Hydrogel system for improved cell graft viability and function in treating Type 1 Diabetes

Victor M. Quiroz[1*], Yuanjia Wang[1*], Sarah Y. Neshat[1], Stephany Y. Tzeng[1], and Joshua C. Doloff[1,2,3]

1. Department of Biomedical Engineering, Translational Tissue Engineering Center, Johns Hopkins University School of Medicine, Baltimore MD
2. Institute of Nanobiotechnology, Johns Hopkins University, Baltimore, MD
3. Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD
***: equally contributing authors**

Introduction: Biomedical devices such as islet-encapsulating systems are used for treatment of type 1 diabetes (T1D). While there have been recent strides in preventing biomaterial-induced fibrosis, challenges remain for using biomaterials as scaffolds due to limitations on cells contained within. This study tests the hypothesis that the proliferation and insulin secretion of insulinoma cells and/or pancreatic islets destined for therapeutic treatment of T1D can be improved by encapsulation in alginate hydrogels of optimized gel%, crosslinking, and stiffness.

Materials and Methods: First, a 2-step crosslinking process was adopted to eliminate regional variation in material architecture. Subsequently, we characterized four types of (G or mannuronic acid (M)-rich) alginate, using 2 gel concentrations and 5 different Barium chloride solution crosslinker concentrations in each case. Rat insulinoma cells (INS-1) were chosen for their insulin secreting ability.

Results and Discussion: Alginates were easily tunable with stiffnesses ranging from 300 Pa to over 5,000 Pa (**Fig. 1a**). This range fits the reported physiological range of pancreatic tissue of 1 to 4 kPa. qPCR-based graft phenotyping performed on INS-1 cells encapsulated in hydrogels of varying stiffness identified a range that maximizes the transcription of insulin (INS1) between 700 and 1000 Pa (**Fig. 1b**). Functional glucose-responsive insulin secretion (GRIS) data shows some agreement with the gene expression analysis though these results will need to be validated through experimental repeats. The fabrication process using $BaCl_2$ was also optimized, as extended exposure has shown to be cytotoxic, and a representative 3D Live/Dead IF microscopy z-stack showing high viability after these optimizations is provided (**Fig. 1c**). We then compared the storage modulus of alginates crosslinked via $BaCl_2$ vs $CaCl_2$ and found that $CaCl_2$-crosslinked hydrogels exhibited a sharp loss in storage modulus over $BaCl_2$ following various *in vitro* incubations (**Fig. 1d**). Despite the higher affinity for the G-G block in alginate, $BaCl_2$-crosslinked hydrogels also exhibited stiffness loss over time. This may be due to exchange of barium with other divalent ions present in the culturing media. Lastly, assays will be repeated using primary islets to confirm a clinically relevant response.

Conclusion: Results suggest it is possible to improve onboard graft function, with implications for graft longevity, productivity, and responsiveness. Such findings have import to those desiring to carry out *in vitro* characterization and optimization of cell carrier systems with high fidelity as well as potential follow-on efficacy testing *in vivo*.

Acknowledgements: We would like to thank the Juvenile Diabetes Research Foundation (JDRF) (Grant 1-PNF-2019-782-S-B) and NIH R25GM109441 (Hopkins PREP) for support of this work.

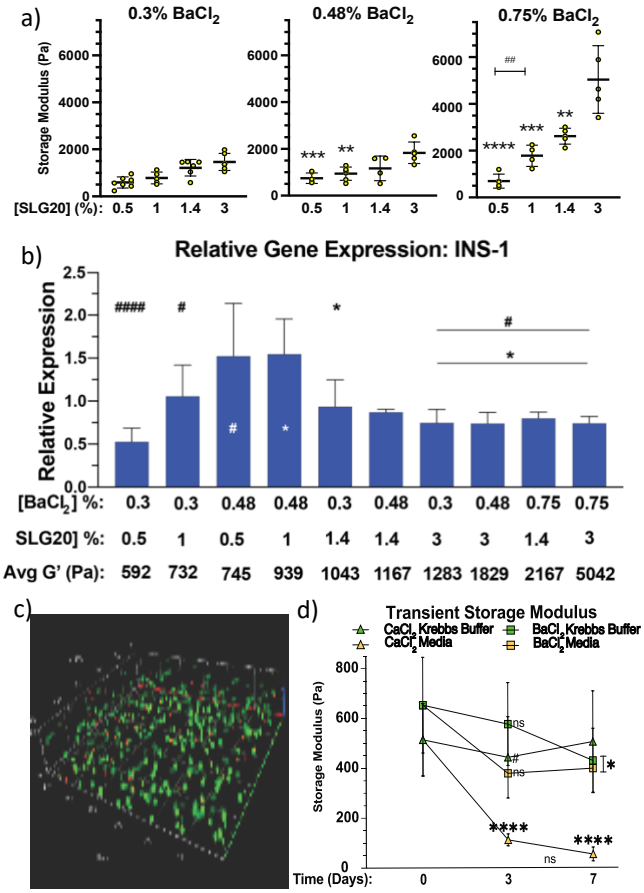

Figure 1 – Matrix tunability affects viability and therapeutic functional output of encapsulated cells. a) Storage modulus (Pa) of hydrogels crosslinked with 0.75, 0.48, and 0.3% $BaCl_2$ using 0.5, 1.0, 1.4, & 3% SLG20 alginate. *Denotes degree of significance in reference the group with the highest storage modulus for each subfigure respectfully. b) Relative expression of INS1 (insulin) at day 3 plotted in order of increasing storage modulus. Error bars, SD. c) 3D z-stack of a representative image of INS-1 cells in alginate. Green cells are alive while red cells are dead. d) Transient Storage Modulus of $CaCl_2$- or $BaCl_2$-crosslinked hydrogels incubated at 37°C and 5% CO_2 in either 1mL of Krebs buffer or 1mL of cell culture media and measured via rheometry on days 0, 3, and 7. One-way ANOVA statistical analysis was performed for each. *, $p \leq 0.001$; **, $p \leq 0.01$; and ***, $p \leq 0.001$.