Manipulation of Protein Sequence and Functionalization to Enhance Cell Interactions with Bioactive Hydrogels

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Statement of Purpose: Poly(ethylene glycol) (PEG) hydrogels are widely utilized biomaterial scaffolds due to their established biocompatibility and tunable properties. Their intrinsic resistance to protein adsorption and cell adhesion provides a "blank slate" into which bioactive agents can be incorporated to illicit desired cell-material interactions. We have established a biomaterial platform based on PEG hydrogels with streptococcal collagen-like proteins (Scl2-1). Scl2-1 has the triple helical structure of collagen, but it lacks integrin binding sites and can be modified to have selective cell adhesion. $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins have been introduced into Scl2-1 to provide a modified Scl2 protein (Scl2-2) that maintains the structure Scl2-1 while promoting endothelial cell (EC) adhesion. Upon functionalization with a photo-crosslinkable PEG linker, Scl2-2 can be incorporated into PEG hydrogels without detriment to bioactivity. We have shown that decreasing acrylate-PEG-N-hydroxysuccinimide (Acr-PEG-NHS) linker density on the Scl2-2 backbone enhances EC interactions with PEG-Scl2-2 hydrogels through reducing steric hindrance around integrin binding sites; however, this negatively impacts the protein stability within the matrix due to hydrolysis of acrylate groups on Acr-PEG-NHS. We hypothesize that a hydrolytically stable PEG linker could increase protein retention within the hydrogel matrix and allow for use of lower functionalization density proteins to enhance and sustain EC interactions. Alternatively, the Scl2-2 sequence can be manipulated to position functionalization sites (lysines) further away from backbone integrin binding sites. This Scl2-2 (Scl2-2S) could allow for use of an increased functionalization density to improve protein maintenance over time with reduced effects of PEG linkers on cell adhesion.

Methods: ***Scl2-2 Functionalization:*** Acrylamide-PEG-Isocyanate (Aam-PEG-I) was synthesized from PEG(3.4k) diamine. Briefly, PEG diisocyanate was synthesized by reacting PEG diamine with hexane diisocyanate. The resulting product was reacted with a 1 molar ratio of aminoethyl acrylamide to produce the di-functional PEG linker. Scl2-2S was synthesized by Genewiz to optimize codon usage for expression. Scl2-2 and Scl2-2S were functionalized with Aam-PEG-I or Acr-PEG-NHS at varying ratios of PEG:NH_2 (1:1; 0.1:1) to produce proteins with high and low PEG linker densities (1X; 0.1X). Bioactive hydrogels were fabricated by combining the functionalized proteins with PEG diacrylamide (PEGDAA) and exposing to UV light to initiate crosslinking. ***EC Interactions:*** ECs were seeded onto the swollen gels in the presence of serum at 10,000 cells/cm^2. After 3 hours, cells were fixed and stained with rhodamine phalloidin and SybrGreen. Representative cell images were obtained using a fluorescent microscope and used to quantify EC adhesion and spreading. EC adhesion strength was measured using a spinning plate apparatus. Briefly, samples were crosslinked to acrylated glass coverslips and seeded with ECs (10,000 cells/cm^2) for 24 hours. Samples were affixed to a spinning disc, lowered into PBS, and spun at a set rotational velocity for 10 minutes to expose adhered cells to a range of shear stresses. Following fixation and staining, fluorescent microscopy was used to determine the maximum shear stress at which ECs remained attached. ***Scl2-2 Retention:*** PEG-Scl2-2 hydrogels were swollen in 0.015 M NaOH for up to 6 days, and CBQCA was run on the retained swelling solutions to measure protein loss and determine protein retention over an accelerated timeframe.

Results: ***Scl2-2 Functionalization:*** FTIR spectroscopy confirmed successful functionalization of Scl2-2 and Scl2-2S with low and high densities of PEG linkers. The functionalized protein spectra contained absorption peaks corresponding to the carbonyl of the amides (C=O, ~1650 cm^{-1}) in the protein backbone and to the ether (C-O-C, ~1110 cm^{-1}) of PEG linker. ***EC Interactions:*** EC adhesion and spreading was significantly increased on PEG-Scl2-2 0.1X hydrogels relative to PEG-Scl2-2 1X hydrogels, with comparable trends seen between the Acr-PEG-NHS and Aam-PEG-I linker systems. ***Scl2-2 Retention:*** All Acr-PEG-NHS-functionalized Scl2-2 was lost after 1 day of swelling in the accelerated solution; however, Aam-PEG-I-functionalized Scl2-2 was retained in PEGDAA hydrogels for 6 days of swelling in accelerated conditions, **Figure 1**.

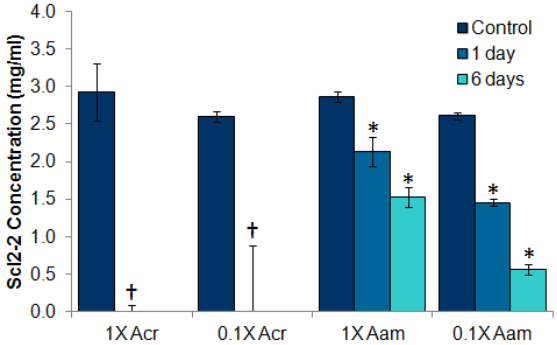

Figure 1. Scl2-2 retention in PEGDAA gels after 6 days of swelling in 0.015 M NaOH. †$p<0.05$ relative to previous time point. *$p<0.05$ relative to previous time point and analogous Acr-PEG-NHS-functionalized system.

Conclusions: Aam-PEG-I provides a PEG linker option for protein functionalization that is comparable to traditional Acr-PEG-NHS. However, the significantly enhanced protein retention with Aam-PEG-I allows for a reduced functionalization density to be used to promote increased cell interactions without detriment to long-term protein levels. Current work focuses on analyzing the effects of PEG linker position with Scl2-2S as an alternate method for improving sustained cell interactions with bioactive hydrogels.

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