Reproducibility and extraction of complex hydrogel geometry fabrication in 3D printed injection molds

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Motivation

Type 1 diabetes is an autoimmune disorder characterized by the destruction of pancreatic islet cells which eliminates the capacity to produce insulin. Common treatments for this disorder don't cure the disease, and existing treatments often result in secondary complications such as blindness and amputation.

Islet cell transplantation via macroencapsulation is a potential therapy to treat type I diabetes in the absence of immunosuppression. One limiting factor of macroencapsulation devices compared to microencapsulation techniques are their large scale, which limits oxygen and nutrients available to islets and limits their viability and function. A smaller geometry leads to better oxygen and nutrient flow for the cells through the hydrogel barrier that encapsulates the islets. Therefore, by designing a device that has an optimal geometry with a better surface area to volume ratio, such as a spiral, we can produce better flow of oxygen and nutrients across the hydrogel barrier to reach the islets inside of the gel.

The goal of this project is to design an injection molding device that can generate hydrogels of complex geometries without damaging cells during fabrication. Injection molding devices are fabricated via a photolithography Formlabs Form 3 3D printer that uses a flexible printing material. The Flexible material will also help with making it easier to take out the mold after it has finished.

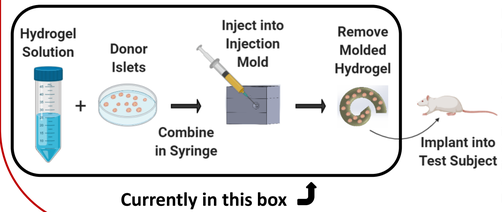


Figure 1. Displays the process of creating complex hydrogel geometries to then be implanted into a test subject. The hydrogel solution is combined with the donor islets and then injected into the mold to then be remove and implanted. We will only be testing what is inside of the black outlined box.

Methods

Flow Modeling:
3D Flow Modeling is performed in Solidworks 2020 Flow Simulation to generate an accurate representation of how the gel injected inside both the 1mm and 2mm diameter spiral will perform. The velocity, pressure and shear rate are measured within the mold. The density and viscosity of the non-Newtonian liquid that is being simulated within the mold are similar to the two hyrdogels that are used for the experiment. The boundary conditions include the inlet mass flow rate is set at 0.01kg/s and temperature of 293.2K; the outlet static pressure have the parameters of pressure being 101325 Pa and temperature being 293.2K. Gravity is considered during the simulation. Once the flow looks like promising results, then the molds are made in Solidworks to then be printed on Formlabs stereolithography resin 3D printers. The photopolymer resin used was the Flexible resin that helps release the molded spiral easier when it has hardened.

Injection molding experiment:
The experiment used all flexible material devices, printed on Formlab's Form 3 3D resin printer. The experiment included 3 groups of different hydrogels (PEG, agarose and alginate). The composition of the PEG hydrogel consists of 5% PEG + adhesive ligand (1mM RGD) & DTT; the composition of the agarose hydrogel consists of 2% agarose solution; the composition of the alginate hydrogel consists of 2% alginate mixed with calcium carbonate and cross linked with D-(+)-Gluconic acid δ-lactone. The trials for the 2mm diameter spiral were: PEG (n=5), Agarose (n=10), Alginate (n=10). The trials for the 1mm diameter spiral were: Agarose (n=10), Alginate (n=9). After each trial in each experiment a picture was taken of the gel on each half of the device. That picture was then examined on ImageJ with a built macro to analyze the complete fill percentage of the spiral and then compared to the other hydrogels.

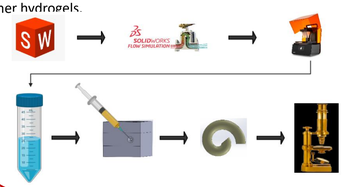


Figure 2: Presents the method of preparing molds in Solidworks, modeling the flow simulation, 3D printing, injecting the hydrogels, extracting them, and then evaluating the fill of the injection molds.

Flow simulation results

1mm Diameter Spiral

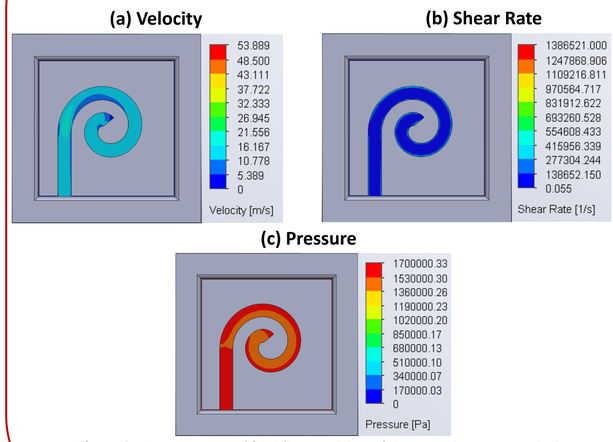


Figure 3: Demonstration of fluid flow modeling of the 1mm diameter spiral. These images represent the (a) velocity (m/s), (b) Shear rate (1/s), (c) Pressure (Pa) of fluid going into the mold and out of the airholes. The blue scale color represent the least and red scale color represents the most for given measurement.

2mm Diameter Spiral

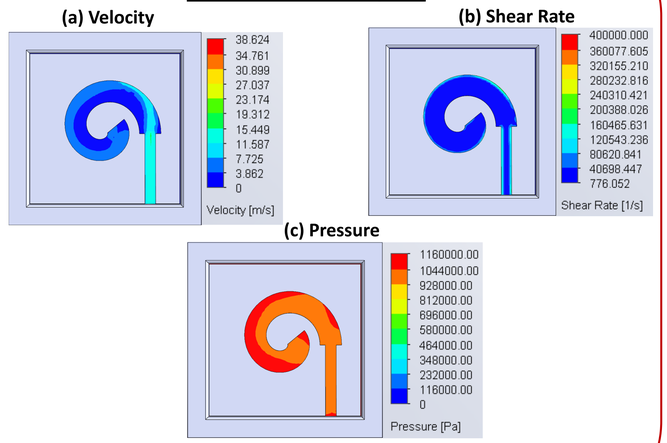


Figure 4: Demonstration of fluid flow modeling of the 2mm diameter spiral. These images represent the (a) velocity (m/s), (b) Shear rate (1/s), (c) Pressure (Pa) of fluid going into the mold and out of the airholes. The blue scale color represent the least and red scale color represents the most for given measurement.

Versatility of injection molded hydrogel geometries

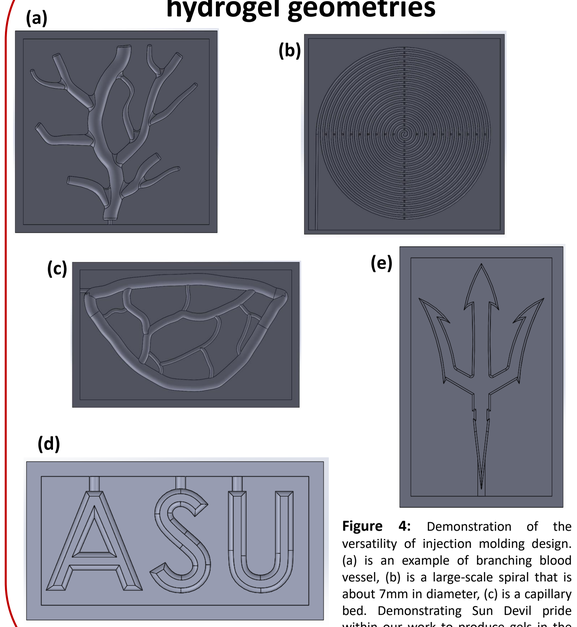
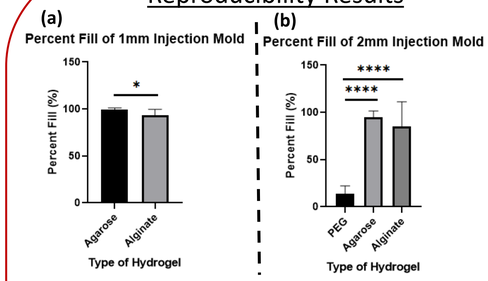


Figure 4: Demonstration of the versatility of injection molding design. (a) is an example of branching blood vessel, (b) is a large-scale spiral that is about 7mm in diameter, (c) is a capillary bed. Demonstrating Sun Devil pride within our work to produce gels in the shape of (d) letters and (e) the ASU pitchfork logo.

Reproducibility Results



(a)

	Successful Extraction of 1mm Diameter Gel	
Trial #	Agarose	Alginate
Trial 1		
Trial 2		
Trial 3		
Trial 4		
Trial 5		
Trial 6		
Trial 7		
Trial 8		
Trial 9		
Trial 10		-

(b)

	Successful Extraction of 2mm Diameter Gel		
Trial #	PEG	Agarose	Alginate
Trial 1			
Trial 2			
Trial 3			
Trial 4			
Trial 5			
Trial 6	-		
Trial 7	-		
Trial 8	-		
Trial 9	-		
Trial 10	-		

Experiment Results

Figure 5: Comparison of percent filling of the injection mold for: (a) a 1mm diameter spiral and running an unpaired t-test, there is a slight significant difference between the alginate/agarose, having a P-value = 0.0120. (b) 2mm diameter spiral and running a one-way ANOVA with a Tukey's multiple comparisons test. There is a significant difference between the alginate/PEG and agarose/PEG, both having a P-value <0.0001. There is no significant difference between the alginate/agarose hydrogels.

	Yes, whole extraction
	Partial, in pieces extraction
	No extraction

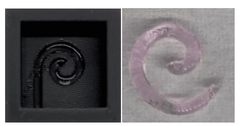
Table 1: Trials that had either a successful, partial or no extraction of the hydrogel after it had been molded. (a) Represents extraction trials for 1mm diameter spiral, (b) Represents extraction trials for 1mm diameter spiral

Extraction Results

1mm Diameter Spiral

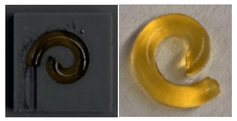
Agarose

Alginate

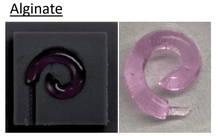


2mm Diameter Spiral

Agarose



Alginate



PEG

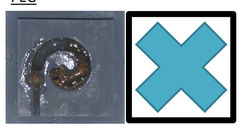


Figure 6: Displays mages of the three different hydrogel materials inside of each the 1mm and 2mm spiral injection molds. The left side images consists of images taken after the hydrogel has been molded and then the right side consists of images extracted on to a petri dish. There was no PEG hydrogel that had a successful extraction out of the mold.

Obstacles Faced/Overcome

- Hydrogel crosslinks too fast to be able to have uniform fill of complete PEG hydrogel [1]
- Limited resources due to the Pandemic.

[1]

Conclusion and Future Studies

The 2mm diameter spiral injection mold design successfully generated hydrogel macroencapsulation devices. The injection molding strategy can be used to generate many different complex hydrogel geometries. The possible shapes are many, but the limit to making these shapes are making sure that the whole inject mold is filled properly. Depending on the type of hydrogel that is used, there is a difference in the fill percentage of the mold itself. PEG hydrogels demonstrated repeatedly that they do not fill the mold. Agarose and alginate filled the mold entirely and with consistency. Increasing trials of the injection molds would improve the data. Demonstrating injection molding of the spiral shape is crucial to move on to larger and more complex shapes. Future studies with the injection molding device include testing bigger and more complex shapes, as well as incorporating cells in the hydrogel to test the cell viability within these molds, which is being performed by my graduate mentor, Amy Emerson. There are many future implications of hydrogel injection molding for not only the treatment of T1 diabetes, but many other diseases that require the applications of cell-based therapies to an extensive patient population.

Acknowledgements

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