We are developing materials to enhance the maturation of cardiomyocytes by utilising patterns found in nature.

We **micropatterned** proteins, in geometries adhering to the golden ratio, on **hydrogels** to study the effects on cardiomyocyte physiology.

Cardiomyocyte Maturation: A Universal Improvement.

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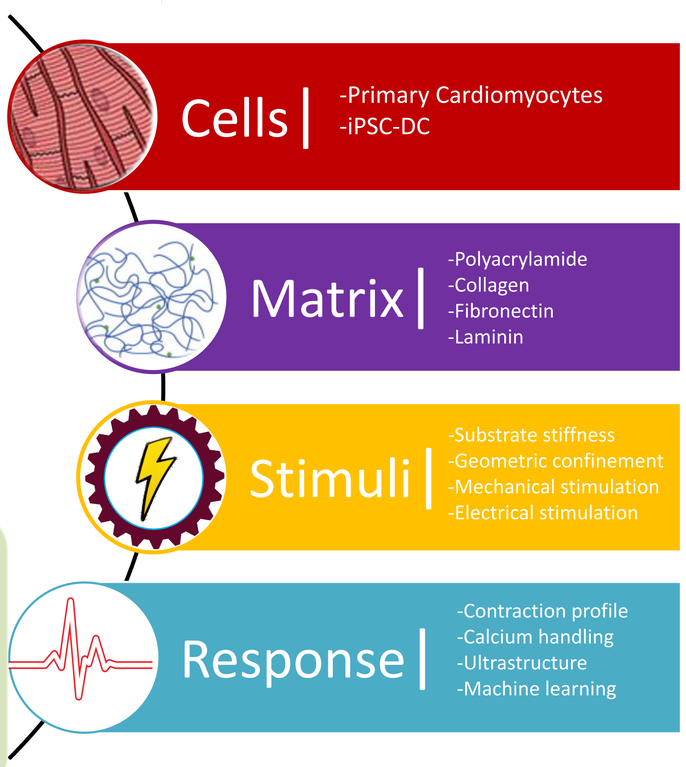
Introduction

- Induced pluripotent stem cell derived cardiomyocytes (iPSC-DC) are frequently used in pre-clinical models[1].
- iPSC-DC have immature phenotypes and many researchers are trying accelerated iPSC-DC maturation[2].
- Electrical, mechanical, and geometrical confinement, stimulates iPSC-DC towards a more matured phenotype [3].
- The desire to create smarter biomaterials for cardiac patches and develop better cardiomyocytes for *in vitro* modeling and drug screening [4].

Aims

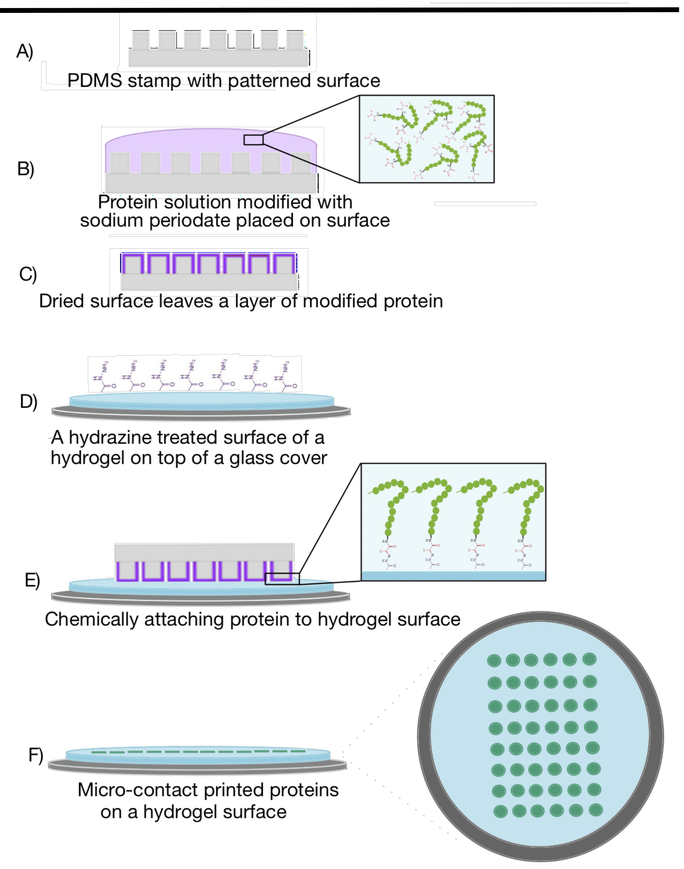
- Find geometries that aid iPSC-DC maturation.
- Develop a non-invasive, contraction-profile based, machine learning platform to assess cardiomyocyte maturation.
- Produce cardiac patches that can aid and reverse the effects of a heart attack.



Methods

- **Polyacrylamide hydrogels** were chosen to microcontact print combinations of ECM proteins because their stiffnesses can be easily tuned to mimic the mechanical properties of native tissues [5].
- Experimenting with different geometries and hydrogels mechanical properties enables fine tuning of cell attachment and physiology.

Figure 1. Schematic of how microcontact printed proteins are adhered to polyacrylamide hydrogels.



Results 1: Cardiomyocyte isolation

- Optical microscopy characterization of cardiomyocyte morphology shows rounded, partially digested cells when using old isolation methods and squared, striated and calcium tolerant cells using new isolation method.
- Loading of ratio-metric calcium dye (FURA-2) into cardiomyocytes shows efficiency of calcium handling during contractions.

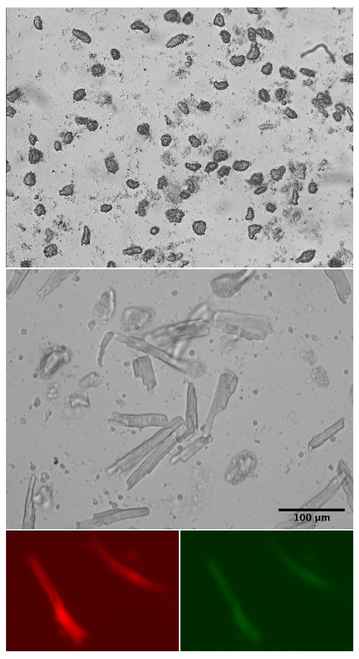


Figure 2: Optical images of freshly isolated adult cardiomyocytes from a mouse. Isolated cells using collagenase solution with mechanical agitation- old method (top). Isolated cells using controlled buffered dissociation, and perfusion techniques – new method (middle), Calcium uptake fluorescence (bottom).

Results 3: Micropatterned geometries effect on ultrastructure

- Optical and transmission electron microscopy (TEM) Characterization was done for cardiomyocytes patterned on different substrates.
- Cardiomyocytes patterned on ECM substrates, at physiologically relevant stiffnesses, showed ultrastructure more like adult cardiomyocytes.
- Mitochondria in cells on ECM substrate are more prevalent and aligned with the sarcomeres and long axis of the cell.

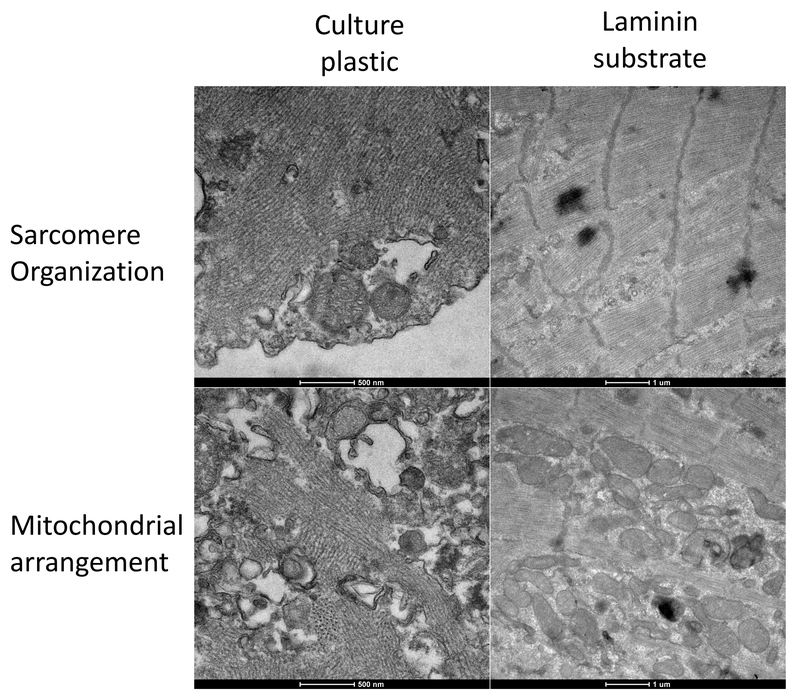


Figure3: TEM micrographs of primary embryonic cardiomyocytes on culture plastic and a laminin ECM substrate.

Results 3: Contractile Profiles

- Non-invasive contraction profiles produced from high-speed, high-resolution videos of cardiomyocytes at different developmental stages.
- Machine learning developed to group results based on contraction profiles.

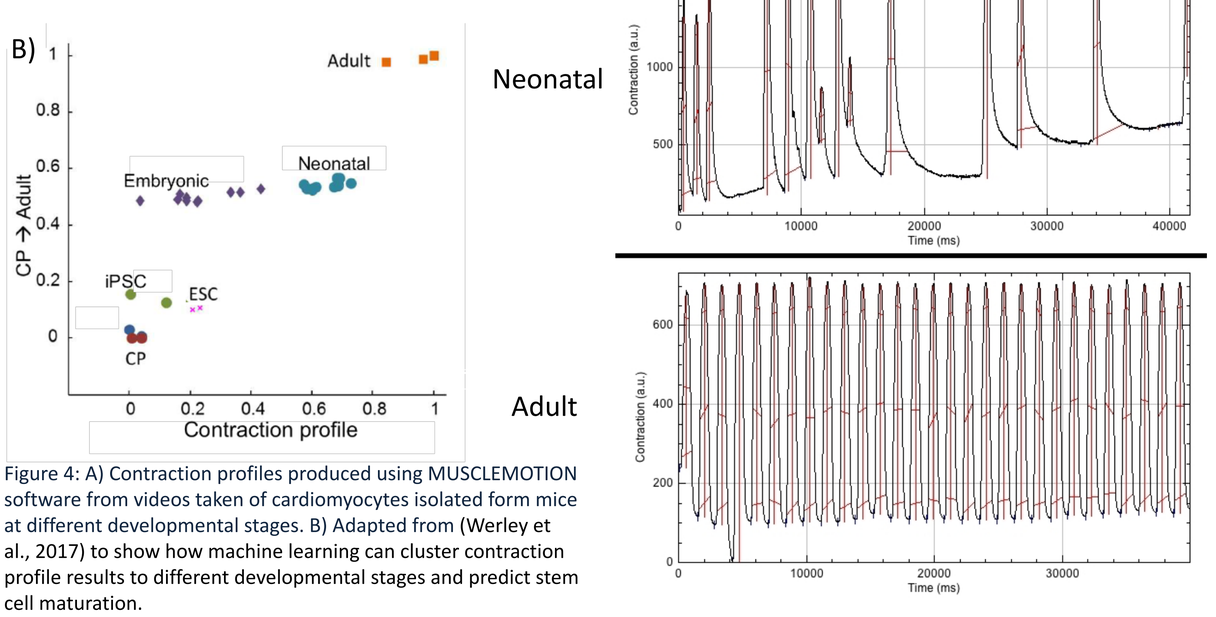


Figure 4: A) Contraction profiles produced using MUSCLEMOTION software from videos taken of cardiomyocytes isolated form mice at different developmental stages. B) Adapted from (Werley et al., 2017) to show how machine learning can cluster contraction profile results to different developmental stages and predict stem cell maturation.

Conclusion

- Here, we demonstrate a new method of isolating pure cardiomyocytes from different developmental stages of murine animals.
- Patterning cardiomyocytes on specific substrates enables maturation of ultrastructural features of cardiomyocytes in vitro.
- Analyzing the contraction profiles of primary cardiomyocytes patterned on an array of substrate materials and geometric confinements, is allowing us to develop a machine learning platform to analyze and predict the maturation states of iPSC-DC.

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