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Research paper

Tri-layered vascular grafts composed of polycaprolactone, elastin, collagen, and silk: Optimization of graft properties

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ARTICLE INFO

Article history:
Received 3 November 2011
Received in revised form
8 February 2012
Accepted 28 February 2012
Published online 14 March 2012

Keywords:
Electrospinning
Vascular graft
Multilayer
Polycaprolactone
Elastin
Silk

ABSTRACT

The purpose of this study was to create seamless, acellular, small diameter bioresorbable arterial grafts that attempt to mimic the extracellular matrix and mechanical properties of native artery using synthetic and natural polymers. Silk fibroin, collagen, elastin, and polycaprolactone (PCL) were electrospun to create a tri-layered structure for evaluation. Dynamic compliance testing of the electrospun grafts ranged from 0.4–2.5%/100 mmHg, where saphenous vein (1.5%/100 mmHg) falls within this range. Increasing PCL content caused a gradual decrease in medial layer compliance, while changes in PCL, elastin, and silk content in the adventitial layer had varying affects. Mathematical modeling was used to further characterize these results. Burst strength results ranged from 1614–3500 mmHg, where some exceeded the capacity of the pressure regulator. Four week degradation studies demonstrated no significant changes in compliance or burst strength, indicating that these grafts could withstand the initial physiological conditions without risk of degradation. Overall, we were able to manufacture a multi-layered graft that architecturally mimics the native vascular wall and mechanically matches the gold standard of vessel replacement, saphenous vein.

1. Introduction

Native artery is an extremely complex multi-layered tissue composed of a number of different proteins and cell types, playing an integral role in the mechanical behavior of the structure. In order to withstand the high flow rate, high pressure, and pulsatile nature of blood flow, an artery is comprised of three distinct layers: intima, media, and adventitia. Each of these layers has a different composition and plays a different physiological role. The intimal layer is the innermost layer of the vessel wall and is made up of a single layer of endothelial cells (EC) on a thin basal lamina and a subendothelial layer made of collagen (COL) type IV and elastin (ELAS). This layer contacts the bloodstream and therefore provides a critical barrier to platelet activation. The thick medial layer is composed of several layers of smooth muscle cells (SMC) in a matrix of COL types I and III, ELAS, and proteoglycans. The outermost adventitial layer is made of fibroblasts and randomly arranged COL type I (Niklason, 1999; Boland et al., 2004; Rhodin, 1980). The major protein components of native artery, COL and ELAS, provide tensile support and prevent vessel rupture, and confer elasticity to

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1751-6161/$ - see front matter
doi:10.1016/j.jmbbm.2012.02.026

the vessel wall (Buttafoco et al., 2006; Berglund et al., 2004). It is the elastic nature of ELAS that dominates the low-strain mechanical response of the vessel to blood flow and prevents pulsatile energy from being dissipated as heat (Huang et al., 2000; Strandness and Sumner, 1975).

The dynamic situation of a blood vessel creates a complicated sequence of events in which the walls are stretched and the inner lumen is sheared. A microenvironment capable of withstanding these forces with minimal energy loss is essential for proper blood vessel functioning. Electrospinning of polymer fibers into a vascular conduit has been demonstrated to be a potential technique that not only creates scaffolds simulating the ECM, but also contributes tailorable mechanical properties and minimal energy loss (McClure et al., 2009).

Currently, the most common vascular materials are Dacron® (polyethylene terephthalate) and expanded polytetrafluoroethylene (ePTFE). Dacron is widely known for its use in aortic and iliac grafts. Its success in these areas of the body is due mainly to larger diameters and flow rates, which are not conducive to thrombus formation and the subsequent decrease in luminal diameter. However, for a small diameter graft of 6 mm or less, the following properties must be attained for successful grafting to occur: biocompatibility, lack of chemical reactivity, very low thrombogenicity, porosity, and mechanical strength, including compliance matching that of native artery and resistance to aneurysm formation (Boland et al., 2004; Kannan et al., 2005; Conte, 1998).

Inner luminal wall thickening, hyperplasia, and subsequent occlusion and loss of blood flow in vascular grafts is one of the most critical concerns when designing a vascular prosthetic. Mechanical problems such as compliance mismatch between the natural vessel and prosthesis contribute to this effect, and are a key component to the creation of a successful vascular graft. Previously, our laboratory performed compliance testing of electrospun polydioxanone and ELAS in an effort to match natural arterial compliance (Sell et al., 2006), demonstrating the advantageous aspects of the addition of ELAS proteins to synthetic biodegradable polymers in a physiological setting.

The biomechanics of vascular grafts is one of the most essential components to its success. Previously, we designed a preliminary study to mimic the vascular architecture, producing a tri-layered vascular analogue (McClure et al., 2010). Blends of PCL, ELAS, and COL were electrospun in all three layers and evaluated through uniaxial tensile testing, compliance, suture retention, and burst strength. Results from this tri-layered study revealed that as the modulus of the middle layer was changed, the compliance values increased to within native arterial range, while burst strength remained strong due to the outer layer, exhibiting a tunable structure with excellent properties. The goal of this current study was to expand off of the previous results. While the first tri-layered study observed PCL, ELAS, and COL blends, its scope was limited to changing only the medial layer, using one crosslinking reagent, and providing no degradation data. The aim here was to change the composition of both the medial and adventitial layers, utilize a new protein, silk fibroin (SF), compare two crosslinking reagents, and measure degradation properties to determine which blend would be considered the most optimal prior to cellular investigation.

PCL was chosen as the synthetic polymer for this study based on promising results from *in vivo* rat studies that have been conducted by Pektok et al., whereby histological analysis revealed rapid enthothelialization of the inner lumen of the graft and becoming confluent at 12 weeks (Pektok et al., 2008). Additionally, vascular tissue contains between 25%–30% COL and 40%–50% ELAS. Therefore, ratios were chosen for each of the three layers based on native extracellular matrix design while keeping mechanical integrity throughout the graft structure.

Electrospun COL has been well established as a protein which promotes cellular attachment; however, debates regarding its composition once dissolved in fluoro-alcohols still exist. Zeugolis et al. found HFP to partially denature COL, however, this group never discussed the amount of time the COL samples were allowed to dissolve in HFP (Zeugolis et al., 2008). Additionally, subcutaneous implantations comparing both electrospun gelatin (denatured COL) and electrospun COL found massive inflammation and encapsulation surrounding gelatin samples while COL electrospun samples experienced a low inflammatory response (Simpson, 2006). Therefore, electrospun COL retains some advantageous properties, differentiating it from gelatin. Electrospun SF has been gaining interest for vascular tissue engineering, displaying mechanical characteristics that were comparable to native artery and cellular characteristics that promoted the adhesion and proliferation of both SMCs and ECs (Soffer et al., 2008). Previous research indicated that combinations of ELAS, COL, and SF required fixation in order to retain proteins within the scaffold matrix (McClure et al., 2008; Barnes et al., 2007; Soffer et al., 2008). A previous experiment (data unpublished) indicated that two cross-linkers, genipin and carbodiimide, fixed the proteins with slightly altered mechanical properties between the two. Therefore, in this study, we aimed to evaluate a range of genipin and carbodiimide crosslinked tri-layered vascular grafts using uniaxial tensile testing, compliance, and burst strength.

To aid in the optimization of graft materials, a mathematical model (data not shown), established in our previous tri-layered experiment, was implemented to help describe circumferential wall stress (CWS) of the individual layers and their mechanical effects on surrounding layers (McClure et al., 2010). Combining mathematical modeling with the results of compliance and burst strength helped determine optimal graft properties and decipher which grafts would be used in further degradation analysis.

2. Materials and methods

2.1. *Protein extraction*

SF was extracted from the cocoons of *Bombyx mori* silk worms (The Yarn Tree) through an established protocol (Jin et al., 2004). Briefly, cocoons were cut into pieces and boiled in a 0.02 M Na_2CO_3 (Sigma Aldrich) solution for 30 min to remove the sericin gum, followed by thorough rinsing in de-ionized water (DI), and drying in a fume hood. The SF was then