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Preliminary Investigation of Electrospun Collagen and Polydioxanone for Vascular Tissue Engineering Applications

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Abstract: *Electrospun collagen alone does not provide the mechanical integrity necessary for tissue engineering applications. Thus, blends of polydioxanone (PDO) and collagen have been electrospun into nanofibrous scaffolds with novel compositions and structures as well as mechanical properties. PDO, a biodegradable synthetic polymer, serves to maintain the mechanical integrity of the scaffold, and collagen (specifically types I and III) is utilized in the electrospun blend to provide the native ultrastructure necessary for the desired cellular interactions. Polymer solutions of PDO and collagen (of one or both types) were electrospun to create randomly oriented, non-woven, fibrous scaffolds that were then analyzed via scanning electron microscopy and uniaxial tensile testing. The addition of collagen to PDO decreased the mean fiber diameter (compared to PDO alone), but there was no specific trend observed with the blended compositions (i.e. an increase in the percentage of collagen did not have a significant effect on mean fiber diameter, which ranged from 210 to 340 nm for the PDO/collagen blends). A comparison of the uniaxial tensile mechanical properties of the electrospun blends of PDO and collagen to those properties of vascular grafts currently in clinical use revealed that these electrospun mats can be tailored to match the basic mechanical properties of those prosthetics. Finally, human dermal fibroblasts were seeded onto some of the electrospun mat compositions. Those scaffolds containing collagen displayed favorable cellular interactions in terms of biocompatibility in that fibroblasts moved into the thickness of those scaffolds (whereas the cells merely migrated on the seeded surface of the PDO-only scaffold). One tissue engineering application of particular interest for such a polymeric blend is an off-the-shelf, bioresorbable, acellular small diameter (<5 mm inner diameter) vascular prosthetic capable of* in situ *arterial tissue regeneration; these materials have the potential to achieve this desired response* in vivo.

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.*

Key words: *electrospinning, collagen, polydioxanone, scaffold, tissue engineering*

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INTRODUCTION

Tissue engineering, or regenerative medicine, is an interdisciplinary field that merges principles and technologies from engineering and life sciences for the purpose of addressing the improvement, repair, or replacement of tissue/organ function [1, 2]. The appeal of the use of collagenous nanofibers in tissue engineering is the ability to mimic the extracellular matrix (ECM) both structurally and compositionally. The ECM creates a dynamic, three-dimensional microenvironment in which cells are maintained. Signals are transmitted between the cell nucleus and the ECM enabling communication between both structures for cell adhesion, migration, growth, differentiation, apoptosis, modulation of cytokine and growth factor activity, and activation of intracellular signaling [3]. It is known that cell-collagen interactions influence cell growth and differentiation depending on how well the cells are able to penetrate the ECM composed of interwoven collagen fibrils [4].

Collagen, the most abundant protein (natural polymer) in the human body, provides the overall structural integrity and strength to tissues [5]. As the major protein of the ECM, collagen provides the cells with the scaffolding for embryologic development, organogenesis, cell growth, and wound repair [4]. There are a minimum of 28 genetically distinct types of collagen. The common feature of the fibrillar types of collagen (including types I and III) is the triple-helical molecular structure with overlapping units that form fibrils exhibiting a periodic pattern of 67 nm end-to-end separations; however, the collagen types differ in specific amino acid composition, tissue location, and function [5]. Collagen fibrils range in diameter from 50 to 500 nm [6]. In many native tissues, type I collagen is the principal structural element of the ECM and is found in skin, bone, cornea, tendon, vessels, fibrocartilage, intestine, dentin, and uterus [3, 5]. Type III collagen is found in skin, ligaments, and blood vessels. The flexibility of blood vessels is partially attributed to type III collagen, and during the early stage of wound healing type III collagen is expressed [5].

Historically, collagen has been used in a variety of tissue engineering applications because of its prevalence in native ECM and because it can be isolated from a variety of sources and is relatively non-immunogenic. Collagen gels and sponges have been prepared from fibrillar and non-fibrillar collagen for use as scaffolds in injectable drug delivery devices, skin substitutes, articular cartilage repair, corneal tissue, and tendon repair [7-9]. In addition to gels, native collagen-rich tissue samples have been de-cellularized for use as scaffolds [7], and freeze-dried solutions of collagen have been prepared as honeycomb-like scaffolds [10]. Electrospinning solutions of collagen to create non-woven meshes for uses as scaffolds in tissue engineering applications has become popular [11-19], and even coating electrospun synthetic fibers with collagen is in practice [20].

Collagenous tissues removed from their natural environment, as well as processed collagenous structures, must be treated to prevent immediate degradation and ensure biocompatibility [21, 22], which is also true of electrospun collagen. There are a variety of cross-linking methods currently utilized. However, cross-linking can result in a leathery material with minimal bioactivity. Thus, our group has explored the electrospinning of biodegradable synthetic polymers with the natural polymers including collagen and elastin

[23]. Polydioxanone (PDO) is one such synthetic polymer that can be incorporated into structures requiring immediate strength such as an acellular vascular prosthetic [24]. PDO is a highly crystalline (55% crystalline fraction), biodegradable polyester that was originally developed for use as a suture (Ethicon, Inc.). PDO exhibits excellent biocompatibility, high strength, flexibility (due to the incorporation of an ester oxygen in the monomer backbone), a predictable degradation rate, and shape memory (that may prove important in kink resistance in a vascular conduit).

Conventional polymer processing techniques cannot produce fibers much less than 10 μm in diameter, which is several orders of magnitude greater than the native ECM. Electrospinning technology allows for the production of continuous micro- to nano-scale polymer fibers, which can be collected in various orientations to create unique structures in terms of composition and mechanical properties [25-27]. Electrospinning offers further advantages including being relatively simple, cost effective, and capable of upscaling for mass production [28]. In electrospinning, the positive output lead of a high voltage power supply is attached to the nozzle of a reservoir containing a polymer solution to charge the solution. The charged solution is attracted to a grounded target some distance away. As the solution leaves the nozzle, a cone of solution (known as a Taylor cone) forms at the nozzle tip. The liquid jet is formed from this cone when electrostatic repulsions in the solution overcome the surface tension of the solution. As it is drawn toward the grounded target, the liquid jet follows a trajectory dictated by both the electrostatic forces in the solution and the electric field. If there are significant chain entanglements among the polymer chains in the solution and the solvent is relatively volatile, evaporation yields the formation of a fiber. The end result is a dry, non-woven fibrous mat. The parameters involved in the electrospinning process that influence the resulting fiber morphology have been investigated [26]. Control of fiber diameter is accomplished by varying the polymer solution concentration (and, hence, viscosity and polymer chain entanglements). Additionally, structures of various shapes, sizes, and thicknesses can be constructed while at the same time precisely controlling fiber orientation (via rate of rotation of the target) and composition (i.e. polymer type or types and concentration).

Our laboratory has previously reported the creation of a layered, electrospun vascular construct of varying ratios of collagen types I and III and elastin involving the seeding of fibroblasts, smooth muscle cells, and human umbilical vascular endothelial cells [14, 29]. This study investigates the electrospining of a blend of collagen types I and III with PDO to characterize the electrospun scaffolds and prove their efficacy in an application such as a vascular graft. The ultimate goal is to investigate if such a blend may serve to enable native cells to regenerate, *in situ*, the three layers of the native grafted artery without the need to engineer the prosthetic in three layers.

MATERIALS AND METHODS

Electrospinning

Collagen types I and III were extracted from fetal calf dermis via homogenization of the skin, suspension in acetic acid, salt precipitation (1.7 M NaCl for type III and 2.5 M

NaCl for type I), and subsequent purification via a series of dissolutions, precipitations, and dialyses [30]. The lyophilized collagen was dissolved in 1,1,1,3,3,3 hexafluoro-2-propanol (HFP, Sigma-Aldrich Chemical Co.) at a concentration of 60 mg/mL. Polydioxanone (PDO, Ethicon, Inc.) was dissolved in HFP at a concentration of 100 mg/mL. PDO alone and blends of the collagen solutions with PDO were electrospun to create randomly-oriented, non-woven, fibrous scaffolds. The blends consisted of the following PDO:collagen ratios: 90:10, 80:20, and 70:30. The compositions are hereafter labeled PDO:collagen type I (PDO:CI), PDO:collagen type III (PDO:CIII), and PDO: collagen types I and III (PDO:CI/CIII), which is a 50/50 blend of collagen type I and collagen type III.

Figure 1 illustrates the electrospinning process (which has been described in detail elsewhere [25]). The apparatus used includes a syringe pump (KD Scientific), a high voltage power supply (Spellman CZE1000R, Spellman High Voltage Electronics Corp.), a syringe (Becton Dickinson, plastic) as the reservoir for the polymer solution to which is attached an 18-gauge blunt-end needle, and a 303 stainless steel mandrel (2.5 cm x 7.6 cm x 0.4 cm) as the collection target. All electrospinning parameters were kept constant, including applied voltage (22 kV), distance between the needle and grounded mandrel (12.7 cm), solution dispensing rate (8 mL/hr), and mandrel translational speed (2 cm/s over a 7.3 cm distance) and rotational speed (500 rpm for random fiber orientation). For each composition, 3 mL of each solution was electrospun.

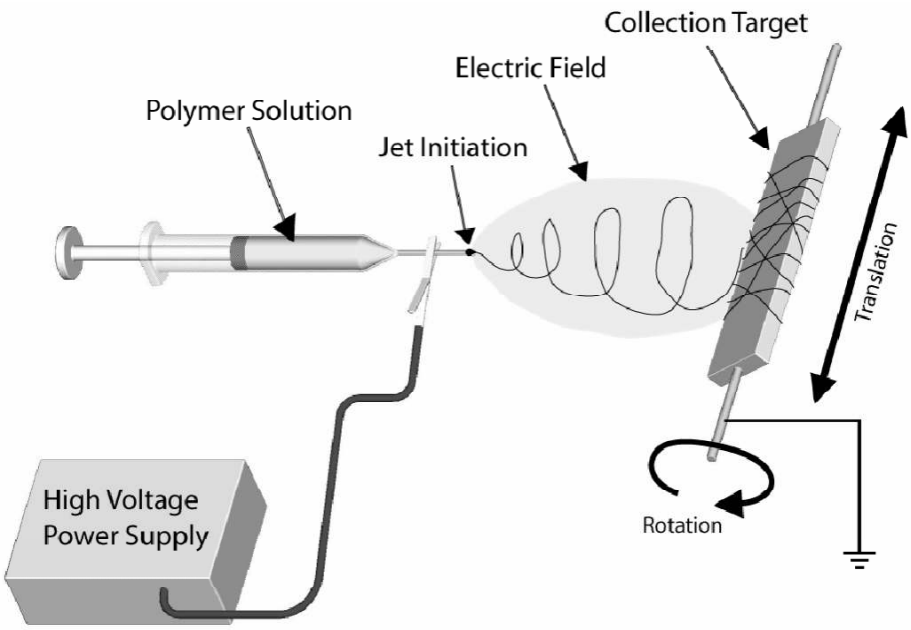

Fig. 1: Schematic of the Electrospinning Process to Illustrate the Basic Phenomena and Process Components

Scaffold Characterization

Fiber morphology was viewed with scanning electron microscopy (SEM) using a JEOL JSM-820 JE electron microscope. Micrographs were digitized with a Hewlett-Packard Scanjet 5550c flatbed scanner and examined with UTHSCSA ImageTool 3.0 imaging software (NIH shareware) to measure fiber diameter. Means and standard deviations were determined from 60 fiber diameter measurements per micrograph, and calibration was made with the scale bar on each micrograph.

Mechanical Testing

Mechanical properties of the electrospun mats were measured in the dry state to show handling properties and in the hydrated state (soaked in phosphate buffered saline, PBS, for approximately 24 hours at room temperature) to show behavior under approximated physiological conditions. Uniaxial tensile testing of the scaffolds on a MTS Bionix 200 mechanical testing system (MTS Systems Corp., Eden Prairie, MN), incorporating a 50N load cell with an extension rate of 10.0 mm/minute to failure, was performed on six "dog-bone" shaped samples (2.67 mm wide at their narrowest width and a gage length of 7.49 mm) punched from each electrospun composition. The mechanical properties recorded include tangential modulus, peak stress, and strain at failure, which were calculated by the MTS software TestWorks 4.0.

Cell Seeding

To evaluate preliminary cell interaction in this feasibility study of PDO and collagen, only collagen type III was utilized. Three circular discs (5.9 mm diameter, 100 μm average thickness) were punched from each of four different ratios of PDO:collagen type III (100:0, 90:10, 80:20, 70:30), disinfected (by soaking in ethanol for 10 minutes, followed by four rinses in PBS), and placed in a 96 well plate; the discs covered the entire surface area of the well. Human dermal fibroblasts (Cascade Biologics) were cultured in Dulbecco's Modified Eagle Medium with Nutrient Mixture F-12 (Invitrogen Corp.) supplemented with 10% fetal bovine serum and 1% penicillin-streptomyocin (10,000 Units/mL each). Each scaffold was seeded with 100 μL of cell/media mixture at a concentration of 50,000 cells/mL. After an incubation period of 45 minutes 100 μL of media was added to each well with cells. The scaffolds were then statically cultured under standard culture conditions (37°C and 5% CO_2) in an incubator. One disc of each ratio was removed after 24 hours for SEM analysis, while the others were left in culture for seven days, fixed in 10% formalin, and processed for histology (Hematoxylin and Eosin staining). The histology slides were analyzed using an inverted microscope (Nikon Eclipse TE300) at a magnification of 20X.

Statistics

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

RESULTS AND DISCUSSION

Scaffold Characterization

Both collagen types I and III have a linear relationship between solution concentration and fiber diameter. Though we have previously reported the successful electrospinning of collagen type III with other polymers, this is the first report of the linear relationship between solution concentration and fiber diameter for electrospun collagen type III alone. Figure 2 gives this relationship. At concentrations greater than 80 mg/mL, the electrospun collagen type III resulted in small fragments of fibers as well as ribbon-like fibers. This linear relationship for collagen type I has been previously shown [14]; however, comparable solution concentrations resulted in larger fiber diameters for collagen type I than for collagen type III.

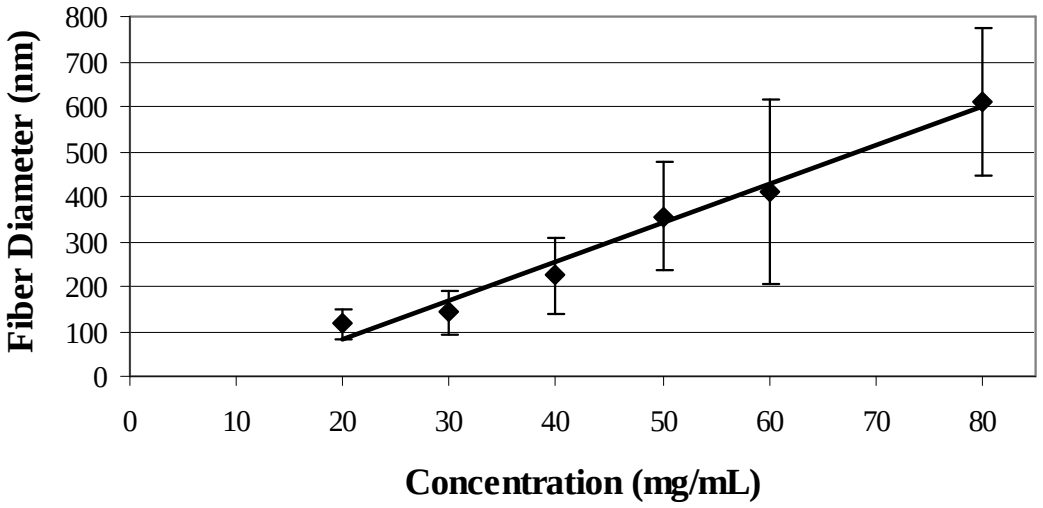

Fig. 2: Linear Relationship between Collagen type III Solution Concentration and Fiber Diameter; the Trendline Shown has an $R^2 = 0.981$.

Blends of PDO and collagen (types I, III, and both I and III) were successfully electrospun to produce randomly organized, non-woven, nano-fibrous mats. Figures 3 and 4 give representative scanning electron micrographs of the dry mats produced. Fiber diameters were taken from these micrographs; these results are presented in Figure 5. The mean fiber diameter of the 100% PDO mat was significantly different from all other mean diameters of the other compositions. Thus, the addition of collagen to the electrospinning solution did have a significant effect on decreasing the fiber diameter. Additionally, the mean fiber diameter for the 90:10 PDO:CI/CIII composition was siginificantly less than all of the following compositions: 80:20 PDO:CI/CIII, 80:20 PDO:CI, 70:30 PDO: CI, 70:30 PDO: CIII.

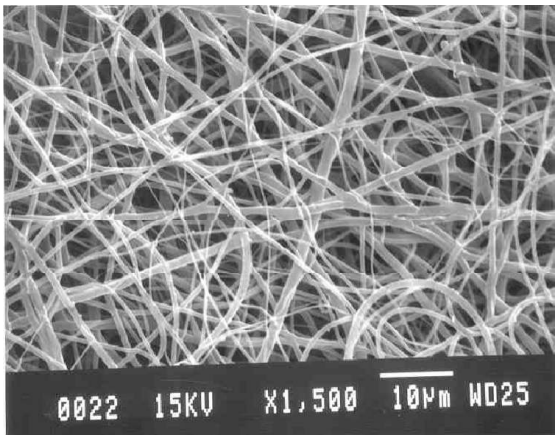

Fig. 3: Scanning Electron Micrograph of the PDO Scaffold Electrospun from a Solution of 100 mg/mL PDO in HFP. Magnification is 1,500X and scale bar is 10 µm.

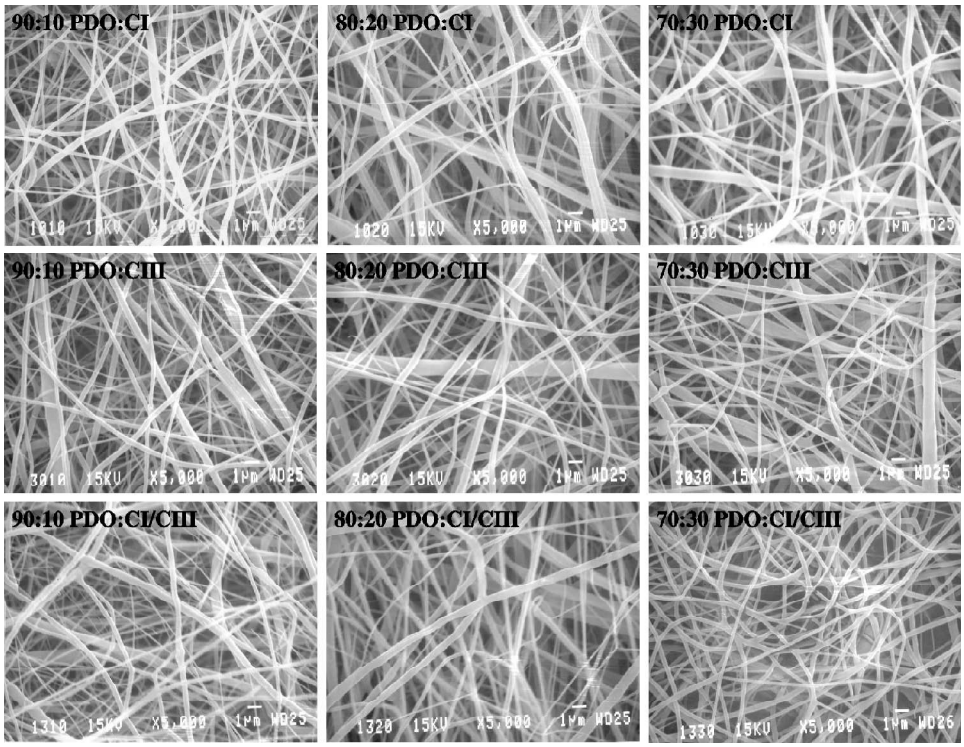

Fig. 4: Scanning Electron Micrographs of the PDO and Collagen Scaffolds Electrospun from Solutions of 100 mg/mL PDO and Varying Ratios of Collagen types I and III (60 mg/mL): PDO and Collagen type I (top row), PDO and Collagen type III (Middle Row), and PDO and Collagen Types I and III (bottom row). The Left, Middle, and Right Columns Show 10, 20, and 30% Collagen Solutions, Respectively. Magnification is 5,000X and Scale Bar is 1 µm.

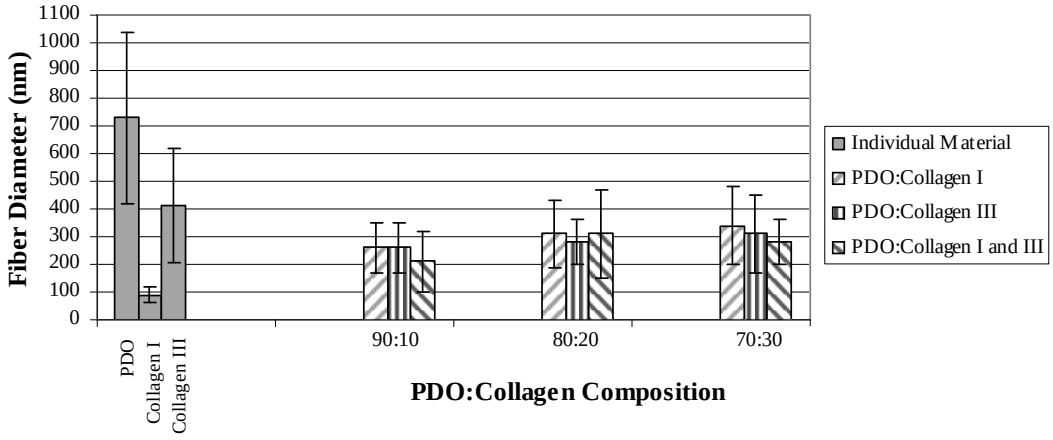

Fig. 5: Fiber Diameters Measured from the Scanning Electron Micrographs of the Electrospun PDO and Collagen Scaffolds. The First set of Bars give the Mean Fiber Diameters of the Individual Materials Electrospun at the Same Concentration used to Make the Blends.

Mechanical Testing

Based on the previous work performed in the laboratory on collagen types I [11, 14] and III [31], the percentage of collagen in an electrospun mat can not exceed 50% by volume without cross-linking of the mat to maintain structural integrity. Thus, in this study collagen compositions were tested only up to 30% by volume to eliminate the need for cross-linking.

Uniaxial tensile testing was performed on the electrospun compositions to determine the materials' mechanical characteristics in both dry and hydrated conditions. Figures 6 through 8 give the results of the mechanical testing. Table 1 gives the mean values of peak stress, tangential modulus, and strain at break for all of the compositions and conditions tested.

In terms of peak stress, the wet 70:30 PDO:CI was significantly different from the dry 70:30 PDO:CI and the wet 70:30 PDO:CIII. The wet 100% PDO was also significantly different from the dry 70:30 PDO:CI and the wet 70:30 PDO:CIII. The dry and wet 90:10 PDO:CI/CIII and the wet 70:30 PDO:CI/CIII were all significantly different from the following: the dry 70:30 PDO:CI, the wet 70:30 PDO:CIII, the dry 80:20 PDO:CI, and the dry 80:20 PDO:CIII.

There was a general trend of increasing tangential modulus for increasing collagen concentration for the dry electrospun mats in all three different PDO:collagen compositions. This trend was not apparent for the wet electrospun mats, except for the PDO:CI blends. Also of interest were the overall larger mean values of modulus for each ratio of PDO:CI compared to the same ratio for the PDO:CIII and PDO:CI/CIII compositions. Collagen type I is known to be less flexible than collagen type III [32], and thus displays this stiffness here. However, when the type I and type III collagens are blended, the type I does not appear to dominate – there is a combined effect of both on

the resulting modulus of the composite. Of the statistically significant differences, there are several to be noted. Most of the composites showed significant differences between the dry and hydrated states except the following: the 100% PDO, the 90:10 PDO:CIII, and the 90:10 PDO:CI/CIII. The dry 100% PDO mat was significantly different from the following dry mats: 70:30 PDO:CI, 80:20 PDO:CI, 70:30 PDO:CI/CIII, 80:20 PDO:CI/CIII, 70:30 PDO:CIII, and 90:10 PDO:CI. When comparing all of the wet mats, there were no significant differences. Additionally, the 70:30 PDO:CI dry was significantly different from all other mats (wet and dry).

The mean strain at break values for all of the hydrated mats were greater than those values for the dry mats of the same composition. Comparing the dry and hydrated mats of the respective compositions, only the 70:30 PDO:CIII demonstrated a significant difference between the dry and wet states. The wet 100% PDO mat is significantly different from all other mats except the dry 100% PDO mat and the wet 70:30 PDO:CIII. Interestingly, there was a negative trend between strain at break and collagen type I concentration, but a positive trend between strain at break and collagen type III concentration. However, there seemed to be no direct relationship between strain at break and the concentration of collagen types I and III together. Additionally, the wet 70:30 PDO:CIII was significantly different from all other mats except the 100% PDO (wet and dry) and the 80:20 PDO:CIII (wet and dry).

These mechanical properties can be compared to the mechanical properties of native collagen and elastin, two major structural components in soft tissues such as blood vessels. Native collagen fibers exhibit a peak stress ranging from 5 to 500 MPa, a modulus ranging from 100 to 2900 MPa, and a percent strain at break ranging from 5 to 50%. Native elastin fibers display the following properties: a peak stress ranging from 0.36 to 4.4 MPa, a modulus ranging from 0.3 to 0.6 MPa, and a percent strain at break ranging from 100 to 200% [33]. The electrospun blends of PDO and collagen, as well as the PDO alone, demonstrated peak stress values that ranged between the upper limit of elastin and the lower limit of collagen. The blends' tangential moduli range is approximately an order of magnitude greater than the upper limit of elastin and an order of magnitude less than the lower limit of collagen; thus, this property for the blends falls between the ranges for the native tissue components. The percent strain at break for most of the blends falls within the upper limit of collagen and the lower limit of elastin. However, the 100% PDO, 80:20 PDO:CIII, and 70:30 PDO:CIII compositions had strain at break ranges within the elastin range or approaching the upper limit of elastin.

For materials proposed for use in a vascular prosthetic, it is critical to compare the mechanical properties to those of native vessel, i.e. femoral artery, and of materials currently used as vascular grafts, the so called "gold standards", which include expanded polytetrafluoroethylene (e-PTFE) and the saphenous vein (Table 1). When comparing the hydrated electrospun samples tested, the mechanical properties of the electrospun materials fell within the ranges of the traditional vascular prosthetic materials, with the exception of strain at break. All of the hydrated electrospun materials attained or exceeded the upper limit of the strain at break of femoral artery. This is encouraging at this time, but more intensive testing is required to determine compliance and dynamic mechanical behavior.

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Table 1
Uniaxial Mechanical Properties of Electrospun Blends of PDO and Collagen as well as e-PTFE (30 mm Internodal Distance) [34], Saphenous Vein [35], and Femoral Artery [36, 37].

		Peak Stress (MPa)		*Tangential Modulus (MPa)*		*Strain At Break (%)*	
Composition	*Diameter (μm)*	*Dry*	*Wet*	*Dry*	*Wet*	*Dry*	*Wet*
100:0 PDO	0.73 ± 0.31	5.8 ± 0.7	4.8 ± 0.6	13.2 ± 3.1	10.4 ± 3.8	185.8 ± 93.4	229.8 ± 117.2
90:10 PDO:CI	0.26 ± 0.09	6.3 ± 0.7	5.7 ± 1.1	25.7 ± 3.0	11.2 ± 1.8	57.5 ± 4.8	89.2 ± 4.6
80:20 PDO:CI	0.31 ± 0.12	6.4 ± 1.0	5.9 ± 1.1	48.3 ± 12.5	15.8 ± 3.7	40.4 ± 8.0	72.8 ± 11.1
70:30 PDO:CI	0.34 ± 0.14	6.7 ± 0.4	4.9 ± 0.5	68.3 ± 6.1	18.0 ± 2.7	27.5 ± 4.7	56.5 ± 11.8
90:10 PDO:CIII	0.26 ± 0.09	5.1 ± 0.9	5.2 ± 0.9	19.1 ± 5.5	8.9 ± 2.6	63.0 ± 8.9	100.1 ± 18.7
80:20 PDO:CIII	0.28 ± 0.08	6.6 ± 0.5	6.1 ± 0.7	22.3 ± 2.9	8.5 ± 1.5	115.6 ± 6.4	153.5 ± 21.3
70:30 PDO:CIII	0.31 ± 0.14	5.8 ± 1.1	6.7 ± 0.8	26.7 ± 4.3	7.6 ± 1.8	110.7 ± 21.4	186.4 ± 18.0
90:10 PDO:CI/CIII	0.21 ± 0.11	4.6 ± 1.3	4.6 ± 1.0	14.1 ± 4.8	7.7 ± 2.7	64.9 ± 11.3	101.1 ± 12.5
80:20 PDO:CI/CIII	0.31 ± 0.16	5.5 ± 0.8	5.0 ± 0.4	27.0 ± 6.2	10.1 ± 1.2	54.4 ± 10.1	88.2 ± 7.6
70:30 PDO:CI/CIII	0.28 ± 0.08	6.2 ± 1.0	4.6 ± 0.8	37.6 ± 11.9	9.0 ± 2.3	53.8 ± 13.8	98.3 ± 18.1
e-PTFE	—	6 – 15		42 – 60		20 – 30	
Saphenous Vein	—	3		43		11	
Femoral Artery	—	1 – 2		9 – 12		63 – 76	

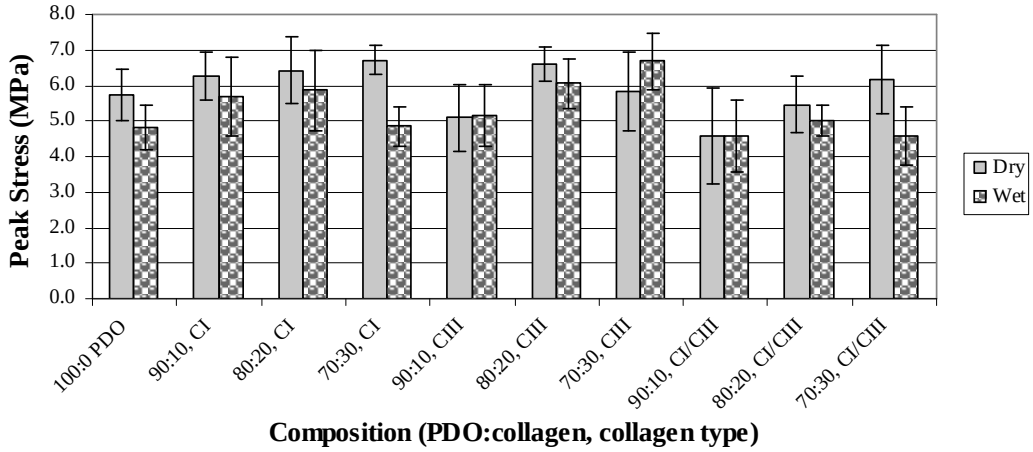

Fig. 6: Peak Stress Determined for Dry and Hydrated Electrospun PDO and Collagen Scaffolds.

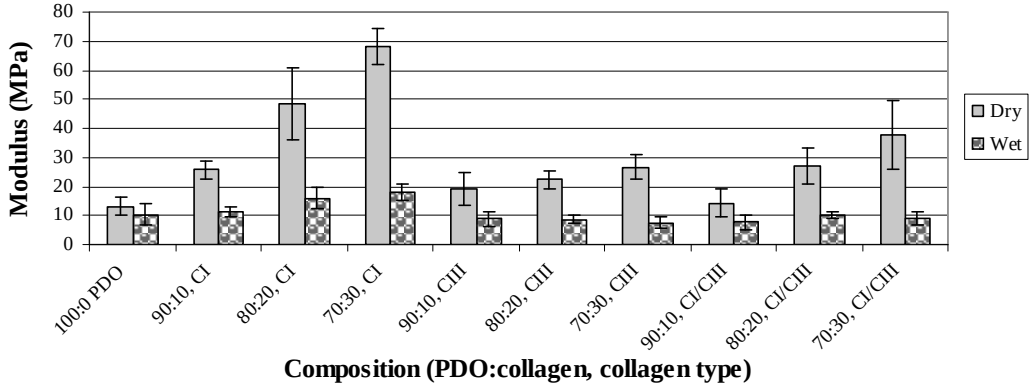

Fig. 7: Tangential Modulus Determined for Dry and Hydrated Electrospun PDO and Collagen Scaffolds.

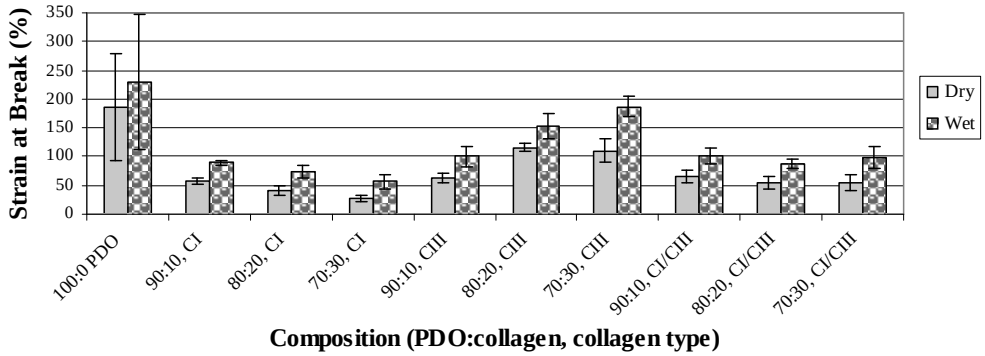

Fig. 8: Strain at Break Determined for Dry and Hydrated Electrospun PDO and Collagen Scaffolds.

Cell Seeding

Figure 9 gives the results of the cell seeding study at day 1 and day 7. Scanning electron micrographs were taken at day 1 to investigate the behavior of the fibroblasts on the surface of the scaffolds. After one day the cells were spreading in a similar fashion irrespective of scaffold material. After seven days in culture, scaffolds were removed for histological processing (Figure 9). The bright field photograph of the 100% PDO scaffold shows the fibroblasts only on the top (seeded surface) of the scaffold. Hoffmann's modulation contrast photographs were taken of the PDO:CIII scaffolds to better depict the distribution of cells through the 100 μm thick scaffold. Fibroblasts infiltrated the thickness of all of the PDO:CIII scaffolds, though the 90:10 and 80:20 compositions appear to have a greater cell density. It is speculated that similar results will be seen with compositions including collagen type I and blends of the collagen types in terms of fibroblast interaction with the scaffolds.

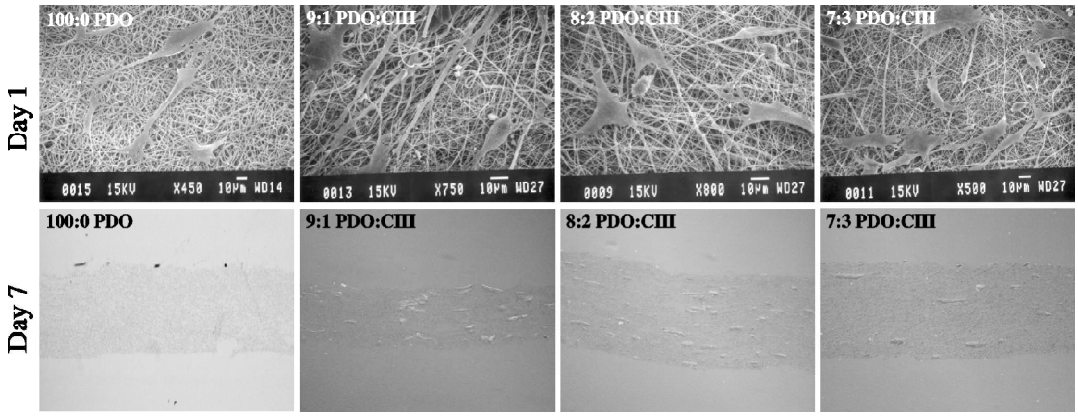

Fig. 9: Results of the Cell Seeding Study on PDO: C III Compositions; Top Row: Scanning Electron Micrographs to Show Fibroblasts on the Surface of the Scaffolds at Day 1 (Magnification between X450 and X800, Scale bar is 10 μm); Bottom Row: Photographs (Bright Field and Hoffmann's Modulation Contrast) of H&E Stained Sections to show even Distribution of Fibroblasts within 104 μm thick Scaffolds at day 7 (Magnification 20X).

CONCLUSIONS

The interest in an off-the-shelf, bioresorbable, acellular small diameter (<5 mm I.D.) vascular prosthetic capable of *in situ* arterial tissue regeneration is twofold: (1) natural or synthetic bioresorbable polymers will degrade and be replaced by native extracellular matrix and (2) the absence of cell seeding and incubation eliminate the requirement of any special storage or handling; thus, the risk of long-term prosthetic rejection is eliminated. Work to achieve this goal has begun with the elecrospinning of blends of PDO and collagen (types I, III, or both) into randomly-oriented, non-woven, nanofibrous mats. The addition of collagen to PDO resulted in mean fiber diameter measurements

on the same order as those of native ECM fibers. The basic mechanical properties of the electrospun blends of PDO and collagen were comparable to clinically used autologous and synthetic vascular grafts. Initial cell seeding studies demonstrated favorable cell interactions on all compositions containing collagen. Thus, further investigations will be pursued in which more in-depth mechanical and cellular analysis will be conducted utilizing electrospun seamless tubes of PDO and collagen as small diameter vascular grafts.

Acknowledgements

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