OPTIMIZATION OF HYDROGEL VISCOELASTICITY TO IMPROVE TRANSPLANTED CELL VIABILITY

Brian Aguado[1], *Sarah C. Heilshorn*[2]

[1] Department of Biomechanical Engineering,
[2] Department of Materials Science and Engineering,
Stanford University, Stanford, CA

Introduction

The use of cell transplantation in cardiovascular and neural tissue engineering has demonstrated promising results as a method to improve tissue function in patients after myocardial infarction or stroke. However, several obstacles prevent these novel cell transplantation procedures from being utilized in a mainstream clinical environment. Outcomes of cell viability with the use of current cell transplantation techniques result in a considerable deficit of live cells after transplantation (typically as low as 5-35%).[1] The need to increase the percentage of live cells post injection is critical to the success of the transplant. Previous studies have correlated the success of symptomatic relief with higher cell viability after transplantation.[2] Parameters of injection including cell density, flow rate, cell-carrier medium, and needle diameter currently rely on trial and error.[3]

Here we study the biophysical variables that affect the overall viability of cells during direct injection procedures. Hydrogels as biomaterials are known to provide an artificial extracellular matrix (ECM), which can provide viscoelastic support for new cell and tissue formation.[4] Recent studies have also indicated cell injection with hydrogels can improve cell viability.[2] We have chosen alginate as a model hydrogel material to determine the impact of cell-carrier viscoelasticity on cell viability during injection. Alginate has been utilized in many pre-clinical injection studies to provide a temporary cellular scaffold and attenuate adverse cardiac remodeling.[5] Alginate, an easy to use and inexpensive biopolymer that undergoes ionic crosslinking, has easily tunable viscoelastic properties, making it attractive for tissue engineering applications. By systematically altering the weight percent, degree of ionic crosslinking, and molecular weight of the alginate biopolymer, we create a series of injectable alginate hydrogels. Using these materials, we investigate the optimal viscoelastic properties to provide proper cell encapsulation and protection from shear stress during injection.

The viscoelastic properties of the various alginate hydrogel formulations are determined using dynamic shear rheometry. Human umbilical vein endothelial cells (HUVEC) are encapsulated within the hydrogels, shear-thinned at constant linear velocity using a syringe pump, and evaluated for acute cell viability. Endothelial cells are a clinically-relevant cell type that has been investigated in pre-clinical studies for treatment of multiple diseases including peripheral artery disease, myocardial infarct, and stroke.[6] These results will be analyzed using idealized fluid flow models to determine which mechanical parameters are most critical to maintaining high cell viability during injection procedures. Together with fluid dynamics models, the experimental results provide mechanistic insight into the optimal hydrogel viscoelastic properties required for improving the viability of transplanted cells. These results are expected to be applicable to the future development of novel hydrogel materials with optimized viscoelastic properties for clinical cell transplantation therapies.

Experimental

Methods and Results. 2.1: Alginate Formulation and Characterization. Alginate biopolymers are composed of long chains of individual sugar residues of G-subunits and/or M-subunits.[4] On a molecular level, four G-subunits coordinate with one calcium ion to form an ionic crosslinking junction. This ratio allows us to consistently adjust the ratio of G-subunits to available calcium ions to produce gels with pre-determined viscoelastic properties. A total of 27 different alginate gels have been made by varying three different parameters: (1) the average molecular weight of a single chain (75,000 g/mol, 145,000 g/mol, and 200,000 g/mol); (2) the alginate concentration (0.25%, 0.50% and 1.00% wt/vol), and (3) the ratio of G-subunits to calcium ions (2:1, 3:1, 4:1). These hydrogels were then characterized by rheometry using an MCR301 Anton Paar rheometer (Ashland, VA).

The dynamic storage and loss moduli of each hydrogel were measured and compared to determine apparent trends in viscoelastic properties. Each variable was independently tuned (keeping the other two variables constant). As expected, increasing the molecular weight of the polymer (while keeping crosslinking stoichiometry and weight per volume concentration constant), resulted in a corresponding increase in storage (G') and loss (G") moduli (Figure 1). Similarly, increasing the alginate weight concentration or the extent of calcium ion crosslinking resulted in increases in G' and G" (data not shown). Across a frequency sweep of 0.1 to 100 sec^{-1}, both G' and G" were found to be relatively independent of frequency, although all values trended up slightly at higher frequencies. Shear rheometry was also utilized to determine the shear-thinning and re-gelation behavior of the alginate hydrogels to simulate injection protocols.

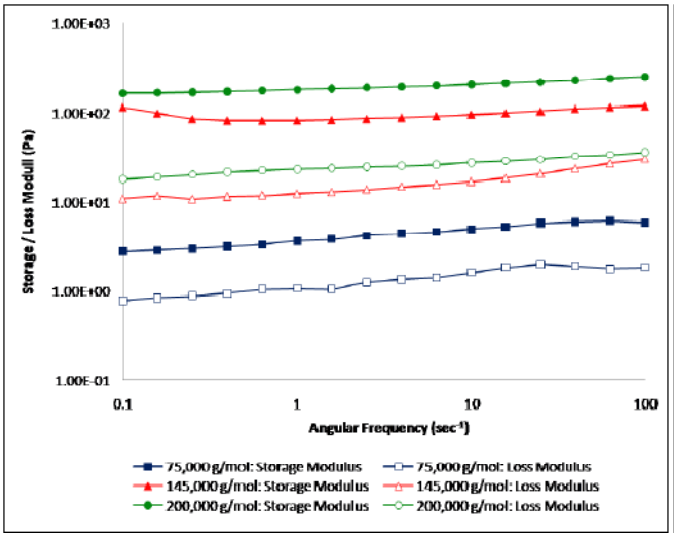

Figure 1. Storage (filled symbols) and loss (open symbols) moduli of alginate hydrogels of varying molecular weight, identical polymer weight concentration, and identical extent of ionic crosslinking.

2.2: HUVEC Encapsulation and Injection. HUVEC (Lonza) were cultured in EGM-2 HUVEC growth medium; this same medium also was used to prepare the calcium chloride solution, while alginate was dissolved in phosphate buffered saline (PBS). HUVEC were first suspended in the calcium-rich medium and then mixed with the alginate solution inside a 28 gauge micro-fine insulin syringe (BD). The syringe was placed on a syringe pump and the mixture was ejected into a Petri dish at a flow rate of 1,000 μL/min. HUVEC viability was determined using a fluorimetric LIVE/DEAD assay (Invitrogen) to detect compromised cell membranes. All experiments were performed in triplicate.

For preliminary experiments, HUVEC were encapsulated in alginate hydrogels spanning the range of plateau G' from ~20-1,000 Pa. In all cases, the encapsulated HUVEC were observed to be evenly dispersed throughout the hydrogels. While negative control solutions (PBS buffer only) resulted in cell viability of 51.7 ± 3.1%, a relatively compliant alginate hydrogel (G'~20 Pa) yielded 91.1 ± 1.1% viability, similar to the viability of encapsulated HUVEC in a non-sheared alginate positive control sample (Figure 2). However, further increasing the hydrogel shear modulus (G'~1,000 Pa) resulted in a decrease in cell viability, 52.6 ± 4.0% (Figure 3). These results clearly demonstrate that an optimal range of hydrogel viscoelasticity exists, which can greatly increase cell viability during high shearing, as might be experienced during cell transplantation therapies.

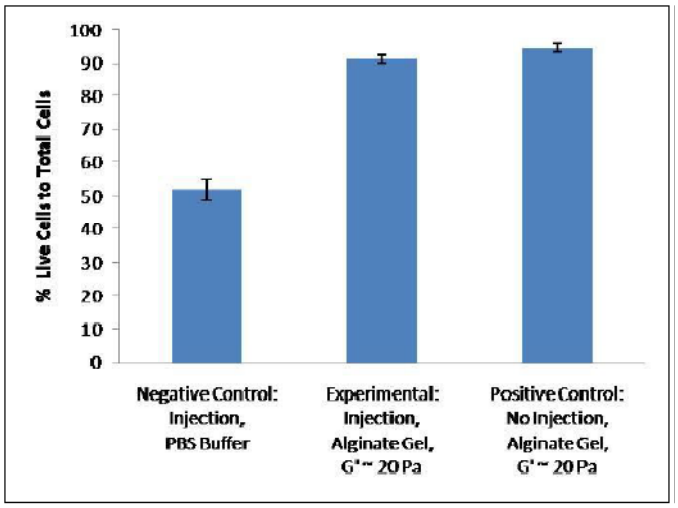

Figure 2. HUVEC viability within various carrier matrices (liquid buffer and a compliant alginate hydrogel, G'~20 Pa) with and without linear shearing through a syringe needle.

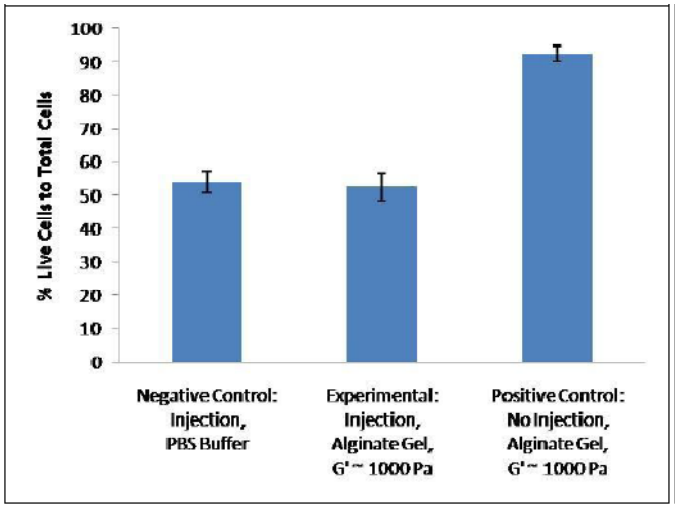

Figure 3. HUVEC viability within various carrier matrices (liquid buffer and a compliant alginate hydrogel, G'~1,000 Pa) with and without linear shearing through a syringe needle.

Results and Discussion

As expected, tailoring the alginate molecular weight, the ratio of G-subunit to Ca^{+2} ions, and the alginate weight concentration in solution allows the formation of multiple alginate hydrogels with specific viscoelastic properties. By taking advantage of the tunable characteristics of alginate hydrogels, quantitative data has been generated describing the potential relationship between the viscoelastic properties of the alginate hydrogel and the viability of sheared HUVEC.

Conclusions

The initial viability data indicates that cell injection using parameters commonly employed in pre-clinical cell transplantation studies (liquid cell carrier injected through a 28 gauge needle at 1,000 µL/min) results in a significant amount of cell death, with only about half of the cells surviving the procedure. Intriguingly, compliant alginate hydrogels (G'~20 Pa) increase cell viability similar to non-sheared controls, while more rigid hydrogels (G'~1,000 Pa) offer no cell viability enhancement. Current work is underway to utilize fluid flow models to estimate the pressure drop and shear stress across a single cell (diameter ~10 microns) located at various radial positions within the syringe needle. Ultimately, this research has demonstrated preliminary promise in the future development of hydrogels with optimized viscoelastic properties for the improvement of efficient cell transplantation therapies.

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References

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