Published in final edited form as:

Mater Res Soc Symp Proc. 2015 ; 1718: .

Biohybrid Fibro-Porous Vascular Scaffolds: Effect of Crosslinking on Properties

Vinoy Thomas^{1,2}, Danna Nozik³, Harsh Patel¹, Raj K. Singh⁴, and Yogesh K. Vohra²

Vinoy Thomas: vthomas@uab.edu

¹Department of Materials Science & Engineering, University of Alabama at Birmingham (UAB), BEC 254, 1720 2nd Avenue South, Birmingham, AL 35294, U.S.A

²UAB Center for Nanoscale Materials and Biointegration, Birmingham, AL 35294, U.S.A

³Brown University, Providence, RI 02912, U.S.A

⁴Vivo Bioscience, Inc. Birmingham, AL 35205, U.S.A

Abstract

Tubular grafts were fabricated from blends of polycaprolactone (PCL) and poly(glycolide -co-caprolactone) (PGC) polymers and coated with an extracellular matrix containing collagens, laminin, and proteoglycans, but not growth factors (HuBiogel™). Multifunctional scaffolds from polymer blends and membrane proteins provide the necessary biomechanics and biological functions for tissue regeneration. Two crosslinking agents, a natural crosslinker namely genipin (Gp) and a carbodiimide reagent namely 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), were used for further stabilizing the protein matrix and the effect of crosslinking was evaluated for structural, morphological, mechanical properties using SEM, DSC and DMA. SEM images and fiber diameter distribution showed fiber-size between 0.2 μm to 1 μm with the majority of fiber diameters being under 500 nm, indicating upper range of protein fiber-sizes (for example, collagen fibers in extracellular matrix are in 50 to 500 nm diameter range). HB coating did not affect the mechanical properties, but increased its hydrophilicity of the graft. Overall data showed that PCL/PGC blends with 3:1 mass ratio exhibited mechanical properties comparable to those of human native arteries (tensile strength of 1–2 MPa and Young's modulus of <10 MPa). Additionally, the effect of crosslinking on coating stability was investigated to assure the retention of proteins on scaffold for effective cell-matrix interactions.

INTRODUCTION

Engineered small-diameter vascular grafts are in high demand as the majority of vascular disease cases involve small-caliber blood vessels [1]. While large-diameter vascular grafts have been successfully synthesized for clinical applications, small-diameter vascular grafts have been less successful due to high incidence of thrombus formation as well as intimal hyperplasia resulting from incompatibility between the mechanical properties of the graft

and native blood vessel [2]. Electrospinning is a facile technique for the fabrication of seamless tubular fibroporous scaffolds as electrospun scaffolds mimic the nano/micro morphological features of native extracellular matrix (ECM) [3, 4]. Scaffolds for vascular tissue engineering must have the bioactivity necessary for cell adhesion/growth and mechanical properties matching those of native blood vessels to withstand the pressure exerted due to blood flow. To this end, electrospun tubular scaffolds were fabricated from biocompatible PCL and fast-degrading PGC blends [5, 6] and coated with a physiological protein matrix, HuBiogel™. In a recently published paper we have shown that the PGC/PCL composition affected both the degradation and mechanical properties of tubular scaffolds such that a 3:1 PCL/PGC blend exhibited miscibility and co-continuous phase with optimized mechanical properties for vascular graft [5]. HuBiogel (HB) is a native matrix of collagens, laminin, and proteoglycan, derived from human amnions [7]. It is free of growth factors and proteases. Bioactive scaffolds can be designed by mixing tissue-specific growth factors. Its hydrogel property is beneficial for nutrient and metabolite exchange. Therefore, tubular grafts were coated with HB to create a biohybrid scaffold. The objective of this study was to evaluate the effect of crosslinking using carbodiimide (EDC) and a natural, crosslinker, genipin (Gp) [8–11], on the properties of the protein incorporated biohybrid graft.

EXPERIMENTAL

PCL and PGC (suture form as Monocryl Plus) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in a 3:1 (w/w) ratio to obtain a 12% (w/v) solution. Electrospun tubular scaffolds were fabricated by initially spinning a sacrificial layer of poly(vinyl alcohol) (PVA) onto a collector (a grounded cylindrical mandrel of 4 mm diameter rotating at 400 rpm) from a 10% (w/v) solution of PVA in water at a rate of 1 mL/h and an electric field strength of 1.5 kV/cm. The needle was driven along the length of the mandrel at a rate of 30 mm/s to guarantee that the polymer fibers would be deposited evenly [5]. After coating the mandrel with a thin layer of PVA, 2.5 mL of the PCL/PGC solution was spun onto it using the procedure described above. The scaffold with the mandrel was then sonicated in water bath for 2 h to dissolve the PVA layer, enabling the easy removal of the PCL/PGC scaffold. Tubular scaffolds (5 cm long) were immersed in a HuBiogel solution (1 mg/mL in the phosphate buffered saline, PBS) for 24 h and then kept at 37 °C for 2 h for gelation. Crosslinking of proteins using both Gp and EDC was carried out in solution-phase [6, 12]. Scaffolds were soaked in 200 mM solutions of Gp in pure ethanol or EDC in pure ethanol for 24 h. After crosslinking the mechanical, structural, and morphological characterization, as well as coating stability studies, were carried out for PCL/PGC, biohybrid (HB coated), and crosslinked scaffolds.

A uniaxial tensile test was performed (n=6) for PCL/PGC, biohybrid, and crosslinked scaffolds and stress-strain curves were plotted for each sample. SEM micrographs were captured for each scaffold to study fiber morphology. Samples were sputter coated with a gold/palladium alloy and micrographs were captured at an accelerating voltage of 5 kV. Micrographs were analyzed using ImageJ software to measure the diameter of the scaffold fibers (n=100) to plot the fiber diameter distribution. DSC scans from 0 °C to 250 °C at a rate of 10 °C/min were conducted to determine the melting temperature and enthalpy of

melting. Effect of crosslinking on the hydration was further studied by measuring PBS uptake by scaffolds from the mass difference between the initial mass (M_1) and mass after hydration in PBS (M_2) for 24h using equation (1).

$$\text{PBS uptake (wt-\%)} = \{(M_2 - M_1)/M_1\} \times 100 \quad (1)$$

The surface wettability of scaffolds was studied using static contact angle testing. Samples were mounted on glass slides in a Keyence VHX digital microscope and were wetted with a 50 μL drop of water. After 10 s, the (inner) angle between the scaffold surface and edge of the water droplet was measured. The stability of the HB coating in aqueous environments was studied by staining scaffolds with picosirius red (PSR) and aging them in PBS [12]. Finally, human umbilical vein endothelial cells (HUVECs) were cultured (6×10^5 cells/ cm^2) onto the scaffolds to study the effect of coating on cell-scaffold interaction.

RESULTS AND DISCUSSION

EDC-crosslinking further increased fiber diameter in the range of 0.935 – 1.415 μm . The apparent density of scaffolds and the density of bulk 3:1 PCL/PGC were used to obtain the estimated porosity of samples (Table I). Effect of crosslinking on the surface wettability and hydration were further studied by measuring the water contact angle and the PBS uptake of scaffolds after hydration for 24 h (Table I). In the case of porous scaffolds, the PBS uptake value is the result of intrusion of PBS in the scaffold pore-volume and bulk absorption by the polymer.

SEM micrographs (Figure 1) were analyzed to determine the fiber diameter distribution and study the fiber morphology of PCL/PGC, biohybrid, and cross-linked scaffolds. The fiber diameter distributions of the scaffolds are shown in Figure 2. The majority of fibers in PCL/PGC scaffolds were found to have diameters in the range of 0.29 – 0.50 μm ; this matches the diameters of collagen fibers in the native extracellular matrix (50 – 500 nm diameter). Coating with HB increased the diameters of fibers; the majority of fibers have diameters in the range of 0.71 – 1.27 μm .

Results from the estimated porosity calculations and PBS uptake studies are in agreement. Coating with HB decreased the porosity of scaffolds, and crosslinking with both EDC- and Gp- resulted in a further decreased porosity. The HB coating was shown to decrease the contact angle of scaffolds; this indicates an increase in hydrophilicity. The increase in fiber diameter due to HB coating might have also contributed to the decrease in surface contact angle. However, crosslinking resulted in an increase in the water contact angle, and therefore, a decrease in surface hydrophilicity and hydration behavior. DSC scans indicated a melting transition temperature at 56 $^{\circ}\text{C}$ for biohybrid, and crosslinked scaffolds, but with less enthalpy of melting for the later (Table I).

The tensile properties of the scaffolds are given in Table II. Mechanical properties of biohybrid scaffolds were not significantly affected by either HB coating or crosslinking. However, EDC-crosslinking resulted in a slight increase in the tensile strength of the biohybrid scaffold, and Gp-crosslinking slightly decreased the elongation at break and increased the Young's modulus of the biohybrid scaffolds. The tensile properties of all

cross-linked scaffolds remain in the range of native human arteries: tensile strength of 1–2 MPa and Young's modulus of <10 MPa. [6]. The minimal effect of crosslinking on tensile properties is likely due to the relatively very small amount (1 mg/mL) of HB incorporation into the synthetic polymer scaffold.

Picrosirius red (PSR) is an acidic azo-dye that reacts with the basic amino acids present in collagen fibrils, staining them (brilliant red). The collagens fraction in HB can therefore be visualized using PSR [12]. Microscope images of scaffolds stained with PSR and washed in PBS are shown in Figure 3. The collagens in the HB coating of biohybrid scaffolds seems to dissolve in PBS washing as indicated by the decreased intensity of PSR after 12 h aging. Retention of red staining indicates presence/retention of collagen fibers in cross-linked scaffolds. EDC-crosslinked scaffolds indicated better coating stability and protein retention than Gp-crosslinked and non-crosslinked scaffolds (Figure 3).

Poly(glycolide-co-caprolactone) has a shorter degradation period (60% reduction in mechanical strength within 3–4 weeks as a suture) than PCL. Being a relatively durable polymer, PCL can provide enhanced viscoelasticity and prevent the early collapse of 3D tubular structure which in turn may inhibit the tissue-regeneration. HuBiogel is a protein cocktail from human basement membrane. The human umbilical vein endothelial cells (HUVECs) were grown to form an endothelial tissue layer (Figure 4) on the HB coated biohybrid PCL/PGC scaffold.

CONCLUSIONS

Tubular scaffolds were successfully fabricated from a blend of PCL and PGC (3:1 mass ratio) and coated with HuBiogel™. Scaffolds were cross-linked with carbodiimide (EDC) and genipin (Gp), and the effect of crosslinking on graft properties was evaluated. It was found that while crosslinking increased the diameter of electrospun fibers, they remained in the upper range of protein fibers found in the ECM. The mechanical properties of crosslinked scaffolds emulate those of native human arteries, which is critical for the grafts to withstand the pressures due to blood flow. Additionally, the decrease in scaffold porosity due to crosslinking of biohybrid grafts was minimal. Finally, the carbodiimide(EDC)-crosslinked grafts have shown higher stability of the HB coating compared to genipin(Gp)-crosslinked grafts and non crosslinked grafts. It is noteworthy that the crosslinking did not alter the mechanical properties drastically. HUVECs grown onto the biohybrid scaffold formed a confluent endothelial tissue layer suggesting the biocompatibility and regeneration potential of HuBiogel enriched vascular graft.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Center For Advancing Translational Sciences of the National Institutes of Health under Award Number R41TR001009. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health (NIH). Danna Nozik acknowledges support from the National Science Foundation (NSF) REU-site under grant no. NSF-DMR-1058974 awarded to UAB and Dr. Thomas acknowledges UAB Faculty start-up fund. The authors would like to acknowledge the imaging support provided by the UAB Scanning Electron Microscopy core Facility at the Department of Materials Science & Engineering. One of the authors (R.K.S) works for Vivo Bioscience Inc., supplying the HB matrix.

REFERENCES

1. Wang X, Lin P, Yao Q, Chen C. World J. Surg. 2007; 31:682. [PubMed: 17345123]
2. Salacinski HJ, Goldner S, Giudiceandrea A, Hamilton G, Seifalian AM, Edwards A, Carson RJ. J. Biomater. Appl. 2001; 15:241. [PubMed: 11261602]
3. Pham QP, Sharma U, Mikos AG. Tissue Eng. 2006; 12:1197. [PubMed: 16771634]
4. Zhang X, Thomas V, Xu Y, Bellis SL, Vohra YK. Biomaterials. 2010; 31:4376. [PubMed: 20199808]
5. Patel HN, Garcia R, Schindler C, Dean DR, Pogwizd SM, Singh R, Vohra YK, Thomas V. Polym Int. 2015; 64:547. [PubMed: 25914444]
6. Zhang X, Thomas V, Vohra YK. J. Mater. Sci. Mater. Med. 2010; 21:541. [PubMed: 19902335]
7. Singh R, Collingsworth P, Alapati V, Chen X, Siegal GP. In Vitro Develop. Biol. 2002; 38:11.
8. Barnes CP, Pemble CW, Brand DD, Simpson DG, Bowlin GL. Tissue Eng. 2007; 13:1593. [PubMed: 17523878]
9. Frohbergh ME, Katsman A, Botta GP, Lazarovici P, Schauer CL, Wegst UG, Lelkes PI. Biomaterials. 2012; 33:9167. [PubMed: 23022346]
10. Zhang K, Qian Y, Wang H, Fan L, Huang C, Yin A, Mo X. J. Biomed. Mater. Res. A. 2010; 95:870. [PubMed: 20824649]
11. Bottino MC, Thomas V, Jose MV, Dean DR, Janowski GM. J. Biomed. Mater. Res. B Appl. Biomater. 2010; 95:276. [PubMed: 20842698]
12. Gee AO, Baker BM, Silverstein AM, Montero G, Esterhai JL, Mauck RL. Cell Tissue Res. 2012; 347:803. [PubMed: 22287042]

Figure 1.
SEM of scaffolds. Scale bar = 20 μm

Figure 2.
Fiber diameter distribution

Figure 3. Optical images (10× magnification) showing stability of protein coating on scaffolds aged 12 h in PBS. Picrosirius red (PSR) dye is used for staining collagens.

Figure 4.

Representative confocal microscopic image of human umbilical vein endothelial cells (HUVECs) cultured on the Biohybrid scaffolds of electrospun PCL/PGC coated with HB for 7 days. The HUVECs subculture at passage 3 was cultured onto the scaffolds with 6×10^5 cells/cm² density in endothelial cell growth media (EGM-2 Lonza Group) at 37 °C under 5% CO₂. DAPI and rhodamine phalloidin staining were used to stain nucleus and actin filaments respectively.

Table I

Porosity, thermal and hydration effects

Sample	Porosity (%)	Contact Angle (degree)	PBS Uptake (wt-%)	Melt Enthalpy (J/g)
PCL/PGC	83	114 ± 2	350 ± 16	47.63 ± 2.4
Biohybrid	80	61 ± 10	298 ± 37	48.90 ± 0.45
EDC-crosslinked	73	95 ± 16	212 ± 29	37.82 ± 1.97
Gp-crosslinked	76	81 ± 14	247 ± 1	42.47 ± 1.48

Table II

Effect of crosslinking on the mechanical properties of scaffolds

Sample	Tensile Strength (MPa)	Young's Modulus (MPa)	Elongation at Break (%)
PCL/PGC	1.73 ± 0.06	4.76 ± 0.87	461.79 ± 22.32
Biohybrid	1.64 ± 0.07	5.33 ± 0.75	529.67 ± 31.53
EDC-crosslinked	1.98 ± 0.16	5.42 ± 1.48	576.93 ± 67.17
Gp-crosslinked	1.64 ± 0.14	6.73 ± 1.40	419.23 ± 22.03