A comparison of degradable synthetic polymer fibers for anterior cruciate ligament reconstruction

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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. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res 93A: 738–747, 2010

Key words: ACL reconstruction; tissue engineering; polymer; fiber scaffold; cell compatibility

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 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. Both autograft and allograft surgical techniques have successful short-term results, but 5-year follow-ups show that patients have instability and pain. Permanent synthetic grafts used for ACL reconstructions have also been of interest, but are now seldom in use. They had satisfactory initial strength, but were prone to rupture and failure due to material degradation, wear debris, foreign-body inflammation, and synovitis. The shortcomings of autografts, allografts and permanent synthetic grafts have encouraged the investigation of a tissue engineered approach to heal the ACL.

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. 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(lactic acid), PLLA, for ACL reconstruction devices. Compared to biological materials such as collagen, processing conditions of synthetic polymer fibers allow for greater control over uniformity and mechanical properties. PLLA, a semicrystalline polymer, is currently one of the most widely investigated and commonly used polymers for medical applications. 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. This debris has been identified up to 8 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. 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. It shows complex phase behavior that could be used to obtain a broader range of properties than that obtainable from PLLA. It also has no known toxic degradation products. 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 non-sterile 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(DTD 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.

MATERIALS AND METHODS

Polymer source

p(DTD DD) was purchased from TyRx Pharma (formerly Advanced Material Design) (Lot # AMD/SP1129001, Monmouth Junction, NJ). Medical grade PLLA (Resomer L-297, Lot #2/0076) in pellet form was purchased from Boehringer Ingelheim (Henley Division, Montvale, NJ).

Fiber processing and properties

Fibers were melt spun using a James plunger fed micro-melt 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. requires polymers to dry in a vacuum oven for 24 h 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 three lots were pooled. All fibers were supplied in spool form.

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 MW of 44.06 g/mole. The Anprolene sterilization cycle was 12 h 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.

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) before testing. Fibers had a 50-mm gage length and were elongated mechanically at a displacement rate of 30 mm/minute 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 crystalization 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 Millennium software (Waters). Two GPC columns (pore size \(10^5\) and \(10^3\) \(\AA\), 30 cm in length) were operated in series at a flow rate of 1 mL/min in THF.

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 \(\sim 1/100\) 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 poststerilization. 24 h 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 min 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\(^{2+}\) and Ca\(^{2+}\), dispase powder (Invitrogen, Grand Island, NY, 35 units/mL)) for 16 h at 4°C. Disperse 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 \(1.0 \times 10^5\) cells/20 \(\mu\)L of complete media, further supplemented with 50 \(\mu\)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 h 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.

Scaffold cell compatibility measurement

To quantitatively analyze in vitro cell attachment and growth, 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxy-
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 lipophylic 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 batches 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 μL along the length of the scaffold. The scaffolds were incubated in tissue culture plates for 4 h to allow cell attachment. Complete media was added to bring the total volume to ~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).

TABLE I
Mechanical Properties of Single Fibers in Dry and Saline Environments

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

Statistical analysis

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

RESULTS

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 I. P(DTD DD) and PLLA fiber batches had an average diameter of 91 ± 2 and 88 ± 3 μm, 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 185 ± 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 (Fig. 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 dry (control) values, respectively. There was a significant difference between the moduli for sterile p(DTD DD) fibers 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% of their dry (control) values for the non-sterile p(DTD DD) and PLLA fibers, respectively (Fig. 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 (Figs. 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°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°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°C, cold crystallization at 99.5°C and $T_m$ at 174.7°C; during cooling there is melt crystallization at 101.6°C and $T_g$ at 56.7°C. The $T_m$ of PLLA decreased from 174.2 ± 0.2 to 168.8 ± 3.2°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. The main melting at 55.5°C is followed by secondary melting at 66.9°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

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

NZW rabbit skin fibroblasts attached and proliferated on both p(DTD DD) and PLLA scaffolds after cell seeding and incubation in a complete media solution (Fig. 7). Initial cell attachment values after 4 h were 500 ± 500 and 1800 ± 2600 cells for p(DTD DD) and PLLA fiber scaffolds, respectively. Viable cells labeled with red fluorescent dye (Fig. 8) were seen distributed throughout the fiber scaffold. After 16 days 20,000 ± 18,000 and 23,000 ± 19,000 cells were
attacked 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 h and 16 day time points ($p = 0.021$) (Fig. 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.

**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., 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 and human ACL. Numerous researchers have successfully used a rabbit model to study various orthopaedic applications. 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:

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

where $P(\varepsilon)$ is the tensile load, $A$ is the cross-sectional area of a single fiber, $E_\varepsilon$ is young’s modulus for a single fiber, $N_o$ is the fiber count, $\varepsilon$ is the applied strain, $L$ is the fiber length. The Weibull shape parameter $m$ and scale parameter $\varepsilon_o$ were measured to be 1.45 and 2.25, respectively, using 500 fiber bundle mechanical data, as shown by Chi. 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 mm$^2$) would potentially sustain loads of a rabbit ACL, 360 ± 20 N. For a human ACL with a breaking load of 2160 ± 157 N, a scaffold composed of 5000 fibers (cross-sectional area of 29.7 mm$^2$) 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 MW 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 MW 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 at 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 (Fig. 9).

The moduli of the ACL’s anteromedial bundle (AMB) and posterolateral bundle (PMB) have been
DEGRADABLE SYNTHETIC POLYMER FIBERS

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. Results confirmed the cell compatibility, as fibroblasts attached and proliferated on both types of scaffold. Cell attachment at 4 h 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 cell compatibility study, there was no significant difference in the number of fibroblasts attached to the two polymers. This comparison underscores the challenge of optimizing strength, degradation rate, and cellular compatibility for an ACL reconstruction scaffold.

CONCLUSION

These data suggest that p(DTD DD) fibers are able to sustain fibroblast attachment and growth and has higher initial strength and lower modulus than PLLA fibers, which is necessary to support the mechanical requirements typically seen on a human ACL. Although the cellular compatibility of PLLA and p(DTD DD) fibers 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 postimplantation.

References