Proceedings of The National Conference
On Undergraduate Research (NCUR) 2020
Montana State University, Bozeman MT
March 26-28,2020

Engineering a Scaffold for Cardiac Tissue Repair

Gabriela Pena Carmona; Elroi Kibret; Keenan Anderson-Fears
Department of Biomedical Engineering
California State University- Long Beach
1250 Bellflower Blvd, Long Beach, CA 90840 USA

Faculty Advisor: Dr. Perla Ayala

Abstract

According to the American Heart Association, heart disease remains the number one leading cause of death in the United States with more than one person having a heart attack every minute. A heart attack occurs when a blood clot blocks the coronary arteries preventing oxygenated blood flow from reaching the heart muscle. Cell death that occurs after a heart attack is repaired by the body with scar tissue that does not have the functional characteristics of myocardial tissue. The objective of the study is to initiate the investigation of a collagen-based scaffold combined with decellularized cardiac matrix that is biocompatible and that supports adhesion and viability of cardiac muscle cells. To prepare the engineered scaffold, Collagen Type I was isolated from porcine skin via a solubilization method. Additionally, decellularized cardiac matrix (dCM) derived from porcine heart tissue was incorporated into the composite scaffold. Significant cell viability and proliferation was observed when HL-1 cardiac cells were cultured on polydimethylsiloxane (PDMS) substrates coated with dCM for 5 days. Cell adhesion and viability was also observed on composite collagen-dCM scaffolds cultured for 3 days. To provide cell organization on the scaffold, the composite construct was molded onto a PDMS molds with parallel grooves. HL-1 cardiac organization was observed on composite scaffolds with micro-grooves. This bioengineered scaffold demonstrates promise for future cardiac repair applications.

Keywords: Scaffold, HL-1, decellularized cardiac tissue

1. Introduction

In the United States approximately 1 million Americans have a new or recurrent heart attack every year with surviving patients possibly developing congestive heart failure[1]. Myocardial infarctions, or heart attacks, are generally caused by the formation of a blood clot in the coronary arteries due to plaque build-up that blocks oxygenated blood flow from reaching the heart muscle. Cell death (apoptosis, autophagy, and necrosis) that occurs due to myocardial infarction is irreversible; however these cells are replaced by scar tissue that does not have the cardiac phenotypic characteristics that allow for the uniform and effective contraction of the heart[2]. Most current therapies are focused on restoring blood flow to the heart, but no treatment currently exists to replace or repair the damaged tissue. In recent years, the regenerative medicine and tissue engineering fields have focused on developing approaches to repair human tissues and organs by replacing diseased tissue with engineered substitutes and/or by stimulating the body repair mechanisms[3]. The field of tissue engineering aims to develop strategies to achieve tissue regeneration by using biomaterials, cells, bioactive molecules, and combinations thereof[4]. In general, soft synthetic and natural polymeric materials can be used to develop these novel strategies for tissue repair and organ replacement. Numerous injectable and implantable technologies for tissue regeneration have been developed[5]. Although some of these have shown promising results in animal models, challenges still remain in the laboratory and the clinic[6]. Restoring and repairing the functionality of myocardial tissue can be facilitated with the use of cardiac engineered scaffolds that can improve functional outcomes after MI[7]. However there is still a clinical need to develop an engineered scaffold that can support cardiac repair and prevent heart failure[8]. The design of effective regenerative strategies requires an understanding of how the biological microenvironment affects cellular behavior[9]. By controlling the microenvironment's properties (ECM components, topography), we can provide

necessary signals for cell proliferation and function. We previously designed robust collagen constructs that showed good integration with the host tissue, but additional work is needed to achieve optimal tissue repair[10]. Control of the extracellular matrix (ECM) composition is also critical for preventing pathological remodeling and supporting regeneration[11]. The ECM has complex biological effects and influences cell proliferation, migration, lineage specification, intercellular signaling, and growth factor presentation[12]. The cardiac extracellular matrix plays a role in a cell's mechanical integrity, growth, attachment, and signaling[13]. Collagen Type 1 is a natural connective tissue polymeric material that supports cell attachment and viability[14]. The optimal scaffold would provide a microenvironment that is similar to the heart's myocardium. Previous studies have also demonstrated the use of decellularized scaffolds for tissue repair[15]. The main purpose of the decellularized cardiac matrix (dCM) is to provide the cells a cardiac microenvironment that also provides physical and mechanical support. Here, we present early studies on the development of a cardiac composite patch. Initial studies demonstrate integration of a collagen sheet with decellularized matrix that supports cardiac cell attachment, proliferation, and organization.

1.1 Present Investigation

The purpose of this study is to design a composite scaffold that is biocompatible and supports the adhesion and proliferation of cardiac cells. We hypothesized that the combination of a Collagen Type 1 with dCM would provide an improved scaffold that promotes cellular viability and proliferation. In this study, we investigate a collagen only scaffold, a collagen+dCM composite scaffold, and the integration of micro-grooves on the scaffold to promote cell organization and alignment. We studied the effect of different concentrations of dCM on cardiac cell viability. First, we investigated cell viability and organization on micro-grooved PDMS wells coated with various concentrations of decellularized matrix. The decellularized cardiac tissue was derived from porcine skin through a sodium dodecyl sulfate (SDS) protocol. Second, we investigated the fabrication of collagen+dCM composite scaffolds and the cell viability and organization of cardiac cells on these scaffolds. Collagen only and collagen+dCM scaffolds were developed on PDMS molds with micro-grooves to promote cell alignment. Collagen Type 1 was isolated and purified from porcine skin using an acid solubilization method. The dCM was incorporated into the collagen scaffold to prepare a composite collagen+dCM patch. HL-1 cardiac cells[16] were seeded on the scaffolds and viability was assessed through fluorescence imaging. The results demonstrated significant viability and organization of HL-1 cells in the micro-grooved collagen only and the collagen+dCM scaffolds.

2. Methodology

2.1 Collagen Type 1 Isolation and Purification

Collagen Type 1 was isolated and purified from porcine skin using an acid solubilization method as previously published[17] following an adapted procedure, previously used for rat tail tendon collagen extraction by Silver and Treslad[18]. Briefly, cleaned frozen porcine skin sheets were minced and placed in 10 mM HCl for 4 hours at room temperature. Soluble collagen was isolated and purified by centrifugation and with vacuum filtration with final 0.2 mm filter membranes. The porcine collagen was precipitated by adding concentrated NaCl with stirring for 1 hour. The collagen solution was centrifuged to recover the collagen. The pellet was re-dissolved in 10 mM HCl overnight and the solution was dialyzed (Spectra/Por Dialysis membrane, molecular weight cut-off 50,000) against a phosphate buffer, 10 mM HCl, and deionized water. The dialyzed solution was frozen a -80˚C overnight and collagen was lyophilized for 5 days.

2.2 Decellularized Extracellular Matrix (dCM)

The porcine derived decellularized cardiac muscle tissue was obtained using a customized sodium dodecyl sulfate (SDS) protocol. Cubed porcine heart tissue was decellularized in a 1% (w/v) SDS solution with penicillin-streptomycin (0.5%v/v) for 2-3 days. The decellularized tissue was rinsed with sterile DI water 3X times for 2 days. The decellularized tissue was then lyophilized for 3-5 days. After lyophilization, the decellularized tissue (10 mg/mL) was incubated with stirring in a pH1 HCl solution with pepsin (5mg/mL) for 48 hours. After incubation, NaOH was added to neutralize the pH of the solution. The decellularized tissue was lyophilized in 1X PBS for 3-5 days.

2.3 PDMS Molds

The PDMS molds were prepared using standard manufacturer's protocol; briefly a 10:1 ratio of base to curate agent was used to prepare the PDMS molds (SYLGARD 184 Silicone Elastomer Kit). Once the polymer solution was combined, it was poured over 3D printed frames that were placed inside prepared aluminum foil wells. The frames used to create micropatterned molds were printed using a Project 6000 3D printer. PDMS molds were prepared using micro-grooved frames. The dimensions of the frames have 8 mm depth, 1cm length, 1cm width and present forty-nine channels (micro-grooves). Each channel is 100 microns in width, depth, and separation from each other. The PDMS molds were left at room temperature for 30 minutes before being cured in an oven for 4 hours in order to eliminate bubbles created during mixing. After cooling to room temperature, the PDMS molds were peeled from the aluminum foil well. Prior to use, the molds were incubated in a 70% ethanol for 30 min, the ethanol was then removed, and the molds were allowed to dry for at least 1 h in a biosafety cabinet to maintain sterilization.

2.4 Cell Seeding

HL-1 cardiac cells[16] were seeded at a density of *100,000* □□□□□/□□^{2}. The cells were first cultured inside T25 flasks according to the provider's protocol (Sigma-Aldrich) for three days then passaged and seeded on prepared molds or scaffolds. Briefly, cardiac muscle cells were seeded on plates pre-coated with 0.02% Gelatin and Fibronectin. HL-1 cells were seeded on PDMS molds with micro-grooves pre-coated with 0.02% Gelatin and Fibronectin, with 0.02% Gelatin/Fibronectin + dCM (0.25mg/mL, final concentration), or with 0.02% Gelatin/Fibronectin + dCM (0.5mg/mL, final concentration) to determine cell viability. HL-1 cells were also seeded on collagen only and collagen +dCM (1.25mg/mL, final concentration) composite constructs on micro-grooved PDMS molds.

2.5 Scaffold Fabrication

Collagen Type 1 only scaffolds were prepared similar to previously described scaffolds[10, 17]. Briefly, isolated porcine Collagen Type I was dissolved in 10 mM HCl (2.5 mg/mL). The collagen solution was gelled by neutralizing it with a phosphate buffer (composed of 4.14 mg/mL monobasic sodium phosphate, 12.1 mg/ml dibasic sodium phosphate, 6.86 mg/mL n-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid sodium salt, and 7.89 mg/mL sodium chloride, at pH 8.0) at 4° C in a PDMS mold for 24 h. Collagen gels were then incubated for 24 h at 37° C. After an additional 24 h incubation at 37° C, gels were rinsed in deionized water, allowed to dry in the PDMS mold overnight to form a collagen sheet. Following drying, the sheet was rehydrated in fiber incubation buffer (FIB, 7.89 mg/mL sodium chloride, 4.26 mg/mL dibasic sodium phosphate, 10 mM Tris, pH 7.4) and incubated for 48 h at 37° C to promote fibrillogenesis. Collagen sheets were rinsed with deionized water and were allowed to dry on the PDMS mold. The collagen+dCM composite patch was developed by incorporating decellularized porcine heart extracellular matrix within the collagen sheet. Lyophilized dCM was added to the sterile phosphate buffer used to neutralize collagen gels (1.25mg/ml, final concentration). Composite sheets were the prepared using the same method as the collagen only sheets.

2.6 Cell Staining and Imaging

HL-1 cell viability was analyzed by Calcein AM staining (Life Technologies) at day 3 or day 5 of culture. Briefly, samples were washed with 1X PBS buffer and then incubated for 30 min with 1X PBS buffer containing Calcein AM. Cells on scaffolds were fixed and stained for F-actin and α-actinin. Briefly, cells were fixed with formalin for 15 minutes (Fisher Scientific, Pittsburgh, PA). Cells were then rinsed and incubated in 0.5% Triton X-100 (Sigma Aldrich) for 15 minutes. Cells were washed 3X with 1X PBS before blocking with 1% bovine serum albumin (BSA) (Sigma Aldrich) for 30 minutes. To stain the cell cytoskeleton (F-actin), cells were rinsed and incubated with phalloidin stain Alexa Fluor 568 phalloidin (Molecular Probes) for 30 minutes at a 1:200 ratio. To stain α-actinin, cells were incubated with a mouse α-actinin IgG (Sigma Aldrich) at room temperature for 1.5 h and incubated with Alexa 488-conjugated goat/mouse anti-mouse IgG (Molecular Probes) for 1 h at room temperature. Nuclei was stained with DAPI. The samples were imaged using an EVOS fluorescent microscope.

3. Results

3.1 Isolation of Collagen Type 1

Collagen Type 1 was isolated purified from porcine skin to fabricate an engineered cardiac patch. The porcine skin was acquired from a provider that has ISO certification and is US FDA compliant (Tissue Source, IN). The purity of the porcine collagen has been assessed by Coomassie in previous studies[17].

3.2 Preparation of decellularized Cardiac Matrix (dCM)

Cardiac decellularized matrix was obtained from a porcine heart acquired from a provider that has ISO certification and is US FDA compliant (Tissue Source, IN). Cubed porcine heart tissue was decellularized and fashioned into a lyophilized powder (Figure 1).

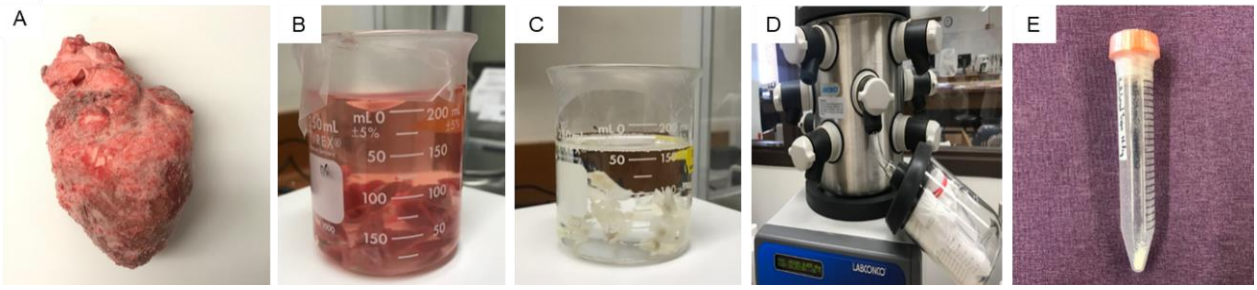

Figure 1. Cardiac ECM decellularization process

Figure 1. Cardiac ECM decellularization process. A) Porcine cardiac tissue is cut into small cubes. B) The tissue is placed in 1% (w/v) SDS for 4-5 d. C) Tissue is rinsed in DI water for 48 h. D) After pepsin treatment and solution neutralization, the tissue is dialyzed and then lyophilized. E) Lyophilized decellularized cardiac matrix (dCM) powder.

3.3 Preparation of Micro-grooved PDMS molds

The frames used to create micropatterned PDMS molds were printed using a Project 6000 3D printer. PDMS molds were prepared using micro-grooved frames. The dimensions of the frames are: 8 mm in thickness, 1cm in length, and 1cm in width (Figure 2). Each channel is 100 µm in width, depth, and separation from each other.

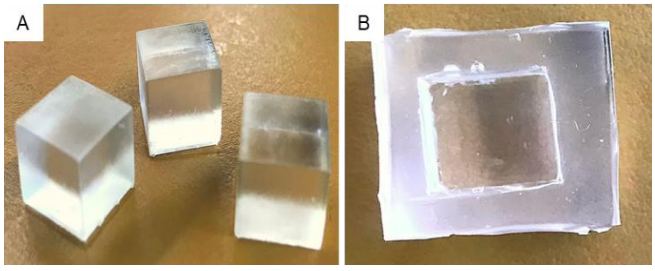

Figure 2. Forming of 3D printed frames

Figure 2. A) 3D printed frames printed using a Project 6000 3D printer. The frames are 1cm^2 in length × width. The frames have 8 mm thickness and contain forty-nine grooves. The grooves have 100 µm width and depth. Each groove is 100 µm apart from each other. B) A PDMS mold is then made with one of the 3D printed frames.

3.4 HL-1 Cardiac Cell Viability on Micro-grooved molds

HL-1 cardiac muscle cells were seeded on sterilized micro-grooved polydimethylsiloxane (PDMS) molds both with and without dCM at two different concentrations for 5 days. Cells stained with Calcein AM demonstrated similar robust viability on micro-grooved surfaces with and without dCM (Figure 3).

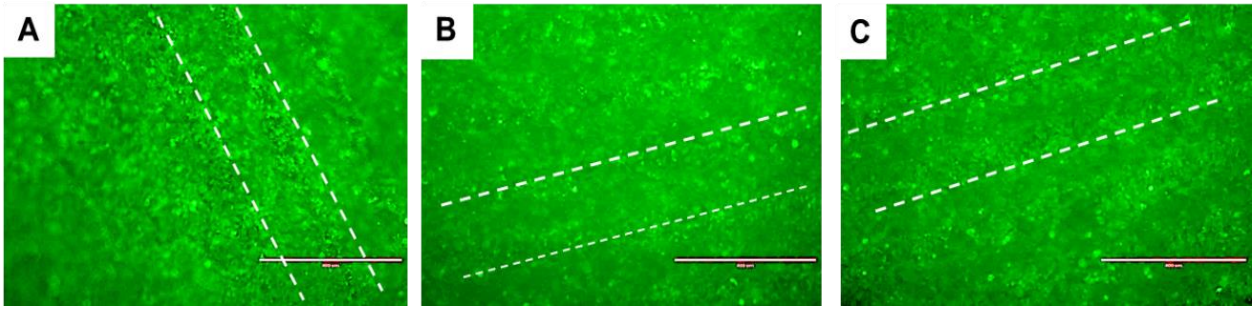

Figure 3. Calcein AM stained cardiac cells on day 5

Figure 3. Cardiac muscle cells were seeded inside grooved PDMS molds for Calcein AM stain at day 5. PDMS molds were pre-coated with (**A**) % 0.02 Gelatin/Fibronectin (G/F), (**B**) G/F added with dCM (0.25mg/mL, final concentration), and (**C**) G/F added with dCM (0.25mg/mL, final concentration). Images were taken at 10x magnification. (Bar = 400 µm, dashed lined = micro-groove direction).

3.5 Fabrication of Collagen and Collagen+dCM Scaffolds

Engineered collagen sheets were fabricated from collagen gels formed from a pH 2 porcine skin derived Collagen Type 1 solution neutralized with a phosphate buffer. Collagen gels (8 mm thickness) were initially cast for 48 hours on micro-grooved PDMS molds. After rinsing, gels were allowed to dry to form sheets that were then incubated for an additional 48 hours in fibril inducing buffer. Sheets were then rinsed and dried overnight. Following drying, sheets were sterilized with 70% and rinsed with sterile DI water before cell seeding. Engineered collagen composite sheets were fabricated in a similar way as the collagen only sheets. The dCM was incorporated in the phosphate added for cross-linking to a final concentration of 1.25 mg/mL. The same steps as the collagen only sheet preparation were followed after addition of the dCM.

3.6 HL-1 Cardiac Cell Viability on Engineered Composite Scaffolds

HL-1 cardiac muscle cells were seeded on sterilized micro-grooved collagen sheets with dCM or without dCM for 3 days. Cells stained with Calcein AM demonstrated similar robust viability on micro-grooved sheets with and without dCM. Cells organized themselves along the grooves in both sample sets (Figure 4). F-actin staining demonstrated cellular cytoskeleton morphology and organization on micro-grooved sheets both with and without dCM (Figure 5).

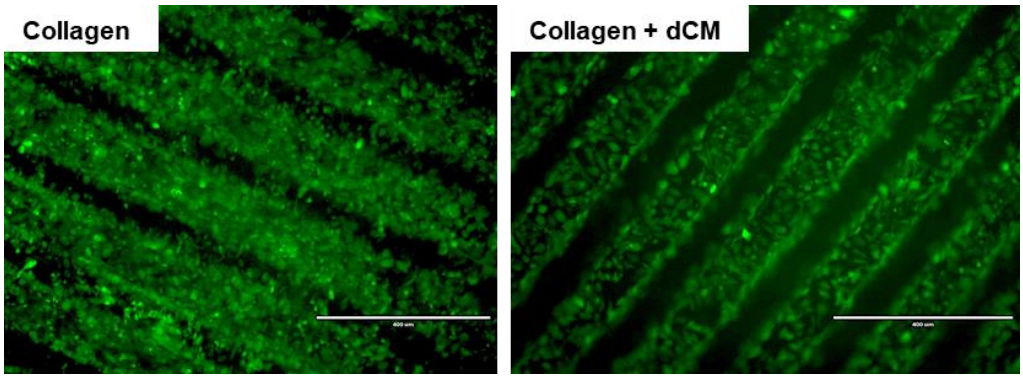

Figure 4. F-actin stained cardiac cells on day 3

Figure 4. Cardiac muscle cells cultured on collagen only and collagen + dCM scaffolds were stained with Calcein AM and imaged on day 3. Images were taken at 10x magnification. (Bar = 400 µm)

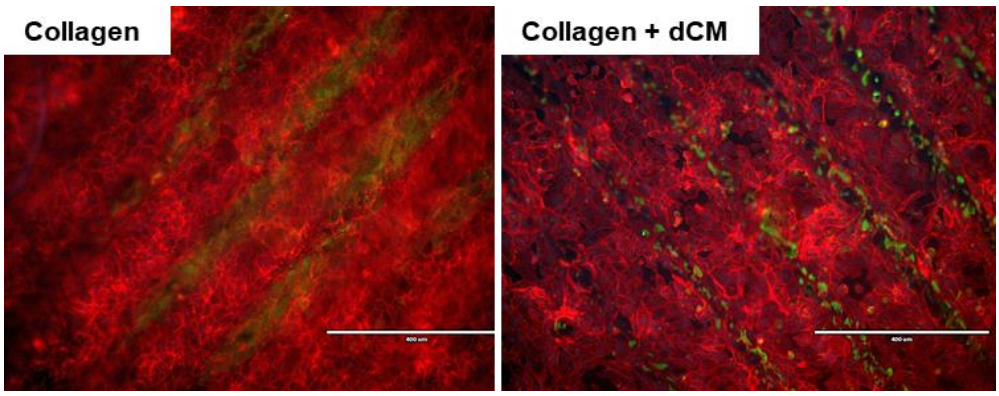

Figure 5. F-actin and α-actinin stained cardiac cells on day 3

Figure 5. Cardiac muscle cells cultured on collagen only and collagen + dCM scaffolds stained for F-actin and α-actinin on day 3. Images were taken at 10x magnification. (Bar = 400 µm)

4. Discussion

We report a bioengineered porcine derived collagen composite scaffold for future cardiac tissue repair applications. Porcine tissue was acquired from an FDA and ISO certified tissue provider. The engineered scaffold consists of a sheet formed with Collagen Type 1 and decellularized cardiac matrix. Collagen Type 1 provides the main structural support and the dCM provides a cardiac matrix microenvironment. The decellularized tissue supported cell proliferation and viability. Additionally, micro-grooves were incorporated to promote cellular alignment and organization. We demonstrate the engineering of a robust scaffold for future cardiac applications. Initial characterization demonstrates significant cardiac cell viability on this scaffold. The composite scaffold provides cell culture support and provides a degradable environment during tissue repair. The study also showed that the grooved microtopography on the scaffold can be used to facilitate cellular structure and organization needed to replicate a striated myocardial wall.

Our studies are congruent with others that have demonstrated that extracellular matrix scaffolds create a supporting regenerative microenvironment and act as an inductive template for tissue repair of various organs[19]. Recent reports have established that cardiac patches derived from decellularized porcine derived through the use of an SDS protocol can provide distinct cell growth and attachment[20, 21]. Within this study, the preparation of dCM within a 1% SDS solution that

was also implemented into a scaffold presented similar results. Collagen based scaffolds in previous studies demonstrate optimal biological and structural computability with ECM[22]. Additional studies have shown that the use of decellularized porcine myocardium can create an ideal cardiac patch due to its microstructure and composition[23]. The dCM can play a significant role in creating the optimal scaffolds for cell delivery in rat myocardial infarction (MI) models. The use of dCM at higher concentration can optimize cellular function and it can also serve as an optimal scaffold for *in vivo* studies. Similarly, a recent *in vivo* study presented that the use of porcine dCM scaffolds can restore the function of the heart following scar formation in wistar rats that underwent acute and chronic myocardial infarctions[24]. The aim of engineering 3D scaffolds is to restore and repair the myocardial contractile cells that become permanently damaged following a heart attack. The use of dCM and collagen are important factors in the development of a scaffold that has the mechanical integrity and chemical composition to stimulate the growth and attachment of cells. These factors are significant for the accelerated clinical applications that are needed for myocardial infarction patients. This scaffold shows great promise for future applications in cardiac repair.

Future work will focus on the characterization of the mechanical strength of these scaffolds. Further quantitative and functional characterization must also be performed in order to establish biocompatibility and effectiveness of this engineered construct.

5. Conclusion

Engineered scaffolds composed of Collagen Type 1 and decellularized cardiac tissue showed robust cardiac cell viability. The micro-grooves facilitated cellular organization and alignment. The bioengineered composite sheet demonstrates great potential to be used in future applications of cardiac muscle repair where structure, mechanical support, and precise matrix components are required.

6. Acknowledgements

This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers UL1GM118979, TL4GM118980, and RL5GM118978. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. This work was also supported by the California State University-Long Beach UROP BUILD Health Research Peer Group, by CSUPERB and by CSULB COE. The authors wish to acknowledge Jeremy Bonifacio who facilitated 3D printing of the frames and Daniel Guerrero and Bryan Vu for creating the Solid Works template for the frames. Thank you to the members of the Therapeutic & Regenerative System Lab.

7. References

1. Thom, T. et al. Heart disease and stroke statistics--2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* **113**, (2006): e85-151 , https://www.altmetric.com/details.php?domain=www.ahajournals.org&citation_id=706696

2. Tian, X.-F., Cui, M.-X., Yang, S.-W., Zhou, Y.-J. & Hu, D.-Y. Cell death, dysglycemia and myocardial infarction. *Biomedical Reports* **1**, (2013): 341-346, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3917009/

3. Krishna, K., Berrocal, R., Rao, K. & Rao, K.R.S. Myocardial infarction and stem cells. *Journal of Pharmacy and Bioallied Sciences* **3**, (2011): 182-188, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3103911/

4. Langer, R. & Vacanti, J.P. Tissue engineering. *Science* **260**, (1993): 920-926, https://science.sciencemag.org/content/260/5110/920

5. Lin, Z. & Pu, W.T. Strategies for cardiac regeneration and repair. *Sci Transl Med* **6**, 239rv231 (2014), https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4280908/

6. Janaswamy, P., Walters, T.E., Nazer, B. & Lee, R.J. Current Treatment Strategies for Heart Failure: Role of Device Therapy and LV Reconstruction. *Curr Treat Options Cardiovasc Med*,(2016): 18, 57, https://pubmed.ncbi.nlm.nih.gov/27488313/?dopt=Abstract&holding=f1000,f1000m

7. Ogle, B.M. et al. Distilling complexity to advance cardiac tissue engineering. *Sci Transl Med* **8**, 342ps313 (2016). https://stm.sciencemag.org/content/8/342/342ps13

8. Zhang, J., Zhu, W., Radisic, M. & Vunjak-Novakovic, G. Can We Engineer a Human Cardiac Patch for Therapy? *Circulation Research* **123**, 244-265 (2018): 244-265, https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.118.311213

9. Hogrebe, N.J., Reinhardt, J.W. & Gooch, K.J. Biomaterial microarchitecture: a potent regulator of individual cell behavior and multicellular organization. *J Biomed Mater Res A* **105**, (2017): 640-661, https://pubmed.ncbi.nlm.nih.gov/27682265/

10. Ayala, P. et al. Engineered composite fascia for stem cell therapy in tissue repair applications. *Acta Biomater* **26**, (2015): 1-12, https://pubmed.ncbi.nlm.nih.gov/26283165/

11. Bowers, S.L., Banerjee, I. & Baudino, T.A. The extracellular matrix: at the center of it all. *J Mol Cell Cardiol* **48**, (2010): 474-482, https://pubmed.ncbi.nlm.nih.gov/19729019/

12. Bonnans, C., Chou, J. & Werb, Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* **15**, (2014): 786-801, https://www.nature.com/articles/nrm3904

13. Chan, B. & Leong, K. Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *European Spine Journal* **17**, (2008): 467-479, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2587658/

14. Domenech, M., Polo-Corrales, L., Ramirez-Vick, J.E. & Freytes, D.O. Tissue Engineering Strategies for Myocardial Regeneration: Acellular Versus Cellular Scaffolds? *Tissue Engineering Part B: Reviews* **22**, (2016): 438-458, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5124749/

15. Johnson, T.D., Braden, R.L. & Christman, K.L. Injectable ECM scaffolds for cardiac repair. *Methods Mol Biol* **1181**, (2014):109-120, https://pubmed.ncbi.nlm.nih.gov/25070331/

16. White, S.M., Constantin, P. & Claycomb, W. Cardiac physiology at the cellular level: use of cultured HL-1 cardiomyocytes for studies of cardiac muscle cell structure and function. *Am. J. Physiol.-Heart Circul. Physiol.* **286**, H823-H829 (2004), https://journals.physiology.org/doi/full/10.1152/ajpheart.00986.2003

17. Ayala, P. et al. Evaluation of a bioengineered construct for tissue engineering applications. *J Biomed Mater Res B Appl Biomater* **106**, (2018): 2345-2354, https://pubmed.ncbi.nlm.nih.gov/29130596/

18. Silver, F.H. & Trelstad, R.L. Type I collagen in solution. Structure and properties of fibril fragments. *The Journal of biological chemistry* **255**, (1980): 9427-9433, https://www.jbc.org/content/255/19/9427

19. Kc, P., Hong, Y. & Zhang, G. Cardiac tissue-derived extracellular matrix scaffolds for myocardial repair: advantages and challenges. *Regen Biomater* **6**, (2019): 185-199, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6683951/

20. Jonas, S. et al. Anisotropic engineered heart tissue made from laser-cut decellularized myocardium. *Scientific Reports* **6** (2016), https://www.nature.com/articles/srep32068

21. Ye, X. et al. Impact of decellularization on porcine myocardium as scaffold for tissue engineered heart tissue. *Official Journal of the European Society for Biomaterials* **27**, (2016): 1-8, https://pubmed.ncbi.nlm.nih.gov/26886818/

22.Chattopadhyay, S., Raines, R.T. & Glick, G.D. Review collagen-based biomaterials for wound healing.(Report)(Reprint). *Biopolymers* **101**, (2014): 821, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4203321/

23. Shah, M., Kc, P., Copeland, K.M., Liao, J. & Zhang, G. A Thin Layer of Decellularized Porcine Myocardium for Cell Delivery. *Scientific reports* **8**, (2018): 16206-16206, https://www.nature.com/articles/s41598-018-33946-2

24. Sarig, U. et al. Natural myocardial ECM patch drives cardiac progenitor based restoration even after scarring. *Acta Biomaterialia* **44**, (2016): 209-220, https://pubmed.ncbi.nlm.nih.gov/27545814/