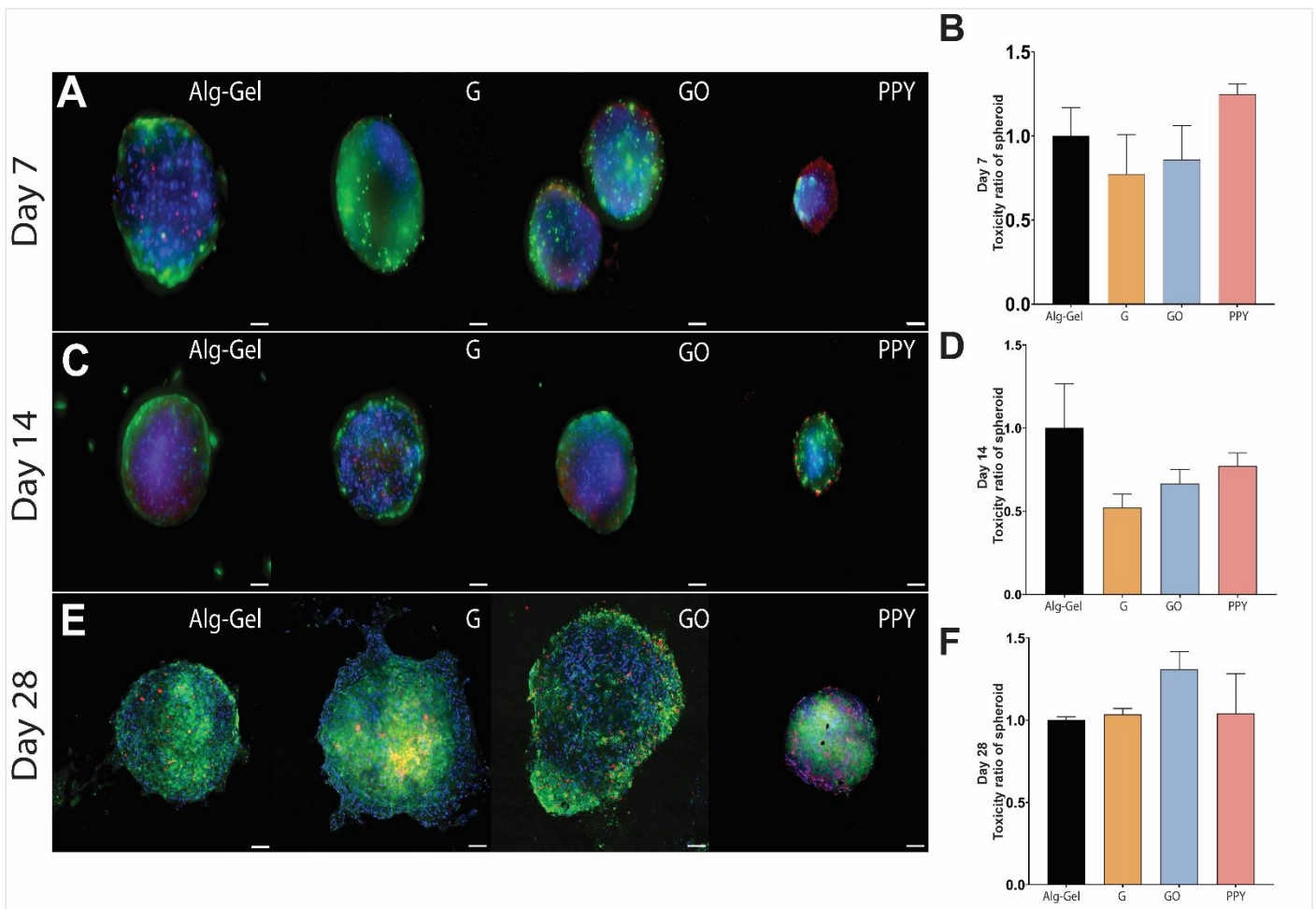


Figure 23 Toxicity ratio of bioprinted cardiac spheroids in conductive hydrogels.

(A, C, E) Representative confocal images showing cell viability of bioprinted cardiac spheroids embedded in conductive hydrogels containing 0.01 g graphene (G), 0.01 g graphene oxide (GO), or 0.025 g polypyrrole (PPY), compared to the control hydrogel (Alg-Gel) at days 7, 14, and 28. (B, D, F) Quantification of the toxicity ratio (dead/live cells) for each hydrogel at days 7, 14, and 28. Data represent mean $\pm$ SEM (n = 10 independent replicates). Statistical significance was determined by one-way ANOVA.

3.2.3 Contractile Activity of CSs Bioprinted in Conductive Hydrogels

The contractile activity of bioprinted cardiac spheroids within conductive hydrogels was assessed on Day 28 to evaluate their long-term functional performance. Contractile activity is a key indicator of the physiological relevance and maturity of engineered cardiac tissues (Vikhorev & Vikhoreva, 2018).

A significant increase in contraction frequency was observed for bioprinted cardiac spheroids within PPY hydrogels compared to the Alg-Gel control ($p < 0.01$; one-way ANOVA with Tukey's post hoc analysis) on Day 28, while no significant differences were found for spheroids in G and GO hydrogels (Figure 24A).

Similarly, the fractional shortening percentage (FS%), which measures the relative change in spheroid diameter during contraction, was significantly higher in PPY hydrogels compared to Alg-Gel ($p < 0.05$) on Day 28, indicating enhanced contractile function (Figure 24 B).

No significant changes in FS% were detected for G and GO hydrogels compared to the control. These findings confirm that among the tested conductive hydrogel formulations, PPY most effectively enhances the contractile performance of bioprinted cardiac spheroids after 28 days in culture.

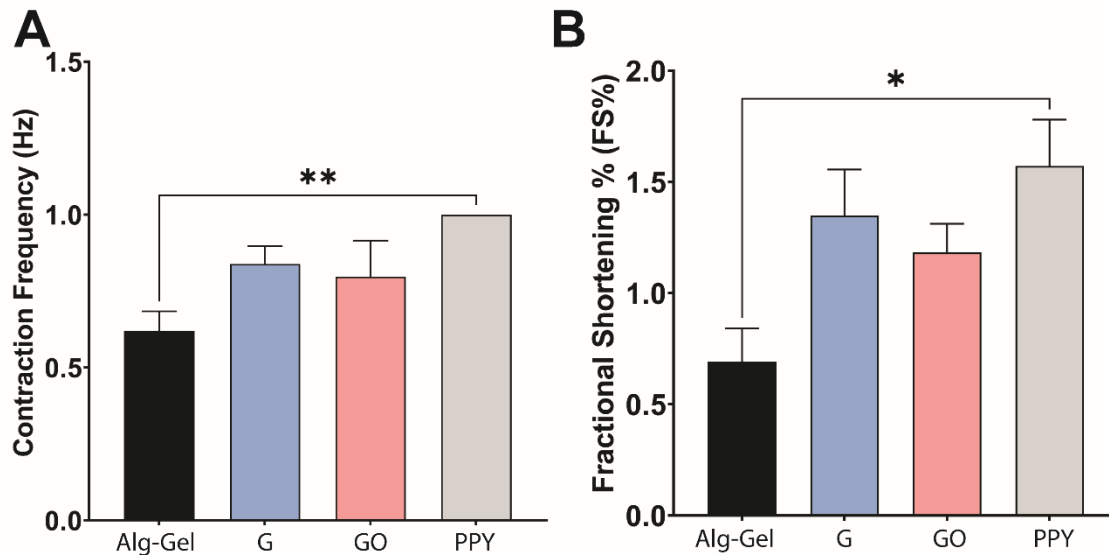


Figure 24. Contractile activity of bioprinted cardiac spheroids in conductive hydrogels. A. Contraction frequency (Hz) of bioprinted cardiac spheroids in conductive hydrogels after 28 days of culture at 37 °C. **B.** Fractional shortening percentage (FS%) of bioprinted cardiac spheroids in conductive hydrogels after 28 days at 37 °C. Contractile motion was recorded using an EVOS M7000 microscope. n = 6 independent replicates. Data represent mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA with Tukey's post hoc test. $p < 0.05$, $p < 0.01$.

3.2.4 Spheroid Immunostaining in Conductive Hydrogels

The cardiac spheroids were bioprinted within conductive hydrogels (Alg-Gel, G, GO, and PPY) and immunostained after 28 days of culture to evaluate whether the different hydrogel compositions influenced the distribution of cell populations within the spheroids. Samples were labelled with antibodies against CD31 (blue, marking cardiac endothelial cells), cardiac troponin T (cTNT; red, marking cardiomyocytes), and vimentin (green, marking cardiac fibroblasts) (Figure 25). For each condition, separate images show vimentin, cTNT, CD31, and a merged composite, allowing clear visualisation of the spatial distribution of each cell type.

The immunostained images confirmed the presence of all three cardiac cell types within the spheroids across all conductive hydrogels, comparable to the Alg-Gel control. No noticeable reduction in fluorescence intensity was observed for any cell type in G, GO, or PPY compared to Alg-Gel, indicating that the conductive hydrogels preserved the multicellular composition of the cardiac spheroids throughout long-term culture. These findings

demonstrate that the addition of conductive polymers did not compromise the structural integrity of the spheroids or alter the distribution of endothelial cells, cardiomyocytes, or fibroblasts.

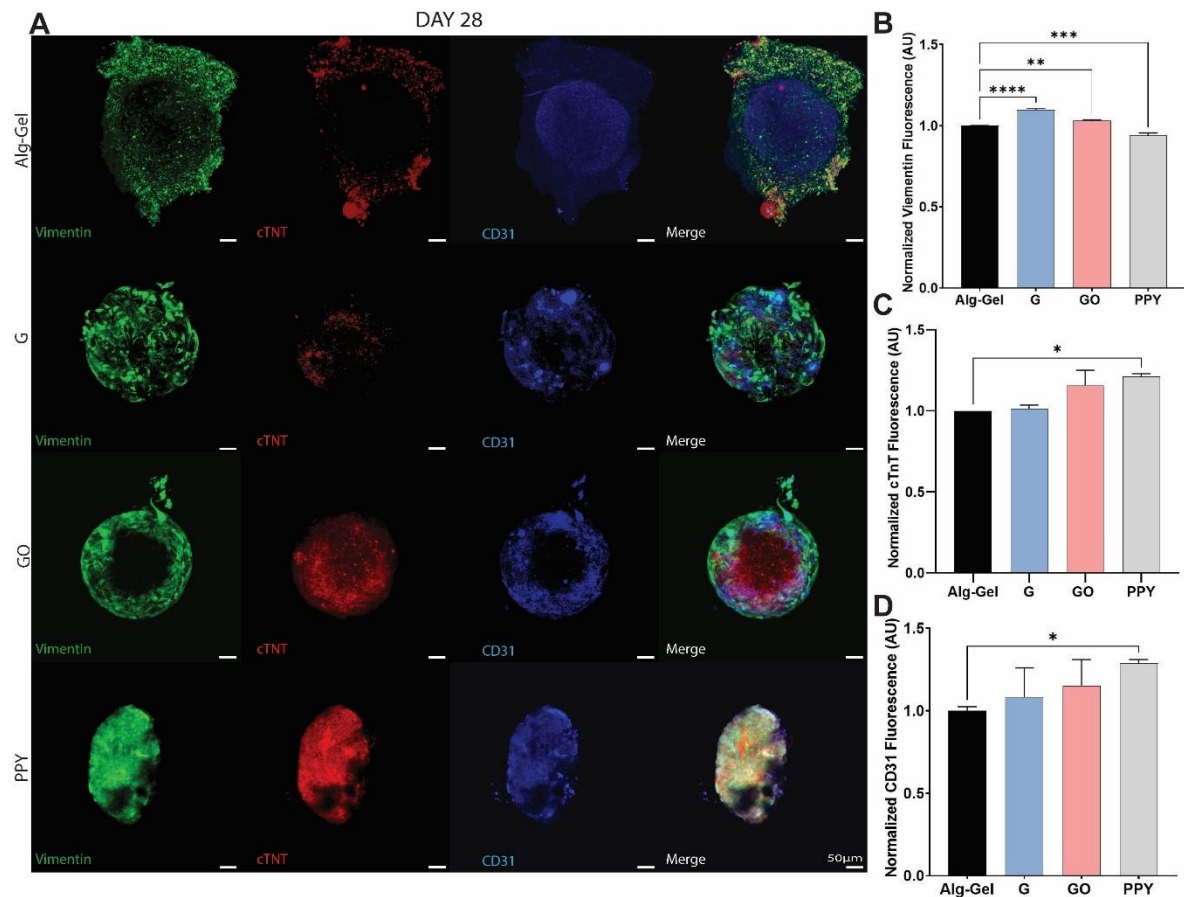


Figure 25. Confocal imaging and quantitative fluorescence analysis of cardiac spheroids cultured within conductive hydrogels at Day 28. **A.** Representative confocal images of cardiac spheroids stained for vimentin (green; fibroblasts), cardiac troponin T (cTnT) (red; cardiomyocytes), and CD31 (blue; endothelial cells) in Alg-Gel (control), graphene (G), graphene oxide (GO), and polypyrrole (PPY) hydrogels. Merge panels show co-localization of all three cell markers. Scale bar: 50 μ m. **B.** Quantification of vimentin fluorescence intensity (AU), showing Alg-Gel and G have significantly higher fibroblast expression compared to GO and PPY (* $p < 0.001$). **C.** Quantification of cTnT fluorescence intensity (AU), showing GO and PPY exhibit higher cardiomyocyte expression compared to Alg-Gel and G.

D. Quantification of CD31 fluorescence intensity (AU), showing PPY has significantly higher endothelial expression compared to Alg-Gel ($p < 0.05$).

3.2.5 Optical Mapping of the Cardiac Spheroid in Conductive Hydrogels

Optical mapping was performed to measure the conduction velocity of cardiac spheroids embedded in conductive hydrogels (G, GO, and PPY) compared to the control Alg-Gel. This technique uses dyes that stain for the action potential and calcium ion influx, which are crucial in initiating and regulating cardiac contraction. The method used the voltage-sensitive dye RH237 for action potential and calcium-sensitive dyes like Rhod-2 AM for calcium signalling. In both GO and PPY, the conduction velocity (mm/ms) was significantly increased compared to Alg-Gel ($p < 0.05$, one-way ANOVA with Tukey's post hoc analysis), as seen in Figure 26F, indicating enhanced electrical propagation and contractility in cardiac cells. The increased conductive velocity in PPY further suggests improved calcium dynamics, contributing to more synchronised cardiac contractions, as seen in Figure 26F.

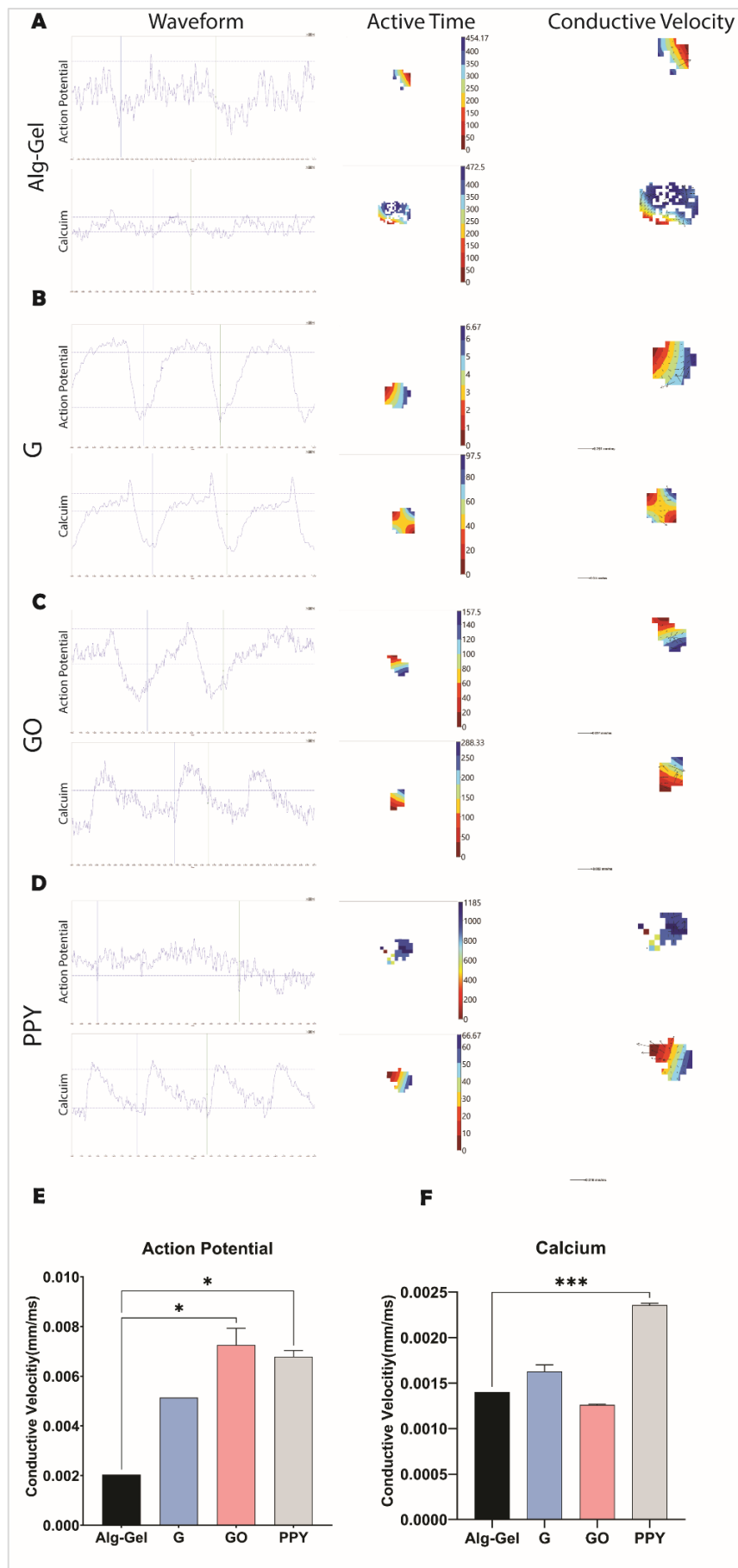


Figure 26. Optical mapping analysis of bioprinted cardiac spheroids in conductive hydrogels.

Cardiac spheroids were bioprinted within conductive hydrogels containing cardiac endothelial cells, cardiomyocytes, and cardiac fibroblasts. Optical mapping was used to record the electrical activity and calcium dynamics within the following conductive hydrogels: 0.1 g graphene (G), 0.1 g graphene oxide (GO), 0.01 g polypyrrole (PPY), and the control hydrogel (4 g Alg-Gel). **(A–D)** Representative raw optical mapping results for each condition: each panel shows waveforms for action potential (top) and calcium signal (bottom), corresponding activation time maps (middle column), and conduction velocity maps (right column) for **A.** Alg-Gel, **B.** G, **C.** GO, and **D.** PPY hydrogels. **E.** Quantification of conduction velocity (mm/ms) for the action potential signal in each hydrogel group. **(F)** Quantification of conduction velocity (mm/ms) for the calcium signal in each hydrogel group. Data were obtained from $n = 3$ independent replicates and are presented as mean $\pm$ SEM. Statistical significance was determined by one-way ANOVA with Tukey's post hoc test (* $p < 0.05$, *** $p < 0.001$).

3.3 Discussion

This chapter demonstrates that conductive hydrogels can effectively support the viability and contractile function of bioprinted cardiac spheroids, highlighting their suitability for cardiac tissue engineering applications. This finding is consistent with Wang et al. (2021), who demonstrated that conductive materials can support the development of bioengineered cardiac tissue. In this study, conductive hydrogels, including G, GO, and PPY, provided a supportive microenvironment that maintained cellular activity and enhanced viability. Additionally, these hydrogels increased electrical conductivity, which is critical for replicating the electrophysiological properties of native cardiac tissue (Lee et al., 2022). Improved conductivity facilitates cell–cell communication and electrical signal propagation, contributing to the observed high cell viability (Gonzalez et al., 2023).

Bioprinted CSs within conductive hydrogels were analysed at days 7, 14, and 28, showing no significant differences in cytotoxicity for G, GO, and PPY compared to Alg-Gel (Figure 23A–F). This confirms that these hydrogels support cardiac spheroid viability by promoting nutrient and oxygen diffusion and efficient waste removal (Elkhoury et al., 2021; Liu Chung Ming et al., 2022; Saberi et al., 2019). This is supported by SEM images showing that

Alg-Gel, G, GO, and PPY exhibited higher porosity than Au and PANI (Figure 12A); therefore, increased porosity enhances oxygen and nutrient diffusion, improving cell viability compared to Au and PANI.

The electrical conductivity of the hydrogels also improves the maturation and contractile function of bioprinted cardiac spheroids (Wang et al., 2021; Züger et al., 2022). The contractile activity, measured on Day 28, demonstrated that PPY significantly increased contraction frequency compared to Alg-Gel, indicating enhanced functional behaviour that more closely mimics native tissue and improves cell–cell communication (Takahashi et al., 2018). Additionally, the fractional shortening percentage was significantly higher in PPY than in Alg-Gel, confirming enhanced contraction and relaxation ability of CSs embedded in PPY (Figure 24B).

The immunostaining of CSs bioprinted in conductive hydrogels provides valuable insights into their structural and cellular organisation (Polonchuk et al., 2017). As shown in Figure 25, all three major cardiac cell types — endothelial cells (CD31), cardiomyocytes (cTNT), and fibroblasts (vimentin) — were present across all hydrogel conditions, comparable to the Alg-Gel control. This confirms that conductive hydrogels preserve the multicellular architecture and support the intended cardiac patch composition (Song et al., 2023)

The optical mapping results further confirmed the functional benefits of conductive hydrogels. Using the voltage-sensitive dye RH237 and calcium-sensitive dye Rhod-2 AM enabled precise measurement of conduction velocity (CV) and calcium handling within the cardiac spheroids (Herron et al., 2012). RH237 integrates into the cell membrane and emits light in response to voltage changes, allowing visualisation of electrical activity patterns (Windisch et al., 1985). This capability facilitates measurement of conduction velocity, action potential duration, and the detection of arrhythmias (Salama & Hwang, 2009). Rhod-2 AM enables imaging of calcium ion movement during spheroid contraction, providing critical insight into excitation–contraction coupling. Thus, optical mapping delivers comprehensive data on both electrical and mechanical cardiac function (Jaimes III et al., 2016).

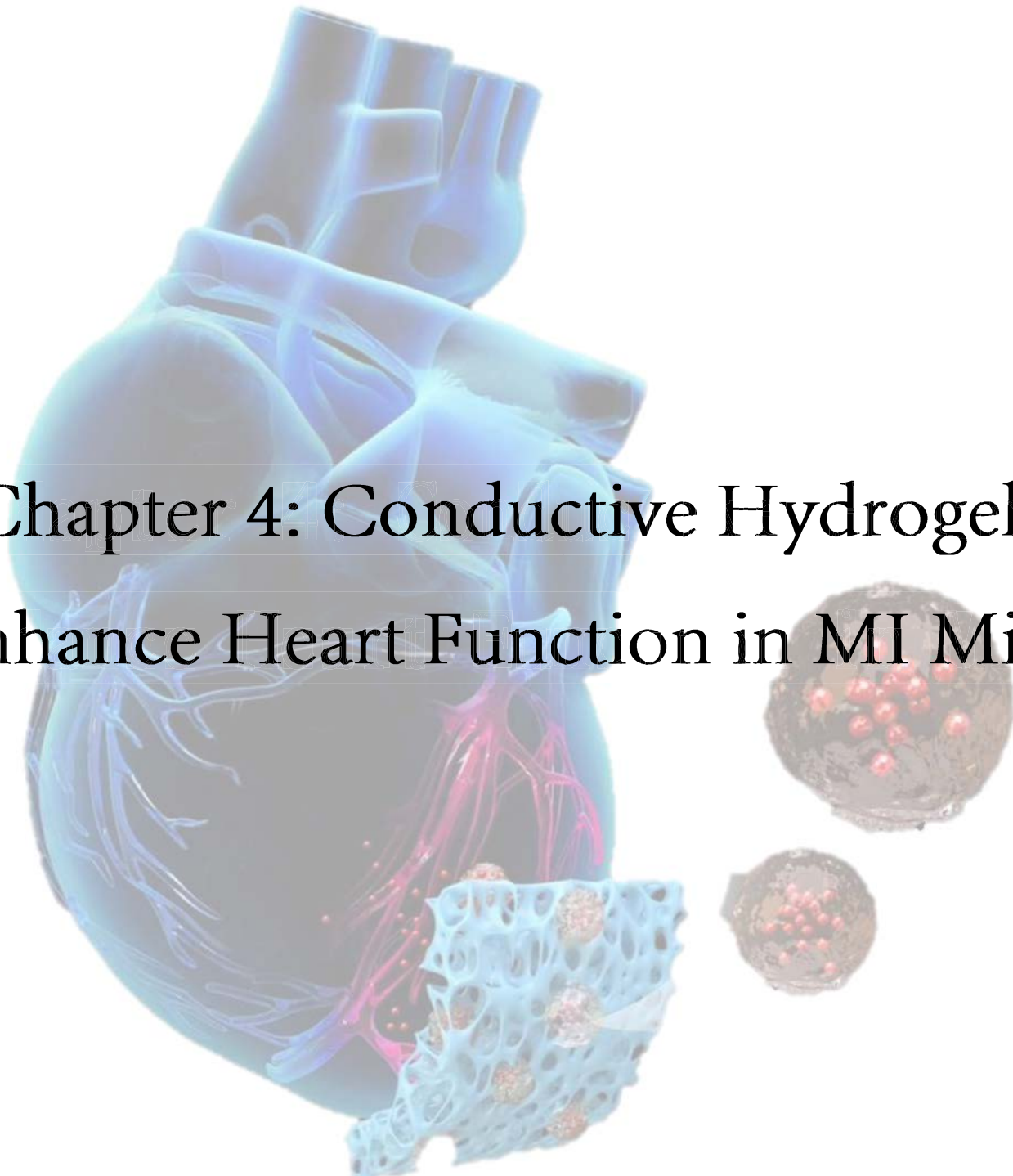
The results demonstrated a significant increase in conduction velocity for the action potential in both GO and PPY compared to Alg-Gel (Figure 26E), indicating enhanced electrical propagation and stronger contractility due to better ion exchange and signal transmission.

Additionally, conduction velocity for calcium signals was significantly increased in PPY compared to Alg-Gel (Figure 26F), suggesting improved calcium dynamics and more synchronised contraction. Together, these findings confirm that combining conductive polymers like GO and PPY with Alg-Gel enhances cell viability, spheroid contractility, and intercellular electrical communication. Improved porosity further supports oxygen and nutrient diffusion, enhancing the synchronised contraction of cardiac cells and mimicking native cardiac behaviour more closely.

Furthermore, a significant increase in conduction velocity was observed for calcium signals in PPY compared to Alg-Gel, suggesting improved calcium ion handling and excitation–contraction coupling in PPY. This indicates that PPY enhances synchronised cardiac cell contraction more effectively than other hydrogels. Overall, conductive hydrogels, particularly those containing GO and PPY, show significant potential for developing cardiac patches to treat myocardial infarction by improving both the electrical and mechanical functions of cardiac tissue (Eisner et al., 2017; Nerbonne & Kass, 2005).

3.4 Conclusion

The study demonstrates that conductive hydrogels such as G, GO, and PPY significantly enhance cell viability, cardiac cell functionality, and the integration of hydrogels with bioprinted CSs. These hydrogels improve electrical conductivity, support oxygen and nutrient diffusion, and reduce cytotoxicity, fostering an environment similar to native cardiac tissue (Kougkoulos et al., 2023). Enhanced porosity in conductive hydrogels further facilitates oxygen and nutrient diffusion, which supports cell function (Zhang et al., 2023). The increased contractile activity and fractional shortening observed in PPY highlight its potential for mimicking native cardiac tissue behaviour and improving cell–cell communication. Immunostaining confirmed the presence of essential cardiac cell types within the spheroids, and optical mapping demonstrated improved electrical activity and synchronised contractile function in PPY. These findings suggest that conductive hydrogels mixed with Alg-Gel could be utilised to develop cardiac patches to treat myocardial infarction by enhancing cell viability, spheroid contractility, and electrical communication.



Chapter 4: Conductive Hydrogels Enhance Heart Function in MI Mice

Chapter 4: Conductive Hydrogel Enhances Heart Function in MI Mice

Abstract

Chapter 4 investigates the therapeutic potential of conductive hydrogel-based cardiac patches for myocardial infarction (MI) repair using 3D bioprinting technology. The study explores the integration of conductive materials, including graphene, graphene oxide, and polypyrrole, into bioprinted patches, with and without cardiac spheroids, for transplantation in a mouse MI model. The aim is to evaluate the efficacy of these patches in improving cardiac function and tissue regeneration. Mice underwent baseline and follow-up echocardiographic imaging at days 0, 7, 14, and 28, while histological analysis was performed at 28 days to assess tissue structure and cellular integrity. These assessments evaluated myocardial function, tissue regeneration, and patch integration with host tissue. Results demonstrate that conductive hydrogels, particularly when combined with spheroids, enhance myocardial function and promote tissue integration, providing insight into the potential of bioprinted cardiac patches as a promising therapeutic strategy for MI. The findings suggest that these hydrogel-based patches could improve long-term cardiac function by supporting cell viability, promoting angiogenesis, and reducing fibrosis. This study contributes to the advancement of cardiac tissue engineering, presenting a promising alternative to traditional heart transplantation.

4 Introduction

Recent advancements in 3D bioprinting and cell culture have enabled the fabrication of epicardial patches for myocardial tissue repair. Whole-heart allotransplantation is the current standard treatment for end-stage heart failure patients (Roche et al., 2023). Bioprinted cardiac patches show promise in improving heart structure and function after myocardial infarction (MI). However, the mechanisms behind these improvements are not fully understood. Emerging evidence suggests that modulating the host's inflammatory response may be more crucial than replacing lost cells. Additional preclinical research is necessary to optimise the engineering of effective cardiac patches (Pezhouman et al., 2023).

Our group has investigated the integration of 3D bioprinting with vascularised cardiac spheroids (VSC) that replicate the human heart microenvironment. These spheroids have been utilised to generate viable, functional heart tissue. We theorise that 3D bioprinting patches

containing VSC can improve cardiac function in myocardial infarction (MI) mice models (Roche et al., 2023; Roche et al., 2021).

Incorporating conductive hydrogels into bioprinted cardiac patches can enhance electrical conductivity, which is essential for synchronising cardiac cell activity and improving tissue integration. These hydrogels can facilitate better electrical signal propagation across the patch, potentially leading to more effective restoration of myocardial function (Ghovvati et al., 2022; Lee et al., 2023).

This experiment, described in this chapter, investigates the efficacy of different conductive materials in enhancing cardiac repair. It presents results from epicardial transplantation of 3D bioprinted conductive hydrogel patches in a myocardial infarction (MI) mice model. The patches were composed of Alg-Gel with and without spheroids; graphene (G) with and without spheroids, graphene oxide (GO) with and without spheroids and polypyrrole (PPY) without spheroids. The control group included sham (healthy control) and untreated MI models. Ultrasound imaging and histology analysis were performed to assess structural and cellular analysis across the different treatment groups.

The research on bioprinted cardiac patches using conductive hydrogels and advanced materials represents a significant advancement towards personalised cardiac therapies. These innovations could reduce the need for heart transplants and lead to more effective, tailored treatments for patients with myocardial infarction (Birla & Williams, 2020). By enhancing the integration and functionality of the bioprinted patches, this project may not only improve cardiac repair but also address the limitations of current therapies, ultimately benefiting MI patient outcomes and quality of life (Wu et al., 2023).

4.1 Materials and Methods

4.1.1 Preparation of Conductive Hydrogels

The hydrogel preparation method used in this study is described in detail in Chapter 2. Specifically, the preparation of conductive hydrogels involving Alg-Gel, G, GO, and PPY is outlined. For a comprehensive understanding of the procedure and protocol, refer to Section 2.1.1 in Chapter 2.

4.1.2 Development of Human Cardiac Spheroids

The preparation of CSs is described in detail in Chapter 3, Section 3.1.3. Briefly, CSs were formed by combining hiPSC-derived cardiomyocytes (hiPSC-CMs, iCells, Fujifilm Cellular Dynamics, Inc., USA), human cardiac fibroblasts (HCFs), and human coronary artery endothelial cells (HCAECs) in hanging drop cultures. Cells were seeded in agar plates using 2.5×10^4 hiPSC-CMs, 6.25×10^3 HCFs, and 6.25×10^3 HCAECs per 20 μ L of custom medium. The CS medium was a 2:1:1 mix of iCells maintenance, HCF, and MesoEndo media, following the manufacturer's protocol(Sharma & Gentile, 2021a) and shown in Figure 27.

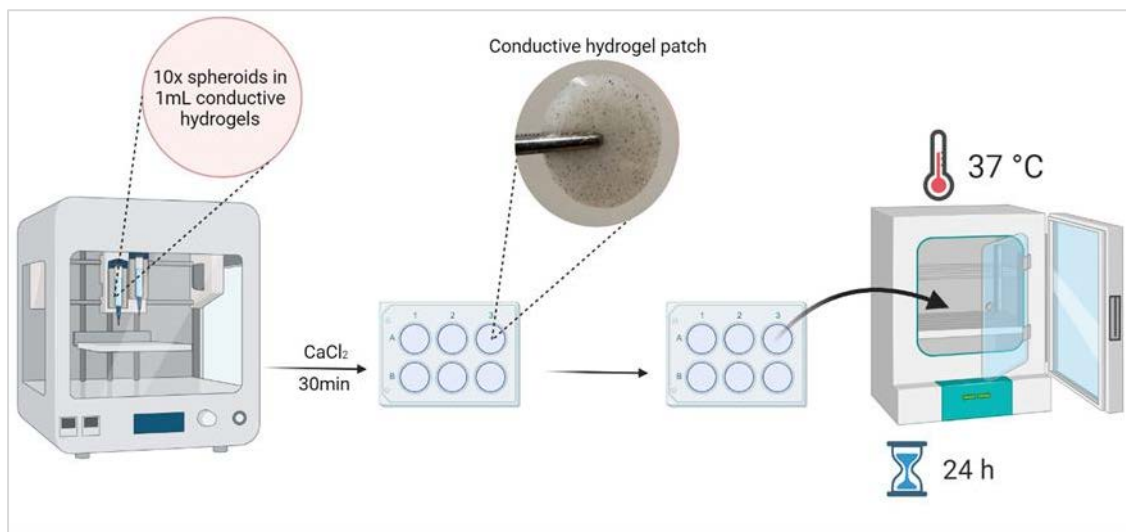


Figure 27. The Bio X6 bioprinter was used to print conductive hydrogel patches measuring 10 x 10 x 0.5 mm, with a layer thickness of 0.2 mm and a 25% infill. The patches were crosslinked with 2 mL of CaCl_2 and cultured at 37°C

4.1.3 3D Bioprinted Conductive Heart Patch

Bioprinting was conducted using a BIO X6 bioprinter (CELLINK Life Science, Boston, MA, USA). Conductive hydrogels were transferred directly into syringes, and the nozzle speed was adjusted during printing to print a 10 x 10 x 0.5 mm patch with 0.2 mm layers and 25% infill. Post-printing, the patch was cross-linked with 2 mL of 2% CaCl_2 in PBS on an ice block and then cultured in individual well plates. Prior to transplantation, each printed patch was carefully cut into four equal sections to ensure proper sizing for the mouse model. The patches were incubated in a custom culture medium with a ratio of 2:1:1 (iCells maintenance media, human

cardiac fibroblast medium, and MesoEndo growth medium) at 37°C until transplantation. The medium was changed every second day (Figure 27).

4.1.4 Conductive Hydrogels Heart Patches for MI Transplantation

Conductive hydrogel cardiac patches were transferred onto the epicardial surface of mouse hearts using an optimised surgical protocol adapted from a previous method (Roche & Gentile, 2020). Mature RAG1 mice (7–8 weeks old) were randomly assigned and underwent baseline echocardiography. Mice were anaesthetised with intraperitoneal ketamine (40 mg/kg) and xylazine (5 mg/kg) and inhaled isoflurane (5 L/min for induction, 0-2 L/min for maintenance). The mice were intubated and ventilated, followed by a left thoracotomy to access the heart.

Experimental groups were assigned using an ethical semi-randomised approach to minimise mortality and optimise group size. All procedures were approved by the University of Technology Sydney Animal Ethics Committee under protocol number ETH20-4883. The nine experimental groups were: 1) SHAM surgery (a suture was passed around the left anterior descending artery (LAD) and tied loosely, without occluding the artery); 2) MI by permanent LAD ligation (MI); 3) Alg-Gel without CSs; 4) Alg-Gel with CSs; 5) G without CSs; 6) G with spheroids; 7) GO without CSs; 8) GO with CSs; 9) PPY without CSs. Postoperative care included subcutaneous buprenorphine (0.08 mg/kg) administered twice daily for the first three days. Mice were monitored daily and maintained under standard postoperative care for 28 days. Cardiac function and tissue regeneration were assessed via ultrasound imaging and histological staining, as shown in Figure 28.

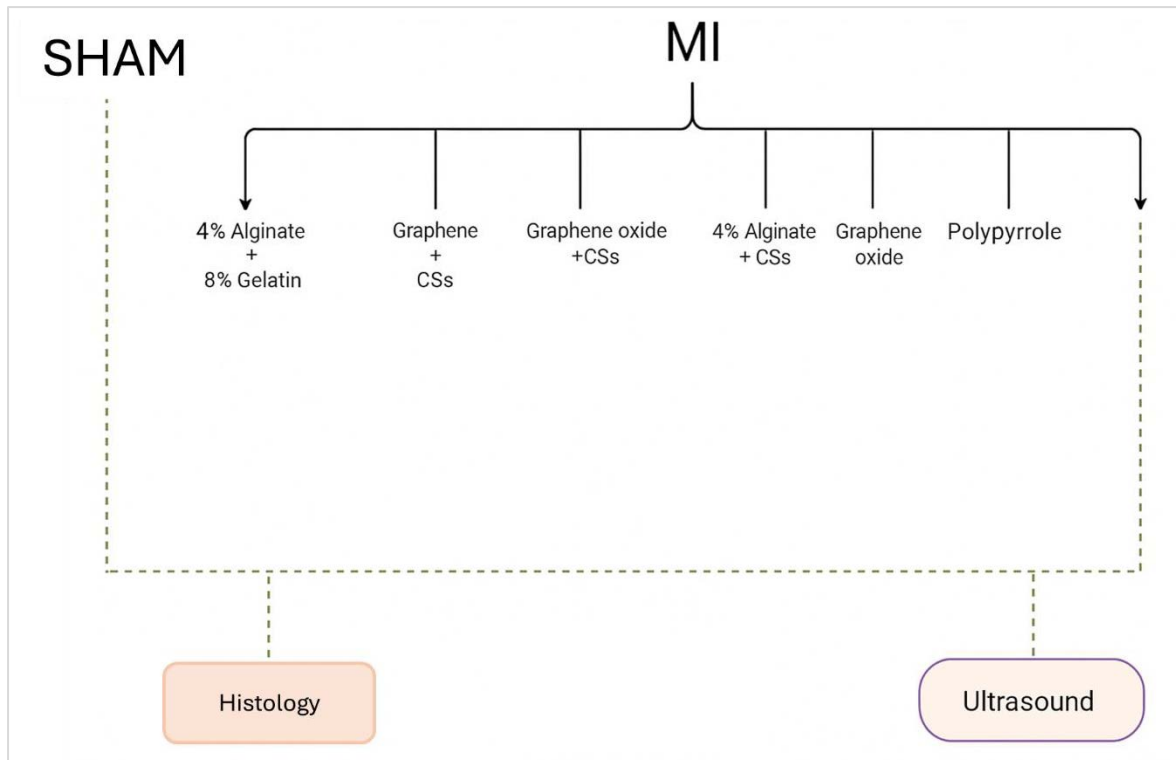


Figure 28. Surgical procedure for transplanting conductive hydrogel cardiac patches onto the epicardial surface of mouse hearts. The nine experimental groups include variations with and without CSs. Postoperative care included ultrasound and histology over 28 days.

4.1.5 Echocardiography of Conductive Hydrogel Patches in Mice

All mice were imaged at baseline, midpoint, and on days 0, 7, 14, and 28 using the Vevo 3100 Preclinical Imaging System (FUJIFILM VisualSonics, Toronto, Canada) following the protocol described in a previous study (Roche et al., 2023). Mice were anaesthetised with isoflurane administered through a nose cone at a rate of 5 L/min for induction and 2 L/min for maintenance. They were then placed on a warming platform in a supine position. B-mode and M-mode echocardiographic data were collected from parasternal long-axis and short-axis views. The long-axis data were analysed using the AutoLV (AI-based analysis) function, and the validity of the analysis was assessed using VisualSonics Data Specialist software, as seen in Figure 29.

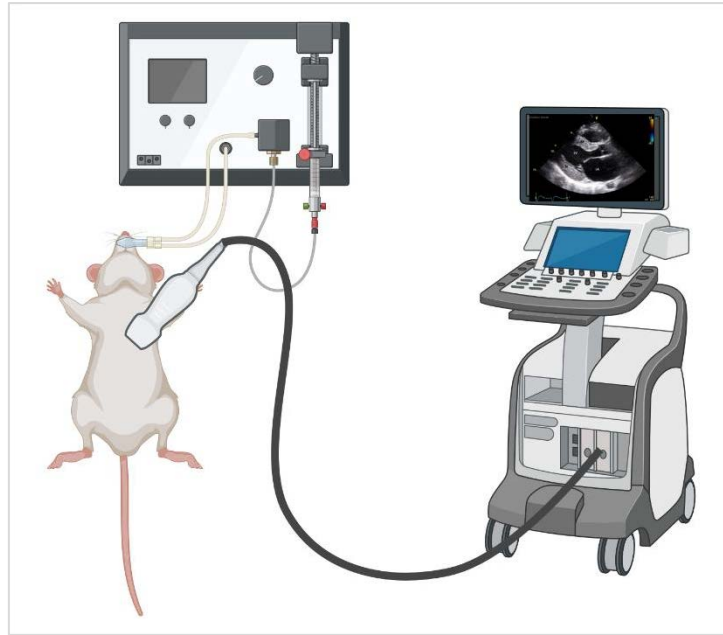


Figure 29. Imaging of mice at day 0, 7, 14 and 28 using the Vevo 3100 system. Mice were anaesthetised with isoflurane and positioned supine. B-mode and m-mode echocardiographic data were collected and analysed with AutoLV, with independent confirmation by a VisualSonics specialist.

4.1.6 Histological Analysis of Conductive Hydrogel Patches in Mice

At 28 days post-procedure, mice were euthanised, and 1 mm transverse slices of the left ventricle (distal to the LAD ligation) were collected for structural analyses. Histological examinations were performed on 4 μm tissue sections using H&E, Picrosirius Red, and Masson's Trichrome stains, as shown in Figure 30.

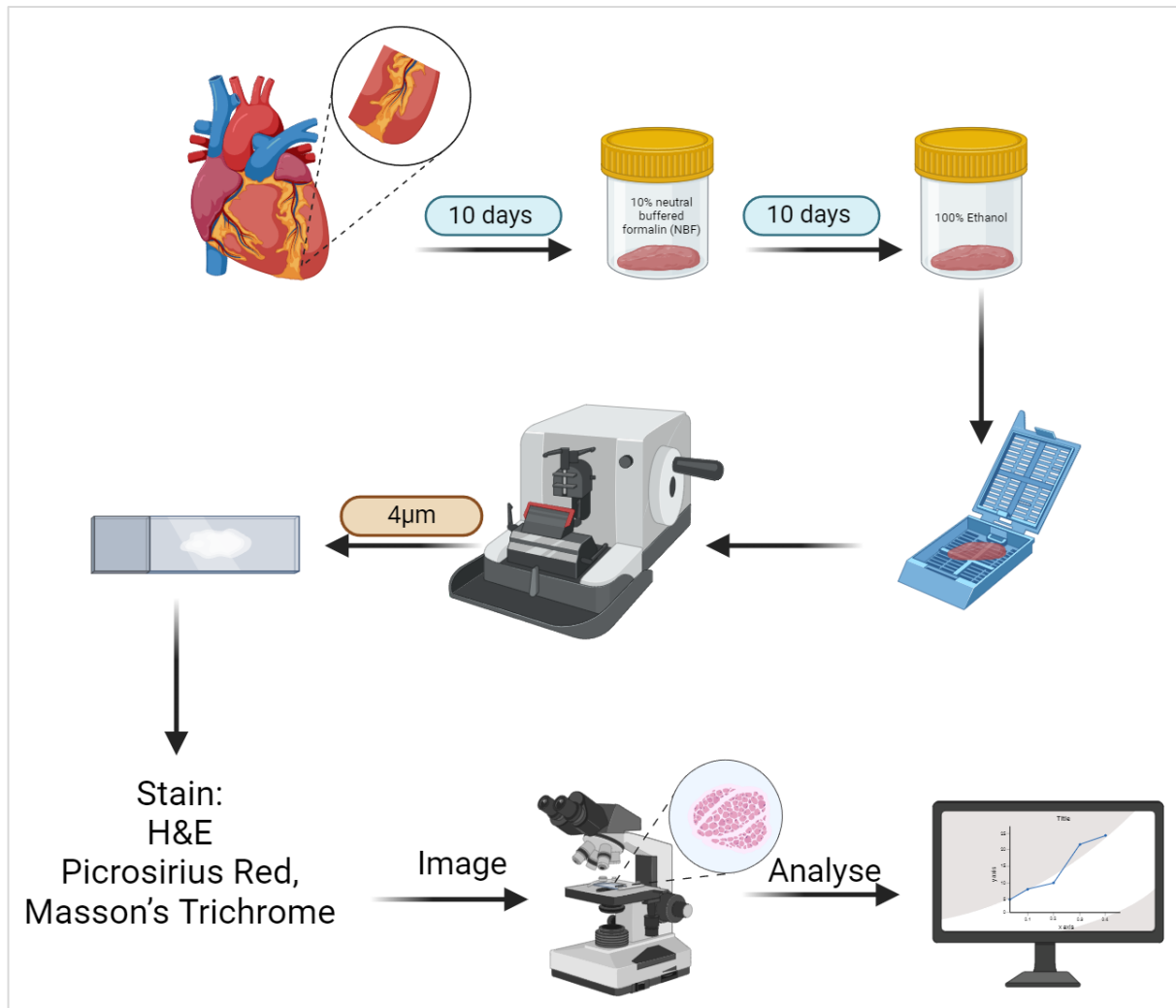


Figure 30. At 28 days post-procedure, 1mm transverse slices of the left ventricle were collected for structural analyses. The sample was kept for ten days in formalin and the following ten days in 100% ethanol. Histological examinations were performed on a 4 µm section using H&E, Picrosirius Red, and Masson's Trichrome stains.

4.1.7 Statistical Analyses

The data was analysed using GraphPad Prism 9.5 software (GraphPad Software, CA, USA). Each experiment was performed in 4 technical replicates (n=4). Significance was determined using one-way ANOVA with Tukey's post Hoc test. The results are presented as mean $\pm$ SEM (standard error of the mean). P-values less than 0.05 were considered statistically significant.

4.2 Result

4.2.1 Echocardiography of Conductive Hydrogel Patches in Mice

This study used echocardiography to evaluate changes in cardiac function in a mouse model by measuring the left ventricular ejection fraction percentage (LVEF%). LVEF is a key indicator of how well the heart contracts following myocardial infarction (MI), representing the percentage of blood ejected from the heart's ventricles with each heartbeat (Murphy et al., 2020). No significant differences in LVEF were observed between experimental groups at baseline (Day 0), whether patches were applied with or without CSs (Figure 31A and 31B). Similarly, by Day 14, there were no significant changes in LVEF across all groups, either without CSs (Figure 31C) or with CSs (Figure 31D).

However, by Day 28, echocardiographic analysis revealed significant improvements in LVEF in animals treated with Alg-Gel ($60.2\% \pm 3.8$), G ($61.9\% \pm 3.5$), and GO ($62.7\% \pm 4.0$) compared to the MI group ($50.0\% \pm 4.4$) (Figure 31E). PPY ($58.2\% \pm 4.3$) also showed a moderate increase but was not statistically different from MI. In groups treated with patches containing CSs, LVEF at Day 28 was significantly improved in G/CS ($61.6\% \pm 3.3$) and GO/CS ($60.9\% \pm 3.8$) compared to the Alg-Gel/CS ($52.4\% \pm 4.1$) and MI ($47.1\% \pm 3.7$) groups ($p < 0.01$ and $p < 0.0001$, respectively) (Figure 31F). SHAM controls remained the highest at $66.3\% \pm 3.0$.

These results suggest that incorporating CSs into conductive patches enhances cardiac function recovery following MI, with G/CS and GO/CS demonstrating significantly better outcomes than the control. Overall, the echocardiography results confirm the benefit of conductive hydrogel patches, particularly those containing G or GO and seeded with CSs, in preserving or restoring cardiac function after MI.

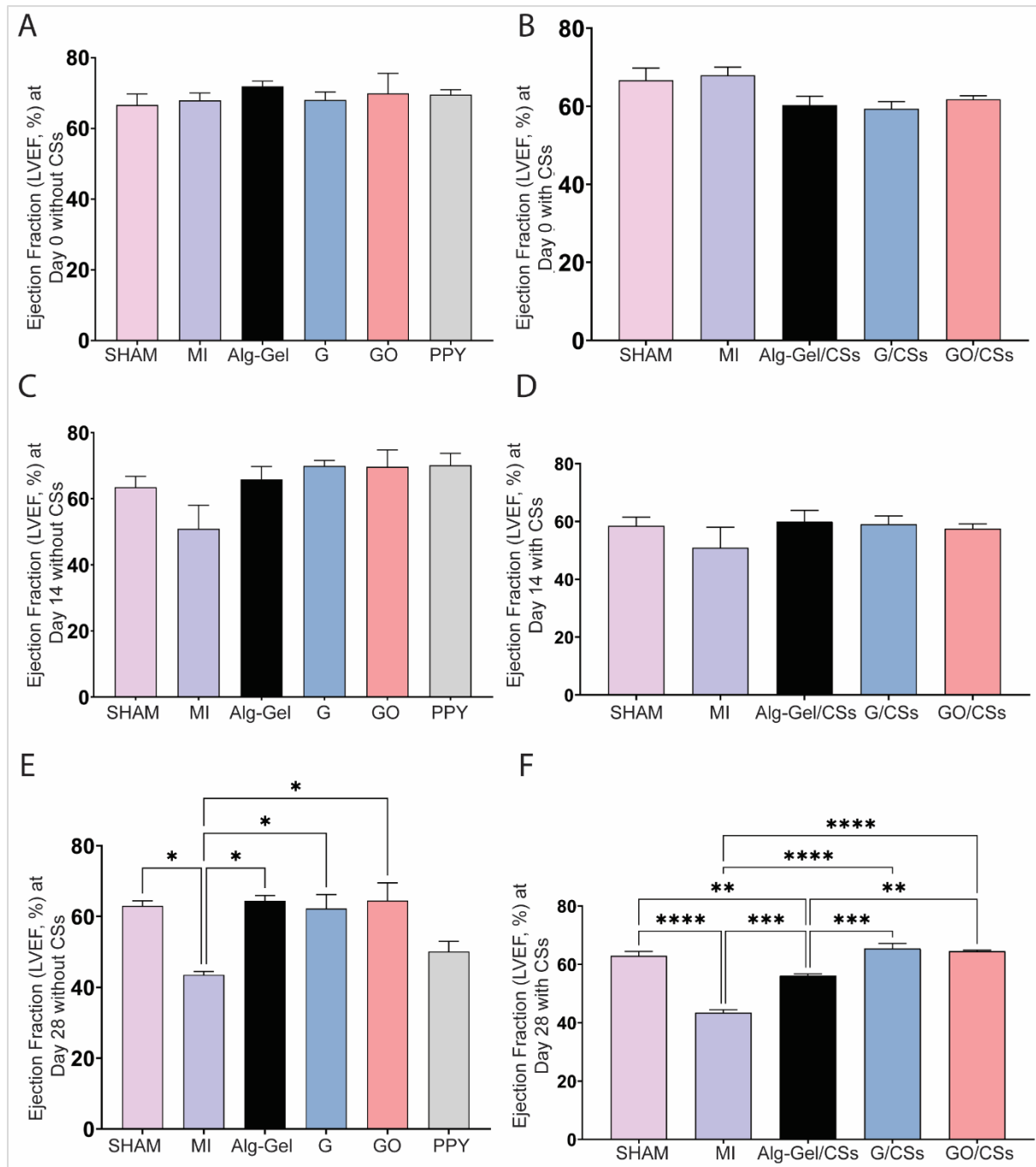


Figure 31. Evaluation of left ventricular ejection fraction (LVEF%) in a mouse model of myocardial infarction (MI) using echocardiography. (A-B) Ejection Fraction on day 7 for mice before surgery. **C.** Conductive hydrogels (Alg-Gel, G, GO) without CSs cardiac patch transplanted in mice at day 14. **D.** Conductive hydrogels (Alg-Gel, G, GO) with CSs cardiac patch transplanted in mice on day 14. **E.** Conductive hydrogels (Alg-Gel, G, GO) without CSs cardiac patch transplanted in mice on day 28. **F.** Conductive hydrogels (Alg-Gel, G, GO) with CSs cardiac patch were transplanted in mice at day 28. Data is presented as mean $\pm$ SEM.

Statistical significance was determined using One-Way ANOVA with Turkey's post hoc analysis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

4.2.2 Histological Analysis of Conductive Hydrogel Patches in Mice

Hematoxylin and Eosin (H&E), Masson's Trichrome, and Picrosirius Red stain were used to assess nuclei area, cell death, inflammation, and fibrosis. Specifically, H&E staining identifies nuclei (blue) and cytoplasm (pink), allowing evaluation of cell death and inflammatory changes. Masson's Trichrome and Picrosirius Red both assess fibrosis by staining collagen, but with different colourations: Masson's Trichrome stains collagen blue and cytoplasm/muscle red, while Picrosirius Red stains collagen red and healthy myocardium yellow under bright-field microscopy (Schipke et al., 2017).

H&E staining results indicate that MI tissue has a significantly higher nuclear area (%) than SHAM in cardiac tissues, both with and without CSs, reflecting pathological remodelling. In Figure 32A, Alg-Gel, G, GO, and PPY significantly reduce the nuclei area in cardiac tissue without CSs, approaching SHAM levels, indicating effective restoration and highlighting their potential use as cardiac patches. Alg-Gel/CS, G/CS, and GO/CS also significantly reduce the nuclei area, approaching SHAM levels, indicating effective restoration of cardiac structure, as shown in Figure 32B.

Fibrosis was assessed using two techniques. Masson's Trichrome staining results (Figure 33A) show that Alg-Gel, G, GO, and PPY (without CSs) reduce collagen content compared to MI. Figure 32B shows that Alg-Gel/CS, G/CS, and GO/CS significantly reduce collagen area compared to MI, bringing levels closer to SHAM and highlighting the enhanced antifibrotic effect of CSs combined with hydrogels. Overall, MI tissue showed a significantly higher collagen area (%) than SHAM, reflecting severe post-MI fibrosis.

Picrosirius Red staining results align with these findings. As shown in Figure 34A, MI and PPY groups had significantly higher fibrosis (%) than SHAM, indicating extensive collagen deposition and remodelling. In contrast, Alg-Gel, G, and GO reduced fibrosis compared to MI, while PPY worsened it. Figure 38B demonstrates that Alg-Gel/CS, G/CS, and GO/CS further reduced fibrosis, bringing levels closest to SHAM. These findings highlight the superior antifibrotic effect of combining conductive polymers with CSs in mitigating post-infarct fibrosis.

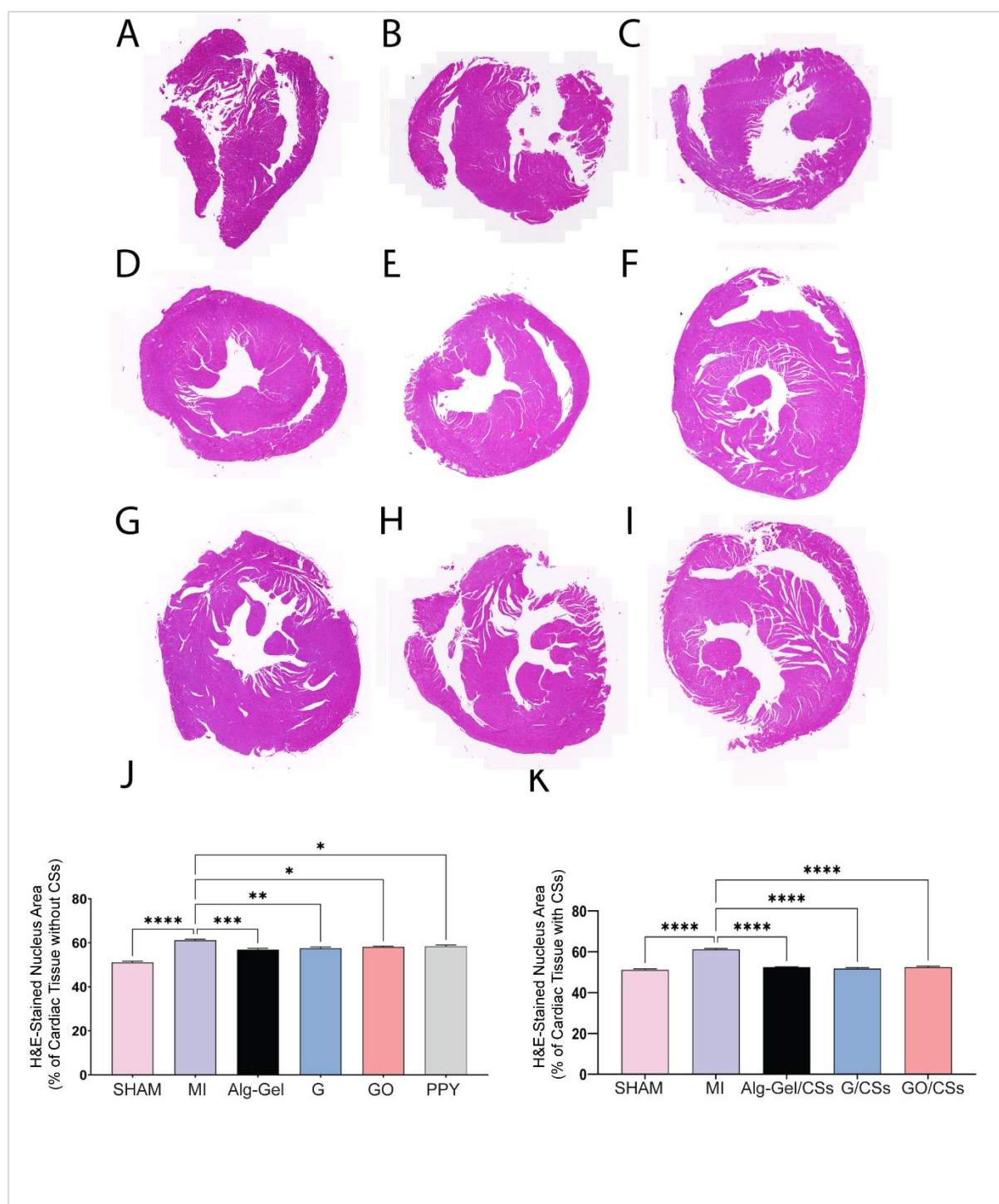


Figure 32. H&E staining of cardiac tissue to assess nucleus areas in mice treated with conductive hydrogels, MI, and the SHAM group. (A–I) Representative H&E-stained heart sections: A. SHAM, B. MI, C. Alg-Gel, D. G, E. GO, F. PPY, G. Alg-Gel/CSs, H. G/CSs, I. GO/CSs. (J). Quantification of nucleus area (%) in cardiac tissue without CSs. (K) Quantification of nucleus area (%) in cardiac tissue with CSs. The nucleus area was measured from H&E-stained sections using ImageJ software by thresholding and quantifying hematoxylin-stained (nuclei)

regions in five sections per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined using One-Way ANOVA with Tukey's post hoc analysis.

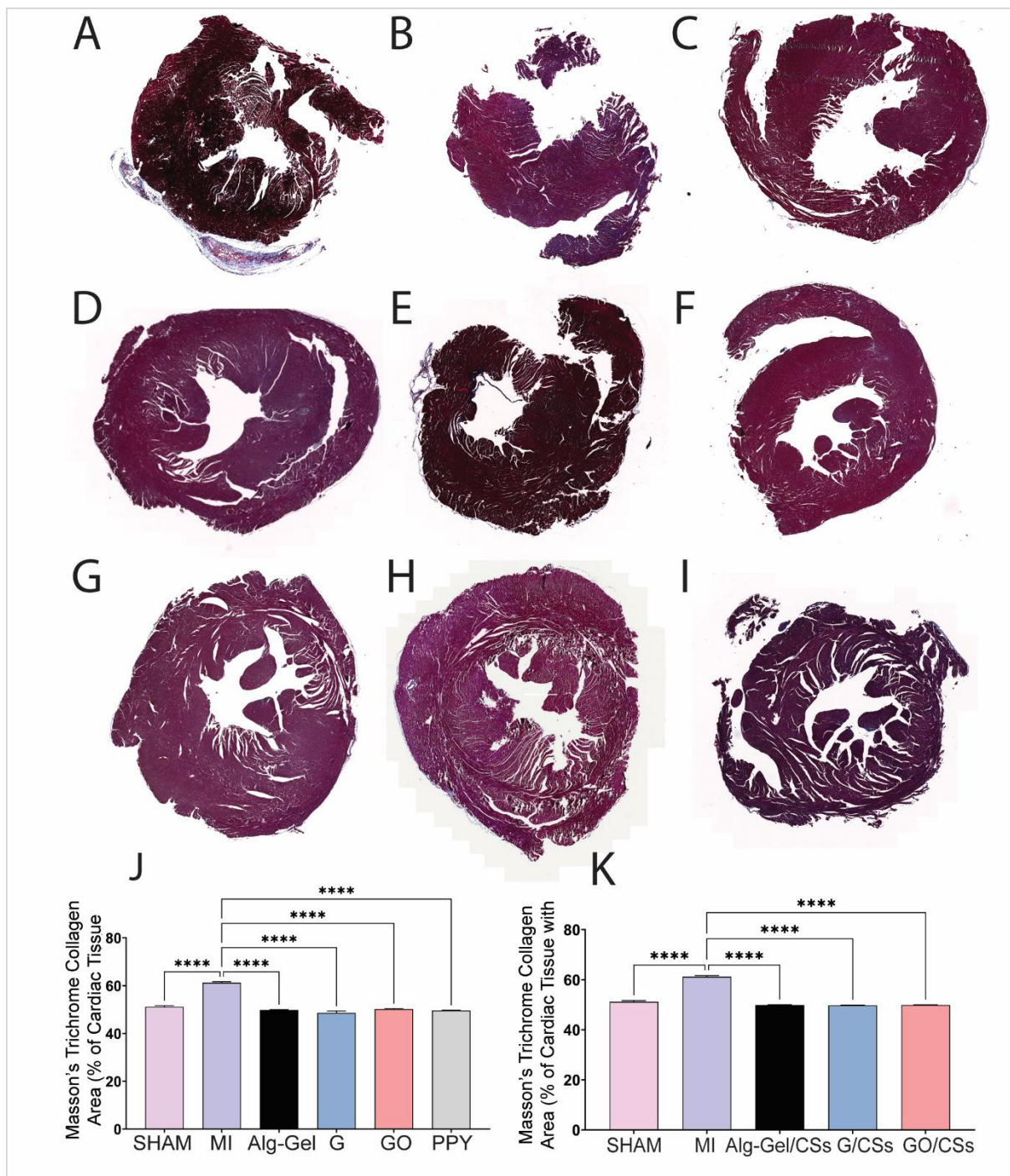


Figure 33. Masson's Trichrome staining of cardiac tissue to assess collagen content in mice treated with conductive hydrogels, myocardial infarction (MI), and SHAM control. (A–I) Representative Masson's Trichrome-stained heart sections: A. SHAM, B. MI, C. Alg-Gel, D. G, E. GO, F. PPY, G. Alg-Gel/CSs, H. G/CSs, I. GO/CSs. J. Quantification of collagen area (%) in

cardiac tissue without CSs. **K.** Quantification of collagen area (%) in cardiac tissue with CSs.

Collagen content was measured from Masson's Trichrome-stained sections using ImageJ software by thresholding and quantifying the blue-stained fibrotic regions in five sections per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA with Tukey's post hoc analysis

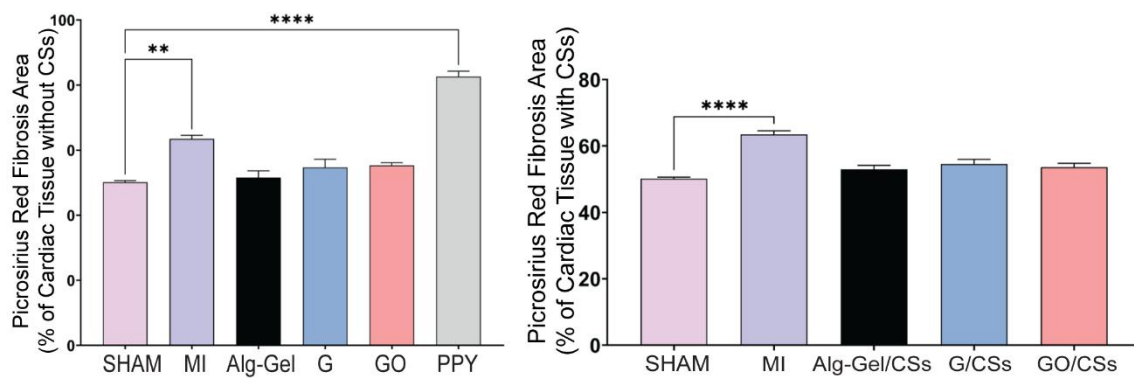
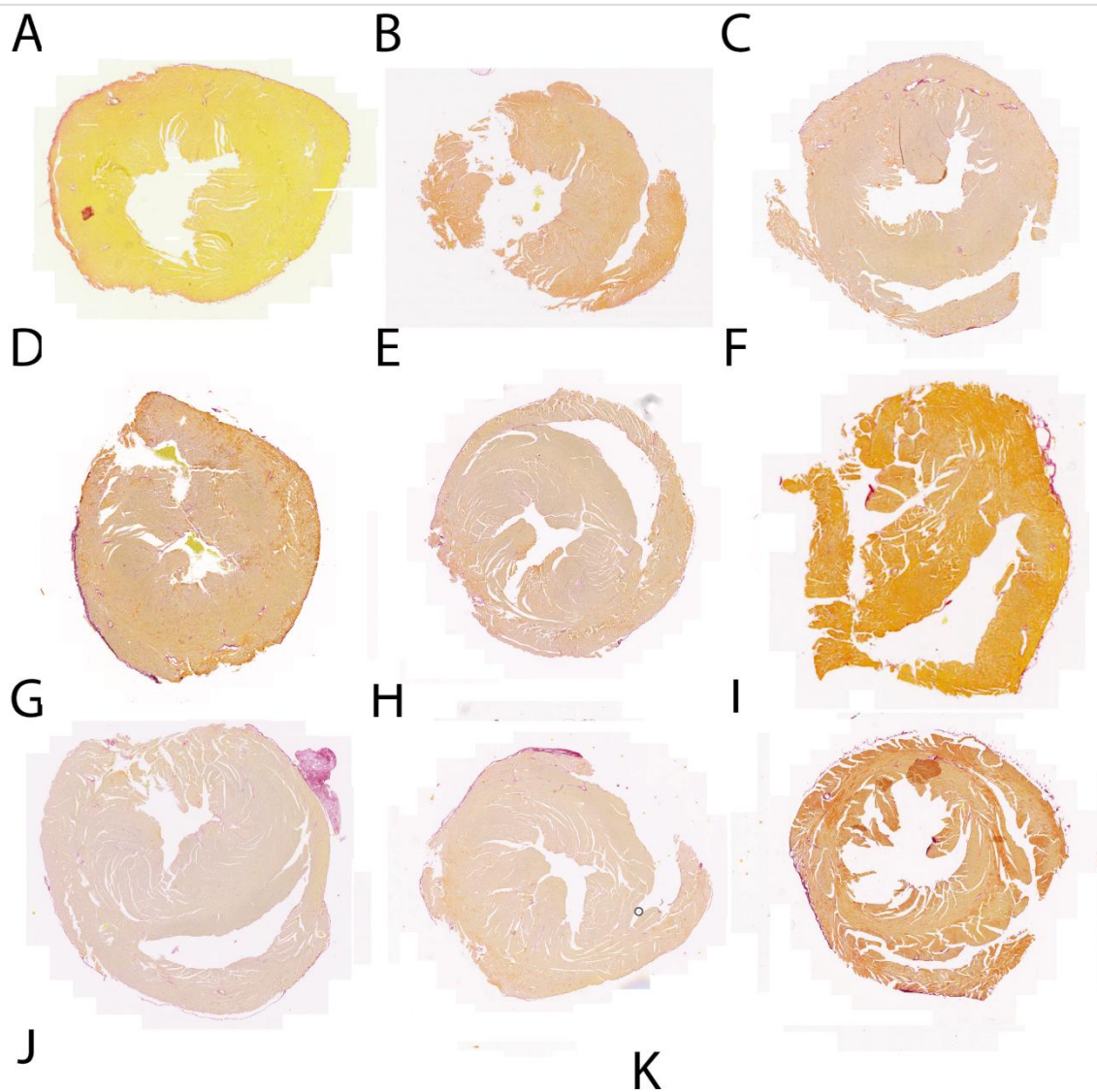


Figure 34. Picrosirius Red staining was used to assess collagen deposition, fibrosis, and tissue integrity in cardiac tissue from mice with myocardial infarction (MI) treated with conductive hydrogels. (A–I) Representative Picrosirius Red-stained heart sections: **A.** SHAM, **B.** MI, **C.** Alg-Gel, **D.** G, **E.** GO, **F.** PPY, **G.** Alg-Gel/CSs, **H.** G/CSs, **I.** GO/CSs. **J.** Quantification of fibrosis area (%) in cardiac tissue without CSs. **(K)** Quantification of fibrosis area (%) in cardiac tissue with CSs. Fibrotic area was measured from Picrosirius Red-stained images using ImageJ software by thresholding and quantifying red-stained collagen-rich regions in five images per group. Data are presented as mean $\pm$ SEM. Statistical significance was determined using one-way ANOVA with Tukey's post hoc analysis.

4.3 Discussion

Echocardiography is a key non-invasive imaging method to evaluate cardiac function by measuring the left ventricular ejection fraction (LVEF%), which reflects how effectively the heart pumps blood. Healthy mice generally show LVEF values between 55%–85% (Paonessa et al., 2015). In this study, echocardiography was performed at baseline (Day 0), Day 14, and Day 28 to assess how conductive hydrogel patches, with or without CSs, influence cardiac recovery following myocardial infarction (MI).

There were no significant differences in LVEF% among the groups at baseline and Day 14 (Figures 31A–D). However, by Day 28, significant improvements were observed in mice treated with G and GO hydrogels, both alone and combined with CSs (Figures 31E–F). These findings suggest that incorporating conductive materials like G and GO into hydrogel patches enhances electrical signal propagation and promotes better synchronisation of cardiomyocyte contraction (Hsiao et al., 2013; Yi et al., 2020). The inclusion of CSs further enhances this functional recovery, highlighting their role in supporting cell–cell interaction and tissue integration. PPY showed only moderate benefit and did not reach statistical significance compared to MI, indicating limited effectiveness in this formulation. Overall, these results confirm that conductive hydrogels, especially G and GO combined with CSs, are promising for preserving and improving cardiac function after MI. This supports their potential application in cardiac tissue engineering as effective bioactive patches that restore myocardial contractility.

To further assess cardiac recovery, histological analyses were conducted using Hematoxylin and Eosin (H&E), Masson's Trichrome, and Picrosirius Red staining. These stains

were selected to evaluate nuclei integrity, collagen deposition, and fibrotic remodelling, respectively.

H&E staining quantified nuclei area as a marker of cellular integrity, using ImageJ software to threshold hematoxylin-stained regions (blue) in five images per group. H&E results revealed that MI tissue exhibited a significantly higher nuclei area (%) compared to SHAM, indicating structural disruption and inflammation (Alonzo et al., 2020; Ojha et al., 2008). Similar to prior findings (Iyer et al., 2016), this study showed that Alg-Gel, G, GO, and PPY significantly reduced nuclei area in cardiac tissue without CSs, restoring it toward SHAM levels (Figure 32J). Likewise, Alg-Gel/CS, G/CS, and GO/CS significantly reduced nuclei area with CSs (Figure 32K), indicating effective tissue restoration. These findings demonstrate that both conductive and non-conductive hydrogels, with or without CSs, help mitigate structural damage and support myocardial regeneration.

Masson's Trichrome staining was used to evaluate collagen content, with blue indicating collagen, dark red indicating nuclei, and red/purple indicating cytoplasm (Ouyang et al., 2010). In previous research, Wang et al. (2020) demonstrated that MI tissue has higher collagen content than SHAM. The collagen area was quantified using ImageJ by thresholding the blue-stained fibrotic regions. The results showed that MI tissue had significantly higher collagen content than SHAM, indicating severe fibrosis. As shown in Figure 33J, Alg-Gel, G, GO, and PPY (without CSs) reduced collagen content compared to MI. With CSs, Alg-Gel/CS, G/CS, and GO/CS, the collagen content was further decreased (Figure 33K), demonstrating an enhanced antifibrotic effect. These results support the role of conductive hydrogels, especially when combined with CSs, in reducing fibrosis after MI. However, MI tissue exhibited a significantly higher collagen area compared to the SHAM, reflecting the severe fibrosis associated with myocardial infarction, which is comparable to the previous research finding conducted by Wang et al. (2020). Awada et al. (2017) assessed fibrosis using Picrosirius Red staining, highlighting the extensive fibrosis extending from the infarcted region to the non-infarcted region in MI rats.

Picrosirius Red staining was used to assess fibrotic remodelling by detecting collagen-rich regions (red), while healthy myocardium appears yellow. Fibrosis area was quantified using ImageJ by thresholding red-stained regions in five randomly selected sections per group. Figures 34J and 34K show that MI and PPY-treated tissue exhibited significantly higher fibrosis

(%) compared to SHAM, while Alg-Gel, G, and GO reduced fibrosis levels. With CSs, Alg-Gel/CS, G/CS, and GO/CS further reduced fibrosis, approaching SHAM levels, indicating a strong antifibrotic effect from the combination of conductive materials and CSs. PPY alone did not reduce fibrosis, contrasting with findings by Cui et al. (2018), who reported benefits with PPY-chitosan.

Overall, the echocardiography and histological findings demonstrate that conductive hydrogels, particularly G and GO, support both functional and structural recovery following myocardial infarction. When combined with CSs, these materials not only improve cardiac function but also reduce fibrotic remodelling and promote tissue regeneration. These results highlight the therapeutic promise of conductive hydrogels for cardiac repair and their potential to advance the field of cardiac tissue engineering.

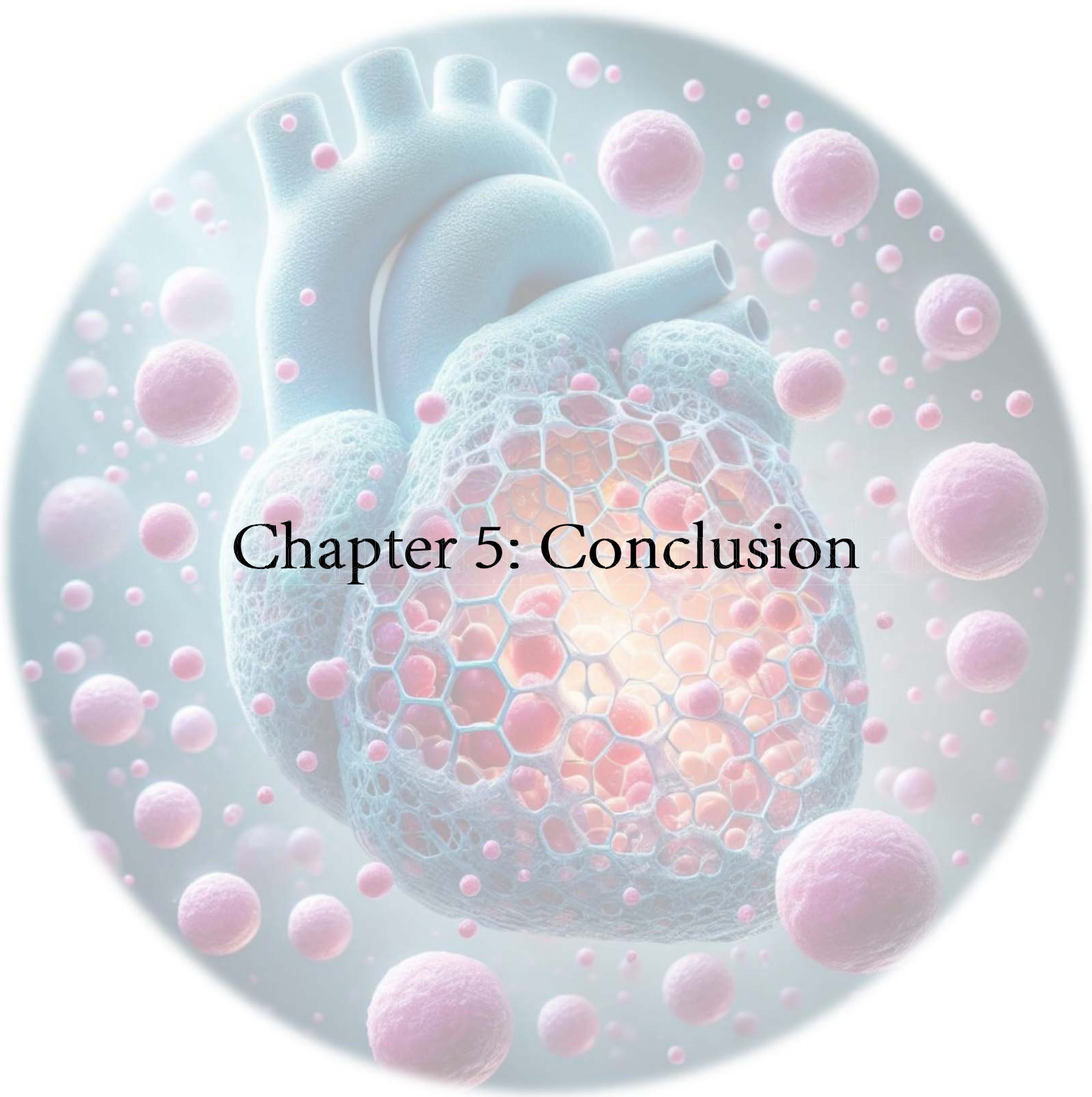
4.4 Conclusion

Conductive hydrogels, particularly G and GO, show significant promise for improving cardiac function following myocardial infarction. By day 28 post-implantation, these hydrogels significantly increased LVEF%, approaching levels observed in the healthy SHAM group. The gradual improvement in LVEF indicates that the benefits of hydrogel integration become more apparent as cardiac tissue heals and interacts with the implanted materials over time.

Histological analyses further support the biocompatibility of these hydrogels for cardiac repair. Techniques such as H&E, Masson's Trichrome, and Picrosirius Red staining demonstrate that the hydrogels help maintain cellular integrity and reduce fibrosis without causing significant inflammation. Notably, variations were observed across formulations. While G and GO hydrogels showed clear reductions in fibrosis, PPY-treated hearts exhibited comparatively greater fibrotic deposition. This may be attributed to the smaller or irregular pore structures of PPY hydrogels, which can limit nutrient diffusion and restrict adequate cell infiltration, slowing tissue remodelling. Additionally, PPY's stronger electrical stimulation may enhance early contractile activity but could also increase local mechanical stress in the infarct region, contributing to prolonged or excessive collagen deposition. These findings highlight the importance of selecting conductive polymers that balance conductivity with structural and biological compatibility to minimise fibrosis and promote optimal healing.

This compatibility, along with the observed improvement in cardiac function, highlights the potential of conductive hydrogels in cardiac tissue engineering. However, this study was conducted in a limited animal model with a short follow-up period; therefore, further research with larger sample sizes, extended monitoring, and validation in clinically relevant models is necessary to confirm these findings and advance towards clinical translation. more moderate porosity and lower inflammatory profile. These findings emphasise that strong in vitro performance does not always ensure favourable in vivo outcomes, and that degradation behaviour and inflammatory responses must be carefully considered when selecting conductive polymers for cardiac patch development.

Overall, this study shows that conductive hydrogels, particularly those containing G and GO, enhance spheroid viability, electrical function, and contractile behaviour, while maintaining favourable biocompatibility. PPY demonstrated strong in vitro performance but poorer in vivo outcomes due to increased fibrosis. These results highlight the importance of balancing conductivity, porosity, degradation rate, and inflammatory response when designing conductive hydrogels for myocardial regeneration. Further long-term in vivo studies are needed to optimise material selection for clinical cardiac patch applications.



Chapter 5: Conclusion

5 Conclusion

This thesis evaluated the potential of conductive polymers (G, GO, PPY, and PANI) in treating myocardial infarction by integrating them with 4% Alg-Gel + 8% Gel (Figures 12–16) to create conductive hydrogels for cardiac tissue engineering. These materials demonstrated promising results, with conductive polymers such as Au, G, GO, and PANI enhancing the electrical conductivity of hydrogels (Figure 14), a critical factor for restoring the electromechanical properties of the heart post-myocardial infarction. Notably, SEM analysis revealed that PPY-based hydrogels exhibited the largest pore size (Figure 12), facilitating superior nutrient exchange compared to other formulations.

While elasticity remained consistent across formulations, rheological analysis identified PANI hydrogels as having a reduced shear modulus (Figure 13), indicative of lower viscosity. Furthermore, G and GO hydrogels outperformed the control (Alg-Gel) in printability and shape fidelity (Figure 15), highlighting their potential for scalable biofabrication. Importantly, the conductive hydrogels retained over 90% of their structure in a cell culture medium (Figure 15), demonstrating excellent durability.

In vitro analysis of conductive hydrogels on CSs revealed no significant difference in cytotoxicity between the conductive hydrogels (G, GO, and PPY) and the control Alg-Gel (Figure 22), indicating excellent biocompatibility overall. However, the PPY hydrogel demonstrated enhanced CS contractile activity compared to the control Alg-Gel (Figure 23). Optical mapping of CSs within GO and PPY hydrogels showed significantly increased conduction velocity compared to the control Alg-Gel (Figure 25), which facilitated improved electrical propagation and calcium dynamics, leading to better cardiac cell contractility and synchronisation.

These findings highlight the potential of conductive hydrogels, particularly those incorporating PPY and GO, to improve the electromechanical properties of cardiac tissue constructs. By enhancing electrical conduction and calcium signalling, these materials could serve as a foundation for developing bioengineered cardiac patches to repair damaged myocardium following myocardial infarction.

This PhD project provides valuable insights into the role of conductive hydrogels in cardiac tissue engineering, demonstrating their potential to enhance electrical conduction and contractility in CSs. These findings contribute to the growing body of research aimed at

developing innovative solutions for myocardial repair and offer a foundation for future advancements in cardiac regenerative therapies.

Moreover, this project evaluated conductive hydrogels using *in vivo* MI mice models. Echocardiography (Figure 35) was used to monitor the left ventricular ejection fraction (LVEF%), with a normal range of 55%–85% in healthy mice. The analysis showed that conductive hydrogels, both with and without CSs, particularly those containing G and GO, demonstrated significant improvements in LVEF by day 28 post-implantation compared to both the myocardial infarction (MI) group and the control group treated with non-conductive hydrogel (Alg-Gel). These findings suggest that the intrinsic properties of G and GO hydrogels, such as enhanced electrical conductivity and improved cell synchronisation, play crucial roles in supporting cardiac function and promoting recovery after myocardial damage.

Furthermore, the histological analysis (Figures 36–38) demonstrated that conductive hydrogels, particularly G, GO, and Alg-Gel, exhibit excellent biocompatibility and integrate effectively with cardiac tissue, making them promising candidates for myocardial repair. H&E staining showed that Alg-Gel, G, GO and PPY hydrogels significantly reduced the nuclei area (Figure 36), aligning it closer to SHAM levels, which overall is indicative of effective cardiac structure restoration. Masson's Trichrome staining (Figure 37) indicated no significant differences in collagen content between the hydrogels and SHAM, while MI tissue showed significantly higher collagen levels, reflecting severe fibrosis. Picrosirius Red staining (Figure 38) confirmed reduced fibrosis in Alg-Gel, G, and GO compared to MI, with Alg-Gel/CS and GO/CS showing the most significant improvements. Overall, G, GO, and Alg-Gel demonstrated strong potential for reducing fibrosis and restoring cardiac structure, supporting their use in cardiac tissue regeneration.

Overall, this project underscores the significant potential of conductive hydrogels, particularly G, GO, and Alg-Gel, as innovative materials for cardiac tissue engineering and myocardial infarction repair. Their ability to enhance electrical conductivity, improve cardiac function, and reduce pathological remodelling was demonstrated through *in vitro* and *in vivo* analyses. Key findings, including improved LVEF, reduced fibrosis, and excellent biocompatibility, establish a strong foundation for their future development. However, challenges such as optimising pore size, balancing mechanical properties, and ensuring long-

term integration within native cardiac tissue remain. Addressing these limitations through further research will be crucial for translating these promising materials into effective clinical therapies, paving the way for advancements in cardiac regenerative medicine.

A circular graphic with a light blue background. In the center is a detailed anatomical illustration of a human heart, showing its major blood vessels. To the right, a hand is shown holding a surgical instrument, possibly a scalpel or forceps, positioned as if performing surgery on the heart. The background of the circle is filled with a complex molecular structure, featuring various sized spheres (red, blue, white) connected by lines, representing a chemical or biological network. The overall theme suggests medical research, surgery, and molecular biology.

Chapter 6: Future Direction

6 Future Direction

Conductive hydrogels in cardiac regeneration present several challenges that must be addressed to advance tissue engineering effectively. One critical challenge is ensuring the biodegradability of the scaffold, allowing for gradual breakdown over time. For a conductive polymer to be used clinically, the scaffolds should degrade at a rate synchronised with cardiac tissue regeneration. Future research should focus on optimising the composition of biodegradable conductive hydrogels to achieve the ideal balance between mechanical strength, electrical conductivity, and degradation rate, ideally matching the tissue remodelling timeframe of 4–8 weeks in small animal models and up to 12 weeks in larger animals (V. Y. Wang et al., 2017). These scaffolds must maintain structural integrity during initial tissue repair while breaking down appropriately to allow natural tissue remodelling and integration (Zhou et al., 2022).

One well-known limitation of conventional conductive polymers is that they are often not biodegradable *in vivo*, which could trigger a persistent inflammatory response due to their prolonged half-lives in the body (Wang et al., 2016). In this study, non-biodegradable conductive polymers were utilised to demonstrate proof of concept for enhanced electrical conductivity and improved cardiac function. However, to translate this technology into clinical applications, future studies should prioritise designing novel biodegradable conductive polymers that retain sufficient conductivity while safely degrading to avoid long-term inflammatory responses.

Another significant challenge is the hydrophobicity of many conductive hydrogels, which can hinder cell adhesion, a critical factor for effective tissue regeneration. To overcome this, conductive hydrogels should be modified to increase hydrophilicity through surface modifications, the addition of hydrophilic groups, or blending with compatible materials. This can be measured using water contact angle tests or swelling ratio analyses, such modifications will improve cell attachment, proliferation, and functionality, ultimately enhancing cardiac repair outcomes (Wang et al., 2021; Zhang et al., 2019). Conductive polymers such as PANI, PPY, GO, and G have shown promise in enhancing cardiac function. However, optimising their properties for better cell integration and compatibility remains an essential area of research. Future work should also measure hydrophilicity changes quantitatively using water contact

angle measurements and surface energy calculations to confirm improvements (Ertl & Frantz, 2005).

The reduced pore sizes of certain hydrogels, such as Au and PANI, may restrict nutrient exchange, potentially impacting cell viability over extended periods; however, GO maintained sufficient porosity and high viability comparable to Alg-Gel in this study (Figure 12A, B). Future work should aim to improve pore size and nutrient exchange in hydrogels with smaller pore structures without compromising the mechanical and electrical properties of the hydrogels. Additionally, while the hydrogels demonstrated good mechanical stability in this study, further investigations are needed to evaluate their long-term performance and functional integration *in vivo*.

Additionally, most research in this field has focused on small animal models like mice. To better translate these findings to human applications, larger animal models, such as pigs or sheep, should be prioritised in preclinical studies, as they closely mimic human cardiac physiology and anatomy. This approach will bridge the gap between small animal studies and human clinical trials, providing more accurate insights into the performance and safety of conductive hydrogels for cardiac tissue regeneration. It is also essential to investigate the immune response and long-term effects of these materials in large animal models before progressing to human trials (Roche et al., 2023).

Future work should also leverage advanced techniques, such as 3D bioprinting, to further optimise and validate these hydrogel formulations in more complex and functional cardiac tissue constructs, expanding on what was demonstrated in this study. Moreover, exploring the scalability of these hydrogels for large-scale production will be crucial for advancing their practical application in regenerative medicine (Chang et al., 2021). This will involve refining hydrogel formulations to balance mechanical properties, electrical conductivity, and ease of manufacturing to meet clinical demands (Guo & Ma, 2018).

In conclusion, the successful clinical translation of conductive hydrogels for cardiac regeneration hinges on addressing limitations such as biodegradability, hydrophobicity, pore architecture, and scalability. By refining hydrogel design, improving scaffold functionality, and conducting comprehensive large-animal and long-term studies, this research can pave the way toward safe and effective treatments for myocardial infarction and other heart conditions.

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