Functionalization of alginate with RGD peptide to enhance viability and function of encapsulated islets

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Introduction: Interactions between extracellular molecules and pancreatic islet-cells have regulatory effects on pancreatic islet survival, insulin secretion, proliferation, morphology, and biochemical signaling[1-2]. Unfortunately, these interactions are disrupted during the islet-isolation process. We hypothesize that islet viability and function can be enhanced by the preservation or reintroduction of these extracellular matrix interactions. Previously, ECM-mimicking recognition sequences have been incorporated into alginate capsules to improve cellular interactions[3-5]. However, chemical conjugation to alginate can be detrimental to encapsulated islets. Here, we conjugate RGD peptide to alginate via a two-step reaction in which alginate is oxidized by sodium periodate and subsequently functionalized with RGD via reductive amination using a non-toxic, but effective, reducing agent[6].

Methods: Pronova UP MVG alginate (NovaMatrix) was oxidized with sodium-(meta)periodate. *Functionalization of alginate with RGD:* Aldehyde groups of oxidized alginate spontaneously tethered to the N-terminus of RGD peptides and picoline-borane complex was added to form a stable secondary amine bond between alginate and RGD. *Quantification of functionalization:* Peptide tethering was quantified with fluorescence imaging by tethering alginate to RGD-anthracenecarboxylic acid (RGD-ant). *Cell encapsulation:* INS-1 cells were suspended in functionalized alginate at a density of 24×10^5cells/mL. The alginate-cell slurry was then dropped into a 100 mM $CaCl_2$ bath using a Nisco encapsulator and a Harvard Apparatus syringe pump.

Results: FTIR spectra of RGD-functionalized alginate confirm the formation of a stable peptide tether with an observed C-N stretch at ~1281 cm^{-1} (Fig 1A). RGD-functionalized alginate can crosslink to form microcapsules that present RGD homogeneously throughout (Fig 1B). Furthermore, the density of incorporated RGD to alginate proportionally increased with peptide concentration and degree of oxidation (Fig 1C and 1D). Viability of encapsulated INS-1 cells was higher in RGD-alginate capsules (79.1±7.2%) than in unmodified alginate (69.1±9.1%) and scrambled RDG-alginate capsules (31.9±6.7%) but was similar to that of control, unencapsulated INS-1 cells (77.7±10.3%) (Fig 1E).

Conclusion: We successfully conjugated RGD to alginate using a cytocompatible technique that allows for efficient and tunable tethering. RGD-functionalized alginate can gel and form capsules that uniformly display RGD. We also observed enhanced viability of INS-1 cells encapsulated in RGD-functionalized alginate capsules compared to unmodified and RDG-scrambled peptide alginate capsule controls. Future studies will look at the effect RGD-functionalization has on islet viability, insulin secretion, and metabolic activity.

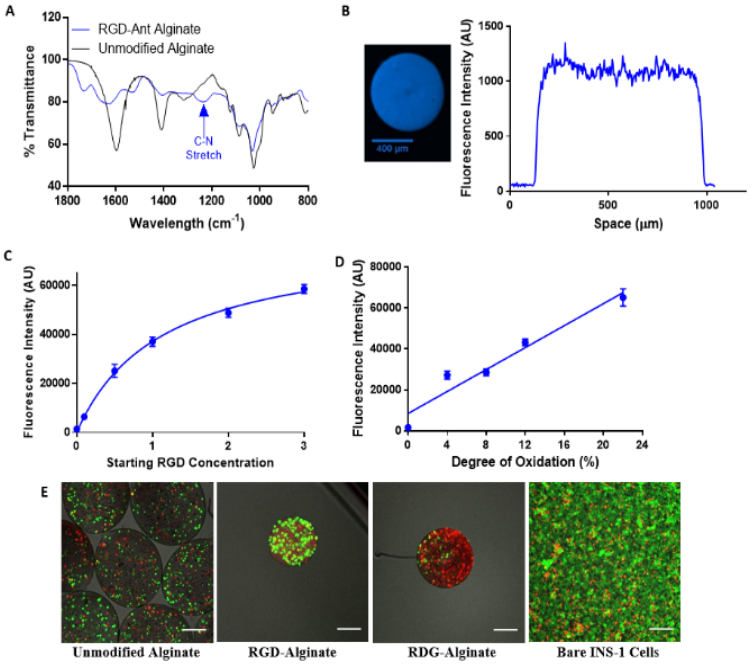

Figure 1. A) FTIR of unmodified alginate and RGD-functionalized alginate. **B)** Confocal image of an RGD-Ant alginate capsule and the presentation of RGD across the capsule (scale bar 400 µm). Fluorescence emission of RGD-ant-functionalized alginates with **C)** varying initial peptide concentrations and **D)** varying oxidation degrees (average ± st dev (n=3)). **E)** Live/dead confocal images of INS-1 encapsulated cells (scale bar 100 µm).

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