Production of Small Scale Microgels for Applications in Cell Encapsulation

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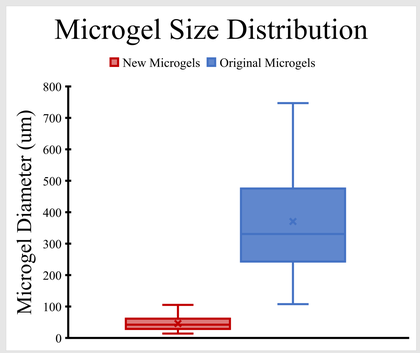
Introduction

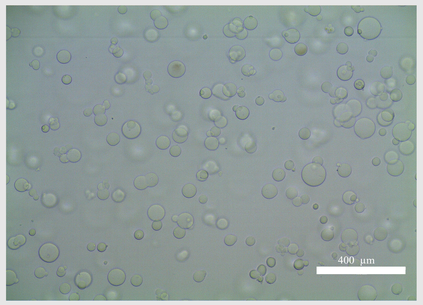
Injuries and degenerative diseases can lead to long recoveries and/or irreversible damage. To alleviate these effects, one could inject specific stem cells into the damaged area to enhance growth and limit inflammation. However, direct injection of cells into the body results in low viability and significant dispersion. Hydrogels have been identified as possible cures for this issue. A degradable structure with encapsulated cells increases viability and stabilizes cell location. Nevertheless, conventional hydrogels lack porosity, mitigating cell growth and penetration of host biology. To address this, Jeong Lab uses novel injectable microporous hydrogels made of gelatin to increase cell proliferation. Microgels are formed through an oil emulsion and then cured with microbial transglutaminase (mTG) to form a bulk hydrogel with interstitial space. My summer research focused on the alteration of microgel diameter. Controlling microgel diameter can influence hydrogel properties such as nutrient transfer, cell morphology, cell spreading, and available surface area.

Microgel Production of Various Sizes through Oil Emulsion

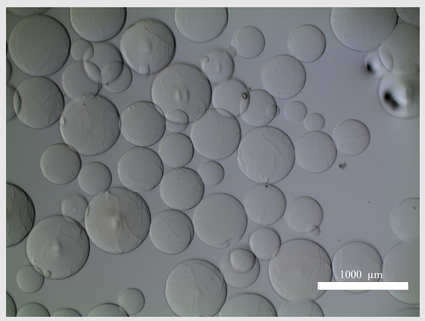


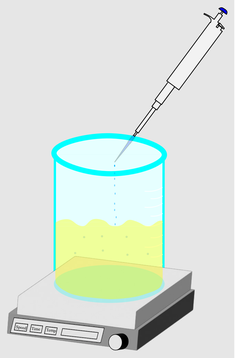
By integrating an emulsifier into the hydrogel emulsion protocol and decreasing gelatin concentration to 5% (from the original 10%), the diameter of the microgels were reduced to an eighth of their original size.

New Microgels



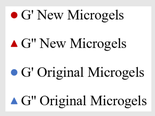
Original Microgels

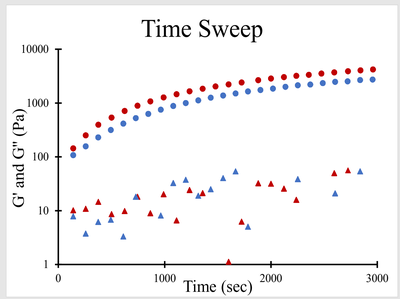


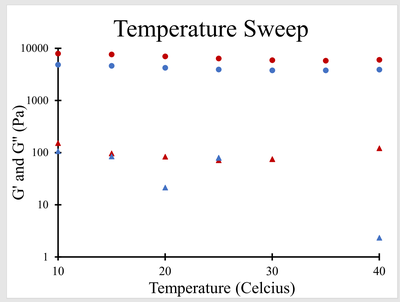


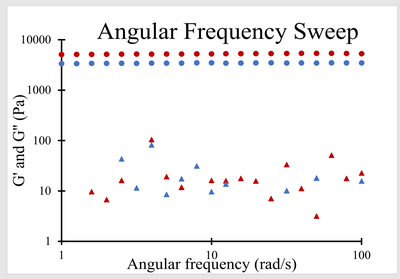
Rheology Comparison of New and Original Microgels

Rheology shows that the new microgels form a stiffer bulk gel compared to the original microgels through gelation and after gelation.



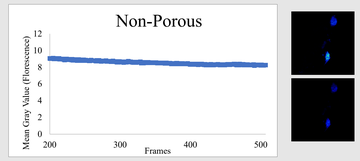




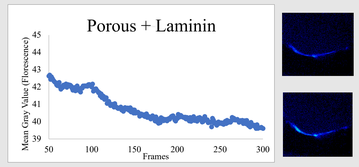


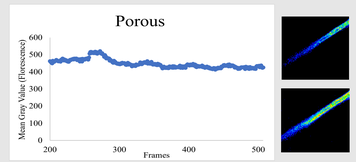
Fluo-8 Calcium Flux Assay after 14 days of Differentiation

The fluo-8 assay shows fluctuations in calcium concentrations, which occur during neuronal firing. Monitoring the gray value of pixels can illuminate the firing of these cells. In this experiment neural stem cells, left to differentiatwere cultured in original microgels to observe possible firing. The results proved that this time frame allowed for the maturation of neurons in the microporous hydrogel.



Imaging of Cells in Different Hydrogel Conditions

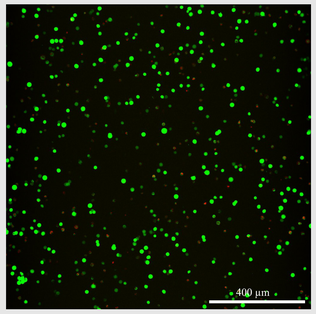




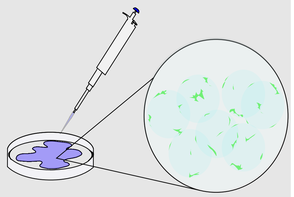
Encapsulation of 3T3 Cells using mTG

I learned encapsulation protocol for porous and non-porous hydrogels using NIH 3T3 cells, which proliferated in the porous environment.

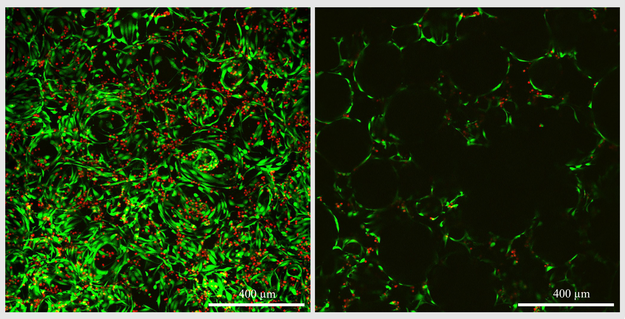
Non-Porous



Live/Dead Staining
(■) = Living Cell
(■) = Dead Cell

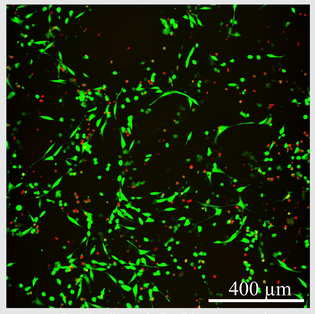


Porous

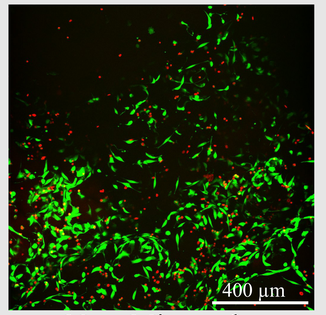


Testing for Cytotoxicity through Cell Encapsulation

Cell viability was similar in both mediums making it feasible that the new microgels could be used for cell encapsulation.



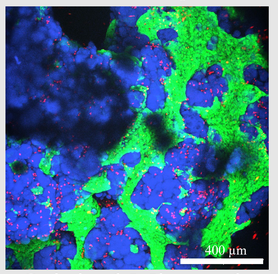
Original Microgels



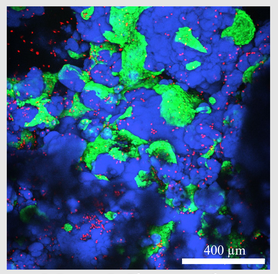
New Microgels

Conclusions and Further Research

- Microgels with significantly reduced diameter can be produced by adding an emulsifier and decreasing gelatin concentration.
- These smaller microgels produce a stiffer bulk hydrogel when compared to their larger counterparts.
- Neural stem cells, left to differentiate for 14 days, can fire when grown in microporous gelatin hydrogels.
- Preliminary results show that cells can grow in these new microgels, while further research must be performed on how they react in this environment.



Original Microgels



New Microgels

Acknowledgments

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