Myofibroblast Differentiation in Cardiomyocyte-Cardiac Fibroblast Bi-Layer Tissues on Tunable Synthetic Fibrous Matrices

Darcy D. Huang[1] (Honors Capstone), Samuel J. DePalma[1], Austin E. Stis[1], Robert N. Kent III[1], Brendon M. Baker[1]

[1]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI

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UNIVERSITY OF MICHIGAN

Introduction

- Cardiac pathologies are the leading cause of death in the United States[1] with fibrosis implicated in many of these diseases.
- A hallmark of cardiac fibrosis is the differentiation of cardiac fibroblasts (CFs) into myofibroblasts (MFs) that synthesize and remodel the cardiac extracellular matrix (ECM).[2]
- Previous studies have shown that modifications to the mechanics of the extracellular environment[3] and cellular crosstalk between cardiomyocytes (CMs) and CFs[4] are integral to this transformation.
- However, many of their *in vitro* settings fail to accurately recapitulate the mechanics and architecture of the native heart, let alone integrate different cell types.
- Here, we developed a novel bi-layer tissue platform (BLT) consisting of layers of CFs and CMs separated by a tunable synthetic fibrous matrix to mimic the ECM mechanics of healthy or diseased tissue states.
- Using this platform, we sought to understand how the synergy between mechanical cues and heterotypic cell-cell communication maintains tissue homeostasis vs. drives cardiac fibrosis.

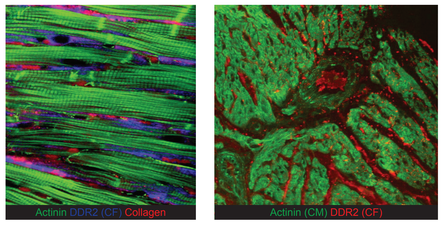

Figure 1. Fluorescent images of native heart tissue sections.[5,6]

Materials and Methods

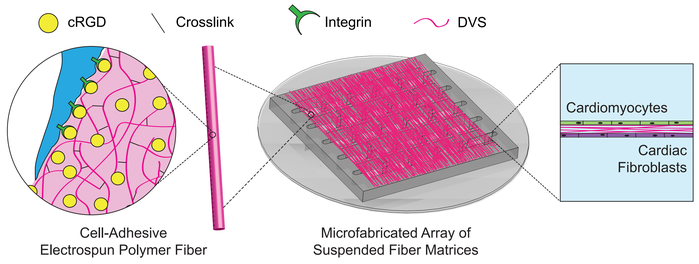

Figure 2. Schematic of the BLT. Monolayers of CMs and CFs are separated by an electrospun dextran vinyl sulfone (DVS) fiber matrix with tunable mechanical properties.

- Substrate Fabrication with Tunable DVS Fiber Matrix: DVS fibers are electrospun onto microfabricated polydimethysiloxane (PDMS) substrates containing an array of square microwells (2×2 mm). Matrix alignment and stiffness were controlled by modulating the fiber collection mandrel speed and UV light exposure, respectively. Fibers were functionalized with the cell-adhesive peptide RGD (CG**RGD**S).
- Bi-Layer Tissue Formation and Culture: First, induced pluripotent stem cell-derived CMs are seeded on top of the matrix and allowed to adhere for 3 days. Then, normal human CFs (NHCFs) are pipetted down the channels of the substrate. The substrate is inverted to allow the NHCFs to adhere to the bottom of the matrix. The next day, the substrates are reoriented upright. Cells are cultured for an additional 6 days in either RPMI B27 with insulin and Primocin or FGM3. Basal media is selectively supplemented with TGF-β1 (10 ng/mL).
- Analysis of MF Differentiation: Immunostaining for α-smooth muscle actin (αSMA) is conducted to assess MF differentiation. Samples are imaged using confocal microscopy and quanitifed using custom MATLAB scripts.

Results and Discussion

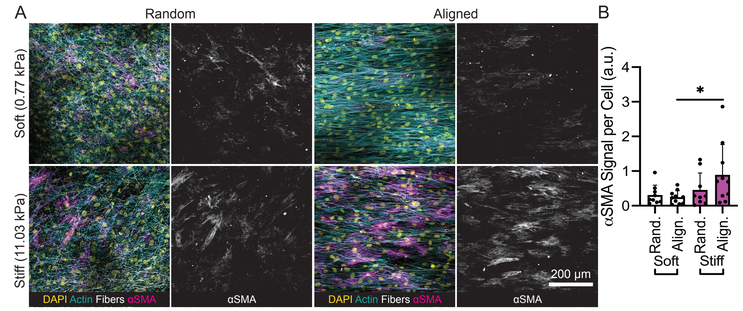

Figure 3. A) Representative fluorescent images of CF monocultures on varying matrix mechanics. B) Quantification of the αSMA signal per cell. * p < 0.05 by two-way ANOVA.

- The effect of varying matrix mechanics on MF differentiation from CF monocultures was studied.
- Aligned, stiff (11.03 kPa) matrices induced significantly higher MF differentiation compared to random, soft (0.77 kPa); aligned, soft; and random, stiff matrices upon the addition of the pro-fibrotic cytokine TGF-β1, supporting the role of both soluble and mechanical cues in MF differentiation.

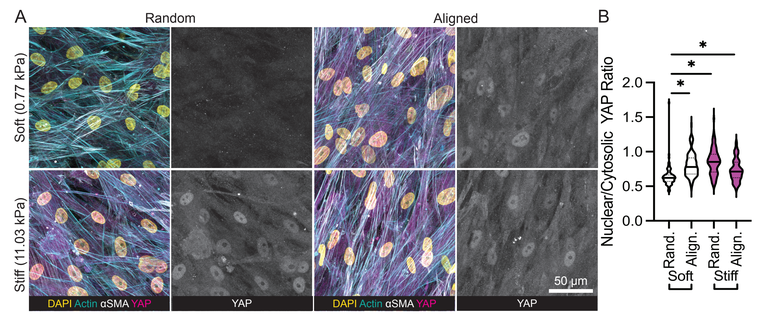

Figure 4. A) Representative fluorescent images and B) quantification of nuclear/cytosolic YAP localization. * p < 0.05 by two-way ANOVA.

- Nuclear localization of YAP was heightened in all conditions except for the soft, random matrices, further supporting altered mechanosensing in these environments.

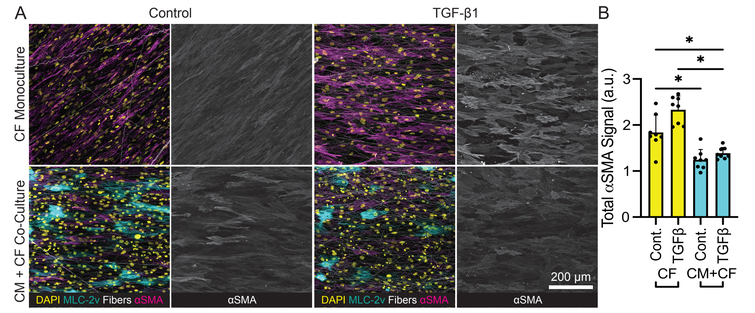

Figure 5. A) Representative fluorescent images of CF monocultures and CM + CF co-cultures. B) Quantification of the total αSMA signal. * p < 0.05 by two-way ANOVA.

- Co-culture tissues were seeded to examine the influence of CM-CF crosstalk on MF differentiation.
- Co-culture significantly decreased MF differentiation even in the presence of exogenous TGF-β1.
- This suggests that protective signaling from CMs acts to impede MF differentiation.

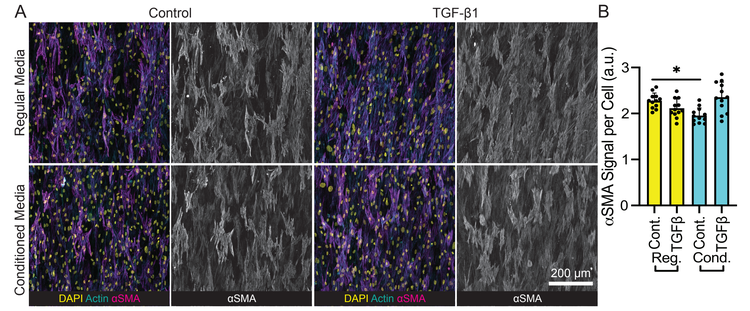

Figure 6. A) Representative fluorescent images of CF monocultures with regular or CM conditioned media. B) Quantification of the αSMA signal per cell. * p < 0.05 by two-way ANOVA.

- CF monocultures were cultured in regular or CM conditioned media to characterize the protective effect of CM + CF co-culture.
- Culture in CM conditioned media led to a small reduction in MF differentiation; however, this effect was negated by the presence of exogenous TGF-β1.
- This suggests that CMs need to be cultured together with CFs to retain their protective effects.

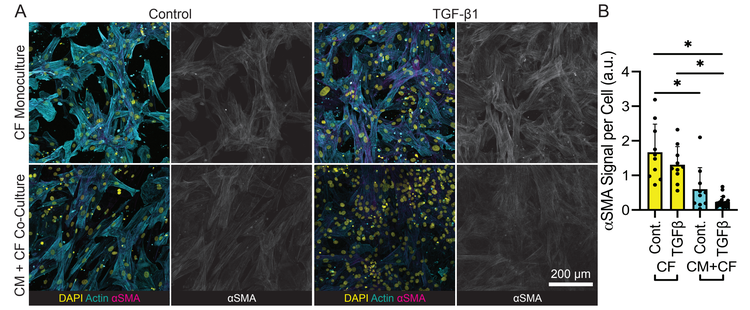

Figure 7. A) Representative fluorescent images of CFs ~300 μm away from CMs. B) Quantification of the αSMA signal per cell. * p < 0.05 by two-way ANOVA.

- CFs were seeded on the PDMS ~300 μm below the CMs on the fibrous matrix to characterize the protective effect of CM + CF co-culture.
- CMs still significantly decreased MF differentiation even with separation.
- This suggests that soluble factors from CMs inhibit MF differentiation.

Conclusions and Future Directions

- Our findings demonstrate that physical cues from the ECM play a critical role in MF differentiation, as supported by altered cellular mechanosensing as a function of matrix architecture and mechanics.
- Interestingly, co-culture with CMs hindered MF differentiation through protective paracrine signaling that necessitates CMs in the vicinity of CFs, potentially due to required reciprocal heterocellular crosstalk.
- Future work will explore the influence of matrix mechanics on CM-CF communication and identify the mechanisms by which CMs inhibit MF differentiation via secretomics analyses and RNA sequencing.

Acknowledgements and References

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