

Crosslinking Hydrogels to Connect Neural Organoids Estrella Garrido, <u>estrellagarrido2025@gmail.com</u>

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Introduction

Lab: Make tissues to model living systems Team: Focus on modeling the brain by designing systems to grow brain organoids that connect Me: Focus on fabricating hydrogels and test them under different conditions

Brain Organoid: A self-organizing threedimensional structure that models certain aspects of the brain (derived from human pluripotent stem cells)

- MTG & gelatin used to form a hydrogel & mimic the environment of the human body
- Hydrogel droplets link cells

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School of Engineering

K-12 STEM Center

Droplet networks are easier for organoids to grow through



Objectives & Impacts

Impact:

- Create complex systems that better mimic the human brain
- Improved drug screening
- Improved Understanding of
- **Neurodegenerative Diseases**
- **Develop personalized treatment**
- Improved perception of human brain development

Objectives

- Fabricate hydrogels that connect neural organoids
- Explore the properties that make gelatin and transglutaminase link together

Methods & Results

Hydrogel needs to go from liquid-to-solid

- Gelatin needs to be melted to avoid clogs or breaking droplet generator •
- MTG can't be hot enough to degrade
- Hydrogel needs to become solid if not cells won't have a substrate to grow on

Methods:

- Warmed up MTG at 4 different temperatures (RT, 37, 50, 65) for 1hr & gelatin at 65 degrees
- Added either MTG or water to gelatin
- Left at RT overnight
- Observed the cross-linking ability of 2 batches of MTG
- Biopsy punch hydrogel to test stiffness in mechanical tester
- Rest was put into oven at 37 degrees to see if it melt or not

Results:

- Batch 1 became stiff under RT (Room Temperature) and 37 degrees Celsius
- Batch 2 became stiff only under RT (Room Temperature)
- Negative conditions broke transglutaminase. Batch 1



Stiffness of gelatin MTG hydrogels after overnight incubation





Hydrogels after taken out of 37° Celsius oven



Batch 2

Future Directions

Next steps:

- Will use batch 1 at 37 degrees to link organoids
- Make hydrogel droplets
- Use hydrogel droplets to connect organoids

Skills Developed

- Learned how to make hydrogels
- Use biopsy punch to take out hydrogels
- Learned how to use calipers to measure diameter & height of the different hydrogels
- Learned to use degasser (to get rid of bubbles in PDMS
- Learned to cut PDMS (cut out droplet generator)
- Used Plasma Bonder (to bind droplet generator and microscope slide)





References

1.Gupta, D., Santoso, J. W. & McCain, M. L. Characterization of gelatin hydrogels cross-linked with microbial transglutaminase as engineered skeletal muscle substrates. Bioengineering 8, 6 (2021).

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