Formulation of pancreatic β-cell encapsulation alginate scaffold for type II diabetes optogenetic therapy

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BACKGROUND

Type II Diabetes Mellitus

Type II Diabetes Mellitus (T2DM) is a disorder characterized by defective insulin secretion by pancreatic β-cells and insulin resistance, or cell rejection of glucose, leading to the inability of those afflicted to properly regulate blood glucose levels, with serious consequences such as organ damage.[1] Affecting 37.3 million people in the United States (11.3% of the national population), current therapies reduce the quality of life of patients and are inconvenient and difficult to administer.[2]

Optogenetics

Optogenetics is a technique in which photoreceptive moieties are genetically incorporated into a biological system such that light is used as a biological activator. It is beneficial for its reversibility and high spatiotemporal control of biological processes and commonly applied to the control of neuronal systems.

Alginate Hydrogels

Hydrogels are gaining traction as scaffolds for cell encapsulation in biomedical applications. Derived from alginic acid, a polysaccharide in seaweed, alginate hydrogels are biocompatible, abundant, and cheap.[3] Calcium ions in solution serve as cross-linking agents to bind alginate polysaccharides and form a gel.

Combining Diabetes Therapy, Alginate Hydrogels, and Optogenetics

The Tzanakakis research group at Tufts University seeks to develop a cell therapy to target T2DM by incorporating photoactivatable adenylyl cyclases into pancreatic β-cells, allowing for the photoinduction of insulin secretion in patients with impaired ability to do so naturally. This project seeks to develop a cell delivery vehicle of encapsulated engineered cells in the form of an alginate-based hydrogel that is both useful for optogenetic application and biocompatible. Such a therapy would be convenient and reduce patient stress, requiring only external illumination for blood glucose level management.

METHODS

Protocol was adapted from *Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties* by Kuo and Ma.

Solution Preparation

3% w/v alginate solution was made by pouring sodium alginate into heated and stirred deionized water. Calcium carbonate ($CaCO_3$) and GDL were dissolved in DI water to generate solutions of varying concentration.$CaCO_3$ and GDL molar ratio was kept at 1:2 to maintain pH neutrality.

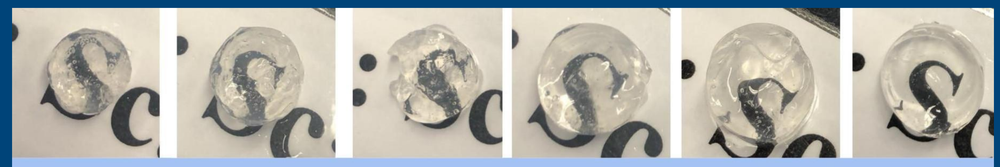
Variations in mixing method and concentration and volumetric ratio between alginate and $CaCO_3$/GDL solution were used for formulation optimization.Mixing methods tested included manual mixing in 24-well plate with a metal spatula and vortexing solutions in a 2 mL vial. Alginate concentrations tested varied from 1% w/v to 3% w/v. $CaCO_3$ concentrations tested varied from 50 mM to 200 mM. Volumetric ratios tested varied from 25% alginate to 85% alginate, with the remaining volume being $CaCO_3$/GDL solution.

Gelation and Mechanical Property Testing

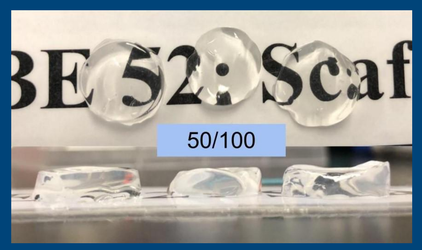
Following combination, the resulting solution was poured into a 24-well plate. Gels were left to gelate for 24 hours before mechanical testing via dynamic mechanical analysis (DMA) machine to determine hydrogels' compressive strength.

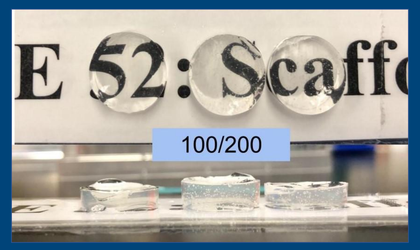
RESULTS

A clear, structurally uniform hydrogel with minimal bubbles was produced with Young's moduli in the range of 3.70 kPa to 4.75 kPa; desired moduli lie within the range of 0.5 (murine pancreas)[4] or 1.06 ± 0.25 kPa (human pancreas)[5] and thus warrant use of concentrations of $CaCO_3$ below 50 mM.

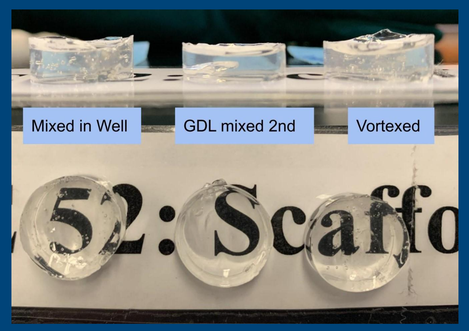


Different ratios of sodium alginate to $CaCO_3$/GDL concentrations were tested for maximum transparency and minimal bubble formation in the gel, leading to the determination that a 50/50 volumetric ratio was optimal.





Different $CaCO_3$ and GDL concentrations were tested for their effect on the structural integrity of the gels. Higher concentrations of $CaCO_3$ yielded firmer gels with higher Young's moduli, due to higher cross-linking density. This ratio also yielded gels that better maintained mold morphology.



3% alginate solution with 50 mM $CaCO_3$ and 100 mM GDL. Different mixing methods and order of mixing were tested to find optimum clarity in samples with minimal bubbles. Mixing GDL in second helped reduce the number of bubbles in the gel, thus resulting in a more transparent sample.

Sample	Young's Modulus (kPa)
50 mM	3.70
75 mM	3.57
100 mM	4.75

DMA compression test results of hydrogels made of 3% alginate with concentrations of $CaCO_3$ ranging from 50-100 mM. 1:2 ratio of $CaCO_3$ to GDL was used.

DISCUSSION

Effect of Calcium Ion Concentration on Hydrogel Young's Modulus

The gels created had an slightly higher Young's Modulus (3.70~4.75 kPa) than that of the human pancreatic tissue (1.06±0.25 kPa). There is an increasing trend in Young's Modulus with the increase in $CaCO_3$ concentration, which is consistent with our expectation, as calcium creates the cross-linking network in alginate. While likely challenging for its expected fragility, given the moduli attained, a hydrogel made with a lower concentration of $CaCO_3$ than 50 mM is likely to provide a better growth environment for pancreatic β-cells that is most similar to their native environment.

Effect of Mixing Method on Hydrogel Transparency

The gels that are made from mixing sodium alginate and $CaCO_3$ first in vortex, then adding the GDL solution to vortex resulted in gels with the best transparency. Although this result is not obtained quantitatively, through qualitative observation, it is evident that the gels made from this method had the least amount of air bubbles. Since the engineered beta cells respond to light, it is crucial that these gels have good transparency. However, as vortexing has the potential to damage resuspended cells, further study on the viability of cells after vortexing is warranted.

Formulation Conclusions

Gels made from 0.5ml 3% w/v sodium alginate, 0.25ml of $CaCO_3$ (50, 75, 100mM), and 0.25ml of GDL (100, 150, 200mM) and mixed by combining alginate and $CaCO_3$ followed by GDL resulted in gels that have excellent transparency and promising mechanical properties.

FUTURE DIRECTIONS

Future experiments would focus on:

- Achieving a gel Young's modulus comparable to pancreatic tissue at either ~500 Pa (murine) or ~1 kPa (human) while maintaining the geometric integrity of the hydrogel
- Further inspection of gelation time, in the interest of maintaining cell viability during encapsulation
- Development of formulation optimal for cell survival, including a gel that can withstand submersion in cell growth media such as DMEM

Once a satisfactory hydrogel is achieved, further studies would include optimization of:

- Mass transfer of nutrients and metabolites through the hydrogel via modeling using COMSOL software
- Cell viability, conducted to ensure that the pancreatic cells can survive on the scaffold

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ACKNOWLEDGEMENTS

We extend our heartfelt gratitude to Emily Edwards, Professor Mess, Professor Tzanakakis, and Rana Said for their guidance and mentorship throughout the semester. Thank you also to Thomas Falcucci, for his generous time and assistance with material property testing, as well as to the Tufts University Department of Chemical and Biological Engineering for funding and support of this project.