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High compliance vascular grafts based on semi-interpenetrating networks

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Abstract

Current synthetic vascular grafts have poor patency rates in small diameter applications (<6 mm) due to intimal hyperplasia arising from a compliance mismatch between the graft and native vasculature. Enormous efforts have focused on improving biomechanical properties; however, polymeric grafts are often constrained by an inverse relationship between burst pressure and compliance. We have developed a new, semi-interpenetrating network (semi-IPN) approach to improve compliance without sacrificing burst pressure. The effects of heat treatment on graft morphology, fiber architecture, and resultant biomechanical properties are presented. In addition, biomechanical properties after equilibration at physiological temperature were investigated in relation to polyurethane microstructure to better predict *in vivo* performance. Compliance values as high as $9.2 \pm 2.7 \text{ \%}/\text{mmHg} \times 10^{-4}$ were observed for the semi-IPN graft while also maintaining high burst pressure, $1780 \pm 230 \text{ mm Hg}$. The high compliance of these heat-treated poly(carbonate urethane) (PCU) and semi-IPN grafts is expected to improve long-term patency rates beyond even saphenous vein autografts by preventing intimal hyperplasia. The fundamental structure-property relationships gained from this work may also be utilized to advance biomedical device designs based on thermoplastic polyurethanes.

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author

Keywords

polyurethanes; silicones; semi-interpenetrating networks; electrospinning; small-diameter vascular grafts

1. Introduction

Approximately 1.4 million patients in the USA are treated with arterial prostheses every year.^[1] Autologous saphenous veins and mammary arteries are the current gold standards for bypass grafts but are unavailable for use in approximately 20% of patients due to disease, trauma, or anatomic abnormalities.^[2, 3] Cadaveric saphenous veins have been explored as an alternative biologic option but concerns regarding processing effects and immunological risks have been raised.^[4–8] Synthetic grafts of expanded polytetrafluoroethylene (ePTFE) and woven polyethylene terephthalate (PET) are viable alternatives in large diameter applications (>4 mm); however, high failure rates in small diameter applications have been attributed to poor compliance matching to native arteries resulting in occlusion at the distal anastomoses.

A compliance mismatch between the graft and artery has been previously reported to cause flow separation and stagnation zones in vasculature downstream from synthetic grafts.^[9] This decrease in mean shear stress experienced along the vascular wall induces a physiological response to increase the arterial wall thickness via intimal hyperplasia in an attempt to restore the target wall shear stress.^[10] Given the known correlation between graft compliance and patency of autologous and synthetic vascular grafts,^[11] this biomechanical property has become one of the key design requirements in the development of improved arterial prostheses. To the best of our knowledge, no known homopolymer has been shown to provide appropriate compliance without sacrificing requisite suture retention strength and/or burst pressure.

Segmented polyurethanes (SPUs) have emerged as a popular choice for several cardiovascular applications including cardiac pacemaker lead coatings^[12–14], arteriovenous shunts^[15, 16], and vascular grafts.^[17, 18] The high tunability of these segmented block copolymers provide multiple mechanisms to tailor graft mechanical properties to enhance clinical performance. Early polyurethane grafts such as the Corvita, Thoratec, and PulseTec vascular grafts were developed as an alternative to the standard Dacron and GoreTex grafts with modest improvements in compliance. However, compliance values were still well below autologous standards.^[19] Newer commercial SPU grafts such as the UCL-NanoTM and Myolink grafts, now available in Europe, have exhibited improved compliance values over traditional synthetic graft options.^[20, 21] Unfortunately, no record of the burst pressure of these grafts is available indicating that it meets minimum standards for implantation. SPU materials have been previously explored in our laboratory as an option for an electrospun reinforcing layer of a multilayered vascular graft.^[22] Despite outperforming current polyurethane grafts in achieving high compliance values by modulating graft architecture with electrospinning parameters, the graft did not retain burst pressure comparable to current grafts.^[20] Furthermore, an inverse correlation between burst pressure and compliance was

still observed making it difficult to match the high burst pressure and high compliance of native arteries.

Although polyurethanes are similar to arteries in their high elastic recovery at low strains and high tensile strength, arteries typically display an earlier and more robust strain hardening region.^[23] Arterial tissue is composed of alternating layers of elastin and collagen with elastin serving as the key contributor at low strains and collagen becoming more dominant at high strains.^[24] The similarity in the SPU stress response may be attributed to the microphase-separated morphology with elastomeric stretching of the soft segment matrix dominant at low strains followed by hard domain rotation/shear and strain-induced crystallization of the soft segment as strain is increased.^[25] To reduce modulus in a polyurethane to improve compliance, a lower hard segment content is typically chosen; however, this also results in reduced tensile strength which has been correlated with lower burst pressures.^[26] Therefore, a new strategy must be employed beyond typical segmental chemistry modifications to better match arterial properties without sacrificing the desirable high elastic recovery and tensile strength of the polyurethane.

Interpenetrating polymer networks (IPNs) have been explored in biomedical devices due to their ability to maintain the desirable properties of multiple materials.^[27–31] Similarly, semi-interpenetrating polymer networks (semi-IPNs) replace one of the covalently crosslinked networks with a linear component which allows for separation without change in chemical composition and ease of processing. For this work, semi-IPNs based on a linear SPU was selected to allow for full use of their attractive characteristics without sacrificing key mechanical properties. A crosslinked silicone network was selected as the second component of the semi-IPN given the prevalence of the material class in biomedical devices^[32–34] and demonstrated biostability.^[35] Previous research has explored employing an SPU coupled with a poly(dimethylsiloxane) (PDMS)-based macromer to improve the compliance of a vascular graft.^[36] The authors attributed the increase in compliance to the proposed plasticization of the physically crosslinked SPU network by the siloxane molecules.

In the present study, we propose to implement this PDMS-SPU semi-IPN design in an electrospun graft to improve compliance while maintaining burst pressure. The effect of semi-IPN chemistry on compliance and burst pressure of electrospun grafts with similar thickness and fiber morphology was determined. Dynamic mechanical analysis (DMA), differential scanning calorimetry (DSC), and attenuated total reflectance (ATR)-FTIR were used to assess microphase morphology and correlate with observed biomechanical properties to identify key structure-property relationships. Finally, the biomechanical properties of these electrospun SIPN grafts were then compared to reported values of current autologous grafts to assess their potential as arterial prostheses.

2. Experimental Section

2.1. Materials

Poly(carbonate urethane) (PCU) Carbothane[®] PC3575A was purchased from Lubrizol (Boston, MA) and used as received. Methacryloxypropyl terminated PDMS and (3-

Acryloxy-2-hydroxypropoxypropyl) terminated PDMS (Figure 1) were purchased from Gelest, Inc. (Morrisville, PA) and used as received. The PDMS-dimethacrylate (PDMS-DMA) macromer had a reported weight average molecular weight (M_w) range of 380–550 Da and the PDMS-diacrylate (PDMS-DA) macromer had a reported M_w range of 600–900 Da. Poly(ethylene glycol) (PEG) with M_w of 35 kDa, N,N'-dimethylacetamide (DMAC), chloroform, and benzoyl peroxide (BPO) were purchased from Sigma Aldrich (Milwaukee, WI) and used as received.

2.2. Electrospun Graft Fabrication

2.2.1. Solution Preparation—PCU pellets were subject to a minimum of 24 hours vacuum drying at ambient temperature prior to use. DMAC and chloroform were stored over molecular sieves prior to solution preparation to avoid minimize water content. PCU solutions were prepared by dissolving the SPU pellets at 18 wt% in DMAC and purged with nitrogen. SIPN solutions were made by first mixing DMAC and chloroform at a 3:1 ratio followed by the addition of 1 wt% thermal initiator, BPO. The PDMS-DMA macromer was then added at 5, 10, or 20 wt% concentrations of the original 18 wt% solute and allowed to dissolve before adding the remaining PCU component. Solution viscosities were adjusted as needed to maintain a viscosity of 10 Pa·s to avoid variation in electrospun architecture. [37] A 25 wt% solution of PEG 35 kDa in chloroform was also prepared for use as a sacrificial inner layer to aid in graft harvesting.

2.2.2 Electrospinning Process—All electrospinning runs were performed inside an enclosed acrylic box to maintain consistent levels of approximately 50% relative humidity and 21°C temperature. Prior to electrospinning PCU or SIPN solutions, a sacrificial layer of PEG was electrospun onto 5 mm mandrels to maintain fibrous morphology on layer while also providing separation between the PCU and mandrel. Briefly, the 25 wt% PEG 35 kDa solution was poured into a 10 mL glass syringe equipped with a blunted 20 gauge needle. The solution was dispensed out of the needle at 0.5 mL/hr using a KDS 100 syringe pump (KD Scientific, Holliston MA) with a distance to collector (DTC) of 52 cm onto the stainless steel mandrel rotating at 500 rpm. A positively charged voltage of 14 kV was applied to the tip of the 20 gauge needle and a negatively charged 2 kV was applied to the mandrel using high voltage sources purchased and used as received from Gamma High Voltage (Ormond Beach, FL). The PEG 35 kDa solution was allowed to spin for 5 minutes before the syringe was replaced with one loaded with Carbothane® or a semi-IPN solution. The PCU and SIPN solutions were subject to the same parameters except the positive voltage was increased to approximately 20 kV and the negative to 5 kV. All PCU/SIPN solutions were electrospun for 2 hours and 15 minutes to achieve a graft thickness of approximately 0.2 mm. Solutions were electrospun for 4–6 hours for mesh thicknesses of 0.4 mm.

2.2.3. Heat Treatment—Electrospun meshes were soaked overnight in deionized water to dissolve the inner electrospun PEG layer and facilitate the removal of the electrospun grafts from the mandrel. The grafts were then dried under vacuum overnight to remove excess water. Finally, the electrospun SIPN grafts were subject to a 12 or 24 hour heat treatment on 5 mm diameter PTFE rods to covalently crosslink the siloxane network. A set of

Carbothane[®] electrospun grafts were also heat treated to isolate the effects of the curing conditions on the electrospun PCU material properties.

2.3. Graft Characterization

Grafts were cut into 4 cm long pieces for all materials testing. Characterization was performed on both as-spun and heat-treated grafts to observed effects of the curing cycle on electrospun fiber architecture and properties. Fiber morphology of electrospun grafts were observed using scanning electron microscopy (SEM). Changes in viscoelastic response were monitored using dynamic mechanical analysis (DMA). Microphase morphology was then further investigated with differential scanning calorimetry (DSC) and ATR-FTIR spectroscopy. Crosslinking of covalent PDMS networks was confirmed with NMR spectroscopy.

2.3.1. Scanning Electron Microscopy (SEM)—Fiber morphology was examined using SEM with a JEOL NeoScope JCM-5000 (Tokyo, Japan) at a 5 kV acceleration voltage. Specimens were coated with approximately 4 nm of gold using a Cressington Sputter Coater 108 (Watford, England) prior to imaging. Similar fiber diameter and junction quality of as-spun grafts were confirmed prior to heat treatment and microphase morphology/ biomechanical analysis.

2.3.2. Microphase Morphology Analysis—Effects of electrospinning and heat treatment on the material properties of the Carbothane[®] and semi-IPN grafts were examined using DMA, DSC, and ATR-FTIR analysis. DMA was conducted with a TA RSA3 on grafts cut into strips of approximately 5 cm in width. Specimens were subject to a temperature ramp from -90°C to 100°C at a rate of $5^{\circ}\text{C}/\text{min}$ while under a 0.1% cyclic strain at 1 Hz frequency. DSC thermograms ($n=3$) were collected on specimens of approximately 10–15 mg which were subjected to a temperature ramp of -90°C to 100°C with the same rate under nitrogen gas using a TA DSC Q100 (Houston, TX). For DSC, all analysis was performed on the first scan to examine processing effects from electrospinning and/or heat treatment. Finally, proposed effects on microphase morphology were corroborated by using ATR-FTIR to examine changes in hydrogen bonding within the hard domains of the electrospun PCU. Briefly, 32 spectral scans of as spun and heat treated electrospun grafts were taken and averaged over the wavenumber range of 4000 to 700 cm^{-1} using a Bruker ALPHA equipped with a Germanium ATR single reflectance module. The carbonyl wavenumber region (1800 to 1600 cm^{-1}) of segmented polyurethanes was monitored to examine changes in the hydrogen bonding of the urethane carbonyl as an indicator of microphase morphology.^[38] Specifically, the free urethane carbonyl absorbance near 1730 cm^{-1} was expected to be reduced while the hydrogen bound carbonyl absorbance at 1700 cm^{-1} would increase with evidence of microphase separation.

2.3.3. Biomechanical Testing—Static compliance of each graft was measured using a method described previously.^[39] Briefly, a nonporous latex tube was inserted inside each graft prior to testing. The grafts were subject to a pressure ramp from 0 to 150 mmHg using deionized water dispensed via a syringe pump connected to the latex tube. A standard in line strain gauge pressure transducer from Merit Medical (South Jordan, UT) was used to

measure intraluminal pressure while the outer diameter of the graft was measured with a Lasermike He-Ne laser micrometer. Compliance (C) was calculated using the following equation:

$$C = \frac{\Delta D}{D_0 \cdot \Delta P} = \frac{D_{120} - D_{80}}{D_{80} \cdot 40}, \quad (1)$$

where ΔP is change in pressure and D_{120} and D_{80} are the graft outer diameters at 120 and 80 mmHg intraluminal pressure, respectively. Compliance values were recorded 5 times on each graft and values from each graft were averaged to obtain the reported value ($n=15$ total). Burst pressure testing was then performed on the same graft by pumping deionized water into the graft at 100 mL/min until the electrospun mesh failed ($n=3$).^[40] The maximum pressure upon failure was measured and recorded using a NoShok high pressure gauge ranging from 0 to 60 psi (Berea, OH), which was then converted to mmHg. Both burst pressure and compliance were performed under standard laboratory conditions (25°C) and simulated physiological conditions (37°C, high relative humidity). Prior to testing at simulated physiological conditions, grafts were equilibrated in water at 37°C overnight.

2.3.4. Confirmation of Silicon Network Formation—Nuclear magnetic resonance (NMR) spectroscopy was utilized to determine the extent of silicone network formation in the semi-IPNs after heat treatment. Briefly, as-spun and heat-treated grafts were dissolved in deuterated chloroform (CDCl_3) at a concentration of 10 mg/mL. Solutions were allowed to settle in order to identify gel fractions, if any, formed by the crosslinked network. Small 1 mL samples were then extracted from the solute fraction and analyzed with ^1H NMR (300 MHz, CDCl_3): PDMS DMA 380 δ 0.08 (s, 3H, -CH₃), 0.52–0.57 (m, 2H, -CH₂-), 1.62–1.73 (m, 2H, -CH₂-), 1.95 (s, 3H, -CH₃), 4.07–4.12 (m, 2H, -CH₂-), 5.54 (s, 1H, =CH₂), 6.10 (s, 1H, =CH₂), PDMS DA 600 δ 0.03–0.12 (m, 3H, -CH₃), 0.47–0.58 (m, 2H, -CH₂-), 1.52–1.68 (m, 2H, -CH₂-), 3.39–3.55 (m, 2H, -CH₂-), 3.85–3.90 (m, 1H, -CH-), 4.18–4.29 (m, 2H, -CH₂-), 5.80–5.97 (m, 1H, =CH₂), 6.07–6.22 (m, 1H, -CH), 6.38–6.53 (m, 1H, =CH₂). Following initial characterization, changes in spectral peaks of the semi-IPNs characteristic only of the silicone component were monitored for potential crosslinking of the PDMS macromer.

2.4. Statistical Analysis

A student's T-test was performed to determine statistical significance between groups of data. All comparisons were made with a 95% confidence interval. ($\alpha=0.05$)

3. Results and Discussion

3.1. Initial Graft Characterization

Synthetic vascular grafts were fabricated by electrospinning 18 wt% solutions of Carbothane[®] dissolved in DMAc or varied blends of Carbothane[®] and up to 20% silicone macromer DMA 380 or DA 600 dissolved in 3:1 DMAc:CHCl₃. All solutions were electrospun for approximately 2–3 hours to produce grafts of approximately 0.2 mm thickness. Similar fiber morphologies of each electrospun fiber mesh were then confirmed with SEM to isolate material composition changes from architectural effects. Following

morphology comparison, all 0.2 mm thick electrospun PCU/PDMS blends were subjected to a 12 hour 50°C heat treatment to facilitate silicone network formation for the overall semi-IPN. Electrospun PCU grafts were also subject to the same heating cycle to observe the effects of heat treatment on PCU grafts.

All as-spun fiber meshes had similar fiber morphologies in both PCU and all PCU/PDMS compositions (Figure 2) which indicates the morphology was unaffected by PDMS macromer content. Heat treatment of all grafts produced fiber junctions with higher percent fusion within the mesh than their as-spun counterparts. The increase in fusion at fiber junctions was attributed to the softening of the fibers during the 12 hours at 50°C. These decreases in fiber interbond arc length^[41] has been cited as a direct source of increases in modulus and ultimate elongation in the circumferential direction of a tubular graft.^[41–43] Furthermore, this softening was enhanced with higher concentrations of the PDMS DMA 380 macromer and to a greater extent in the 10/90 DA 600 system. Given our recent report on the effect of increased fusion on graft biomechanical properties, compliance was expected to decrease and burst pressure was expected to increase with this change in fiber architecture.^[26]

3.2. Graft Biomechanical Properties

Compliance and burst pressure measurements were conducted on 0.2 mm thick electrospun Carbothane[®] grafts and semi-IPN grafts with varied levels of PDMS DMA 380 or PDMS DA 600 macromer. Prior to heat treatment to crosslink the silicone macromers, compliance of as-spun grafts increased and burst pressure decreased with the presence of the silicone macromer (Table 2). This was attributed to the low glass transition temperature (T_g) of the silicone macromer^[44] and the disruption of soft segment crystallinity and hydrogen bonding in the polyurethane.^[36] An exception to this trend was observed in the 10/90 PDMS DA 600 grafts which exhibited an increase in compliance and an increase in burst pressure over as-spun Carbothane[®] grafts. This unexpected phenomenon was attributed to premature initiation of PDMS DA 600 crosslinking during the electrospinning process. A low level of crosslinking prior to heat treatment was confirmed by the loss of the acrylate peaks in the NMR spectra of the as-spun graft as compared to the polymer solution. This effect was likely not observed by the methacrylate components due to the lower reactivity of methacrylate groups compared to acrylates,^[45, 46] which limited the possibility of radical initiation in the PDMS DMA grafts. A 12 hour heating cycle at 50°C was then applied to grafts to covalently crosslink the unreacted silicone macromers. Carbothane[®] grafts were subjected to the same treatment to monitor changes to the polyurethane component of the semi-IPN meshes.

3.2.1. Effect of Heat Treatment on Carbothane[®] Grafts—Interestingly, both compliance and burst pressure of Carbothane[®] grafts increased following heat treatment. Compliance increased from 3.8 ± 1.0 to 4.7 ± 0.8 %/mmHg $\times 10^{-4}$ and burst pressure increased from 1190 ± 100 to 1470 ± 70 mm Hg. Changes in chemical structure, fiber architecture, and polyurethane microphase morphology after heat treatment of the Carbothane[®] grafts were investigated to determine the cause of the observed improvements in biomechanical properties. No changes in structure were observed in proton NMR spectra

and SEM analysis of the fiber morphology displayed minimal effects of heat treatment (Figure 2). Microphase morphology before and after heat treatment was then examined using DMA. The storage modulus plot of the as-spun Carbothane[®] exhibited a broad T_g of the polycarbonate soft segment at approximately -9°C and an additional melting transition temperature (T_m) at 39°C , Figure 3a. In comparison to the storage modulus plot of a cast Carbothane[®] film, the electrospun graft displayed a much broader T_g with a strong melting peak at a lower melting temperature (39°C vs 63°C). Eceiza et al. reported a similar transition at 45°C in the storage modulus plot of a poly(carbonate urethane) and assigned it to melting of crystalline soft domains.^[47] Therefore, it was hypothesized that alignment of soft segment chains during electrospinning enhanced crystallization and limited phase separation, as evidenced by the increased breadth of the T_g . Similar behavior was observed in electrospun polycaprolactone-based polyurethanes as compared to the cast film.^[48]

Following heat treatment, a reduction in soft segment crystallization ($H = 18 \pm 2$ vs $H = 12 \pm 3$) in the Carbothane[®] DSC thermogram (Figure 4) was accompanied by reduced breadth of the soft segment T_g in the storage modulus plot indicative of improved phase separation (Figure 3A).^[49, 50] Infrared spectroscopy has previously been used to assess phase separation from an analysis of the extent of hard segment hydrogen bonding.^[51] However, this assessment was inconclusive in the PCU given the convolution of the urethane carbonyl region with the carbonyls of the polycarbonate-based soft segment. Nevertheless, the DSC and DMA analyses provides strong evidence that the improvement in biomechanical properties of the Carbothane[®] grafts was due to reduced soft segment crystallinity and enhanced microphase separation after heat treatment. Specifically, reduced crystallinity was expected to enhance the flexibility of the soft segment matrix and reduce modulus which has been linked to compliance.^[26] The enhanced burst pressure was attributed to the increase in phase separation and order of the hard domains. Similar annealing effects have resulted in increased polyurethane tensile strength^[52] which has been correlated with increased burst pressure.^[26]

3.2.2. Effect of Heat Treatment on Semi-IPN Grafts—The effect of heat treatment on the compliance and burst pressure of semi-IPN grafts varied based on the macromer chemistry and macromer concentration. Biomechanical properties of 5/95 PDMS DMA 380 grafts were unchanged after heat treatment. The enhanced phase separation observed in the Carbothane[®] control grafts upon heat treatment was not observed, Figure 3B. In contrast, heat treatment of the 10/90 PDMS DMA 380 graft resulted in a substantial increase in compliance beyond previously reported saphenous vein values^[11] but no effect on burst pressure. Finally, the 20/80 PDMS DMA 380 graft exhibited a small increase in burst pressure and a substantial decrease in compliance. The observed differences in biomechanical properties as a function of macromer content and chemistry were hypothesized to be a result of differences in fiber architecture, silicone network formation and polyurethane phase morphology. Fiber architecture changes were assessed with SEM, as described above, with particular attention given to the extent of fiber fusion. Silicone network formation was assessed using proton NMR of the silicone sol fraction present in semi-IPNs after heat treatment (supplemental material). Finally, the effect of silicone

macromer on phase morphology and soft segment crystallinity was assessed using DMA and DSC, respectively.

An increase in fiber fusion was observed with increasing DMA 380 concentration, Figure 2. We have previously demonstrated that increasing fusion can result in enhanced burst pressure and decreased compliance. Although not statistically significant, there is an overall trend of increasing burst pressure with increasing concentration of the DMA 380, Table 2. There was little improvement observed in burst pressure with heat treatment for any of the DMA 380 semi-IPN grafts. The degree of crosslinking assessed by the silicone present in the NMR spectra of the sol fraction was low for both the 5/95 and 10/90 semi-IPNs. It was hypothesized that this low level of crosslinking was insufficient for mechanical reinforcement. The 20/80 PDMS DMA 380 graft did exhibit more crosslinking; however, the network formation remained insufficient to cause a significant increase in burst pressure. In contrast, minimal silicone in the sol fraction of the heat treated 10/90 600 DA semi-IPN graft indicated excellent crosslinking and a corollary increase in burst pressure. The improved network formation and burst pressure of the DA 600 semi-IPN graft is likely due to the increased reactivity of the acrylate and higher molecular weight of the silicone macromer. Following heat treatment, the 10/90 DA 600 semi-IPN graft also exhibited the lowest storage modulus drop of all the thin grafts (Figure 3c) as well as most significant microphase separation and highest ordered soft segment crystallinity in its DSC thermogram (Figure 4). Finally, the degree of fiber fusion of the 10/90 PDMS DA 600 grafts following heat treatment appeared to be more similar to Carbothane[®] which had minimal effects on biomechanical properties (Figure 2). As a result, the graft exhibited improved biomechanical properties as compared to its as-spun counterpart and the Carbothane[®] control. Overall, the increased compliance of the semi-IPN grafts was attributed to the silicone macromer disrupting soft segment crystallinity of the polyurethane, as described above. Indeed, a substantial decrease in soft segment crystallinity was observed for all semi-IPN grafts (Figure 4).

3.2.3. Effect of Thickness on Carbothane[®] Grafts—In order to increase the burst pressure of the Carbothane[®] grafts to match saphenous vein values, the graft thickness was increased from 0.2 to 0.4 mm. Our laboratory has previously demonstrated that increasing graft thickness via longer electrospinning times can be effectively used to enhance burst pressure.^[22] Increased thickness of the Carbothane[®] grafts resulted in a modest increase in burst pressure from 1190 ± 100 to 1330 ± 70 mm Hg and decrease in compliance from 3.8 ± 1.0 to 3.2 ± 0.1 %/mmHg $\times 10^{-4}$. Initial 12 hour heat treatment of 0.4 mm thick Carbothane[®] specimens produced no changes in graft properties or DMA plots (results not shown) indicating longer heat treatment times were needed. Increasing the duration of heat treatment to 24 hours increased the compliance (5.1 ± 0.5 %/mmHg $\times 10^{-4}$) and burst pressure (2260 ± 160 mm Hg) of Carbothane[®] grafts to values that exceeded reported autologous vein properties (saphenous vein compliance: 4.4 ± 0.8 %/mmHg $\times 10^{-4}$ ^[53] and burst pressure: 1680 ± 307 mmHg^[54]). Additional heat treatment times (48 and 72 hours) did not result in additional improvements in graft biomechanical properties (data not shown).

3.2.4. Graft Performance under Physiological Conditions—The DMA storage modulus plots of the grafts displayed thermal transitions between 30°C and 40°C. Therefore, it is reasonable to assume that the performance of the grafts under standard testing temperature of 25°C would differ from physiological conditions (37°C). To address this issue, the biomechanical properties of the most promising grafts, Carbothane® (0.4 mm thickness) and 10/90 PDMS DA 600 (0.2 mm thickness), were equilibrated in water at 37°C overnight and then tested in a warm room that was held at a constant 37°C and 100% relative humidity. The results were then compared with previous results obtained under standard laboratory conditions (20°C, ~50% humidity).

As-spun grafts of both materials were found to exhibit substantial increases in compliance (Figure 5) after equilibration in water and testing at 37°C. The increase in compliance was attributed to partial melting of the crystalline soft segment, as described above with heat treatment. Interestingly, this almost doubling of compliance at 37°C was not accompanied by a significant change in burst pressure as observed with grafts after heat treatment. Both sets of heat-treated grafts displayed large decreases in burst pressure at physiological conditions with more modest increases in compliance. The large increase in burst pressure of heat-treated grafts observed at 20°C (Carbothane®: 2260 vs 1330 mm Hg; semi-IPN: 2360 vs 2070 mm Hg) is likely due to a combination of polymer morphology and increased fusions. Given that testing under physiological conditions did not significantly alter fiber architecture, the decrease in heat-treated graft burst pressures at 37°C was attributed to changes in polymer morphology. Specifically, we hypothesized that a further reduction of the soft segment crystallinity ($T_m = 36 \pm 1^\circ\text{C}$) occurred during incubation at 37° which caused the observed decrease in graft burst pressures. The less substantial decrease in the semi-IPN could then be attributed to the added reinforcement of the silicone network and the increased melting temperature of the soft segment in the semi-IPN ($45 \pm 1, 56 \pm 2^\circ\text{C}$). Overall, the improved compliance of both grafts under the more physiological conditions while maintaining acceptable burst pressures suggests favorable performance during implantation may be expected. Based on the established correlation with compliance and 5 year patency rates from previous in vivo studies, these Carbothane® and 10/90 PDMS DMA 380 grafts are expected to exceed previous graft options (Figure 6).^[11]

4. Conclusions

Carbothane® electrospun grafts displayed morphological changes after heat treatment that improved graft compliance and burst pressure values beyond previously reported autologous vein properties. The range of biomechanical properties of the PCU grafts was then expanded with the incorporation of a silicone network. Biomechanical properties of the most promising grafts were then assessed under physiological conditions. Although the graft burst pressure dropped significantly due to disruption of soft segment crystallization, the values remained comparable to autologous veins. Compliance values for both grafts showed additional improvements at 37°C. Given the known correlation of graft compliance and patency, the improved compliance of both the heat-treated Carbothane® and the 10/90 PDMS semi-IPN grafts provide promising alternatives to current synthetic grafts and autologous veins as arterial replacements. We are currently utilizing these grafts as the outer

layer of a multilayer design with the luminal layer composed of a thromboresistant and bioactive hydrogel.^[55]

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Figure 1.
Chemical structures of PDMS macromers

Figure 2. Scanning electron micrographs of 0.2 mm thick electrospun grafts comparing fiber morphology before and after heat treatment (50°C, 12 hours)

Figure 3.

DMA storage modulus plots of 0.2 mm thick electrospun grafts comparing effects of (A) heat treatment (12 hours at 50 $^{\circ}\text{C}$) on Carbothane $^{\circ}$, (B) PDMS macromer content on heat treated specimens, and (C) PDMS macromer M_w on heat treated specimens

Figure 4.

DSC thermograms of 0.2 mm thick electrospun Carbothane® and heat treated PCU/PDMS macromer blends (12 hours, 50°C)

Figure 5.

(A) Compliance and (B) burst pressures of 0.4 mm Carbothane® and 0.2 mm 10/90 PDMS DA 600 grafts testing at 20°C (room temperature) and 37°C (body temperature); †data taken from *in vivo* saphenous vein data from literature; *p<0.05 from 20°C.

Figure 6.

Projected patency rates of heat treated 0.4 mm thick Carbothane® and 0.2 mm 10/90 PDMS DA600 semi-IPN grafts based on linear relationship observed in previous *in vivo* data as reported by Salacinski et al.[1] The patency data for the different grafting materials used in the femoro-popliteal position used in this correlation were obtained from several recent large studies. Figure adapted with permission from “The Mechanical Behavior of Vascular Grafts: A Review,” by HJ Salacinski, S Goldner, A Giudiceandrea, G Hamilton, AM Seifalian, A Edwards, and RJ Carson, *Journal of Biomaterials Applications* 2001, 15(3), 241–278. Copyright Sage Journals.

Table 1

Electrospun semi-IPN compositions

	Percentage Carbothane® 3575A	Percentage PDMS Macromer	Solvent	BPO Initiator Content
PCU	100%	0%	DMAc	0%
5/95 DMA 380	95%	5% DMA 380	3:1 DMAc:CHCl ₃	1%
10/90 DMA 380	90%	10% DMA 380	3:1 DMAc:CHCl ₃	1%
20/80 DMA 380	80%	20% DMA 380	3:1 DMAc:CHCl ₃	1%
10/90 DA 600	90%	10% DA 600	3:1 DMAc:CHCl ₃	1%

(DMAc = N,N'-dimethylacetamide, CHCl₃ = Chloroform, BPO = Benzoyl Peroxide)

Table 2

Biomechanical properties of as spun and heat treated electrospun grafts.

		Carbothane®	5/95 DMA 380	10/90 DMA 380	20/80 DMA 380	10/90 DA600
As Spun	Compliance [%/mmHg x 10 ⁻⁴]	4.2 ± 1.1	7.2 ± 1.3	4.6 ± 0.6	5.5 ± 1.4	5.1 ± 0.4
	Burst Pressure [mm Hg]	1190 ± 100	1100 ± 40	990 ± 220	900 ± 130	2070 ± 20
Heat Treated	Compliance [%/mmHg x 10 ⁻⁴]	5.1 ± 0.9	6.5 ± 0.8	8.0 ± 0.9 ^{a)}	4.0 ± 0.6 ^{a)}	7.0 ± 2.1 ^{a)}
	Burst Pressure [mm Hg]	1470 ± 70 ^{a)}	1250 ± 150	910 ± 100	1040 ± 140	2320 ± 150 ^{a)}

average ± standard deviation; n=15 for compliance, n=3 for burst pressure; graft thickness = 0.2 mm, 50°C, 12 hours,

^{a)} p<0.05 from as-spun

Table 3

Comparison of biomechanics properties of 0.4 mm thick Carbothane[®] grafts and 0.2 mm thick 10/90 PDMS DA 600 semi-IPN grafts tested at room (20°C) and physiological temperature (37°C)

	As Spun		Heat Treated	
	20°C	37°C	20°C	37°C
Carbothane [®]	Compliance [%/mmHg x 10 ⁻⁴]	3.8 ± 0.3	6.7 ± 1.5 ^{a)}	7.2 ± 1.4 ^{a)}
	Burst Pressure [mm Hg]	1330 ± 70	2260 ± 160	1530 ± 140 ^{a)}
10/90 PDMS DA 600	Compliance [%/mmHg x 10 ⁻⁴]	5.1 ± 0.4	9.2 ± 2.1 ^{a)}	9.2 ± 2.7 ^{a)}
	Burst Pressure [mm Hg]	2070 ± 20	2320 ± 150	1780 ± 230 ^{a)}

average ± standard deviation; n=15 for compliance, n=3 for burst pressure, 50°C treatment,

* p<0.05 from 20°C