Cardiomyocytes are the cells that make up heart muscle, and sarcomeres are the main contractile units within a cardiomyocyte.

Sarcomere structure and organization plays a main role in cardiomyocyte function.

Hypoplastic Left Heart Syndrome (HLHS) is a congenital heart defect that leads to underdevelopment of the left side of the heart.

CM microtissues can reveal differences between healthy and HLHS sarcomere structure/alignment, and allow early diagnosis and treatment testing.

### Objective & Impact of Professor’s Research

To Engineer a physiologically relevant, patient specific model of HLHS to assess cardiomyocyte function by measuring sarcomere structure, calcium handling, and contractility.

- Find key differences between healthy and HLHS cardiomyocytes in order to gain a better understanding of HLHS. My individual objective was to fabricate hiPSC-CM microtissues on microcontact printed substrates and quantify sarcomere formation.

### Introduction

- Cardiomyocytes are the cells that make up heart muscle, and sarcomeres are the main contractile units within a cardiomyocyte.
- Sarcomere structure and organization plays a main role in cardiomyocyte function.
- Hypoplastic Left Heart Syndrome (HLHS) is a congenital heart defect that leads to underdevelopment of the left side of the heart.
- CM microtissues can reveal differences between healthy and HLHS sarcomere structure/alignment, and allow early diagnosis and treatment testing.

### Methods

#### 1. Human Induced Pluripotent Stem Cell Derived Cardiomyocytes (hiPSC-CMs)

- Fix Cells onto cultured substrates
- Permeabilization - allows antibody to access the protein
- Blocking - Reduces non-specific antibody binding
- Primary Antibody - Binds to target protein
- Secondary Antibody - Binds to primary antibody with fluorophore
- Mounting and Imaging

#### 2. Microcontact Printing of PDMS Culture Surfaces

1. Incubate 30x30 PDMS stamp with FN protein
2. Stamp on PDMS coated glass coverslip
3. Seed hiPSC-CMs on patterned surface

#### 3. Staining Cells with Antibodies

1. Fix Cells - Secures cells on substrate
2. Permeabilization - allows antibody to access the protein
3. Blocking non-specific antibody binding
4. Primary Antibody - Binds to target protein
5. Secondary Antibody - Binds to primary antibody with fluorophore
6. Mounting and Imaging

### Results

![Figure 1](image1.png)

**Figure 1.** 30umx30um lanes stained with fibronectin, made from PDMS microcontact printing.

![Figure 2](image2.png)

**Figure 2.** 30x30 lanes, microcontact printed, seeded with hiPSC-CMs.

### Limitations:

- Cells often stick together instead of forming lanes
- Sarcomere quantification was done by hand

### Future Directions

1. Calcium imaging using lanes to assess contractile function
2. Quantify and compare sarcomere structure, expression of cardiac genes, and metabolism.
3. Compare responses to stretch, hypoxia, and clinically-relevant drugs.

### Skills Learned and Advice to Future SHINE Participants

- **Learned how to:**
  - Fabricate micropatterned PDMS stamps and polyacrylamide gels
  - Fix cells onto cultured substrates
  - Stain cell proteins with antibodies
  - Quantify sarcomere appearance

- **Advice:**
  - Make the most out of SHINE by going to every event and taking advantage of every opportunity
  - Don’t be afraid to ask questions, your mentor is your biggest resource
  - Make friends and connections, and have fun!

### Citations