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Electrospun Polyglycolic Acid-Poly(Carbonate-Urea) Urethane Scaffold as a Hybrid Tissue Engineered Vessel

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Abstract

Alternative small diameter vascular grafts are needed for patients that require surgical revascularization in patients lacking autologous vein. In this study, a Polyglycolic Acid (PGA)-Thermoplastic Polyurethane (TPU) electrospun scaffold seeded with human dermal fibroblasts was placed in a biomimetic perfusion system to generate a hybrid tissue engineered vessel. The outer layer was an electrospun PGA that was co-electrosprayed with sacrificial polyethylene oxide (PEO) microparticles to increase porosity. The PGA-TPU scaffold remained static for 1 week, then circumferential strain amplitude was incremented from 1% to 5% over 6 weeks. The hybrid tissue engineered vessel had an outer cellular layer with collagen deposition replacing the biodegradable PGA and the inner residual polyurethane layer remained relatively acellular. The tensile properties of the hybrid tissue engineered vessel demonstrated a significant reduction in the elastic modulus compared to the PGA-TPU scaffold, but the ultimate tensile strength, extension to break, and burst pressure remained stable. Fourier-transform infrared spectroscopy confirmed the degradation of the PGA and a reduction of polyurethane crosslinking in the hybrid TEV compared to the PGA-TPU. Thus, a biomimetic perfusion system can be used to evaluate the biocompatibility of an electrospun polyurethane scaffold in vitro, to understand the mechanical changes of the polyurethane scaffold after exposure to circumferential stretch, and to generate a hybrid tissue engineered vessel with suitable characteristics for implantation.

Keywords: Electrospun • Polyurethane • Tissue engineering • Vascular graft

Introduction

An ideal small diameter vascular graft (<6 mm) substitute continues to be a major challenge for the treatment of patients with advanced cardiovascular disease in need of a bypass procedure. The conduit of choice for small diameter arterial bypass grafts is autologous vein, although up to 20%-30% of patients do not have an adequate saphenous vein because of small size, varicosities, or prior harvest. The currently available alternative grafts, expanded Polytetrafluoroethylene (ePTFE) or cadaveric vein, have poor outcomes due to early graft failure from thrombosis or anastomotic intimal hyperplasia. Tissue engineering has the potential to create a more biologically compatible graft that has biomechanical properties more similar to a native artery and promote tissue ingrowth with remodeling. Over the past 2 decades, there has been exciting progress on the development of tissue engineered vascular grafts into human clinical trials. An alternative fabrication method to obtain a three-dimensional fibrous scaffold is electrospinning. Electrospinning is attractive because it is a versatile technique that can generate a nanofiber or microfiber scaffold to mimic native extracellular matrix. The main advantages of electrospinning are the fine-tuning capability to control fiber properties such as to adjust electrospinning parameters, to use a wide variety of synthetic or natural proteins, and to modify the structure while electrospinning with adjunctive methods (i.e. increase porosity with a porogen). Electrospun scaffolds have been gaining interest for use as a vascular graft, and have been evaluated in animal models as a bypass conduit [1]. Polycaprolactone is the synthetic polymer of choice for electrospun vascular scaffolds because of the long-term degradation and desirable mechanical properties as an electrospun scaffold. Polycaprolactone has also been electrospun with a variety of extracellular matrix proteins, most often collagen, to enhance biocompatibility. Electrospun scaffold composed of PCL or PCL with collagen have been implanted in animal models as a vascular graft with primarily a strategy of direct implantation or a brief preconditioning of the lumen with endothelial cells. Most animal studies report positive results on graft patency and cell ingrowth with remodeling, but there are concerns of a chronic inflammatory response with polycaprolactone.

Thermoplastic Polyurethanes (TPU) have been clinically used as vascular grafts, and are increasingly being investigated as a biomaterial for electrospun scaffolds. Thermoplastic polyurethanes

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are a very appealing polymer because the block chemistry composed of hard and soft segments provides the ability to tailor the polyurethanes biocompatibility, mechanical properties, and degradation rates. Polyurethane electrospun scaffolds are a promising biomaterial because of their high elastic properties and biocompatibility. Despite these favorable properties, conventionally fabricated polyurethane vascular grafts are susceptible to hydrolytic and enzymatic degradation that leads to a loss of biostability. Electrospun polyurethane vascular grafts have been shown to maintain biostability in vivo and support cell migration with extracellular deposition. The aim of the study was to evaluate an electrospun hybrid scaffold of PGA-TPU in a bioreactor to create a hybrid tissue engineered vessel. We hypothesized that a bi-layer electrospun scaffold with an outer PGA layer and an inner TPU layer would be biocompatible and have favorable biomechanical properties as a vascular graft. The PGA-TPU scaffolds were seeded with fibroblasts and cultured in a biomimetic system with circumferential stretch over 7 weeks. The PGA-TPU scaffolds and tissue engineered vessels with residual polyurethane were characterized by scanning electron microscopy, histology of the hybrid tissue engineered vessel, biomechanical properties, and chemical analysis [2].

Materials and Methods

Scaffold fabrication

The Polyglycolic Acid-Polyurethane (PGA-TPU) scaffold was fabricated using a custom electrospinning set-up with blunt needles at 180-degree geometry (for the PEO). PGA (Teleflex, Wayne, PA) polymer was dissolved in 1,1,1,3,3,3-fluoro-2-propanol (HFIP) (Oakwood Chemical, Estill, SC) to form a concentration of 14% weight per volume (w/v). Two aliphatic polycarbonate-based thermoplastic polyurethanes (TPU) were used for the study: Carbothane™ PC3595A (Lubrizol, Wilmington, MA) and Carbothane™ PC3572D (Lubrizol) were separately dissolved in HFIP to form a concentration of 12% w/v. Polyethylene oxide (PEO, Mn=8000, Sigma-Aldrich, St. Louis, MO) was dissolved in chloroform to form a concentration of 120% w/v. The polymers were electrospun in layers while co-electrospraying PEO, with the TPU on the inner surface (50 minutes) and an outer layer of (40 minutes). The PGA or TPU solution was injected through a 15-gauge blunt tipped needle by a syringe pump (Infusion Syringe Pump, Richmond, CA) at a constant rate of 2.5 ml/hr. The PEO solution was injected through 15-gauge blunt tipped needle by a syringe pump at a constant rate of 2 ml/hr. A positive high voltage power supply (ES30P-5W, Gamma High Voltage Research Inc, Ormand Beach, FL) of 16 kilo-Volts (kV) was applied to the blunt tipped needles, and a negative 1 kV was attached to the mandrel. The distance from the mandrel to the tip of the needles was 14 cm for the PGA, 16 cm for the TPU, and 16 cm for the PEO. The mandrel diameter was 4.5 mm and rotated at 250 rotations per minute. The PEO was removed from the scaffolds by rinsing three times for 20 minutes in deionized water, and then air dried for 2 days.

Cell culture

Human dermal fibroblasts (hDF, Lonza, Walkersville, MD) were maintained in Dulbecco's Modified Eagle Medium (ThermoFisher Scientific, Waltham, MA) supplemented with 5% fetal bovine serum (ThermoFisher), 100 U/mL penicillin (Sigma). The cells were incubated at 37°C and 5% CO_2. The media was changed every 3 days, and cell were passaged when confluent and harvested for use at passage.

Polyurethane hybrid tissue engineered vessel in bioreactor system

The electrospun PGA-TPU scaffold was placed over a 4.5 mm silicone tube (Saint Gobain, Malvern, PA). The ends of the PGA scaffold were sutured to a dacron cuff (LeMaitre, Burlington, MA) using 4-0 prolene suture (Ethicon, Cincinnati, OH). The 8 cm electrospun PGA/TPU scaffold was threaded through the side-arms of a glass bioreactor and attached to connectors on the external side-arms of the bioreactor to keep the silicone tubing and scaffold taut. The bioreactor and the scaffold were disinfected by placing into a 100% ethanol solution for 30 minutes, and allowed to dry overnight with additional UV radiation treatment for 10 minutes. The electrospun scaffolds were seeded with 10 million human dermal fibroblasts at passage 5 suspended in 1 mL of media. The culture media for the bioreactor consisted of DMEM (ThermoFisher) supplemented by 15% FBS (ThermoFisher), 100 U/mL of penicillin (Sigma), MEM non-essential amino acid (Sigma), 5 µg/mL Insulin (Sigma), 50 µg/mL ascorbic acid (Sigma), and 10 ng/mL fibroblast growth factor. The scaffolds remained static for 7 days, then the bioreactor was connected on one external side-arm to tubing with sterile water passing through the bioreactor to create a hydraulic system and capped on the other external side-arm. The tubing was passed through a sterile filter, then attached to a solenoid valve (Automation Direct, Atlanta, GA) and the amount of compressed air was controlled by a pressure regulator (Automation Direct). The frequency of the solenoid valve was 0.67 Hz with 15% duty cycle. The strain amplitude was increased from 1% to 2.5% to 5% every 2 weeks over the six-week culture period with stretch. One-half the bioreactor media was changed every 4 days [3].

Characterization of the electrospun scaffold

The electrospun PGA-TPU scaffold was prepared for evaluation by Scanning Electron Microscopy (SEM) on the inner and outer surface of the PGA-TPU scaffold and the inner surface of the Tissue Engineered Vessel with Polyurethane (TEV-TPU). The samples were sputter-coated with gold for 1 minute. The images were acquired using a SEM (Hitachi S-2700). The SEM images were analyzed with Image J to determine the average fiber diameter and pore size of the outer (PGA) and inner (TPU) surfaces. Ten random fibers or pores per image were measured on four separate images to calculate the mean and the standard error of the mean.

Histology of the hybrid tissue engineered vessel

Hybrid tissue engineered vessels were harvested from the bioreactor and fixed in 10% neutral buffered formalin (Sigma) for 4 hours, then embedded in optimal cutting temperature compound (Finetek, Torrance, CA) by placing into 2-butanol bath in liquid nitrogen. Sections of 8-µm thickness were stained with hematoxylin and eosin (Vector, Burlingame, CA) and Masson's Trichrome (Polyscience, Warrington, PA). The histological images were obtained with an Olympus BX51 microscope. The outer cell layer and

cell infiltration along with the residual TPU thickness were measured by evaluating four samples with six measurements per sample.

Mechanical testing of the PGA-TPU and the hybrid tissue engineered vessel

Uniaxial tensile testing was performed on the PGA-TPU scaffolds and the TEV-TPU. A ringlet of the scaffold or engineered vessel had stainless steel hooks placed through the vessel that were secured by compressive grips. The length and the width were measured using digital calipers, and the thickness was obtained from the histology frozen cross-sections. An Instron 3342 system was used with a 100 N load cell. The ringlets were extended at a rate of 0.5 mm/second until failure. Strain was calculated based on the change in length of the scaffold or tissue divided by the initial length of the tissue. The stress was calculated as force divided by the initial cross-sectional area (multiplied by 2 to account for the two sides of the engineered vessel). The ultimate tensile strength was the tensile strength at failure (n=4 in duplicates). The elastic modulus was determined as the slope of the linear region of the stress-strain curve. (n=4 in duplicates). Burst pressure was obtained by placing the PGA-TPU scaffold or TEV-TPU over a 12 mm non-compliant Bard Atlas® angioplasty dilation catheter (Bard, Tempe, AZ). The balloon pressure was monitored using a high-pressure gauge (0-60 PSI, Automation Direct) connected to a manual insufflator. Burst pressure was recorded as the maximum pressure at the PGA-TPU electrospun scaffold or TPU-TEV failure (n=4).

Results

Electrospun scaffold characterization

The PGA-TPU scaffold was fabricated in layers by electrospinning an inner layer of the TPU with a transition to the PGA while simultaneously electrospraying sacrificial PEO microspheres as shown in Figure 1A. The electrospun scaffold was transferred to the bioreactor (Figure 1B) to generate a hybrid tissue engineered vessel. The sacrificial PEO microspheres were incorporated into the inner and outer electrospun layers to increase the pore size and porosity of the PGA scaffold.

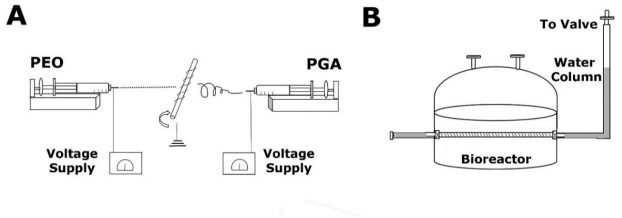

Figure 1: Schematic of the electrospinning set-up and the biomimetic tissue culture system. A) Electrospinning set-up with simultaneous electrospraying of PEO microparticles while electrospinning PGA or TPU; B) Biomimetic system with electrospun scaffold over silicone tubing filled with sterile water exiting the bioreactor side-arm to a fluid filled column of tubing and an air interface with a sterile filter leading to a solenoid valve connected to a pressure regulator.

The inner layer composed of the TPU Carbothane™ PC3595A or Carbothane™ PC3572D are shown in Figure 2 as the scaffold (2A) and as the hybrid tissue engineered vessel (2B). The morphology of the TPU fibers in Figure 2 are randomly oriented, and were identical after culture in the bioreactor. The fiber diameter of the inner TPU layer was similar for both types of TPU as the PGA-TPU (3595 1.52± 0.05 μm and 3572: 1.78 ± 0.07 μm) scaffold compared to the hybrid TEV-TPU (3595: 1.57 ± 0.04 μm and 3572 1.65 ± 0.06 μm). The outer PGA layer of the PGA-TPU scaffold had a fiber diameter of 1.37 ± 0.03 μm. The pore area of the PGA-TPU scaffold had a significantly greater pore area for the PGA (51.9 ± 1.4 μm2) compared to either of the polyurethanes (3595: 33.3 ± 1.4 μm2 and 3572: 32.1 ± 1.5 μm2). The scaffold thickness after removal of the PEO microparticles was 400-500 μm for PGA-TPU 3595 and 500-600 μm for PGA-TPU 3572 scaffolds.

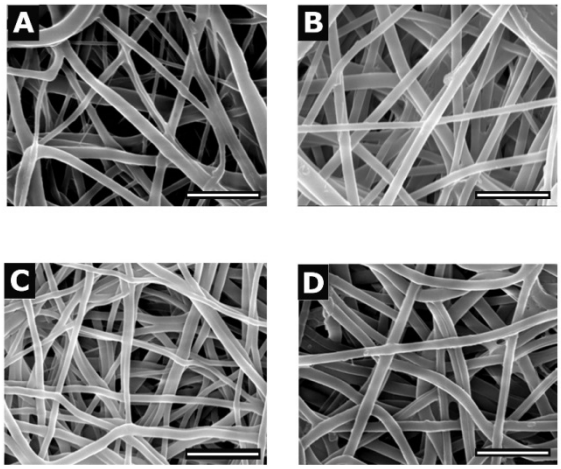

Figure 2: Scanning electron microscopy (SEM) of polyurethane fibers. The inner layer of the Carbothane™ PC3595A of the electrospun scaffold; A) and the hybrid tissue engineered vessel; B) The inner layer of the Carbothane™ PC3572D of the electrospun scaffold; C) and the hybrid tissue engineered vessel; D) from the biomimetic system. The inner Carbothane™ electrospun fibers and pore sizes were similar in the scaffold compared to the hybrid tissue engineered vessel. Scale bar =10 μm.

Hybrid tissue engineered vessel morphology

The histology of the hybrid human tissue engineered vessel with the residual polyurethane in the bioreactor was shown in Figure 3. The H and E stain showed the outer PGA layer mostly degraded fibers with a cellular layer while the inner TPU layer for both of the polyurethanes had minimal cellular infiltration (Figure 3). The scaffold was well integrated, and the two layers were fused together without evidence of separation. The outer cellular layer demonstrated scaffold remodeling with the formation of a denser layer of collagen on both TEV-TPU, and some collagen deposition into the scaffold (Figure 3B and 3D). The cell infiltration for each hybrid TEV-TPU was the following distance: Carbothane™ PC3595A ranged from 45 μm.

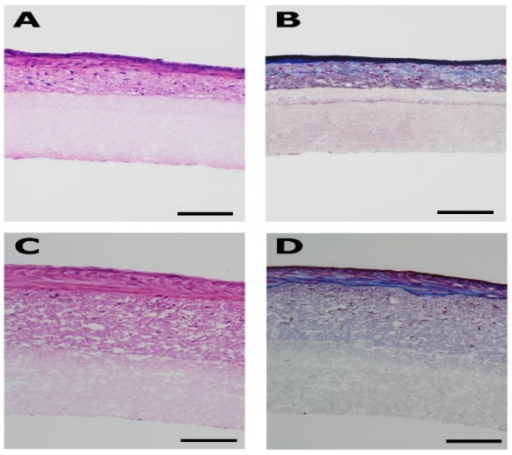

Figure 3: Hybrid Tissue Engineered Vessel with Carbothane™ PC3595A; A) Hematoxylin and Eosin; B) Masson's Trichrome stain. Hybrid TEV with Carbothane™ PC3572D; C) Hematoxylin and Eosin; D) Masson's Trichrome stain. There is an outer cellular layer with collagen formation and cell infiltration into the residual TPU. Scale bar =100 μm.

Mechanical properties of the electrospun scaffold and hybrid tissue engineered vessel

The tensile properties of the PGA-TPU scaffold and TEV-TPU were shown in the representative stress-strain curves in Figure 4. The stress-strain curves of the PGA-TPU were typical for a composite material with a more rigid phase related to the PGA and a more elastic phase from the TPU, and after formation of the TEV-TPU the curve was dependent on the polyurethane mechanics. The burst pressure was similar for both types of TPU (Carbothane™ PC3595A and Carbothane™ PC3572D) was similar for the electrospun PGA-TPU scaffold and the TEV-TPU. The burst pressure was obtained over a non-compliant angioplasty balloon because of the permeability of the scaffold at higher pressures. The diameter of the PGA-TPU and the TEV-TPU was observed to stretch with some shortening of the construct as the pressure was increased.

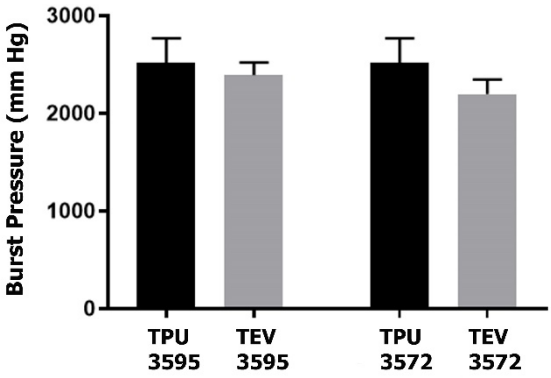

Figure 4: Burst Pressure of the of the electrospun PGA-TPU Carbothane™ PC3595A compared to the hybrid TEV; burst pressure of the PGA-TPU Carbothane™ PC3572D compared to the hybrid TEV-TPU.

Discussion

Tissue engineering is an emerging technology to address the need for a small diameter vascular graft with properties similar to a native artery. A tissue engineered vessel would harness the regenerative capacity of the patient to remodel the implanted graft to form a functional neoartery. In this study, we designed an in vitro method to develop a hybrid tissue engineered vessel by seeding dermal fibroblasts on a PGA-TPU electrospun scaffold in a biomimetic system. The hybrid tissue engineered vessel consisted of an outer cellular layer composed of extracellular matrix with a residual inner polyurethane layer. The combination of electrospinning and polyurethanes could be used to create a wide number of vascular grafts with modifiable features for both the polyurethane and electrospinning. Polyurethanes have been investigated as a biomaterial for vascular grafts because of the compliant nature of the polymer. A challenge with early generations of polyurethane-based vascular grafts was the unstable biodegradation and the potential for an inflammatory response of the degradation byproducts [4]. The first generation of Poly(ester urethanes) degraded from hydrolysis, and next generation Poly(ether urethanes) were susceptible to oxidation by free radicals from macrophages or foreign body giant cells. Poly(carbonate urethanes) have been shown to be less sensitive to oxidative degradation with better long-term biostability compared to poly(ester urethanes). Carbothane™ was selected as the polyurethane because of the non-toxic aliphatic isocyanate, the commercial availability, and the range of hardness of the polyurethane. Two Carbothane™ polyurethanes were evaluated in this study, PC3595A had a lower shore hardness compared to PC3572D. The shore hardness rating, measures the depth of indentation in the material with an applied force, is based on the properties of the resin and not on the electrospun scaffold. The ultrastructural properties of the two types of electrospun Carbothane™ scaffolds in this study were similar in terms of fiber diameter.

Electrospun polyurethane scaffolds have demonstrated biocompatibility as vascular grafts to support cell growth in vitro and in vivo. A potential limitation to the use of polyurethanes as an electrospun scaffold for a vascular graft is the lack of cell infiltration into the electrospun material and the hydrophobic nature of the polyurethane. The use of extracellular matrix proteins has been shown to increase cell attachment, and will degrade over time to increase porosity. An adjunctive approach to increasing porosity that can easily be adapted to a standard electrospinning technique is a sacrificial porogen such as a polyetheylene oxide microparticle. Sacrificial PEO microparticles have been shown to increase the porosity and enhance cell migration into an electrospun scaffold, and this technique was performed in this study. A hybrid tissue engineered vessel with a cell-based extracellular matrix and residual electrospun polyurethane scaffold was generated in a biomimetic perfusion system. In this study, circumferential stretch was transmitted to the electrospun scaffold through an inner silicone tubing with a hydraulic system. There has been description of solenoid valves with a pressure regulator to apply compressed air to stretch an inner latex tube, however, most tubing has some gas permeability. A hydraulic system is a more controlled system that prevents the unregulated direct force of the compressed air into contact with the scaffold. This unique biomimetic culture system

provided independent control of the frequency set by the programable solenoid valve and the circumferential stretch controlled by an air pressure regulator. In this biomimetic system, the frequency and duty cycle were maintained at same rate while the circumferential stretch was increased every two weeks from 1% to 2.5% then to 5% over the six-week culture period. The values of the circumferential stretch and frequency were based on previous studies to tissue engineer vessels in a bioreactor system to stimulate collagen deposition. The PGU-TPU scaffold supported dermal fibroblast growth on the outer layer of the PGA portion of the scaffold, but the fibroblasts did not appear to consistently migrate into the polyurethane layer. The lack of cell infiltration into the polyurethane layer was attributed to the smaller pore area of the polyurethane and the hydrophobic nature of the polyurethane. Although we observed the limited cell infiltration into the polyurethane electrospun scaffold *in vivo*, others have reported cell infiltration with small pore size (<10 μm) of an electrospun polyurethane in an in vivo animal model. The cell migration may be enhanced in a more complex in vivo environment with multiple cell types, including inflammatory cells that could break down with scaffold and promote cell infiltration with the release of cytokines [5].

Further studies are needed to optimize the structure of the electrospun scaffold, and the use of animal models to test the hybrid tissue engineered vessels with residual polyurethane in an animal model. In addition to the commercially available polyurethanes that have been used in biomedical applications, custom polyurethanes can be synthesized for specific mechanical and degradation characteristics. The structure of the electrospun scaffold could be varied from a bilayer approach with a polyurethane and a biodegradable polymer to a blended composition with polyurethane integrated throughout the electrospun scaffold. In addition to a hybrid tissue engineered vessel with cell-based extracellular matrix and residual electrospun polyurethane, natural polymers (collagen, fibrin, elastin, etc) could be electrospun into the scaffold to eliminate the need for a cell-based extracellular matrix. An animal model with an implantation into the arterial system is needed to test the in vivo response of the hybrid tissue engineered vessel with residual polyurethane. The rodent model is often used a first screening for a vascular graft, and can provide valuable data on the inflammatory response and remodeling.

Conclusion

A hybrid tissue engineered vessel with residual polyurethane has biocompatibility and mechanical properties for a new type of vascular graft. The electrospun scaffold can support human dermal fibroblast cell growth and deposition of a cell-based collage matrix with the structural support of polyurethane. Our findings suggest the biomimetic system can be used to generate hybrid tissue engineered vessels with residual polyurethane or used as a method to understand the mechanical changes of an electrospun polyurethane scaffold exposed to physiological conditions. The electrospun polyurethane scaffolds in combination with a biomimetic perfusion system may help to develop new hybrid tissue engineered vessels and provide insight for the transition to a cell-free vascular graft in the future.

Disclosure Statement

No conflict of interest or competing financial interests.

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