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Tissue Engineering of the Anterior Cruciate Ligament Using a Braid-Twist Scaffold Design

Joseph W. Freeman, Ph.D.^{1,4}, Mia D. Woods, B.S.¹, and Cato T. Laurencin, M.D., Ph.D.^{1,2,3}

¹ Department of Orthopaedic Surgery, University of Virginia

² Department of Biomedical Engineering, University of Virginia

³ Department of Chemical Engineering, University of Virginia

⁴ School of Biomedical Engineering and Sciences, Virginia Tech

Abstract

The anterior cruciate ligament (ACL) is the most commonly injured intra-articular ligament of the knee. The insufficient vascularization of this tissue prevents it from healing completely after extreme tearing or rupture, creating a need for ACL grafts for reconstruction. The limitations of existing grafts have motivated the investigation of tissue engineered ACL grafts. A successful tissue engineered graft must possess mechanical properties similar to the ACL; to date no commercially available synthetic graft has achieved this. To accomplish this goal we have combined the techniques of polymer fiber braiding and twisting to design a novel poly L-lactic acid (PLLA) braid-twist scaffold for ACL tissue engineering. The scaffold is designed to accurately mimic the biomechanical profile and mechanical properties of the ACL. In this study, braid-twist scaffolds were constructed and compared to braided scaffolds and twisted fiber scaffolds.

The addition of fiber twisting to the braided scaffold resulted in a significant increase in the ultimate tensile strength, an increase in ultimate strain, and an increase in the length of the toe region in these constructs over scaffolds that were braided. Based on the findings of this study, the braid-twist scaffold studied was found to be a promising construct for tissue engineering of the anterior cruciate ligament.

Keywords

anterior cruciate ligament (ACL); poly (L-lactic acid) (PLLA); scaffold; polymer; tissue engineering

Introduction

The anterior cruciate ligament (ACL) is the most commonly injured ligament of the knee. Annually, more than 200,000 patients are diagnosed with ACL disruptions [Beynon and Fleming, 1998; Pennisi 2002; Albright et al. 1999] and approximately 150,000 ACL surgeries are performed [Cameron et al., 2000; Cooper et al., 2002]. The ACL is the major intra-articular ligament of the knee and is critical to normal kinematics and stability. Due to

Corresponding Author: Cato T. Laurencin, M.D., Ph. D., University Professor, Lillian T. Pratt Distinguished Professor and Chair of Orthopaedic Surgery, Professor of Biomedical Engineering, Professor of Chemical Engineering, The University of Virginia, 400 Ray C. Hunt Drive, Ste 330, Charlottesville, VA 22903, Telephone: (434) 243-0250, Fax: (434) 243-0252, E-mail: Laurencin@virginia.edu

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inherently poor healing potential, ACL ruptures do not heal and surgical replacement is often required.

Ligaments are dense, highly ordered tissues composed of proteins (such as collagen types I, III, and V and elastin), proteoglycans, water, and cells. Ligaments contain a hierarchical structure with increasing levels of organization including collagen molecules, fibrils, fibril bundles, and fascicles that are organized along the long axis of the tissue. The collagen fibrils display a periodic change in direction called a crimp pattern. The fascicles contain collagen fibrils, proteoglycans, and elastin. In addition, the ligament is surrounded by a sheath of vascularized epiligament [Amiel et al., 1990].

Due to the arrangement of their components, ligaments display three stages of behavior when placed under strain (Figure 1). First there is an area where the ligament exhibits a low amount of stress per unit strain (low slope) labelled the non-linear or toe region. When force is first applied to the tissue it is transferred to the collagen fibrils resulting in lateral contraction of fibrils and the straightening of the crimp pattern. Following this area is the linear region, which displays an increase in slope. Once the crimp pattern is straightened, the force is directly translated into collagen molecular strain [Silver, 1994; Diamant, 1972]. The collagen triple helix is stretched and interfibrillar slippage occurs between crosslinks leading to an increase in stress per unit strain [Amiel et al., 1990; Mosler et al., 1985]. The yield and failure region is the last area; it displays a decrease in slope and represents the defibrillation of the ligament [Silver, 1994]. In this area, the collagen fibers in the ligament fail by defibrillation causing a decrease in slope and tissue failure [Amiel et al., 1990; McBride et al., 1988]. In order to successfully restore the functionality of the knee, an ACL replacement must display the same biomechanical behavior as normal ACL tissue.

Traditionally, ACL injuries have been treated with biological grafts (autografts or allografts) [Amiel et al., 1990]. Both autografts and allografts possess good initial mechanical strength and promote cell proliferation and new tissue growth. However, they suffer from a number of disadvantages. Autografts inherently require additional surgery which has been known to cause donor site morbidity, increased recovery time, and possible pain at the harvesting site [Cartmell and Dunn, 2004]. Allografts are limited in supply, could potentially transmit disease or bacterial infection, and may elicit an unfavorable immunogenic response from the host [Cartmell and Dunn, 2004; Cameron et al., 2000; Vunjak-Novakovic et al., 2004].

Alternatively, attempts have also been made to use synthetic materials in ligament replacements. Non-degradable synthetic materials that have been used for ACL repair include carbon fibers, polyethylene terephthalate (Leeds-Keio ligament), polypropylene (Kennedy Ligament Augmentation Device), and polytetrafluoroethylene (Gore-Tex) [Snook, 1983; Silver, et al., 1991; Smith et al., 1993; Arnoczky, 1983; Amiel et al., 1990]. Some of these synthetic ligament replacements have been conditionally approved by the FDA for testing and augmentation but are not recommended in the U.S. for primary ACL repair [McPherson et al., 1985]. These synthetic devices fail due to fragmentation, stress shielding of new tissue, fatigue, creep, and wear debris [Vunjak-Novakovic et al., 2004; Noyes and Grood 1976], problems which can eventually lead to arthritis and synovitis [Pennisi, 2002; McPherson et al., 1985].

Problems with past synthetic replacements have caused tissue engineering to emerge as a superior approach to the replacement, repair, and regeneration of damaged tissues. The advantage of this method over other previously used methods lies in its interdisciplinary approach toward tissue repair. Tissue engineering is the application of biological, chemical, and engineering principles toward the development of substitutes for the repair or restoration of tissue function [Laurencin et al., 1999]. A successful tissue engineered scaffold must be biocompatible, display similar mechanical behavior (shape of the stress-strain and stress

relaxation response), have mechanical properties that are similar to or greater than the tissue it is regenerating, promote tissue ingrowth, and degrade at a rate that does not cause stress shielding or rupture of the new tissue.

Our laboratory has developed a tissue engineered scaffold based on a braid-twist method of construction. Braiding is a technique that has been used to create products designed to bear axial loads, supply reinforcement, or serve as protective covers [Kawabata, 1989]. The simplest braids are composed of sets of yarns that follow circular paths in opposite directions with a sequence of crossovers that cause the yarns to interlace forming a fabric [Kawabata, 1989]. These structures can transfer large loads and provide extension [Cooper, 2002]; their design makes them shear resistant and conformable [Cooper, 2002].

The twisting of fibers is used frequently in the textile industry to form yarns that can withstand the weaving or knitting process [Hudson et al., 1993]. Both the twisting direction and degree of twisting affect yarn strength, abrasion resistance, and flexibility [Hudson et al., 1993]. Low twist produces weaker yarns that pull apart more easily; these yarns may develop protrusions on their surfaces from abrasions [Hudson et al., 1993]. As the amount of twist is increased the strength and level of abrasion resistance of the yarns are increased. If the yarns are wound too tightly (and the fibers become more perpendicular to the long axis of the yarn) the strength and abrasion resistance decrease [Hudson et al., 1993].

The structure of this scaffold is also similar to the organization of a native ligament and is designed to mimic the biomechanical behavior of the ACL (display a toe region and linear region when placed under increasing load). It combines two previously conceived scaffold design methods, fiber twisting [Altman et al., 2002; Chen et al., 2003; Vunjak-Novakovic et al., 2004] and fiber braiding [Cooper et al., 2005; Lu et al., 2005]. In work by Altman et al., twisted fiber scaffolds were constructed from bundles of silk fibers wound into strands that were wound again into cords and arranged to form the matrix [Altman et al., 2002]. The scaffolds demonstrated a toe region followed by a linear region. The braided scaffold produced by Cooper et al. prevented sudden failure with a three-dimensional (3-D) braiding technique that reinforced the scaffold [Cooper et al., 2002; Cooper et al., 2005; Lu et al., 2005]. It was composed of poly (L-lactic acid) (PLLA), a poly α -hydroxyester that has been approved by the FDA for implantation in other biomedical devices.

In this study it was hypothesized that combining fiber twisting with fiber braiding in scaffolds will cause an increase in toe region length, strain at failure, and maximum load when compared to braided fiber scaffolds.

Materials and Methods

PLLA fibers obtained from Albany International (Lot # 5248–49, Albany International, Mansfield, MA) were used to make three types of scaffolds; a braided scaffold, a twisted fiber scaffold, and a braid-twist scaffold (Figure 2). The purchased PLLA fibers (diameter of 0.295 ± 0.044 mm) are composed of 30 smaller microfibers (with diameters similar to collagen fibers, approximately 12 ± 0.6 μ m in diameter) plied together, similar to the arrangement of collagen in ligament tissue. PLLA was chosen because it is FDA approved for use in medical devices and has been used for previous scaffolds produced by our laboratory [Cooper et al., 2002; Cooper et al., 2005; Lu et al., 2005]. Before constructing the scaffolds, the PLLA fibers were cut into 160 mm lengths. These fibers were used to make the three types of scaffolds.

The braided scaffolds were made using the following procedure (Figure 2a):

- Nine groups of six 160 mm length PLLA fibers were selected.

- The PLLA fibers were combined to form three yarns. Each yarn was composed of eighteen 160 mm length PLLA fibers.
- These three yarns were braided together to form one scaffold.

The twisted fiber scaffolds were made using the following procedure (Figure 2b):

- Nine groups of six 160 mm length PLLA fibers were selected.
- Each group of six fibers was twisted in a counter-clockwise manner to form a fiber bundle (a total of nine fiber bundles/scaffold).
- Three of these bundles were twisted around one another counter-clockwise to form a yarn (a total of three yarns/scaffold).
- The three yarns were twisted together clockwise to form one scaffold.

The braid-twist scaffolds were made using the following procedure (Figure 2c):

- Nine groups of six 160 mm length PLLA fibers were selected.
- Each group of six fibers was twisted in a counter-clockwise manner to form a fiber bundle (a total of nine fiber bundles/scaffold).
- Three of these bundles were twisted around one another counter-clockwise to form a yarn (a total of three yarns/scaffold).
- These three yarns were braided together to form one scaffold.

After construction each scaffold was bound to a rectangular cardboard frame with epoxy to aid in loading the scaffolds for mechanical testing, prevent slipping of the scaffold during testing, and act as a precautionary measure to prevent unbraiding or untwisting (Figure 2d). The epoxy was not allowed to touch the portion of the scaffold inside of the frame and the gage length used during mechanical testing represents the middle third of the scaffold. These precautions were taken to ensure that the epoxy did not affect the results of the mechanical tests.

The number of fibers in each group was chosen based on projected scaffold size. The fibers and yarns in this study were twisted in the same direction; this typically is not done in the rope industry because this may lead to kinking and unbalanced yarns (future studies will have fibers and yarns that are twisted in opposite directions). The braiding and twisting angles were chosen to represent scaffolds ranging in degree of twisting and braiding angle. Fiber twisting was done using a Conair twisting device. The fibers were twisted for various time periods (1, 3, and 5 seconds); the twisted fibers were then photographed to obtain the angle of the fibers twisted for each time period. Yarns of fibers were braided by hand at various stitches per inch (2, 4, and 6 stitches/inch). The braids were then photographed to obtain the angle of the fibers. Photographs were taken during each step of scaffold preparation (2 pictures per step) using a Nikon Coolpix camera at a magnification of 3X and an Epson Perfection 2450 Photo Scanner. The scaffolds were placed under a 0.2 N load while the pictures were taken. Lines parallel to the stitches, twists, and long axes were placed onto the pictures using Microsoft Word and the angles were measured with a protractor (3 angles per picture). This procedure yielded 6 measurements for every angle calculated. The braiding and twisting angles were formed from the intersection between the line perpendicular to the scaffold long axis and the direction of the fibers (Figure 3).

Scaffolds were identified by the type of structure (braided scaffold, twisted fiber scaffold, or braid-twist scaffold, as seen in Figure 2, the number of stitches per inch, and the twisting angle. For example, if the twisted fiber scaffold had fibers twisted to 60°, fiber bundles twisted to 72°, and yarns twisted to 68° the scaffold was labeled “twist 60-72-68”. If the braid-twist scaffold had fibers twisted to 78°, fiber bundles twisted to 83°, and yarns braided at 6 stitches

per inch the scaffold was labeled “6 braid 78–83”. If the braided scaffold had yarns that were braided at 4 stitches per inch the scaffold was labeled “4 braid”. Consistency in braiding and twisting angles between scaffolds was maintained by braiding the fibers a specified number of stitches per inch (to achieve constant braiding angles) or twisting the fibers at a constant rate for specified time periods (to achieve constant twisting angles).

Scaffold Characterization

The different scaffold types were characterized by mechanical properties analyzing four samples in each group (n=4); the properties measured were modulus, ultimate tensile stress (UTS), strain at failure, and length of toe region. The modulus was measured by calculating the slope of the linear region. The length of the toe region was measured manually as a percentage of the gage length. The toe region extended from the beginning of the test until the point where the linear region began (noted by the presence of a constant slope larger than the initial slope).

Measurements were made manually from Microsoft Excel. The mechanical properties of the scaffolds were examined under tension using an Instron mechanical testing system (Model 5544, Instron Corp. Canton, MA). Scaffolds were placed into phosphate buffered saline (PBS) for 20 min prior to testing in order to hydrate the samples (mimicking the *in vivo* environment); tests performed in our lab have shown that the diameters of these fibers equilibrate after soaking in PBS for 10 min (Figure 4). During testing, the samples were hydrated with PBS; the cardboard frame was cut in half to allow the scaffold to be pulled freely. Samples were preloaded to 0.2 N and tests were performed using a gage length of 40 mm; samples were tested at a strain rate of 12%/sec until failure occurred. This strain rate was chosen in order to compare these scaffolds to scaffolds used in other studies [Pioletti et al, 1999]. Prior to testing the cross sectional areas of the scaffolds were measured using digital calipers with 0.1 N of pressure (a solid cross section was assumed). Areas were taken from 5 tightly wound scaffolds held straight under a 0.2 N force; cross-sectional areas were measured at three places along the length of the scaffolds and the average was recorded.

In order to analyze the affect of braiding angle on the failure rate of the scaffolds (braided and braided twisted), a free body diagram was drawn to identify the forces placed on the yarns (Figure 5). The yarns in a scaffold were modeled as solid rods; force estimates were based on a tensile load of 40 N and the braiding angles observed in this study. The forces were estimated using the following equations:

$$T_S = T_{By} = T_B \cos \theta, \quad T_B = \frac{T_S}{\cos \theta} \quad (1)$$

As seen in Figure 5, the y-component of the force placed on the yarn, T_B , (T_{By}) is equal to the tensile force, T_S . T_S is the force placed on the scaffold by the Instron. The force B is the force placed on the yarn by the rest of the braid in the x direction (preventing the braid from becoming completely straight when pulled), and θ is the angle between the longitudinal axis and the tensile force placed on the fiber (T_B). The value of T_B can be multiplied by the number of bundles in the scaffold in order to calculate the amount of force placed on the scaffold, T_S . Balancing the forces in the x and y directions (Equations 2 and 3) allows us to solve for the remaining forces. Rearranging the equations gives us the value of B (Equation 2).

$$B = 2 T_S \sin \theta \quad (2)$$

In order to solve for B we must solve for the angle θ . The relationship between the angle θ and the braiding angle (λ) is listed in Equation 3.

$$\theta = 90 - \lambda \quad (3)$$

Data Analysis

All means and standard deviations from collected data were calculated using Microsoft Excel. All data was analyzed for significance using a one-way ANOVA with a post hoc analysis performed via Tukey test with SPSS software.

Results

Scaffold characteristics

The braiding and twisting angles for the scaffolds are listed in Table 1; the braiding angles apply to both braided and braid-twist scaffolds, the twisting angles apply to both twisted and braid-twist scaffolds. Braiding produced structures with braiding angles of 78°, 69°, and 61° after braiding with 2, 4, and 6 stitches/inch respectively (Table 1). The braiding angle decreased with an increase in the number of braiding stitches/inch. Twisting the PLLA fibers produced scaffolds with fiber bundles twisted to 78°, 69°, and 60°, yarns twisted to 83°, 72°, and 62°, and scaffolds twisted to 79°, 68°, and 62° (Table 1).

Mechanical properties

An example of the data obtained from the mechanical tests can be seen in Figure 6. The results of the mechanical tests may be seen in Supplementary Figures 1–4. Data from the stress-strain tests were used to calculate the modulus, length of the toe region, UTS, and ultimate strain. All scaffolds failed in the mid-substance region.

The 6 braid 60–72 and 6 braid 60–62 scaffolds had lower moduli, 428.2 ± 60.7 MPa and 424.6 ± 129.4 MPa respectively, than three of the four twisted scaffolds (twist 78–83–79 with 796 ± 247.9 MPa, twist 78–83–68 with 772.3 ± 21.9 MPa, and twist 69–72–79 with 888.2 ± 60.7 MPa) (Supplementary Figure 1) ($p \leq 0.05$) and the braided scaffolds (810.2 ± 233.5 MPa for 2 braid, 757 ± 264.1 MPa for 4 braid, and 768.8 ± 115.9 MPa for 6 braid) (Supplementary Figure 1) ($p \leq 0.05$).

The twist 69–72–79 scaffolds had a significantly longer toe region ($6.5 \pm 0.001\%$) than the braids ($1.45 \pm 0.09\%$ for 2 braid, $1.70 \pm 0.62\%$ for 4 braid, and 1.99 ± 0.02 for 6 braid), shown in Supplementary Figure 2. Three of the braid-twist scaffolds displayed significantly longer toe regions ($4.0 \pm 0.001\%$ for 4 braid 60–72, $2.72 \pm 0.058\%$ for 6 braid 60–72, and $3.0 \pm 0.001\%$ for 6 braid 60–62) than three of the twisted scaffolds (twist 78–83–79 with $1.53 \pm 0.712\%$, twist 78–83–68 with $1.0 \pm 0.0003\%$, and twist 78–83–62 with $0.2 \pm 2.6 \times 10^{-9}\%$) and all of the braided scaffolds (Supplementary Figure 2) ($p \leq 0.05$). There was an increase in the length of the toe region ($p \leq 0.05$) with increased braiding angle and the addition of fiber twisting to the braided structure (Supplementary Figure 2).

The ultimate tensile stress (UTS) of the 2 braid 60–62 and 4 braid 60–72 scaffolds (70.9 ± 7.1 MPa and 81.6 ± 1.6 MPa respectively) were comparable to that of the twist 69–72–79 scaffolds (80.9 ± 6.9 MPa) (Supplementary Figure 3). The 4 braid 60–72 scaffolds displayed a significantly higher UTS ($p \leq 0.05$) than the 6 braid 60–72 and 6 braid 60–62 scaffolds (51.7 ± 8.8 MPa and 44.3 ± 3.6 MPa respectively) and the braided scaffolds (Supplementary Figure 3). The UTS values of all of the scaffolds were greater than ($p \leq 0.05$) or comparable to the UTS of human ACL (38 ± 9 MPa) (Supplementary Figure 3) [Silver 1994].

The addition of fiber twisting increased the ultimate strain of the braided scaffolds ($p \leq 0.05$) except for the 6 braid 60–62 (Supplementary Figure 4). The ultimate strains of the 2 braid 60–62 and 4 braid 60–72 scaffolds were significantly larger ($p \leq 0.05$) than those for all of the braids, the 6 braid 60–72 scaffolds, and 6 braid 60–62 scaffolds (Supplementary Figure 4).

The data also showed that the combination of the twisted fibers and a braided structure can be detrimental to graft strength; this occurred in only in the braid 60–72 and 6 braid 60–62 scaffolds. Both the 6 braid 60–72 and 6 braid 60–62 samples had lower UTS values than the 4 braid 60–72 scaffolds (Supplementary Figure 3). When comparing just the braided and braid-twist scaffolds the 4 braid 60–72 scaffolds had a larger ultimate strain than the 6 braid 60–62 scaffolds (Supplementary Figure 4). These results may be due to increased forces placed on the fibers due to the increase in braiding angle when combined with the high twisting angles.

Discussion

In order to successfully replace and regenerate new ACL tissue, a graft must be able to induce cell adhesion and proliferation, sponsor tissue growth, and display mechanical behavior comparable to the native ACL tissue. In order to achieve the latter criteria, the scaffold design discussed in this paper combined two previously studied techniques, the multilayered twisting of fibers and fiber braiding.

In order to avoid mechanical rupture, an ACL replacement must have strength that is similar to or exceeds that of the ligament. It must possess a modulus similar to that of the ACL. Ligaments display toe regions, and it was of interest to us to study the design of tissue engineered constructs exhibiting toe regions of similar length to that of native tissue. The structures described in this paper combined two techniques used in the textiles industry: fiber twisting and fiber braiding. Scaffolds developed using fabric-based techniques are an improved option for ligament tissue engineering because fabrics are flexible and generally display non-linear behavior [Kawabata, 1989].

The aim of this study was to explore a novel ligament scaffold design by combining these two methods of scaffold production and to select the best scaffolds for replacement. The properties of the scaffolds examined in this study can be fine tuned, by varying the number of fibers incorporated, the degree of twisting, or braiding angle. By adjusting braiding and twisting angles we were able to alter the scaffold mechanical behavior and create scaffolds with toe region lengths and moduli that were similar to, or exceeded, the ACL.

Data from previous studies indicate that human ligaments such as the ACL and MCL (medial collateral ligament) have toe regions that range in length from 2.0% to 4.8% strain, as seen in Supplementary Figure 2, [Ambrosio et al., 1998; Bonifasi-Lista et al., 2005; Dienst et al., 2002; Moon et al., 2006; Thornton et al., 2002]. These comparisons may not be fair considering these studies used strain rates that differ from the rate used in this study. The toe regions in a study using bovine ACLs tested at a similar strain rate (10%/sec) were $\approx 3\%$ [Pioletti et al., 1999]. Some of the scaffolds tested in this study, have toe regions that are equal to or larger than those seen in the previously mentioned studies (Supplementary Figure 2).

Based on our design criteria (toe region length, modulus, and UTS), the 4 braid 60–72 scaffolds studied were best suited for ACL replacement and regeneration. These scaffolds degrade by bulk erosion and hydrolysis through hydrolytic de-esterification into lactic acid; this process leads to a decrease in elastic modulus and UTS over time. To counteract degradation, further scale-up of the ACL scaffold will necessitate moduli and UTS levels that are larger than those displayed by native ACL tissue. The braid-twist scaffold design allows for modulation of mechanical properties by varying braiding and twisting angle and numbers of fibers. In our planned scale-up experiments, fibers and yarns will be twisted in opposite directions in an effort to minimize large standard deviations in the mechanical data.

As seen in the data, by adjusting the braiding angle (changing the number of braid stitches per inch) and the twisting angle, the length of the toe region and UTS of the PLLA scaffold was altered. As the angle decreased the fibers moved further away from the longitudinal axis (the

loading axis). The application of force to the scaffold aligned the fibers along the loading axis and then stretched them (similar to what has been seen in ligaments); this created the toe region. Therefore, as the angle decreased (as measured by the method shown in Figure 3) the length of the toe region increased because more force was used in aligning the fibers with the loading axis instead of stretching the fiber. Through careful adjustment the combination of these techniques led to a longer toe region when compared to braids (Supplementary Figure 2). The physiological relevance of obtaining a toe region in these scaffolds will need to be explored via *in vivo* studies.

The combination of braiding and twisting can also lead to premature scaffold failure as seen in the 6 braid 60–72 and 6 braid 60–62 samples. As the braiding angle decreases (in both braided and braid-twist scaffolds), the angle λ (seen in Figure 5) decreases and the quantity $\sin(90-\lambda)$ increases. This amplifies the values of T_B , T_S , and B (Figure 7). Therefore, as the braiding angle decreases, the forces along the fiber bundles and where the bundles change direction increase (Figure 7). This increase explains the early failure of the braid-twist scaffolds with the highest braiding angle (6 braid 60–72 and 6 braid 60–62). The combination of the increase of tensile force in the fiber bundles and forces normal to the bundles caused by the increased braiding angle can cause premature failure of the scaffold as apparent in the decreased strength (UTS) and ultimate strain of the 6 braid 60–72 and 6 braid 60–62 scaffolds.

This finding is significant because it demonstrates the importance of fiber arrangement in these scaffolds; in this case there is a balance between braiding angle, twisting angle, and the forces placed on the fibers. The data shows that the 4 braid 60–72 scaffold represents a limit with regard to the braiding angle and twisting angle. Above this limit, the forces due to scaffold architecture are large enough to cause premature scaffold failure.

Representing the yarns as rods does not account for the individual fibers that make up the yarns or their flexibility. If the flexibility of the yarns was included it might serve to dampen the compressive force B , but this force may also produce creep at contact points between yarns, ultimately creating another mode of failure.

Changes in mechanical behavior are important for scaffold viability. In the braid-twist scaffolds, the increased length of the toe region allows the scaffold to be strained a greater amount without directly stretching the individual polymer fibers. This increase in length is due to the stretching out of the braids or twists, similar to the stretching of the crimp pattern in native ligaments.

This study describes the stress-strain tests performed on scaffolds constructed using 3 different fabrication techniques. According to this study, the 4 braid 60–72 scaffold was the best of the tissue engineered scaffolds in this study; it has a modulus and UTS that exceed the same properties of the ACL (necessary to prevent failure during degradation) and has a toe region of similar length to natural ligament. For a complete characterization of the effects of combining fiber braiding and twisting, further investigations will explore whether this scaffold is appropriate for ACL replacement and regeneration using cyclic loading and stress relaxation tests.

This new scaffold has shown the potential to mimic the biomechanical behavior of the ACL. After further refinement, it is believed that this type of scaffold could be used to produce tissue engineered scaffolds for successful ACL regeneration. The techniques of fiber braiding and twisting used in this study can be combined with 3-D braiding, as seen in our earlier ACL scaffolds [Cooper, 2002; Cooper et al., 2005; Lu et al., 2005] to develop scaffolds with increased strength and mechanical behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Stress-strain behavior of ligament. The graph displays the 3 staged behavior of ligament (toe region, linear region, and yield region).

Figure 2. Schematics for braid scaffold production (a), twisted fiber scaffold production (b), braid-twist scaffold (c), and technique used to mount scaffolds for testing (d).

Figure 3. Diagram describing how twisting and braiding angles of scaffolds were measured alongside pictures of actual scaffolds.

Figure 4. Diameters of PLLA fibers (n=6 at each time point) while soaking in PBS for up to 30 min. The diameter equilibrates after 10 min.

Figure 5.
The free body diagram used to calculate the forces placed on the braids.

Figure 6.
Examples of the load-strain behavior of a twisted fiber scaffold, braid-twist scaffold, and braided scaffold. Each plot notes the toe region (1), and linear region (2).

Figure 7. The calculated tensile force (T_s) and normal force (B) placed on the yarns. As the number of braiding turns/inch increases, the braiding angle decreases. The chart shows that as the braiding angle decreases the tensile force and normal force placed on the yarns increases.

Table 1

The braiding angles and twisting angles associated with each braided scaffolds, braided-twisted fiber scaffolds, and twisted fiber scaffolds are listed below. The twisting angles are arranged into structures (fiber bundles, yarns, and scaffolds).

Scaffold levels	Twisting Angles (degrees)		
Fibers twisted to form Fiber Bundles	78 ± 3.4	69 ± 4.0	60 ± 4.5
Fiber Bundles twisted to form Yarns	83 ± 2.1	72 ± 2.3	62 ± 4.5
Yarns twisted to form Scaffolds	79 ± 1.4	68 ± 3.8	62 ± 4.5
Scaffold	2 braid	4 braid	6 braid
Braiding Angle (degrees)	78 ± 1.8	69 ± 2.7	61 ± 3.4