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Regional and Fiber Orientation Dependent Shear Properties and Anisotropy of Bovine Meniscus

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Abstract

Imaging of meniscal tissue reveals an extracellular matrix comprised of collagen fibrils arranged in circumferential bundles and radially aligned tie fibers, implicating structural material anisotropy. Biochemical analyses demonstrate regional disparities of proteoglycan content throughout the meniscal body, a constituent known to affect the shearing response of fibrocartilagenous tissue. Despite this phenomenological evidence and previous mechanical testing implicating otherwise, the meniscus is often modeled as a homogenous, transversely isotropic material with little regard for regional specificity and material properties. The aim of this investigation was to determine if shear stress response homogeneity and directionality exists in and between bovine menisci with respect to anatomical location (medial and lateral), region (anterior, central, and posterior) and fiber orientation (parallel and perpendicular). Meniscus explants were subjected to lap shear strain at 0.002 sec^{-1} with the circumferential collagen fibers oriented parallel or perpendicular to the loading axis. Comparisons were made using a piecewise linear elastic analysis. The toe region shear modulus was calculated from the first observed linear region, between 3%-13% strain and the extended shear modulus was established after 80% of the maximum shear strain. The posterior region was significantly different than the central for the extended shear modulus, correlating with known proteoglycan distribution. Observed shearing anisotropy led to the use of an anisotropic hyperelastic model based on a two-fiber family composite, previously used for arterial walls. The chosen model provided an excellent fit to the sample population for each region. These data can be utilized in the advancement of finite element modeling as well as biomimetic tissue engineered constructs.

Keywords

Mechanical properties; hyperelastic; anisotropic; glycosaminoglycans; radial tie fibers

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5. Conflict of interest statement

The authors recognize no conflicts of interest.

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1. Introduction

The diarthrodial knee joint contains two fibrocartilagenous c-shaped menisci, separating the articulating tibiofemoral surfaces (Messner and Gao, 1998). Both the medial and lateral menisci function dynamically to aid in force distribution, stability and as a lubricating surface (Ahmed and Burke, 1983, Chevrier, et al., 2009, Fithian, et al., 1990, Shoemaker and Markolf, 1986, Walker and Erkman, 1975). Their proficiency in attenuating complex loading pathways and arresting articular cartilage degradation stems from an intricate structure and mechano-responsive biochemistry (Fithian, et al., 1990, McDevitt and Webber, 1990, Messner and Gao, 1998). Joint trauma can compromise the function of the meniscus leading to attrition of the articulating joint surfaces resulting in osteoarthritis, a degenerative disease that is fast approaching epidemic levels (Ratzlaff and Liang, 2010). Proposed mechanisms to impede degradation include the development of tissue engineered replacements which seek to recreate the native biomaterial (Buma, et al., 2004). The advancement and implementation of these constructs hinges on the ability to accurately mimic the native meniscus (Setton, et al., 1999).

The meniscus is a multiphasic material composed of interstitial water, collagen, proteoglycans, and fibrochondrocytes (AufderHeide and Athanasiou, 2004, McDevitt and Webber, 1990). Meniscal ultrastructure consists of three distinct fibrous layers. The superficial layer, which contacts the articulating joint surfaces, is comprised of randomly orientated fibers providing a smooth lubricating surface. Past the superficial layer is the lamellar layer, with randomly orientated fibers as well as distinct radially aligned fibers at the periphery. Lastly, the deep layer contains circumferentially oriented fibers intermixed with radially aligned tie fibers (McDevitt and Webber, 1990, Peterson and Tillman, 1997, Rattner, et al., 2010, Skaggs, et al., 1994). Previous work reveals that the two identified fiber families are unique in both biochemistry and structural organization (Kambic and McDevitt, 2005, Melrose, et al., 2005, Naumann, et al., 2002, Rattner, et al., 2010). Electron microscopy analysis of various menisci across a wide variety of vertebrate species reveals that the circumferential fibers are primarily collagen I arranged in ~5 μm bundles which are grouped together in organized arrays with a distinct crimping pattern, characteristic of tensile-load bearing structures (Fithian, et al., 1990, Rattner, et al., 2010). In contrast, the radial fibers are a co-localization of type I and II collagens arranged in ~10 μm parallel bundles (Rattner, et al., 2010, Skaggs, et al., 1994). This morphology evokes an anisotropic material response relative to collagen fibril orientations (Anderson, et al., 1991, Bursac, et al., 2009, Fithian, et al., 1990, Leslie, et al., 2000, Proctor, et al., 1989, Zhu, et al., 1994).

In addition to intrinsic fiber orientation, collagen fiber and proteoglycan interactions are believed to influence shear properties of the meniscus (Anderson, et al., 1991, Sweigart and Athanasiou, 2001, Zhu, et al., 1994). Heterogenic proteoglycan distribution throughout the meniscus is a presumed adaptation to joint stress (Mow, et al., 1992). For example, glycosaminoglycans, a proteoglycan side-chain responsible for water retention, have been shown to be greater in percent dry weight in the medial meniscus over the lateral as well as anterior over the central and posterior regions (Bursac, et al., 2009). Analysis of canine menisci showed significantly less glycosaminoglycans in the central region of the medial meniscus, compared to the lateral (Stephan, et al., 1998). These findings encourage evaluating shear performance as a function of anatomical location. Though previous torsional shear testing of bovine meniscus has elucidated material anisotropy no distinction between anatomical site was made (Zhu, et al., 1994). To further understand how meniscal tissue responds to loading, this study seeks to conclude if anatomical and regional dependencies dictate shear performance in accordance with structural and biochemical cues.

Lastly, although previous work has shown bovine meniscus to exhibit material anisotropy, constitutive modeling of the meniscus has often been relegated to transverse isotropy (Donahue, et al., 2003, Spilker, et al., 1992). Recent work correlating histological and mechanical data of arterial walls, which possess a similar multiple fiber family structure as the deep zone of the meniscus, has resulted in novel material formulations. These models superimpose transversely isotropic contributions of each fiber family and the isotropic matrix in order to coincide with observations and satisfy the polyconvexity condition within the framework of invariant theory (Balzani, et al., 2006, Holzapfel, et al., 2000, Schroder and Neff, 2003). Thus, in this study the feasibility for applying this anisotropic constitutive model to meniscal tissue will be examined.

Augmented comprehension of stress distributions within knee soft-tissues will incalculably aid in knee biomechanics and etiopathology; potentially leading to novel joint trauma and osteoarthritis preventative measures (Mow, et al., 1992).

2. Methods

2.1. Specimen Preparation

Pairs of menisci were harvested from nine skeletally mature bovine, obtained from a local abattoir. Bovine menisci were chosen for the present study due to availability and tissue size, as the larger menisci facilitates generating multiple tissue explants from specific regions. Previous studies of the aggregate and shear modulus have found similarity between human and bovine tissue (Sweigart, et al., 2004). No distinction was made between left or right hind limbs for this study. Whole stifle joints were stored at -20°C then dissected at room temperature, while continuously hydrating the exposed tissue surfaces with 0.9% saline solution.

The menisci were sectioned into six regions; medial anterior (MA), medial central (MC), medial posterior (MP), lateral anterior (LA), lateral central (LC), and lateral posterior (LP) (Figure 1). A 6.35 mm diameter cylindrical punch was used to extract meniscal explants from the fibrous tissue body. The explants were then trimmed until only the deep layer of the tissue remained so that fiber orientations were primarily circumferential and radial; resulting in an average specimen length of 3.5 mm. An ink line was applied on the longitudinal axis of each explant as a visual aid to ensure uniform shearing of the tissue. Previous studies have directly applied cyanoacrylate to soft-tissue; however, cite special care being taken to minimize adhesive penetration using a multi-axis loading device (Dokos, et al., 2000, Dokos, et al., 2002). Here, dyed nitrocellulose applied to the traction surfaces was found to effectively block pores and inhibit penetration of cyanoacrylate fixative. Serial sectioning of these surfaces showed total penetration less than 10% of specimen length. Lastly, the meniscal explants were adhered to glass slide covers and hydrated for 10 minutes in a saline bath. Cyanoacrylate fixative was used to adhere the glass slide covers to the parallel platens of a lap shear fixture with collagen fibers orientated perpendicular (Pe) or parallel (Pa) to the direction of the shear load. The removal of the superficial and lamellar layers allows for distinction of the circumferential fiber direction with the naked eye (see Figure 2) (Mow, et al., 1992).

2.2. Test setup

A servo-hydraulic uniaxial testing system was utilized to apply simple shear to the explants (Model 8872, Instron Corp, Canton, MA). For our test fixture the top shear platen was mounted to a 44.5N load cell ($\pm 0.445\text{N}$) (Futek, LSB303) and displacement between the shear platens was tracked via a linear variable differential transformer (LVDT) ($\pm 0.001\text{mm}$) (Omega, LD320-2.5) mounted directly adjacent to the test specimen. To ensure specimens

underwent simple shear, the shearing platens are allowed free rotation relative to one another (see Figure 3). Additionally, images were captured during testing to monitor changes in the ink lines with respect to load, however only the LVDT was used to record deformation. Meniscal explants were strained at a very low rate of 0.002 sec^{-1} , so as to minimize fluid effects (Proctor, et al., 1989), until material or fixative failure.

2.3. Deformation homogeneity

Others have examined simple shearing of fiber-reinforced bio-composites and have utilized various methods to either ensure homogenous deformation or circumvent this concern using inverse FE methods to obtain material parameters (Dokos, et al., 2002, Gardiner and Weiss, 2001, Weiss, et al., 2002). In reviewing of simple shearing of rubbers and biological tissues by Horgan and Murphy the aspect ratio of 1:4, with the sample height between shearing platens being smaller, is recommended in order to ensure homogeneity of shear stresses up to the shearing modulus. Though it is admitted that based on differing and well-qualified assumptions the interpretation of the stress field remains a bit ambiguous (Horgan and Murphy, 2011). Here an experimental ratio closer to 1:2 was utilized due to tissue size constraints and consideration for a small amount of fixative surface penetration. Pilot work using digital image correlation examined the shear deformation homogeneity of the meniscal explants. A random speckle pattern of India ink was applied to the surface of the test specimen using a gravity-fed spray gun backed by 10 psi of air pressure. Inked specimens were sheared and a charge coupled device camera (MicroPix Ltd, West Sussex, UK) recorded images at 7.5 fps. Digital image correlation software (Opticist 0.948, www.opticist.org) was used to process and visualize shear deformation. The 6 parameter zero-normalized sum of squared differences algorithm was utilized to process 21 pixel^2 subset displacements as per recommendation of Pan et al. as it has proven to be robust and reliable (Pan, et al., 2009).

2.4. Piecewise linear elastic analysis

All data sampled were processed using a second order Savitzky-Golay smoothing function to aid in regional identification. Experimentally measured force, V , was converted to shear stress, τ , using specimen diameter, d , Eq. (1). The shear strain was calculated using the specimen length, L , and change in displacement, δx , recorded from the LVDT, Eq. (2). The toe region shear modulus was calculated from the first observed linear region, Eq. (3), between 3%–13% shear strain and the 2nd, extended shear modulus, was established after 80% of the maximum shear strain until material or fixative failure. All data processing was performed using custom MATLAB scripts (version 7.6 (R2008a), Natick, MA).

$$\tau = V / ((\pi/4)d^2) \quad (1)$$

$$\gamma = \delta x / L \quad (2)$$

$$G = \tau / \gamma \quad (3)$$

2.5. Anisotropic Hyperelastic Modeling

The meniscus was considered a hyperelastic material (See Appendix 1). To account for both the circumferential and radial tie fibers the material is considered an isotropic matrix

reinforced with 2 orthogonal unique collagen fiber families (Rattner, et al., 2010, Skaggs, et al., 1994) represented by the direction vectors a_i , $i = 1, 2$ with $|a_i| = 1$. These vectors result in the fiber family structure tensors $M_i = a_i \otimes a_i$, depicting the material anisotropy (Balzani, et al., 2006, Holzapfel, et al., 2000). Due to the presence of hydrophilic glycosaminoglycans the material is considered incompressible thus, similar to Balzani et al. (Balzani, et al., 2006), a decoupled strain-energy potential can be written as

$$\psi = \psi^{iso} + \psi_1^{aniso} + \psi_2^{aniso} + \psi^{vol} \quad (4)$$

where ψ^{iso} and ψ_n^{aniso} $n=1, 2$ represent the isochoric matrix and fiber contributions, respectively, and ψ^{vol} is a purely volumetric contribution. Particularized forms of ψ are such that the isotropic matrix is modeled using the classic neo-Hookean model, Eq. (5), and a polynomial function, Eqs. (6, 7), is leveraged to encapsulate the observed stiffening effect such that

$$\psi^{iso} = \frac{c}{2} (I_1 - 2) \quad (5)$$

$$\psi_1^{aniso} = \sum_{a=1}^2 \left[\alpha_1^a (J_4^a - 1)^{\alpha_2^a} \right], \psi_2^{aniso} = \sum_{a=1}^2 \left[\alpha_3^a (K_3^a - 2)^{\alpha_4^a} \right] \quad (6, 7)$$

$$\psi^{vol} = \frac{p}{2} (I_3 - 1) \quad (8)$$

where $J_4^a = \text{tr} [C M^a]$ and $K_3^a = I_1 J_4^a - J_5^a$ are so-called mixed invariants which control the square of the stretch in the fiber direction and deformation of an area element with a normal perpendicular to the fiber direction, respectively, with $J_5^a = \text{tr} [C^2 M^a]$, and p is an indeterminate Lagrange multiplier due to the aforementioned incompressibility (Schroder and Neff, 2003). The implementation of these explicit invariants satisfies the polyconvexity condition in the sense of Ball (Balzani, et al., 2006), thus ensuring a physically reasonable and numerically stable material model. The parameters $c > 0$ and $\alpha_i^a > 0$ $|i=1, 3$ are stress-like material parameters for the matrix and fiber families, respectively, and $\alpha_j^a > 0$ $|j=2, 4$ are dimensionless parameters. Material parameters were determined using all test points for a given group aggregated into a single sorted dataset and fit simultaneously, yielding a single set of parameters (Morrow, et al., 2010). The parameters α_1^a & α_2^a are not identified as they do not influence the shear responses examined in the present study. Curve-fitting was performed using a nonlinear least squares optimization routine implemented in a custom MATLAB script.

2.6. Statistical analysis

A three-way blocked ANOVA was performed for each group of moduli to make anatomical location, region, and fiber orientation comparisons. Identified significant differences between regions for the extended shear modulus were further investigated using a post-hoc t-test. P-value less than 0.05 was considered significant.

3. Results

Digital image correlation revealed a largely homogenous deformation pattern with slight warping primarily near the edges of the traction surfaces (Figure 4). This warping appeared to penetrate ~10% into the specimen's surface; however this did not result in any apparent discrepancy between the relative platen displacement and DIC computed deformation.

There were significant differences between the fiber orientation groups for the toe region modulus with the perpendicular group being more compliant (Figure 5). While the extended shear modulus did not show significant differences between the two different fiber orientation groups, significant regional differences were noted. Post-hoc t-tests revealed an explicit distinction between the central and posterior regions for the extended shear modulus (Figure 5). These findings dictated grouping the data by region and orientation in preparation for hyperelastic curve-fitting, yielding a total of six datasets.

The utilized strain-energy density function qualitatively fit the trend of the experimental data (Figure 6) for each of the regions. Additionally, the root mean square error for each group was relatively low with an upper bound of 0.018 MPa (Table 1), indicative of the ability of the function selected to characterize the sample population.

4. Discussion

The results of this study reinforced that, for small strains, the meniscus exhibits anisotropic behavior (Anderson, et al., 1991, Zhu, et al., 1994). Similar to the theory of others, the lower observed toe modulus for the perpendicular specimens may be due to the sheaths of radial tie fibers (RTF) facilitating slip between circumferential fiber layers, analogous to boundary slip along shear surfaces in composite materials (Mow, et al., 1992, Rattner, et al., 2010). RTF's in bovine medial menisci have previously been shown to be more abundant and thicker in the posterior region, resulting in higher tensile stiffness when compared to anterior and central (Skaggs, et al., 1994). This trend was repeated for large shear strains as the posterior region was significantly stiffer than the central region. This could be in part due to take up of the shear load by the larger RTF's as the meniscus is subjected to large shear deformations.

Biochemical analyses have shown that proteoglycan presence and quality has a dramatic effect on shear properties in articular cartilage (Zhu, et al., 1994). Despite the meniscus possessing an eight-fold lower concentration of proteoglycans than articular cartilage (Adams and Muir, 1981) their presence may still have a significant contribution to the observed shear properties. Examination of regional differences in bovine menisci has shown that proteoglycan presence is greatest in the posterior region (Eyre and Wu, 1983) and total GAG presence in canine tissue is lower in the central region (Adams and Muir, 1981), correlating with mechanical findings here. Others have postulated that molecular cross-linking of proteoglycans and the collagen fibrils could be inducing a stiffening behavior, hence resulting in the observed regional differences (Weiss, et al., 2002). Clearly, the mechanisms dictating shear performance appear reliant on both the meniscus ultrastructure and interaction between the extracellular matrix constituents, facilitating the aforementioned layer sliding (Mow, et al., 1992).

Expanding on the observed material anisotropy from the small strain region a modified decoupled strain-energy density function was utilized to describe the material response for the sampled population. The chosen model, based on prior work with anisotropic vascular tissue, readily expedites investigation of complex loading mechanisms and microstructure by means of coupling deformation and structure tensors in the strain-energy density function. An additional advantage is the recent incorporation of anisotropic hyperelastic

modeling in commercially available FE software (ABAQUS, Simulia, Providence, RI), which includes the model this analysis is derived from (Holzapfel, et al., 2000). The groundwork performed here should act as a compass for those seeking a material model to emulate the anisotropy and inhomogeneity of the meniscus, both of which are crucial aspects in understanding how this structure functions (Mow, et al., 1992, Setton, et al., 1999).

Experimental techniques utilized in this study pose several limitations. The noted ideal aspect ratio was not adhered to, thus true homogeneity cannot be assured throughout. Digital texture correlation techniques revealed a fairly homogenous distribution in the specimen midsubstance; however, there is evident inhomogeneous warping of the pixel subsets (Figure 4). This effect is observed more often near the traction surfaces, presumably due to adhesive penetration and not a direct effect of tissue ultrastructure. As the primary aim of this study was to assess if regional structure and biochemistry disparities exhibit similar trends in practice, the piecewise linear metrics chosen are employed comparatively rather than definitively. Additionally, the purposefully simplistic loading scenario of explants examined here was exploited to simplify the continuum analysis; however, physiologically and pathologically relevant loading could also be introduced via modification of the deformation tensor. Also, special care was taken to remove the majority of randomly oriented fibers to aid in parameter determination; however, the structure tensors could easily be adapted to incorporate imaging data that assesses the relative fiber angles for a given spatial location throughout the meniscus. Lastly, it is acknowledged that sparse oblique tie fibers do branch from the main radial fibers; however, their influence was not considered in this analysis as it is presumed that the primary axes of interest are explicitly circumferential and radial, which have been rigorously explored in the literature.

Novel to this work is regard for regional and anatomical location of meniscus material response due to shear loading, borne from supporting biochemical analyses linking proteoglycan distribution and mechanical performance. The findings indicate region specific shearing properties should be taken into consideration when attempting to model meniscus material behavior. Additionally, conventional modeling has often regarded the meniscus as a transversely isotropic material; however, the observed shearing anisotropy violates this presumed material symmetry. A structurally driven mechanical model was shown to encapsulate this behavior and is proposed as a viable candidate for those seeking to model knee joint behavior. Continued study of experimental meniscus biomechanics coupled with advanced modeling techniques will undoubtedly forge novel understanding of joint etiology and reparative techniques.

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Appendix 1

Kinematics and hyperelasticity

The deformation tensors for the experimental loading scenarios as a function of shear strain γ_{ij} are

$$\mathbf{F}_1 = \begin{bmatrix} 1 & \gamma_{12} & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}, \mathbf{F}_2 = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & \gamma_{32} & 1 \end{bmatrix}$$

Additionally, the important strain-like right Cauchy-Green tensor, C , is introduced where

$$C := F^T F$$

The meniscus is considered a hyperelastic material which implies that the Cauchy stress σ is defined as

$$\sigma = 2J^{-1} F \frac{\partial \Psi(C)}{\partial C} F^T$$

where ψ is the Helmholtz free-energy function and $J = \det(F) > 0$ is the local volume ratio (Holzapfel, 2001).

Figure 1.

Anatomical location and regional identification for left leg of a test specimen. Regions were defined by measuring the circumference of each meniscus and marking three equidistant points determined by the arc length, thus accounting for differences in meniscal sizes. Two explants were extracted from each region, one for each of the two fiber orientation deformation cases. Superficial and lamellar surfaces were removed in order to minimize the presence of randomly oriented collagen fibers.

Figure 2.

A) Surface of meniscus appears homogenous to the naked eye due to the small randomly oriented fibrils. B) Removal of superficial and lamellar layer reveals circumferentially aligned collagen fiber bundles. C) Transverse sectioning of the meniscus shows radially aligned tie fibers (white arrows).

Figure 3.

Schematic of lap shearing fixture. Both platens are allowed to freely rotate about the single same dimension. An adjacent LVDT is used to measure relative displacement of the platens.

Figure 4.

Digital texture correlation deformation mesh of parallel and perpendicular specimens. Gaps in parallel mesh are due to indistinguishable speckling in that region. Shear deformation appears more homogenous within the specimen midsubstance. Inhomogeneous deformation is observed closer to the applied traction surfaces, presumably due to adhesive penetration.

Figure 5.

The parallel and perpendicular fiber orientations are significantly different from one another for small strains (Toe Modulus). The central and posterior regions are significantly different from one another for large strains (Extended Shear Modulus). * = $p < 0.05$

Figure 6.

Cauchy stress vs. shear strain for anterior parallel samples. Open symbols represent experimental data and solid line is the fit using the anisotropic hyperelastic constitutive equation (Eq. 4). The chosen model accurately describes the trending behavior for all datasets considered. Root mean square error = 0.001.

Table 1

Material parameters from the hyperelastic anisotropic analysis for specimens subjected to simple shear. The strain-energy density function utilized accurately describes the material behavior across all groups as demonstrated by low RMSE values. Superscript a defines the fiber family ($a=1$:Circumferential, $a=2$:Radial) which the according parameter belongs to.

Region	Orientation	Parameters [MPa]			RMSE
		c	$\sigma^{a,3}$	$\sigma^{a,4}$	
Anterior	Parallel $\mu=1$	0.609	0.282	1.586	0.001
	Perpendicular $\mu=2$		0.299	1.603	0.018
Central	Parallel $\mu=1$	0.543	0.236	1.577	0.013
	Perpendicular $\mu=2$		0.214	1.540	0.012
Posterior	Parallel $\mu=1$	0.544	0.171	1.501	0.012
	Perpendicular $\mu=2$		0.247	1.573	0.010