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A Comparison of Degradable Synthetic Polymer Fibers for Anterior Cruciate Ligament Reconstruction

Nick Tovar^{1,2}, Sharon Bourke³, Michael Jaffe⁴, N. Sanjeeva Murthy³, Joachim Kohn³, Charles Gatt¹, and Michael G. Dunn¹

¹Orthopaedic Research Laboratory, UMDNJ-RWJMS, New Brunswick, New Jersey, USA

²Department of Biomedical Engineering, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA

³Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, Piscataway, New Jersey, USA

⁴Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, New Jersey, USA

Abstract

We compared mechanical properties, degradation rates, and cellular compatibilities of two synthetic polymer fibers potentially useful as ACL reconstruction scaffolds: poly(desaminotyrosyl-tyrosine dodecyl dodecanedioate)(12,10), p(DTD DD) and poly(L-lactic acid), PLLA. The yield stress of ethylene oxide (ETO) sterilized wet fibers was 150 ± 22 MPa and 87 ± 12 MPa for p(DTD DD) and PLLA, respectively, with moduli of 1.7 ± 0.1 MPa and 4.4 ± 0.43 MPa. Strength and molecular weight retention were determined after incubation under physiological conditions at varying times. After 64 weeks strength decreased to 20 and 37% of the initial sterile fiber values and MW decreased to 41% and 36% of the initial values for p(DTD DD) and PLLA, respectively. ETO sterilization had no significant effect on mechanical properties. Differences in mechanical behavior may be due to the semicrystalline nature of PLLA and the small degree of crystallinity induced by mesogenic ordering in p(DTD DD) suggested by DSC analysis. Fibroblast growth was similar on 50-fiber scaffolds of both polymers through 16 days *in vitro*. These data suggest that p(DTD DD) fibers, with higher strength, lower stiffness, favorable degradation rate and cellular compatibility, may be a superior alternative to PLLA fibers for development of ACL reconstruction scaffolds.

1. INTRODUCTION

The knee is the largest and most complex joint in the human body. Its stability is largely dependent on the ability of the anterior cruciate ligament (ACL) to resist anterior tibial translational loads (primary) and rotational loads (secondary) 1· 2. The ACL is composed of

Corresponding Author: Nick Tovar Graduate Student Department of Orthopaedic Surgery UMDNJ-RWJMS 51 French Street, PO Box 19 Orthopaedic Surgery Room 411 New Brunswick, NJ 08903 Phone: 732-235-8720 nicktovar@gmail.com. Sharon Bourke, Ph.D. 1 Millennium Way Branchburg, NJ 08876 908-947-1172 SBourke@lifecell.com. Michael Jaffe, Ph.D. Professor Medical Device Concept Laboratory 111 Lock Street CHEN Building Room 202 Newark, NJ 07103 973-596-6497 Michael.Jaffe@njit.edu. N. Sanjeeva Murthy, Ph.D. Associate Research Professor New Jersey Center for Biomaterials 145 Bevier Road Piscataway, NJ 08854 732 445 0488 fax 732 445 5006 murthy@biology.rutgers.edu. Joachim Kohn, Ph.D. Director New Jersey Center for Biomaterials 145 Bevier Road Piscataway, NJ 08854 732 445 0488 fax 732 445 5006 joachim@rutchem.rutgers.edu. Charles Gatt, M.D. Chairman and Program Director Department of Orthopaedic Surgery UMDNJ-RWJMS 51 French Street, PO Box 19 Orthopaedic Surgery Room 425 New Brunswick, NJ 08903 Phone: 732-235-7869 gattcj@umdnj.edu. Michael G. Dunn, Ph.D. Associate Professor Department of Orthopaedic Surgery UMDNJ-RWJMS 51 French Street, PO Box 19 Orthopaedic Surgery Room 424 New Brunswick, NJ 08903 Phone: 732-235-7972 dunnmg@umdnj.edu.

dense fibrous connective tissue that attaches the femur to the tibia 2–5. It has a large amount of collagen fibers arranged in a hierarchical pattern giving it high tensile strength 6–7. This organization, however, has its limitation and will tear under high tensile and torsional forces 8–9. ACL tears will not heal without surgical intervention due to low blood supply and ligament retraction from the synovial tissue that envelops a tear 10–12.

Surgical reconstructions of the ACL using an autograft or allograft have been an option for the last 30 years. Autogenous tissue for ACL reconstruction has been associated with knee pain, donor site weakness and decreased range-of-motion 13–14. Allogenic tissue concerns include risk of disease transmission and immunogenic response by the host 15–16. Both autograft and allograft surgical techniques have successful short-term results, but five-year follow-ups show that patients have instability and pain 17. Permanent synthetic grafts used for ACL reconstructions have also been of interest, but are now seldom in use 18. They had satisfactory initial strength, but were prone to rupture and failure due to material degradation, wear debris, foreign-body inflammation and synovitis 19–22. The shortcomings of autografts, allografts and permanent synthetic grafts have encouraged the investigation of a tissue engineered approach to heal the ACL 20–23–27.

The tissue engineering approach involves implantation of a degradable ACL reconstruction scaffold to temporarily support mechanical loads and promote tissue ingrowth as the scaffold degrades, leading to regeneration or restoration of the ACL. Some of the requirements of an ACL reconstruction scaffold are cell compatibility, degradability, and functional strength 23. As neoligament tissue forms along the scaffold and the fibers gradually degrade and lose strength, neoligament tissue should bear the mechanical loads and remodel. This study is a continued step towards formulating and testing such a device.

In addition to our extensive work on collagen fibers, our laboratory is comparing fibers from two degradable synthetic polymers, poly(desaminotyrosyl-tyrosine dodecyl dodecanedioate) (12,10), p(DTD DD) and poly(L-lactic acid), PLLA, for ACL reconstruction devices 28–30. Compared to biological materials such as collagen, processing conditions of synthetic polymer fibers allow for greater control over uniformity and mechanical properties 31. PLLA, a semicrystalline polymer, is currently one of the most widely investigated and commonly used polymers for medical applications 32. But, there are clearly established disadvantages when a massive PLLA device comes in contact with bone as in the case of an ACL scaffold. As PLLA degrades the byproducts, such as lactic acid and debris, can contribute to local pH changes and bone resorption 33–38. This debris has been identified up to eight years after implantation. PLLA can be made into a strong material by increasing its crystallinity, but highly crystalline PLLA is known to cause foreign body reactions 39. The purpose is therefore to identify materials that could replace PLLA while providing similar mechanical performance and degradation properties. P(DTD DD), first developed by Kohn's group, is a tyrosine-derived polyarylate with low crystallinity and some liquid crystalline characteristics 40. It shows complex phase behavior that could be used to obtain a broader range of properties than that obtainable from PLLA 41–43. It also has no known toxic degradation products 44–45. This suggests that p(DTD DD) may be a good replacement for PLLA while having the potential for better overall cell compatibility - especially during long-term degradation.

We hypothesize that p(DTD DD) (a) is capable of withstanding processing conditions to form fibers whose mechanical properties and degradability are not affected by sterilization; (b) fibers have yield loads and moduli within the range required to construct a tissue engineered scaffold for clinical use; (c) fibers can sustain fibroblast attachment and growth. PLLA fibers were fabricated and used for comparison since PLLA is a well-studied degradable polymer with a history of use in FDA-approved devices. In order to investigate

our hypothesis we focused on three objectives. The first objective was to compare the initial mechanical properties of p(DTD DD) and PLLA fibers, and determine effects of ethylene oxide (ETO) sterilization on fiber properties. The second objective was to compare strength retention and molecular weight (MW) retention for sterile and nonsterile fibers incubated in physiological conditions for various times. Differential scanning calorimetry (DSC) was also performed to compare changes in the melting and crystallization curves. The third objective was to compare cell attachment and growth on sterile p(DTDD DD) and PLLA fiber scaffolds. By comparing initial mechanical properties, strength retention, MW retention, and cell compatibility of p(DTD DD) and PLLA fibers, we determined the relative potential of developing these materials into degradable ACL reconstruction scaffolds.

2. MATERIALS AND METHODS

2.1. Polymer source

P(DTD DD) was purchased from TyRx Pharma, Inc. (formerly Advanced Material Design) (Lot # AMD/SP11292001, Monmouth Junction, NJ). Medical grade PLLA (Resomer L-297, Lot #2/0076) in pellet form was purchased from Boehringer Ingelheim (Henley Division, Montvale, NJ).

2.2. Fiber processing and properties

Fibers were melt spun using a James plunger fed micromelt spinner (Charlotte, NC) with a single 1 mm diameter die, located at the Medical Device Concept Laboratory of the New Jersey Center for Biomaterials (Newark, NJ). The process as described by Jaffe *et al* 31 requires polymers to dry in a vacuum oven for 24 hours at 120 °C under flowing N₂. The polymers were then placed in the barrel gradually heated from 100 °C to 140 °C, and 160 °C to 200 °C, for p(DTD DD) and PLLA, respectively. The molten polymer, forced through the die, solidifies under ambient temperature under a take up speed of 96 m/min and 72 m/min for p(DTD DD) and PLLA, respectively. The fibers were then spun by 2 drums. For p(DTD DD) the first drum speed was 4.0 m/min and the second drum speed was 8 m/min at 50 °C. For PLLA the first drum speed was 3.0 m/min and the second drum speed was 10 m/min at 90 °C. For each polymer, three individual fiber lots were fabricated under identical processing conditions, and data from the 3 lots were pooled. All fibers were supplied in spool form.

2.3. Fiber sterilization and incubation

An Anprolene Sterilization Tray System (Model AN72C, Andersen Products, Haw River, NC) with 5 cc ampules of ETO (Model AN71, Andersen Products, Haw River, NC) was used to sterilize p(DTD DD) and PLLA fibers. ETO ampules had a molecular weight of 44.06 g/mole. The Anprolene sterilization cycle was 12 hours with 2 additional hours for purging the liner bag. The entire sterilization cycle took place at room temperature under a ventilated hood to ensure that the operator was not exposed to more than the permitted level. Dosage was verified with an Exposure Indicator (Model AN85, Andersen Products, Haw River, NC). Post-sterilization aeration of the materials was performed in a vacuum chamber at room temperature for 2 weeks to remove any residual ETO. Sterile and non-sterile single fibers of p(DTD DD) and PLLA were then incubated in phosphate buffered solution (PBS) with a pH of 7.4 at 37 °C for intervals of 0, 0.01, 1, 2, 4, 8, 16, 24, 32 and 64 weeks. Since this was the first time these fibers were subjected to degradation characteristics, we tested them for over a year and collected data to represent long, medium and short term degradation time points.

2.4. Single fiber mechanical testing and characterization

A modified version of American Society for Testing and Materials (ASTM) D3822, Standard Test Method for Tensile Properties of Single Textile Fibers, was used to mechanically load individual fibers until failure. P(DTD DD) fibers (sterile n=15 and non-sterile n=15) and PLLA fibers (sterile n=15 and non-sterile n=15) were initially tested in a dry (control) environment. P(DTD DD) fibers (sterile n=15 and non-sterile n=15) and PLLA fibers (sterile n=15 and non-sterile n=15) incubated from 0.01 to 64 weeks were also tested in a wet (environmental chamber filled with distilled water at 37 °C) environment. Fiber diameters were measured using a laser micrometer (Z-mike model 1202B, Dayton, OH) prior to testing. Fibers had a 50 mm gauge length and were elongated mechanically at a displacement rate of 30 mm/min until failure using a MTS model 658.25 (MTS Systems Corporation, Eden Prairie, MN).

A DSC machine (Universal V2.6D TA Instruments) was used to calculate the melting temperature (T_m) and crystallization temperature (T_c) for p(DTD DD) fibers (sterile n=6 and non-sterile n=6) and PLLA (sterile n=6 and non-sterile n=6) fibers. Gel permeation chromatography (GPC) (1100 series, Hewlett Packard, CA) in tetrahydrofuran (THF) calibrated with polystyrene standards was used to determine MW of p(DTD DD) single fibers (sterile n=6 and non-sterile n=6) and PLLA single fibers (sterile n=6 and non-sterile n=6). This chromatographic system consists of a Waters model 410 pump, a Waters model 410 refractive index detector, and a PerkinElmer model 410 computerized data station equipped with Millenium software (Waters). Two GPC columns (pore size 10^5 and 10^3 Å, 30 cm in length) were operated in series at a flow rate of 1 ml/min in THF.

2.5. Scaffold preparation and cell seeding

Sterile p(DTD DD) scaffolds (N=3) and PLLA scaffolds (N=3) consisting of 50 parallel fibers were used to test for cell compatibility through *in vitro* fibroblast attachment and growth. This scaled-down scaffold restricts material use, time of preparation and is approximately 1/100 the size of an ACL reconstruction device in a human model. Scaffolds were tied at ends with cotton gauze thread to maintain uniformity. The total length of the scaffold was 1.5 cm with 1 cm length between threads. All scaffolds were ETO sterilized and aerated for 2 weeks post-sterilization. 24 hours prior to cell seeding sterilized p(DTD DD) and PLLA scaffolds were soaked in Dulbecco's Modified Eagle Medium (DMEM; Sigma St. Louis, MO), supplemented with 10% fetal bovine serum, 1% glutamine, 1% Hepes buffer solution, 2% antibiotic/antimycotic, and 0.4% gentamicin (complete media).

Full dermis skin samples were harvested from the hinder part of a New Zealand White rabbit using general anesthesia and sterile surgical procedures per Institutional Animal Care and Use Committee (IACUC) approved procedures. Samples were placed in betadine scrub for 10 minutes and transferred via cold Hanks Buffered Saline Solution (HBSS; Sigma, St. Louis, MO). To isolate fibroblast cells, samples were then placed in 6-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) with 8 ml of filtered sterile dispase solution (HBSS w/o Mg^{2+} & Ca^{2+} , dispase powder (Invitrogen, Grand Island, NY, 35 units/ml)) for 16 hours at 4 °C. Dispace activity was then stopped with Iscove's Modified Dulbecco's Medium (Hyclone, Logan, UT) and the tissues were minced with a sharp blade and placed into 250 ml Tissue Culture Flask (Becton Dickinson, Franklin Lakes, NJ) along with 200 units/ml of collagenase (Sigma, St. Louis, MO) and complete media. Flasks were incubated at 37 °C in a 5% CO_2 incubator and used within the second passage. Sterile p(DTD DD) and PLLA scaffolds were placed in untreated 24-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ). Seeding was performed by pipetting a concentrated cell suspension of 1.0×10^5 cells/20 μ l of complete media, further supplemented with 50 μ g/ml of L-ascorbic acid, along the length of the scaffold. To prevent disruption of cells from the scaffold during

the initial seeding, cells were allowed to attach for 4 hours prior to applying complete media. Complete media with L-ascorbic acid was then added to bring the total volume to approximately 1 ml. Medium was changed every other day.

2.6. Scaffold cell compatibility measurement

In order to quantitatively analyze *in vitro* cell attachment and growth, 3-(4,5-dimethyl-thiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Cell Titer 96, Promega, Madison, WI) was used. MTS assay is a colorimetric method in which metabolically active cells react with MTS tetrazolium compound and convert it into formazan dye. This dye is absorbed at 490 nm and when compared to a standard curve is directly proportional to the number of viable cells. MTS tetrazolium compound was added to all plates and incubated for 3 hours to allow for equilibration of the dye before absorbencies were read by an Emax Precision Microplate Reader using SOFTmax Plate Reader 1993 software (Molecular Devices Corp., Sunnyvale, CA). Cellular attachment and growth were determined 4 hours after seeding (initial attachment) and after 4, 8 and 16 days of incubation *in vitro* (N=3). Four days was chosen to allow cells time to proliferate and subsequent time points were doubled to obtain a growth curve on the materials.

2.7. Fluorescent labeling

Fluorescent labeling is a qualitative method for analyzing the cells on the scaffolds. Viable cells were labeled with PKH26-GL (Sigma, St. Louis, MO), a red fluorescent lipophilic dye that emits at a wavelength of 567 nm. The manufacturer's suggested protocol was followed. PKH26-GL stains the membrane of viable cells and is distributed amongst cells when mitosis occurs. Sterile p(DTD DD) and PLLA fiber scaffolds were placed in 24-well tissue culture plates and seeded with labeled cells by pipetting a concentrated cell suspension of 1.0×10^5 cells/20 μ l along the length of the scaffold. The scaffolds were incubated in tissue culture plates for 4 hours to allow cell attachment. Complete media was added to bring the total volume to approximately 1 ml. Labeled cells were visualized using a Nikon Eclipse TE300 inverted fluorescent microscope (Micro Optics, Cedar Knolls, NJ) with imaging software (IPLabs Image Analysis, Fairfax, VA).

2.8. Statistical analysis

Table data and graphs are presented in the form of mean $\pm$ standard deviation. Comparison among control, sterile and non-sterile groups was performed using a one-way analysis of variance (ANOVA) ($p < 0.05$) to determine significant differences. Comparison between the 2 polymer types were performed with an unpaired Student's t-test (mean) ($p < 0.05$) to determine significant differences.

3. RESULTS

3.1. Objectives 1 and 2: Initial mechanical properties and *in vitro* degradation studies

The mechanical properties of single fibers in dry (control) and PBS environments are summarized in Table 1. P(DTD DD) and PLLA fiber batches had an average diameter of 91 ± 2 and 88 ± 3 μ m, respectively. No statistically significant differences were detected in fiber diameters ($p < 0.05$). Mechanical testing of control sterile single fibers resulted in an average yield stress of 184 ± 12 MPa (1.09 ± 0.09 N) and 118 ± 9 MPa (0.68 ± 0.08 N) for p(DTD DD) and PLLA, respectively. Yield stress values for both sterile polymer fibers remained over 100 MPa up to 24 weeks. At 32 weeks, p(DTD DD) and PLLA fiber yield strengths decreased to 49% (91 ± 9 MPa; $p = 0.003$), and 71% (84 ± 7 MPa; $p = 0.003$), of their initial values, respectively. After 64 weeks of incubation fiber strength decreased to 20% and 37% of their initial values for sterile p(DTD DD) and PLLA fibers (Figure 1).

Initial moduli for control dry sterile p(DTD DD) and PLLA fibers were 2.6 ± 0.3 and 5.2 ± 0.3 GPa, respectively. When tested wet (at 37°C) the moduli decreased to 65%, 1.7 ± 0.1 ($p < 0.05$), and 86%, 4.4 ± 0.3 ($p = 0.009$) GPa, of their initial dry values, respectively. There was a significant difference between the moduli for sterile p(DTD DD) at the 32 and 64 week time points as it increased from 1.7 ± 0.2 to 2.7 ± 0.4 GPa ($p = 0.001$).

There were no statistically significant differences between the mechanical properties of sterile and non-sterile fibers. Average control yield stress values of non-sterile p(DTD DD) and PLLA fiber were 191 ± 11 ($p = 0.981$) and 125 ± 13 MPa ($p = 0.828$), respectively. After 64 weeks of incubation fiber strength decreased to 29% and 31% for the non-sterile p(DTD DD) and PLLA fibers, respectively (Figure 2).

Similar to yield stress values, a general downward trend was observed with MW vs. incubation time, as expected. There was a significant difference between the MW at the 32 (53 ± 3 kDa) and 64 (35 ± 2 kDa) week time points for sterile p(DTD DD), $p = 0.001$, and between 24 (97 ± 15 kDa) and 64 (53 ± 17 kDa) week time points for sterile PLLA fibers, $p = 0.017$. After 64 weeks sterile p(DTD DD) and PLLA MW values decreased to 41% and 36% and non-sterile polymers decreased to 39% (43 ± 15 kDa) and 30% (35 ± 2 kDa) of the initial MWs, respectively (Figure 3 and 4).

There was a significant decrease in T_c for sterile p(DTD DD) from 39.8 ± 0.1 to 38 ± 0.1 $^\circ\text{C}$ ($p = 0.001$) between the 32 and 64 week time points. There was also a significant increase in T_m for sterile p(DTD DD) from 59.1 ± 2.6 to 63.5 ± 0.2 $^\circ\text{C}$ ($p = 0.01$). This increase in T_m contrasts with the decrease in T_m observed in sterile PLLA. Figures 5 and 6 show the DSC scans of sterile p(DTD DD) and PLLA fibers at 16 weeks, respectively. The DSC of PLLA is straightforward; during heating there is a T_g at 60.3 $^\circ\text{C}$, cold crystallization at 99.5 $^\circ\text{C}$ and T_m at 174.7 $^\circ\text{C}$; during cooling there is melt crystallization at 101.6 $^\circ\text{C}$ and T_g at 56.7 $^\circ\text{C}$. The T_m of PLLA decreased from 174.2 ± 0.2 to 168.8 ± 3.2 $^\circ\text{C}$ ($p = 0.043$) between the 32 and 64 week time point. The interpretation of DSC scan of p(DTD DD) is more complex because of the liquid crystalline characteristics that could be present in this polymer 41–43. The main melting at 55.5 $^\circ\text{C}$ is followed by secondary melting at 66.9 $^\circ\text{C}$, beyond which the polymer loses its birefringence and remnants of any order. Although p(DTD DD) passes through various types of ordering as it is heated into the isotropic state, the main endothermic peak at 55.5 $^\circ\text{C}$ can be regarded as the main melting peak, T_m . Upon cooling, the polymer crystallized at 39.3 $^\circ\text{C}$.

3.2. Objective 3: Fibroblast growth on scaffolds *in vitro*

Rabbit skin fibroblasts attached and proliferated on both p(DTD DD) and PLLA scaffolds after cell seeding and incubation in a complete media solution (Figure 7). Initial cell attachment values after 4 hours were 500 ± 500 and 1800 ± 2600 cells for p(DTD DD) and PLLA fiber scaffolds, respectively. Viable cells labeled with red fluorescent dye (Figure 8) were seen distributed throughout the fiber scaffold. After 16 days $20,000 \pm 18,000$ and $23,000 \pm 19,000$ cells were attached to p(DTD DD) and PLLA scaffolds, respectively. There was a 3 fold increase in cell number for both p(DTD DD) and PLLA scaffolds between the 4 and 16-day time points according to MTS Assay readings and there was a significant difference between the average values between the 4 hour and 16 day time points ($p = 0.021$) (Figure 7). There was no significant difference ($p = 0.305$) found between the number of cells populating p(DTD DD) and PLLA scaffolds after 16 days.

4. DISCUSSION

The purpose of this study was to evaluate the performance of p(DTD DD) fibers as a possible biomaterial for an ACL reconstruction scaffold, with results compared to fibers

made from PLLA, a well-studied degradable polymer with a history of use in FDA-approved devices. We hypothesized that p(DTD DD) (a) is capable of withstanding processing conditions to form fibers whose mechanical properties and degradability are not affected by sterilization; (b) fibers have yield loads and moduli within the range required to construct a tissue engineered scaffold for clinical use; (c) fibers can sustain fibroblast attachment and growth. Results of this study support this hypothesis.

Fibers were fabricated from raw polymers by a melt spinning process. Ideal processing would result in a fiber of sufficient strength and cell compatibility to support ACL reconstruction. Fiber strength depends primarily on the polymer structure, MW, alignment of polymer chains along the load axis, and crystallinity, which in turn depend on processing conditions such as temperature and draw ratio. When a single fiber in a scaffold breaks, the load originally carried by this fiber is transferred to the intact fibers. This sudden increase in load promotes more fiber breaks in the scaffold which is dependent on the distribution of fibers. As pointed out by Peirce *et al* 46, we cannot assume that the strength of a scaffold is equal to the average strength exhibited by the component fiber. Assuming fibers follow the two-parameter Weibull distribution, we calculated the number of fibers needed in a scaffold to sustain loads typically seen on a rabbit 47 and human 48 ACL. Numerous researchers have successfully used a rabbit model to study various orthopaedic applications 29, 49–54. This calculation was attained by using Chi's load strain formula for a bundle, based on Coleman's survival function for a single filament to a bundle 55, 56:

$$P(\varepsilon) = AE_f \varepsilon N_o \exp \left[-L \left(\frac{\varepsilon}{\varepsilon_o} \right)^m \right]$$

Where $P(\varepsilon)$ is the tensile load, A is the cross-sectional area of a single fiber, E_f is young's modulus for a single fiber, N_o is the fiber count, ε is the applied strain, L is the fiber length. The Weibull shape parameter m and scale parameter ε_o were measured to be 1.45 and 2.25, respectively, using 500 fiber bundle mechanical data, as shown by Chi 55. Inserting the Weibull parameters back into the equation it can be calculated that p(DTD DD) fiber scaffolds composed of 750 fibers (cross-sectional area of 4.5 mm²) would potentially sustain loads of a rabbit ACL, 360 ± 20 N 47. For a human ACL with a breaking load of 2160 ± 157 N 48, a scaffold composed of 5000 fibers (cross-sectional area of 29.7 mm²) would potentially suffice.

The strength retention and MW of non-sterile and ETO sterilized fibers were evaluated through 64 weeks of incubation in PBS with a pH of 7.4 at 37 °C. It was found that ETO sterilization did not significantly affect p(DTD DD) or PLLA fiber mechanical properties and MW. Strength retention decreased to 20 and 37% and MW decreased to 41 and 36% for ETO sterilized p(DTD DD) and PLLA fibers, respectively. P(DTD DD) yield stress values remained significantly higher ($p=0.01$) up to the 8 week time point in which yield stress values were 49% higher than PLLA, 128 ± 7 MPa versus 86 ± 12 MPa. The decrease in T_c of p(DTD DD) between the 32 and 64 week time points is consistent with the observed decrease in yield strength, and could be attributed to the decrease in the molecular weight due to degradation. Lower T_c and the increased T_m could both be indicative of the mesogenic ordering of the p(DTD DD) chains that contributes to the higher modulus at 32–64 week time points. No such ordering is observed in PLLA as indicated by decrease in T_m . While the decrease in T_m could be attributed to decrease in crystalline order (decrease in size or increase in the number of defects) in PLLA, the increase in T_m suggests aging-induced reordering of the mesogenic chains in p(DTD DD). This is consistent with the observed increase in the modulus of p(DTD DD) fibers at the 64 week time point despite a decrease in molecular weight associated with degradation (Table I).

Values at the 64 week time point were used to predict the time in which the strength retention and MW would reach 0%. At 86 and 99 weeks strength retention would reach 0% and 127 and 124 weeks MW retention would reach 0% for p(DTD DD) and PLLA, respectively. The MW of the materials decreased over time due to degradation of the backbone chain, caused by the polymer's hydrophilic sensitivity, which decreased the strength of the polymer. This is ideal since our goal is to have the material degrade, allowing the neoligament tissue to take on the axial load gradually over time. A graphical analysis of strength retention versus MW curve confirmed that the result was linear (Figure 9).

The moduli of the ACL's anteromedial bundle (AMB) and posterolateral bundle (PMB) have been measured at 283 ± 114 and 155 ± 120 MPa, respectively 57. If the modulus of the implant exceeds that of the neoligament, stress shielding may occur and the load-deprived neoligament tissue may not mature. It is therefore important that the moduli be low enough to allow for normal neoligament remodeling, with strengths high enough to protect the scaffold from failure in the early postoperative period. P(DTD DD) was stronger than PLLA and had a significantly lower modulus at both the initial (1.7 ± 0.1 vs. 4.4 ± 0.3 GPa, $p=0.001$) and 64 week (2.7 ± 0.4 vs. 4.5 ± 1.2 GPa, $p=0.005$) time points.

An ACL reconstruction device has to maintain a population of fibroblasts to achieve deposition of new extracellular matrix. We evaluated cell compatibility of a sterile scaffold in complete media by determining cell attachment and growth, which are affected by surface characteristics and geometry 58-59. Results confirmed the cell compatibility, as fibroblasts attached and proliferated on both types of scaffold. Cell attachment at 4 hours showed no significant difference between p(DTD DD) and PLLA. There was an upward trend up to the 16 day time point and once again no significant difference between the two polymers. In contrast when comparing our 16 day time point to the 14 day time point of a 14 collagen scaffold by Caruso *et al*, collagen had approximately three times the population of fibroblasts, but lacked the strength of the synthetic polymers 60. This comparison underscores the challenge of optimizing strength, degradation rate, and cellular compatibility for an ACL reconstruction scaffold.

5. CONCLUSION

These data suggest that p(DTD DD) is able to sustain fibroblast attachment and growth and has higher initial strength and lower modulus than PLLA, which is necessary to support the mechanical requirements typically seen on a human ACL. Although the cellular compatibility of the PLLA and p(DTD DD) are similar, the changes in the mechanical behavior of the two materials during incubation in PBS have different profiles. These differences are attributed to the semicrystalline nature of PLLA and the small degree of crystallinity induced by mesogenic ordering in p(DTD DD). These fundamental structural differences as well as favorable degradation products in p(DTD DD) encourage us to proceed to the next phase, an *in vivo* ACL reconstruction model. We will monitor parameters such as strength retention, tissue ingrowth, and neoligament formation and remodeling as a function of time post-implantation.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.

Figure 8.

Figure 9.

Table 1

Material	Average Properties	Dry (Control)	Saline (Initial)	Saline 64 Weeks
Non-Sterile P (DTD DD)	Yield σ (MPa)	191 ± 11	144 ± 17	55 ± 24
	Yield ϵ (%)	9.8 ± 1.2	9.6 ± 1.4	3.6 ± 2.0
	Young E (GPa)	2.7 ± 0.2	1.8 ± 0.2	2.8 ± 0.8
	MW (kDa)	90 ± 6	85 ± 2	35 ± 2
Non-Sterile PLLA	Yield σ (MPa)	125 ± 13	95 ± 16	38 ± 21
	Yield ϵ (%)	3.4 ± 0.4	2.8 ± 0.6	3.0 ± 2.3
	Young E (GPa)	5.4 ± 0.2	4.8 ± 0.3	4.6 ± 0.8
	MW (kDa)	144 ± 2	145 ± 7	43 ± 15
Sterile P(DTD DD)	Yield σ (MPa)	185 ± 12	150 ± 22	37 ± 20
	Yield ϵ (%)	10.1 ± 1.0	10.5 ± 1.6	2.6 ± 1.2
	Young E (GPa)	2.6 ± 0.3	1.7 ± 0.1	2.7 ± 0.4
	MW (kDa)	86 ± 3	85 ± 3	35 ± 2
Sterile PLLA	Yield σ (MPa)	118 ± 9	87 ± 12	44 ± 23
	Yield ϵ (%)	3.2 ± 0.3	2.8 ± 0.4	1.8 ± 1.1
	Young E (GPa)	5.2 ± 0.3	4.4 ± 0.3	4.5 ± 1.2
	MW (kDa)	148 ± 6	149 ± 5	53 ± 17