Incorporation of Collagen-Mimetic Proteins into Bioactive Hydrogels

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Statement of Purpose: Tissue engineered vascular grafts (TEVGs) have the potential to restore function when conventional grafts are unavailable or fail. Although there have been significant advances in TEVGs, several obstacles still remain before they can be considered viable clinical alternatives. Past research has included the use of collagen-based hydrogels to aid in tissue repair. Collagen's multitude of receptor binding motifs increases the risk of thrombosis and has limited their use to grafts pre-seeded with endothelial cells prior to use. This study introduces the use of a Streptococcal collagen-like protein Scl2.28, termed Designer Collagens (DCs). This protein forms a triple helix similar to collagen but lacks collagen's numerous receptor binding motifs and risk of thromobosis. This provides a biological blank slate that can then be used to insert specific integrin binding sites using site-directed mutagenesis of the DC protein. Although the DC proteins show great promise in cardiovascular applications, their use was previously limited due to the low mechanical properties of the protein and potential coating destabilization in physiological conditions. To address this limitation, we have developed the synthetic methodology to functionalize Scl2 proteins with photocrosslinking sites to enable incorporation into a three dimensional hydrogel matrix. In this study three DC proteins were functionalized with acrylate end groups for incorporation into poly(ethylene glycol) hydrogels. The DC proteins used are DC1 which has no receptor binding motifs, DC2 which has the integrin binding motifs α1β1 and α2β1, and DC3 which has the integrin binding motif α2β1. Studies were then performed to confirm that functionalization did not disrupt the triple helix conformation and bioactivity.

Methods: *Synthesis:* Chemicals were purchased from Sigma-Aldrich (Milwaukee, WI, USA) and used as received. Acrylated PEG-(*N*-hydroxy succinimide)(NHS) was purchased from JenKem Technology (Beijing, China). PEGDA was synthesized and purified according to the method developed by Hahn[2]. FTIR and NMR spectroscopy were used to verify end group functionalization. The DCs were functionalized via the coupling lysine amino side groups with Acr-PEG-NHS(3500) according to the method developed by West[1]. FTIR spectroscopy and 10% SDS-PAGE gels were used to verify functionalization of DCs and denaturing conditions were used to confirm the retention of the triple helix.

Integrin Binding: Recombinant integrin I-domains α1 and α2 binding to the functionalized DCs was determined using ELISA-type binding assays. Bound integrin was detected using anti-His HRP conjugate. Collagen type I was used as a positive control for integrin biding.

Results: *Synthesis:* Successful synthesis of PEG-DA (data not shown) and functionalized DC was verified with FTIR spectroscopy, **Figure 1**. Specifically, an absorption peak corresponding to the stretching vibration of an ester carbonyl (C=O) group not found in the spectrum of PEG diol was observed in the spectrum of PEG diacrylate at 1728 cm^{-1} due to the esters introduced during functionalization. An absorption peak corresponding to the stretching vibration of an amide carbonyl (C=O) group not found in the PEG diol and PEG-DA spectra was observed in both the DC control and acrylated DC spectra at

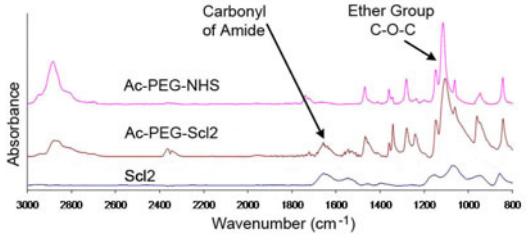

Figure 1: FTIR spectra showing PEG functionalized Scl2

1652 cm^{-1} due to the amides in the backbone of the proteins. In addition to FTIR spectroscopy, percent acrylation of the PEG was performed using NMR analysis and was greater than 85%. Functionalization of DC was further confirmed using SDS-PAGE, **Figure 2**. Specifically, in the columns containing the functionalized DCs an upward smeared band can be seen in comparison to the control, indicating an increase in molecular weight with functionalization. Furthermore, SDS-PAGE confirmed the retention of the triple helix following functionalization. The functionalized Scl2s retained the ability to migrate as multimers on SDS-PAGE analysis (lanes 5-8), whereas heat denaturation results in monomers with molecular weights at ~ 35kd

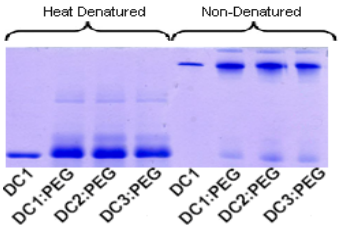

Figure 2: Coomassie-stained 10% PAGE analysis of functionalized DC proteins, non-denaturing and denaturing conditions.

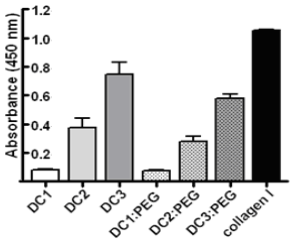

Figure 3:. ELISA-type binding assays demonstrate the retention of integrin binding after functionalization.

Integrin Binding: Functionalization resulted in a modest decrease in integrin binding, but the reduction was not statistically significant, **Figure 3.**

Conclusions: We have successfully functionalized DC proteins with photocrosslinking sites for incorporation into 3D hydrogels matrices. The functionalization did not disrupt the triple helix conformation or integrin binding of the DC proteins. Current studies are investigating the effects of material variables on endothelial cell behavior. The potential of this research is to provide an increase in controlled bioactivity to tissue engineered vascular grafts.

[1] Hahn M. Biomaterials
[2] West JL. Advanced Materials 18, 2679-2684 (2006).