Exploring the Synergistic Connection between Glucose Metabolism and Mechanosensitivity in Macrophage Function

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Statement of Purpose: Encapsulated islet transplantation (EIT) is a tissue engineered therapy that targets exogenous insulin dependance and regulates glucose levels in Type 1 diabetic patients.[1] This therapy consists of encapsulated whole donor islets, specifically the insulin-secreting β cells from islets, in a semipermeable polymeric membrane. The capsule protects the cells from immune cells, leukocytes, while promoting islet function and viability. While researchers have seen success in diabetic animal models, EIT therapies are currently not a clinically relevant, long-term solution for insulin dependance, due to rejection by the patient's immune system.[2] Macrophages are a primary contributor to the orchestration and severity of foreign body response.[3] As phagocytes and antigen-presenting cells, macrophages respond to EIT capsules, engaging with the foreign object and secreting proinflammatory cytokines like tumor necrosis factor (TNF-α) and interlukin-6 (IL-6), degrading enzymes, and chemokines to protect the body from the invader. Recent studies have shown substrate stiffness affects macrophage function.[4,5] However, due to dysregulation of glucose maintenance in diabetic patients, varying from normoglycemic (80-120 mg/dL) to hypoglycemic (<80 mg/dL) or hyperglycemic (>120 mg/dL) conditions, it is imperative to determine if this glucose dysregulation will affect the mechanosensitivity of the macrophages as they respond to EIT biomaterials. The objective of this study is to explore the relationship between glucose metabolism and mechanosensitivity and its impact on macrophage function.

Methods: 1, 13, and 280 kPa polyacrylamide gels were synthesized on 22x22mm glass coverslips.[4,6,7] Compositions for each stiffness are as follows: 15% acrylamide + 1.2% bis (280 kPa), 8% acrylamide + 0.2% bis (13 kPa), and 3% acrylamide + 0.2% bis (1 kPa). Gels were activated with 0.5 mg/mL sulfo-SANPAH and coated with 0.1 mg/mL fibronectin for cell attachment. 1e5 RAW264.7 macrophages were seeded onto each gel in a 6-well plate and incubated overnight at 37°C to adhere. Macrophage-attached gels are transferred to a new well and media is changed to begin study. Cells were cultured under either 450 mg/dL (hyperglycemic – HG) or 100 mg/dL (normoglycemic – LG) conditions, with or without 0.5 µg/mL salmonella typhosa LPS included for stimulation. Samples were assayed for TNF-α, IL-10 and IL-6 cytokine secretion via ELISA.

Results: Changes in glucose concentration from LG (100 mg/dL) to HG (450 mg/dL) resulted in a difference in how macrophages respond to stiffness in cytokine expression. For TNF-α expression, shown in **Figure 1**, when stimulated with LPS, significant mechanosensitivity trends were only seen in HG conditions with no changes seen in LG conditions specifically after 3 days of LPS stimulation. Interestingly, this trend was reversed in unstimulated conditions. Significant mechanosensitivity changes were only seen in LG conditions. The mechanosensitivity trends indicated increased inflammatory cytokine secretion in 1 kPa substrates compared to 280 kPa substrates. When HG conditions were compared to LG conditions under the same stiffness, significant changes in TNF-α expression were only found in 1 kPa cultured macrophages. Similar trends were observed for IL-10 and IL-6 secretion for HG conditions on 1 kPa compared to LG. However, mechanosensitivity trends when compared under the same glucose concentration were not as significant as TNF-α findings.

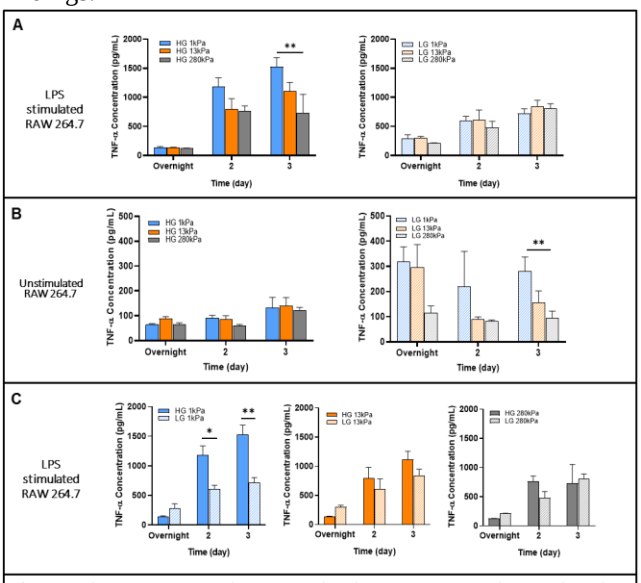

Figure 1: A) TNF-α expression by LPS stimulated RAW264.7 macrophages cultured on 1, 13, and 280 kPa polyacrylamide gels under 450 mg/dL (HG) and 100 mg/dL (LG) glucose conditions. Samples were taken pre-transfer (overnight) and after transfer and stimulation (day 2 and day 3). B) TNF-α expression by unstimulated RAW264.7 macrophages under the same parameters as LPS samples. C) Comparison between HG and LG samples cultured on the same stiffness. N=3 biological replicates were conducted for all samples. Statistical analysis was conducted using ANOVA and Student t test, where $p < 0.05$ indicated statistical significance. *$p < 0.05$ and **$p < 0.005$. Multiple comparisons were done after ANOVA using Tukey tests.

Conclusion: Glucose metabolism was determined to influence the mechanosensitivity of RAW264.7 macrophages through cytokine secretion, a key cellular function. Current studies will investigate macrophage polarization, gene expression, and glucose uptake to understand the mechanism of this relationship and determine ideal stiffness for encapsulation design.

References: 1. Vaithilingam V. Rev. Diabet. Stud. (2017; 14:51-78) 2. Yang HK. Transplantation. (2016; 100:334-343) 3. Sheikh Z. Materials. (2015; 8:5671-5701) 4. Adlerz KM. Eur. Biophys. J. (2016; 45:301-309) 5. Mennens SFB. Results Probl. Cell Differ. (2017; 62:209-242) 6. Wang YL. Meth. Enzymol. (1998; 298:489-496) 7. Stroka KM. Blood (2011; 1632-1640)