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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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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.

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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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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 endothelialization 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 cross-linked 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 Na₂CO₃ (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

dissolved in a LiBr (Fisher Scientific) solution at 60 °C for 4 h. This solution was then dialyzed against deionized water for 3 days using 3500 MWCO dialysis tubing (Fisher Scientific). The SF solution was then frozen and lyophilized to provide a pure SF for electrospinning.

COL type I was extracted from 6 month bovine corium through an acetic acid based process previously described (Barnes et al., 2007). Briefly, tissue was homogenized, suspended in acetic acid, and subsequently purified via a series of dissolutions, precipitations (using NaCl (Fisher Scientific) and Tris (Fisher Scientific)), and dialyses (Barnes et al., 2007). Following the final dialysis, COL solutions were frozen and lyophilized.

2.2. Electrospinning

The electrospinning setup consisted of a syringe pump (KD Scientific), a high voltage power supply (Spellman CZE1000R, Spellman High Voltage Electronics Corp.), a plastic Becton Dickinson syringe used as the polymer reservoir, attached to an 18-gauge blunt-tip needle. Concentrations of 150 mg/ml of PCL (MW: 125,000, Lakeshore Biomaterials), 200 mg/ml of soluble ELAS from bovine neck ligament (Elastin Products Co. Inc.), 70 mg/ml of COL I (6 month bovine corium), and 80 mg/ml of SF were dissolved in 1,1,1,3,3,3 hexafluoro-2-propanol (TCI America). ELAS-based scaffold materials were blended in ratios of 45-45-10, 55-35-10, 65-25-10 PCL-ELAS-COL and PCL-ELAS-SF, while SF- and COL-based scaffold materials were blended in ratios of 45-10-45, 55-10-35, and 65-10-25 PCL-ELAS-COL and PCL-ELAS-SF. To evaluate material properties, blended scaffolds were electrospun onto rectangular mandrels measuring 2.5 cm wide × 10.2 cm long × 0.3 cm thick to create electrospun sheets with random fibers. The parameters for electrospinning were kept constant with an applied voltage of 25 kV, air gap distance (distance between the needle and rotating drum) of 15 cm, and solution dispensing rate of 4 ml/h.

To create vascular tubes with random fibers, each polymer mixture was loaded into a 3 ml plastic Becton Dickinson syringe with an 18 gage blunt tip needle. Insulated Tygon tubing was then run from the needle tip to a 3-1 (input-output) nozzle charged to 25 kV at an air gap distance of 15 cm. Fig. 1 displays the 3-1 nozzle setup. The solutions were sequentially electrospun onto a 2 mm diameter cylindrical mandrel rotating at 500 RPM with a translational speed of 6 cm/s over a distance of 12 cm. Flow rates and volumes were as such: the material for the intima was kept constant at 100-0-0 PCL-ELAS-COL and electrospun at a rate of 4 ml/h and a volume of 0.25 ml followed by a transition combining both intimal and medial syringes for 0.1 ml at 2 ml/h each for a combined flow rate of 4 ml/h. The intimal syringe was then shut off and the medial layer was allowed to spin for 0.6 ml at 4 ml/h followed by a transition between the media and adventitia for 0.1 ml of polymer solution at 2 ml/h each for a combined flow rate of 4 ml/h. Finally, the media was stopped and the adventitia was allowed to spin for 0.3 ml at 4 ml/h.

Medial layers were 45-45-10, 55-35-10, and 65-25-10 PCL-ELAS-COL and PCL-ELAS-SF. Adventitial layers were 45-10-45, 55-10-35, and 65-10-25 PCL-ELAS-COL and PCL-ELAS-SF.

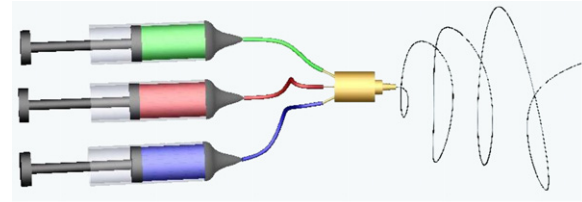


Fig. 1 – Schematic displaying how the 3-1 nozzle is used in this experiment.

Vascular tubes were created using each possible combination and cross-linked using both EDC and GEN to determine layered graft behavior based on differences in protein type and cross-linker type.

2.3. Crosslinking

Scaffolds were cross-linked using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, FlukaBiochemika) or genipin (GEN, Wako Pure Chemical Industries, Ltd.). Using EDC, a 5 mM crosslinking solution was prepared in 90% ethanol (Fisher Scientific) and allowed to cross-link for 18 h followed by a 0.1 M Na₂HPO₄ rinse for 1 h and a PBS rinse for 1 h.

Using GEN, a 10 mM crosslinking solution was prepared in 90% ethanol (Fisher Scientific) and scaffolds were placed in the cross-linking solution for a period of 72 h (Sung et al., 2003), followed by a PBS rinse for 2 h.

2.4. Material properties of individual layers

Uniaxial tensile testing was performed on six samples ($n = 6$) from one electrospun sheet hydrated in PBS for 24 h. “Dog-bone” shaped samples were punched from electrospun mats (2.75 mm wide at their narrowest point with a gage length of 7.5 mm) and tested on a MTS Bionix 200 testing system with a 100 N load cell (MTS Systems Corp.) and an extension rate of 10.0 mm/min. Peak stress, modulus, strain at break, and energy to break were calculated using TestWorks version 4.

2.5. Burst strength

Burst strength testing on six samples ($n = 6$) from six different electrospun grafts was completed using a device designed in accordance with Section 8.3.3.3 of ANSI/AAMI VP20-1994. Tubes, 2–3 cm in length, were hydrated in PBS, fitted over 1.5 mm diameter nipples attached to the device, and secured with 3-0 silk suture. Air was introduced into the system, increasing the pressure at a rate of 5 mm Hg/s until the tubes burst. Results are reported as the pressure in mm Hg at which tubes ruptured. Fig. 2 depicts the burst strength device.

2.6. Dynamic compliance

Dynamic compliance was determined for six 2 mm inner diameter tubular grafts ($n = 6$) taken from six different electrospun grafts at a length of 3 cm under simulated physiological conditions in accordance with Section 8.10 of ANSI/AAMI VP20-1994. The specimens were tested in an Intelligent Tissue



Fig. 2 – Image of the custom burst strength device. In this image, air flows from a pressure regulator into the graft. Graft pressure is measured with a pressure transducer.

Engineering via Mechanical Stimulation (ITEMS™) Bioreactor developed by Tissue Growth Technologies (Minnetonka, MN) filled with PBS at 37 °C, Fig. 3. The bioreactor provided a cyclic (1 Hz, representing 60 beats per minute) pressure change to the inside of the graft; pressure levels of 120/80 mm Hg (systolic/diastolic) were investigated. Specimens were secured at either end to a nipple with 3–0 silk suture and placed in the bioreactor chamber. PBS then filled the chamber and was run continuously on the outside of the graft to maintain a temperature of 37 °C and a pressure of 0 mm Hg. Simultaneously, PBS was pumped through the inside of the graft and an actuator in the bioreactor created the difference in pressure. Prior to compliance measurements for each pressure level, all grafts were allowed to stress relax for 30 min or 1800 cycles.

Internal pressure was measured with a pressure transducer capable of measuring dynamic pressure up to 200 ± 2 mm Hg, while the external diameter was recorded with a laser micrometer system with an accuracy of ± 0.001 mm. Prior to insertion into the bioreactor, the grafts were soaked in PBS at 37 °C for 24 h. The following equation was used to calculate compliance for each specimen:

$$\% \text{ Compliance} = \frac{(R_{P2} - R_{P1})/R_{P1}}{(P_2 - P_1)} \times 10^4 \quad (1)$$

where R is the internal radius, P_1 is the lower internal pressure, and P_2 is the higher internal pressure.

2.7. Degradation

Grafts were tested for degradation using a 55 mL rotating cell culture system (RCCS, Synthecon, Inc.). First, chosen grafts were electrospun according to the protocols mentioned above. Second, 55 mL of Dulbecco's Modified Eagle Medium F12 (DMEM F12, Invitrogen) supplemented with 10% fetal bovine serum (Hyclone) and 1% penicillin/streptomycin (Invitrogen) was placed in each bioreactor. Twelve tri-layered vascular grafts ($n = 4$) from one polymer blend group were placed in one bioreactor to create three separate timed groups: 1, 2, and 4 weeks at 37 °C with 5% CO₂. Media was changed once every 7 days to remove any degradation products and replace

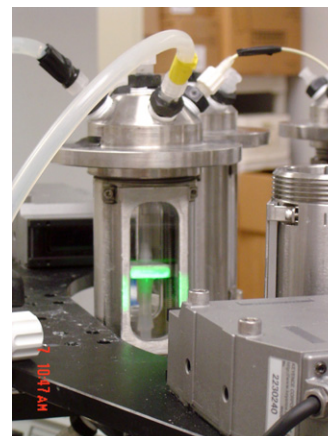


Fig. 3 – Image of the ITEMS bioreactor used to measure compliance.

enzymatic capabilities. At each time period, grafts were taken out of culture and tested for compliance and burst strength in accordance with the above protocols.

2.8. Statistical analysis

Unless otherwise stated, all statistical analysis was based on a Kruskal–Wallis one-way analysis of variance on ranks and a Tukey–Kramer pair-wise multiple comparison procedure ($\alpha = 0.05$) performed with the JMP® IN 4.0.3 statistical software package (SAS Institute, Inc.).

3. Results

3.1. Material properties: PCL–Elastin–Collagen

The primary goal of assessing material properties was to analyze how combinations of PCL, ELAS, COL, and SF interact with one another when PCL–ELAS–COL and PCL–ELAS–SF were electrospun in different ratios. Crosslinked scaffolds were investigated for differences between EDC and GEN to understand if the crosslinking procedures caused a dramatic change in the overall scaffold properties due to chemical crosslink or crossbridges that would form. Tables 1 and 2 display peak stress, modulus, strain to break, and energy to break. Looking at Table 1, COL-based scaffolds, peak stress values for EDC crosslinked scaffolds with PCL at volumetric ratios of 65% were significantly different ($p < 0.05$) from their GEN counterparts. A common trend amongst the GEN crosslinked scaffolds was a decrease in peak stress in comparison to the EDC. Although none of the other crosslinked scaffolds were different from one another, they each possessed a decrease in average peak stress, excluding the 45–10–45 PCL–ELAS–COL scaffolds. Additionally, as ELAS incrementally increased from 25% to 45%, it gradually caused a decrease in peak stress with differences ($p < 0.05$) between 65–25–10, 55–35–10, and 45–45–10 regardless of crosslinking reagent or scaffold protein.

Modulus values are especially important to determine a scaffold's stiffness. They can be good determinants or

Table 1 – Material property results PCL–ELAS–COL.

	Xlink	Peak stress (MPa)	Modulus (MPa)	Strain to break (% Elongation)	Energy to break (N mm)
45-45-10	EDC	0.4 ± 0.1	2.5 ± 0.5 ^b	48.0 ± 5.0 ^a	0.52 ± 0.17
45-45-10	Genipin	0.3 ± 0.1	2.6 ± 0.33	29.0 ± 3.0	0.23 ± 0.07
55-35-10	EDC	1.0 ± 0.1	9.5 ± 1.61	43.0 ± 7.0	0.98 ± 0.31
55-35-10	Genipin	0.8 ± 0.1	7.2 ± 2.25	42.0 ± 3.0	0.88 ± 0.30
65-25-10	EDC	2.3 ± 0.1 ^a	10.1 ± 0.63	112.0 ± 11.0	10.45 ± 1.39 ^a
65-25-10	Genipin	2.0 ± 0.1	10.4 ± 0.60	120.0 ± 13.0	9.35 ± 1.60
45-10-45	EDC	1.0 ± 0.2	6.6 ± 0.85 ^b	33.0 ± 5.0 ^a	0.76 ± 0.37
45-10-45	Genipin	1.1 ± 0.2	7.1 ± 0.79	49.0 ± 6.0	1.49 ± 0.41
55-10-35	EDC	1.2 ± 0.3	9.7 ± 2.76	29.0 ± 8.0	0.55 ± 0.33
55-10-35	Genipin	1.1 ± 0.2	8.1 ± 1.94	30.0 ± 4.0	0.49 ± 0.11
65-10-25	EDC	1.5 ± 0.2 ^a	13.5 ± 3.08	43.0 ± 9.0	1.90 ± 0.61
65-10-25	Genipin	1.2 ± 0.4	11.8 ± 5.51	40.0 ± 8.0	1.38 ± 0.93

^aIndicates a significant difference between EDC and GEN.^bIndicates a significant difference between 65% and 45% PCL content scaffolds.**Table 2 – Material property results PCL–ELAS–SF.**

	Xlink	Peak stress (MPa)	Modulus (MPa)	Strain to break (% Elongation)	Energy to break (N mm)
45-45-10	EDC	1.1 ± 0.2 ^{a,b}	4.1 ± 0.6 ^b	50.0 ± 12.0	0.9 ± 0.5 ^b
45-45-10	Genipin	0.8 ± 0.2	3.9 ± 0.6	40.0 ± 6.0	0.7 ± 0.2
55-35-10	EDC	1.4 ± 0.8	11.3 ± 1.1	50.0 ± 10.0	2.1 ± 0.6
55-35-10	Genipin	1.2 ± 0.1	9.6 ± 1.8	50.0 ± 3.0	1.9 ± 0.4
65-25-10	EDC	1.4 ± 0.1	13.2 ± 1.0	70.0 ± 4.0 ^a	4.1 ± 0.8 ^a
65-25-10	Genipin	1.2 ± 0.1	12.7 ± 2.7	50.0 ± 3.0	2.1 ± 0.5
45-10-45	EDC	0.9 ± 0.1 ^{a,b}	9.1 ± 2.0 ^b	50.0 ± 8.0	2.0 ± 0.3 ^b
45-10-45	Genipin	0.7 ± 0.1	9.2 ± 1.3	50.0 ± 7.0	1.7 ± 0.3
55-10-35	EDC	0.8 ± 0.1	9.3 ± 1.1	30.0 ± 3.0 ^a	1.0 ± 0.1 ^a
55-10-35	Genipin	0.7 ± 0.1	8.1 ± 1.6	150.0 ± 5.0	3.1 ± 0.3
65-10-25	EDC	1.2 ± 0.1	10.2 ± 1.9	50.0 ± 5.0 ^a	2.3 ± 0.3 ^a
65-10-25	Genipin	1.1 ± 0.1	10.7 ± 0.8	150.0 ± 33.0	4.3 ± 0.8

^aIndicates a significant difference between EDC and GEN.^bIndicates a significant difference between 65% and 45% PCL content scaffolds.

predictors of how well a vascular material will behave under physiological pressures. Examining the results, 65-10-25 PCL–ELAS–COL scaffolds exhibited the highest stiffness, where EDC was the highest and GEN was the lower of the two, but were not statistically significant. Average moduli for GEN scaffolds were also lower than their EDC counterparts for 55-10-35 and 55-35-10, but again not significant. Trends amongst the scaffolds also demonstrated that for both COL- and ELAS-based scaffolds 65% PCL content was significantly different ($p < 0.05$) from 45% PCL content, a result similar to the previous study.

Strain to break values for all scaffolds ranged from 1.2 to 0.29 mm/mm. The higher strain values were present in scaffolds with increased PCL content for ELAS-based scaffolds. Averages for these scaffolds illustrated how the addition of proteins to a scaffold significantly decreased ($p < 0.05$) strain values in upwards of 75%. Differences ($p < 0.05$) between EDC and GEN occurred for 45-45-10 and 45-10-45 scaffolds. Where GEN cross-linking caused the strain values to decrease for ELAS-based scaffolds and increase for COL-based scaffolds. Again, incremental changes in PCL content displayed significant differences ($p < 0.05$) between 65-25-10 and 45-45-10, and 65-10-25 and 55-10-35.

Energy to failure data demonstrated significant differences ($p < 0.05$) between 65-25-10 EDC and GEN scaffolds. These scaffolds displayed a decrease in energy to break when GEN was used as the crosslinker, decreasing from 10.5 to 9.4 N mm. For almost every case, the energy to break values decreased when GEN was used as the crosslinker. Moreover, as PCL content decreased energy to break values also decreased with significant differences between 65-25-10 and 45-45-10, and 55-10-35 and 45-10-45.

3.2. Material properties: PCL–Elastin–Silk

According to Table 2, overall average values for peak stress and modulus demonstrated decreases when crosslinked with GEN, while average values for strain to break exhibited decreases for ELAS-based scaffolds, 45-45-10, 55-35-10, and 65-25-10, but had increases for SF-based scaffolds, 55-10-35 and 65-10-25. Specifically looking at the peak stress values, significant differences ($p < 0.05$) existed among 65-25-10, 45-45-10, and 45-10-45 EDC and GEN scaffolds with marked decreases of 0.2, 0.3, and 0.2 MPa, respectively. In addition to the differences between scaffold crosslinkers, there were differences between groups of scaffolds. ELAS- and SF-based scaffolds with 65% PCL content were different ($p < 0.05$)

from those containing 45% PCL, each with a decrease in peak stress.

Moduli displayed average values for all GEN crosslinked ELAS-based scaffolds which were lower than their EDC counterparts. For example, 65-25-10 EDC scaffolds decreased from 13.2 to 12.7 MPa, 55-35-10 EDC scaffolds decreased from 11.3 to 9.6 MPa, and 45-45-10 EDC decreased from 4.1 to 3.9 MPa. SF-based scaffolds, however, did not display the same trends with values for 65-10-25 and 45-10-45 increasing from 10.2 to 10.7 MPa and 9.1 to 9.2 MPa, respectively, when crosslinked with GEN. None of the values for either ELAS or SF-based scaffolds were significantly different from one another. Staying consistent with Table 1, differences did exist between the moduli of scaffold groups with 65% and 45% PCL.

For strain to break, the majority of the results are in the range of 0.4–0.7 mm/mm, except for the 65-10-25 and 55-10-35 scaffolds crosslinked with GEN. Significant differences ($p < 0.05$) between EDC and GEN scaffolds existed only for 65-25-10, 65-10-25, and 55-10-35, where values decreased from 0.7 to 0.5 mm/mm for 65-25-10 and increased from 0.3 to 1.5 mm/mm for 55-10-35 and from 0.5 to 1.5 mm/mm for 65-10-25. The results seen here could be attributed to the low amount of ELAS present within the scaffold. The ELAS present in the 45-10-45 scaffold may not interact with the PCL and SF in the same specific way as both the 55-10-35 and 65-10-25 scaffolds due to the higher concentration of SF protein in the polymer solution.

Energy to break revealed decreases in the amount of energy absorbed by the scaffolds when crosslinked with GEN for all ELAS-based scaffolds, while the SF-based scaffolds of 55-10-35 and 65-10-25 displayed increases in the amount of energy absorbed. This can be attributed to the large strain to break values seen by these scaffolds, increasing the area under the curve as the scaffolds continued to elongate under a constant strain rate. Significant differences ($p < 0.05$) between EDC and GEN occurred for the 55-10-35, 65-10-25, and 65-25-10 PCL-ELAS-SF scaffolds, clearly demonstrating that a change is occurring when a different crosslinker is used, and that this change is dependent on the ratios of PCL to ELAS and to SF. Additionally, scaffold groups were again different ($p < 0.05$) from each other with differences between 65-25-10 and 45-45-10 and between 65-10-25 and 45-10-45. This showed that as ELAS or SF was incrementally increased their energy to break decreased with average values decreasing by 3.2 N mm and 2.6 N mm for ELAS and SF-based scaffolds, respectively.

3.3. Compliance

Mean values for PCL-ELAS-COL vascular grafts, Fig. 4, displayed average results in the range of 0.4%–1.8%/100 mm Hg, and individual values went as high as 2.4%/100 mm Hg. While results demonstrated values not within the range of native artery, they did provide some insight into crosslinking dependence. GEN grafts, on average, had higher compliance values than those crosslinked with EDC. This is especially evident for grafts electrospun with a 45-45-10 PCL-ELAS-COL medial layer, where grafts with a 45-10-45 and 55-10-35 GEN crosslinked adventitial layers are significantly higher ($p < 0.05$) than their EDC counterparts with values of 1.4

and 1.3%/100 mm Hg, respectively, compared to 0.6% and 0.4%/100 mm Hg for EDC, respectively. In the 55-35-10 medial layer group, both 45-10-45 and 55-10-35 GEN crosslinked adventitial layer grafts are significantly different ($p < 0.05$) from the same EDC crosslinked grafts, portraying values of 1.2% and 1.4%/100 mm Hg compared to 0.7% and 0.6%/100 mm Hg. The 65-25-10 medial layer group did not display any significant differences between EDC and GEN crosslinked grafts. Moreover, the GEN crosslinked grafts exhibited significant differences ($p < 0.05$) as the medial layer changed from 45-45-10 to 65-25-10, where combinations of 45-45-10 medial layer with a 45-10-45 adventitial layer were significantly different ($p < 0.05$) from combinations of 65-25-10 medial layer with a 45-10-45 adventitial layer. This was the same case for combinations containing the 55-10-35 adventitial layer.

Looking at Fig. 5, grafts with an adventitial layer of 45-10-45 PCL-ELAS-SF tend to increase in compliance as PCL content is increased in the medial layer where average compliance of 45-45-10 medial layers with EDC and GEN crosslinking increase from 0.6 and 0.3%/100 mm Hg, respectively, to 0.9 and 1.3%/100 mm Hg. Furthermore, GEN crosslinked 45-45-10 medial layer was significantly different ($p < 0.05$) from 55-35-10 medial layer. These results could indicate that the amount of stiffness seen within the compliance results is due to SF, and in order to overcome the stiffness caused by SF higher amounts of PCL must be added to the solution. In the 55-10-35 adventitial layer group, there were no significant changes for EDC crosslinked scaffolds as PCL content was increased; however, GEN crosslinked scaffolds have significant differences ($p < 0.05$) between the 45-45-10 medial layers, 1.4%/100 mm Hg, and 65-25-10 medial layers, 0.3%/100 mm Hg. Additionally, EDC 45-45-10 medial layers with 55-10-35 adventitial layers are significantly different ($p < 0.05$) from GEN crosslinked grafts. Finally, in the 65-10-25 adventitial layer group EDC crosslinked 45-45-10 medial layer grafts have the highest compliance of the three medial layers and amongst the whole group with a value of 1.0%/100 mm Hg. GEN crosslinked 45-45-10 medial layer grafts have the second highest compliance in the group with a value of 0.9%/100 mm Hg. Therefore, there were no significant differences between the EDC and GEN 45-45-10 medial layer grafts when combined with 65-10-25 adventitial layers. Furthermore there were no significant differences between any of the EDC and GEN crosslinked grafts in the 65-10-25 group.

3.4. Burst strength

The burst strength of all the PCL-ELAS-COL grafts in Fig. 6 exceeded the standard 1600 mm Hg that is associated with saphenous vein (L'Heureux et al., 1998). Notably, the only grafts that possessed values which increased as the PCL content of the medial layer was increased were the grafts in the 45-10-45 adventitial layer group. While EDC crosslinked grafts did not significantly change as the stiffness of the medial layer was increased, GEN crosslinked grafts did change ($p < 0.05$) increasing from a value of 1614 mm Hg, 45-45-10, to a value of 2403 mm Hg, 65-25-10. These increases due to an increase in medial layer stiffness were also seen in the 55-10-35 adventitial layer group for both EDC

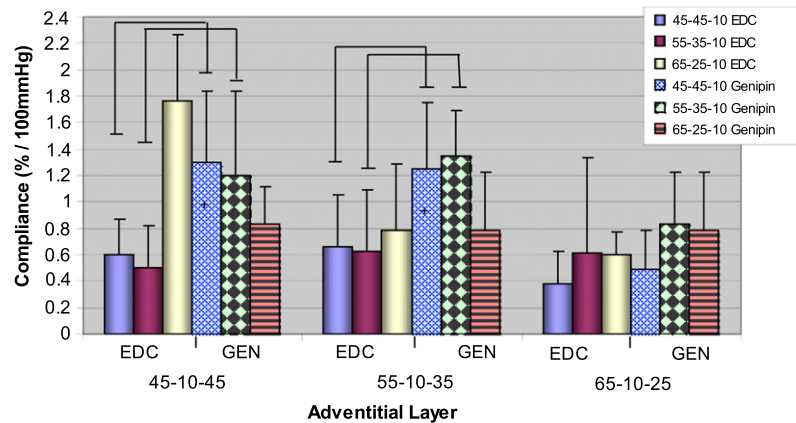


Fig. 4 – Compliance values determined from external radii at arterial pressures of 120/80 mm Hg for PCL-ELAS-COL tri-layered grafts. + indicates significant difference between 45-45-10 and 65-25-10 medial layer. Bars indicate significant differences between samples.

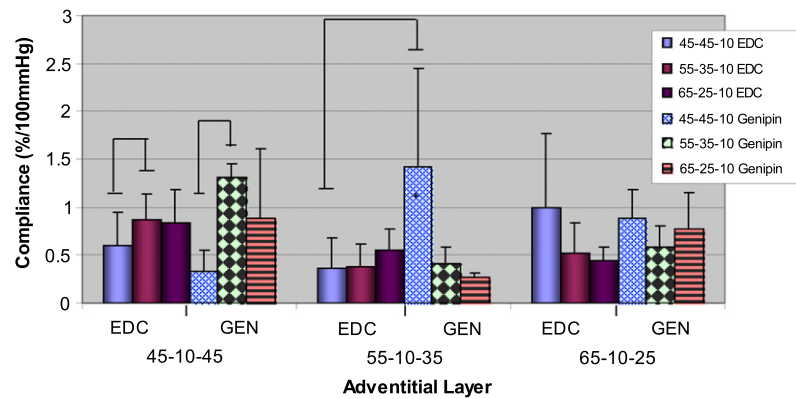


Fig. 5 – Compliance values determined from external radii at arterial pressures of 120/80 mm Hg for PCL-ELAS-SF tri-layered grafts. + indicates significant difference between 45-45-10 and 65-25-10 medial layer. Bars indicate significant differences between samples.

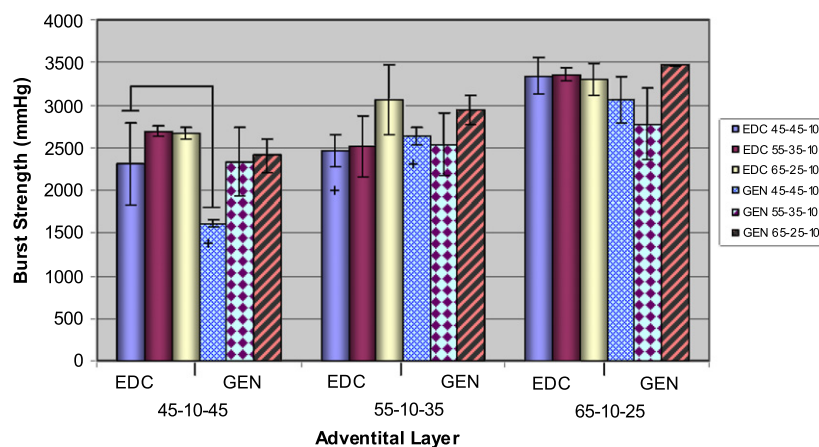


Fig. 6 – Burst pressure results for tri-layered specimens with changes in the both the medial layers and adventitial layers for PCL-ELAS-COL grafts. + indicates significant difference between 45-45-10 and 65-25-10 medial layer. Bars indicate significant differences between samples.

and GEN crosslinking. For this group, 45-45-10 and 65-25-10 EDC crosslinked scaffolds increased from an average value of 2465–3059 mm Hg, respectively, and 45-45-10 and

65-25-10 GEN crosslinked scaffolds increased from 2635 to 2946 mm Hg, respectively ($p < 0.05$). Looking at the 65-10-25 adventitial group, the results for EDC crosslinked grafts

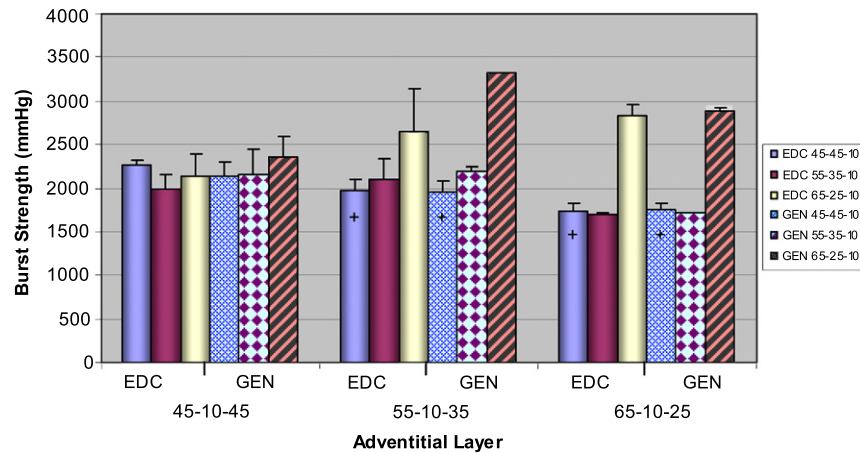


Fig. 7 – Burst pressure results for tri-layered specimens with changes in the both the medial layers and adventitial layers for PCL-ELAS-SF grafts. + indicates significant difference between 45-45-10 and 65-25-10 medial layer.

were all very similar and exhibited no significant differences. Lastly in Fig. 6, there is a gradual trend from the 45-10-45 to the 65-10-25 adventitial group, indicating that as PCL the adventitial layer becomes stiffer the burst pressure increases which reinforced the results from the material properties.

Examining Fig. 7, PCL-ELAS-SF burst strength results revealed very different results from PCL-ELAS-COL. Studying the 45-10-45 adventitial layer group, it was evident that neither EDC nor GEN produced significantly different results. The 55-10-35 adventitial layer grafts demonstrated gradual increases in burst strength as the medial layer incrementally increased in PCL content for both EDC and GEN crosslinked grafts. Again for this 55-10-35 group, there were no differences between EDC and GEN crosslinked grafts. However, there were differences as the medial layer increased its stiffness, where 45-45-10 grafts were significantly different ($p < 0.05$) from 65-25-10 grafts for both EDC and GEN crosslinking. Examining the 65-10-25 adventitial group, there were again differences ($p < 0.05$) between the 45-45-10 and 65-25-10 medial layer combinations for both EDC and GEN crosslinking.

3.5. Compliance and burst degradation

Degradation was carried out over a period of 4 weeks to determine if there were any immediate degradation characteristics which would impact compliance and burst strength properties. Fig. 8(A) and (B) depict the compliance results for grafts containing COL and SF, respectively. Fig. 8(A) demonstrated a significant increase ($p < 0.05$) in compliance for 65-10-25 adventitial layer grafts with a 45-45-10 medial between week 0 and week 2 and between week 1 and week 2. Additionally, there was a significant increase ($p < 0.05$) in compliance from week 1 to week 2 for grafts with a 45-10-45 adventitial layer and 45-45-10 medial layer. However, week 4 compliance results were not different from weeks 0 and 1. Interestingly, the average values for the 55-10-35 adventitial layers with either a 45-45-10 or a 55-35-10 medial layer incrementally increased from week 0 to week 2, and compliance results for 55-35-10 medial layer displayed a significant difference from week 0. Furthermore, both the

compliance of the 65-10-25 adventitia 45-45-10 media and 55-10-35 adventitia 55-35-10 media grafts were within the range of native artery.

Fig. 8(B) shows that SF grafts had significant decreases ($p < 0.05$) in compliance between week 1 and 2 for 55-10-25 adventitial layer grafts with 45-45-10 medial layers. Additionally, the 65-10-25 adventitia with a 45-45-10 media increased in average compliance from week 0 to week 2; however, the difference was not significant. Average compliance values for SF grafts did not reach into the range of native artery. Nonetheless, they did display fairly consistent values amongst the grafts. This would indicate that there was no degradation over the course of two weeks whereas it seems that in Fig. 8(A) COL containing grafts may have experienced slight amounts of degradation.

Fig. 9(A) and (B) describes the burst strength properties of grafts degrading over a 4 week period. It was expected that if any degradation was happening to the grafts, then a decrease in burst strength would have occurred. In the case of the PCL-ELAS-COL grafts in Fig. 9(A), there was a significant decrease ($p < 0.05$) between week 0 and 2, week 0 and 1, and week 0 and 4 for 65-10-25 adventitia with 45-45-10 media. Additionally, grafts with a 55-10-35 adventitia and a 55-35-10 media were significantly different ($p < 0.05$) between week 1 and week 2. Furthermore, 45-10-45 adventitia 45-45-10 media grafts significantly increased ($p < 0.05$) in burst strength from week 0 to week 4 but there were no differences amongst weeks 1, 2, and 4.

Fig. 9(B) reveals a significant decrease ($p < 0.05$) in burst strength for grafts with a 55-10-35 adventitia and a 45-45-10 media between week 0 and week 4, while 45-10-45 adventitia with a 55-35-10 media significantly increased in burst strength from week 0 to week 2. Finally, 65-10-25 adventitia with a 45-45-10 media significantly decreased ($p < 0.05$) between week 1 and week 2, and the average burst pressure from week 4 was still lower than that of week 1 but similar to week 0.

If a correlation is made between the compliance and burst results, then we could determine whether or not degradation was truly occurring or if the differences were mere coincidence. Observing the PCL-ELAS-COL grafts, the

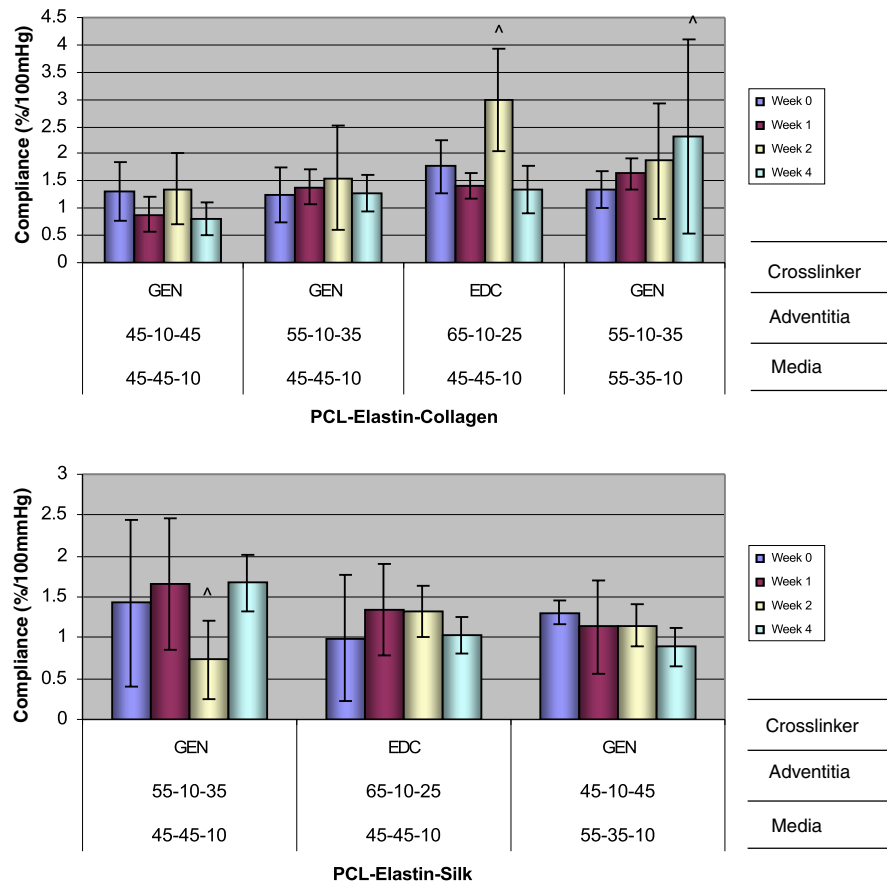


Fig. 8 – Compliance values for a 4 week degradation study determined from external radii at arterial pressures of 120/80 mm Hg. (a) Top graph represents PCL-ELAS-COL tri-layered grafts and (b) bottom graph represents PCL-ELAS-SF tri-layered grafts. ^ indicates a significant difference from weeks 0 and 1.

only graft to display a possible difference in properties due to degradation was the 55-10-35 adventitia with a 55-35-10 media due to a simultaneous increase in compliance and decrease in burst strength from week 0 to week 4. For PCL-ELAS-SF grafts, there was no correlation to indicate that degradation had occurred within a group of grafts.

4. Discussion

4.1. Material properties

The properties of individual layers provide some clues as to how a multi-layered vascular graft should perform under physiological conditions. First, peak stress indicates how much stress or pressure can be applied to the structure prior to rupturing. Second, modulus indicates how stiff the layer is going to be, which will contribute to the overall compliance and burst properties of the graft. Third, strain to break indicates how far a material will yield prior to failure, providing insight into either brittle or ductile failure. Fourth, energy to failure displays information about how much energy a material can absorb prior to rupture and are good determinants of scaffold toughness.

For a native artery, material properties are extremely important in order to be able to decipher the specifics of a

tissue. Previous studies involving muscular arteries were not based on the experimental data of the individual layers (Wang et al., 2006; Fung and Liu, 1995), but instead considered vessel walls as a homogenous material (Takamizawa and Hayashi, 1987; Fung et al., 1979). However, due to the non-homogeneity of native arteries, current studies have aimed to analyze the biomechanics of each individual layer either as a two layer model or as a three layer model (Holzapfel et al., 2005, 2004; Lu et al., 2004). Two layer models typically leave the intima and media intact and separate the adventitia, while three layer models carefully separate the intima, media, and adventitia. These studies have demonstrated the specific biomechanics in each layer through the use of uniaxial tensile testing. The results from these tests are then utilized to determine composition, how the tissue behaves, and eventually aid in the development of an equation to predict how a native artery will behave under different physiological circumstances. Determining the specific mechanical properties of each electrospun material assists in the overall design of a tri-layered vascular graft.

GEN is a crosslinker that has not received a great deal of attention and has been used in only a handful of studies. One of the hypotheses made in this study was that the long molecular structure of GEN would result in longer strain to breaks and lower elastic moduli and lower peak

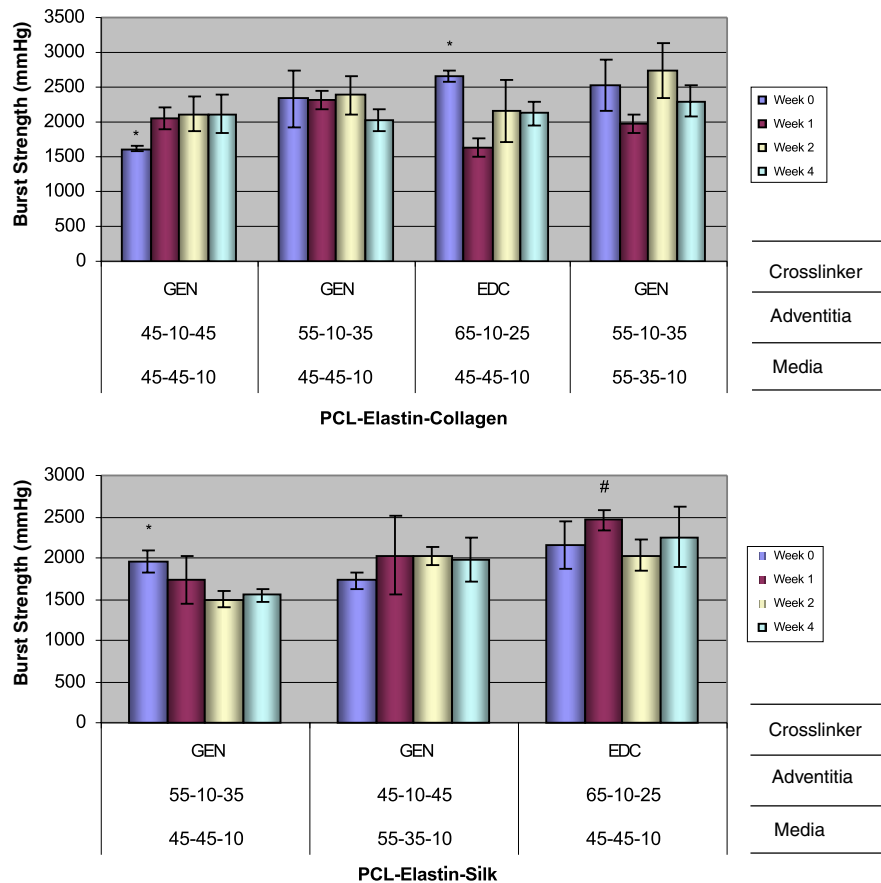


Fig. 9 – Burst pressure results for tri-layered specimens with changes in the both the medial layers and adventitial layers during a 4 week degradation study. (a) Top graph represents PCL–ELAS–COL tri-layered grafts and (b) bottom graph represents PCL–ELAS–SF tri-layered grafts. * indicates a significant difference from week 4. # represents a significant difference from week 2.

stress values. In a study by Vieth et al., scaffolds were created using recombinant ELAS-based polypeptide with either pyrroloquinoline quinone (PQQ), another zero-length crosslinker, or GEN (Vieth et al., 2007). The results from the study demonstrated that PQQ-crosslinked sheets exhibited a lower modulus and peak load, and a higher strain to break than GEN crosslinked sheets. In another study, COL-based constructs were crosslinked using EDC, GEN, and formaldehyde (Madhavan et al., 2010). Here, they found that COL gels crosslinked with GEN had a higher strain to break than EDC crosslinked constructs, while modulus and peak stress remained similar. Both these ELAS and COL gel studies demonstrated that crosslinking displays a time dependence and is essential to scaffold material properties.

Comparing these studies to ours, both Vieth and Madhavan tested gelled constructs which have a very different microstructure than electrospun fibers. Additionally, our study utilizes a synthetic polymer, which dominates the mechanical environment. The decreases seen in peak stress are most likely due to the combination of PCL, ELAS, and COL. The interaction of these polymers in solution created a different fiber composition for each construct with different chain entanglements and molecular weights. In the ELAS-based constructs we have a decrease in peak stress for 45-45-10-65-25-10. However, in the COL-based materials, there is a

slight increase in the 45-10-45 scaffolds for GEN crosslinking, but then as the content of COL decreases the ELAS is able to take on more of the mechanical loading. When this ELAS is crosslinked with GEN, it takes on a lower peak stress.

Energy to failure results demonstrated differences between the crosslinkers. For almost every case, the energy to failure values decreased when GEN was used as the crosslinker, excluding the 45-10-45 scaffolds. Differences between EDC and GEN for energy to failure values were found for only the 65-25-10 PCL–ELAS–COL scaffold. Nevertheless, based on the tensile test results, differences between the crosslinking reagents could not be conclusively drawn. Since the moduli were similar for both EDC and GEN structures, EDC crosslinked structures were chosen to be used in the modeling equations so as to simplify the data presented in the models.

Native tissue has been studied relentlessly, using mechanical properties and mathematical modeling of coronary intima, media, and adventitia to gain information that explains how the arterial wall reacts under physiological conditions (Wang et al., 2006; Holzapfel et al., 2005; Roy et al., 2005; Pandit et al., 2005; Lu et al., 2004; Holzapfel et al., 2004; Fung and Liu, 1995; El-Kurdi et al., 2008). Many of these models use a strain–energy function in a quasi-linear viscoelastic equation. The model used in the previous study (McClure et al., 2010)

was also used here to determine how materials will behave with one another. The results from the compliance and burst strength data were tested and subsequently compared to the model predictions.

4.2. Compliance and its relation to modeling

It is commonly agreed upon that compliance mismatch between native artery and an implanted graft is one of the fundamental causes of intimal hyperplasia and long-term graft occlusion. This is the predominant reason why ePTFE grafts possess a very low patency rate for small diameter blood vessels, and for the use of saphenous vein as the coronary bypass “gold standard”. Although native vein contains an altered ECM in comparison to artery, it retains a tri-layered structure, has an endothelial lining, and will adapt itself well to the new physiological pressures imposed on its wall.

An off-the-shelf vascular graft would be ideal for bypass graft cases because it would not involve tissue excision from a vascular site, and a tri-layered vascular graft would be ideal because of its tailorability. In this case, we tested how the stiffness of a biodegradable material affects compliance values through changes to the medial and adventitial layer. The results from this study exhibited some differences from the trends seen in the preliminary study (McClure et al., 2010), where medial layer stiffness was changed while intimal and adventitial layers remained constant.

In Fig. 4, PCL–ELAS–COL blends, EDC crosslinked grafts displayed results opposite what was found in the preliminary study; compliance increased as ELAS content decreased regardless of the adventitial layer. GEN crosslinked grafts, all manufactured in the same manner as EDC grafts, exhibited trends like those seen in the preliminary study. When a linear regression analysis was performed to quantify the trends between compliance and adventitial layer changes, the 45-45-10 medial layers contained an R^2 value of 0.91 while 55-35-10 medial layers contained an R^2 value of 0.59; an indication that both medial layers displayed adequate increases in stiffness as the adventitial layer increased in stiffness. The results of the GEN crosslinked scaffolds matched the predicted data from the mathematical analysis. However, EDC crosslinked scaffolds did not match the predicted model, where a stiffer medial layer would produce a lower compliance. The data did match when the adventitial layer stiffness increased. For example, the 65-25-10 medial layer exhibited an R^2 value of 0.86 in addition to the 45-45-10 medial layer displaying an R^2 value of 0.56. 55-35-10 medial layer displayed an R^2 value of 0.68, but its trend line increased in compliance as the adventitial layer increased in stiffness.

Fig. 5 displays the results from the PCL–ELAS–SF vascular grafts. According to the mathematical model, the SF grafts in combination with the 55-10-35 adventitial layer should prove to be beneficial with a high compliance while 45-10-45 and 65-10-25 incrementally increased in stiffness. Unfortunately, all of the EDC crosslinked grafts from this 55-10-35 group failed to produce desirable compliance values. Moreover, the only graft which possessed attractive compliance results from this grouping was the GEN crosslinked 45-45-10 medial layer. The GEN crosslinked group also produced results that matched

that mathematical model where the compliance decreased as the medial layer stiffened. This is demonstrated through a linear regression analysis where the R^2 value is 0.83. It also appeared that 65-10-25 adventitial layer grafts displayed results that matched the modeling data for both EDC and GEN, where the compliance decreased as the medial layer gained more PCL. When these results were analyzed further for the 65-10-25 group, EDC grafts displayed regression R^2 values of 0.85 while the GEN crosslinked group had an R^2 value of 0.12. Based on the linear regression analysis GEN grafts did not satisfy the mathematical model despite their decrease in compliance.

4.3. Burst strength and its relation to modeling

Burst strength of the all grafts demonstrated ample ability to withstand physiological pressures. The bursting properties also exhibited how changing the medial or adventitial layers of the graft material influenced its final burst pressure. In Fig. 6, grafts within the 45-10-45 adventitial layer group displayed, on average, the lower burst pressures of the three adventitial groups, the lowest belonging to GEN 45-45-10 at 1614 mm Hg. Furthermore, grafts within the 65-10-25 adventitial layer group showed averages around 3000 mm Hg. As the adventitial layers were incrementally increased with PCL, the burst pressure incrementally increased as well. This was shown through linear regression analysis where medial layers of 45-45-10 had an R^2 value of 0.94, and both 65-25-10 and 55-35-10 had a value of 0.99. Additionally, as the medial layer was increased burst values rose. Linear regression proves this with R^2 values of 0.94, 0.89, and 0.99 for 45-10-45, 55-10-35, and 65-10-25 adventitial groups, respectively. Therefore, PCL–ELAS–COL grafts, under burst pressure, exhibited values which were in agreement with the mathematical model, demonstrating that, as PCL content increased and the material stiffened, there was a subsequent relief of stress, allowing other layers to take on more pressure and thereby increasing burst strength.

Observing Fig. 7, PCL–ELAS–SF grafts demonstrate they do not fit with the mathematical model. Both the EDC and GEN 45-45-10 and 55-35-10 medial layers with increasing adventitial stiffness displayed a decrease in burst strength. This was echoed by their R^2 values of 0.99 and 0.47 for EDC 45-10-45 and 55-10-35, and R^2 values of 0.99 and 0.69 for GEN 45-10-45 and 55-10-35, respectively. Interestingly, both 45-10-45 cross-linked blends displayed higher correlation values while 55-10-35 revealed lower correlation values. Both EDC 65-25-10 and GEN 65-25-10 medial layers had a positive increase in burst strength with an R^2 value of 0.93 and 0.30, respectively. This clearly demonstrates that burst pressure in fact decreased for adventitial layers of 45-10-45 and 55-10-35, indicating that as less SF was introduced to the scaffold, the overall graft stiffness decreased. While higher SF content could explain the changes in burst strength trends compared to COL, increases in medial layer stiffness had a direct affect on burst pressure. This indicated a consistency amongst higher ELAS content layers. This was shown through the R^2 values of 0.88 and 0.73 for EDC 55-10-35 and 65-10-25 adventitial layers and values of 0.80 and 0.88 for GEN 45-10-45 and 55-10-35 values, respectively.

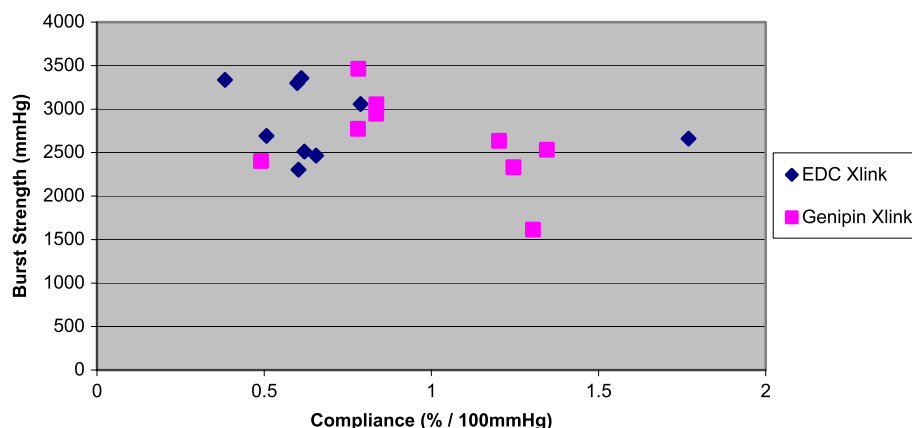


Fig. 10 – Burst strength vs. compliance to determine best overall PCL-ELAS-COL tri-layered grafts for future testing.

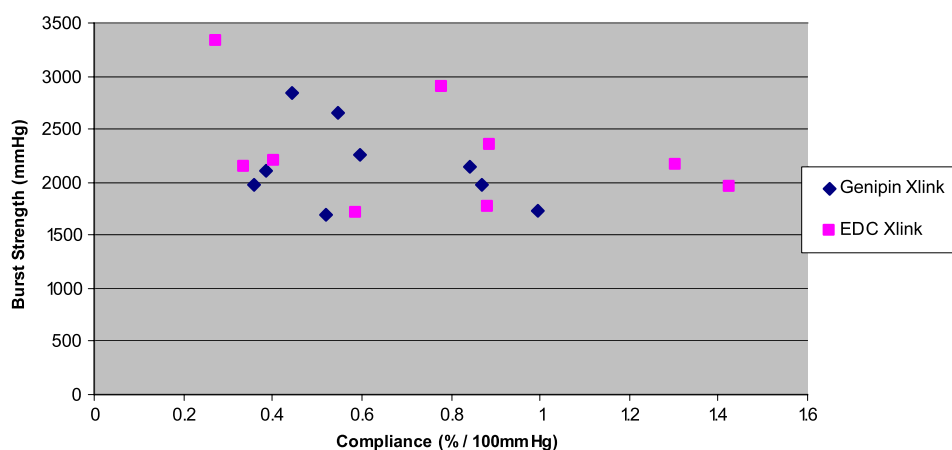


Fig. 11 – Burst strength vs. compliance to determine best overall PCL-ELAS-SF tri-layered grafts for future testing.

4.4. Choosing the best grafts: compliance vs. burst strength

A total of 36 different grafts and crosslinking combinations were used in this experiment. In order to narrow the data from the COL group and from the SF group, burst strength and compliance were plotted together. These results are displayed in the scatter plot below in Figs. 10 and 11. An interesting feature of Fig. 10 displays the majority of EDC crosslinked scaffolds clustered together at lower compliance values and higher burst strength. This is equally evident in Fig. 11. Some of these grafts have burst strength values around 1600 mm Hg, yet they fail to attain higher compliance values. This unpredictable behavior in Fig. 11 can be attributed to the SF. Prior to the beginning of the experimental design, preliminary studies were done to determine if SF would be a good candidate for vascular testing. These tests demonstrated desirable results with high compliance values and adequate burst pressures. This has been further proven in several experiments which have researched SF and electrospun SF (Zhang et al., 2008; Soffer et al., 2008).

GEN crosslinked scaffolds displayed the majority of the top results for both Figs. 10 and 11. For Fig. 10, a cut-off point was made at 1.3%/100 mm Hg compliance. In Fig. 11,

a cut-off point was made at 1.0%/100 mm Hg. These cut-off marks provided the best choice results for COL- and SF-based materials. For COL, grafts with combinations of (1) GEN 45-45-10 with 45-10-45, (2) GEN 45-45-10 with 55-10-35, (3) GEN 55-35-10 with 55-10-35, and (4) EDC 45-45-10 with 65-10-25 PCL-ELAS-COL were found to contain the best compliance and burst strength properties. For SF, grafts with combinations of (1) GEN 45-45-10 with 55-10-35, (2) GEN 55-35-10 with 45-10-45, and (3) EDC 45-45-10 with 65-10-25 PCL-ELAS-SF were found to contain the best compliance and burst strength properties. Each of these grafts were electrospun again and examined for degradation characteristics.

4.5. Compliance and burst degradation

When biodegradable materials are implanted *in vivo*, they experience both hydrolytic and enzymatic degradation. PCL is a polymer that has been shown to degrade, *in vitro*, over a period of 12 months. Studies have been performed *in vivo* with electrospun PCL vascular grafts implanted in the descending aorta of rats (Pektok et al., 2008). This study found that PCL, at both molecular weights of 120 and 80 kDa, began to degrade after 3 weeks implantation and had lost 20% of

its original molecular weight after 24 weeks of implantation. It was concluded that the degradation characteristics were most likely due to the cleavage of the ester linkages. Other studies (Bolgen et al., 2005), have revealed a molecular weight degradation of 30% after just 3 months.

While PCL degradation is mainly due to hydrolysis, crosslinked ELAS, COL, and SF must undergo enzymatic degradation. In a study done by Mercuri et al., a comparison was done between glutaraldehyde (GLUT), EDC, and periodate fixations for a glycosaminoglycan (GAG) aortic leaflet (Mercuri et al., 2007). Once fixed, these GAG scaffolds were placed in degrading enzymatic media. Results determined that fresh leaflets and GLUT crosslinked leaflets were significantly digested. However, EDC crosslinked scaffolds significantly resisted enzymatic digestion. Therefore, crosslinking mechanisms can significantly alter digestion time for the molecular bonds within ELAS and COL proteins. Moreover, SF proteins have also been found to degrade by way of proteolytic digestion (Lu et al., 2011), which can take in excess of 12 months.

According to our experiment, it appears that a 4 week period did not mechanically degrade the grafts. In some cases, there may have been some batch to batch variations which could have been caused by degradation, non-homogeneity from the electrospinning process, or non-homogeneity in the cross-linking process. For example, there was an increase in compliance and a slight decrease in burst strength for the EDC crosslinked 65-10-25 adventitia with a 45-45-10 media PCL-ELAS-COL after 2 weeks. However, week 4 compliance results revealed similarities to weeks 0 and 1. Meanwhile, 55-10-35 adventitia 55-35-10 media PCL-ELAS-COL grafts showed a gradual increase in compliance and a decrease in burst from week 0 to week 4, which could demonstrate degradation. Nonetheless, week 2 burst strength for these same grafts was the highest of the 4 values. While other PCL-ELAS-COL scaffolds seemed to slightly increase in compliance, yet they did not yield lower burst strength values. PCL-ELAS-SF grafts did not indicate that any degradation had occurred amongst the scaffolds.

5. Conclusion

In conclusion, COL blended samples displayed overall better mechanical properties when compared to SF blended samples. Furthermore, GEN crosslinked samples were consistently similar to the model predictions. The results from the compliance and burst strength data proved to change the overall graft properties with incremental changes in scaffold protein. From these experiments, grafts with the best combined compliance and burst properties were chosen to be tested in a 4 week degradation experiment. This experiment displayed some signs of graft degradation, increasing compliance without a loss of burst strength below 1600 mm Hg, but none were conclusive. Based off of the results from the degradation study, three grafts would be of future interest to evaluate the cellular and *in vivo* characteristics of the graft materials, EDC crosslinked 45-45-10 medial 65-10-25 adventitial PCL-ELAS-COL, GEN crosslinked 55-35-10 medial 55-10-35 adventitial PCL-ELAS-COL, and GEN crosslinked 45-45-10 medial 55-10-35 adventitial PCL-ELAS-SF.

REFERENCES

- Barnes, C.P., Pemble, C.W., Brand, D.D., Simpson, D.G., Bowlin, G.L., 2007. Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol. *Tissue Eng.* 13, 1593–1605.
- Berglund, J.D., Nerem, R.M., Sambanis, A., 2004. Incorporation of intact elastin scaffolds in tissue-engineered collagen-based vascular grafts. *Tissue Eng.* 10, 1526–1535.
- Boland, E.D., Matthews, J.A., Pawlowski, K.J., Simpson, D.G., Wnek, G.E., Bowlin, G.L., 2004. Electrospinning collagen and elastin: preliminary vascular tissue engineering. *Front. Biosci.* 9, 1422–1432.
- Bolgen, N., Manceloglu, Y.Z., Acatay, K., Vargel, I., Piskin, E., 2005. In vitro and in vivo degradation of non-woven materials made of poly(epsilon-caprolactone) nanofibers prepared by electrospinning under different conditions. *J. Biomater. Sci. Polym. Ed.* 16, 1537–1555.
- Buttafoco, L., Kolkman, N.G., Engbers-Buijtenhuijs, P., Poot, A.A., Dijkstra, P.J., Vermes, I., Feijen, J., 2006. Electrospinning of collagen and elastin for tissue engineering applications. *Biomaterials* 27, 724–734.
- Conte, M.S., 1998. The ideal small arterial substitute: a search for the Holy Grail? *FASEB J.* 12, 43–45.
- El-Kurdi, M.S., Hong, Y., Stankus, J.J., Soletti, L., Wagner, W.R., Vorp, D.A., 2008. Transient elastic support for vein grafts using a constricting microfibrillar polymer wrap. *Biomaterials* 29, 3213–3220.
- Fung, Y.C., Fronek, K., Patitucci, P., 1979. Pseudoelasticity of arteries and the choice of its mathematical expression. *Am. J. Physiol.* 237, H620–H631.
- Fung, Y.C., Liu, S.Q., 1995. Determination of the mechanical properties of the different layers of blood vessels in vivo. *Proc. Natl. Acad. Sci. USA* 92, 2169–2173.
- Holzappel, G.A., Gasser, T.C., Ogden, R.W., 2004. Comparison of a multi-layer structural model for arterial walls with a Fung-type model, and issues of material stability. *J. Biomech. Eng.* 126, 264–275.
- Holzappel, G.A., Sommer, G., Gasser, C.T., Regitnig, P., 2005. Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling. *Am. J. Physiol. Heart Circ. Physiol.* 289, H2048–H2058.
- Huang, L., McMillan, R.A., Apkarian, R.P., Pourdeyhimi, B., Conticello, V.P., Chaikof, E.L., 2000. Generation of synthetic elastin-mimetic small diameter fibers and fiber networks. *Macromolecules* 33, 2989–2997.
- Jin, H.J., Chen, J., Karageorgiou, V., Altman, G.H., Kaplan, D.L., 2004. Human bone marrow stromal cell responses on electrospun silk fibroin mats. *Biomaterials* 25, 1039–1047.
- Kannan, R.Y., Salacinski, H.J., Butler, P.E., Hamilton, G., Seifalian, A.M., 2005. Current status of prosthetic bypass grafts: a review. *J. Biomed. Mater. Res. Part B* 74, 570–581.
- L'Heureux, N., Paquet, S., Labbe, R., Germain, L., Auger, F.A., 1998. A completely biological tissue-engineered human blood vessel. *FASEB J.* 12, 47–56.
- Lu, X., Pandit, A., Kassab, G.S., 2004. Biaxial incremental homeostatic elastic moduli of coronary artery: two-layer model. *Am. J. Physiol. Heart Circ. Physiol.* 287, H1663–H1669.
- Lu, Q., Zhang, B., Li, M., Zuo, B., Kaplan, D.L., Huang, Y., Zhu, H., 2011. Degradation mechanism and control of silk fibroin. *Biomacromolecules* 12, 1080–1086.
- Madhavan, K., Belchenko, D., Motta, A., Tan, W., 2010. Evaluation of composition and crosslinking effects on collagen-based composite constructs. *Acta Biomater.* 6, 1413–1422.
- McClure, M.J., Sell, S.A., Barnes, C.P., Bowen, W.C., Bowlin, G.L., 2008. Cross-linking electrospun polydioxanone-soluble elastin blends: material characterization. *J. Eng. Fibers Fabr.* 3, 1–10.

- McClure, M.J., Sell, S.A., Simpson, D.G., Bowlin, G.L., 2009. Electrospun polydioxanone, elastin, and collagen vascular scaffolds: uniaxial cyclic distension. *J. Eng. Fibers Fabr.* 4, 18–25.
- McClure, M.J., Sell, S.A., Simpson, D.G., Walpoth, B.H., Bowlin, G.L., 2010. A three-layered electrospun matrix to mimic native arterial architecture using polycaprolactone, elastin, and collagen: a preliminary study. *Acta Biomater.* 6, 2422–2433.
- Mercuri, J.J., Lovekamp, J.J., Simionescu, D.T., Vyavahare, N.R., 2007. Glycosaminoglycan-targeted fixation for improved bio-prosthetic heart valve stabilization. *Biomaterials* 28, 496–503.
- Niklason, L.E., 1999. Replacement arteries made to order. *Science* 286, 1493.
- Pandit, A., Lu, X., Wang, C., Kassab, G.S., 2005. Biaxial elastic material properties of porcine coronary media and adventitia. *Am. J. Physiol. Heart Circ. Physiol.* 288, H2581–H2587.
- Pektok, E., Nottelet, B., Tille, J.C., Gurny, R., Kalangos, A., Moeller, M., Walpoth, B.H., 2008. Degradation and healing characteristics of small-diameter poly(epsilon-caprolactone) vascular grafts in the rat systemic arterial circulation. *Circulation* 118, 2563–2570.
- Rhodin, J.A.G., 1980. Architecture of the vessel wall. In: Bohr, D.F., Somlyo, A.P., Sparks, H.V. (Eds.), *Handbook of Physiology* Bethesda. American Physiological Society, MD, pp. 1–31.
- Roy, S., Silacci, P., Stergiopoulos, N., 2005. Biomechanical properties of decellularized porcine common carotid arteries. *Am. J. Physiol. Heart Circ. Physiol.* 289, H1567–H1576.
- Sell, S.A., McClure, M.J., Barnes, C.P., Knapp, D.C., Walpoth, B.H., Simpson, D.G., Bowlin, G.L., 2006. Electrospun polydioxanone–elastin blends: potential for bioresorbable vascular grafts. *Biomed. Mater.* 1, 72–80.
- Simpson, D.G., 2006. Dermal templates and the wound-healing paradigm: the promise of tissue regeneration. *Expert. Rev. Med. Dev.* 3, 471–484.
- Soffer, L., Wang, X., Zhang, X., Kluge, J., Dorfmann, L., Kaplan, D.L., Leisk, G., 2008. Silk-based electrospun tubular scaffolds for tissue-engineered vascular grafts. *J. Biomater. Sci. Polym. Ed.* 19, 653–664.
- Strandness, D.E., Sumner, D.S., 1975. *Hemodynamics for Surgeons*. Grune & Stratton, Inc., New York.
- Sung, H.W., Chang, W.H., Ma, C.Y., Lee, M.H., 2003. Crosslinking of biological tissues using genipin and/or carbodiimide. *J. Biomed. Mater. Res. Part A* 64, 427–438.
- Takamizawa, K., Hayashi, K., 1987. Strain energy density function and uniform strain hypothesis for arterial mechanics. *J. Biomech.* 20, 7–17.
- Vieth, S., Bellingham, C.M., Keeley, F.W., Hodge, S.M., Rousseau, D., 2007. Microstructural and tensile properties of elastin-based polypeptides crosslinked with genipin and pyrroloquinoline quinone. *Biopolymers* 85, 199–206.
- Wang, C., Garcia, M., Lu, X., Lanir, Y., Kassab, G.S., 2006. Three-dimensional mechanical properties of porcine coronary arteries: a validated two-layer model. *Am. J. Physiol. Heart Circ. Physiol.* 291, H1200–H1209.
- Zeugolis, D.I., Khew, S.T., Yew, E.S.Y., Ekaputra, A.K., Tong, Y.W., Yung, L.-Y.L., Hutmacher, D.W., Sheppard, C., Raghunath, M., 2008. Electro-spinning of pure collagen nano-fibres—just an expensive way to make gelatin? *Biomaterials* 29, 2293.
- Zhang, X., Baughman, C.B., Kaplan, D.L., 2008. In vitro evaluation of silk fibroin scaffolds for vascular cell growth. *Biomaterials* 29, 2217–2227.