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Suture-reinforced electrospun polydioxanone–elastin small-diameter tubes for use in vascular tissue engineering: A feasibility study

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Abstract

This study characterizes the cross-linking of electrospun elastin and the mechanical properties of suture-reinforced 1.5 mm internal diameter electrospun tubes composed of blended polydioxanone (PDO) and soluble elastin. Several tube configurations were tested to assess the effects of reinforcement on tube mechanical properties. Between the electrospun layers of each double-layered prosthetic, zero, one or two 6-0 sutures were wound, maintaining 1 mm spacing with a pitch of 9°. Single-layered tubes without suture were also examined. Samples were cross-linked and tested for compliance and burst strength. Compliance decreased significantly ($p < 0.05$) and burst strength significantly increased ($p < 0.01$) with reinforcement. Uncross-linked tubes were also tested to determine the effects of cross-linking. Results demonstrated that cross-linking significantly decreases burst strength ($p < 0.01$), while decreases in compliance for cross-linked tubes were not significant. Cross-linked suture-reinforced PDO–elastin tubes had burst pressures more than 10 times greater than normal systolic pressures and exhibited a range of compliance values, including those matching native artery. These tubes display many characteristics of the "ideal" small-diameter graft, having mechanical properties that can be tailored to match those desired in vascular replacement applications.

Keywords: Electrospinning; Vascular grafts; Polydioxanone; Elastin; Cross-linking

1. Introduction

There is a tremendous need for a functional, biocompatible, small-diameter vascular graft in the population at large. The American Heart Association reports that nearly one million deaths in 2003 were due to cardiovascular disease, while nearly 84 million people suffered from some form of the disease. In that year, it is believed that 13.2 million people suffered from coronary artery disease alone [1]. These statistics emphasize and validate current research efforts into developing a small-diameter vascular replacement.

The current "gold standard" in bypass surgery is the use of autologous saphenous vein or internal mammary artery; however, these vessels are not always a good option, as they may be occluded or diseased, especially if the patient already suffers from some sort of peripheral vascular disease. Peripheral vascular options beyond autologous vessels include the use of synthetic vessels like expanded poly(tetrafluoroethylene) (e-PTFE) and knitted or woven poly(ethylene terephthalate) (PET) grafts (Dacron®).

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These materials have demonstrated adequate performance for internal diameters (ID) larger than 6 mm, where high flow rates and low resistances make thrombus formation (i.e. acute graft failure) unlikely. However, when these synthetic grafts are used for small-diameter vascular replacements (<6 mm ID), they encounter much lower flow scenarios and are more likely to promote thrombus formation and intimal hyperplasia [2–5].

Recent work with bioresorbable and natural polymers has opened a new avenue for exploration in vascular tissue engineering [6]. In small-diameter applications, vascular grafts made with biodegradable polymers that are non-thrombogenic are desirable over other synthetics, such as e-PTFE, because over time, the body degrades and can replace the graft with a newly regenerated vascular structure indistinguishable from native vessels [2,5]. In addition, some synthetic biodegradable polymers, like polydioxanone (PDO), have mechanical strength capable of withstanding pulsatile flow [6–8]. Additionally, a graft that degrades and is replaced with newly formed tissue over time avoids the formation of a permanent, impenetrable acellular fibrotic capsule. Sanders et al. have demonstrated a reduction in fibrous capsule formation with decreasing scaffold fiber diameter [9]. They have also demonstrated that scaffolds fabricated by electrospinning show no initial signs of the formation of a fibrotic capsule, even when the scaffold is composed of a non-resorbable polymer [10].

We believe that the use of electrospinning to create nanofibrous structures is an excellent option for use in numerous tissue engineering applications, including vascular graft fabrication. The process of electrospinning has been described in detail previously [2,6,7,11–15]. Briefly, in our prototype system, the positive lead of a high voltage power supply is attached to the needle of a polymer-filled syringe, which is mounted to a syringe pump. A grounded mandrel is placed a set distance from the needle tip. When a large voltage is applied, the resulting electric potential draws a liquid polymer jet out of the needle tip. As the jet travels through the air, solvent evaporation leaves behind a dry fiber, which collects on the mandrel as a non-woven fibrous structure.

Using this procedure, we have successfully produced scaffolds in an assortment of shapes and sizes using a variety of synthetic and natural polymers, including polydioxanone, collagen, elastin, fibrinogen and hemoglobin [2,6,7,12–15]. One important advantage to this polymer processing technique is that scaffolds produced are composed of submicron diameter fibers in the same size range as native extracellular matrix (ECM) fibers [2,16].

Previous work in our laboratory and by others has demonstrated that PDO, a synthetic bioresorbable polymer, offers several advantages over the more traditional bioresorbable polymers like poly(glycolic acid) (PGA), poly(lactic acid) and poly(lactic-co-glycolic acid), because it has a slower resorption rate and induces a lower inflammatory response [7,17]. In addition, vascular prostheses made of PDO have been shown to be less thrombogenic than both PGA and Dacron® synthetic grafts [18]. However, like with other synthetic polymers, the use of PDO alone does not promote cell infiltration in vivo [6], leading the body to treat PDO as foreign. The endpoint of the body's response to any internal material identified as "foreign" is the formation of fibrotic tissue around the implant. This indicates the need for a more bioactive graft than is possible with synthetic polymers alone.

We have previously demonstrated that cells interact more readily with electrospun scaffolds containing natural polymers like collagen and elastin [2,16,19]. Scaffolds composed of these polymers are "bioactive", that is, they more closely mimic the composition of the native ECM, thus enabling cells to function as though they were interacting in a native environment. In vitro results of testing on electrospun blends of PDO and elastin show that the incorporation of elastin into these bioresorbable structures offers increased bioactivity [6], thus there is the potential for promoting in situ regeneration. These PDO–elastin composites have the mechanical integrity and strength of PDO combined with the bioactivity and minimal energy loss (due to elasticity) of elastin, and preliminary testing has shown that this polymer blend has significant potential for use as a vascular replacement.

Avoiding the formation of aneurysm in these composite tubes is critical if they are to have value as a potential vascular replacement. Preliminary in vivo data in our laboratory recently demonstrated a slight tendency to form an aneurysm in 1.5 mm tubes electrospun from 100% PDO after 6–12 weeks in a rat aortic replacement model. Because electrospun pure elastin lacks mechanical integrity, it is likely that the inclusion of elastin in small-diameter tubes will increase the possibility of rupture or aneurysm formation. Also, the potential for uneven degradation of implanted biodegradable polymers is another issue that raises some concern [20]. In order to potentially alleviate these concerns by providing additional mechanical strength and structure, we have designed and fabricated dual-layered 1.5 mm ID electrospun PDO–elastin tubes with either one or two 6-0 PDS™II bioresorbable polydioxanone sutures wound around the tubes between the layers. The purpose of this study was to characterize the mechanical properties (i.e. compliance and burst strength) of these reinforced tubes to determine if they meet the basic mechanical requirements for use in vascular prosthetic applications.

2. Materials and methods

To examine elastin cross-linking, soluble elastin from bovine neck ligament (Elastin Products Co., Inc.) was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, TCI America) at 250 mg ml^{-1} prior to electrospinning onto a rectangular mandrel (2.5 cm × 10.2 cm × 0.3 cm) followed by cross-linking using one of three cross-linking agents: a 50-fold molar excess (200 mM for 250 mg ml^{-1} elastin) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Sigma–Aldrich) in ethanol for 18 h [21],

50% glutaraldehyde (GLUT, Fisher Scientific) vapor (at both 100 °C and 25 °C for 30 min and 1 h, respectively) and ethylene–diglycidyl ether (EGDE, Sigma–Aldrich) for 24 h [22].

The concentration of free primary amine groups present in cross-linked electrospun pure elastin was determined using 2,4,6-trinitrobenzenesulfonic acid (TNBS, Research Organics, Inc.). Electrospun elastin scaffolds were cut, massed and cross-linked using the different reagents listed above. Samples were placed in 15 ml centrifuge tubes along with $NaHCO_3$ (1 ml, 4 wt./vol.%) and TNBS (1 ml, 0.5 wt./vol.%), followed by incubation at 40 °C for 2 h. An additional 3 ml of 6 M HCl was then added to the centrifuge tubes followed by incubation at 60 °C for 2 h. Finally, samples were diluted with 9 ml of phosphate-buffered saline (PBS) and cooled to room temperature, and the absorbance at 345 nm was measured using a SPECTRAmax® PLUS^{384} microplate spectrophotometer (Molecular Devices). The degree of cross-linking was expressed as the percent loss in free amino groups after cross-linking (as in Ref. [21]) and is calculated as follows:

$$\%\ \text{Cross-linking} = 1 - \left[\frac{\text{Abs}_c/\text{Mass}_c}{\text{Abs}_{nc}/\text{Mass}_{nc}}\right]$$

where subscripts c and nc indicate cross-linked and uncross-linked samples, respectively.

Prior to electrospinning onto a 1.5 mm diameter cylindrical mandrel of length 12 cm, PDO (Ethicon, Inc.) and soluble bovine elastin were each dissolved in HFP at concentrations of 60 and 200 mg ml^{-1}, respectively, and then blended at a ratio of 70:30 (by vol.) PDO:elastin. Two milliliters of the mixture were drawn into a Becton-Dickinson syringe fitted with an 18-gauge blunt-tip needle, and dispensed using a KD Scientific syringe pump at a rate ranging from 5.25 to 6 ml h^{-1}. During electrospinning, a voltage of 24 kV was applied to the needle via the positive lead of a Spellman CZE1000R high voltage power supply (Spellman High Voltage Electronics Corp.); the mandrel remained grounded. All other electrospinning parameters were kept constant: the air gap distance was 12.7 cm, the translation of the mandrel was ±3.75 cm, and the rotational and translational speeds of the mandrel were 500 RPM and 2 cm s^{-1}, respectively.

Midway through electrospinning, 6-0 PDS^{TM}II monofilament suture (diameter = 115 μm, Ethicon, Inc.) was wound around the tube to provide additional mechanical strength to the tubes, maintaining a spacing of 1 mm and a pitch of 9°. This was accomplished using a device designed in our laboratory, shown in Fig. 1, that enables the user to wind fine fibers/threads (such as suture) around a small-diameter tube or mandrel while maintaining control of pitch and spacing [19,23]. Double-layered tubes were wound with two sutures, applied between the layers, such that one suture was oriented in a right-hand helix and the other in a left-hand helix, resulting in a 6 cm double-layered tube. Each layer of the tube was electrospun from 1 ml of the PDO–elastin blend. Several other tube configurations were also fabricated and examined in order to better characterize the effects of the suture on the mechanical properties of the tubes. Double-layered tubes with no suture (DL0S) and double-layered tubes with one suture (DL1S) were tested in addition to the original design having two sutures (DL2S). Finally, single-layered tubes with no suture winding (SL) were electrospun from 1 ml of polymer blend in order to investigate the properties of the inner layer alone.

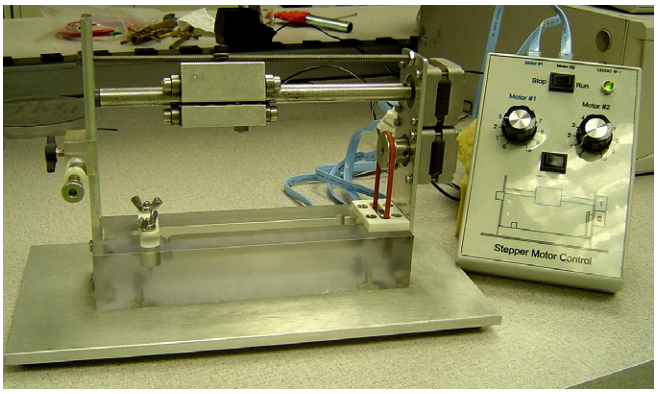

Fig. 1. Apparatus and control interface used to wind suture onto small-diameter mandrels. Varying of the translational and rotational speeds of the linear actuator/suture guide and mandrel, respectively, allow for control of suture spacing and pitch during application [19,23].

Using a method developed by Barnes et al. for cross-linking collagen [21], tubes were cross-linked for 18 h using a 50-fold molar excess of EDC in ethanol (167 mM for 200 mg ml^{-1} elastin), followed by a 2 h rinse in 0.1 M sodium phosphate. Tubes were then soaked in PBS for a minimum of 1 h and tested for compliance and burst strength. To investigate the effects of cross-linking, some DL2S tubes were hydrated in PBS for a minimum of 1 h and tested without being cross-linked.

Dynamic compliance testing was performed on segments (2–3 cm in length) of the 1.5 mm ID tubes in accordance with section 8.10 of ANSI/AAMI VP20:1994 [24] using an Intelligent Tissue Engineering via Mechanical Stimulation (ITEMS^{TM}) bioreactor developed by Tissue Growth Technologies (Minnetonka, MN). The testing chamber was filled with PBS at room temperature after samples were loaded, and the bioreactor provided a 1 Hz sinusoidal cyclic pressure differential of 120 mm Hg/80 mm Hg to the inside of each tube. Compliance was calculated as previously described [6], using the equation

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

where $P1$ and $P2$ represent the low and high pressures measured during sampling, and R_{P1} and R_{P2} represent the internal radii of the tube at pressures $P1$ and $P2$, respectively. Results are reported as percent compliance per 100 mm Hg.

Burst strength testing was completed using a device designed in accordance with section 8.3.3.3 of ANSI/AAMI VP20:1994 [24]. Tubes, 2–3 cm in length, were hydrated in PBS, fitted over 1.5 mm diameter nipples attached to the device, and secured with 2-0 silk suture. Air was introduced into the system, increasing the pressure at a rate of 5–10 mm Hg s^{-1} until the tubes burst. Results are reported as the pressure in mm Hg at which tubes ruptured.

Statistical analysis of all data was performed using the JMP™ 5.0 statistical software package (SAS Institute, Inc.) by first using Bartlett's test for homogeneity of variances, followed by a one-way analysis of variance (ANOVA) for all data and a Welch (non-parametric) ANOVA for non-homogeneous data. To examine statistically significant pairwise differences, the Tukey–Kramer and Wilcoxon rank-sum tests were employed as appropriate. In all evaluations, $p < 0.05$ indicated statistically significant differences.

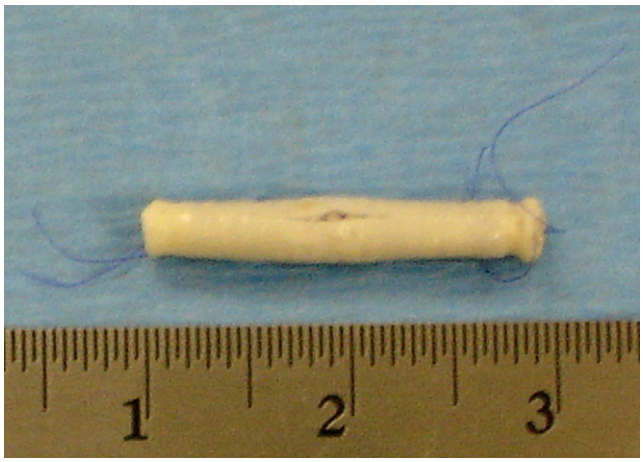

Fig. 3. Hydrated EDC cross-linked 1.5 mm ID electrospun PDO–elastin DL2S tube shown after completion of burst strength testing.

3. Results

Blended PDO and soluble elastin were successfully electrospun into 1.5 mm ID seamless, fibrous tubular constructs, as shown in Figs. 2 and 3. Scanning electron micrographs (SEMs) of DL1S tubes were taken using a Zeiss EVO 50 XVP (Nano Technology Systems Division, Carl Zeiss). Fig. 2A demonstrates the double-layered nature of the tubes, where a fine gap, indicated by the arrow, is visible along with an embedded suture. One DL1S sample was cut longitudinally in order to examine the packing of the electrospun matrix around the suture. This is seen in Fig. 2B, where arrows indicate the location of adjacent suture winds; it is notable that the two layers of the electrospun construct are relatively tightly packed, with a small gap between. This is further supported by Fig. 2C, which

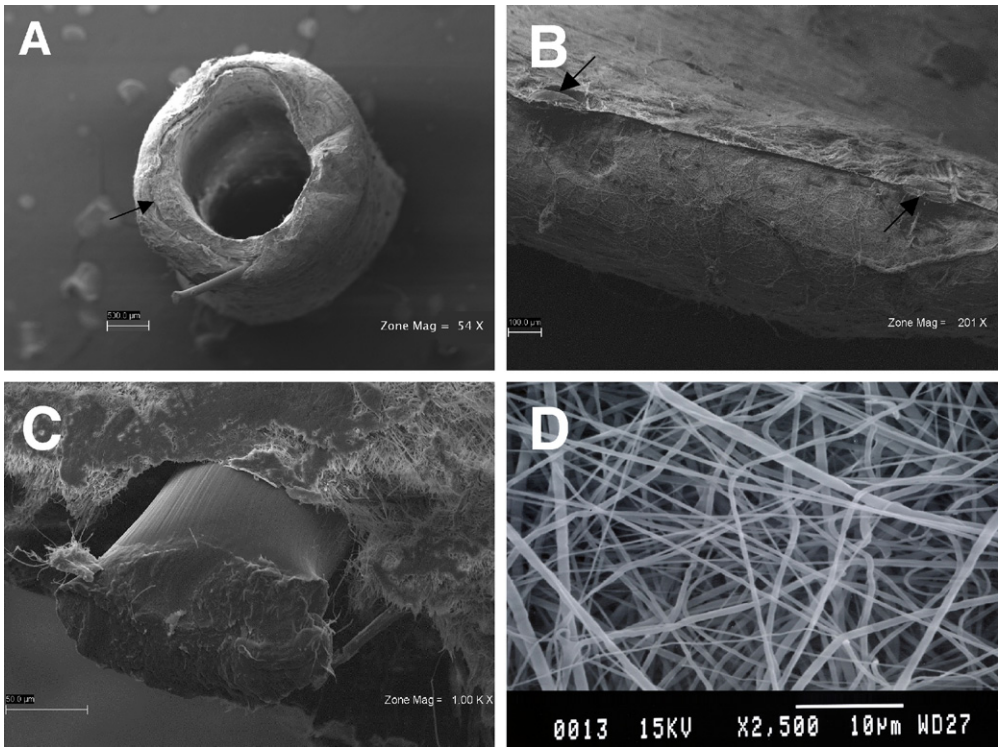

Fig. 2. (A) End-view SEM of a DL1S tube at 54× magnification, showing the PDS™II suture embedded between layers of the double-layered structure. The arrow indicates the fine gap between layers. Scale bar = 500 μm. (B) Longitudinal cross-section of a DL1S tube at 201× magnification demonstrating the compact nature of the two layers. Arrows indicate location of the suture embedded between layers. Scale bar = 100 μm. (C) 1000× magnification SEM showing the fibrous nature of the electrospun PDO–elastin and the close packing of the fibers around the PDS™II suture. Scale bar = 50 μm. (D) High-magnification SEM (2500×) showing the fibrous nature of electrospun 70:30 (by vol.) PDO–elastin. Scale bar = 10 μm.

shows a single suture at 1000× magnification, surrounded tightly by the fibrous electrospun matrix. Fig. 2D. shows electrospun PDO–elastin (70:30 by vol.) at 2500× magnification, demonstrating the fibrous nature of the blend. Fig. 3 shows a 2 cm segment of a hydrated DL2S tube after EDC cross-linking and completion of burst strength testing.

Results of the TNBS assay examining the cross-linking of electrospun elastin ($n = 9$) are illustrated in Fig. 4, where 200 mM EDC provided a percent cross-linking mean of 45.1%, while the mean for 50% heated GLUT vapor was 17.4%. Scaffolds cross-linked in both the EGDE and 50% room temperature GLUT vapor groups dissolved immediately in the $NaHCO_3$ solution prior to incubation, indicating minimal to no cross-linking; means for percent cross-linking for these reagents were not calculated. Statistical analysis indicates a statistically significant difference ($p < 0.01$) in percent cross-linking between the 200 mM EDC and 50% heated GLUT vapor groups. Given these results, EDC is the cross-linking reagent of choice for this study.

Results of compliance testing on EDC cross-linked 1.5 mm ID tubes ($n = 8$ with 10 replicates) are shown in Fig. 5 and range from 0.80%/100 mm Hg (DL2S) to 3.05%/100 mm Hg (SL). Statistical analysis indicates significant differences between DL2S tubes and all other groups ($p < 0.05$), indicating that tube compliance decreases with reinforcement. Average tube wall thicknesses (given as mean ± standard deviation) were measured to be 0.52 ± 0.06 mm for DL2S, 0.46 ± 0.17 mm for DL1S, 0.34 ± 0.10 mm for DL0S and 0.22 ± 0.07 mm for SL tubes.

Burst strength testing results ($n = 5$) are graphically represented in Fig. 6; values ranged from 916 mm Hg (SL) to 2347 mm Hg (DL2S). Statistical analysis indicates

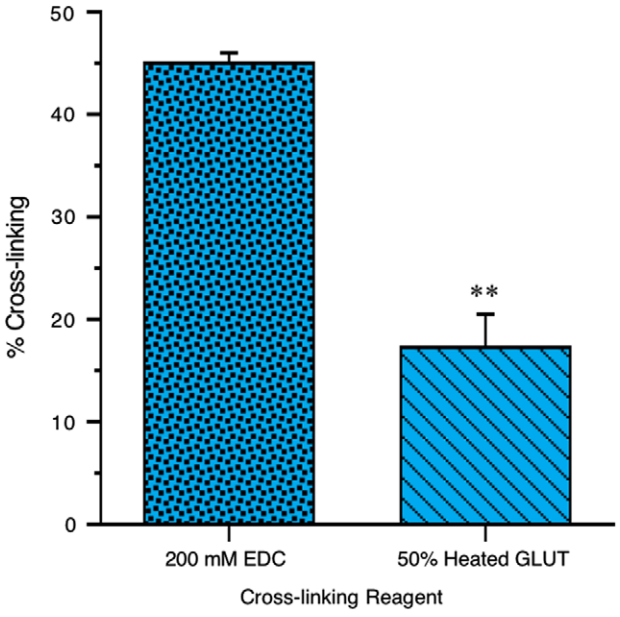

Fig. 4. Results of TNBS assay measuring free amine groups following cross-linking, expressed as percent cross-linking ($n = 9$). Asterisks indicate a statistically significant difference exists between the two groups ($^{**}p < 0.01$). Error bars indicate standard errors of the mean.

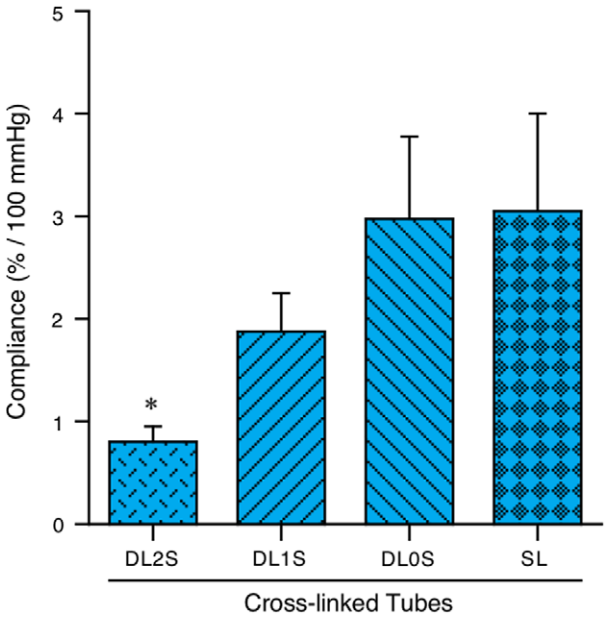

Fig. 5. Results of compliance testing on EDC cross-linked 1.5 mm ID electrospun PDO–elastin tubes ($n = 8$). The asterisk indicates that pairwise statistically significant differences exist between DL2S and all other groups ($^{*}p < 0.05$). Error bars indicate standard errors of the mean.

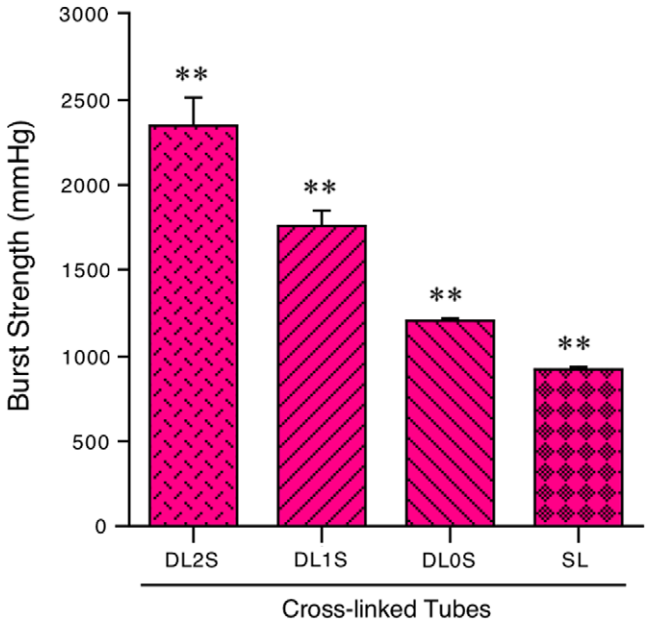

Fig. 6. Results of burst strength testing on EDC cross-linked 1.5 mm ID electrospun PDO–elastin tubes ($n = 5$). Asterisks indicate statistically significant differences exist between all pairs ($^{**}p < 0.01$). Error bars indicate standard errors of the mean.

significant differences ($p < 0.01$) between all pairs. As expected, burst strength increases with increasing reinforcement. In addition, and more importantly, reinforced tubes all exhibit burst pressures an order of magnitude larger than normal systolic pressure.

In Fig. 7, results of testing on uncross-linked DL2S tubes are compared with EDC cross-linked DL2S results

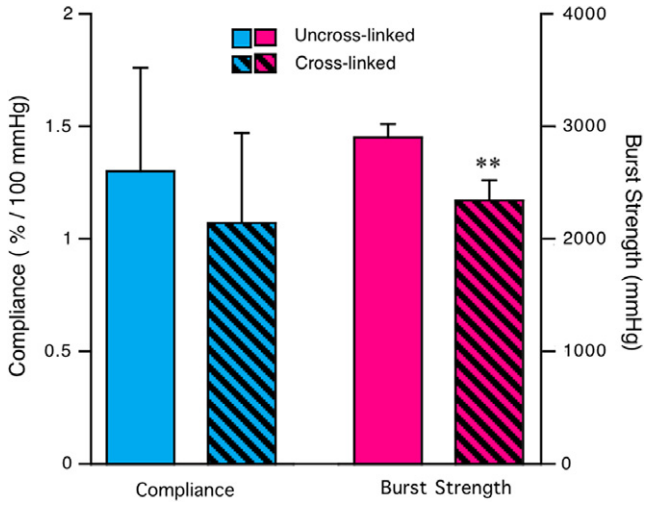

Fig. 7. Compliance and burst strength results for EDC cross-linked ($n = 8$) and uncross-linked ($n = 3$) DL2S tubes. Asterisks indicate statistically significant differences ($^{**}p < 0.01$). Error bars indicate standard errors of the mean.

from above to assess the effects of cross-linking on tube mechanical properties. It is important to note that the performance of uncross-linked DL2S tubes was poor. Of the fourteen 2 cm segments manufactured, only three survived testing; the remaining samples delaminated with minimal handling. Means of both compliance (1.3%/100 mm Hg) and burst strength (2906 mm Hg) for the uncross-linked tubes ($n = 3$ with 10 replicates) were larger than means of the same parameters for cross-linked tubes. Statistical analysis indicates no changes in compliance with cross-linking; however, there is a statistically significant decrease in burst strength with cross-linking ($p < 0.01$).

4. Discussion

Native elastin cross-linking renders a highly hydrophobic protein from soluble tropoelastin molecules. The initial reaction is an oxidative deamination of lysine residues by the enzyme lysyl oxidase to produce allysine, also known as α-amino adipic δ-semialdehyde [25]. All subsequent reactions are spontaneous and involve the condensation of closely positioned lysine and allysine residues to produce cross-links such as allysine aldol, lysinonorleucine, merodesmosine and tetrafunctional crosslinks unique to elastin, such as desmosine and isodesmosine, both of which are highly insoluble domains [25]. However, the soluble bovine elastin used in this study does not retain the ability to form the native crosslinks through the natural process described above; instead, the natural molecular regions of the carboxyl and amine groups on the soluble elastin allow for the formation of synthetic cross-links by a cross-linking reagent [26].

Weight sampling, SEM characterization and uniaxial tensile testing completed in our laboratory (unpublished data) provide evidence that uncross-linked elastin does not stay within hydrated electrospun scaffolds. The presence of elastin is desired in order to achieve and maintain desired mechanical properties and to provide bioactivity. Therefore, it is necessary to cross-link electrospun scaffolds containing elastin.

Glutaraldehyde treatment is one of the only commercially viable processes that has received widespread acceptance. It is readily available, inexpensive and forms aqueous solutions that can effectively cross-link tissue in a relatively short period of time, making it extremely useful. GLUT is a five-carbon aliphatic molecule with an aldehyde at each end of the chain. This aldehyde is able to interact with amine groups to form chemical bonds [27]. EGDE is a polyether oxide which, in previous work, has been applied to a variety of tissues including bovine pericardium and artery. The epoxy functionality predominantly reacts with the amine group on lysine, much like GLUT [27]. EDC, on the other hand, is somewhat different. Here, the carboxyl functional groups of aspartic and glutamic amino acids on the protein undergo a three-step chemical reaction in which the carboxyl group is esterified, eventually resulting in the formation of acyl azides, which, in turn, react and couple with adjacent amine groups on other amino acids in the elastin [26–29].

We have demonstrated that both GLUT at room temperature and EGDE did not sufficiently cross-link pure elastin scaffolds; therefore, we have discounted them as adequate cross-linking agents for electrospun scaffolds containing elastin. In comparing the heated GLUT with the EDC in ethanol, it is clear that EDC has a larger percent cross-linking mean than heated GLUT. Given these results and concerns regarding the toxicity of GLUT [30], we have chosen to pursue EDC as the cross-linking reagent for this study.

It is widely believed that the poor performance of current synthetic options in small-diameter vascular replacement applications is due in part to compliance mismatch between the replacement and native vessels [31,32]. Compliance mismatch can lead to more turbulent blood flow and eventually intimal hyperplasia. Efforts to minimize or eliminate hyperplasia have included a variety of techniques, from cell-seeding [33] to heparin coating of grafts [34], and even administration of immunosuppressive drugs to decrease neointimal proliferation [35], with mild to no success. Most current small-diameter synthetic vascular grafts actually decrease in compliance over time following implantation [31]. However, bioresorbable vascular grafts have been shown to increase in compliance over time due to graft resorption and the in-growth of tissue more compliant than the graft itself [31]. Based on published results regarding the interactions of different cell types (human umbilical vein endothelial cells, aortic smooth muscle cells, and dermal fibroblasts) with electrospun materials [2,6,16], we speculate that the use of a bioactive graft (containing natural polymers, such as elastin) will result in enhanced endothelial and smooth muscle cell infiltration and migration, leading to quicker and more efficient regeneration and

less time for changes in compliance to occur, as compared with PDO alone. The scope of the work reported here is focused on the design of such a bioactive graft with mechanical properties that can be tailored to achieve compliance matching with the native vessel.

We have constructed a variety of electrospun small-diameter prostheses with a range of compliance values, ranging from those matching e-PTFE to nearly 1.2 times the compliance of native artery, as shown in Fig. 8A (values for artery, vein, and e-PTFE from [6].) Fig. 8B compares burst pressures of our 1.5 mm ID tubes to burst pressures of decellularized porcine carotid artery (from Ref. [36]), decellularized canine saphenous vein (from Ref. [37]) and e-PTFE (average of the range reported in Ref. [32]).

A comparison of the standard deviations in compliance for our 1.5 mm ID tubes indicates that tubes without suture have much larger standard deviations (greater than 2.25%/100 mm Hg) than reinforced tubes (less than 1.05%/100 mm Hg). This indicates that suture-reinforcing dominates the mechanical properties of the randomly oriented electrospun fibers, resulting in more consistency and less variability in compliance between constructed tubes. This ability to produce tubes having a reliable compliance allows for the customizing of tube mechanical properties in order to achieve compliance matching in any vascular replacement application.

We have also demonstrated the ability to control burst strength via suture-reinforcement. Typically, there is a negative correlation between compliance and burst strength, often resulting in a design trade-off in which compliance matching is set aside in order to maintain extremely high tube burst pressures. Indeed, with our reinforced PDO–elastin tubes, we also see a strong negative correlation (Pearson's $r = -0.9870$) between compliance and burst strength, as demonstrated in Fig. 9.

It is worthwhile to note the performance of the EDC cross-linked DL1S electrospun PDO–elastin tubes, in particular. These tubes maintain physiologically unachievable burst pressures while simultaneously possessing compliance close to typical arterial compliance. Accordingly, the DL1S tube may offer promise as a viable prosthesis for use in treating both peripheral vascular disease and coronary artery disease.

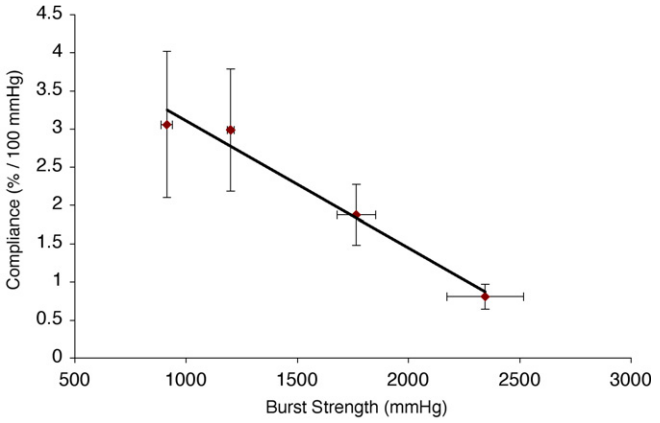

Fig. 9. Strong linear correlation between compliance and burst strength for EDC cross-linked tubes. Pearson's correlation coefficient: $r = -0.9870$. Error bars indicate standard errors of the mean.

With regard to results indicating that cross-linking of the DL2S tubes resulted in a decrease in burst strength and not an increase, it is important to note that the process of cross-linking does not cross-link between electrospun elastin fibers. It is hypothesized that the cross-linked structures are stiffer and less elastic. The cross-linked elastin, unable to stretch to accommodate increased pressure, ruptures more readily, and thus the tube ruptures more readily. In addition, it should be re-emphasized that the elastin used in these tubes is soluble, thus it dissolves into the PBS solution when uncross-linked tubes are hydrated, leaving only PDO fibers in the hydrated uncross-linked tubes. Therefore, the burst strength results for uncross-linked tubes are essentially burst strength results of 100%

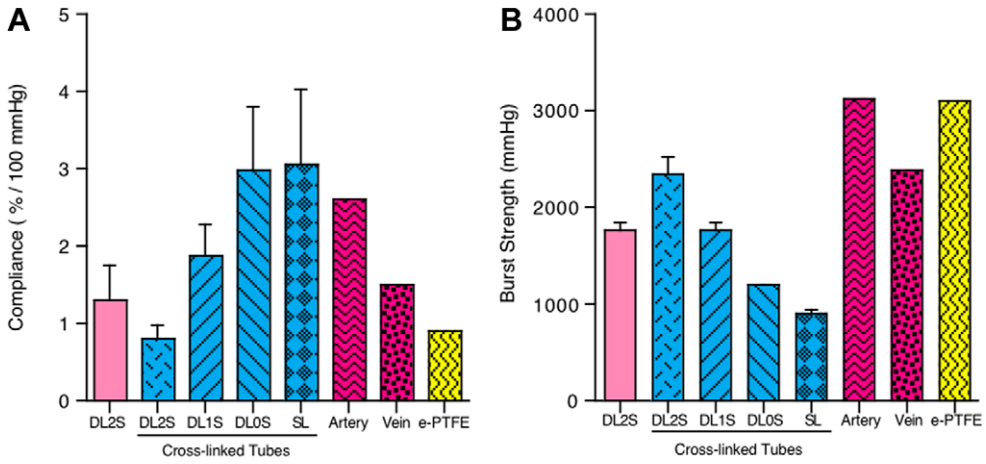

Fig. 8. Comparisons of (A) compliance and (B) burst strength for uncross-linked PDO–elastin tubes, EDC cross-linked PDO–elastin tubes, carotid artery, saphenous vein and e-PTFE. The compliance values for artery, vein and e-PTFE are from Ref. [6]; the burst pressures are from Refs. [36] (artery), [37] (vein) and [32] (e-PTFE). Error bars indicate standard errors of the mean.

PDO tubes, which, having no mechanically weaker elastin fibers present, burst at significantly higher pressures.

Much work in tissue engineering today is focused on endothelial cell seeding of grafts, graft surface modification, and the growing of new tissue in vitro [3,4,38]. With regard to the latter of these, the promise of successfully growing a functional artery in an artificial environment is still some time from becoming a reality. While cell-seeding tissue engineering approaches have met with some success, there are many issues yet to be resolved. In order to harvest autologous cells to use in culture, the patient may have to undergo additional invasive procedures prior to the vascular replacement operation. Also, timing is often critical, as many patients do not have the luxury of time required to culture cells and grow them to form the replacement vessel before entering into surgery. Surgeons are demanding an immediately available, ''off-the-shelf'' acellular vascular prosthetic capable of simultaneously functioning as a replacement vessel and promoting the formation of a neo-artery.

The search for the ''ideal'' small-diameter vascular graft has been called the tissue engineering equivalent of the ''Search for the Holy Grail'' [2,39]. This ideal prosthesis should have many characteristics, including ease of surgical handling, flexibility with kink resistance, biocompatibility (non-thrombogenicity and limited immunogenicity), compliance matching with native artery and resistance to aneurysm formation [5]. In this study, we have demonstrated the ability to fabricate small-diameter bioresorbable tubes that meet several of these criteria. Our acellular PDO–elastin tubes are easy to manufacture, can be cross-linked, sterilized, freeze-dried and packaged, and require no special handling when compared with currently available synthetic prostheses. They are kink-resistant (due to the shape memory of PDO), and have the ability to match compliance with native artery yet still maintain a large safety factor in burst pressure.

We are just beginning to assess the in situ performance (rat aorta model) of these prostheses. In these preliminary studies, cardiovascular surgeons have indicated that these 1.5 mm ID tubes have ''excellent'' suture ability and suture retention. Further in vivo investigations in animal models will focus on mechanical performance of the grafts as degradation and cell-infiltration occur, with particular emphasis on three key factors: patency/non-thrombogenicity, propensity to form aneurysm and tendency to induce intimal hyperplasia. Studies exploring the integration of cells/tissue with the grafts in vitro are also underway. Additionally, exploration of other tube design parameters may be warranted, including possibly varying suture spacing and pitch, in order to identify design characteristics that meet mechanical requirements necessary for optimal in situ regeneration.

5. Conclusion

Using electrospinning, we have successfully fabricated suture-reinforced 1.5 mm ID seamless bioresorbable tubes of blended PDO and elastin for potential use in vascular tissue engineering. Reinforcing of these tubes with biodegradable suture allowed for maintenance of high burst strength while also permitting compliance to be tailored to meet specific needs. We have also examined cross-linking reagents and demonstrated the superior cross-linking achieved by EDC. Of the reinforced 1.5 mm ID tube designs studied, the DL1S configuration simultaneously demonstrated physiologically unachievable burst pressure with compliance nearly matching that of native artery. These acellular bioactive grafts are easy to manufacture, handle, package and store, and represent a promising option for an off-the-shelf small-diameter vascular replacement. These prostheses have the potential to fill a significant void in the treatment of both peripheral vascular disease and coronary artery disease and, as such, continued research on these tubes is warranted.

Disclosure

Several authors have United States and International patents pending concerning technology presented in this manuscript, and this technology has been licensed to NanoMatrix, Inc., of which several authors have a financial interest.

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