



Multi Chamber Culture Insert for Connected Neuronal Aggregates

Team: Greta Mahlke (Co-Leader), Sam Brandt (Co-Leader), Eli Von Holtum (Communicator), Sophia Mazer (BWIG), Safia Syed (BSAC), Sri Krishnan (BPAG)

Client: Dr. Jason Brant

Advisor: Prof. Mehmet Orman

October 2nd 2026

Overview

- Problem Statement
- Background Research
- Competing Designs
- Product Design Specifications
- Preliminary Designs
- Design Matrices
- Future Work
- Acknowledgements
- References



Problem Statement

- Well plates and petri dishes, are readily accessible but fail to model the complex physiological environments of the human body.
- Important for the study of auditory pathways due to its dynamic environment.
- Design a device that allows for growth and interaction of neuronal cell aggregates while allowing for independent treatment of aggregates.



Figure 1: 12-Well Plate [1]

Client Background



*Figure 2: Client, Dr. Jason A. Brant,
[2]*

Client: Dr. Jason A. Brant

- MD at WashU, Fellowship and Residency at UPenn
- Practicing physician at UW Health w/ double board certifications in General Otolaryngology and Neurotology
- **Main focus:** Improving hearing restoration through a hybrid clinical-bioengineering approach
- **Request:** Develop a cell-culture insert that partitions a well plate into three or four isolated chambers maintaining independent culture media w/ a neuronal cell aggregate seated at base of each chamber and adjacent aggregates connected through ECM-filled channels.

Background

- Micro Tissue Engineered Neural Networks (μ -TENNs) are implantable brain pathways which help bypass areas of gliosis [3]
 - Used for CNS reconstruction and allows for network replacement – considered “living scaffolds” [3]
- 3% agarose molded into glass cylindrical tubes with an acupuncture needle in the middle to create a central channel for axonal growth [3]
- Cortical & brainstem neurons were harvested from E18 rat fetuses and then developed into neuronal aggregates [4]

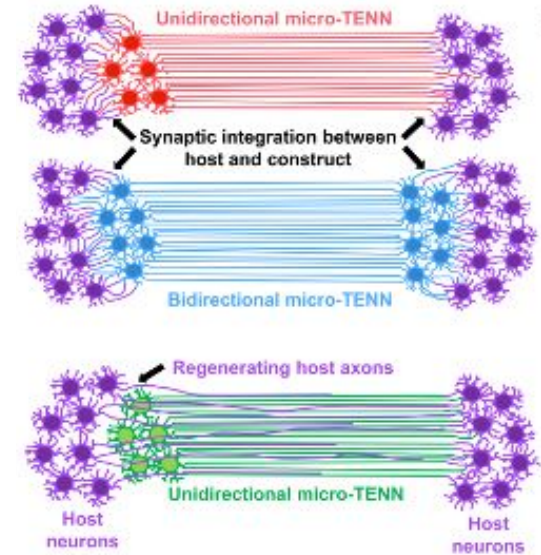


Figure 3: Principles of Micro-Engineered Tissue Networks [3]



Competing Solutions

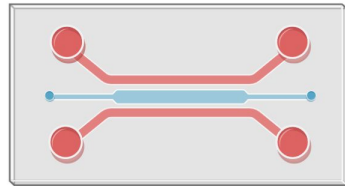
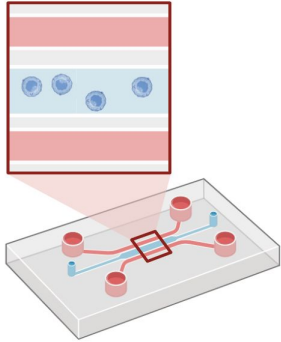


Figure 4: A depiction of a microfluidic device.

Microfluidic devices

- Studies designed around fluid flow or transport
- Adaptable, yet not optimized for this project [4]

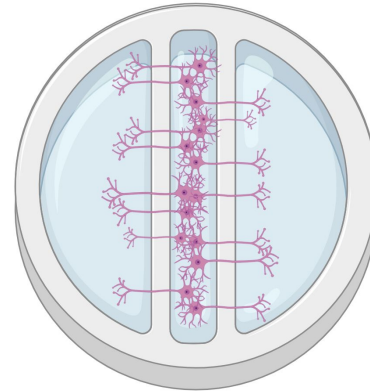


Figure 5: A depiction of a Campenot chamber.

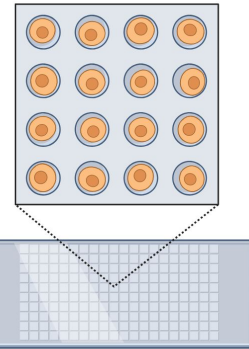


Figure 6: A depiction of a microwell array.

Campenot chambers

- Compartmentalized neuronal cultures good for drug treatment
- Studies focus on somas and axons
- Not as compatible with linear μ -TENNs [4]

Microwell arrays

- Suitable for positioning neuronal aggregates
- Highly adaptable
- Requires modification to incorporate linear architecture [5]



Design Specifications

Device Basics

- Three to four media separated chambers
 - No media transfer between chambers
- Chambers connected by ECM channels
 - 100-300 um in diameter
- Neuronal aggregates sit in each chamber
 - Must not float into media
- Allow for post-treatment imaging of cells

Success Criteria

- Neuronal aggregates must grow into the ECM columns
 - 50-70% within 7 days
- 90% of constructs must be recovered, intact, allowing imaging
- No measurable media transfer between chambers

Design Matrix

Design Criteria (Weight)	Glass slip w/ PDMS Cover		Milled Well Plate		PDMS w/ divots for agarose	
	Raw Score (X/5)	Weighted Score	Raw Score (X/5)	Weighted Score	Raw Score (X/5)	Weighted Score
Aggregate Stability/Positio ning (30)	3/5	18	5/5	30	4/5	24
Media Isolation (25)	3/5	15	5/5	25	3/5	15
Imaging Ability/Cell Recovery (20)	4/5	16	5/5	20	2/5	8
Ease of Use (10)	4/5	8	4/5	8	3/5	6
Ease of Fabrication/ Reproducibility (10)	3/5	6	2/5	4	3/5	6
Cost (5)	4/5	4	1/5	1	5/5	5
Total (100)	67		88		64	

Table 1: Design Matrix



Design 1: Glass with PDMS Cover

- 2 Pieces:
 - Glass coverslip (bottom)
 - PDMS cover (top)
- Glass Coverslip:
 - Circular glass
 - 3 Aggregates on glass bottom
 - Aggregates connected through μ -TENNs
- PDMS cover:
 - Indentations encapsulate the aggregates/columns
 - Through holes above each aggregate connect aggregate to isolated media chamber

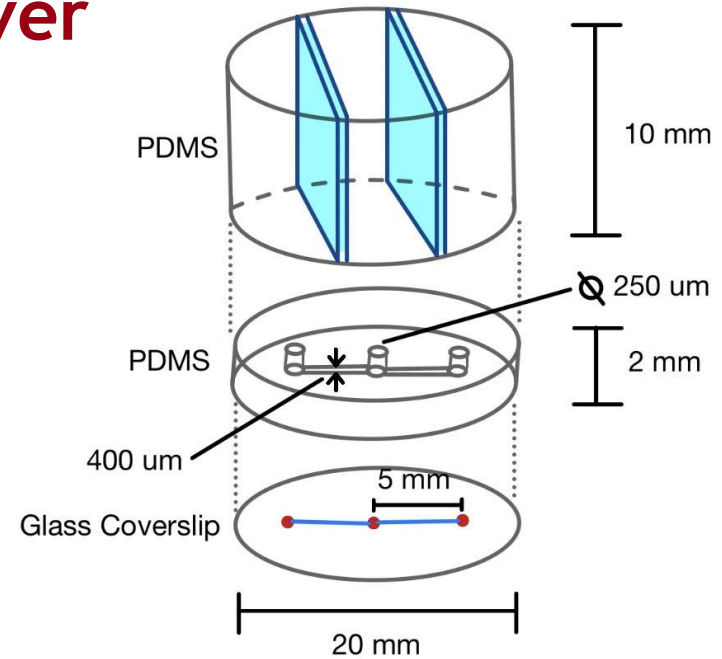


Figure 7: Glass w/ PDMS Cover Design

Design 1 Matrix

Table 2: Glass Slip with PDMS Cover Design Matrix

Criteria	Weight	Raw Score	Weighted Score
Aggregate Stability/Positioning	30	3/5	18
Media Isolation	25	3/5	15
Imaging Ability/Cell Recovery	20	4/5	16
Ease of Use	10	4/5	8
Ease of Fabrication/Reproducibility	10	3/5	6
Cost	5	4/5	4
Total (out of 100):			67



Design 2: Milled 384 Well Plate

- 384 Well Plate
- Milling Modifications:
 - Bottom of plate milled to create channel connecting wells
 - Through holes in each well
 - Allows addition of ECM, Neurons, Culture Media
- Distance between aggregates can be changed by changing number of wells between

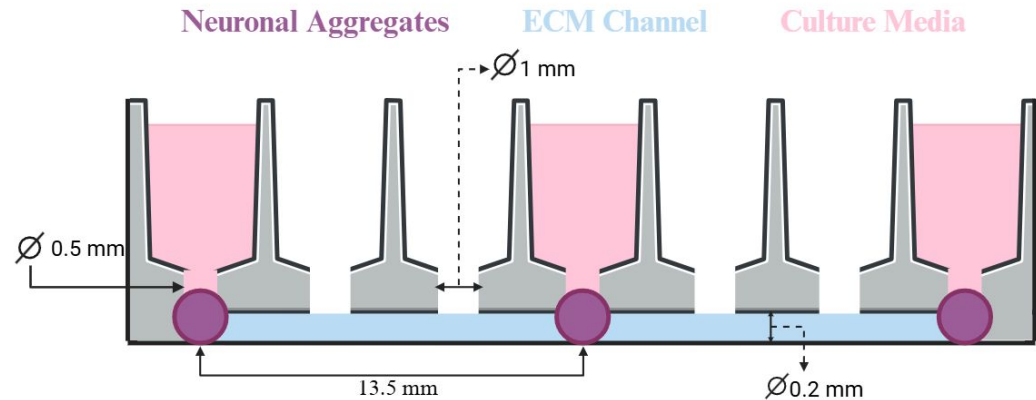


Figure 8: Milled 384 Well Plate Design

Design 2 Matrix

Table 3: Milled Well Plate Design Matrix

Criteria	Weight	Raw Score	Weighted Score
Aggregate Stability/Positioning	30	5/5	30
Media Isolation	25	5/5	25
Imaging Ability/Cell Recovery	20	5/5	20
Ease of Use	10	4/5	8
Ease of Fabrication/Reproducibility	10	2/5	4
Cost	5	1/5	1
Total (out of 100):			88



Design 3: PDMS with Divots for Agarose

- Neuronal aggregates and agarose columns sandwiched between two pieces of PDMS
- Both pieces have divots to fit aggregate/column geometry
- Through holes above aggregates to reach media
 - Additional PDMS piece can be fixed above to increase media volume

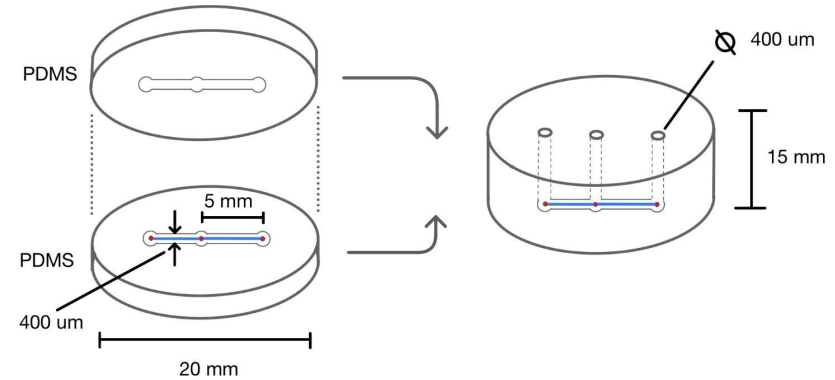


Figure 9: PDMS with Divots for Agarose Design

Design 3 Matrix

Table 4: PDMS with Divots for Agarose Tubes Design Matrix

Criteria	Weight	Raw Score	Weighted Score
Aggregate Stability/Positioning	30	4/5	24
Media Isolation	25	3/5	15
Imaging Ability/Cell Recovery	20	2/5	8
Ease of Use	10	3/5	6
Ease of Fabrication/Reproducibility	10	3/5	6
Cost	5	5/5	5
Total (out of 100):			64



Final Design: Milled 384 Well Plate

Three culture wells connected by an ECM-filled channel at the plate base

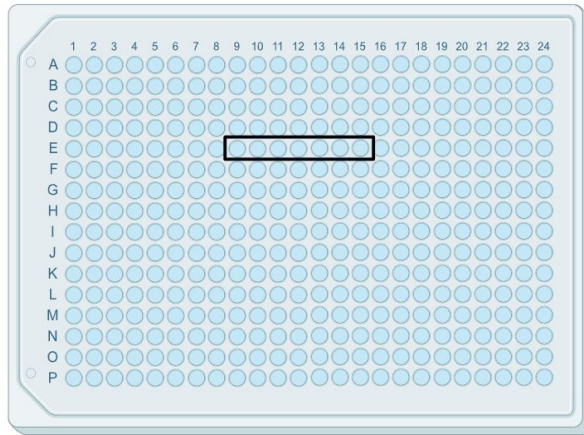
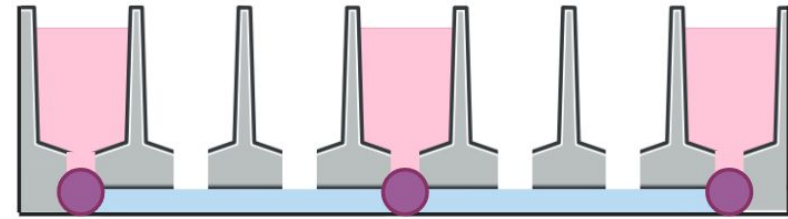


Figure 10: Plate location of culture configuration



Neuronal Aggregates ECM Channel Culture Media

Figure 11: Culture configuration

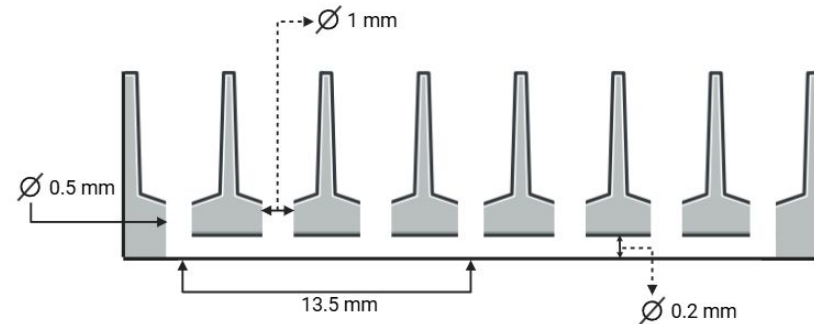


Figure 12: Fabrication geometry

Final Design : Loading and Culture Sequence

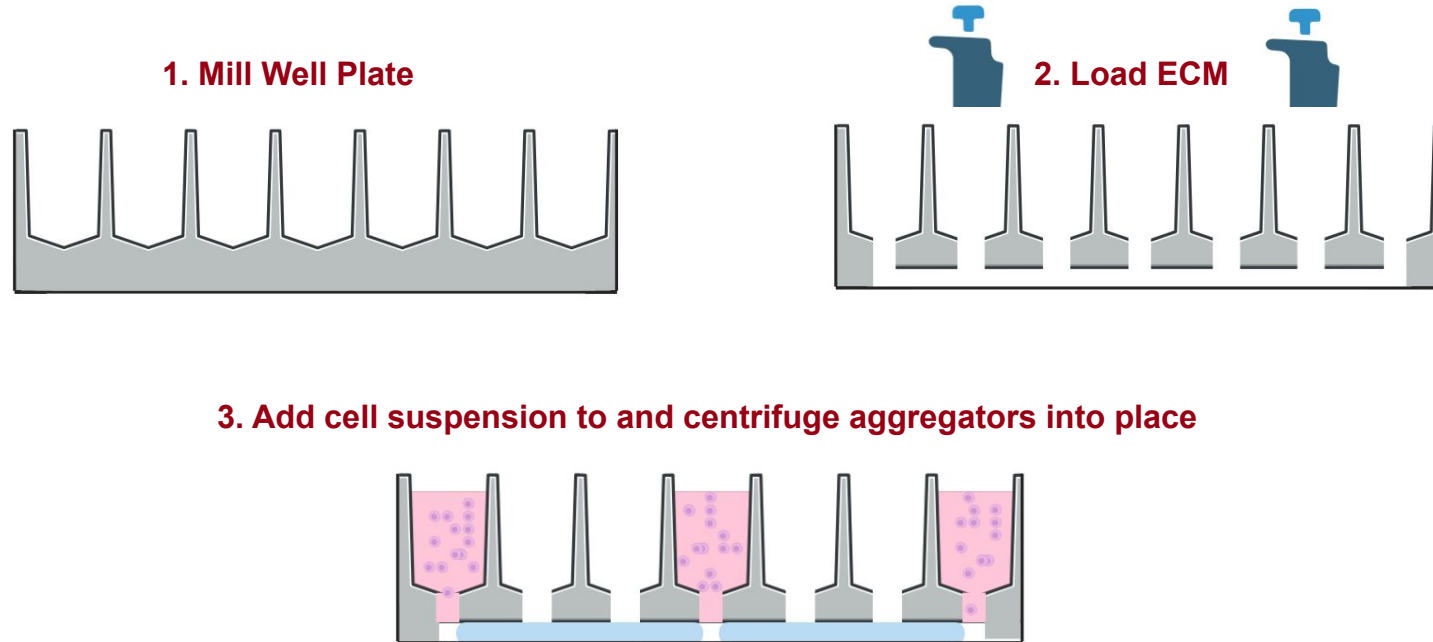
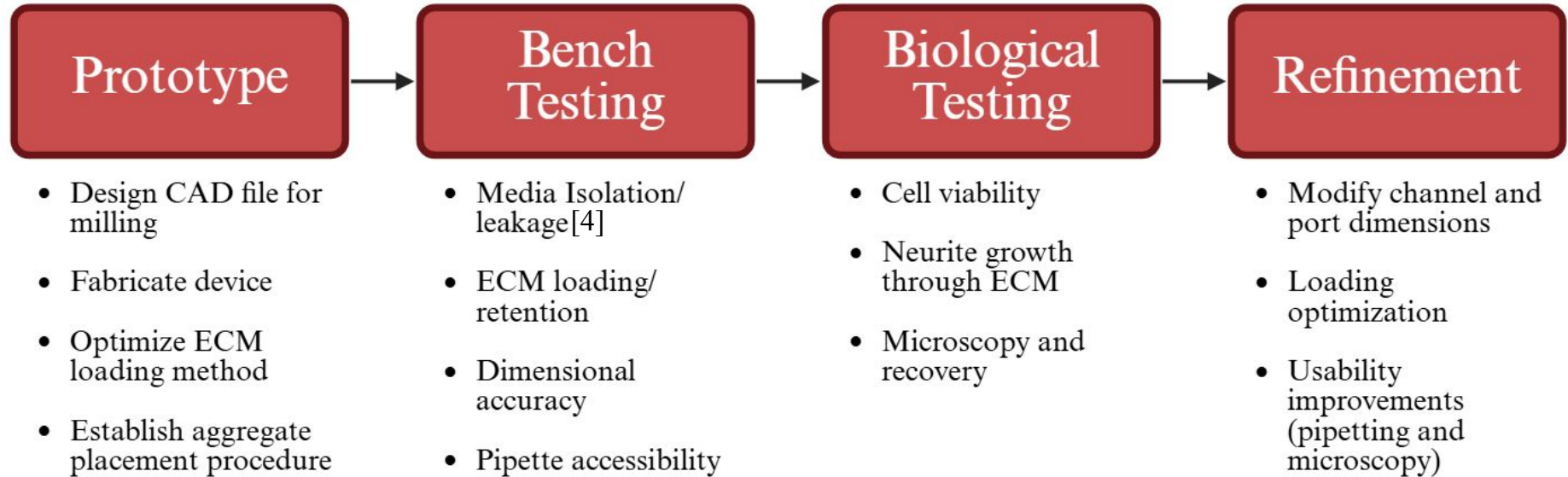


Figure 13: Loading and Culture Sequence



Future Work and Testing



Acknowledgments

We would like to thank:

- Dr. Jason Brant - Client
- Dr. Gunnar Quass - Research collaborator
- Jordan Mumm - Research Specialist
- Prof. Mehmet Orman
- Dr. John Puccinelli
- UW-Madison Department of Biomedical Engineering
- Department of Otolaryngology - Head and Neck surgery



References

- [1] “Costar® 12-well Clear Not Treated Multiple Well Plates, Bulk Pack, Sterile,” Corning.com, 2026.
<https://ecatalog.corning.com/life-sciences/b2c/US/en/Microplates/Assay-Microplates/96-Well-Microplates/Costar%C2%AE-Multiple-Well-Cell-Culture-Plates/p/3737>
- [2] “Brant Lab,” Brant Lab, Mar. 24, 2026. <https://brantlab.otolaryngology.wisc.edu/> (accessed Sept. 30, 2026).
- [3] L. A. Struzyna et al., “Rebuilding Brain Circuitry with Living Micro-Tissue Engineered Neural Networks,” *Tissue Eng Part A*, vol. 21, no. 21–22, pp. 2744–2756, Nov. 2015, doi: 10.1089/ten.tea.2014.0557.
- [4] A. M. Taylor, M. Blurton-Jones, S. W. Rhee, D. H. Cribbs, C. W. Cotman, and N. L. Jeon, “A microfluidic culture platform for CNS axonal injury, regeneration and transport,” *Nature Methods*, vol. 2, no. 8, pp. 599–605, July 2005, doi: 10.1038/nmeth777.
- [5] J. H. Bae, J. M. Lee, and B. G. Chung, “Hydrogel-encapsulated 3D microwell array for neuronal differentiation,” *Biomed. Mater.*, vol. 11, no. 1, p. 015019, Feb. 2016, doi: 10.1088/1748-6041/11/1/015019.





DEPARTMENT OF

Biomedical Engineering

UNIVERSITY OF WISCONSIN-MADISON