Engineering PDMS Pillars for Compartmentalization of Motor Neurons and Skeletal Muscle Myotubes

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Introduction

In Professor McCain's Laboratory of Living Systems Engineering, the primary focus is the development of platforms that can be used to create safe and effective cures for human diseases. In order to successfully reach their goal, they engineered micro-scale models that mimic both healthy and diseased human tissues. Of the different tissues that make up the body, the two that are given the most focus are cardiac and skeletal muscle tissue. During my seven weeks in SHINE, I have been studying skeletal muscle tissue and have done research on the engineering process of a physiologically relevant human "Organ on a Chip" system that would resemble physiological skeletal muscles.

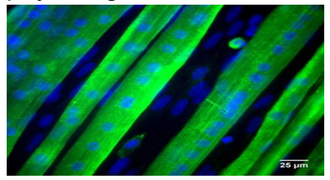

Figure 1. Engineered skeletal myotubes. PC: Archana Bettadapur, Gio Suh, Holly Huber, Alyssa Viscio, and Evelyn Wang.

Objective & Impact of Professor's Research

The overall objective of the research being conducted is to improve current neuromuscular cell models so that they may better represent muscles in the body and in neuromuscular diseases. My goal during the seven weeks was to construct a chamber that can hold motor neurons in pillars, allowing axons to branch out from the main neuron body and innervate myotubes to form neuromuscular junctions. Ideally, our system will imitate the function of healthy and diseased neuromuscular tissues.

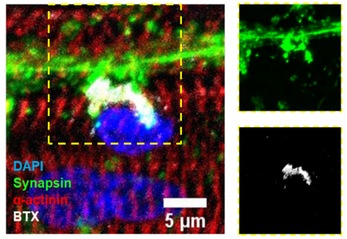

Figure 2. Engineered neuromuscular junctions. Motor neurons (green) innervate skeletal muscle myotubes (red) at clusters of acetylcholine receptors (white). PC: Jeffrey Santoso

Methods

During the seven weeks, I designed different chamber systems that would be able to wedge motor neuron aggregates between the pillars. After designing the chambers using Tinkercad, I was able to make a master mold of them using PDMS, a flexible and transparent polymer that hardens when baked. Using the master PDMS mold, we made a final version of the mold using PDMS again.

PDMS Master Protocol

Ratio of Base to Curing Agent is 5:1
mold release agent = detergent in 75% ethanol

1. Place a cup on scale and tare it to set weight to 0g
2. Carefully pour Base into the cup
3. Weigh the Base and divide it by 5 and add that amount of the Curing Agent
4. Add 1 drop of food coloring in the cup
5. Place the cup in the mixer and set the dial to match total weight
6. Mix and de-gas the contents for 2 minutes
7. Vacuum the molds with PDMS for 30 minutes or until there are no visible bubbles
8. Place in 100°C oven for 1 hour
9. Let cool and remove hardened master from the mold
10. Pre-treat the master with 35ml of mold release agent in a petri dish for 30 minutes in the vacuum
11. Air dry the master and place it on a new petri dish
12. Repeat steps 1-3 and 5-9,

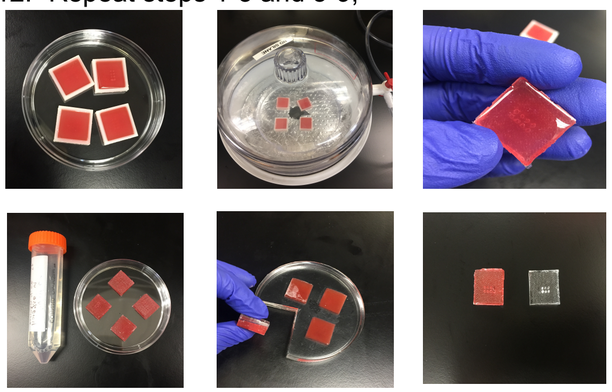

Figure 3. PDMS protocol. From left to right (top to bottom) are steps 4, 5, 9, 10, and 12 of the PDMS protocol used for the camber prototypes that were designed. PC: Jaylene Lopez

Results

During my first week, I designed a total of five different chambers. My original porotype was 22mm in length, width, and height. We attempted to make a mold of the chamber and discovered that it would not be possible to remove the hardened PDMS because the chamber was too high and there would be a possible break of one of the cylinders. From there, I designed four other chambers that varied in height but remained the same in terms of length and width. We were able to conclude that the chamber of 5mm in height with cylinders that are 1mm tall worked best for molding.

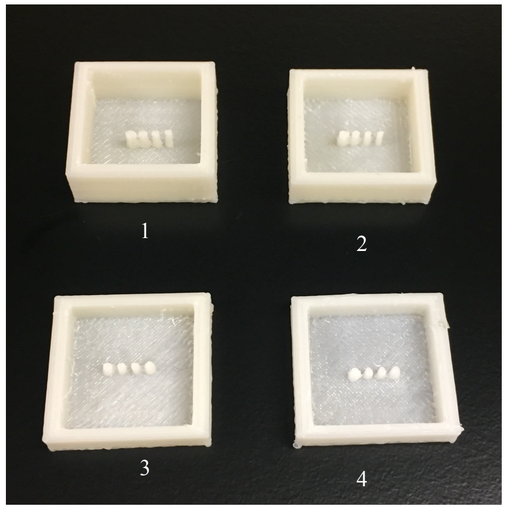

Figure 4. Chamber Prototypes. All 4 chambers are made up of boxes and cylinders of the same length and width, the only difference is height. (1) Box: 10mm, Cylinders: 5mm (2) Box: 8mm, Cylinders 4mm (3) Box: 6mm, Cylinders: 3mm (4) Box: 5mm, Cylinders: 2.5mm. PC: Jaylene Lopez

Figure 5. First chamber prototype. The chamber is made of a box and cylinders that are 22mm in length, width, and height. PC: Jaylene Lopez

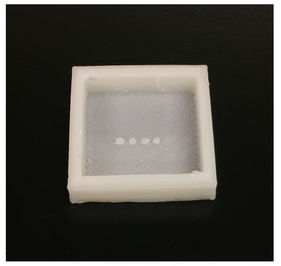

Figure 6. Finalized chamber. The chamber is made of a box that is 22mm in length and width, meanwhile its height is 5mm. The cylinders inside are 1mm in length, width, and height. PC: Jaylene Lopez

Future Steps

The next step is to test the cylinders in the chamber and their ability to hold the motor neurons in place. This will be done using dyed polymer particles the size of the motor neurons to mimic what they would do. If successful, the same procedure will be performed with the cells being cultured and muscle junction will be analyzed.

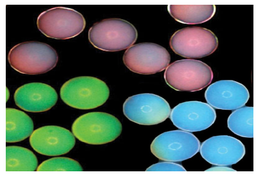

Figure 7. Dyed particles. Polymers prepared to produce colors on imaged structures when using a microscope. Adapted from "Brighter Inks, Without Pigment" by many Morone, 2014. Copyright 2014 by President and Fellows of Harvard College

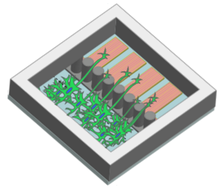

Figure 8. Proposed design for pillar-based separation of motor neurons and skeletal muscular films. PC: Jeffrey Santoso

Relation to STEM Coursework

While shadowing my mentor and working in Dr. McCain's laboratory, I was given the opportunity to experience how PhD students conduct research. I was also able to explore the laboratory and expanded my knowledge of the inner workings of biomedical engineering. The integration of engineering and biological studies in the laboratory has allowed me to create a chamber for the "Organ on a Chip" platform which mimic both healthy and unhealthy muscles in the broad study being conducted by those in the lab.

Acknowledgements

Dr. Katie Mills, Dr. Megan Herrold, Professor McCain, Jeffrey Santoso, SHINE staff, Laboratory of Living Systems Engineering