Tracking Electrical Signals in Neural Tissue

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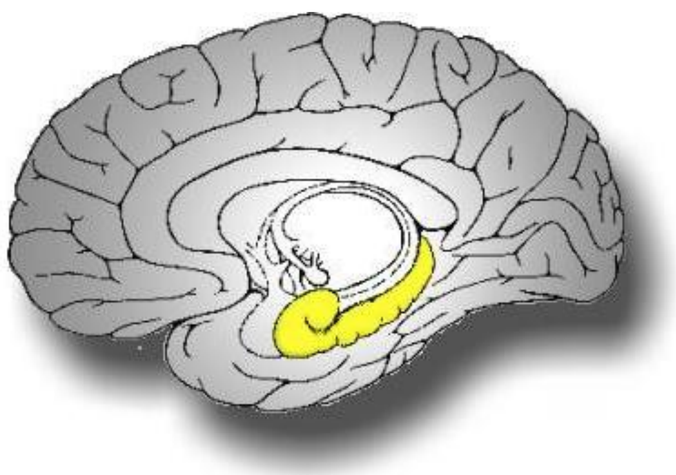
ABSTRACT

- The brain remains one of the most unknown mysteries, with nearly 100 billion neurons within the brain, understanding their interconnectedness and mechanics is not an easy task. Although, developing tools to study neural cell cultures we are able to identify variations in neurons without having to work within a complex brain, thus making it easier to identify variations due to disease. However, methods that revolve around growing neurons on two dimensions may lead to unnatural differences in electrical signaling. In order to achieve results that simulate neurons in the brain we plan to encapsulate each neuron in a gel microsphere and closely pack the spheres together to control how they grow in three dimensions. We have designed various microfluidic droplet generators that will allow reproducible cellular encapsulation. In an effort to form reliable droplets within our generators we need a surfactant to stabilize an aqueous in oil droplet, and a gel that is suitable for neuronal culture. Achieving these intermediary steps toward successfully encapsulating our neurons will bring us one step closer to studying electrical signals in a way that is consistent with their natural environment within the brain, in turn, allowing us to view an effective model to understand debilitating neurological ailments.



Hippocampus in human brain

BACKGROUND

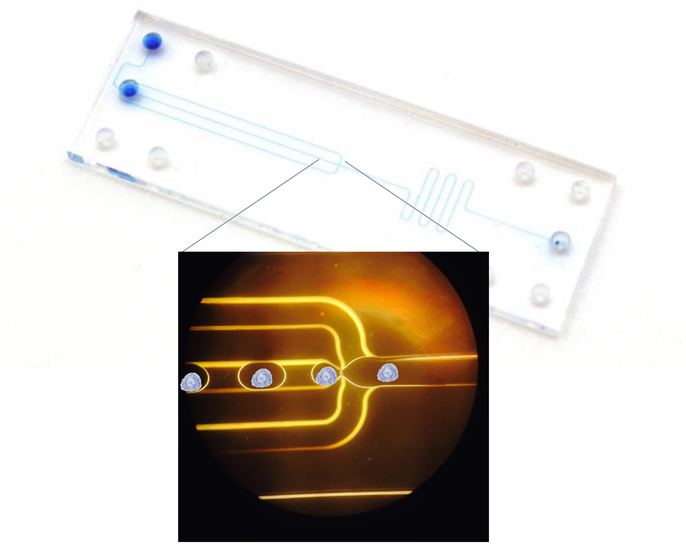
- Tracking electrical signals of neurons in three-dimensions is a newly pioneered way of studying electrical patterns in the most realistic simulation of their natural environment.
- Research of the past has studied electrical signaling relying heavily on the use of plating neurons onto an array without the use of vertical pillars to detect a wider scope of signaling. Our methods rely on utilizing pillars for further detection.
- Studying neurons as it has been done in the past (two-dimensional manner) affects neuron morphology, therefore, their electrical signaling.
- In order to achieve these results our mission will be to use a neuronal encapsulation technique, which requires droplet formation around individual neurons displaced in gel and ultimately transported to a multi-electrode array for the tracking of electrical signals.

- In preparation for this procedure we will need to test two things prior to the plating of neurons within our array.
1. Construct a microfluidic droplet generators that successfully encapsulate neurons and does not decrease survival rates.
2. Optimize our gel and surfactant combination to provide the most reproducible droplets for encapsulation.

- After we accomplish these preliminary objectives we can move into the tracking of electrical signals throughout a network of neurons growing on a multi-electrode array.
- The multi-electrode array is comprised of 120 electrodes as well as 16 pillar electrodes. The purpose of utilizing this array is to electrically stimulate areas of neurons and to record how that stimulation moves through a network of neurons.

OBJECTIVES

- Dissect rat/mouse for hippocampal neurons.
- Determine the best surfactant and gel combination for encapsulation.
- Use CAD to create a microfluidic droplet generators to test alternate designs.
- Collect encapsulated neurons and transfer and incubate them on a multi-electrode array.

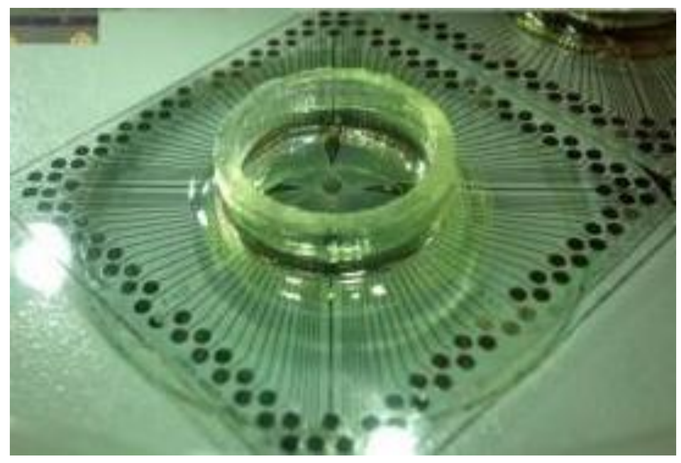


Microfluidic droplet generator, zoomed encapsulation site (40x magnification).

METHODS

- After rat/mouse dissection and removal of the hippocampus our objective is to dissociate the neurons so that they can be run through a droplet generator.
- Using two syringe pumps we draw up one syringe full of a gel and surfactant combination and a syringe full of neurons in media.
- One of our goals being to utilize a gel and surfactant combination that provides the best reproducible droplets. In this search we used Span 80 and OPOE.
- When our syringes have been filled with the appropriate mixtures they are then pumped into a microfluidic chip and monitored under a microscope for encapsulation.
- In our objectives we also want to determine how effective our microfluidic designs are and if they decrease the neuronal lifespan.
- In order to understand the effectiveness of the chip we have to run a control experiment without the use of encapsulation. Isolating the experimentation to a control group of neurons being incubated and neurons passed through a chip and observing the long-term results.

- After identifying the proper chip design we will need to practice our encapsulation technique. Establishing the best infusion speeds for highly reproducible encapsulated neurons.
- Following encapsulation we will need to collect our droplets in a vial and ultimately transfer them to a multi-electrode array to mature in incubation.

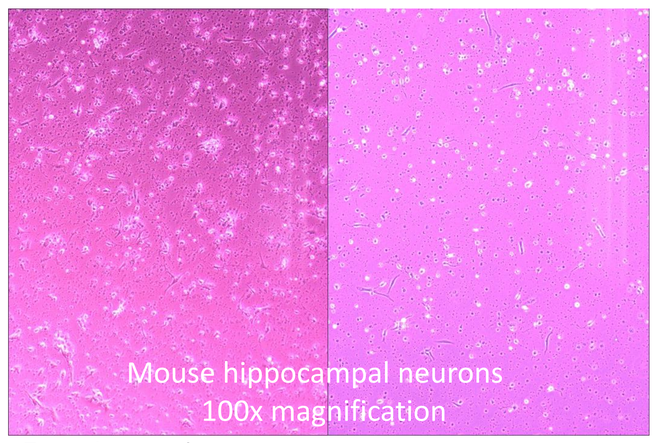


Multi-electrode array with 16 pillar electrodes located in the center

- When the neurons mature and extended their prostheses for connections we can set up a stimulations pattern that will allow for the tracking of electrical signals within neuronal networks.

RESULTS

- Testing our chip against the control revealed that our current chip design diminishes neuronal life span. Neurons after being passed through the chip did not survive beyond 3 days.



Experimental neurons *Control neurons*

- Our CAD designs of a microfluidic device were unsuccessful. The two layers that they are printed in were not capable of creating a strong enough seal to reliable infuse liquids.
- In an effort to produce droplets we found that our current surfactants were incompatible for our experiment. The surfactants we used were optimal for an oil in aqueous droplet, however, our experiment requires the opposite.
- Due to not accomplishing our preliminary goals we were not able to yet employ our multi-electrode array and record electrical signals.

CONCLUSIONS

- In the future we will need to test plasma bonding between our two chips for increased seal strength.
- Change our surfactant to perfluoropolyether, which has been reported to have greater coalesce prevention properties and reliable biocompatibility.
- In accomplishing these preliminary goals we will be closer to successfully tracking electrical signals on a multi-electrode array in a way that is consistent within our brains.