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

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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-Dibenzocyclooctyne (DBCO) macromer to a 4-arm thiol-functionalized RGD with a PEG-Maleimide (MAL) macromer

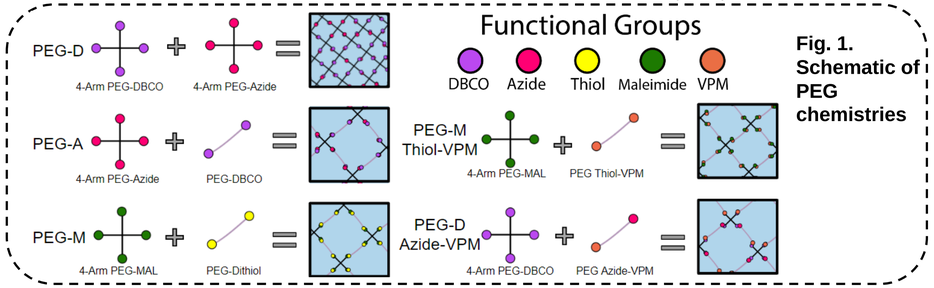

Fig. 1. Schematic of PEG chemistries

Methods

Gel Fabrication

- Preparation of 15 µl PEG hydrogels (n=3) with or without 0.001 M RGD in DPBS(-)(-)
- Conjugation of thiol- or azido- RGD (Arginine, Glycine, and Aspartate) to Alexa Fluor 647 NHS ester (Invitrogen) 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 DBPS (-)(-) 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 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

Results

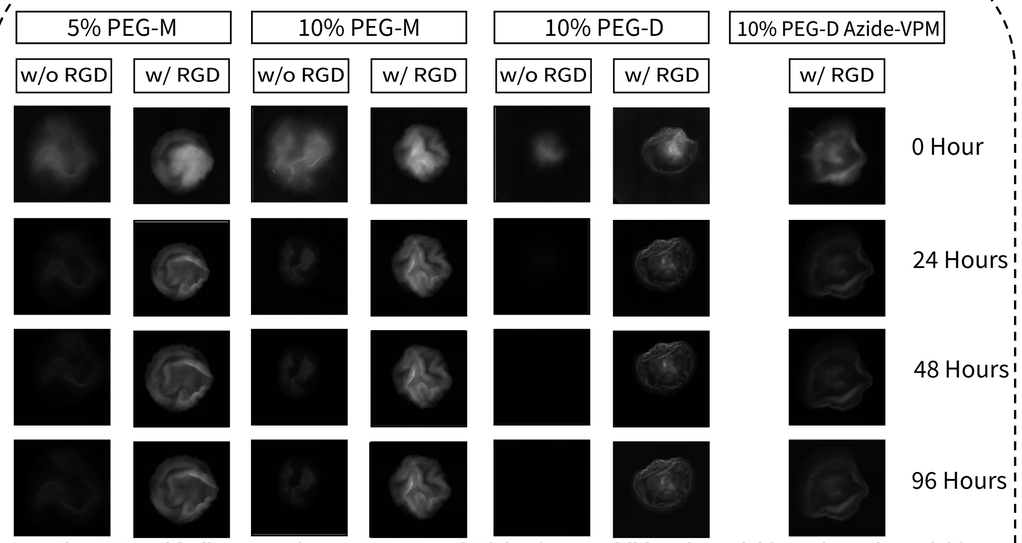

Fig. 2. RGD binding retention to SPAAC and Michael-type addition degradable and nondegradable hydrogels. Fluorescence images of PEG with or without RGD at 0, 24, 48, and 96 hours post-fabrication. Each image represents an individual gel chosen as a representative image. n=3/group. Additional gels with and without RGD will be 5% PEG-D, 5% PEG-D Azide-VPM, 10% PEG-D Azide-VPM (w/out RGD), 5% PEG-A, and 10% PEG-A .

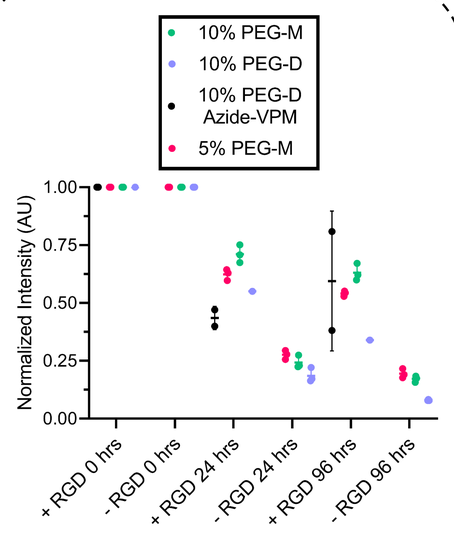

Fig 3. RGD binding efficacy over time of SPAAC and Michael-type addition degradable and nondegradable hydrogels. Fluorescent intensity over time for PEG-hydrogels with and without RGD. Data represented as mean +/- SD. n=1-3.

Conclusion

- 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

References

[1]"JDRF Official Statement on 2022 CDC National Diabetes Statistics Report," JDRF. https://www.jdrf.org/press-releases/jdrf-statement-on-2022-cdc-national-diabetes-statistics-report/ .
[2] V. Pathak, N. M. Pathak, C. L. O'Neill, J. Guduric-Fuchs, and R. J. Medina, "Therapies for Type 1 Diabetes: Current Scenario and Future Perspectives," Clin Med Insights Endocrinol Diabetes, vol. 12, p. 1179551419844521, May 2019, doi: 10.1177/1179551419844521.
[3]N. Kumar, H. Joisher, and A. Ganguly, "Polymeric Scaffolds for Pancreatic Tissue Engineering: A Review," Rev Diabet Stud, vol. 14, no. 4, pp. 334–353, 2017, doi: 10.1900/RDS.2017.14.334.
[4]A. Espona-Noguera et al., "Review of Advanced Hydrogel-Based Cell Encapsulation Systems for Insulin Delivery in Type 1 Diabetes Mellitus," Pharmaceutics, vol. 11, no. 11, p. 597, Nov. 2019, doi: 10.3390/pharmaceutics11110597.
[5]S. L. Bellis, "Advantages of RGD peptides for directing cell association with biomaterials," Biomaterials, vol. 32, no. 18, pp. 4205–4210, Jun. 2011, doi: 10.1016/j.biomaterials.2011.02.029.

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