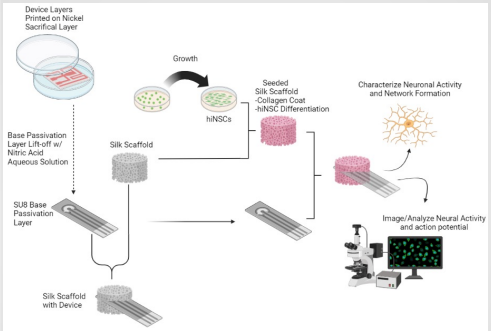
Development of Bioelectronic Scaffolds for Hybrid Brain Tissue

Diamond Mensah[1], Enrique Rodriguez[1] , Zainab Olushoga[1], Brain Timko[1]
[1] Tufts University School of Engineering, Department of Biomedical Engineering

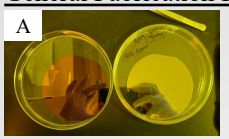
Abstract

Bioelectric sensors are a popular tool for quickly assessing cellular physiologic status, metabolic changes, and recording real-time electrophysiology of active tissue. The Timko Lab has previously designed a flexible 3D bioelectronic scaffold multielectrode array (BioES) meant to be embedded with neural cells. When embedded within the hybrid engineered tissue scaffolds, these bioelectronic devices could provide stable, long-term readouts of tissue function. Our research aims to develop and characterize the hybrid tissue scaffold environment and confirm the biocompatibility with the bioelectronic device. This type of integration has not previously been achieved and could open a new range of possibilities for the field of bioengineering.

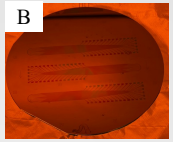
Experimental Summary



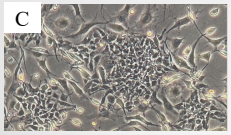
General Fabrication Images



(A) Nickel Sacrificial Layer Production A 10mm silicon wafer without (left) and with (right) 200nm Nickel layer



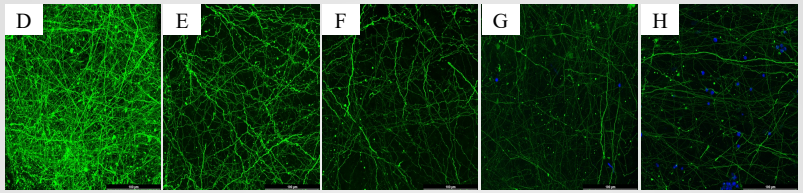
(B) Developed SU8 Photoresist after exposure to UV. SU8 photoresist cross-links under UV exposure and leaves pattern after development in acetate-based solvent



(C) Undifferentiated iNSCs in colonies. Stem cells were thawed from -80C and seeded onto a layer of Mouse Embryonic Fibroblasts with daily feedings of FGF to pre-mature differentiation

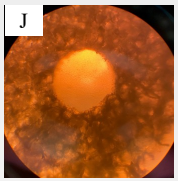
Project Aims	Project Goals	Assay	Threshold of Success	Outcome
Aim 1.1	*Assess structural integrity and compatibility of empty silk scaffold and SU-8 top layer*	*Integration using surgical adhesive – "Kwiksil"* *Leave model at room temp. for >6 hours and observe degradation* *Lift model gently and hold in air for ~1 minute and observe if the adhesive fails*	*After 6-hours of the silk scaffold being adhered to the passivation layer, we will observe no degradation and a maintained structural integrity after slight agitation*	*SU8 layer and Silk Scaffold will be compatible with glue, and we can move forward to the cell integration*
Aim 1.2	*Characterize in vitro Brain Model*	*Flou-4 AM Calcium* *Calcian AM Viability* *Beta III Tubulin (tuj1)*	*>12 events / minute* *70-80% Viability*	*Ability to identify the distinct baseline nature of our brain model to compare to future integrations*
Aim 1.3	*Embed the SU-8 layer of the BioES into the in vitro brain model*	*Thermofisher Live Dead* *Repeat Aim 1.2 Assays*	*>60% Viability* *Same Thresholds*	*Determined compatibility of SU8 layer with our brain model*
Aim 2	*Generate Seeded Scaffolds with an embedded 3-layer BioES*	*Repeat Assays in Aim 1.3* *Connect BioES to PCB Board and Computer*	*Same Thresholds* *Any voltage and Electrical Readings (no set values)*	*Complete compatibility and seeded neurons throughout the scaffolds and full BioES*

Results

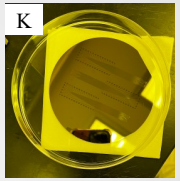


(D) – (H) Beta III Tubulin (tuj1) Imaging Results Imaging results of Green, Neuron-Specific class III beta-tubulin for neuronal cell bodies, dendrites, axons, and axonal terminations in 5 week differentiated iNSCs in silk, donut scaffolds **(D)** & **(H)** Additional Blue, DAPI staining for nuclei/DNA. 100um Scale Bar. Overlay of 100 Z-Stacks

(I) Completed, Unintegrated Brain Model in 48-well Plate



(J) Seeded & Coated Silk Scaffold under 10x Magnification



(K) Completed Base Passivation Layer of BioES

Discussion

In our development of hybrid brain tissues, we encountered many obstacles, including the cleanroom used to fabricate the BioES being down for several months. Nonetheless, we made strides to prepare others to take on this project. Both the BioES device and brain were well-established components, but we found that the protocols needed to be optimized to fit this project's unique needs. As a result, we focused on optimizing procedures for the device as well as methods for characterizing our brain organoids. Making strides towards Aim 1.1 and 1.3 we were able to fabricate the passivation layer with SU-8. We also were able to successfully make the brain models and characterize their network formation using Beta III Tubulin stains and calculate relative network density.

Future Work

Based on our progress so far in the project, there are several future directions that could be pursued in this field. Below are some possible routes:

1. Test and optimize the calcium AM assay to quantify the spontaneous firing.
2. Develop more efficient fabrication protocols for the BioES.
    1. Design liftoff protocols for single layer liftoff.
    2. Optimize thermal evaporator nickel deposition for a more even nickel layer
    3. Test out Sterilization protocols
    4. Evaluate Transfer protocols.
3. Integrate the BioES and Brain Scaffolds; ensuring the scaffolds and devices are biocompatible.
    1. Conduct live-dead assays as well as confocal studies to show neurons local to the SU8.
    2. Conduct Beta Tublin Assays to compare neuron density before and after.
    3. After integration, identify the neuron firing behavior sign calcium fluorescence assays after creating the protocol.
    4. Analyze the action potentials of the neurons

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