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Fabrication and Characterization of Electrospun Templates from Polydioxanone and Its Co-Polymers

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William's paper received a *Quaesitum* best paper award.

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Faculty Sponsor

Dr. Gary Bowlin

Abstract

Electrospinning is a process through which tissue regeneration templates are fabricated. Since every tissue within a biological system is subjected to unique physical stresses and strains, implantable biomaterials, such as electrospun templates, must possess certain properties that can withstand the biomechanical forces of a native microenvironment. The aim of this study was to electrospin new co-polymers of polydioxanone (PDO) and evaluate their resultant mechanical properties. Results show that each electrospun co-polymer exemplifies some unique physical and mechanical properties which are polymer specific and can be tailored to a specific physiological need.

Introduction

Tissue engineering is the process by which a biofactor, such as a cell, gene, or protein, is transplanted within a porous degradable material (a scaffold) for the formation of a new viable tissue [1]. Within the last twenty years, a new tissue engineering technology called electrospinning has emerged with promising prospects for creating templates through which biological systems can regenerate tissue. The process of electrospinning involves applying a voltage source to a polymer solution, which is stored in a syringe. Due to the potential difference between the liquid surface and target, nano- to microfibers extrude from the syringe and collect on a grounded target opposite the syringe. As the extruded fibers collect, mats composed of nonwoven, randomly distributed fibers are formed which are biomimetic of native extracellular matrix (ECM). These electrospun templates can be fabricated with an array of finely-tuned physical and chemical properties which, as a result, create efficacious biomaterials for diverse applications in guided tissue regeneration. Ultimately, the polymer selection, the diameter of electrospun fibers, and the mechanical integrity of the template all influence the utility of electrospun materials.

When implanted in the body, electrospun templates initiate a host tissue-specific response. Soluble blood serum proteins adhere to the surface of the biomaterial first, followed by the recruitment of neutrophils, macrophages, and other inflammatory cells, resulting in acute inflammation and the foreign body response [2]. Ideally, electrospun templates will initiate a host tissue-specific response without triggering this large-scale inflammatory foreign-body response. By fabricating electrospun templates with appropriate polymers, the scaffold can degrade at a rate such that the native ECM will regenerate and replace the scaffold as it is broken down. Electrospun tissues are advantageous biomaterials due to their biomimetic properties and their tailorable biodegradability. Polydioxanone (PDO) is a synthetic polymer that is commonly used in electrospinning due to its ideal degradation rate, its tailorable material properties, and its non-toxic degradation products. Other non-toxic polymers include poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) which have different mechanical properties, degradation rates, and degradation products which are appropriate for select tissue regeneration applications.

In this study, a variety of recently released PDO co-polymers from the drug device company Bezwada Biomedical Engineering LLC were characterized and fabricated for electrospinning applications. We electrospun PDO and three PDO co-polymers with PLA and PGA to explore their fabrication parameters for electrospinning and evaluated the resultant mechanical

properties. The purpose of this work was to explore the applicability of these co-polymers as electrospun tissue engineering templates. To do this, we electrospun each polymer to create templates composed of a range of fiber diameters and performed mechanical testing on each template. From this testing data, we evaluated if electrospun PDO/PGA, PDO/PLA, and PDO/PGA/PLA co-polymers were mechanically comparable to PDO. Additionally, we explored if mechanical properties changed when templates were composed of different fiber diameters and how these properties differed with the direction of testing. We hypothesized that these co-polymers could be electrospun, that they would have similar mechanical properties to PDO, and that the mechanical properties of each of these templates would be fiber diameter and orientation-dependent. Through this study, we demonstrated that these polydioxanone co-polymers can be electrospun to form templates with diverse, tailorable mechanical properties.

Materials and Methods

Template Fabrication and Characterization

In this study, PDO, PDO/PLA (90/10) (i.e. a co-polymer of 90% polydioxanone and 10% poly(lactic acid)), PDO/PGA (90/10), and PDO/PGA/PLA (90/5/5) were dissolved overnight in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Oakwood Products, Inc., Pro. No. 003409) at different concentrations. Each solution was loaded into a 5-mL syringe (Becton, Dickinson, and Company) and attached to an 18-gauge blunt needle (Becton, Dickinson, and Company, Pro. No. 305196), which was connected to the positive lead of a Spellman CZE1000R power source (Spellman High Voltage Electronic Corp.). The syringe was placed on a syringe pump (Model No. 78-01001, Fisher Scientific). Fibers were collected on a grounded, stainless steel 200 x 750 x 5 mm rectangular mandrel, rotating at 1250 RPM and translating 6.5 cm/s over 13 cm. Polymer concentration, applied voltage, flow rate, and airgap for each electrospun template were optimized to produce templates composed of a range of fiber diameters. The templates (thickness 0.06 - 0.18 mm) were stored in a desiccator at 25 °C until analysis.

To characterize the templates by scanning electron microscopy, each template was sputter coated with 5 nm of gold-palladium in an argon gas field. The templates were then imaged using a FEI Nova NanoSEMTM 650 with a field emission gun at +20 kV and a working distance of 5 mm. The inside and outside surfaces of each template were imaged at 1000x to ensure the templates were composed of non-fused, non-beaded fibers. If the SEM image revealed a template composed of beaded or fused fibers, the template was discarded, and the fabrication and characterization process

was repeated until optimal electrospinning parameters were found. The fiber diameters of each template were quantified by analyzing the SEMs in FibraQuant 1.3 software (nanoTemplate Technologies, LLC). A minimum of 200 semi-automated random measurements were taken per image to generate averages and corresponding standard deviations.

Mechanical Testing

After fabrication and SEM characterization, the electrospun templates were cut into dogbone shaped samples (2.75 mm at the narrowest point) in two directions: parallel and perpendicular to the electrospinning axis of rotation. The thickness of each specimen was measured using calipers (Mitutoyo Absolute, Pro. No. 547-516), and the dogbone shape samples were loaded into a TestResources frame (model no. 220Q with 111 N load cell) with a gauge length of 7.5 mm. Uniaxial tensile testing was then performed ($n \geq 7$ punches of each orientation) at a strain rate of 10 mm/min, which is the standard for electrospun template uniaxial tensile testing, until failure to evaluate the ultimate tensile stress (UTS), Young's modulus, and maximum percent elongation as a function of average fiber diameter for each polymer type [3]. Stress strain data was recorded by the XY software associated with the testing frame, and then exported to Microsoft Excel.

Analysis

Data from Microsoft Excel was used to generate stress strain curves, which were then used to obtain UTS, modulus of elasticity, and maximum percent elongation for each mechanically tested sample. The data were analyzed with a Kruskal-Wallis analysis of variance and Dunn's multiple comparison procedure. Mechanical data was tested for significance between each polymer concentration and between each punch orientation. All analyses were performed in GraphPad Prism 6 at a significance level of 0.05.

Results

Electrospinning and Fiber Diameter Analysis

Electrospinning of each polydioxanone co-polymer was possible over a range of concentrations (Table 1). The maximum and minimum concentrations for electrospinning each polymer was determined, and then two intermediate concentrations were selected to provide a complete range of polymer concentrations. Whereas PDO has been electrospun at concentrations as low as 42 mg/mL and as high as 167 mg/mL, the Bezwada PDO only produced electrospun templates with uniform fibers for concentrations ranging from 60 mg/mL to 160 mg/mL [4].

The optimized electrospinning parameters for each of the polymers are shown in Table 1.

	Concentration (mg/mL)	Voltage (kV)	Flow Rate (mL/h)	Airgap Distance (cm)
PDO	60	+24	2.75	12.7
	80	+24	3.00	12.7
	120	+25	5.00	12.7
	160	+26	4.50	15.2
PDO/PGA	60	+27	3.50	12.7
	80	+24	3.75	12.7
	120	+26	3.50	17.8
	150	+24	2.81	19.1
PDO/PLA	70	+13	0.40	11.4
	80	+23	4.00	12.7
	120	+25	5.00	14.0
	155	+25	2.20	17.8
PDO/PGA/ PLA	60	+21	1.75	8.9
	80	+23	4.00	12.7
	120	+28	4.00	17.8
	150	+27	1.90	17.8

Table 1. Electrospinning parameters optimized for four concentrations of PDO and its co-polymers.

Using the optimized electrospinning parameters, each polymer was used to fabricate templates on the grounded rectangular mandrel. Representative SEM images of each template are shown in Figures 1 through 4. Ideally, each electrospun template is composed of an array of fibers which are approximately the same diameter and are non-fused and non-beaded. This is important in the fabrication of electrospun templates for guided tissue regeneration because the extracellular matrix is a fibrous mesh of relatively smooth fibers. Since the purpose of electrospun templates is to mimic the ECM, the templates should, as closely as possible, recreate the micro-environment that would be found in the native biological tissue.

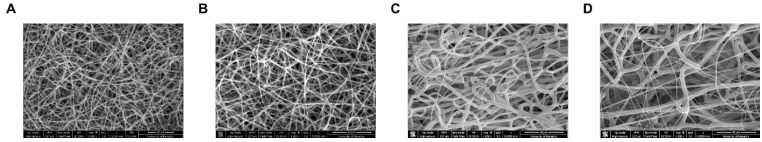

Figure 1. Representative SEMS of PDO templates fabricated with a concentration of (A) 60 mg/mL, (B) 80 mg/mL, (C) 120 mg/mL, and (D) 160 mg/mL (images taken at 1000x, scale bars = 20 μm (A) and 30 μm (B-D)).

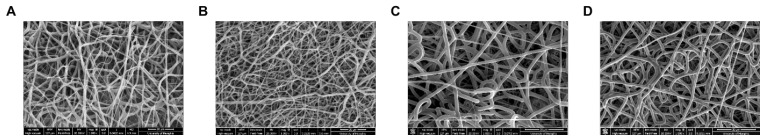

Figure 2. Representative SEMS of PDO/PGA templates fabricated with a concentration of (A) 60 mg/mL, (B) 80 mg/mL, (C) 120 mg/mL, and (D) 150 mg/mL (images taken at 1000x, scale bars = 20 μm (A) and 30 μm (B-D)).

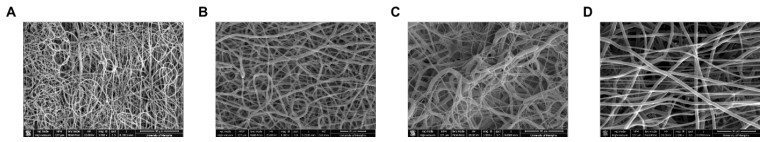

Figure 3. Representative SEMS of PDO/PLA templates fabricated with a concentration of (A) 70 mg/mL, (B) 80 mg/mL, (C) 120 mg/mL, and (D) 155 mg/mL (images taken at 1000x, scale bars = 20 μm (B) and 30 μm (A,C,D)).

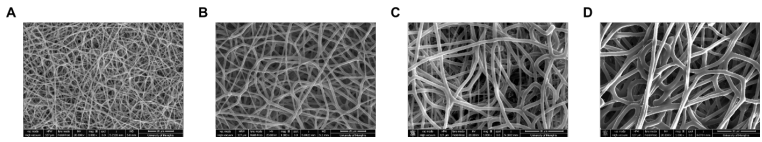

Figure 4. Representative SEMS of PDO/PGA/PLA templates fabricated with a concentration of (A) 60 mg/mL, (B) 80 mg/mL, (C) 120 mg/mL, and (D) 150 mg/mL (images taken at 1000x, scale bar = 20 μm (A-B) and 30 μm (C-D)).

The results from fiber diameter analysis (Figure 5) indicate a positive correlation between fiber diameter and increasing polymer concentration. However, for some of these polymers, fiber diameter did not continue to increase at the highest polymer concentrations. These exceptions include PDO/PGA and PDO/PGA/PLA, which were not statistically significant from the 120 mg/mL fiber diameters at their highest respective concentrations. When either co-polymer was fabricated at a concentration higher than 150 mg/mL, wet, fused fibers were formed. This implies that maximum fiber diameter for PGA co-polymers may not be as tailorable by changing polymer concentration as for other polymers. Additionally, each polymer was characterized by a unique range of fiber diameters at its low, intermediate, and high concentrations with PDO/PLA yielding consistently smaller fibers and PDO/PGA/PLA yielding consistently larger fiber diameters than PDO.

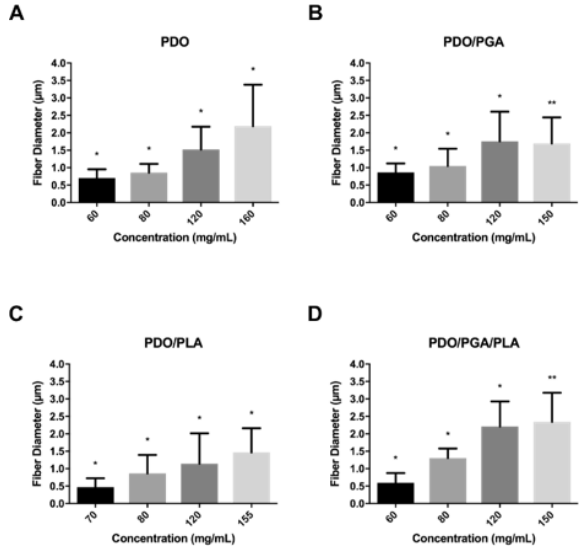

Figure 5. Fiber diameter vs. concentration histograms for (A) PDO, (B) PDO/PGA, (C) PDO/PLA, and (D) PDO/PGA/PLA at concentration. * indicates a significant difference from all other concentrations. ** indicates a significant difference from all concentrations except 120 mg/mL ($p < 0.05$).

Mechanical Testing Data

Uniaxial tensile was performed on dogbone-shaped punches oriented parallel and perpendicular ($n \geq 7$ each orientation) to the axis of rotation of each polymer at each concentration. Each sample was tested to failure to generate stress-strain data, which was used to determine UTS (Figure 6), Young's Modulus (Figure 7), and maximum-percent elongation (Figure 8). As shown in Figure 6, the mechanical strength of PDO and PDO/PGA templates do not show a patterned correlation between UTS and concentration. PDO/PLA and PPP templates exhibit a significant negative correlation between UTS and concentration. Additionally, there were more significant differences between the axial and perpendicular groups for PDO/PLA and PPP compared to PDO and PDO/PGA, suggesting that the UTS of electrospun PLA co-polymers may be orientation-dependent.

For Young's Modulus, shown in Figure 7, there were few significant trends between polymers or between concentrations of the same polymer. However, potentially due to deposition patterns, Young's modulus of 120 mg/mL PDO was significantly greater than all other PDO templates. By visually inspecting the SEM images for PDO (Figure 1), a more curled, overlapped fiber morphology for PDO 120 mg/mL can be seen. These overlapped fibers may have functioned as additional supports in the template, thereby increasing the yield stress of that template. Additionally, PDO was the only polymer that showed orientation-specific statistical significance, which was shown at both 80 mg/mL and 120 mg/mL.

Results for maximum percent elongation (Figure 8) show testing orientation-dependent results for PDO and each of its co-polymers. Axial data for each polymer displayed significantly larger strain capabilities for PDO/PLA at three concentrations, for PDO and PDO/PGA at two concentrations, and for PPP at one concentration as compared to their corresponding perpendicular data. Additionally, the data below consistently display greater maximum percent elongation for PDO co-polymers than for PDO alone (excluding perpendicular PDO/PLA at 70 mg/mL and 155 mg/mL). No consistent concentration-dependent trends were apparent in these data, which may indicate that maximum template strain is not fiber-diameter-dependent.

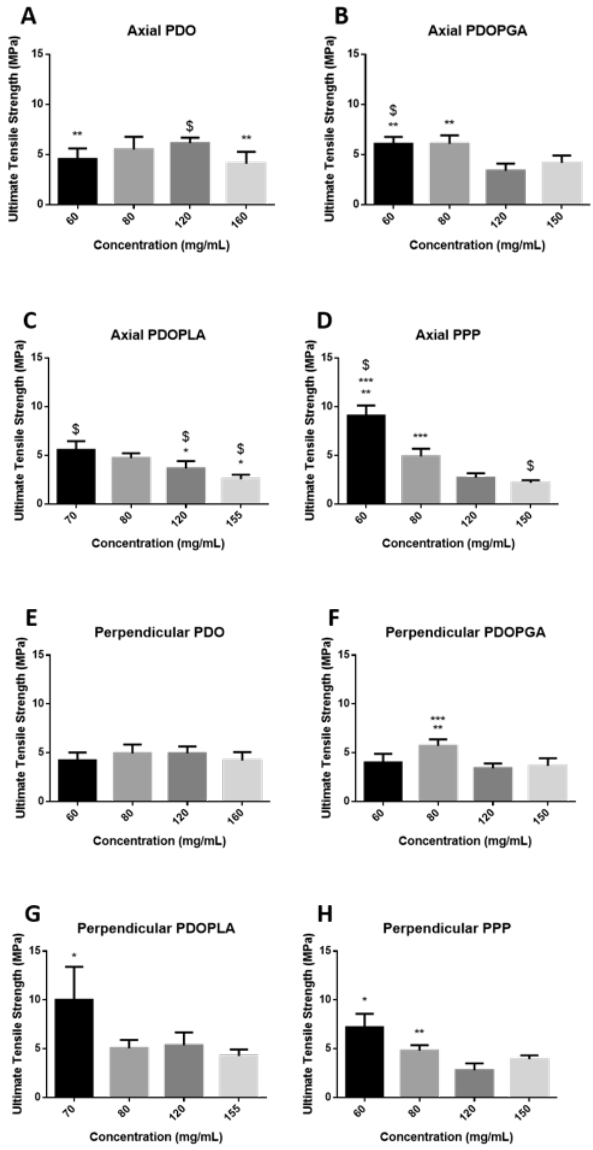

Figure 6. Ultimate tensile strength vs. concentration for each polymer at each concentration (Axial data A-D; Perpendicular data E-H). * indicates a significant difference from all other concentrations. ** indicates a significant difference from 120 mg/mL. *** indicates a significant difference from 150 mg/mL. $ indicates a significant difference between axial and perpendicular ultimate tensile strength of the same template (i.e. orientation specific statistical significance) ($p < 0.05$).

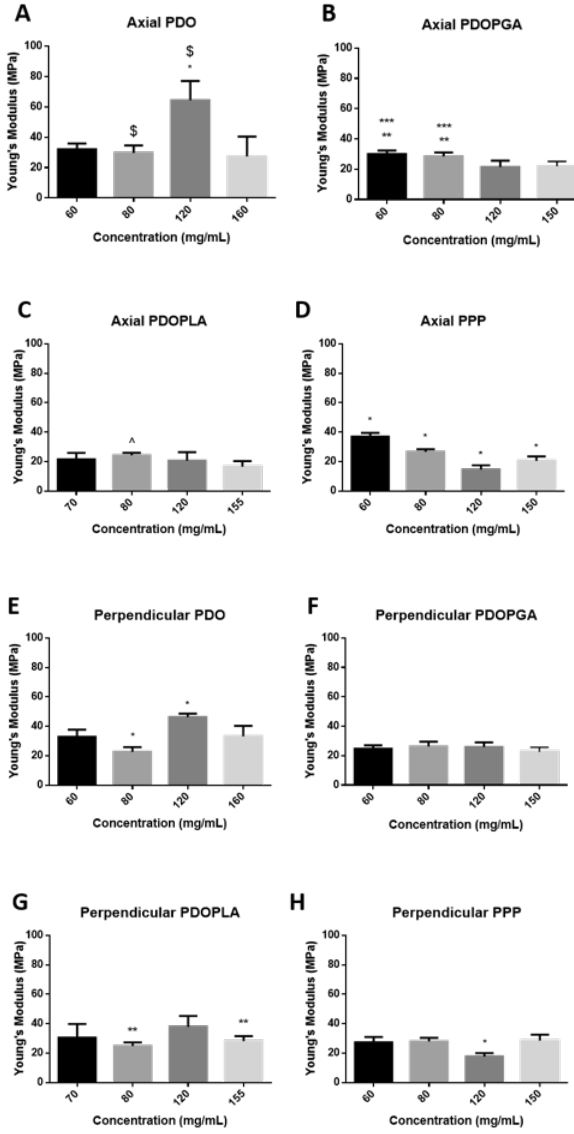

Figure 7. Young's modulus vs. concentration for each polymer at each concentration (Axial data A-D; Perpendicular data E-H). * indicates a significant difference from all other concentration. ** indicates a significant difference from 120 mg/mL. *** indicates a significant difference from 150 mg/mL. ^ indicates a significant difference from 155 mg/mL. $ indicates orientation specific statistical significance between axial and perpendicular Young's Modulus of the same template ($p < 0.05$).

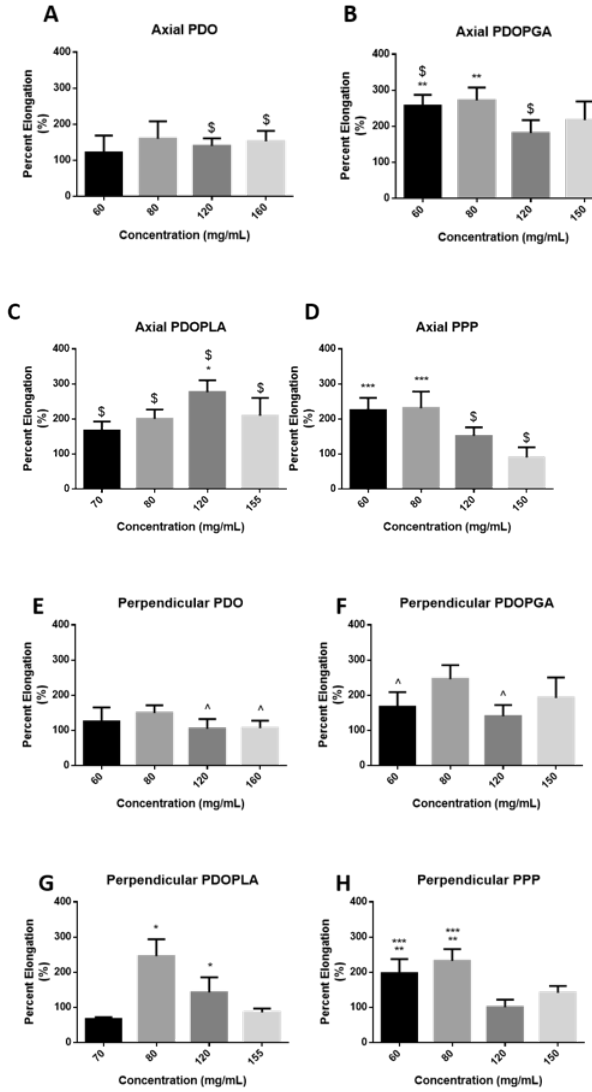

Figure 8. Maximum percent elongation vs. concentration for each polymer at each concentration (Axial data A-D; Perpendicular data E-H). * indicates a significant difference from all other concentrations. ** indicates a significant difference from 120 mg/mL. *** indicates a significant difference from 150 mg/mL. ^ indicates a significant difference from 80 mg/mL. $ indicates orientation-specific statistical significance between axial and perpendicular elongation data of the same template ($p < 0.05$).

Discussion

The goal of this project was to create electrospun templates of PDO co-polymers that were biomimetic of natural ECM and possess variable mechanical properties which could withstand the biomechanical forces required of different biological tissues. As displayed in the above results, each co-polymer can be electrospun over a range of concentrations to create scaffolds of tailorable physical and mechanical properties. Each polymer exhibited fabrication parameters similar to those of PDO as shown in Table 1. Concentration ranges, however, did have to be reduced for each co-polymer. This indicates a reduction in electrospinning breadth when PDO is co-polymerized. Despite these variations in concentration range, each polymer maintained a positive correlation between increasing polymer concentration and increasing fiber diameter. Exceptions included PDO/PGA and PPP which could not be fabricated at their highest concentration to produce large diameter templates that were significantly different from their corresponding 120 mg/mL templates. This disparity between highest polymer concentration and largest fiber diameter may indicate that electrospun co-polymers with PGA plateau in fiber diameter at a certain concentration threshold. Additionally, each polymer was characterized by a unique range of fiber diameters at low, intermediate, and high concentrations. PDO/PLA yielded consistently smaller fibers than PDO, while PPP yielded consistently larger dimeter fibers than PDO. Together these data indicate that a range of concentrations can be fabricated for each PDO co-polymer to create templates of tailorable fiber diameter.

In addition, mechanical testing revealed distinct properties among the different polymers. The UTS for PDO was consistently near 5 MPa for both axial and perpendicular orientation samples. These values for UTS did not display polymer concentration (thus fiber diameter) dependency. Similarly, PDO/PGA did not show a consistent trend between UTS and concentration, but the UTS values were lower than those of PDO alone. For co-polymers that included PLA, a general negative correlation was observed between UTS and concentration. Maximum UTS averages for each of these co-polymers were approximately twice that of the highest PDO and PDO/PGA UTS averages. Differences were further manifest for PLA co-polymers by considering the statistically significant differences between perpendicular- and axial-oriented punches. PDO and PDO/PGA only demonstrated significantly different UTS data for axial- and perpendicular-orientation punches for one concentration each. PDO/PLA and PPP templates displayed orientation-specific significance for three and two concentrations, respectively. These polymer specific differences may be due

to the use of PLA as a building block of each co-polymer, the effect of this, however, needs further investigation.

Unlike UTS, Young's Modulus did not vary considerably for each polymer. Two concentrations of PDO exhibited orientation-specific Young's Moduli, but these were the sole templates which exhibited this orientation dependency. Excluding the 120 mg/mL PDO sample, all PDO and PDO co-polymers templates had similar Young's Moduli with template averages ranging from 16.16 ± 2.15 MPa and 32.48 ± 0.57 MPa. As these co-polymers are 90% PDO, these results indicate that co-polymerization does not impact template stiffness at such small concentrations of PGA and PLA.

Percent elongation data varied vastly from polymer to polymer. PDO had the lowest average percent elongation, which was 50% less than those of the co-polymers, indicating that co-polymerizing PDO with PGA and/or PLA increases the maximum possible strain for electrospun templates, potentially due to differences in PLA and PGA monomer structure from PDO such as crystallinity. Percent elongation results also indicate greater direction dependency for each polymer compared to UTS and Young's Modulus. At least two concentrations of each polymer displayed orientation-specific significance differences with a trend towards greater percent elongation for axial punches compared to the corresponding perpendicular punches. This indicates the likely presence of some fiber anisotropy in each electrospun template as the axially-oriented punches consistently had greater maximum strain than perpendicular punches from the same template. Another possible confounder which may have caused this direction-dependency could be deposition differences on the periphery of a rectangular mandrel compared to the center of the mandrel. Fibers travel slightly further in the electrospinning process to come in contact with the center of the mandrel than with the rest of the mandrel. This slight difference may have created an area of weakness in the center of each punch taken perpendicular to the axis of rotation by decreasing fiber deposition at these loci, but it would not create a corresponding area of weakness in punches taken parallel to the axis of rotation. Together these data indicate that electrospun PDO co-polymers exhibit some mechanical characteristics that are unique and some characteristics that are shared with electrospun PDO.

Since different physiological tissues are subjected to different loads, stress, and strains, it is imperative that tissue engineered constructs exemplify different mechanical properties for the different applications that they will have. Electrospun constructs have utility in everything from vascular grafts to cartilage regeneration and dental implants. These unique force requirements of each tissue are dependent on mechanical characteristics

like those considered in this project. The variations in UTS and maximum percent elongation exemplified by these co-polymers of PDO indicate that each template is mechanically unique, and therefore may be ideal for a certain application which is dependent on these mechanical properties.

Conclusion

This project demonstrated that co-polymers of PDO from Bezwada Biomedical LLC can be electrospun for tissue engineering applications. The resultant electrospun templates of each PDO co-polymer share some mechanical properties, such as Young's Modulus, with PDO, but they have some properties such as UTS and maximum percent elongation that differ. This work holds significance for the field of guided tissue regeneration in that it demonstrates that three new PDO co-polymers have mechanical properties that are polymer-specific and tailorable to specific applications and physiological needs. Ultimately, these electrospun co-polymers of PDO may more accurately meet the stress-strain demands required for certain application than any currently available electrospun polymers.

Funding

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