Evaluation of Multilayer Vascular Grafts Based on Collagen-Mimetic Hydrogels

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Statement of Purpose The urgent clinical need for small-caliber prostheses has led researchers to explore new biomimetic strategies to generate grafts that more closely match native vessels. Given that platelet aggregation and smooth muscle cell proliferation may be mediated by controlling endothelial cell (EC) growth and phenotype, the development of materials that direct appropriate EC behavior would have a significant impact on small vessel repair and replacement. To this end, we have generated a novel biomaterial platform based on Designer Collagens, a collagen-mimetic protein originally derived from group A Streptococcus, Scl2.28. In addition to having the triple helical structure of native collagen and allowing for recombinant expression, Scl2 has several features desirable for cardiovascular applications. Specifically, its low thrombogenecity and selective cell adhesion achieved by engineering the protein to only display selected receptor-binding sequences via site-directed mutagenesis. The ability to tailor both the bioactivity and biomechanical properties of these hydrogels will provide unique control over endothelialization of the graft. However, a clinically-relevant vascular graft must also be able to withstand the peak arterial stresses and provide appropriate suture retention strengths. Matrix properties which promote graft endothelialization may not match those appropriate to sustaining arterial loads. We propose to address these conflicting scaffold requirements by developing multilayer scaffolds based on the bonding of the bioactive hydrogel layer to a polyurethane electrospun mesh. The properties of the luminal gel layer will be tailored to direct desired EC migration/phenotype, and the polyurethane mesh sleeve will be tuned to achieve desired burst pressure, compliance matching, and suture retention. The initial evaluation of our design indicates its potential as a viable, off-the-shelf graft with long-term patency.

Methods: The Scl2 proteins were first functionalized with acrylate-PEG-N-Hydroxysuccinimide to enable incorporation into a three dimensional hydrogel matrix via photocrosslinking sites. Bioactive hydrogels were then fabricated by combining the functionalized Scl2 proteins with poly(ethylene glycol) diacrylate and exposing to UV light to initiate crosslinking. ***EC Adhesion:*** EC were harvested, resuspended in media containing 10% FBS, and seeded onto the swollen Scl2-containing gels at 6,000 cell/cm2. After 3 hours, cells were fixed with paraformaldehyde and stained with rhodamine phalloidin and SybrGreen. Representative cell images were obtained using fluorescent microscope. ***Thrombogenecity:*** The thromboresistance of Scl2 containing hydrogels were evaluated relative to collagen-coated tissue culture polystyrene (TCPS) and pure TCPS positive controls. In brief, whole blood from Yucatan miniature pigs was collected and flowed over the surfaces using a parallel plate flow chamber for 2 minutes at a shear rate of 2000 1/s or in a bioreactor for 6 hours. Assessment of platelet adhesion was conducted via 1) measurement of the levels of lactate dehydrogenase (LDH) released from adherent cells following imposed cell lysis, and 2) manual counts of adherent mepacrine-labeled cells. Qualitative analyses of platelet aggregation were also conducted using brightfield microscopy. ***Multilayer Graft Fabtication:*** A rotating mandrel electrospinning set-up was used to fabricate the reinforcing fiber meshes of poly(ether urethane) urea (PEUU). Briefly, a 5 mm ID electrospun PEUU mesh was placed in a cylindrical mold, after which an inner glass mandrel (3 mm OD) was inserted and the hydrogel solution was pipetted between the mandrel and the pre-wetted mesh. Biomechanical testing (compliance, suture retention, burst pressure) of the resulting grafts was then performed according to established protocols.

Results: Endothelial cell adhesion on the Scl2 containing hydrogels were comparable to collagen-based hydrogels (pos control), **Figure 1**. In addition, platelet adhesion under flow was statistically less than collagen coated TCPS and comparable to the PEG hydrogel, **Figure 2**. Mechanical properties of the multilayer composites were comparable to current clinical grafts, **Table 1**.

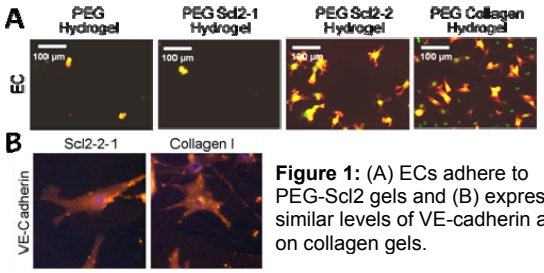

Figure 1: (A) ECs adhere to PEG-Scl2 gels and (B) express similar levels of VE-cadherin as on collagen gels.

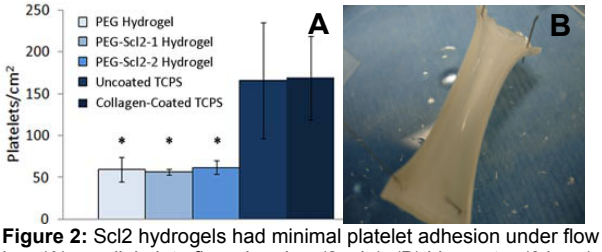

Figure 2: Scl2 hydrogels had minimal platelet adhesion under flow in a (A) parallel plate flow chamber (2 min); (B) bioreactor (6 hour).

Table 1: Mechanical properties of multilayer constructs are comparable to autologous grafts used in bypass surgeries.

Property	Composite	Autologous Grafts
Suture Retention	306 + 21 gf	196 + 2 gf[1]
Burst Pressure	1404 ± 40 mmHg	1680 ± 307 mmHg[2]
Compliance	5.2 ± 0.5 $mmHg^{-1}$ X 10^{-4}	4.7 $mmHg^{-1}$ X 10^{-4}

Conclusions: Scl2 hydrogels provide a non-thrombogenic intimal layer that promotes EC adhesion. Composite grafts reinforced with electrospun polyurethane meshes have mechanical properties comparable to current autologous grafts. Initial evaluation indicates that this multilayer vascular graft design has great potential as an off-the-shelf graft for small diameter arterial prostheses.