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BIAXIAL MECHANICS OF MUSCULOSKELETAL TISSUE AND FIBER-REINFORCED SCAFFOLDS

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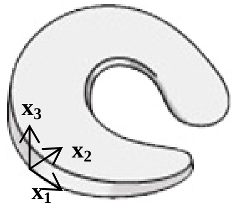
INTRODUCTION

Biaxial tensile testing is the primary experiment used to functionally evaluate cardiovascular fiber-reinforced tissues [1, 2], but has not been widely applied to musculoskeletal tissues. The in situ geometry of many musculoskeletal tissues does not meet uniaxial tensile boundary conditions of freely contracting edges and large aspect ratios. In addition, biaxial tests load the sample through a larger domain of strain configurations as are experienced in situ. In contrast, uniaxial tests represent just a single path within that domain. It has been shown in cardiac tissue and grafts that model parameters determined from biaxial experiments can be used to predict uniaxial behavior, but that uniaxial tests do not predict the biaxial behavior well [1, 2]. Therefore, biaxial mechanical testing is important to address musculoskeletal tissue function.

The knee meniscus, a wedge-shaped semi-lunar fibrocartilage (Fig 1), is an example of a musculoskeletal tissue that undergoes multi-axial physiological loading. However, the meniscus has not been studied in biaxial loading. The uniaxial tensile properties of the meniscus are highly anisotropic [3, 4] and strongly dependent on its fiber-reinforced structure of circumferentially oriented collagen fibers interspersed with radial tie fibers. Since the meniscus is avascular, tears do not heal and current treatments include removal of the torn segment which can predispose the patient to osteoarthritis [5, 6]. Tissue engineering has been proposed for patients with meniscal tears. A tissue engineered meniscus will require recapitulation of the fiber-reinforced architecture. We and others have applied electrospinning to fabricate aligned and nonaligned nanofibrous scaffolds [7, 8, 9].

Fig 1 Meniscus schema showing axis orientation



The objective of this study was to evaluate the uniaxial and biaxial mechanics of free and fixed boundary conditions for meniscus tissue and for aligned and nonaligned nanofibrous scaffolds.

METHODS AND MATERIALS

Sample Preparation: Bovine meniscus tissue was acquired from a local abattoir (n=3). The surface layer was first removed, and then a square planar section aligned with the circumferential (x_1) and radial (x_2) axis was taken from the bottom of the meniscus. The sample was cryo-microtomed to 1.9 ± 0.2 mm thickness and cut into square samples. Sandpaper was glued on the edges of the sample and sutures were placed through the sandpaper and tissue. The square sample length within the sandpaper was 8.6 ± 1.5 mm. Markers were airbrushed onto the sample for optical strain analysis [10].

Nanofibrous scaffolds were produced from a 14.3% w/v solution of polyε-caprolactone (PCL) as previously described (n=3-4/group) [7]. Alignment was achieved by electrospinning with deposition onto a rotating mandrel. Nonaligned scaffolds were electrospun onto a stationary plate. Square samples were cut from a scaffold of 0.93 ± 0.15 mm thickness, with care taken to note the fiber direction. Sandpaper was glued onto the sample edges (2 sides for a uniaxial sample and all 4 sides for biaxial sample) and sutures were placed as above. Samples were airbrushed as above. The square sample length within the sandpaper was 10.2 ± 1.5 mm.

Mechanical Testing: Testing was performed on a custom built biaxial instrument, based on the design by Sacks [11]. The system consists of four stepper motors, linear translation tables, a high resolution camera for optical strain analysis, and other custom hardware. The system is controlled and data is acquired using Labview (National Instruments). Samples were placed into the instrument by inserting sutures over pulleys attached to the translation tables. Force and displacement were recorded throughout the test. Approximately 10 images were acquired for each 1% applied strain.

The meniscus was first tested in uniaxial tension. The tissue was preloaded to 0.5N along the fiber direction and held for 10 minutes, followed by a uniaxial test at 0.1%/s to a displacement of 4 mm (approximately 2.5% tissue strain). This strain was within the linear-region and was kept low to prevent tissue damage. The sample was unloaded and allowed to recover for one hour before biaxial testing. For the biaxial test, a 0.5N preload was applied in the fiber direction and a 0.2N preload in the transverse direction. These preload values were selected based on preliminary experiments to ensure that sutures were taut but tissue remained within the toe-region. The fiber direction was displaced at 0.1%/sec and the transverse direction was displaced at a rate that corresponded to fixed zero strain in that direction.

The nanofibrous scaffolds were tested either in uniaxial or biaxial loading. For these samples, repeated tests were not performed. The aligned scaffold was loaded in the fiber direction. In uniaxial tension a 0.2N preload was applied and held for 5 minutes, followed by a 0.1%/sec displacement to failure. In biaxial tension a 0.2N preload was applied to both axes for 5 min, followed by a 0.1%/sec displacement in the fiber-aligned direction, while the transverse direction was displaced at a rate that corresponded to fixed zero strain in that direction.

Two-dimensional Lagrangian strain (E) was calculated from optical images using Vic2D (Correlated Solutions, Inc). A square central region (~25% of gauge length) was analyzed for tissue strain. The effective Poisson's ratio was calculated as the ratio of transverse/longitudinal strain for uniaxial tests. An exponential equation was used to fit the meniscus tissue stress-strain response, $\sigma = A(e^{B*E}-1)$, where σ is calculated as the force applied divided by the area. The slope was calculated in the toe region as A*B, and in the linear-region at a strain level where both tests were within the linear region of the stress-strain curve. A linear regression was applied to the σ-E response of the scaffolds. A t-test was performed to compare the slope from the uniaxial and biaxial tests, with a paired test used for meniscus. Significance was set at $p = 0.05$.

RESULTS

In this study, bovine meniscus samples were tested both uniaxially and biaxially. The effective Poisson's ratio was 1.49 ± 0.65. The non-linear meniscus stress-strain response was well fit by the exponential function (avg $R^2 = 0.992$, Fig 2A). The curve-fit parameters from the uniaxial testing were: A = 0.08 ± 0.03 MPa and B = 84.7 ± 9.1. The curve-fit parameters for the biaxial testing were: A = 0.06 ± 0.05 MPa and B = 130.5 ± 2.7. The linear region slope was three times higher with biaxial testing compared to with uniaxial ($p = 0.01$, Fig 3A). No differences were found in toe-region slope with either testing modality ($p = 0.8$). To maintain near-zero strain in the transverse direction, the maximum stress applied was 0.61 ± 0.59 MPa (n = 3). Measured transverse strains were non-zero, with a maximum value of -0.44 ± 1.14 %.

A similar testing regime was carried out for nanofibrous scaffolds. The effective Poisson's ratio was 0.11 ± 0.04 for aligned and 0.47 ± 0.10 for nonaligned scaffolds. The linear region slope was 2.6 times higher for both the aligned and the nonaligned scaffolds with biaxial testing compared to with uniaxial ($p<0.001$, Fig 3). To maintain near-zero strain in the transverse direction the maximum stress applied was 0.07 ± 0.03 MPa (n = 9).

DISCUSSION

This study measured the biaxial mechanics of meniscus tissue for the first time, as well as the biaxial and uniaxial mechanics of aligned and nonaligned PCL scaffolds. Biaxial tests were performed by holding the transverse side at a ~0% strain, while applying a

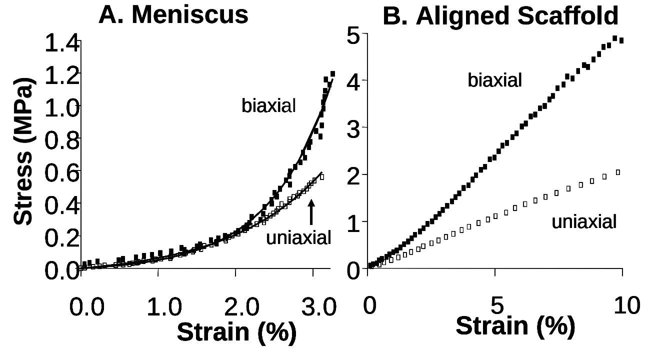


Fig 2 Representative stress-strain plots for (A) meniscus and (B) fiber-aligned scaffolds tested in uniaxial and biaxial tension.

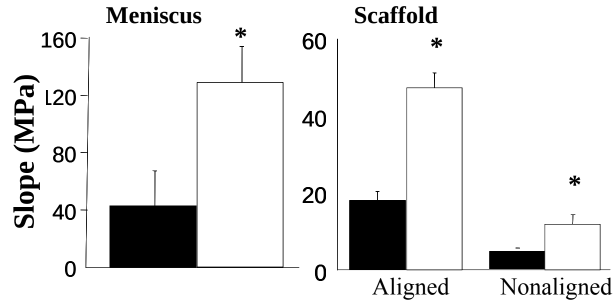


Fig 3 Uniaxial (solid) and biaxial (open) linear-region slopes for meniscus and fiber-aligned and nonaligned scaffolds. * $p \leq 0.01$.

displacement in the fiber-aligned direction. This fixed transverse profile was selected to represent physiologic loading of the meniscus, where the in situ geometry is more similar to a fixed transverse boundary. In contrast, a uniaxial tensile test, which has stress-free boundary conditions along the transverse side, has large contractions along that edge, as demonstrated by the high Poisson's ratio (~1.5 for the meniscus). Other stretch ratios, up to equi-biaxial, which are often applied to cardiovascular tissues, would apply more transverse deformation than could be expected for meniscus, and most other musculoskeletal tissues. Indeed, preliminary studies with transverse tensile strains experienced early failure (not shown).

The meniscus uniaxial modulus in the circumferential direction (43 ± 24 MPa) was similar to previously reported values**.** The modulus for the aligned and nonaligned scaffolds (13 and 5 MPa, respectively) was comparable to that of previous studies from our group [7]. As expected, for the transverse-fixed boundary in biaxial tests, the linear-region slope increased for all samples compared to that found with uniaxial testing.

This study is limited as the feedback control was not fully in place to achieve fixed transverse strain; therefore, samples experienced a small non-zero transverse strain. Improvements to the instrument for strain feedback control will be made in the future. Future work will also characterize tissue biaxial properties for a wider range of physiological strain ratios and apply constitutive modeling to the data.

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