Utilizing Injection Molding to Generate Complex Three-Dimensional Cell Encapsulation Geometries

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Motivation

As cell-based treatments for various disorders approach the clinic, there is a pressing need to generate cell macroencapsulation devices that can protect the cells from the host immune response. However, the risks of chronic systemic immunosuppression outweigh the benefits of cell-based therapies for the majority of these patients. Encapsulation has shown great potential in being a safe and effective way to deliver cells to patients while potentially eliminating the need for immunosuppression. Macroencapsulation has shown the greatest promise for translation due maximal retrievability however a critical issue in macroencapsulation device design is oxygen transport to encapsulated cells. This work uses recently developed hydrogel-based injection molding process to generate complex cell encapsulation geometries to address oxygen transportation utilizing fluid flow simulation to determine pressure regimes within the device during injection to determine cell viability based on rheological properties of diverse hydrogel.

Methods

Modeling

Using SolidWorks, the molds were constructed and tested through SolidWorks Computational Fluid Dynamics Flow Simulation to determine static pressure and pressure profile of the mold to determine weak points and flaws.

Hydrogel Preparation

The hydrogels that were used were sodium alginate, agarose, and polyethylene glycol (PEG). Alginate was prepared at a 4% (w/v) concentration in PBS(-)(-) then crosslinked with a solution of calcium carbonate (60 mM) and Gluconic delta-lactone (120 mM) at 1:1 ratio. Agarose was prepared at a 2% (w/v) concentration in PBS (+)(+). PEG was prepared at a 10% (w/v) concentration using 20kDa PEG-maleimide powder and DPBS then crosslinked with DTT (154.25 g/mol) at a 1:1 ratio.

Rheology

The rheology data was obtained using an Anton Paar Physica MCR 101 with a 25mm sandblasted plate geometry. The data was obtained using rotational analysis with a shear rate of 0.001-1000 1/s over 30 points. The sodium alginate data was taken at 37°C using 490µL and agarose was taken at 100°C using 490µL.

SolidWorks Flow Simulation Parameters and Selection

Inlet boundary condition was set with a volumetric flow rate into the mold at 30 µL/s and an outlet boundary condition set to a static pressure of 101.3 kPa. The fluid selection chosen was a custom material under non-newtonian fluids subcategory. Density (ρ) values were obtained from the literature. Alginate (2% w/v) was given the parameter of ρ = 1010 kg/m^3 , a power law viscosity profile with consistency coefficient and power index. Agarose was given the parameters of ρ = 1010 kg/m^3, a power law viscosity profile with consistency coefficient and power index. PEG (5 w/v) was given the parameters ρ = 1016 kg/m^3, a power law viscosity profile with consistency coefficient 1 Pa*s and a power index of 1

Injection Molded Hydrogel Fabrication

The fabrication processed is imaged below in Figure 1. This process begins with a computer-aided design model of the injection mold, which is 3D printed using a stereolithography printer. The hydrogel is prepared and injected into the mold. Finally, the hydrogel is extracted after allowing for crosslinking.

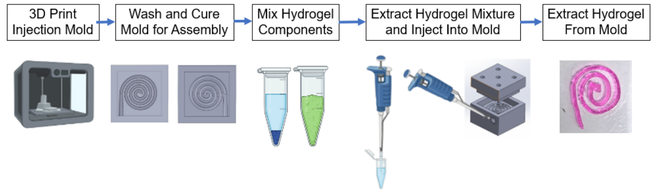

Figure 1. The process of the printing, injecting and extraction of encapsulation devices. Using Formlabs Form 3 stereolithography 3D printer we constructed our experimental mold designs. Printed with a 50 µm resolution and made with a flexible resin.

Rheology

Previous literature shows PEG behaves as a newtonian, n=1 and k=1 Pa*s.

Power Law for Best Fit:

$$\eta = k\dot{\gamma}^{n-1}$$

Power-law Coefficients Based on Viscosity vs Shear Rate		
	2% Alginate	2% Agarose
Consistency coefficient (k)	1.03	0.176
Power index (n)	0.85	0.61

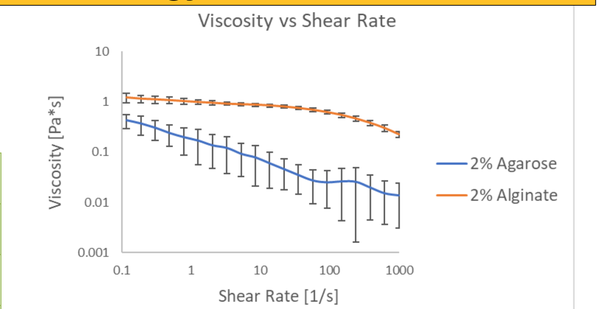

Figure 2. Viscosity vs. Shear rate using rotational analysis at 37C. Using power law to best fit for coefficients used to construct a viscosity profile

Fluid Flow Simulation Output

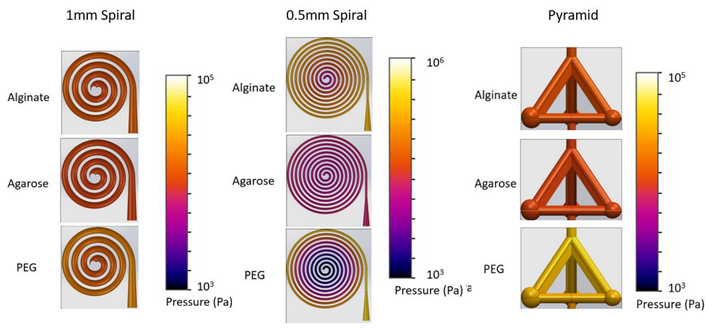

Figure 3. To generate these hydrogels, we need to model the pressure dynamics of the injection molds during injection. By doing this we ensure that the pressure is evenly distributed and there are no blank or problematic areas in our design and that our design is viable for cell survivability during injection. Flow rate is at 30 µL/s and a viscosity profile is made using the values in Figure 3. Maximum pressure tolerance is 200 MPa.

Hydrogel Molding Reproducibility

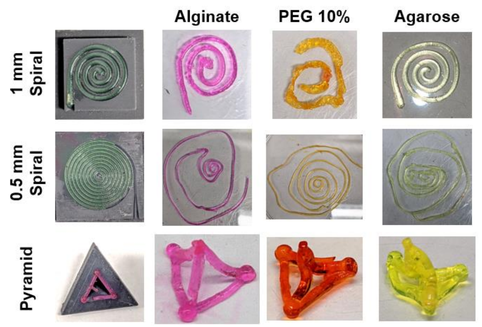

Figure 4. The successful extraction of the encapsulation device in it's designed entirety is paramount. If the gel is broken, incomplete or malformed it will underperform in it's expected parameters or compromise the encapsulation of the cells resulting in the therapeutic unable to be delivered. In these images we show how the hydrogel is formed with in the mold and what they are expected to look with extraction.

Hydrogel Molding Reproducibility

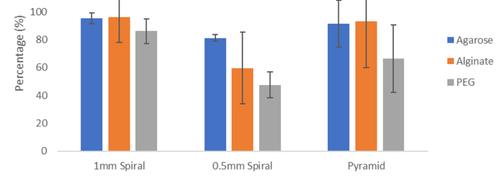

Figure 5. Average fill percentage of hydrogels per mold design. n=10 gels/group. Data was analyzed by t-test used to compare two groups within the geometries with significant difference.
* P < 0.05.
0.5mm Spiral one p-value:
Agarose vs Alginate: 0.023*
Agarose vs PEG: 0.005*
Pyramid one tail p-value:
Alginate vs Agarose: 0.31
Alginate vs PEG: 0.002*

Conclusion

With proof of concept with a simple three-dimensional shapes and a greater complex three-dimensional hydrogel we will expand into replicating biological structures that are present in the body such as blood vessels and alveoli to further expand on our injection molding technique and to show greater diversity for these hydrogels. Utilizing our rheological library and flow simulation we will verify our work for cell viability *in vivo.* With the success of this technique, we will be able to scale up production of macroencapsulation devices to better deliver and develop treatments that have cell-based approaches.

Acknowledgment

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