Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Recapitulate Engraftment *In Vitro*

Katherine Chavez[1], Xining Gao[2, 3], Christopher Chen[3,4]

[1]Sharon High School, Sharon MA 02067; [2]Harvard-MIT Program in Health Sciences and Technology, Institute for Medical Engineering and Science, Massachusetts Institute of Technology; [3]Wyss Institute for Biologically Inspired Engineering, Harvard University; [4]Department of Biomedical Engineering, Boston University

Introduction

- In the US, a myocardial infarction occurs every 40 seconds[1], which leads to permanent impairment of the heart due to the low regeneration rate of cardiomyocytes[2]
- Engrafting human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) holds promise for remuscularization; however, graft-associated arrhythmia has been observed in *in vivo* non-human primate studies[3]
- Our lab has developed a 2D *in vitro* model platform to study how iPSC-CMs integrate and engraftment into cardiac tissue
- **The work here aims to test the feasibility of using sarcomere alignment as a means to assess structural integration.**

Methods

Substrate Fabrication: To enable high resolution imaging, we fabricated polydimethylsiloxane (PDMS) coated glass bottom dishes.

1. Thin glass coverslips were spin-coated with a small drop of uncured polydimethylsiloxane (PDMS); rotational acceleration caused PDMS to accelerate outward upon spinning, coating the cover slip uniformly (Fig. 1)
2. PDMS-coated glass coverslips were then affixed to 35mm dishes with a hole at the base such that the PDMS coating faces the dish's interior
3. PDMS dishes were then cured at 60°C overnight

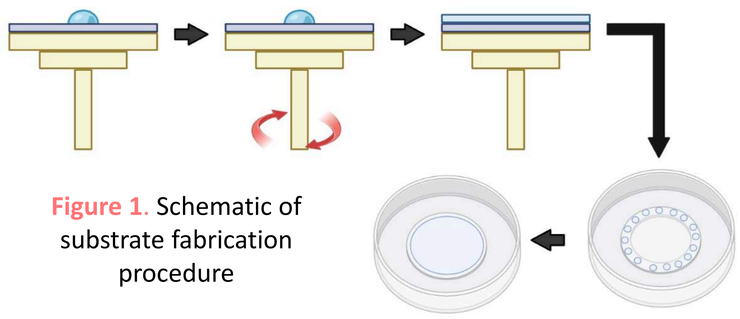


Figure 1. Schematic of substrate fabrication procedure

Micropatterning and seeding: To generate 2D host tissues, dishes were microcontact printed with a fibronectin pattern depicted below (Fig. 2) to confine iPSC-CM attachment and control cell alignment.

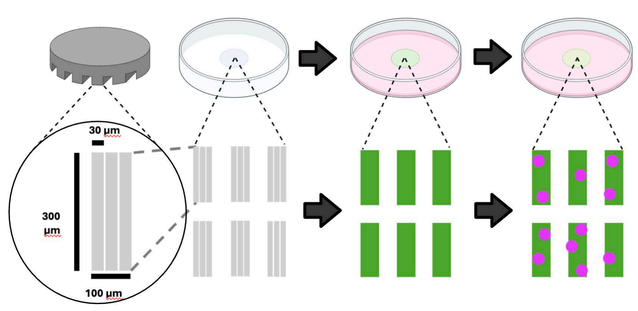


Fig 2. Schematic of microcontact printing and seeding

1. PDMS "stamps" were created with a rectangular array pattern. Each rectangle measures 300µm by 100µm and contains internal alignment cues (30µm lines spaced 5µm apart).
2. Stamps are incubated with a fibronectin solution for one hour, dried, and lightly placed onto the PDMS-coated dishes to transfer the fibronectin pattern.
3. Prior to seeding, dishes are incubated with a 0.2% Pluronics F-127 solustion to block cell attachment outside the fibronectin pattern.
4. iPSC-CMs with GFP-tagged TTN were seeded onto the dishes densely and cultured for 14 days to generate confluent, "host" cardiac tissues.
5. "Graft" iPSC-CMs with mApple-tagged ACTN2 were sparsely seeded over the host tissues to ensure that 1-3 cells landed on each tissue.

Imaging and analysis: TTN-GFP concentrated around the z-disc illuminated a "ladder" denoting the sarcomeres, the directionality of whose "rungs" (discs) were measured via ImageJ to determine sarcomere alignment.

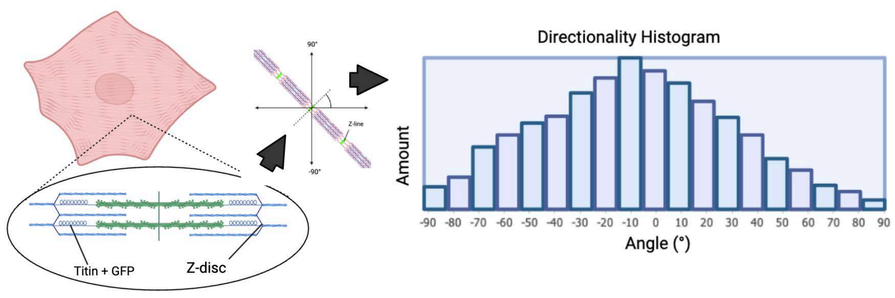
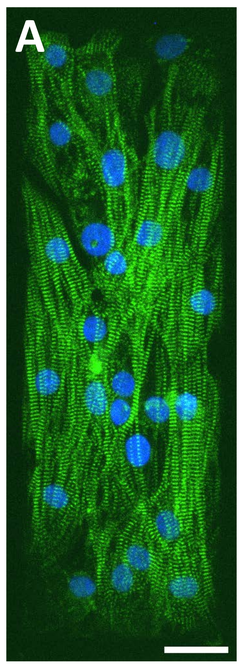


Fig 3. Schematic of z-disc directionality histogram generation

1. Tissues were fixed and imaged with a spinning disk confocal microscope on Day 5 after engraftment. TTN-GFP reflects the z-discs, indicating the direction of sarcomeres (protein complexes that act during contraction and units constituting strand-like myofibrils within cardiomyocytes).
2. Images underwent thresholding and sharpening to produce a segmented skeleton.
3. ImageJ directionality analysis determined the angle of z-discs with respect to the horizontal axis.
4. ImageJ outputs the number of segments at particular angles to generate a representative directionality histogram.

Non-engrafted, Control Cardiac Tissue



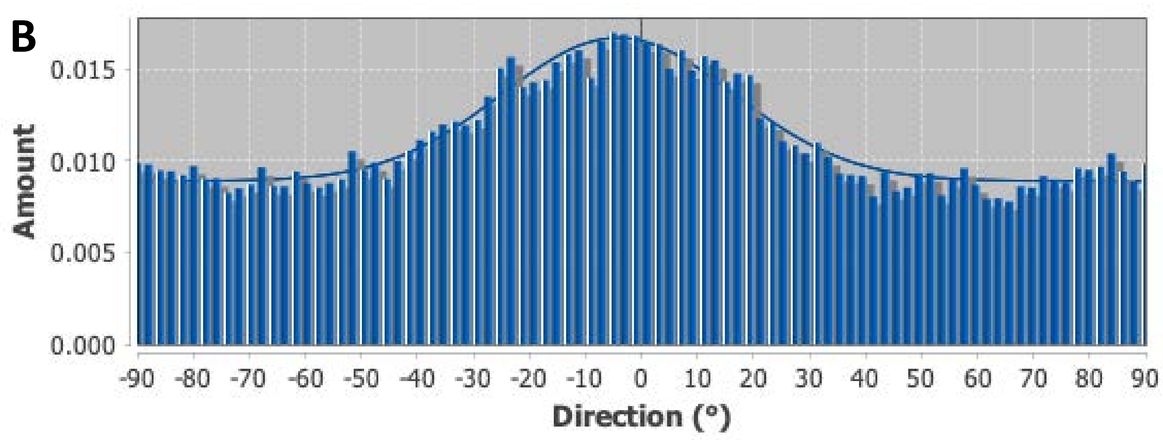
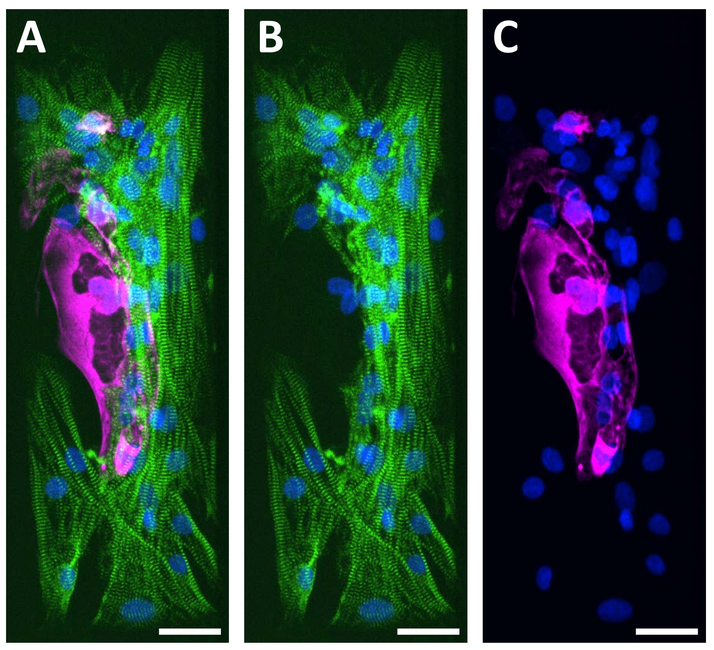


Fig 4. A) Composite image of control host tissue that did not receive graft iPSC-CMs. *Blue: DAPI, green: host TTN-GFP, scale bar: 30µm.* B) Histogram of host cardiomyocyte z-disc directionality.

Sarcomeres visually appear to align in the vertical axis with our fibronectin pattern. The z-disc directionality histogram supports this observation, containing a relatively tall, smooth, clear-cut peak at 0°, and a plateaus as it departs from 0°.

Poor Structural Integration



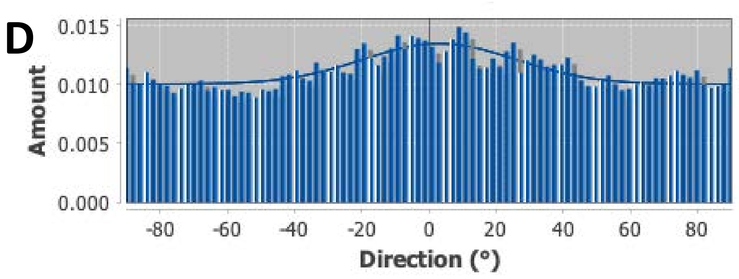
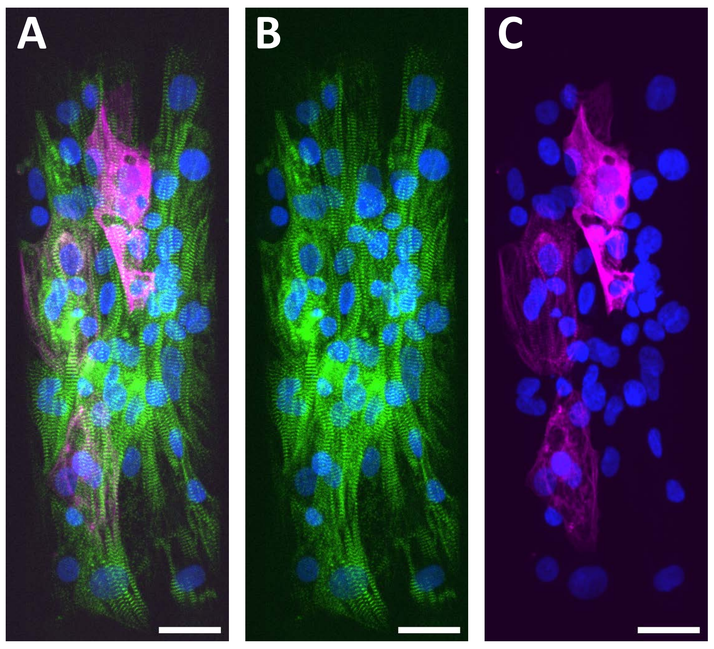


Fig 5. Composite images of A) CMs engrafted on host tissue, B) isolated host tissue, and C) isolated engrafted CMs. *Blue: DAPI, green: host TTN-GFP, magenta: graft ACTN2-mApple, scale bars: 30µm.* D) Histogram of host cardiomyocyte z-disc directionality.

Engrafted and host CMs are visually distinct. The host tissue's sarcomeres bend to adapt the original structure around the shape of the engrafted cell. The directionality analysis shows more spread and a less distinguishable peak to support this observation.

Successful Structural Integration



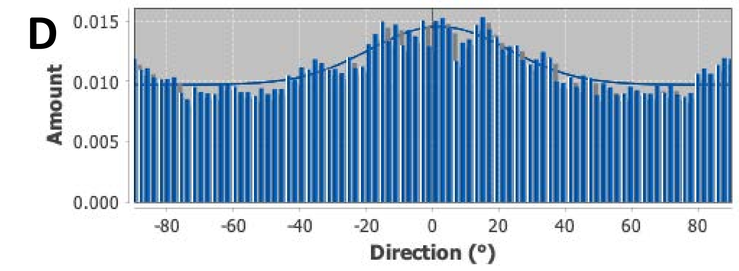


Fig 6. Composite images of A) CMs engrafted on host tissue, B) isolated host tissue, and C) isolated engrafted CMs. *Blue: DAPI, green: host TTN-GFP, magenta: graft ACTN2-mApple, scale bars: 30µm.* D) Histogram of host cardiomyocyte z-disc directionality.

The engrafted CMs appear to stretch across the existing tissue, adapting themselves to the host rather than intruding on it. The host structure retains its vertical alignment. The resultant histogram contains a relatively tall peak with more uniformity, resembling that of the control. This suggests more success in structural integration of engrafted CMs.

Conclusions

We observe two disparate conditions among the engrafted tissue: grafts dislodge within (Fig. 4A) or inlay atop (Fig. 5A) the host cardiac tissue. The precedent data establish host sarcomeric alignment as a feasible metric for engraftment success. The current image processing platform remains insufficient to clearly identify individual z-discs; in the future, we plan to reanalyze these samples with SarcGraph, an improved sarcomere analysis tool developed by our collaborators in the Lejeune Lab. Moreover, further experimentation will supply a fuller data set to assess how such conditions affect integration. Our platform, if successful, holds promise for improving safety and efficacy of engraftment therapy.

References

1. American Heart Association
2. Derks et al. *bioRxiv* 2023
3. Liu et al. *Nature Biotechnology* 2018

Acknowledgements

I am endlessly grateful to Boston University and the RISE program for providing this research opportunity. I would like to thank Xining Gao for her expert mentorship, Professor Christopher Chen for hosting me, and the rest of the Chen Lab for their support.

Funding: NSF ERC EEC-1647837 (CELL-MET), XG also acknowledges funding from NSF GRFP.