Manipulation of Designer Collagen Sequence to Enhance Cell Interactions with Bioactive Hydrogels

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Statement of Purpose: Poly(ethylene glycol) (PEG) hydrogels are a widely used biomaterial due to their biocompatibility and highly tunable properties. PEG hydrogels are inherently resistant to protein adsorption and cell adhesion which permits controlled introduction of bioactive agents and corollary control of cell interactions. We coupled Streptococcal collagen-like protein (Scl2) into PEG-based hydrogels utilizing conjugation chemistry developed in our lab. Scl2 has a triple helix similar to collagen, but lacks integrin binding sites. Introduction of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrin binding sites into Scl2 using site-directed mutagenesis provided a modified protein (Scl2-2) that promotes selective cell adhesion. The Lys residues of Scl2-2 were conjugated to a photo-crosslinkable PEG linker for incorporation into PEG hydrogels. Previous studies demonstrated that decreasing functionalization density of Scl2-2 enhanced endothelial cell (EC) interactions by reducing steric hindrance around integrin binding sites. However, this reduction in functionalization density resulted in reduced protein retention in the PEG network. We hypothesized that reducing the number of Lys near the integrin binding sites of the protein could result in improved EC interactions without sacrificing protein retention. Since retention of the triple helix is vital to retain integrin binding, efforts were also taken to create a more stable triple helix in conjunction with altering functionalization sites. This was feasible due to an algorithm published by Persikov et. al. JBC, 2005. Frequency of triplet presence in eukaryotes was also taken into consideration when re-designing the triple helical domain. The current study characterizes the stability of the triple helix and effect on cell interactions with the new protein that we termed Synthetic Collagen (SC)-based hydrogel.

Methods: ***Protein Re-design and hydrogel fabrication:*** To generate a more thermally stable collagen, two triplets, GKDGKD, were mutated to GDRGER. To tailor functionalization of Scl2-2, 14 single Lys residues were replaced by Arg residues in GKD triplets. Arg at the X position of GXY triplets exists at the same frequency in eukaryotic collagens as replaced Lys residues and are similarly thermally stable. The mutations were introduced by gene synthesis (Genewiz). Scl2-2 and SC were then functionalized with acrylate-PEG-N-hydroxysuccinimide (Acr-PEG-NHS) with 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 (10% w/v) PEG-diacrylamide (3.4kDa) solutions and exposing to UV light to initiate crosslinking. ***Circular Dichroism:*** Spectra of protein in 20 mM Acetic acid were recorded on a Jasco J720 spectropolarimeter in a thermostatically controlled cuvette with a 0.5-mm path length. For thermal transition experiments, the ellipticity at 220 nm was monitored as the sample temperature was increased from 25 to 50°C, with an average temperature slope of 10°C/h. ***EC Interactions:*** ECs were seeded onto the swollen gels at 10,000 cells/cm^2. After 3 hours, cells were fixed and stained with rhodamine phalloidin, and SybrGreen. Cell images were obtained using a fluorescent microscope and used to quantify EC adhesion and spreading.

Results: ***Circular Dichroism:*** A marked increase in melting temperature from 37°C to 42°C was observed, **Figure 1.** These results indicated that protein stability predictions may be used to stabilize the triple helices.

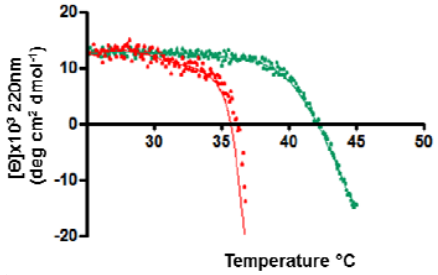

Figure 1. Thermal transitions of Scl2-2 (red) and SC (green).

Protein Functionalization: FTIR spectroscopy confirmed successful functionalization of Scl2-2 and SC with low and high densities of PEG linkers. The functionalized protein spectra contained absorption peaks corresponding to the carbonyl of the amides (~1650 cm^{-1}) in the protein backbone and ether (~1110 cm^{-1}) of PEG linker.

EC Interactions: There was a marked increase in cellular adhesion on the SC gels. Indeed, the high functionalization density of SC had similar adhesion to the low functionalization density of Scl2-2, **Figure 2.** EC spreading was comparable for Scl2-2 and SC at the 0.1X functionalization density. However, spreading for the 1X functionalization density was increased for SC gel.

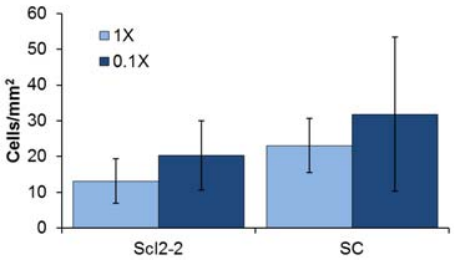

Figure 2. 3 hour EC adhesion on PEGDAA gels.

Conclusions: This study demonstrates the successful modification of the Scl2-2 protein and that this new protein can be used to generate bioactive hydrogels. The modification of the SC protein enhanced both stability and cell interactions with the bioactive hydrogel. Future studies will determine if the observed enhancement in cell interaction is due to reduced steric hindrance around the integrin binding site, increased stability of the triple helix, or a combinatory effect. Overall, this new Synthetic Collagen shows strong promise in the generation of bioactive hydrogels for a variety of tissue scaffolds.