Bioprinting microfabricated anisotropic myobundles towards the scaled generation of 3D cardiac tissue

ENGINEERING HONORS PROGRAM
UNIVERSITY OF MICHIGAN

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Introduction

- The excessive extracellular matrix (ECM) remodeling that occurs following myocardial infarction (MI) causes tissue stiffening and leads to the formation of disorganized and non-contractile scar tissue in place of previously functional myocardium.[1]
- The field of cardiac tissue engineering aims to create 3D cardiac tissues to model the healthy and diseased heart and investigate regenerative therapies.[2]
- In the healthy myocardium, cardiomyocytes (CM) form anisotropic cell bundles interspersed with capillaries and cardiac fibroblasts; we aim to recapitulate this structure *in vitro* for a regenerative therapy following MI (Figure 1A.)
- Previously in our lab, we used a small-scale microfluidic platform to generate elongated hydrogels with aligned synthetic fibers and CMs, or 'myotubes' (Figure 1B-C).

Figure 1. A) Schematic of myocardial tissue bundle structure and organization.[3] B) Fabrication process schematic of aligned myotubes. C) Schematic of myotubes embedded in a bulk hydrogel to create mm-scale anisotropic cardiac tissue.

- 3D bioprinting is rapidly emerging as a powerful technology in translational medicine and can be used to generate tissues at higher throughput.
- This work aims to develop a scalable approach towards creating 3D vascularized cardiac patch to regenerate diseased cardiac tissue using 3D bioprinting.

Specific Aims

1. **Perform a literature review** of the current state of bioprinting techniques for cardiac tissue.
2. **Identify reliable manufacturing parameters** for elongated fibrin-collagen composite hydrogels, including optimization of fibrin and collagen concentrations, printer parameters, and bioink material properties.
3. **Assess fiber alignment** in bioprinted hydrogels through image analysis to achieve reliable alignment.
4. Examine the role of bioink, fibers, and crosslinker composition on **viability and spreading of cells *in vitro***.

Materials and Methods

Bioink formulation: 4 mg/mL fluorescent collagen solution was prepared in Milli-Q water, HEPES, and $NaHCO_3$ and mixed with 10 mg/mL fluorescent fibrinogen in PBS containing dextran vinyl sulfone (DVS) electrospun fibers in equal parts.

Freeform Reversible Embedding of Suspended Hydrogels (FRESH)[4]: LifeSupport (Fluidform) bath was prepared according to manufacturer, then transferred to a 6-well plate to a depth of approximately 5 mm. Various concentrations of collagen bioinks were prepared. Print translation speed was 900 mm/min. After crosslinking, the gelatin bath was melted at 37°C to release prints.

Single Extrusion Scaffold (SES) Bioprinting[5]: 1 wt% agarose troughs were casted from SLA 3D printed molds. Each well was filled with 40 µL of crosslinking solution (1 U/mL thrombin and 6.3 mM NaOH in 1X PBS.) After printing, devices were crosslinked at 37°C for 30 minutes and hydrated in PBS. FibrilTool plugin in ImageJ was used for fiber alignment analysis.[6]

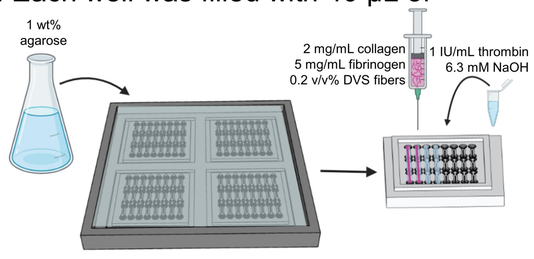
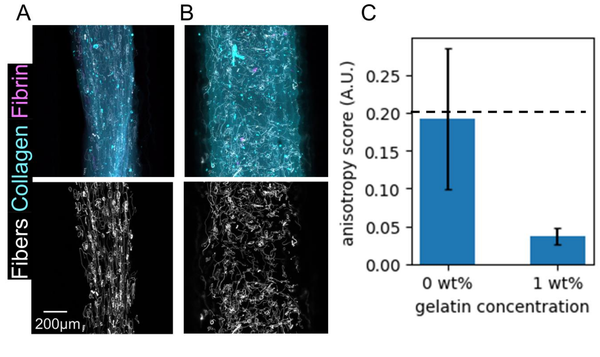


Figure 2. Schematic of agarose scaffold creation.

Results and Discussion

- Using FRESH bioprinting, a gelatin microparticle bath acts as a shear thinning material that structurally supports the print until the support bath is melted at 37°C.
- The final print thickness (500 µm vs 1 mm), extrusion speed (1.87, 2.5, and 3.125 mm/min), and bioink material was modulated (Figure 3A).
- **Result**: The gelatin topography on the surface of the prints that microparticle bath, created a visible hindered nanoscale fiber alignment (Figure 3B.)



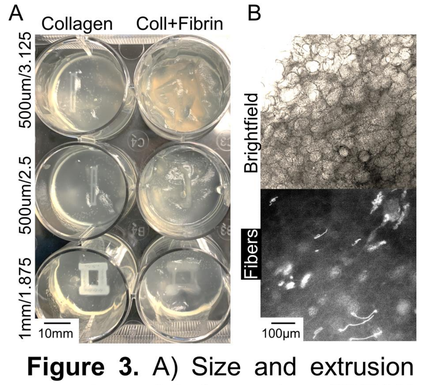


Figure 3. A) Size and extrusion speed modulation using FRESH printing prior to bath dissolution. B) Crosslinked print topography and fibers after bath dissolution.

Figure 4. Representative images of SES prints with (A) and without (B) 1 wt% gelatin, printed at 200 mm/min and with 22G needle. (C) Average alignment scores quantified for 0 wt% and 1 wt% gelatin concentration.

- Using SES, 1 mm agarose troughs were utilized to support the prints. The effect of the following factors on fiber alignment was determined: **bioink viscosity, needle size, extrusion rate, translation speed,** and **crosslinking bath height**.
- Lower viscosity (0% gelatin) prints were more anisotropic (Figure 4). Smaller needle size and high translation speeds resulted in more anisotropic and smaller diameter gels (Figure 5).

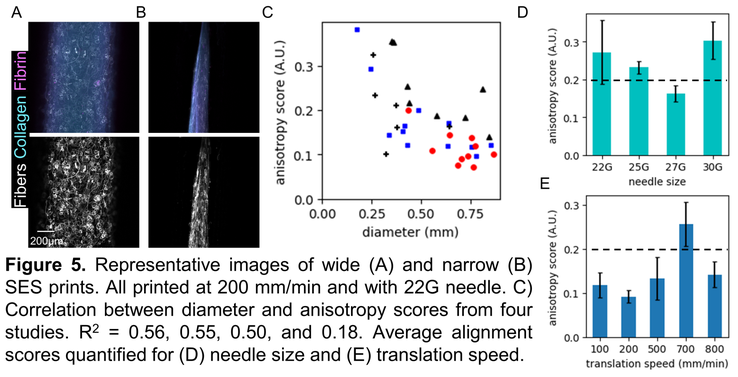


Figure 5. Representative images of wide (A) and narrow (B) SES prints. All printed at 200 mm/min and with 22G needle. C) Correlation between diameter and anisotropy scores from four studies. R^2 = 0.56, 0.55, 0.50, and 0.18. Average alignment scores quantified for (D) needle size and (E) translation speed.

Conclusions

- Bioprinting parameters have been quantitatively optimized, as summarized in Table 1.

Table 1. Optimized printing parameters for SES Bioprinting.

Parameter	Optimal Result	Experimental Range
Needle size	30G (0.16 mm I.D.)	22G – 30G
Translation Speed	700 mm/min	50 – 800 mm/min
Extrusion Volume	0.18 mm/print	0.1 – 0.5 mm/print
Bath volume	30 uL	0 – 70 uL

- No statistical significance of optimal parameters was achieved for some studies; more samples are needed.
- Aim 4 was not achieved due to inconsistent alignment; more studies are required to assess reliability.
- Future work will establish a sterile bioprinting setup and incorporate one or multiple model cell types, e.g. induced pluripotent stem cell-derived CM or CF, and will assess viability and cell spreading *in vitro.*

Acknowledgement

Thank you to the Engineering Honors Program for their support. This work was supported by the National Science Foundation (EEC-1647837, CBET-2033654) and Graduate Research Fellowship Program (DGE1256260).

References

1. Schroer A, *J. of Cell. Sci.* (2015) **2.** Karbassi E, *Nat. Rev. Cardiol. (*2020) **3.** 5. Brown R, *Annu. Rev. Pharmacol. Toxicol.* (2005) **4.** Hinton T, *Sci. Adv.* (2015) **5.** Ahrens J, *Adv. Mater.* (2022) **6.** Boudaoud, A. et al. *Nat Protoc* (2014).