Development of a 3D printed conductive biopolymer for cardiac tissue engineering

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DEVELOPMENT OF A 3D PRINTED CONDUCTIVE BIOPOLYMER FOR CARDIAC TISSUE ENGINEERING

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Doctoral Program in Materials Science and Engineering

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Dedication

This work is dedicated to my husband, for his constant encouragement in pursuing this degree.

To my mom and brother, for their love and support throughout this process.

And to my uncle, Dr Tony Payan, for inspiring me to pursue a doctoral degree.

In Memory of Piedad Alvarado, 1949-2019

Per Aspera Ad Astra
DEVELOPMENT OF 3D PRINTED CONDUCTIVE BIOPOLYMER FOR CARDIAC TISSUE ENGINEERING

by

BRITANNY L STARK, BSc.

DISSERTATION

Presented to the Faculty of the Graduate School of The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

DEPARTMENT OF METALLURGICAL, MATERIALS AND BIOMEDICAL ENGINEERING
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Abstract

Cardiovascular disease (CVD) is the leading cause of death in the US, with approximately 859,000 deaths each year. The major contributor to CVD is Acute Myocardial Infarction (AMI), which causes the death of approximately 25% of the cardiomyocytes present in the left ventricle of the heart. After AMI, the adult human heart has a very limited regenerative capacity. Moreover, the electrical propagation of the myocardium is severely disrupted, making the heart more susceptible to failure and patient death. However, current pharmacological treatments do not address the loss of cardiomyocytes and the disruption of electrical propagation in the heart. Tissue engineering provides a potential solution to regenerate damaged myocardium using biomaterials as a scaffold to re-introduce healthy cardiomyocytes to the heart. To achieve this, an electrically conductive polymer that is biocompatible would promote cardiomyocyte viability, lineage-specific function, and tissue-specific organization while helping to re-establish the electrical propagation of native myocardium. Additionally, incorporation of patient-specific cardiomyocytes and automated, rapid manufacturing techniques would improve clinical translation of these engineered tissues for repair of damaged myocardium. To address these issues, my work aims to develop an electrically conductive and 3D printable hydrogel composed of a novel thiophene-conjugated, photocurable Methacrylated Hyaluronic Acid (MeHA). Thiophene has been shown to be chemically stable, biocompatible and an excellent conductor, while MeHA is a chemically versatile, hydrophilic, and biocompatible hydrogel that is ideal for this application. The goal of this project was to develop a novel biomaterial that is electrically conductive, biocompatible, and can be processed with 3D stereolithography printing. The hypothesis of my project is that by combining photocurable MeHA hydrogel, with 3-Thiopheneacetic Acid (3TAA), a 3D printable conductive biopolymer can be developed for use in cardiac tissue engineering applications. The overall approach for my work was completed in three aims: 1) Materials characterization and biocompatibility testing of a 3D SLA printed polymer, 2) Development of a photocurable conductive hyaluronic acid-based polymer for cardiac tissue engineering using thiophene as a conductor, and 3) Optimization of photopolymerization of conductive biopolymer for 3D SLA printing of myocardial tissue models.
# Table of contents

Dedication ........................................................................................................................................ iii

Acknowledgements ........................................................................................................................... v

Abstract ............................................................................................................................................. vii

Table of contents ............................................................................................................................... viii

List of Tables ...................................................................................................................................... x

List of Figures ................................................................................................................................... xi

Chapter 1 Introduction ......................................................................................................................... 1
  1.1 Rationale ...................................................................................................................................... 1
  1.2 Specific aims .............................................................................................................................. 3
  1.3 Dissertation outline ................................................................................................................... 4

Chapter 2 Literature Review .............................................................................................................. 5

Chapter 3 Materials Characterization and Biocompatibility testing of 3D stereolithography printed polymer ..................................................................................................................................................... 12
  3.1 Introduction .............................................................................................................................. 12
  3.2 Materials and Methods ........................................................................................................... 14
    3.2.1 3D SLA Printing ................................................................................................................ 14
    3.2.2 Mechanical testing: tensile and impact testing .............................................................. 14
    3.2.3 Scanning Electron Microscopy: Fracture Analysis ......................................................... 15
    3.2.4 Contact Angle .................................................................................................................. 15
    3.2.5 Cell culture ....................................................................................................................... 16
    3.2.6 Propidium Iodide and Flow Cytometry Analysis .......................................................... 16
    3.2.7 UV-VIS Spectrophotometry Analysis ............................................................................ 16
    3.2.8 Live-Dead Assay .............................................................................................................. 17
    3.2.9 Statistical Analysis ............................................................................................................ 17
  3.3 Results and Discussion .............................................................................................................. 18
    3.3.1 Mechanical properties of the printed polymer .............................................................. 18
    3.3.2 Failure analysis of tensile specimens ........................................................................... 21
    3.3.3 Surface free energy and wettability analysis using contact angle ............................... 23
    3.3.4 Biocompatibility and cytotoxicity studies .................................................................. 25
  3.4 Conclusion ................................................................................................................................ 30

Chapter 4 Development of a conductive hyaluronic acid-based polymer for cardiac tissue engineering .................................................................................................................................................... 32
  4.1 Introduction .............................................................................................................................. 32
  4.2 Materials and Methods ........................................................................................................... 33
    4.2.1 Methacrylated Hyaluronic Acid and LAP ..................................................................... 33
    4.2.2 Doping of 3-Thiopheneacetic Acid .............................................................................. 34
Chapter 5 Optimize photopolymerization of conductive biopolymer for 3D SLA printing of myocardial tissue models ................................................................. 53
  5.1 Introduction ......................................................................................... 53
  5.2 Materials and Methods ................................................................. 54
    5.2.1 3D MSLA printer, print settings and CAD designs ......................... 54
    5.2.2 Rheology of 3D printed hydrogel .................................................. 54
    5.2.3 Immunostaining of 3D printed iPSC cardiomyocytes ......................... 54
  5.3 Results and Discussion ................................................................. 55
    5.3.1 3D printing of MeHA 3TAA using Prusa SL1S printer ....................... 55
    5.3.2 Rheology of 3D printed MeHA 3TAA ............................................. 56
    5.3.3 3D printing of MeHA 3TAA with iPSC cardiomyocytes ...................... 59
  5.4 Conclusions ..................................................................................... 63

Chapter 6 Conclusions and Future directions .............................................. 65
  6.1 Conclusions ..................................................................................... 65
  6.2 Future directions ............................................................................. 67

References ............................................................................................ 69

Vita ........................................................................................................ 75
List of Tables

Table 3.1 Average tensile strength per post-print day. ................................................................. 19
Table 3.2 Average impact resistance per post-print day. ............................................................... 20
Table 3.3 Average contact angle measurements .......................................................................... 24
Table 4.1 Antibodies used for immunofluorescence staining ....................................................... 38
Table 4.2 Average conductivity measurements for Doped 3TAA ............................................... 42
Table 4.3 Average conductivity measurements for MeHA and MeHA 3TAA ............................. 42
Table 4.4 Average storage modulus, loss modulus and loss tangent for MeHA and MeHA 3TAA. ................................................................................................................................. 45
Table 5.1 Average measurements of complex modulus for 3D-printed and non-3D printed MeHA 3TAA. .......................................................................................................................... 57
List of Figures

Figure 1.1 Methacrylated Hyaluronic Acid chemical reaction ........................................ 3
Figure 3.1 Shows print orientation and build for (a) tensile and (b) impact samples ............ 15
Figure 3.2 Tensile test of printed polymer resin, (a) shows the tensile specimen pre-print, (b) shows the tensile specimen post-print, and (c) shows a statistical comparison in tensile strength, n=3. .......................................................................................................................... 18
Figure 3.3 Impact test of printed polymer resin, (a) shows Izod sample pre-test, (b) shows Izod sample post-test, and (c) shows statistical analysis of impact strength, n=3. ......................... 20
Figure 3.4 SEM images of tensile fracture surface, where the black arrows indicate the mirror and mist regions, and the white arrows indicate the extensive hackle region. Days 0 (a), 3 (b), 7 (c) and 30 (d) are shown. .................................................................................................................. 22
Figure 3.5 Contact angle of printed polymer resin, (a) shows experimental set up, (b) shows statistical comparison in contact angle across post-print days, and (c) shows statistical comparison in angles after ethanol baths. n=3 (**p<0.001, ****p<0.0001) ....................... 23
Figure 3.6 Shows flow cytometry data of exposed cells, (a) indicates the population of the negative control, (b) indicates the population of the positive control, and (c) indicates the exposed population, (d) & (e) show the unstained and stained results, bright field images of HL-60s (f) unexposed and (g) exposed to the printed polymer are also shown. .......................... 26
Figure 3.7 Live-dead assay of images of cells exposed to printed polymer resin for 3 and 7 days. ................................................................................................................................. 27
Figure 3.8 UV-VIS of cell media after exposure to printed polymer-resin, (a) shows a statistical comparison between absorbance points, (b) shows the UV-VIS spectra for day 7, and (c) for day 1 .................................................................................................................................. 29
Figure 4.1 Expected chemical reaction between MeHA and 3TAA. .......................................................... 35

Figure 4.2 NMR data for (a) MeHA and (b) MeHA 3TAA (n =3). Label 1 indicates the HA backbone, label 2 indicates the methacrylate group and label 3 shows the thiophene addition. .. 41

Figure 4.3 Statistical comparison in conductivity measurements for (a) 3TAA and (B) MeHA 3TAA. n=4 (****p<0.0001) ................................................................................................................................. 43

Figure 4.4 Amplitude sweep of (a) MeHA and (b) MeHA 3TAA hydrogels................................................. 44

Figure 4.5 Statistical comparison in (a) storage and loss modulus, and (b) loss tangent between MeHA and MeHA 3TAA ......................................................................................................................... 46

Figure 4.6 MTT Assay statistical comparison in absorbance between MeHA and MeHA 3TAA. .................................................................................. 47

Figure 4.7 Immunostaining of AC-16 in MeHA and MeHA 3TAA and (a) 10X and (b) 40X magnifications. ......................................................................................................................... 48

Figure 4.8 Cell diameter of AC-16s in MeHA and MeHA 3TAA. ................................................................. 49

Figure 4.9 Immunostaining of hiPSC cardiomyocytes in MeHA and MeHA 3TAA at (a) 10X and (b) 40X magnifications. ......................................................................................................................... 50

Figure 5.1 Pictogram showing the post-print process of the 3D printed hydrogel. ......................... 56

Figure 5.2 Statistical comparison of storage and loss modulii between 3D printed MeHA 3TAA and non-3D printed MeHA 3TAA. ............................................................................................................. 57

Figure 5.3 Statistical comparison of storage and loss moduli between MeHA and 3D printed MeHA 3TAA. ........................................................................................................................................... 59

Figure 5.4 Depiction of the experiment set-up for 3D printing hiPSC cardiomyocytes in MeHA 3TAA .................................................................................................................................................. 60
Figure 5.5 Brightfield images of 3D printed MeHA 3TAA with hiPSC derived cardiomyocytes.

Figure 5.6 Immunostaining of Day 5 3D printed hiPSC derived cardiomyocytes encapsulated in MeHA 3TAA in (A) 10X and (b) 40X magnifications.
Chapter 1 Introduction

1.1 Rationale

Cardiovascular disease (CVD) is the leading cause of death in the United States, with 859,000 deaths each year\(^1\). The disease is also taxing on the healthcare system, with $229 billion in expenses every year\(^1\). Moreover, according to the American Heart Association, Acute Myocardial Infarction (AMI) is the major contributor to CVD mortality\(^2\). AMI causes cell death of as much as 25% of the cardiomyocytes present in the left ventricle of the heart, and the adult human heart has a very limited regenerative capacity\(^3\). Despite the considerable advances in the treatments of CVDs, such as surgical interventions, pharmacological treatments, and ventricular assist devices, the need to solve the loss of cardiac tissue remains\(^4\).

For instance, a common treatment for AMI, heart transplantation, poses risks, such as a lack of donors and organ rejection by the immune system\(^3\). Furthermore, current pharmacological approaches for CVD aim to reduce blood volume or increase cardiac contractile force to prevent a recurring AMI, but none of them repair the loss of cardiomyocyte\(^5\)–\(^7\).

One of the currently proposed solutions are cardiogenic therapies to stimulate regeneration of cardiomyocytes, however, an ongoing challenge for these therapies involve limiting the growth pathways of non-cardiomyocytes\(^6\)\(^,\)\(^8\). Another proposed solution is to use scaffold-free cell therapies that include injected stem cell-derived cardiomyocytes, but these result in ventricular arrhythmias and teratomas post-injection\(^9\). These are all technologies that would prove very difficult to translate into a clinical setting.

The use of biomaterials and tissue engineering can potentially solve all of this. This technology offers the potential to create functional cardiac constructs that can mimic native myocardium. These engineered tissues aim to restore normal heart function, thereby addressing
the limitations of donor organ shortages and other complications associated with traditional therapies\textsuperscript{10}. Moreover, hydrogels have gained substantial recognition as biomaterials due to their tunable physicochemical properties, biocompatibility, and ability to provide structural and mechanical support for cells. There are studies that show improved retention, survival and engraftment of cardiomyocytes when using hydrogels as a scaffold\textsuperscript{11–13}. These types of biomaterials, while very useful, still have room for improvement. A major point is electrical conductivity. Following AMI, the heart’s remaining cardiomyocytes are uncoupled and isolated, which leads to the disruption of electrical signaling propagation within the heart. The introduction of non-conductive hydrogels into the heart through injection or transplantation, while extremely useful, does not address the issue of electrical uncoupling of functional cardiomyocytes\textsuperscript{14–16}. Thus, integrating electrical conductivity into these biomaterials can ultimately improve the propagation of electrical signals in the heart while addressing the lack of cardiomyocytes after AMI.

In the present project, a conductive biopolymer will be developed for use in cardiac tissue engineering. The hydrogel to be used as a scaffold is Methacrylated Hyaluronic Acid (MeHA). Hyaluronic acid (HA) is a major component of the extracellular matrix (ECM), and it plays a role in cellular maintenance and crosstalk. In tissue engineering, it has proved extremely useful due to its biocompatibility, hydrophilicity, and chemical versatility\textsuperscript{17,18}. Adding a Methacrylate group to HA allows the material to be UV cross linkable for scaffold design and 3D printing, the chemical process for the methacrylate addition has been well established in previous literature\textsuperscript{19–21}, the HA molecule is reacted with methacrylic anhydride in DI water for 24 hours at 5°C, see Figure 1.1 for a schematic of this reaction.
The proposed method of incorporating conductivity into the hydrogel is by adding thiophene via esterification, a well-established chemical reaction that refluxes carboxylic acid and an alcohol with a catalyst. Thiophene is a colorless heterocyclic compound that becomes charged when treated with an oxidizing agent via a process called doping. This compound has good chemical stability and electrical conductivity, without making the material too stiff, which makes it ideal for this application.

The development of a conductive HA hydrogel using thiophene represents a promising avenue for advancing the field of cardiac tissue engineering. By addressing the electrical conductivity issue while preserving the desirable properties of HA-based hydrogels, this approach has the potential to significantly enhance the regenerative potential of cardiac constructs and contribute to the development of novel therapies for CVDs.

1.2 Specific aims

Aim 1. Materials characterization and biocompatibility testing of 3D stereolithography printed polymer.

Aim 2. Develop a photocurable conductive hyaluronic acid-based polymer for cardiac tissue engineering using thiophene as a conductor, and test biophysical properties.
Aim 3. Optimize photopolymerization of conductive biopolymer for 3D SLA printing of myocardial tissue models.

1.3 DISSERTATION OUTLINE

Chapter 2 provides a literature review of two representative papers that address the integration of electrical conductivity properties into hydrogels to be used as scaffolds for cardiac tissue engineering development.

Chapter 3 addresses Aim 1. This chapter aims to characterize the mechanical and biocompatible properties of a polymer resin that is photocurable and used in 3D SLA printing. The material characterization of this resin provides insight into photo-cross linkable properties of resins and into 3D SLA printing technologies. These insights are then used in the subsequent development of a 3D printable conductive hyaluronic acid-based polymer for cardiac tissue engineering.

Chapter 4 addresses Aim 2. This chapter presents the development of a conductive hyaluronic acid-based printed polymer. Methacrylated Hyaluronic Acid will be used in combination with 3-thiopheneacetic acid to produce a novel hydrogel with conductive properties. The conductivity, mechanical and biocompatibility properties will be discussed in this chapter.

Chapter 5 addresses Aim 3. This study will optimize the novel hydrogel developed in chapter 4 for use in 3D SLA printing. The mechanical and biophysical properties of hydrogel will be tested and discussed in this chapter.

Chapter 6 summarizes the conclusions obtained from this work and provides the future directions in which this project can be taken.
Chapter 2 Literature Review

The use of biomaterials for cardiac tissue engineering is a novel yet promising solution for heart repair after AMI. However, one of the challenges of using a biomaterial as a scaffold is the lack of conductivity, which has the potential to disrupt an already altered electrical propagation after myocardial infarction. Thus, several studies have aimed to solve this problem by incorporating electrical conductivity into the hydrogels.

In the first paper titled “Development of Electrically Conductive Double-Network Hydrogels via One-Step Facile Strategy for Cardiac Tissue Engineering”, Boguang Yang et al have developed a double network hydrogel that consists of a rigid hydrophobic layer of crosslinked Poly(thiophene-3-acetic acid) (PTAA) and a flexible hydrophilic layer of photo crosslinked Methacrylated Aminated Gelatin (MAAG). They have chosen PTAA due to its good chemical stability and electrical conductivity. Moreover, PTAA is expected to undergo biodegradation over time and shows good biocompatibility that supports adhesion and proliferation. On the other hand, they chose gelatin due to its capability of mimicking the extracellular matrix and because it contains the RGD sequence that promotes cell adhesion and proliferation. They chose a photocurable gelatin with a methacrylate group so that it would not degrade too rapidly.

In this study, they used brown adipose-derived stem cells (BADSCs) as seed cells for cardiac tissue engineering because of their cardiomyogenic differentiation potential. They synthesized 3-thiophene methyl acetate in the presence of FeCl₃ via oxidative-coupling polymerization to create the PTAA network, and they modified the aminated gelatin with methacrylic anhydride to make a photo-cross linkable MAAG network. Hence, one layer is chemically crosslinked while the other one is photo crosslinked. After this, they
homogeneously mixed dimethyl sulfoxide (DMSO) with the PTAA and MAAG and introduced CDI (a carboxylic acid activator) and Irgacure 2959 (a photoinitiator). Subsequently, the solution was exposed to UV light, enabling the synchronous formation of the double network. The PTAA network crosslinks through the activation of the carboxylic groups mediated by the CDI, while the MAAG network is photo crosslinked facilitated by the addition of Irgacure 2959 and the UV light exposure. The ratio of PTAA to MAAG network can be adjusted by changing the amount of each compound that is added into the mix; this allows the mechanical properties of the hydrogel to be modified as necessary.

Furthermore, by modifying the ratio of PTAA to MAAG networks, it is possible to tailor the electrical conductivity of the double network, where the PTAA contributes to the conductivity, and the MAAG enhances the capacitance of the hydrogel. Notably, the conductivity of the double network remains consistent with the conductivity range observed in native myocardium.

Additionally, the double network also showed good biocompatibility in both in vitro and in vivo environments. Moreover, the hydrogel improved cardiac differentiation of BADSCs, which was further improved by the application of electrical stimulus.

In summary, this study underscores the value of incorporating conductive elements into hydrogels to meet the requisite properties for cardiac tissue engineering. As demonstrated by the modulation of its mechanical and conductive properties, and by its efficient survival and proliferation of BADSCs. While the presented research is commendable, there are compelling reasons to consider bettering it by adopting a simpler manufacturing method, and a more patient-specific approach to allow for clinical translation. The manufacturing process of this hydrogel impedes the implementation of patient-specific cardiac constructs. By integrating
patient-specific data, such as MRIs and CT scans into the manufacturing process, it is possible to create a tailored cardiac construct that can more closely mimic the patient’s heart, accounting for variations in size, shape, and pathology. Incorporating technologies such as 3D printing would allow for the precise manufacturing of patient-specific constructs. Moreover, 3D printing can streamline the manufacturing process, reducing the labor-intensive aspects involved in the described methods. This would allow for reproducibility and scalability, which are essential for clinical translation.

In conclusion, while the development of the HEDN hydrogel is promising, advancing the field of cardiac tissue engineering could greatly benefit from embracing 3D printing and patient-specific approaches. These advancements can enhance the precision, functionality, and overall effectiveness of engineered cardiac constructs.

In another paper titled “Polypyrrole-incorporated conductive hyaluronic acid hydrogels”, Jong Cheol Yang et al. have developed a conductive hydrogel using a composite of hyaluronic acid and Polypyrrole. In this study, they decided to use Hyaluronic Acid due to its biodegradability, biocompatibility, and bio reabsorption; while Polypyrrole was selected for its excellent conductivity, long-term stability, and biocompatibility. The synthesis involved creating N-(3-aminopropyl) pyrrole through a reaction with anhydrous ethyl ether and lithium aluminum hydride. The HA sodium salt was added to this solution, and the pH was adjusted to 5.5, resulting in a Py-HA solutions with a 20% degree of substitution of Py subunits.

To fabricate the composite hydrogels, Pyrrole was polymerized within the PyHA using an oxidizing agent to induce crosslinking. Different concentrations of Pyrrole-PyHA were prepared. For this study, they used NIH 3T3 fibroblasts to culture within the hydrogels.
The mechanical properties of the hydrogels showed a Young’s modulus that decreased with higher Pyrrole concentrations. They theorized that this may be due to insufficient covalent bond formation and poor stability of mechanical and electrical properties of the composite hydrogel.

Regarding the conductivity of the hydrogels, conductivity values were slightly below the conductivity range of native myocardium, with the potential for improvement with increased Pyrrole concentration. However, similar to the mechanical properties, the conductivity declined with high Pyrrole concentrations.

The in vitro studies revealed the cells attached and proliferated without any issues, which showed the hydrogels were biocompatible. However, no studies were done on the benefits of the conductivity added to the hydrogel, and they did not use a cell line that uses electrical propagation, so the conductivity effectiveness remains inconclusive. Additionally, the use of pyrrole as a conductor was not stable at higher concentrations, and lower concentrations do not produce hydrogels that are conductive enough to match native myocardium. Overall, while the study presents a unique approach to creating a conductive hydrogel using Hyaluronic Acid, using Pyrrole as a conductor poses challenges in the mechanical and conductive properties of the material.

The research conducted in the two aforementioned studies shows that conductivity in hydrogels is possible and beneficial. However, it is evident that more progress needs to be made in this field to harness the full potential of conductive hydrogels.

In another study, Basara et al\textsuperscript{24} introduce a novel composite construct designed to offer both conductive properties and a topographical structure conducive to the growth and function of human induced pluripotent stem cell-derived cardiomyocytes (iCMs). The constructs are
intricately crafted by 3D printing conductive titanium carbide (Ti3C2Tx) MXene in predefined patterns on polyethylene glycol (PEG) hydrogels, utilizing aerosol jet printing at a cellular level resolution. Following the seeding of iCMs onto these constructs, the culture for one week reveals no signs of cytotoxicity. The outcomes of the investigation underscore the pivotal role of the 3D-printed Ti3C2Tx MXene in aligning iCMs, resulting in a notable increase in MYH7, SERCA2, and TNNT2 expressions. Moreover, the engineered patches exhibit improved synchronous beating and conduction velocity, showcasing the promise of 3D-printed Ti3C2Tx MXene in developing physiologically relevant cardiac patches for effective myocardial infarction treatment.

This investigation underscores the potential of cardiac patches as promising candidates for treating acute myocardial infarction (AMI) by fostering the regeneration of cardiomyocytes. However, a notable challenge arises from the intricate and delicate manufacturing process associated with the hydrogel used in these patches. The complexity involved in production may hinder the seamless translation of this material into clinical applications. Despite this, it is crucial to highlight the study's revelation of the distinct advantages conferred by 3D printing in the realm of cardiac tissue engineering. The demonstrated significance lies not only in the creation of cardiac patches but also in its pivotal role in enhancing tissue organization. This emphasis on the benefits of 3D printing serves as a valuable insight, offering potential avenues to address the challenges associated with the intricate hydrogel fabrication process, ultimately paving the way for more feasible clinical applications.

In another study by Song et al they developed a conductive hydrogel made of conductive polyacrylic acid (PAA). The resulting POG1 hydrogel exhibits remarkable
mechanical properties, including substantial stretchability (>500% strain) and compressibility (>85% strain), with a modulus akin to mammalian heart tissue. Significantly, the hydrogel demonstrates self-healing capabilities and stable conductivity even under substantial deformations. The incorporation of PAA nano-channels within POG1 imparts microscopic ultra-homogeneous conductivity, distinguishing it from other electronic conductors-embedded hydrogels. Notably, cardiomyocytes (CMs) seeded in the POG1 hydrogel display significantly oriented sarcomeres compared to counterparts in other conductive hydrogels. The engineered cardiac patch (ECP) based on POG1 proves effective in mitigating left ventricular remodeling and restoring heart function upon in vivo implantation, offering a novel and biocompatible strategy for ionic conductive hydrogel-based cardiac therapy with notable myocardial infarction repair capabilities.

While this hydrogel is promising, its clinical feasibility also remains lacking. The ease of manufacturing would greatly make this material easy to translate into a clinical setting.

In conclusion, the studies discussed in this analysis contribute valuable insights into the development of conductive hydrogels for cardiac tissue engineering, shedding light on their potential benefits and challenges. The first paper explores a double network hydrogel combining Poly(thiophene-3-acetic acid) (PTAA) and Methacrylated Aminated Gelatin (MAAG), demonstrating promising mechanical and conductive properties. However, suggestions for simplifying the manufacturing process and adopting a patient-specific approach for clinical translation are highlighted. The second paper introduces a conductive hyaluronic acid hydrogel incorporating Polypyrrole, emphasizing the need for careful consideration of Pyrrole concentration due to its impact on mechanical and conductive properties. While biocompatibility is demonstrated, the study prompts further exploration of
the effectiveness of conductivity in the context of electrical propagation. Lastly, the third study introduces a novel 3D-printed Ti3C2Tx MXene composite for cardiac patches, showcasing enhanced cell alignment, gene expression, and functional outcomes. Despite the promise, the intricate manufacturing process presents a challenge for clinical translation. In the broader context, the research collectively underscores the potential of conductive hydrogels for cardiac therapy while emphasizing the need for advancements in manufacturing simplicity, patient-specific approaches, and further exploration of their clinical feasibility.

Building upon these insights, my project aims to combine the advantages of hyaluronic acid, which is well-known for its biocompatibility and chemical versatility, with the electrical conductivity properties of thiophene. My strategy is to develop a conductive biopolymer tailored for patient-specific cardiac tissue engineering applications. The goal is to create a hydrogel that maintains electrical conductivity, mechanical integrity, and biocompatibility for a versatile and effective biomaterial for cardiac tissue regeneration. In this pursuit, my project aligns with the broader objective of advancing cardiac tissue engineering.
3.1 INTRODUCTION

3D stereolithography (3D SLA) is a commercial 3D printing process that uses photosensitive resin and UV light to selectively cross-link the resin layer by layer. This process combines cost-effectiveness, remarkable precision, and a smooth surface finish, that make it an ideal candidate for applications in the biomedical field, including bioanalytical devices, and tissue engineering\textsuperscript{26–28}.

However, a critical challenge associated with 3D SLA printing lies in the selection of suitable resins. While this technology has demonstrated its prowess in creating intricate structures, some of the resins employed within the industry have been shown to hinder cell adhesion and proliferation\textsuperscript{29}, even when the manufacturer claims the material holds biocompatible properties that match those of the ISO 10993 standard\textsuperscript{30}. The inherent conflict between achieving structural integrity and biocompatibility demonstrates the need for rigorous material characterization and exploration of novel resins.

The present study shows a thorough evaluation of the commercially available Formlabs Clear V4 resin, with two main objectives: to determine the mechanical properties of the material, and to gauge its biocompatibility. The overarching aim is to establish whether this new resin holds the potential as a reliable candidate for use in the development of bioanalytical devices. The hypothesis of this study is that these resins have the potential to emerge as biocompatible materials.
The rationale for this study extends beyond material characterization; this study seeks to contribute to the ongoing evolution of microfluidics, and the broader field of bioanalytical devices. As 3D SLA printing continues to revolutionize the creation of complex structures, this research acts as a pivotal link in the chain, working to ensure that the materials used align with the demands of biocompatibility and mechanical integrity.

Moreover, this study has the potential to offer valuable insights into the development of resins tailor-made for 3D SLA printing, serving as a building block for pioneering novel hydrogels for use in tissue engineering. This prospect holds promise for revolutionizing the treatment of heart-related conditions, where patient-specific solutions and biocompatible materials are paramount.

In short, this research endeavors to be not just a snapshot of material compatibility but a catalyst for innovation. By combining 3D SLA printing technology with adequate materials, the aim is to enable a future where patient-specific, biocompatible, and mechanically robust constructs can be crafted with ease. These constructs, whether in the realm of microfluidics or cardiac tissue engineering, hold the potential to reshape the landscape of healthcare and biomedical engineering, bringing us one step closer to personalized, precise, and effective therapeutic solutions.
3.2 MATERIALS AND METHODS

3.2.1 3D SLA Printing

A Formlabs Form 3B SLA 3D printer with Formlabs Clear V4 (Formlabs, INC, Sommerville MA, USA) resin was used to print all test samples. The printer has a laser power of 250mW and operates with a UV Laser of 405nm. The layer thickness can range from 25 to 300µm, with an x/y resolution of 25µm. All STL files of the tested samples were created using SolidWorks and exported to Preform for slicing and printing. Post-print processing required all partes to be washed with 90% Isopropanol in a proprietary Formlabs Washer system, and subsequently cured using a Formlabs Cure system that uses a UV light of 405nm at 60°C for 15 minutes according to manufacturer specifications.

3.2.2 Mechanical testing: tensile and impact testing

Tensile testing was carried out following the specifications outlined in the ASTM D638 standard, utilizing Type V samples, and performed using the MTS Criterion C.44 tensile tester with a mechanical extensometer. The primary objective of these tests was to determine the material's tensile strength and the temporal evolution of its mechanical properties. For this purpose, all test specimens were subjected to aging at ambient room temperature under atmospheric conditions for a range of post-print days 0, 1, 3, 7, and 30. Additionally, all tensile samples were printed at a 0° angle from the print bed, figure 3.1a shows the orientation of the tensile specimen and the build direction of the print.

Concurrently, impact testing was conducted according to the ASTM D256 standard, utilizing Izod type samples, and executed on a Tinius Olsen Model Impact 104 tester. The focus of these impact tests was to establish the material's impact energy across time. Similar to the tensile samples, all specimens designated for impact testing were subjected to aging at
ambient room temperature under atmospheric conditions for a range of post-print days 0, 1, 3, 7 and 30. All impact samples were printed at a 0° angle, with the V-notch in the sample in a perpendicular direction to the blow of the impact tester, figure 3.1b shows the print orientation, print build and impact direction.

![Print Orientation and Build](image)

Figure 3.1 Shows print orientation and build for (a) tensile and (b) impact samples.

### 3.2.3 Scanning Electron Microscopy: Fracture Analysis

Scanning Electron Microscopy (SEM) analysis of the tensile specimens’ fracture surface was performed on the tested samples of post-print days 0, 1, 7 and 30 for fractography assessment. The SEM used was a Hitachi SU-3500 SEM (Hitachi High-Technologies Corp, Tokyo, Japan) operating at a vacuum pressure of 30Pa and accelerated voltage of 15kV.

### 3.2.4 Contact Angle

Contact angle analysis was done on a printed square of 1cmx1cmx0.5cm, using a droplet of De-Ionized (DI) water, according to ASTM standard D7490. Like the tensile and impact testing, the contact angles were aged at ambient room temperature under atmospheric conditions for a range of post-print days 0, 1, 7 and 30. Additionally, an ethanol bath was applied for 10 and 30 minutes on samples that were aged for 1 day under the same conditions.
Three different measurements on three different printed samples were taken. An image of the droplet of DI water on the sample was taken using a Canon SX620 HS camera, and the angle was measured using ImageJ (NIH).

3.2.5 Cell culture

Cell lines used for this study were Human Umbilical Vein Endothelial Cells or HUVECs [ATCC, CRL-1730] grown in Endothelial Growth Medium-2 (Lonza) at a seeding density of $1 \times 10^5$ cells/cm$^2$; and acute myeloid Leukemia cell line HL-60 [ATCC, CCL-240] grown in RMPI 1640 medium with 20% FBS at a seeding density of $1 \times 10^5$ cells/cm$^2$.

3.2.6 Propidium Iodide and Flow Cytometry Analysis

A Propidium Iodide (PI) (ThermoFisher) assay was done to determine cell death when a cell culture is exposed to the printed polymer resin. The cell line HL-60 [ATCC, CCL-240] was cultured in a 96-well plate with RPMI 1640 and 20% FBS medium. The cell culture was then exposed to a printed polymer cube of 1cm$^3$ for a period of seven days. After which the cells were stained with PI and analyzed using flow cytometer Gallios (Beckman Coulter, Miami, FL, USA). The PI was excited using 488nm laser and the signal was captured by an FL-2 detector.

3.2.7 UV-VIS Spectrophotometry Analysis

Ultraviolet-Spectrophotometry (UV-VIS) analysis was conducted to analyze the presence of leached chemicals from the printed polymer when in contact with biological samples. The Human Umbilical Vein Endothelial (HUVEC) cell line [ATCC, CRL-1730] was cultured at a seeding density of $1 \times 10^5$ cells/cm$^2$ in Endothelial Growth Medium-2 (Lonza) on a 3D printed and a conventional petri dish coated with 0.2% Gelatin (Sigma, Darmstadt,
After days 1 and 7 in culture, the cell media was analyzed using the UV-VIS functionality of a NanoDrop OneC (ThermoFisher, Waltham, MA, USA).

### 3.2.8 Live-Dead Assay

Live-Dead Assay was performed to determine cytotoxicity levels for cell cultures when exposed to the printed polymer resin. For this experiment, HUVECs [ATCC, CRL-1730] were cultured in conventional petri dishes coated with 0.2% Gelatin (Sigma, Darmstadt, Germany), in Endothelial Growth Medium-2 (Lonza) at a density of $1 \times 10^5$ cells/cm$^2$. These cultures were exposed to a printed polymer square of 1cmx1cmx3cm for 3 and 7 days, except for one, used as a control. All cells were then detached using Trypsin (ThermoFisher, Waltham, MA, USA) and stained with the LIVE/DEAD Viability/Cytotoxicity Kit (ThermoFisher, Waltham, MA, USA). This kit consists of a green fluorescent calcein-AM to indicate alive cells, and a red-fluorescent ethidium homodimer-1 to display dead cells. The stained cells were imaged using the fluorescent microscope Leica Thunder Imager 3D (Leica Microsystems, Germany).

### 3.2.9 Statistical Analysis

All quantitative measurements were performed in triplicate samples, unless otherwise stated. All values are expressed as mean ± Standard Deviation (SD). A One-way ANOVA with a post-hoc Tukey test was used to compare treatment groups and $p<0.05$ was used to assess statistical significance using GraphPad Prism.
3.3 RESULTS AND DISCUSSION

3.3.1 Mechanical properties of the printed polymer

The 3D printed tensile specimen can be seen in Figure 3.2a before testing and in Figure 3.2b post-testing. The average tensile strength is presented in Table 3.1, while Figure 3.2c provides a statistical comparison of tensile strength across days 0, 1, 3, 7, and 30 post-print.

The tensile strength of the polymer stays consistent over time, this indicates that the polymer resin is stable across a period of 30 days and presents no degradation when aged at standard temperature and pressure (STP).

Figure 3.2 Tensile test of printed polymer resin, (a) shows the tensile specimen pre-print, (b) shows the tensile specimen post-print, and (c) shows a statistical comparison in tensile strength, n=3.
Table 3.1 Average tensile strength per post-print day.

<table>
<thead>
<tr>
<th>Post-Print Day</th>
<th>Average tensile strength (N/mm²)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>54.6</td>
<td>3.98</td>
<td>7.29</td>
</tr>
<tr>
<td>Day 1</td>
<td>49.2</td>
<td>1.68</td>
<td>3.41</td>
</tr>
<tr>
<td>Day 3</td>
<td>55.7</td>
<td>2.92</td>
<td>5.24</td>
</tr>
<tr>
<td>Day 7</td>
<td>56.4</td>
<td>3.69</td>
<td>6.55</td>
</tr>
<tr>
<td>Day 30</td>
<td>73.1</td>
<td>9.03</td>
<td>12.4</td>
</tr>
</tbody>
</table>

In the case of the impact testing, the impact samples can be seen in Figure 3.2a before testing and Figure 3.2b post-testing. The average impact strength across the samples can be seen in Table 3.2, and Figure 3.2c shows a statistical comparison between impact energy across all timepoints. Similar to the tensile test data, the impact test data indicates no statistically significant changes in impact strength over time, which further indicates that the printed polymer resin stays stable across a period of 30 days when aged at STP.
Table 3.2 Average impact resistance per post-print day.

<table>
<thead>
<tr>
<th>Post-Print Day</th>
<th>Average impact resistance (J/m²)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>15.2</td>
<td>1.00</td>
<td>6.60</td>
</tr>
<tr>
<td>Day 1</td>
<td>13.7</td>
<td>1.50</td>
<td>10.9</td>
</tr>
<tr>
<td>Day 3</td>
<td>14.0</td>
<td>1.49</td>
<td>10.6</td>
</tr>
<tr>
<td>Day 7</td>
<td>14.5</td>
<td>0.78</td>
<td>5.38</td>
</tr>
<tr>
<td>Day 30</td>
<td>14.4</td>
<td>1.94</td>
<td>13.5</td>
</tr>
</tbody>
</table>

Figure 3.3 Impact test of printed polymer resin, (a) shows Izod sample pre-test, (b) shows Izod sample post-test, and (c) shows statistical analysis of impact strength, n=3.
Tensile and impacting testing, conforming to internationally recognized standards, facilitated the comprehensive assessment of the material’s mechanical performance at different time points. This approach ensured that the mechanical behavior assessment was a dynamic exploration, allowing for insights into the materials strength over time.

### 3.3.2 Failure analysis of tensile specimens

A comprehensive examination of the tensile samples' fracture surfaces was conducted for specimens tested on post-print days 0, 1, 7, and 30. Figure 3.4 provides the SEM images, offering detailed insights into the characteristics of these fracture surfaces.

In all SEM images, a consistent fracture mode is observed, characterized by a distinctive pattern that is attributed to brittle materials. It starts with an angled crack-initiation site, originating at the corner of each sample. Subsequently, the fracture progression exhibits features denoted as "mirror-mist-hackle." Notably, the mirror and mist regions are relatively limited in extent, while the hackle feature predominates the fracture surface of the material.
This specific fracture mode is widely present in brittle materials\textsuperscript{34,35}, which shows a distinct mode of structural failure that is indicative of the material's response to applied stress. This type of fracture mode corresponds to that of thermoset polymers, which tend to be brittle.
in nature. These SEM observations serve as a valuable resource for characterizing the material's response to mechanical forces, contributing to a deeper comprehension of its mechanical properties.

### 3.3.3 Surface free energy and wettability analysis using contact angle

The average contact angle measurements for the printed polymer resin are summarized in Table 3.3. In figure 3.5a a visual representation of the experimental set-up is shown, while Figure 3.4b offers a comparative analysis of contact angle measurements across different timepoints of post-print days 0, 1, 3, 7 and 30.

![Figure 3.5](image.png)

Figure 3.5 Contact angle of printed polymer resin, (a) shows experimental set up, (b) shows statistical comparison in contact angle across post-print days, and (c) shows statistical comparison in angles after ethanol baths. n=3 (**p<0.001, ****p<0.0001)
Table 3.3 Average contact angle measurements.

<table>
<thead>
<tr>
<th>Post-print day</th>
<th>Contact angle</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>56.7</td>
<td>4.47</td>
<td>7.89</td>
</tr>
<tr>
<td>Day 1</td>
<td>53.1</td>
<td>4.67</td>
<td>8.80</td>
</tr>
<tr>
<td>Day 3</td>
<td>58.4</td>
<td>2.45</td>
<td>4.20</td>
</tr>
<tr>
<td>Day 7</td>
<td>54.0</td>
<td>4.56</td>
<td>8.44</td>
</tr>
<tr>
<td>Day 30</td>
<td>71.1</td>
<td>9.60</td>
<td>13.5</td>
</tr>
</tbody>
</table>

The contact angle remains relatively stable over the initial seven-day period, which indicates a consistent surface free energy of the material during this time frame. However, on day 30 there is a statistically significant increase in the contact angle measurements. This change in angle reflects a critical transformation in the material’s surface properties.

Typically, a contact angle of less than 90° signifies a hydrophilic material\(^{36}\). However, the increase in contact angle over time suggests a noteworthy alteration in the surface free energy of the polymer, resulting in a reduction in its hydrophilicity and thereby, its capacity for capillary flow.

Nonetheless, it is crucial to note that while the material’s hydrophilicity decreases over time, it still retains vital hydrophilic properties. These properties are fundamental in the context of microfluidic devices, where capillary flow is the main mechanism for fluid transport.

Moreover, the contact angle measurements conducted after an ethanol bath of 10 and 30 minutes, revealed no substantial difference between the treatment and the control groups. This finding underscores the material’s robustness in withstanding a common sterilization technique without compromising its wettability. The material’s ability to maintain consistent surface free energy, even after sterilization, is of paramount importance in the context of
bioanalytical devices. It ensures that the material can endure the sterilization process without hindering its capacity to facilitate capillary flow while safeguarding against the risk of contamination.

In summary, the printed polymer resin is capable of capillary flow, especially in the first seven days after it has been printed and even after sterilization with ethanol baths.

### 3.3.4 Biocompatibility and cytotoxicity studies

The PI assay yields compelling results, indicating no discernible difference between the cells that were exposed to the printed polymer, and the cells that were not exposed. The absence of significant cell death post-exposure to the printed polymer resin showcases the non-cytotoxic nature of the printed polymer, as illustrated in Figure 3.6 through flow cytometry analysis.
Figure 3.6 Shows flow cytometry data of exposed cells, (a) indicates the population of the negative control, (b) indicates the population of the positive control, and (c) indicates the exposed population, (d) & (e) show the unstained and stained results, bright field images of HL-60s (f) unexposed and (g) exposed to the printed polymer are also shown.
This is further reinforced by the Live-Dead Assay experimental results. Figure 3.7 provides the images of the assay’s outcomes, where it can be seen the ratio of live to dead cells is similar for both the cell culture exposed to the printed polymer, and the unexposed cell culture. These findings further confirm that the polymer is not cytotoxic to biological samples and is therefore not inducing cell death.

Figure 3.7 Live-dead assay of images of cells exposed to printed polymer resin for 3 and 7 days.
Furthermore, the UV-VIS data indicates that the printed polymer does not leach chemicals into the cell culture it comes into contact with. The UV-VIS spectra of the cells that were in contact with the printed polymer and the cells that were not in contact exhibit no discernible distinction, as can be seen in Figures 3.8b and 3.8c. Additionally, a statistical analysis between the absorbance data of the exposed and unexposed cells was conducted, and it yielded no significant difference (Figure 3.8a).
Figure 3.8 UV-VIS of cell media after exposure to printed polymer-resin, (a) shows a statistical comparison between absorbance points, (b) shows the UV-VIS spectra for day 7, and (c) for day 1.
It is worth noting that these biocompatibility experiments consider the disparities between adherent and non-adherent cell lines. By utilizing HL-60s, this study probed the interaction of the printed polymer with non-adherent cell lines, while employing HUVECs allowed to gauge its interaction with adherent cell lines. This inclusive approach ensures that the material’s biocompatibility is evaluated across a spectrum of cellular environments, bolstering its potential for diverse applications.

3.4 Conclusion

The primary objective of this study was to conduct a comprehensive material characterization of a commercially available clear polymer resin. To achieve this, mechanical properties were rigorously assessed through tensile and impact testing, yielding essential insights into the material’s durability and suitability for the intended purpose. Additionally, these findings serve as a reference point for the shelf life of bioanalytical devices utilizing this material.

Moreover, contact angle measurements determined that the material can effectively support capillary flow for up to 30 days post-print, with its optimal performance occurring during the initial seven days post-print. This observation further confirms the material’s potential utility in bioanalytical devices, albeit within a recommended shelf life of seven days post-print. Beyond this timeframe, a significant decline in capillary flow is anticipated. Furthermore, the material’s compatibility with common sterilization techniques was assessed, revealing no adverse impact on its wettability. This finding solidifies its utility for bioanalytical devices, assuring that the material remains consistent and reliable even after sterilization procedures.
In terms of biocompatibility, the PI assay demonstrated the material’s benign interaction with cell cultures, revealing minimal cell death upon exposure. Similarly, live-dead assays confirmed that the material does not induce apoptosis, which affirmed the material’s non-cytotoxic nature. Finally, UV-VIS data analysis corroborated the material’s biocompatibility, as the data showed the printed polymer does not leach any chemicals into cell cultures it comes into contact with. This evidence underscores the material’s biocompatibility, which further bolsters its suitability for diverse applications in the realm of bioanalytical devices.

Essentially, the material’s performance characteristics, coupled with its safety and sterilization compatibility, position it as a promising candidate for diverse applications, with the potential to revolutionize the field of bioanalytical devices. As technology continues to advance, these findings provide a solid foundation for harnessing the full potential of this material, promoting innovation, and enhancing the quality of bioanalytical devices for various scientific and medical purposes.
Chapter 4 Development of a conductive hyaluronic acid-based polymer for cardiac tissue engineering

4.1 INTRODUCTION

Acute Myocardial Infarction (AMI) severely disrupts the intricate electrical signaling system of the heart due to the nonconductive fibrotic scar tissue that appears after an AMI. This disruption extends its adverse influence to the remaining viable cardiomyocytes, causing electrical uncoupling, and consequently, ventricular dysfunction. The repercussions of this disruption are far-reaching, potentially leading to arrhythmias and, ultimately, heart failure\textsuperscript{37,38}.

Addressing this formidable challenge, the development of a conductive biopolymer emerges as a promising solution\textsuperscript{9}. Research has shown that conductive biomaterials have the potential to enhance cardiac function after implantation, offering a glimmer of hope in the quest to mitigate the effects of AMI\textsuperscript{39–41}. However, these potential breakthroughs have been impeded by the complexity of manufacturing processes, which have yet to achieve clinical feasibility.

In this study, a Methacrylated Hyaluronic Acid (MeHA) hydrogel (Sigma) was conjugated with 3-thiopheneacetic acid to create a semi-synthetic conductive polymer for cardiac tissue engineering. Hyaluronic Acid, an abundant glycosaminoglycan of the human extracellular matrix, is known for its chemical versatility, biocompatibility, and ability to promote angiogenesis\textsuperscript{42–46}. Moreover, the addition of a Methacrylate group to HA allows the formation of a hydrogel via photopolymerization with a photoinitiator.
In order to add conductivity for the intricate domain of cardiac tissue engineering, we introduce 3-thiopheneacetic acid into the MeHA matrix. Thiophene, a heterocyclic compound known for its low cytotoxicity can undergo chemical conjugations with ease\(^\text{47}\). It possesses the unique capability of being rendered conductive through a process known as doping, adding a layer of versatility to our polymer. This polymer will also contain the photo initiator LAP (Sigma-Aldrich) to be later optimized for 3D SLA printing.

The core hypothesis for this study is the combination of a well-established esterification technique and 3D printing methodology, will result in the development of a conductive biopolymer for cardiac tissue engineering applications. The rationale of this study is to provide the tissue engineering field with an easily replicable, conductive polymer that faithfully mimics the conductive attributes of native myocardium, offering a promising pathway for cardiac health restoration.

### 4.2 Materials and Methods

#### 4.2.1 Methacrylated Hyaluronic Acid and LAP

The hydrogel used in this study is Methacrylated Hyaluronic Acid (MeHA) (Sigma), characterized by a molecular weight falling within the range of 100-150kDa, with a degree of substitution in the range of 20-25%.

To facilitate the crosslinking process, the photoinitiator Lithium Phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) (Sigma) was used in this study. The LAP is added to the MeHA solution at a concentration of 20µL per mL of MeHA, the gel crosslinks at approximately 30-60 seconds when exposed to a UV light of 405nm in wavelength.
4.2.2 Doping of 3-Thiopheneacetic Acid

For conductivity grafting into the MeHA matrix, the 3-thiopheneacetic acid (3TAA) (Sigma) molecule was used. This compound has a molecular weight of 142.18Da. To introduce the impurities that initiate the doping of 3TAA, pure Iodine (Sigma) was added, according to previous literature\textsuperscript{48–50}.

A total of 12mg of 3TAA was dissolved in deionized (DI) water, with 2mg of pure Iodine. This amount was determined to be at the solubility limit for 3TAA in 10mLs of water. Moreover, all hydrogels were prepared at the solubility limit because it is at 3 molar excess of MeHA. This solution was allowed to mix over a 24-hour period.

4.2.3 MeHA/3TAA Conjugation and crosslinking

After the doping process, the 10mL 3TAA/I\textsubscript{2} solution is mixed with 100mg of lyophilized MeHA. The 3TAA in solution with water will be at 3 molar excess of MeHA, previous reported studies indicate the use of molar excess for chemical modification of Hyaluronic Acid hydrogels.\textsuperscript{51–55} To catalyze the interaction, 1mg 1,1’-Carbonyldiimidazole (CDI) (Sigma) is introduced, this chemical compound is widely used in hydrogels as a catalyst.\textsuperscript{56–58} This is allowed to react for 24 hours while continuously mixing using a platform shaker (IBI Scientific).

Subsequently, the solution undergoes filtration using an 8kDa dialysis membrane (ThermoFisher) for another 24 hours against DI water, to remove any unreacted molecules. This results in a homogenous clear mixture that can be UV-cross linkable using a photoinitiator.
The expected chemical reaction equation for this conjugation is shown in figure 4.1, where MeHA is conjugated with 3TAA using CDI as a catalyst.

![Chemical Reaction Equation](image)

Figure 4.1 Expected chemical reaction between MeHA and 3TAA.

To crosslink the MeHA 3TAA hydrogel, the same process as the MeHA crosslinking was followed. LAP is added to the MeHA 3TAA solution at a concentration of 20µL per mL of MeHA. After this, all gels were crosslinked using a UV light of 405nm in wavelength for 30-60 seconds.

### 4.2.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

For chemical analysis of the MeHA 3TAA hydrogel, Proton NMR was conducted across three different batches of the hydrogel. The instrument used was the Avance III HD NanoBay NMR Spectrometer (Bruker-Spectrospin). This NMR is equipped with a 400MHz magnet, and a 5mm PA BBO probe.

For sample preparation, all batches were lyophilized at -80°C for a period of 3 days, and later resuspended in 99.8%-grade Deuterium Oxide (D2O) (Sigma). The solution was placed in 400MHz 5mm diam. Wilmad NMR tubes (Sigma).

All the data obtained was analyzed using MNova NMR Software (Mestrelab Research, Spain).
4.2.5 Zetasizer system

For all conductivity measurements, the Zetasizer Advance Dynamic Light Scattering (DLS) (Malvern Panalytical, United Kingdom) system was used.

The 3TAA/I₂ solution was tested three times, across three different batches, to ensure robustness and accuracy. This solution was juxtaposed with the 3TAA in solution with DI water, as well as DI water alone.

Additionally, the MeHA 3TAA hydrogel was tested three times, also across three different batches, and compared with MeHA and DI water alone.

4.2.6 Mechanical Testing using Rheology

To determine the mechanical properties of the hydrogel after its conjugation with 3TAA, rheology was conducted using a SmartPave 92 Dynamic Shear Rheometer (Anton Paar, Austria).

An amplitude sweep of the MeHA and MeHA 3TAA hydrogels was performed six times each to establish the Linear Viscoelastic Range (LVER) of the material. The shear strain rate of the test was set to a range spanning from 0.1 to 10%.

After establishing the LVER of the materials, a frequency sweep was conducted to determine the complex modulus of the materials. Using the amplitude sweep data, the shear strain set for the frequency sweep was 1%, and the frequency was set to 1.99Hz. This test involved three different crosslinked hydrogels across three different batches.

4.2.7 Cell culture

Cell lines used for this study were all human cardiomyocytes. AC-16 cell line [Millipore, SCC109] were cultured in Dulbecco’s modified Eagle’s medium F-12 containing 2mmol/L L-glutamine, 12.5% FBS, and 1X penicillin-streptomycin.
Human induced-Pluripotent Stem Cell (GM25267*D line, Corriell Institute) derived-cardiomyocytes were cultured in Essential 8 media and differentiated according to previously reported methods\textsuperscript{59}.

### 4.2.8 MTT Assay

To establish biocompatibility of the MeHA 3TAA hydrogel, an MTT Assay (R&D Systems) was conducted. This assay was done with the AC-16 [Millipore, SCC109] human cardiomyocyte cell line. This experiment was done across a period of 3, 7 and 14 days in culture with MeHA and MeHA 3TAAA, using round bottom 96-well plates and a seeding density of 1x10\textsuperscript{5} cells/mL.

The cells were stained after each period with MTT, and allowed to react for 24 hours, after which they were washed using the provided detergent from the manufacturer for another 24 hours. After staining, a plate reader was used to measure the absorbance of the solution at 590nm. All data was normalized by subtracting the blanks from the signals obtained. Two different types of blanks were used, blank with no cells and no hydrogel, and blanks with hydrogels and no cells. Equation 1 below shows the formula for data normalization.

\[
\text{Absorbance} = \frac{\text{Hydrogel with cells} - \text{Blank}}{\text{Hydrogel without cells} - \text{Blank}}
\]

### 4.2.9 Immunostaining

To further establish biocompatibility of the hydrogel, an immunostaining process was performed after culturing AC-16s [Millipore, SCC109] for a period of 3, 7 and 14 days in media. Additionally, human-induced Pluripotent Stem Cell (hiPSC) derived cardiomyocytes were
cultured for a period of 7 and 14 days in RPMI B27. AC-16s were cultured at a seeding density of 1x10^5 cells/cm², and iPSCs cardiomyocytes at a seeding density of 3x10^6 cells/mL.

Both cell lines were cultured in a Transwell (ThermoFisher) and a 12-well plate in both MeHA and MeHA 3TAA. The cells were fixed with PFA, and the hydrogels were permeabilized with 0.1% Triton (ThermoFisher). Two different antibodies were used for staining, the primary antibody was CTnT, and the secondary antibody was F-Actin 555 and Alexa Fluor 488. During the first antibody stain, 2% BSA was added to prevent non-specific binding of CTnT. Before imaging, DAPI is added to the hydrogels to stain the nucleus of the cells. For a comprehensive overview, Table 4.1 shows the antibodies and fluorescent markers, along with the color they fluoresce, for all stains used in this procedure.

After staining, the hydrogels were imaged using a Leica Thunder 3D Imager (Leica Microsystems, Germany).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Concentration</th>
<th>Color</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTnT</td>
<td>1:200μL</td>
<td>Green</td>
<td>Cardiac Troponin T binds tropomyosin to regulate calcium binding and contractility of striated muscle present in cardiac cells.</td>
</tr>
<tr>
<td>F-Actin</td>
<td>1:200μL</td>
<td>Red</td>
<td>Binds to the N-terminal region of the actin filaments present in muscle cells</td>
</tr>
<tr>
<td>DAPI</td>
<td>2 drops/mL</td>
<td>Blue</td>
<td>Binds to adenine-thymine-rich regions in DNA. Effectively showing cell nuclei.</td>
</tr>
</tbody>
</table>

Furthermore, an image analysis of the cell circumference was taken on the AC-16 images, to quantify the difference in cell size between cells encapsulated in both hydrogels. This analysis
was done using a machine learning algorithm developed by David Chambers and his team at Southwest Research Institute\textsuperscript{60}.

4.2.10 hiPSC cardiomyocytes beat rate

Videos of the spontaneously beating hiPSC derived cardiomyocytes were taken using a Leica Thunder 3D Imager with a DLS 9000GT camera. The beat rate of the hiPSC derived cardiomyocytes encapsulated in the MeHA and MeHA 3TAA hydrogels was calculated using the average displacement over time. This calculation was made using MTrackJ from ImageJ (NIH). From the average displaced, the beat per minute was calculated based on the peaks of displacement.

4.2.11 Statistical Analysis

All quantitative measurements were performed in triplicate samples, unless otherwise stated. All values are expressed as mean ± Standard Deviation (SD). A One-way ANOVA with a post-hoc Tukey test, and a Two-way ANOVA were used to compare treatment groups; and p<0.05 was used to assess statistical significance using GraphPad Prism.

4.3 Results and Discussion

4.3.1 NMR Spectroscopy shows 3TAA conjugation to MeHA

The Proton NMR data shows the conjugation of a thiophene group to the MeHA compound. Figure 4.2a shows the NMR of the MeHA as it appears in literature\textsuperscript{61}, and 4.2b shows the MeHA 3TAA NMR spectra for three different batches. In Figure 4.2a, the peaks for the backbone of Hyaluronic Acid can be seen between 4 and 3ppm (labelled 1). The peaks that appear
between 6.5ppm and 5.5ppm, and between 2ppm and 2.5ppm (labelled 2) show the methacrylate group in MeHA.

In Figure 4.2b, the same peaks from the MeHA can be seen but there are an additional four peaks present. These peaks show between 7ppm and 7.5ppm, and at 9ppm, and they belong to the protons present in 3-thiopheneacetic acid\textsuperscript{62}.

The presence of the 3TAA peaks indicate that the compound has attached to the MeHA effectively.

Furthermore, the ratio of 3TAA to MeHA was calculated using a method previously described in literature\textsuperscript{63,64} and it was found to be approximately 3.61\%.
Figure 4.2 NMR data for (a) MeHA and (b) MeHA 3TAA (n = 3). Label 1 indicates the HA backbone, label 2 indicates the methacrylate group and label 3 shows the thiophene addition.
4.3.2 DLS Data shows improved conductivity

The conductivity measurements taken with the DLS system show a significant increase in conductivity after doping the 3TAA with Iodine. For a comprehensive overview, Table 4.2 delineates the average conductivity measurements, presenting a clear contrast between 3TAA, doped 3TAA, and DI water as control.

Table 4.2 Average conductivity measurements for Doped 3TAA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conductivity (µS)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water</td>
<td>4.58</td>
<td>2.31</td>
<td>50.5</td>
</tr>
<tr>
<td>I₂ in DI water</td>
<td>27.4</td>
<td>5.60</td>
<td>20.4</td>
</tr>
<tr>
<td>Undoped 3TAA</td>
<td>317.8</td>
<td>3.69</td>
<td>1.2</td>
</tr>
<tr>
<td>1mg/mL 3TAA</td>
<td>603.8</td>
<td>37.0</td>
<td>6.13</td>
</tr>
<tr>
<td>2mg/mL 3TAA</td>
<td>553</td>
<td>86.02</td>
<td>15.6</td>
</tr>
</tbody>
</table>

Moreover, the conductivity measurements for the MeHA 3TAA hydrogel showcased a similar trend, revealing a significant surge in conductivity when compared to the MeHA material in isolation. This insight can be seen in Table 4.3, which shows the average conductivity measurements for MeHA and MeHA 3TAA.

Table 4.3 Average conductivity measurements for MeHA and MeHA 3TAA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conductivity (µS)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeHA</td>
<td>1333.7</td>
<td>61.6</td>
<td>4.62</td>
</tr>
<tr>
<td>MeHA 3TAA</td>
<td>2499</td>
<td>119.8</td>
<td>4.80</td>
</tr>
</tbody>
</table>
To offer a perspective on these findings, Figure 4.3a shows a statistical comparison in conductivity measurements between DI water, I₂ in DI water, and two different concentrations of 3TAA in DI water. In a similar fashion, Figure 4.3b shows a statistical comparison between DI water, MeHA and MeHA 3TAA. These analyses paint a compelling picture of the improvement in conductivity achieved through the chemical synthesis of MeHA and 3TAA.

**Figure 4.3 Statistical comparison in conductivity measurements for (a) 3TAA and (B) MeHA 3TAA. n=4 (****p<0.0001)**
4.3.3 Mechanical properties after 3TAA conjugation

The amplitude sweep of both hydrogels revealed a slight difference in the LVER of the material. After conjugation, the MeHA 3TAA shows a slight decline in complex modulus compared to the unconjugated MeHA. Figure 4.4 shows the amplitude sweep for both MeHA (4.4a) and MeHA 3TAA (4.4b).

Figure 4.4 Amplitude sweep of (a) MeHA and (b) MeHA 3TAA hydrogels.
In the case of the frequency sweep, the data shows the conjugated MeHA 3TAA has a significantly lower complex modulus compared to that of the MeHA. In Table 4.4 the average storage and loss modulus for both hydrogels are shown. Figure 4.5a shows a statistical comparison between storage and loss modulus data, with a statistically significant difference between the two.

Table 4.4 Average storage modulus, loss modulus and loss tangent for MeHA and MeHA 3TAA.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Modulus</th>
<th>Measurement (Pa)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeHA</td>
<td>Storage</td>
<td>34.9</td>
<td>2.56</td>
<td>10.2</td>
</tr>
<tr>
<td>MeHA 3TAA</td>
<td>Storage</td>
<td>26.7</td>
<td>6.9</td>
<td>25.9</td>
</tr>
<tr>
<td>MeHA</td>
<td>Loss</td>
<td>9.03</td>
<td>0.63</td>
<td>6.70</td>
</tr>
<tr>
<td>MeHA 3TAA</td>
<td>Loss</td>
<td>14.7</td>
<td>3.40</td>
<td>23.1</td>
</tr>
<tr>
<td>MeHA</td>
<td>Loss Tangent</td>
<td>0.23</td>
<td>0.01</td>
<td>4.35</td>
</tr>
<tr>
<td>MeHA 3TAA</td>
<td>Loss Tangent</td>
<td>0.54</td>
<td>0.01</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Furthermore, the loss tangent between both hydrogels is significantly different. MeHA 3TAA shows a higher loss tangent compared to MeHA, Figure 4.5b shows a statistical comparison. Loss tangent is an indicator on the ratio of energy lost to energy stored during cyclic deformation\(^6\). This indicates that the addition of 3TAA into the MeHA hydrogel has increased its ability to absorb energy without elastically returning it. In theory, this means that the MeHA 3TAA is potentially more viscous compared to the MeHA hydrogel.
4.3.4 Biocompatibility tests show no changes after 3TAA conjugation

MTT Assays show that there is no statistically significant difference between the AC-16 cells that are in 3D culture in the MeHA and in the MeHA 3TAA. Figure 4.6 shows the results of the absorbance data obtained after days 3, 7 and 14 in-culture. Both hydrogel cultures show similar absorbance across time, which indicates that the MeHA 3TAA does not affect cell viability.
When it comes to immunostaining, the AC-16s show no difference between the MeHA and the MeHA 3TAA cultures, as can be seen in figure 4.7. Furthermore, figure 4.8 shows the quantitative analysis, which demonstrates that the cell diameter is not significantly different between both hydrogels. This means that the 3TAA group conjugation to MeHA is not affecting the original biocompatibility properties of the hydrogel.
Figure 4.7 Immunostaining of AC-16 in MeHA and MeHA 3TAA and (a) 10X and (b) 40X magnifications.
Figure 4.8 Cell diameter of AC-16s in MeHA and MeHA 3TAA.
Similarly, the hiPSC cardiomyocytes followed the same trend. No difference can be seen between the MeHA and MeHA 3TAA hydrogels, as shown in figure 4.9.

Figure 4.9 Immunostaining of hiPSC cardiomyocytes in MeHA and MeHA 3TAA at (a) 10X and (b) 40X magnifications.
4.3.5 Beat rate analysis shows iPSC cardiomyocytes are able to beat in the conjugated hydrogel

The average displacement calculated for the movement of the cardiomyocytes was 23.75 µm for MeHA 3TAA, and 3.959 µm for MeHA. The displacement indicates the beat rate of the cardiomyocytes. The beat calculation using average displacement has been used in literature before. The average beat per minute was calculated to be 80 bpm for the MeHA 3TAA hydrogel and 70 bpm for MeHA.

4.4 CONCLUSIONS

This study sought to address the challenge posed by AMI, a condition that disrupts the electrical signaling system of the heart, which leads to arrhythmias, ventricular dysfunction and ultimately, heart failure. The nonconductive fibrotic scar tissue that arises after an AMI creates a barrier against the normal electrical propagation in the heart, leaving viable cardiomyocytes in a state of electrical uncoupling and dysfunction. Because of this, the need for a conductive biopolymer emerged as a therapeutic tool to resolve the electric impedance of the fibrotic scar tissue in the native myocardium.

Prior research had shown that conductive biomaterials hold great promise in enhancing cardiac function after implantation. However, much remains to be improved in terms of the conductive properties of the material, and clinical feasibility. In response, this study set out to synthesize a MeHA hydrogel with a doped solution of 3TAA, resulting in a semi-synthetic conductive polymer for cardiac tissue engineering.

The result of this study showed that 3TAA can be doped in its monomer form, without the need to be polymerized. Previous studies only talked about the polymerized version of 3TAA,
Poly(3TAA), and how it can be doped to introduce conductive properties. While P3TAA has excellent conductive properties once doped, this study proved that its monomer, 3TAA, can also be doped to the same results.

Furthermore, when conjugated with MeHA, it was shown that the conductivity of the hydrogel was significantly improved, while retaining the original biocompatible properties of the hydrogel.

When it comes to the beat rate of hiPSC derived cardiomyocytes, there was improvement in the average displacement of these cells in the MeHA 3TAA hydrogel compared to the MeHA. A higher beat rate is also seen in the modified hydrogel. While further studies need to be done in this regard, this shows promising data for the conductivity applications of the hydrogel.

In conclusion, this study proved that it is possible to conjugate 3TAA and MeHA to create a photo-cross linkable conductive biopolymer for cardiac tissue engineering. Furthermore, adding this modification to the original hydrogel MeHA does not alter its biocompatibility properties, and shows promising improvements in beat rates for cardiomyocytes.
Chapter 5 Optimize photopolymerization of conductive biopolymer for 3D SLA printing of myocardial tissue models.

5.1 INTRODUCTION

The advent of 3D printing technologies has ushered in a new era of rapid prototyping, streamlined design processes, and unparalleled manufacturing precision. These remarkable attributes make 3D printing an ideal candidate for applications in tissue engineering, where the demand for patient-specific designs and prototyping holds immense promise. In a notable milestone, Mirdamadi et al. demonstrated the ability to 3D print a full-sized adult human heart based on MRI scans, showcasing the potential of their Freeform Reversible Embedding of Suspended Hydrogels (FRESH) technology\textsuperscript{73}.

However, despite the tremendous progress in 3D printing, the choice of materials for this revolutionary technique remains a formidable challenge. For applications in tissue engineering, these biomaterials must possess a unique blend of qualities, encompassing bioresorbable, bioactivity, biocompatibility, and mechanical robustness\textsuperscript{74,75}. Considering these challenges, the current study capitalizes on the previously developed Me-HA/thiophene composite, harnessing its potential to advance the field of cardiac tissue engineering.

The central hypothesis in this investigation is that the 3D stereolithography printing capabilities, coupled with the conductivity of the MeHA 3TAA hydrogel, will empower the creation of tissue constructs characterized by precise shapes and structures. The rationale for this study is developing cardiac tissue that can be meticulously tailored to match the anatomical features of specific regions within the heart. By providing a platform for patient-specific treatments
and region-specific modeling, this study seeks to elevate the field to new heights, promising to enhance the depth and scope of research within cardiac tissue engineering.

5.2 MATERIALS AND METHODS

5.2.1 3D MSLA printer, print settings and CAD designs

The 3D printer used for these experiments was the Prusa SL1S Speed MSLA printer. This printer has a high-resolution Monochrome LCD screen that displays the layers onto the print bed and a UV LED panel that cures the resin at a 405nm wavelength.

All CAD designs were made using SolidWorks, and the slicing software used was the Prusa Slicer. All slicing was adjusted at a layer height of 0.01 µm, and a layer curing time of 60 seconds.

5.2.2 Rheology of 3D printed hydrogel

To determine the mechanical properties of the 3D printed hydrogels, a SmartPave 92 Dynamic Shear Rheometer (Anton Paar, Austria) was used. Four different prints of the MeHA and the MeHA 3TAA were tested immediately after printing. A circle design of 1mm in thickness and 10mm in diameter was used for the test.

5.2.3 Immunostaining of 3D printed iPSC cardiomyocytes

Immunostaining of 3D printed MeHA 3TAA with iPSC cardiomyocytes was done to evaluate the effectiveness of 3D printing tissue models and its effect in live cells. The procedure was done by printing discs of 3cm in diameter and 10mm in thickness, with MeHA 3TAA resin and iPSC cardiomyocytes. After printing, the gels were transferred to Transwell inserts in 12-well plates and cultured for 5 days in RPMI B27 medium.
Following culturing, the cells were fixed with PFA, and permeabilized using 0.1% triton. They were first stained using CTnT in solution with BSA to prevent non-specific binding, and later stained with F-Actin and DAPI. For a reference of the stains and their use, please refer to Error! Reference source not found. in the previous chapter.

All images were taken using a Leica Thunder 3D Imager (Leica Microsystems, Germany).

5.3 RESULTS AND DISCUSSION

5.3.1 3D printing of MeHA 3TAA using Prusa SL1S printer

Illustrated in Figure 5-1 is the 3D printing procedure of the MeHA 3TAA hydrogel. In the depiction presented in figure 5-1a, a step-by step progression can be seen, showcasing the process of removing the hydrogel form the print bed after printing. This pictogram shows the hydrogel in its solidified state, offering a visual journey through the stages of post-print operation.

Furthermore, figure 5-1b shows the outcome of the 3D printing process by displaying the printed hydrogel. Notably, this visual insight allows to perceive the hydrogel’s thickness, providing confirmation of the successful 3D print. The depiction of this printed hydrogel not only affirms the efficacy of the printing process, but also serves as a visual reference for assessing the precision and fidelity achieved in translating the hydrogel into a 3D SLA printable material.
Figure 5.1 Pictogram showing the post-print process of the 3D printed hydrogel.

5.3.2 Rheology of 3D printed MeHA 3TAA

Figure 5-2 shows a visual representation of the statistical comparison that showcases a transformation in the mechanical properties of the MeHA 3TAA after the 3D printing process. An examination of the storage and loss modulus, detailed in Table 5-1 for average values, underscores a substantial divergence between the non-3D printed and 3D printed hydrogels. This disparity signifies a marked enhancement in strength resulting from the 3D printing of MeHA 3TAA. These implications are profound, especially in the context of introducing engineered tissue into the native myocardium\textsuperscript{76,77}.
Table 5.1 Average measurements of complex modulus for 3D-printed and non-3D printed MeHA 3TAA.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Modulus</th>
<th>Measurement (Pa)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeHA 3TAA</td>
<td>Storage</td>
<td>26.7</td>
<td>6.9</td>
<td>25.9</td>
</tr>
<tr>
<td>3D printed MeHA 3TAA</td>
<td>Storage</td>
<td>46.6</td>
<td>1.85</td>
<td>3.98</td>
</tr>
<tr>
<td>MeHA 3TAA</td>
<td>Loss</td>
<td>14.7</td>
<td>3.40</td>
<td>23.1</td>
</tr>
<tr>
<td>3D printed MeHA 3TAA</td>
<td>Loss</td>
<td>8.74</td>
<td>1.09</td>
<td>12.5</td>
</tr>
</tbody>
</table>

The significance of this discovery becomes even more apparent when considering the potential implications for modulating the mechanical characteristics of the hydrogel. The ability
to strengthen the material through 3D printing stands as an important breakthrough, opening avenues for refined control over the material’s strength, which remains an important factor when integrating engineered tissue into the intricate environment of the native myocardium.

Taking this exploration further, Figure 5-3 shows a statistical comparison between the original MeHA, and the 3D printed MeHA 3TAA. Notably, the introduction of the 3TAA induces a discernible loss in strength in the biomaterial, as was seen in the previous study. This phenomenon is not only reversed but actually improved upon after the 3D printing process. The depicted significant differences in storage and loss modulus between the two materials accentuates the transformative impact of 3D printing, with the 3D printed material showing higher values in storage modulus and lower values in loss modulus. This evidence underscores the multifaceted benefits of 3D printing, not only amplifying the material strength but also fin-tuning its mechanical properties in a manner that holds immense promise for advancing tissue engineering.
5.3.3 3D printing of MeHA 3TAA with iPSC cardiomyocytes

The 3D printing of the MeHA 3TAA with iPSC-derived cardiomyocytes was done in sterile conditions inside a cell culture hood, as depicted in Figure 5-4a. The accompanying Figure 5-4b showcases the resultant gels with the cells, on the print bed.
Post-print analysis, as illustrated in Figure 5-5, reveals a discernible cellular directionality, suggesting an alignment of the cells that was facilitated by the 3D printing process. This alignment appears to occur in a single-line fashion, corresponding to the printer’s layer by layer approach.
This observed cell orientation bears significance in the realm of cardiac tissue engineering, presenting a distinct advantage over other manufacturing processes. The intricate structure and organization of myocytes significantly influence both the electrical propagation and mechanical properties of the heart. The apparent ability of 3D printing to arrange cells in a specific direction underscores its potential to precisely mimic the native architecture of cardiac tissue. This inherent capability positions 3D printing as a compelling technology for advancing cardiac tissue engineering.

Furthermore, Figure 5-6 provides additional insights, showcasing the immunostaining for day 5 of the 3D printed hydrogels. The figure illustrates that 3D printing the hydrogel with cells does not compromise their culturing and growth. This evidence reinforces the robust compatibility of the 3D printing process with cellular viability, affirming its potential for seamlessly integrating living cells into complex tissue structures. These findings further bolster the argument...
for the efficacy of 3D printing in cardiac tissue engineering, emphasizing not only its architectural precision but also its biocompatibility, crucial for the successful development of functional and physiologically relevant cardiac constructs.

Figure 5.6 Immunostaining of Day 5 3D printed hiPSC derived cardiomyocytes encapsulated in MeHA 3TAA in (A) 10X and (b) 40X magnifications.
5.4 CONCLUSIONS

The transformative impact of 3D printing technologies on tissue engineering is undeniable, ushering in crucial advancements that have propelled the field to new heights. One of the most remarkable feats enabled by this technology is the ability to 3D print full-size models of organs, such as the heart, marking a paradigm shift in our approach to replicating complex anatomical structures. The unparalleled advantages of 3D printing are due to its capacity for high-throughput, high-resolution, and reproducible tissue constructs that can be finely tuned to meet patient-specific needs. While these technological strides have surpassed expectations, a critical frontier in the domain of cardiac tissue engineering lies in the exploration of suitable materials for use with 3D printing technologies.

Constructing cardiac tissues that authentically replicate the intricate environment of the heart presents an ongoing challenge. The ideal materials for this purpose must not only be mechanically tunable but also possess electrical conductivity. Studies indicate that materials exhibiting electrical conductivity outperform their counterparts, demonstrating enhanced adhesion, alignment, organization, and maturation of cardiomyocytes.

In this study, a novel Methacrylate Hyaluronic Acid hydrogel, conjugated with 3-thiopheneacetic acid, emerges as a promising candidate for 3D stereolithography (SLA) printing. Leveraging a 3D SLA Prusa MSLA printer operating at a wavelength of 504nm, the hydrogel consistently yielded solid and stable prints. Rheological data revealed that the young’s modulus of these 3D printed hydrogels surpassed that of constructs produced without a 3D printer, suggesting the potential for tunable mechanical properties through 3D printing.
Moreover, the hydrogel was strategically combined with induced pluripotent stem cell (iPSC)-derived cardiomyocytes to assess its compatibility with biological samples. The resulting hydrogels exhibited a distinctive cellular arrangement, hinting at the printer's automatic alignment of cells in a directional pattern, mirroring the cellular organization found in native tissues. Immunostaining imaging further affirmed the resilience of the cells through the printing process, highlighting their retained biological activity.

In conclusion, this study underscores the feasibility of 3D SLA printing for a conductive hydrogel, showcasing superior properties compared to hydrogels developed without the aid of 3D printing. The findings not only contribute to the evolving landscape of cardiac tissue engineering but also spotlight the potential of 3D printing technologies to revolutionize the fabrication of functional and biocompatible tissue constructs.
Chapter 6 Conclusions and Future directions

6.1 CONCLUSIONS

Chapter 3 discusses the materials characterization of a polymer resin that uses 3D SLA printing. In the pursuit of advancing bioanalytical devices, the material characterization study of a commercially available resin has revealed promising attributes. Rigorous mechanical testing unveiled insights into the material's durability, offering a valuable reference for the shelf life of bioanalytical devices. The material's proficiency in supporting capillary flow for up to 30 days post-print, along with its compatibility with sterilization techniques, establishes it as a reliable choice within a recommended seven-day post-print shelf life. Biocompatibility assessments confirmed the material's benign interaction with cell cultures, highlighting its non-cytotoxic nature and further affirming its suitability for diverse applications.

In essence, this study proved that this polymer resin has the potential to provide a foundation for promoting innovation and enhancing the quality of bioanalytical devices. As material development continues to advance, the study sought to point out the importance of using technologies such as 3D SLA printing, with bioanalytical devices to further advance the field.

Shifting focus to cardiac tissue engineering, the study in chapter 4 aimed to address the impediments posed by AMI-induced fibrotic scar tissue. The synthesis of a MeHA hydrogel with a doped solution of 3TAA resulted in a semi-synthetic conductive polymer. Notably, this study demonstrated the feasibility of doping 3TAA in its monomer form, a departure from prior research focused on polymerized versions. The conjugation of 3TAA with MeHA improved the conductivity of the hydrogel while maintaining its original biocompatible properties. This
breakthrough holds significant promise for cardiac tissue engineering, offering a conductive biopolymer that can enhance cardiac function without compromising biocompatibility.

Expanding the horizon to the realm of 3D printing technologies in tissue engineering, the study in chapter 5 showcased the previously mentioned novel Methacrylate Hyaluronic Acid hydrogel for 3D stereolithography (SLA) printing. The hydrogel exhibited superior mechanical properties compared to non-3D printed constructs, presenting a path for tunable properties through 3D printing. The strategic combination of the hydrogel with iPSC-derived cardiomyocytes showcased automatic cell alignment, mirroring native tissue organization. This study not only contributes to the evolving landscape of cardiac tissue engineering but also underscores the potential of 3D printing technologies to revolutionize the fabrication of functional and biocompatible tissue constructs.

In conclusion, these three studies collectively unveil the potential of advanced materials, conductive biopolymers, and 3D printing technologies to reshape the landscape of bioanalytical devices and cardiac tissue engineering. The material characterization study provides a foundation for reliable bioanalytical devices, the conductive biopolymer study offers a breakthrough for addressing cardiac impairment, and the 3D printing study highlights the transformative potential of this technology in tissue engineering. Together, these findings pave the way for biomedical innovation, promising enhanced quality, and efficacy in various scientific and medical applications.
6.2 Future directions

This project harbors three compelling potential avenues, each poised to significantly advance the field of cardiac tissue engineering. Delving into these directions will not only shed light on the capabilities of the innovative MeHA 3TAA material but also pave the way for transformative applications in the realm of bioengineering.

The first avenue explores the functionalization of MeHA 3TAA through the incorporation of the RGD sequence, a strategy known to enhance cardiomyocyte attachment and grafting. Building upon the findings from previous studies that demonstrated improved cell adhesion and retention by functionalizing Hyaluronic acid hydrogels with the RGD peptide sequence, this project seeks to elevate the potential of MeHA 3TAA for creating fully functional cardiac tissues. The synergistic effect of enhanced cardiomyocyte attachment, facilitated by the RGD sequence, combined with the innate conductive properties of MeHA 3TAA holds the promise of developing cardiac constructs with unprecedented functionality.

Expanding upon this, the integration of the improved MeHA 3TAA with 3D SLA printing emerges as a powerful platform for crafting fully tunable cardiac constructs. This entails meticulous control over cellular alignment, attachment, and engraftment, marking a significant leap towards the precision engineering of functional cardiac tissues. The amalgamation of advanced biomaterial functionalization and cutting-edge 3D printing technologies opens the door to the creation of bespoke cardiac structures tailored to meet the specific requirements of individual patients.
The second direction envisages in vivo experiments involving fully tuned MeHA 3TAA printed hydrogels. Drawing inspiration from prior studies that employed various in vivo experiments using tissue constructs\textsuperscript{93–95}, this avenue promises to provide crucial insights into the efficacy of the material, particularly when the hydrogel has been pre-functionalized with the RGD peptide sequence. These experiments serve as a critical bridge between laboratory-scale success and real-world applicability, offering a comprehensive understanding of how the material performs within the complex biological milieu.

Lastly, within the realm of 3D printing, the third direction harnesses the unique printing capabilities of MeHA 3TAA to create patient-specific constructs. This approach takes into account the inherent variations in size, shape, and mechanical strength present among different patients' native myocardium. By tailoring cardiac constructs to adapt to these individualized parameters, this approach minimizes the probability of failure and enhances the overall efficacy and compatibility of engineered cardiac tissues.

In summary, these three envisioned directions not only showcase the versatility of MeHA 3TAA but also underscore its potential to revolutionize cardiac tissue engineering. From enhanced functionalization and precision 3D printing to rigorous in vivo experimentation, this project has the capacity to propel the field forward, offering innovative solutions that can be customized to meet the unique needs of each patient. As we delve into these directions, we anticipate uncovering novel insights that will contribute to the ongoing evolution of cardiac tissue engineering practices.
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Vita

Brittany L Stark (born Brittany L Payan Baca) was born in Parral, in the state of Chihuahua, Mexico. She moved to El Paso, TX in January of 2014 to pursue higher education. She finished her bachelor’s degree in Biological Sciences with a minor in Mechanical Engineering in May 2019 from the University of Texas at El Paso. After this, she decided to go into her PhD in the Fall of 2020. She graduated from the University of Texas at El Paso with a PhD in Materials Science and Engineering in December 2023.

Through her higher education years, she has found a passion in materials science and engineering, and she hopes to contribute to the engineering world with her dedication and hard work.

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