ABSTRACT
In order to facilitate locomotion and limb movement many animals store energy elastically in their tendons. The formation of crosslinked collagen fibers in tendons results in the conversion of weak, liquid-like embryonic tissues into tough elastic solids that can store energy and perform work. Collagen fibers in the form of fascicles are the major structural units found in tendons. The purpose of this paper is to review the literature on collagen self-assembly and tendon development and to relate this information to the development of elastic energy storage in non-mineralizing and mineralizing tendons. Of particular interest is the mechanism by which energy is stored in tendons during locomotion. In the turkey, much of the force generated by the gastrocnemius muscle is stored as elastic energy during tendon deformation and not within the muscle. As limbs move, the tendons are strained, causing the collagen fibers in the extracellular matrices to stretch. Through the analysis of turkey tendons, collagen fibers, and a molecular model, it is hypothesized that elastic energy is stored in the flexible regions of the collagen molecule. Data from the molecular model, mineralized fibers, and turkey tendons show that the presence of calcium and phosphate ions causes an increase in elastic energy stored per unit strain. Based on the theoretical modeling studies, the increase in stress with strain is a result of the initiation of stretching of the rigid regions of collagen molecules.

INTRODUCTION
Elastic energy storage is an extremely important mechanical characteristic of collagenous tissues; tendons and ligaments are examples of musculoskeletal tissues that store and transmit energy elastically during mechanical deformation [1-3]. Tendons are necessary for the transference of energy from contracting muscles to bones, leading to the movement of limbs. In the turkey, much of the force generated by the gastrocnemius muscle is stored as elastic energy during tendon deformation and not within the muscle. In animals during normal gait, the body is decelerated as the foot lands on the ground, causing kinetic energy to be stored as strain energy in the muscles and tendons that are stretched by the impact with the ground. Elastic recoil in the tendons converts most of the stored energy into kinetic energy as the foot leaves the ground [1, 2]. In contrast to the energy storage function of tendon, ligaments absorb energy during movement in order to protect joints from damage. The anterior cruciate ligament (ACL) prevents damage to the knee by absorbing energy during movement that could lead to anterior translation of the tibia.
It has been found that the energy storage ability of collagenous tissues can be altered through processes such as crosslinking and mineralization [4, 5]. Some musculoskeletal tissues, such as the turkey tendon, mineralize during aging [6]. It has been proposed that mineralization is an efficient means for preserving elastic energy storage while providing the increased load bearing capability required for the locomotion of adult birds [5].

The mechanical properties of developing tendons change quickly before the onset of locomotion. It has been reported that the ultimate tensile strength (UTS) of developing chick extensor tendons increases from about 2 MPa at 14 days of development to 60 MPa 2 days after birth [7, 8]. This rapid increase in UTS is associated with increases in collagen fibril lengths [7, 8]. This increase in fibril length occurs through the formation of crosslinks between collagen fibrils. Studies on self-assembled type I collagen fibers show that the UTS and stiffness is decreased in the absence of crosslinks [9].

Although the macromolecular basis for elastic energy storage in these tissues has been recognized, the molecular reasoning for this behavior has not been as thoroughly investigated. The results of studies based on viscoelastic measurements, microscopic observations, and the amino acid sequence of type I collagen indicate that the molecule contains flexible regions [10, 11, 12, 13]. The results of an amino acid sequence analysis conducted by Silver et al. [14] indicate that regions of the molecule lacking proline and hydroxyproline are more flexible than regions containing the sequence glycine–proline–hydroxyproline (Gly–Pro–Hyp). The study calculated the flexibility of the type I collagen fibril using the area under the conformation plots of different pairs of amino acids in a microfibril. The results of the study indicate that the most flexible areas are the a1, b2, and c3 bands (Fig. 1). These bands, because of their flexibility, could theoretically store elastic energy during tensile deformation.

In this paper, a model is discussed that relates molecular strain at the microfibrillar level to the ability of collagen fibrils to store elastic energy. We report methods for estimating the elastic spring constants for the positive staining bands of type I collagen fibrils and for calculating the amount of elastic energy stored per unit strain. Data reported suggests that elastic
energy storage is consistent with a model in which energy is stored during stretching of the positive staining bands of type I collagen molecules in a quarter-staggered microfibrillar array.

MATERIALS AND METHODS
Molecular models of the type I collagen molecule and a type I collagen microfibril were constructed in order to predict the amount of elastic energy stored during stretching using methods previously reported [15]. The methods are listed here in brief. All molecular modeling was performed on a Silicon Graphics Octane2 workstation using SYBYL (v6.7, v6.8) software developed by TRIPOS Associates Inc.

The type I collagen molecule template consisted of nine repeats of the amino acid sequence Gly–Pro–Hyp in each chain using the dimensions of a previously calculated poly (Gly–Pro–Pro) model [16]. The model incorporates parameters based on experimental data and agrees with crystallographic data. The type I collagen sequence was split up into sections based on the positive staining banding pattern and the amino acid sequence of each section was substituted into the template with two sequences of Gly–Pro–Hyp per chain at the beginning and end of each section; previous studies have shown that this triplet maintains the triple helix formation [12, 17]. Each section of the molecule was then minimized in batch using the Powell method (a conjugate gradient minimization method for large molecules).

The final length of each section was set to an additional 0%, 1%, 2%, 3%, and 5% of the original length at minimization. These values for molecular strain are based on a study that estimates a molecular strain of 1% for every macroscopic strain of 10% in tendon [18].

All of the sections were then arranged into the quasi-hexagonal packing pattern (Fig. 1) creating sections of the microfibril that were strained 1%, 2%, and 3%; these sections were minimized again. The arrangement of these sections was based on earlier observations [19, 20, 21, 22, 23]. The resulting steric energies for every section of the collagen molecule and microfibril were computed by SYBYL.

Calcium and phosphate ions were placed around the quasi-hexagonal molecular pattern in order to observe the possible effects of their presence on molecular strain. These "mineralized" models were minimized to their lowest energy states in the same manner as the earlier microfibrils. After the minimizations were finished, all steric energies were recorded.

**Molecular model for elastic energy storage**

The spring energy stored per unit strain in a type I collagen fiber was calculated in the following manner. The steric energy, in kcal/mol, for each section of the collagen microfibril was calculated using SYBYL. The difference in energy between the values for the strained sections (1%, 2%, 3% and 5% strain) and the unstrained sections was calculated and divided by the difference in length (in angstroms) and multiplied by Avogadro's number (6.023×10²³ mol⁻¹) to obtain an elastic spring constant in kcal/Å for 1%, 2%, 3% and 5% strained fibril sections.

The spring constants were arranged in increasing order and the total displacement of the section pieces were summed until the total of the strains of each section were equal to the desired total microfibrillar strain (e.g. 1%, 2%, and 3%); only the contributions of strains with positive spring constants were considered in spring constant calculations. The spring constants associated with the strains for each section were added together in series using equation 1.
\[ R_{mic} = \frac{1}{\sum_{i=1}^{n} \frac{1}{n_i R_i}} \]  

\( R_{mic} \) is the spring constant for the entire microfibril at a particular strain (1, 2, and 3%), \( n \) is the number of times the band appears in the microfibril, and \( R_i \) is the spring constant for each section of the microfibril that contributes to the total strain.

In order to calculate the elastic energy stored in a collagen fiber, the spring constants for the microfibrils were converted from kcal/Å into J/m. These values were then multiplied by the average dimensions of fibers tested in our laboratory to yield data that could be compared with mechanically tested fibers.

**Preparation of Self-Assembled Collagen Fibers**

Mechanical data used in this study was obtained from a previous study that measured the mechanical properties of self-assembled collagen fibers [15]; the type I collagen used in this study was obtained from rat-tail tendons and extruded into fibers as previously described [24]. Self-assembled fibers were mineralized in a dual chamber bath as described earlier [25]; fibers for mineralization were placed in dialysis tubing which was filled with 0.05 M Trizma solution at a pH of 7.4 and mounted in a frame placed between buffered CaCl₂ and K₂PO₄ solutions. The fibers were allowed to mineralize in the bath for 4 or 7 days at 20°C.

Following mineralization, the fibers were removed from the tubing and frame, washed in phosphate buffered saline (PBS) at pH 7.4 and 20°C and allowed to dry by draping them between two wooden stands overnight.

**Mechanical Testing of Self-Assembled Collagen Fibers**

The mineralized and unmineralized collagen fibers were tested on an MTS Tytron 250 using the technique described earlier [24]. Both mineralized and unmineralized fibers were attached to vellum paper frames, with a gauge length of 20 mm. The dry diameter of each fiber was measured using a light microscope equipped with a calibrated eyepiece. The fiber mounted on the vellum frame was soaked for 30 min in a PBS solution at 20°C. After 30 min, the wet fiber diameter was measured using the same method described above.

![Figure 2](image-url)  

**Figure 2.** Data from incremental stress-strain test on unmineralized collagen fibers.
The samples were immersed in a bath of PBS at 20°C throughout the test to keep the collagen fibers hydrated. Before testing, slits were cut along the sides of the frame to allow the fibers to be pulled without interference from the supporting frame. The fibers were strained to a final strain at a rate of 10%/min, allowed to relax to equilibrium, which was defined as a relaxation rate less than $4 \times 10^{-4}$ g/min., and then another strain increment was applied equal to the first strain increment; this process was repeated until failure.

Each tensile test yielded an incremental stress–strain curve (Fig. 2) consisting of a stress before relaxation (total stress), the stress at equilibrium (the elastic component of the stress), and the viscous stress (the difference between the total stress and the elastic stress) [14]. Data from incremental stress–strain tests was analyzed and the elastic energy stored was calculated by using the area under each elastic stress–strain curve; the stress at each strain (N/m²) was multiplied by 0.5 times the distance strained (in meters) in order to calculate energy per unit area (J/m²). This conversion is based on the relationship between Joules and Newton-meters (100 J = 100N•m). These values were compared with one another using a Student’s t-test (a probability of \( p < 0.05 \) indicated a significant difference between the mean values) and standard deviations.

**Mechanical Measurements on Mineralized Turkey Tendons**
Mechanical data for turkey tendons mineralized to different extents was obtained from an earlier study by Silver et al. [14]. In that study, the gastrocnemius tendons were removed from turkeys sacrificed at 12, 13, and 14 weeks of development. Tendons were strained until failure using incremental stress-strain tests as described above. Elastic energy stored during mechanical testing was calculated using the area under the elastic stress-strain curves for strains up to 20% as described above.

**RESULTS**

**Molecular Model for Elastic Energy Storage**
The calculated amount of energy stored obtained using the two computer models (with and without ions) can be seen in Figure 3A. At 10% strain the unmineralized model stored 531.53 J/m² of energy, 722.19 J/m² at 20% strain, and 1038.07 J/m² at 30% strain. The model representing mineralized collagen fibers stored 1601.40 J/m² at 10% strain, 1758.46 J/m² at 20% strain, and 2623.39 J/m² at 30% strain.

![Figure 3. Plots display the elastic energy calculated from spring constants of unmineralized (0 days) and mineralized (7 days) model (A) and elastic energy vs. strain for unmineralized, 4 day mineralized, and 7 day mineralized collagen fibers (B).](image-url)
Table 1. Data from incremental stress-strain tests for collagen fibers

<table>
<thead>
<tr>
<th>Mineral content-weight fraction (n)</th>
<th>UTS (MPa)</th>
<th>Ultimate strain</th>
<th>Total slope (MPa)</th>
<th>Elastic slope (MPa)</th>
<th>Viscous slope (MPa)</th>
<th>Max elastic stress (MPa)</th>
<th>Max viscous stress (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (12)</td>
<td>0.36 ± 0.067</td>
<td>0.42 ± 0.162</td>
<td>0.71 ± 0.134</td>
<td>0.31 ± 0.085</td>
<td>0.40 ± 0.0943</td>
<td>0.16 ± 0.042</td>
<td>0.19 ± 0.047</td>
</tr>
<tr>
<td>0.296 (9)</td>
<td>0.41 ± 0.042</td>
<td>0.15 ± 0.036</td>
<td>1.78 ± 0.264</td>
<td>0.65 ± 0.110</td>
<td>1.22 ± 0.387</td>
<td>0.15 ± 0.001</td>
<td>0.28 ± 0.020</td>
</tr>
<tr>
<td>0.704 (11)</td>
<td>0.79 ± 0.110</td>
<td>0.14 ± 0.065</td>
<td>3.95 ± 0.551</td>
<td>2.40 ± 1.283</td>
<td>1.97 ± 1.257</td>
<td>0.39 ± 0.021</td>
<td>0.54 ± 0.213</td>
</tr>
</tbody>
</table>

Elastic Energy Storage in Self-Assembled Collagen Fibers

Mechanical data from stress-strain tests is listed in Table 1. The elastic stress at each strain increment was converted into elastic energy; the results of these calculations can be seen in Figure 3B. The maximum amount of elastic energy stored in the control fibers is 805.53±212.078 J/m² at 50% strain, the maximum amount of energy stored in 4 day mineralized fibers, 255.49±43.822 J/m² at 20% strain, and 701.51±37.866 J/m² at 18% strain for fibers mineralized for 7 days. An increase in elastic energy stored is seen when the mineralized values are compared to the unmineralized value at approximately the same strain (20%), 108.17±54.197 J/m² for the control group, 255.49±43.822 J/m² when mineralized for 4 days, and 701.51±37.866 J/m² when mineralized for 7 days (p < 0.05).

Microfibril Flexibility

According to the molecular model, the most flexible regions without the addition of ions are the a2 band, a3 band, a4 band, area between the b1 and b2 bands, area between the c1 and c2 bands, and the d band. After calcium and phosphate ions were added to the model the most flexible areas were the a3 band, a4 band, area between the b1 and b2 bands, c1 band, c2 band, d band, and area between d and e1 band (Table 2).

Tendon Mechanical Analysis

Data from the elastic stress-strain curves for mineralized turkey tendons are listed in Table 3. In general, the elastic stress-strain curves for tendons with low mineral content (0.000) have lower moduli (51.6 MPa) while those with higher mineral content (above 0.2) have a higher modulus (133 MPa).

Table 2. Flexible areas found with molecular model

<table>
<thead>
<tr>
<th>Flexible with ions</th>
<th>Flexible without ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>a2 band</td>
<td>a3 band</td>
</tr>
<tr>
<td>a3 band</td>
<td>a4 band</td>
</tr>
<tr>
<td>a4 band</td>
<td>between b1 and b2 bands</td>
</tr>
<tr>
<td>between b1 and b2 bands</td>
<td>c1 band</td>
</tr>
<tr>
<td>between c1 and c2 bands</td>
<td>c2 band</td>
</tr>
<tr>
<td>d band</td>
<td>d band</td>
</tr>
<tr>
<td></td>
<td>between d and e</td>
</tr>
</tbody>
</table>
Table 3. Elastic strain data of turkey tendons

<table>
<thead>
<tr>
<th>Sample age</th>
<th>Mineral content (weight fraction)</th>
<th>Elastic modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>0.000</td>
<td>62.1</td>
</tr>
<tr>
<td>13 weeks</td>
<td>0.215</td>
<td>116.5</td>
</tr>
<tr>
<td>14 weeks</td>
<td>0.000</td>
<td>51.6</td>
</tr>
<tr>
<td>14 weeks</td>
<td>0.295</td>
<td>133</td>
</tr>
</tbody>
</table>

DISCUSSION

Figure 4. Diagram of the collagen triple helix showing areas considered the most flexible in Hofmann et al. (1984) (top) and the molecular model of the collagen microfibril (bottom)

Evaluation of Flexible Regions of the Type I Collagen Microfibril
The areas of the collagen molecule found to be the most flexible are listed in Table 2. An earlier study by Hofmann [14] identifies the areas from the 1st a2 band to the 1st e2 band, the 2nd a4 band to the 2nd a2 band, the 3rd b2 band to the 3rd a4 band, the area between 4th c1 and c2 bands to the 4th c2 band, and the 4th c3 band to the 5th c2 band as flexible. All of these areas agree with the molecular model except for the area from the 1st a2 band to the 1st e2 band, see Figure 4. This discrepancy occurred because, when placed into a microfibril, this area would be surrounded by less flexible regions making the entire area less flexible, agreeing with the molecular model.

The flexible regions located in this study have amino acid sequences that lack proline or hydroxyproline in the Gly-X-Y triplet; the lack of proline or hydroxyproline allows the molecule to adopt different conformations beyond the rigid and straight triple helical conformation; this leads to the formation of voids in the microfibril. It has been noted in other studies [16, 19] that the lack of proline and hydroxyproline residues causes variations in type I collagen molecular structure. The lack of proline and hydroxyproline in these regions adds to the inherent flexibilities of these sections and allows the charged groups to move away from each other or bond with oppositely charged groups or atoms in the helical backbone.

Comparisons Between Elastic Energy Storage in Model and Self-Assembled Fibers
Plots of elastic energy stored versus strain for both the molecular model and self-assembled type I collagen fibers show an increase in energy stored with an increase in strain. The trend in elastic energy stored for 1%, 2%, and 3% strains in an unmineralized computer model follows the same trend as the elastic energy stored in the unmineralized fibers for strains up to 30% (Fig. 5).
There also seems to be an inflection point in both sets of data (although more data points would need to be taken for the model to prove the presence of a change in slope) (Fig. 5). In the molecular model the low slope region represents elongation of areas with very low spring constants; the high slope region is due to the elongation of regions of the collagen microfibril with slightly larger spring constants. If this is a true inflection point, this same process could also occur in the self assembled collagen fibers, a smaller amount of energy is stored in the early stages of strain (more flexible regions have lower elastic energy spring constants) until the less flexible regions begin to stretch (less flexible regions have higher elastic energy spring constants). The energies due to bond stretching, angle bending, and torsion all increase with increased molecular strain.

Figure 5. Model of elastic energy storage vs. strain curves for unmineralized collagen fibers (A), model of elastic energy storage vs. strain curves for 4 day mineralized collagen fibers (B), and model of elastic energy storage vs. strain curves for 7 day mineralized collagen fibers (C).

In both the molecular model and the reconstituted collagen fiber data, the area of low energy storage is smaller in relation to the area of increased energy storage when the collagen is mineralized (Fig. 5). In the model, this is due to calcium and phosphate ions limiting the movement of some of the flexible regions through the formation of bonds between amino acid side chains, causing them to require more energy to stretch therefore increasing their rigidity. This causes fewer areas of the collagen molecule to be recruited for the strains seen in this model. The same phenomena may be part of the cause of similar behavior in the collagen fibers tested in this study. The data from the molecular model shows that the presence of calcium and phosphate ions alters the elastic behavior of type I collagen causing an increase in elastic energy stored per unit strain. This is consistent with the theory that the binding of calcium and phosphate ions to collagen raises the elastic spring constant of the collagen molecule by preventing the stretching of the flexible regions [26]; the model shows that the phosphate ions form bonds with atoms in the charged areas of the collagen fibril.
To further test the accuracy of the model the values for elastic energy calculated by the molecular model were converted into elastic moduli (Table 4). The moduli for the high slope region (1.62 MPa) and the average modulus (1.30 MPa) for the model are comparable to the elastic modulus seen by Pins et al. [24] in uncrosslinked collagen fibers (1.819 ± 0.344 MPa) (Table 4). Moduli from other studies are much higher because they contain crosslinks, which raise the elastic modulus [27, 28] (Table 4). The model presented in this study does not contain crosslinks. The modulus listed here represents the modulus from the elastic stress, which is lower than that from the total stress. The total stress is used in other studies to produce the moduli of the collagen fibers.

Table 4. Moduli from computer model and type I collagen in other studies

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Source</th>
<th>Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freeman and Silver</td>
<td>Computer model (high slope)</td>
<td>1.620</td>
</tr>
<tr>
<td>Freeman and Silver</td>
<td>Computer model (average slope)</td>
<td>1.301</td>
</tr>
<tr>
<td>Pins et al., 1997 [24]</td>
<td>Uncrosslinked collagen fibers</td>
<td>1.819±0.344</td>
</tr>
<tr>
<td>Pins et al., 1997 [24]</td>
<td>Crosslinked collagen fibers</td>
<td>383.0±111.7</td>
</tr>
<tr>
<td>Dunn et al., 1993 [27]</td>
<td>Crosslinked collagen fibers</td>
<td>75-110</td>
</tr>
<tr>
<td>Gentleman et al., 2003 [28]</td>
<td>Crosslinked collagen fibers</td>
<td>270-485</td>
</tr>
</tbody>
</table>

Tendon Development and Mechanical Properties

Data from Tables 1 and 3 show that turkey tendons are able to bear larger stresses than self-assembled collagen fibers and therefore store more elastic energy than the collagen fibers and molecular model from this study. This difference is most likely due to the absence of proteoglycans and far lower amount of crosslinks in the collagen fibers when compared to the turkey tendons. The turkey tendons may have more crosslinks and/or mineral crystals that act as crosslinks than the self-assembled collagen fibers giving turkey tendons larger ultimate tensile stresses and the ability to bear larger stresses per unit strain than the collagen fibers.

The presence of crosslinks and proteoglycans in collagenous tissues has been shown to augment their load bearing capabilities [29, 30, 31]. Specifically, a relationship has been proposed between the presence of crosslinks and proteoglycans in tendon and an increase in the mechanical properties of the tissue [29]. The increased number of crosslinks in turkey tendons combined with the presence of proteoglycans (there are no proteoglycans in the collagen fibers in this study) may cause turkey tendon to have mechanical properties that are superior to those of collagen fibers.

Assumptions and Limitations

The molecular model presented in this study is not meant to provide a model for mineral nucleation or mineralization. Neither is it meant to be a substitute for the mechanical testing of type I collagen. It has been constructed as a means to display the possible effects of molecular composition and stiffness on the behavior of tissues and how the presence of the ions present during nucleation can affect molecular stiffness.

A number of assumptions and simplifications were used in both the building of the molecular model and its comparison to the collagen fibers in order to minimize computational time. These assumptions have been listed and thoroughly explained earlier [15]; the major assumptions are listed here in brief (Table 5).
### Table 5. List of Assumptions used in model

<table>
<thead>
<tr>
<th>Assumption</th>
<th>Reasoning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of water in model</td>
<td>The effect of water would be universal and therefore be nullified when comparing the changes in energy. Program accounts for the effect of solvent by using a distance dependant dielectric function.</td>
</tr>
<tr>
<td>No interactions between neighboring fibrils</td>
<td>Water between microfibrils in collagen fibers makes these interactions negligible. The larger number of interactions inside of the microfibril make those outside of the microfibril negligible.</td>
</tr>
<tr>
<td>Calcium and phosphate ions never infiltrated the microfibrils</td>
<td>In a mineralization model by Landis [32] calcium phosphate forms in the gap region of a collagen microfibril and spreads along the microfibril surface.</td>
</tr>
</tbody>
</table>

### Applications

Knowledge from the study of elastic energy storage in tissue can be applied to the field of tissue engineering, specifically ligament reconstruction. As mentioned earlier, ligaments store and dissipate elastic energy in order to protect joints. Ligaments have poor healing potential, therefore when a ligament is badly damaged replacement is frequently recommended. In the United States there are no products approved for ligament reconstruction. This has created an opportunity for a variety of researchers to investigate the use of scaffolds as ligament replacements.

Results from this study show that the presence of different amino acid residues changes the molecular stiffness of the collagen fibril because of the structure of the amino acid or the interactions between neighboring amino acids. This could be exploited by producing block copolymers with monomers that have different flexibilities depending on their side chains.

Another ligament scaffold being developed combines two approaches to ligament scaffold engineering and knowledge from elastic energy storage studies. This scaffold combines braiding with twisted fiber architecture. This twisted fiber braid adopts two characteristics which allow ligaments to store elastic energy at the fiber level. The use of a braided architecture gives the scaffold added stability [33]. As load is transferred to the braid, the fibers, which are woven throughout the length of the braid, transfer this force through out the entire scaffold. This allows elastic energy to be transferred effectively and uniformly throughout the scaffold similar to the way that crosslinked collagen fibers transfer force throughout ligaments and tendons. The second characteristic is a function of the braid structure and the twisted fibers. The addition of twist to the fibers and each turn of the braid add a change in angle to the fibrillar arrangement [33, 34]. This is similar to what is seen in ligament and tendon. As load is placed on the scaffold it becomes extended. The force first begins to straighten out the “crimp” placed on the fibers as a result of the twisting and braiding. Both of these arrangements create a “toe” region similar to what is seen in the stress-strain behavior of tendons and ligaments.

In a study performed in our laboratories, tensile tests were performed on groups of two-dimensional (2-D) poly (L-lactic acid) (PLLA) braids. One group consisted of scaffolds formed using a regular 2-D braiding technique, the other group of scaffolds used the 2-D twisted braid architecture. A comparison of a regular braid and a braid with a twisted fiber architecture (twisted braid) shows that twisting the fibers yields a lower overall modulus and a longer strain at failure (Fig. 6). Twisting the fibers also leads to an increase in the length of the toe region (Fig. 6 and Table 6).
Figure 6. Stress-strain curves from two scaffolds, one regular 2-D braid and one twisted 2-D braid.

Table 6. Length of the toe regions of different scaffolds

<table>
<thead>
<tr>
<th>Type of Scaffold (n)</th>
<th>Length of toe region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular 2-D Braid (4)</td>
<td>2.038±0.5765</td>
</tr>
<tr>
<td>Twisted 2-D Braid (4)</td>
<td>3.685±0.2456</td>
</tr>
</tbody>
</table>

The increase in toe region length increases the level of elastic energy that can be stored repeatedly in the scaffold, protecting the implant from failure due to fatigue. It also increases the amount of strain at failure. This allows a greater amount of energy to be stored in the scaffold before failure.

The application of elastic energy storage techniques to biomaterials will help produce devices with appropriate mechanical properties for their applications. This will reduce the number of devices that fail due to rupture and fatigue. If combined with tissue engineering techniques, the use of elastic energy storage techniques has the potential to produce a new generation of devices that will be able to store and transfer energy efficiently, preventing device failure and restoring normal movement and joint function.

ACKNOWLEDGEMENTS

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