Characterization of Adhesive Peptide Binding Efficacy in SPAAC and Michael-Type Addition PEG Hydrogels to Support Encapsulated Pancreatic Islet Cell Viability

Keven Sepulveda, Biomedical Engineering
Mentor: Dr. Jessica D. Weaver, Assistant Professor
School of Biological and Health Systems Engineering

Motivation

• In 2019, the CDC estimated the total population of Americans living with Type I diabetes to be 1.84 million [1].
• Islet cell transplantation is a promising treatment option for blood glucose regulation but is hindered by a lack of pancreatic donors, the need for immunosuppressants, and high percentage of transplant rejections [2].
• Synthetic, nondegradable polyethylene glycol (PEG) hydrogels provide a three-dimensional (3D) hydrophilic matrix to protect pancreatic beta cells from immune system attack (T-cell mediated) [3].
• Hydrogels can be designed to promote passive nutrient diffusion and prevent hypoxia via biofabrication such as injection molding [4].
• RGD is a cell adhesion peptide composed of 3 amino acids (arginine-glycine-aspartate) that improves cellular proliferation and attachment [5].
• This research characterizes strain-promoted azide-alkyne cycloaddition (SPAAC) chemistry suitability for cell encapsulation versus a Michael-type addition chemistry by comparing binding efficacy of an Azide-functionalized RGD with a 4-arm PEG-Dibenzylocyclooctyne (DBCO) macromer to a 4-arm thiol-functionalized RGD with a PEG-Maleimide (MAL) macromer.

Methods

Gel Fabrication
• Preparation of 15 μL PEG hydrogels (n=3) with or without 0.001 M RGD in DPBS(-)(-)
• Conjugation of thiol- or azide- RGD (Arginine, Glycine, and Aspartate) to Alexa Fluor 647 NHS ester (Invivogen) for 1 hour at RT, then RGD via Thiol or Azide group binds to hydrogel.
• Hydrogels formed with 5% and 10% polymer densities (w/v)
• Controls—hydrogels with AF 647 dye but no RGD
• AF 647 is 1% of hydrogel volume
• Hydrogels formed in 24-well plate, gelled for 5-10 min, then incubation in 500 mL DPBS(-)(-) at 37 C in cell incubator
• DPBS changed prior to imaging

EVOS FL Auto Live Cell Imaging System (Microscope)
• Fluorescence imaging (with Cy5 LED cube), 4x oil objective
• Tile image of full hydrogel at 0, 24, 48, and 96 hours

Image J Analysis
• Select standard line across full length of hydrogel and measure fluorescent intensity
• Compare AU fluorescent intensity over time for each hydrogel
• We plan on collecting more data for additional groups prior to conducting a two-way ANOVA with post-hoc Tukey test in GraphPad Prism

Fig 1. Schematic of PEG chemistries

Results

• FI decreased for all gel groups; however, greatest loss of FI was observed after 24 hours
• Comparisons to control group demonstrate that RGD is binding to the hydrogel
• Data shows some dye retention despite no chemical binding to hydrogel via RGD
• Polymer density impacted PEG-MAL groups by visually having a slightly higher retention than gels in the same category.
• This data suggests SPAAC hydrogels may experience lower cell viability outcomes due to lower RGD binding efficacy compared to Michael-type addition hydrogels

Conclusion

References

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