Development of an Electromechanical System for Stimulating Heart Cells and Tracking their Motion

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Introduction

Heart cells are unique in that their micro-environment has both electrical and mechanical stimulation.

To mimic this environment novel devices, including ITO based electrical stimulation systems, and unidirectional and bidirectional mechanical straining systems, were developed [1]. Additionally, live cell tracking software was developed in python to track the cell's response to stimulation.

Methods

All test were done using H9-C2 embryonic rat myoblasts. This project has three distinct parts : electrical, mechanical, and software.

Since the PET sheet which the ITO is set on is inelastic, the electrical and the mechanical systems were tested separately.

Gelatin was used as a coating for the electrical straining system, as collagen has trouble adhering to the hydrophobic ITO.

Mechanical Design

The mechanical stimulation devices were fabricated with 3D printing and laser cutting. The PDMS well was molded from a resin 3d printed template. There were two devices tested, a uniaxial and a biaxial straining system. (Figures 1 and 2 respectively)

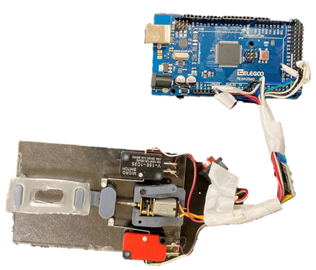


Figure 1: Uniaxial device, with range of 5mm at frequency 0.125 Hz

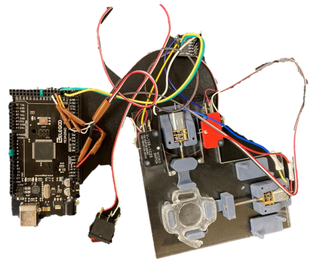


Figure 2: Biaxial device, with range of 5mm at 0.125 Hz in the x and 2.5mm at 0.0625 Hz in the y

Electrical Design

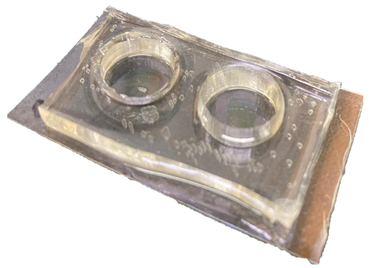


Figure 3. PDMS mold bonded to ITO film on PET sheet used for electrical stimulation trials

The electrical stimulation device was fabricated using ITO coated PET film. (Figure 3) We cultured H9-C2 embryonic rat myoblasts in four cultures, two stimulated and two unstimulated. They were stimulated via a signal generator attached with alligator clips at 60 Hz and 0.6v in a sinusoidal wave form.

Software Design

The cell analyzer application (Figure 4) is written in python using OpenCV. It analyzes videos of cell cultures growing to produce useful metrics including position and area for each cell detected. The code breaks the video down into individual frames. Each frame is edited to improve the algorithm's accuracy. The cell boundaries are then isolated from the background with edge detection. Every contour is then detected within the image and trimmed down through a heuristic into objects that fit specified criteria of size and shape. Each detected object/cell has a central position and area recorded with an ID number. The tracker then analyzes the frames and updates the cells' information overtime. Once every frame of the video has been analyzed, it exports the data (Figure 5).

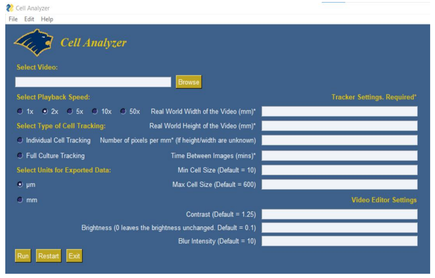


Figure 4. Snip of the GUI input menu of the Cell analyzer.

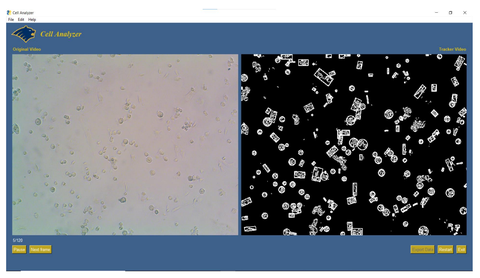
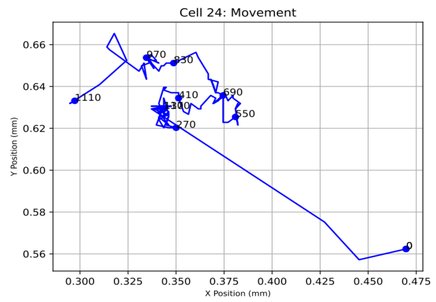
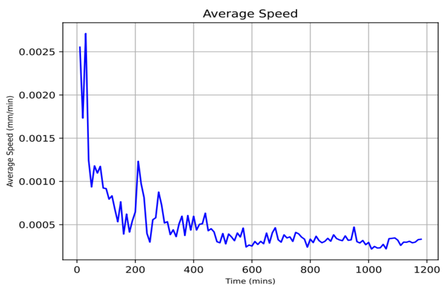


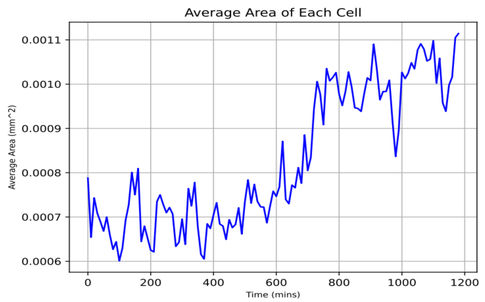
Figure 5. Snip of the GUI output of the Cell analyzer.

Discussion Software



Three outputs from the cell tracking software are displayed in figures 6-8. These outputs help to characterize the behavior of the culture in various ways.
The detriment of this software is that to use it, the video be high resolution and recorded over a long period. Several issues arise due to these factors. For instance, the incubator vibrates slightly while operating causing the video to lose focus over time. The clarity of the bottom of the dish also impacts the quality of results.





Figures 6,7,8. The graphs show the movement of a single cell (top left), the average are of the culture with time (top right) and the average velocity or speed with respect to time (bottom)

Discussion Stimulation

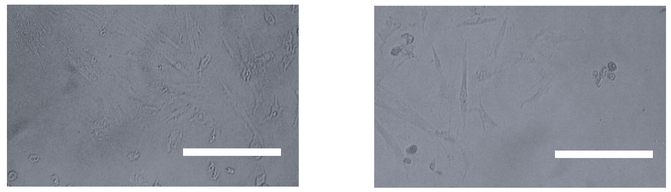


Figure 9. Collagen sealing (left) vs Agarose (right). Scale bar: 200 µm

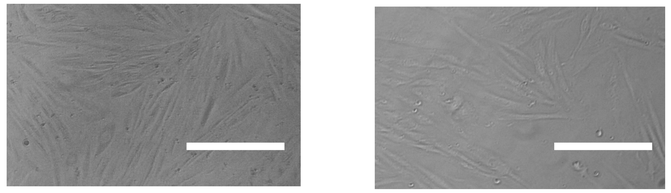


Figure 10. Collagen coating (left) vs gelatin coating (right) Scale bar: 200 µm

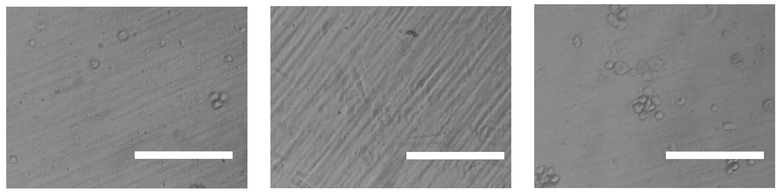


Figure 11. Collagen coating (left) vs gelatin coating on ITO(right.) Scale bar: 200 µm

Figure 12. Copper tape on ITO. Scale bar: 200 µm

From previous research it was found that mechanical stimulation causes cells to un-adhere from the plate. Coats of collagen and agarose gel were tested determine the cell reaction to remediate the issue. The temperatures necessary for the agarose gel coating application result in cell death making collagen the favored option. (figure 9) For cultures on ITO, collagen did not support good cell adhesion, so gelatin was tested as an alternative. (figure 10,11) Copper tape was tested as a method for electrical application, but cells respond poorly to copper in the media. (figure 12) Testing of mechanical stimulation is ongoing.

Future Plans

We plan to switch to a different set of motors for mechanical stimulation which can operate at a higher frequency to better replicate the natural frequency of the heartbeat. Testing will also investigate silver nanofilm as an alternative to ITO. It is also sought to incorporate both these systems simultaneously to see what the combined effects are. Continued work is planned on the recognition algorithm of the software to improve accuracy.

Acknowledgments

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References

[1] Moon, SH., Cho, YW., Shim, HE. et al. Electrically stimulable indium tin oxide plate for long-term in vitro cardiomyocyte culture. Biomater Res 24, 10 (2020). https://doi.org/10.1186/s40824-020-00189-0

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