Project Title: Development of Bioelectronic Scaffolds for Hybrid Brain Tissue
Team Members: Zainab Olushoga, Diamond Mensah, Enrique Rodriguez
PI / Mentor(s): Dr. Brian Timko, Dr. David Kaplan

Project Description:
Bioelectronic devices embedded within the hybrid, engineered tissues could provide stable, long-term readouts of tissue function. We will achieve and optimize flexible, porous scaffolds that provide signals from up to 32 spatially-distinct locations. We will then embed these scaffolds within a 3D brain tissue model to obtain readouts of neural activity. Time permitting, we will apply algorithms to quantify changes in synaptic connectivity over time.

Engineering Design Elements:

1. *What are the objectives of the project and the criteria for selecting them?*

   The objective of the project is to embed a bioelectronic device into a 3D brain tissue model. We plan for the neurons(iNSCs) to grow in the scaffold and for the bioelectronic device to be integrated and form a hybrid brain tissue. Once this is accomplished we expect to be able to measure neural activity.

2. *What system, component, or process is to be designed?*

   A novel hybrid brain tissue that contains a bioelectronic embedded into the tissue. We are tasked with figuring out the best way to embed and track signals for the neurons in the hybrid tissue.

3. *What need does it fulfill (clinical, research, etc.)?*

   Allows for monitoring of electronic signals in live organoids, specifically in brain tissue. The monitoring is essential for understanding tissues that have systemic signalings such as cardiac tissue or brain tissue. Using the bioelectronic chips will allow for the creation of tissues that are more representative of actual tissues.

4. *What scientific, math, and/or engineering methods will be applied?*

   Currently, we are still exploring which methods will be applied for the integration of both parts of this project. We will be using soldering to connect the bioelectronic device to the PCP board to measure the voltage of the neurons.

5. *What realistic constraints (cost, safety, reliability, aesthetics, ethics and social impact, etc.) are to be considered?*

   Considering that individually both components of our project are well reviewed and established constraints are mainly surrounding the integration of the two components. This will be the first time evaluating so we will have to evaluate the constraints throughout our project. We believe that a realistic concern may be sterility since we are going to have to bring devices from the clean room on 200 boston ave to scitech labs in order to create the integrated system. Another

concern was the size of the device and the scafold, specifically we changed our approach from cutting a normal sized scaffold in half to stacking 2 normal sized scaffolds on top of one another using an adhesive.

6. What alternative solutions or changes to the plan will be considered?

   We have considered embedding the bioelectronic prior to the scaffold being salt leeched, so we won't have to worry about the adhesive working well or degrading over time. Another problem is we may have to seed more cells than normal since we are now using 2 scaffolds to mke one hybrid scaffold meaning there should be double the surface area.

7. What are the planned tests and what are the quantitative milestones that will demonstrate the achievement of the objectives?

   Do a comparison of scaffold degradation with and without the device. Immunostaining and images to see neurons. We would also conduct a neural activity assay. To test the adhesive success we are considering performing a tensile stress test on the scaffolds and compare the stress strain curves of the scaffolds with and without adhesive. Where a successful adhesive will have similar properties to a nonadhesive scaffold.

8. Competition: what else is going on in the field that would compete with the project plans?

   As of right now, there is no competition in this field. The combination of both the bioelectronic device and the tissue model is new to the field. We even attended bioelectronic hybrid tissue talks at BMES and no one is doing work like this in the field currently.

Introduction/Background:

The bioelectronic sensor we plan to use for our projects is a flexible scaffold that is designed to interface with neural cells. The sensors record real-time electrophysiology of electric active tissue. These readings allow us to characterize action potentials and cellular networks. Additionally, we can monitor ion channel states, and evaluate neural pathways. Specifically, the sensor is attached to a multielectrode array that was designed in the Timko Lab. The multielectrode array is rigid so it will not contour to the tissue meanwhile the sensor is flexible to allow for it to integrate with the active tissue, promoting cell-to-cell communications. Our main focus is the integration of the hybrid brain model. Separately these two innovations are well known and researched however it's unknown how these would work together jointly. We expect that sandwiching the scaffold around the sensors would promote the growth of neurons around the sensors. However, there is not much research on hybrid model integration.

Methods:

Exp 0 Make the scaffolds and implant them into devices without neurons.

1) Create silk scaffold donuts.
2) Prepare the flexible bioelectronic device.
3) Cut silk scaffold in half and input the bioelectronic
4) Use a surgical adhesive
5) Put in media and observe and compare degradation or lack of degradation of the scaffold.

Exp 1 Culture seed scaffold and make sure it is viable without bioelectronics and then also with bioelectronic.

- After seeding the differentiated neurons into the scaffold we will perform cell viability assays to see how neurons are functions and compare

Exp 2 Take some images to characterize the neurons' immunofluorescence and imaging etc.

-

Exp 3 Gather signals from scaffold and neurons and perform more assays

- See how the neurons signal each other

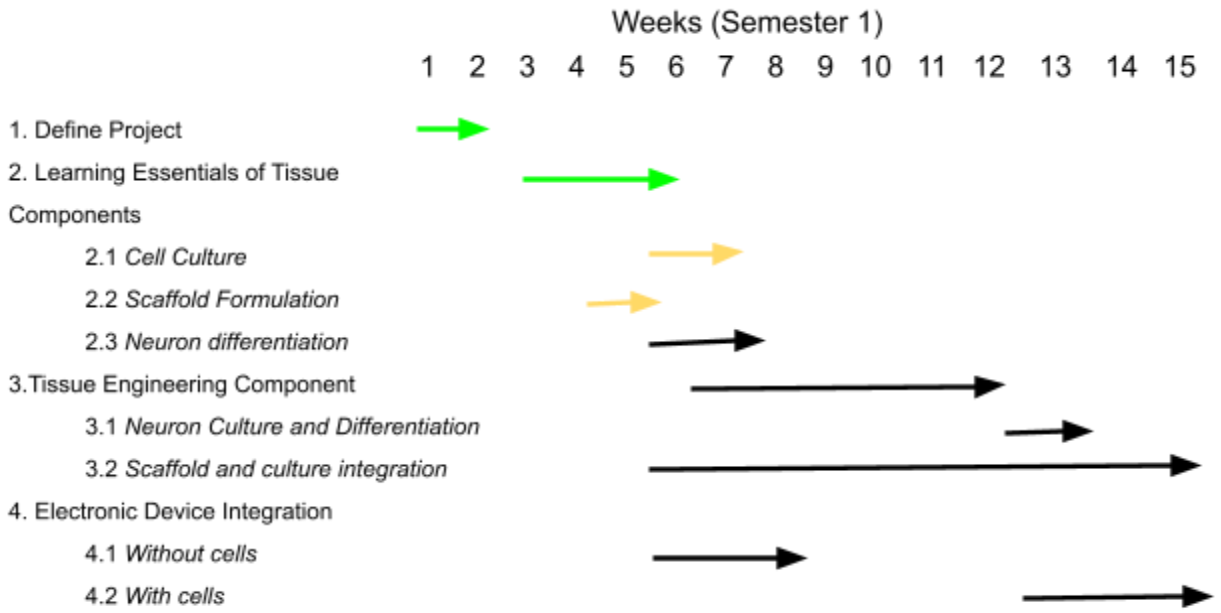
Results (future):
N/A

Discussion (future-work):
N/A

Participation:
We all are working together as much as possible on the project. We all worked together in order to fabricate the silk scaffolds.

> **Bi weekly Report (10/14/22):** This block we all took turns with changing the water when we were doing dialysis and silk scaffold preparation. Diamond submitted the order forms for our cell media. Zainab diluted our silk after we had concentrated it post dialysis, so that it reached the proper concentration. We all worked together to sieve salt and prepare the silk for salt leeching. We observed Marilyn complete seeding her neurons on her own scaffolds, nd coating her scaffolds with collagen as well.
>
> **Bi weekly Report (10/28/22):**

Timeline:



Green = Completed
Yellow = In progress

Bibliography:

1. Cote, M. A. Development and Characterization of a Bioelectronic Scaffold for a Hybrid Brain Model. (Tufts University, 2022).
2. Feiner, R. et al. Engineered hybrid cardiac patches with multifunctional electronics for online monitoring and regulation of tissue function. Nat Mater 15, 679–685 (2016).
3. Li, Q. et al. Cyborg Organoids: Implantation of Nanoelectronics via Organogenesis for Tissue-Wide Electrophysiology. Nano Lett. 19, 5781–5789 (2019).
4. Liaudanskaya, V. et al. Modeling controlled cortical impact injury in three-dimensional brain-like tissue cultures. Adv Healthc Mater 9, e2000122 (2020).
5. Zhou, T. et al. Syringe-injectable mesh electronics integrate seamlessly with minimal chronic immune response in the brain. Proceedings of the National Academy of Sciences 114, 5894–5899 (2017).
6. Dingle, Y.-T. L. et al. Functional Characterization of Three-Dimensional Cortical Cultures for In Vitro Modeling of Brain Networks. iScience 23, 101434 (2020).

Plan for Cell Culture as of (10/11/22) EXP0:

Cut scaffold donuts the week of the 17th of October.
Start MEFs on the 17th so they are confluent by the 24th
Coat the Scaffolds with collagen ECM on the 24 & 25 and then be ready for seeding on the 26th.
Seed iNSCs the week of Halloween (seed iNSCs, tentatively by October 21st)
Change media every day until they are confluent.
Seed onto scaffold at 26 th October, and then hard deadline is the 29 th October .

NF3 for cell viability.
2 MEFs into 2 plates of iNSCs
100 million cells = 50 scaffolds (if we want to do 2 donuts per sandwich we get 50)
Or if we cut scaffolds in half to sandwich the device in between, we would have 100 samples.

Plans for Devices EXP1:
Make another meeting regarding the device with timko 10/17/22.

To Do:
~~Fill out the order forms by the end of this week (10/14/22)~~
Meet with Timko and Rotimi about when we can implant device (10/13/22).
Have a meeting with Kaplan and Timko and other collaborators scheduled for thursday October 20th.