Acta Biomaterialia 8 (2012) 1010–1021

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Multilayer vascular grafts based on collagen-mimetic proteins

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ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form 14 October 2011
Accepted 11 November 2011
Available online 20 November 2011

Keywords:
Vascular grafts
Scl2 proteins
Hydrogels
Electrospinning
Thrombogenicity

ABSTRACT

A major roadblock in the development of an off-the-shelf, small-caliber vascular graft is achieving rapid endothelialization of the conduit while minimizing the risk of thrombosis, intimal hyperplasia, and mechanical failure. To address this need, a collagen-mimetic protein derived from group A Streptococcus, Scl2.28 (Scl2), was conjugated into a poly(ethylene glycol) (PEG) hydrogel to generate bioactive hydrogels that bind to endothelial cells (ECs) and resist platelet adhesion. The PEG-Scl2 hydrogel was then reinforced with an electrospun polyurethane mesh to achieve suitable biomechanical properties. In the current study, initial evaluation of this multilayer design as a potential off-the-shelf graft was conducted. First, electrospinning parameters were varied to achieve composite burst pressure, compliance, and suture retention strength that matched reported values of saphenous vein autografts. Composite stability following drying, sterilization, and physiological conditioning under pulsatile flow was then demonstrated. Scl2 bioactivity was also maintained after drying and sterilization as indicated by EC adhesion and spreading. Evaluation of platelet adhesion, aggregation, and activation indicated that PEG-Scl2 hydrogels had minimal platelet interactions and thus appear to provide a thromboresistant blood contacting layer. Finally, evaluation of EC migration speed demonstrated that PEG-Scl2 hydrogels promoted higher migration speeds than PEG-collagen analogs and that migration speed was readily tuned by altering protein concentration. Collectively, these results indicate that this multilayer design warrants further investigation and may have the potential to improve on current synthetic options.

1. Introduction

Approximately 1.4 million patients require arterial prostheses each year in the USA alone [1]. Existing options for vascular grafts have limited clinical success with an associated cost exceeding US$25 billion. The current gold standards, autologous saphenous veins and mammary arteries, are not available for up to 20% of patients due to disease, trauma, or anatomic abnormalities [2,3]. Cadaveric saphenous vein allografts are readily available but require processing prior to implantation, which induces damage to the endothelial layer and a loss of properties. In addition, allografts have associated immunological concerns [4–8]. Synthetic grafts made of polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE) are viable options for large diameter applications (>4 mm); however, thrombogenicity and low compliance cause re-occlusion in small-caliber vessels [9,10]. Such complications limit the use of these synthetic grafts in coronary artery bypass surgeries, which account for one-third of the arterial prosthesis procedures performed each year [1,11]. The urgent clinical need for off-the-shelf, small diameter vascular prostheses has prompted researchers to investigate biomimetic grafts with properties that more closely match those of native blood vessels [12–15].

For a graft to be effective as an off-the-shelf arterial prosthesis, it must avoid cell harvesting and construct pre-culture, which delay treatment and increase cost [16–19]. Thus, generation of the luminal endothelial cell (EC) layer critical to inhibiting platelet aggregation and smooth muscle cell hyperproliferation must occur following implantation. Therefore, rapid endothelialization has been identified as a critical element in the development of off-the-shelf vascular prostheses to prevent small-caliber graft reocclusion [16,20]. In vivo graft endothelialization appears to involve EC migration from graft anastomoses as well as endothelial progenitor cell adhesion and migration. Thus, the ability to promote cell migration is critical to graft endothelialization. Collagen was

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doi:10.1016/j.actbio.2011.11.015

initially studied as a graft material because of its inherent binding sites for ECs and smooth muscle cells. However, native collagen is thrombogenic and collagen scaffolds generally lack the requisite mechanical properties for arterial replacement [18,19]. An approach that circumvents many of the problems associated with animal-derived collagen combines extracellular-matrix (ECM) derived peptides with synthetic polymer scaffolds. Although this strategy has the potential to generate a non-thrombogenic graft with tunable bioactivity and mechanical properties, high cost and purity concerns associated with large scale solid-phase peptide synthesis limit its utility [21,22]. Additionally, short peptide sequences lack the triple helical conformation of native collagen, which has been shown to affect endothelium remodeling processes [23–25]. A small-caliber graft that incorporates the bioactivity of native collagen while circumventing thrombogenicity concerns and providing appropriate mechanical properties would represent a significant advance in off-the-shelf graft design.

To this end, we have recently developed a novel biomaterial platform based on a collagen-mimetic protein derived from group A Streptococcus, Scl2.28 (Scl2-1). Scl2-1 has several properties that make it desirable for cardiovascular applications. It contains the Gly-Xaa-Yaa (GXY) motifs that form collagen's characteristic triple-helix but lacks hydroxyproline, which eliminates the need for costly post-translational modifications [26,27]. This enables facile recombinant expression of *Escherichia coli* and eliminates the batch variability concerns associated with native collagen as well as the need for expensive solid-phase synthesis of ECM peptides [28,29]. Perhaps the most novel aspect of these proteins is that they can be designed to induce selective cell adhesion and function. Scl2-1 proteins act as a biological blank slate in that they resist cell adhesion even in the presence of serum and display low platelet aggregation. However, specific receptor binding motifs can be readily inserted into the Scl2-1 sequence by site-directed mutagenesis [28].

In native vessels, collagen-based $\alpha1\beta1$ and $\alpha2\beta1$ integrin binding motifs have been shown to modulate EC adhesion and phenotype [30]. We therefore inserted the GFPGER motif into the Scl2-1 sequence, since it has previously shown to mediate binding by $\alpha1\beta1$ and $\alpha2\beta1$ integrins [31,32]. This modified Scl2-1 (Scl2-2) maintains the triple helical structure and low platelet aggregation of Scl2-1 while promoting EC adhesion and spreading [33]. Until recently, the use of Scl2 proteins was limited to coatings due to their inability to self-assemble into stable three-dimensional structures. To address this limitation, we have developed a synthetic methodology to incorporate Scl2 proteins into poly(ethylene glycol) (PEG) based hydrogels [33]. PEG hydrogels were selected for this application due to their resistance to protein adsorption, which underlies their thromboresistance and isolates cell–material interactions of PEG-Scl2 hydrogels to the adhesion sites introduced by the Scl2 proteins [34]. In addition, the modulus of PEG hydrogels can be broadly tuned by altering PEG molecular weight, concentration, or functionality [35]. Pelham and Wang found that cells on flexible substrates exhibited reduced spreading and increased motility compared to cells on rigid substrates [36]. Thus, the ability to control substrate modulus allows for further manipulation of cell adhesion, phenotype, and migration [37–39].

The tunability of both bioactivity and mechanical properties of PEG-Scl2 hydrogels offers unique control over the endothelialization of the graft. However, properties that promote endothelialization may not be consistent with those that are sufficient to withstand physiological loading [36–39]. To address this issue, we have chosen to pursue a multilayered graft comprising a luminal PEG-Scl2 hydrogel layer designed to induce rapid endothelialization and a reinforcing mesh sleeve designed to provide bulk strength, compliance matching, and suture retention. Thus, each component can be individually tuned to achieve improved outcomes without detriment to other design goals and then bonded together into composite grafts. In generating the reinforcing mesh sleeve, segmented polyurethanes (SPUs) were selected due to their established biocompatibility, durability, and fatigue resistance [40]. Electrospinning was chosen for fabricating the SPU mesh sleeve, since this process produces fibrous, porous scaffolds with mechanical properties which can be broadly tailored via modification of electrospinning parameters [41]. Despite having high burst pressures and suture retention strengths, current synthetic vascular grafts have low 2 year patency rates (40–50%) due to their low compliance values [42]. The ability to tune the biomechanical properties of electrospun SPUs to improve matching to those of native vasculature is important in preventing intimal hyperplasia and thrombosis-induced failure in small diameter grafts.

In the present study, multilayer grafts were fabricated and characterized to assess their potential as off-the-shelf small-caliber vascular prostheses. The maintenance of graft integrity as well as biological and mechanical properties following vacuum drying and rehydration was evaluated to obtain an initial evaluation of the capacity of the fabricated grafts to undergo the processing associated with long-term storage. The stability of the interlayer bonding and graft mechanical properties following 4-week exposure to physiological flow was then investigated. Additionally, the capability to tune graft burst pressure, suture retention strength, and compliance by varying electrospun mesh thickness was assessed. The thromboresistance of the luminal PEG-Scl2 hydrogels was tested through a series of in vitro, whole blood tests. Finally, the ability to control EC adhesion and migration through modification of the PEG-Scl2 hydrogel biochemical landscape was explored. Cumulatively, these studies indicate that the proposed multilayer design has significant potential as an off-the-shelf vascular prosthesis.

2. Materials and methods

2.1. Materials

All chemicals were used as received and purchased from Sigma–Aldrich (Milwaukee, WI) unless otherwise noted.

2.2. Scl2 functionalization

The Scl2.28 sequence was amplified and purified as previously described [33]. Scl2-1 served as a negative control with no binding sites for $\alpha1\beta1$ or $\alpha2\beta1$. Scl2-2 is a variant of this protein containing the sequence GFPGER that was generated by site directed mutagenesis as previously described [26]. Scl2 proteins and a rat tail collagen type I control were functionalized with photoreactive crosslink sites according to a protocol adapted from Sebra et al. [43]. Scl2 proteins include ~9% lysine groups that allow for bioconjugation through the established NHS-lysine ε-amino group reaction. Briefly, the proteins were reacted with acrylate-PEG-N-hydroxysuccinimide (Acr-PEG-NHS, MW 3500, Jenkem Technologies USA, Allen, TX) in 50 mM sodium bicarbonate buffer (pH 8.5). The Acr-PEG-NHS:NH_2 molar ratio was 1:1, and the reaction was allowed to proceed with stirring for 24 h at room temperature. Basic byproducts were removed via dialysis against 0.1 M hydrochloric acid for 24 h, and further purification was carried out with dialysis against deionized water for 24 h (MWCO = 20,000). Functionalization of the modified proteins was confirmed with Fourier transform infrared (FTIR) spectroscopy and sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS–PAGE), as previously shown [33].

2.3. Preparation of bioactive PEG-Scl2 hydrogels

Poly(ethylene glycol) diacrylate (PEGDA) was synthesized according to a method adapted from Hahn et al. [44]. Briefly, 4 M