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NANOINDENTATION OF HUMAN MENISCAL SURFACES

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Abstract

Menisci are crescent shaped fibrocartilaginous structures which support load distribution of the knee. The menisci are specifically designed to fit the contour of the femoral condyles, aiding to disperse the stresses on the tibial plateau and in turn safeguarding the underlying articular cartilage. The importance of the meniscal superficial layer has not been fully revealed and it is suspected that this layer plays a pivotal role for meniscal function. In this study, both femoral (proximal) and tibial (distal) contacting meniscal surfaces were mechanically examined on the nano-level among three distinct regions (anterior, central and posterior) of the lateral and medial menisci. Nanoindentation testing showed no significant differences among regions, surfaces or anatomical locations, possibly elucidating on the homogeneity of the meniscal superficial zone structure ($E_{\text{Instantaneous}}$: 3.17–4.12 MPa, $E_{\text{Steady-State}}$: 1.47–1.69 MPa). Nanomechanical moduli values were approximately an order of magnitude greater than micro-scale testing derived moduli values. These findings validate the structural homogeneity of the meniscal superficial zone, showing that material properties are statistically similar regardless of meniscal surface and region. Understanding the mechanical behavior of meniscal surfaces is imperative to properly design an effective meniscal replacement.

Keywords

meniscus; nanoindentation; superficial layer; surface; modulus

1 Introduction

Human knee menisci are semi-lunar shaped fibrocartilaginous structures that provide support, load distribution, and lubrication within the joint, ultimately aiding to attenuate stresses at the tibiofemoral site (Messner, et al., 1998; Shrive, et al., 1978; Walker, et al., 1975). The structural integrity of the menisci has been shown to be instrumental in conserving the underlying articular cartilage (AC) thereby slowing the progression of osteoarthritis (OA) (Andriacchi, et al., 2004). Menisci transmit approximately 70% of the

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Conflict of Interest Statement

The authors affirm that they have no financial affiliation or involvement with any commercial organization that has direct financial interest in any matter included in this manuscript

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load through the knee by supporting hoop/circumferential stresses as well as axial stresses (Ahmed, et al., 1983; Seedhom, 1976; Walker, et al., 1975).

Meniscal composition is inhomogeneous and biphasic, consisting of varying types of collagen fibrils and proteoglycans within the solid matrix of the meniscal body (McDevitt, et al., 1990; Petersen, et al., 1998; Roughley, et al., 1981), and 70% interstitial fluid (Brindle, et al., 2001; Fithian, et al., 1990). Type I collagen fibrils are predominately circumferentially oriented within the menisci, however additional varying collagen fiber orientation layers help maintain the intrinsic structure of the meniscus (McDevitt, et al., 1990; Petersen, et al., 1998). The superficial layer (tibial and femoral AC contacting surfaces), of the meniscus is approximately 150–200 μm thick (Ling, et al., 2012) and is composed of a tight meshwork of randomly oriented collagen fibrils with 10 μm diameter Type I collagen fibers (Figure 1). This network of taut, ‘mat-like’ collagen fibrils is believed to be similar to that of the AC tangential fiber layer (Aspden, et al., 1981; Petersen, et al., 1998; Setton, et al., 1993). It has previously been shown that both flow-dependent and -independent viscoelastic mechanisms rely on the strength of the cartilage superficial zone (Setton, et al., 1993). Moreover, recent investigations of the meniscal superficial layer suggest a stiffer superficial layer in meniscus compared to cartilage (Lai, et al., 2010). Interestingly, the depth dependent properties were inconsistent in the menisci with half of the meniscal samples ($n=6$) displaying uniform compressive strains throughout the superficial layer thickness, and half displaying depth dependency (Lai, et al., 2010). Others have suggested that the contacting meniscal superficial layer may serve a major role to uphold meniscal integrity (Andriacchi, et al., 2004; Newman, et al., 1989; Walker, et al., 1975).

Previous mechanical testing on human menisci has been performed on the macro-level, either using fiber aligned coupon samples for tension tests or meniscal disc samples for confined/unconfined compression (Chia, et al., 2008; Fithian, et al., 1990; Tissakht, et al., 1995; Whipple, et al., 1985). It is unknown if this macro-scale testing can isolate the superficial zone behavior to determine a structure-function relationship at a local level. Nanoindentation provides a physiologically relevant testing procedure to determine the viscoelastic material properties of the meniscus on the scale of the meniscal constituents and has been shown to be applicable for mechanical testing of cartilage-like hydrated tissues (Ebenstein, et al., 2004; Ebenstein, et al., 2006). Lastly, research has shown studying cartilage material properties with a spherical indenter tip (2.5 μm radius) is effective in detecting stiffness changes following elastase digestion of collagen (Stolz, et al., 2004).

To date, human menisci have not been characterized on the nano-scale. Sweigart et al. examined regional (anterior, central and posterior) differences in the proximal and distal meniscal surfaces at the micro-scale (Sweigart, et al., 2004). They reported no differences in proximal versus distal layer, but significant differences did exist in material properties among regions (Sweigart, et al., 2004). Given the importance of the superficial zone and previous investigations on the structure of this zone in the human meniscus, the objective of this study was to quantify and compare the viscoelastic nano-mechanical properties of human menisci on both the proximal (femoral contacting) and distal (tibial contacting) surface. While it has been shown that there are significant regional differences when testing meniscal surfaces on the micro-level, it is hypothesized that the proximal and distal meniscal surfaces will have no regional differences as previous structural studies suggest a homogenous superficial layer (Lai, et al., 2010; Ling, et al., 2012; Petersen, et al., 1998). To isolate the superficial zone nanoindentation will be used on both the proximal and distal surfaces of the anterior, central and posterior regions of lateral and medial human menisci. Nanoindentation will provide an improved understanding of the mechanical behavior at the nano-scale and thus given additional insight into the behavior and role of this superficial zone.

2 Materials and Methods

2.1 Specimen preparation

Eight healthy cadaveric knees (7 males, 1 female, ages 50–77, avg. age 59) (NDRI, Philadelphia, PA) were procured, wrapped in 0.9% saline solution soaked gauze and frozen at -20°C until the time of dissection. Both lateral and medial menisci were cut at the meniscal attachment site, and trisected into anterior, central and posterior regions for regional comparisons (Figure 1) (Chia, et al., 2008; Fithian, et al., 1990; Sweigart, et al., 2004; Tissakht, et al., 1995; Whipple, et al., 1985). Once each meniscus had been trisected, 2 mm wide sections were cut through the cross-section from each region; one sample for proximal mechanical testing and one for distal mechanical testing. Samples were trimmed so that they could be placed under the nanoindenter tip such that indentation was perpendicular to the surface (Figure 1). Nitrocellulose was applied to the face opposite of indentation followed by double-stick tape to facilitate adhesion between the sample and the well puck. Saline solution was filled into the well to keep the specimen fully hydrated throughout the test.

2.2 Testing Procedure

Nanoindentation was performed on both proximal and distal meniscal surfaces using a Nano Indenter (Agilent Technologies, Santa Clara, CA) with a $300\mu\text{m}$ diameter spherical ruby tip (Agilent Nano Measurements, Indianapolis, IN). Fifteen indents were performed on each meniscal sample, spatially randomizing indent locations, normal to the surface (Figure 1). Indents were spaced at least $250\mu\text{m}$ apart to avoid effects from subsequent indents (Ebenstein, et al., 2004). Creep nanoindentation was performed using a trapezoidal loading sequence with a 5 second rise time and 1 mN hold for 70 seconds. These testing parameters were chosen based on preliminary testing results and to limit surface penetration to approximately $10\mu\text{m}$.

Resultant displacement-time data (Figure 2), was curve-fit, using Matlab Optimization toolbox (Math-works, Natick, MA), to determine both instantaneous and steady-state material properties. Material properties are examined within the framework of Hertzian contact (Lee, et al., 1960). Displacement-time history is described by integrating over the ramp and hold phase using an explicit creep compliance function $J(t)$ and loading profile, $P(u)$, over the entire ramp and loading phase as generalized by (Oyen, 2006; Oyen, 2011; Oyen, 2005)

$$h^n(t) = k_g \int_0^t J(t-u) \frac{dP(u)}{du} du \quad (1)$$

where n is the power law tip geometry factor ($n = 3/2$ for spherical) and k_G are geometrical constants. Assuming linear viscoelastic behavior, a Prony series is used to describe material creep where

$$J(t) = C_0 + \sum_{i=1}^j C_k e^{-t/\tau_k} \quad (2)$$

Experimental data can then be fit to the explicit solution of the integral using (1) and (2) and assuming a constant loading rate, yielding

$$h^{3/2}(t) = A_0 - \sum_{i=1}^j A_k e^{-t/\tau_k} \quad (3)$$

where $j = 2$ provided a quality fit to experimental data. Explicit creep function parameters are then determined using

$$C_0 = \frac{8A_0 \sqrt{R}}{3P_{Max}} \quad (4)$$

$$C_k = \frac{8A_k \sqrt{R}}{3P_{Max}(RCF_k)} \quad (5)$$

where RCF is the ramp correction factor for creep accrued during the loading phase as described by

$$RCF_k = \frac{\tau_k}{t_R} [e^{t_R/\tau_k} - 1] \quad (6)$$

and t_R is the length of the ramp phase. Creep compliance coefficients are then utilized to compute instantaneous, G_0 , and steady-state shear modulus, G_∞

$$G_0 = \frac{1}{2(C_0 - \sum_{i=1}^j C_k)} \quad (7)$$

$$G_\infty = \frac{1}{2C_0} \quad (8)$$

Shear modulus computed from an assumption of incompressibility can then be converted to a shear modulus with a constant and compressible assumption for Poisson's ratio (Oyen, 2006; Oyen, 2011).

$$G_{0,\infty}^v = 2G_{0,\infty}(1-\nu) \quad (9)$$

Assuming that $\nu = 0.38$ for the human meniscus (Hu, et al., 2001), the shear modulus is converted to elastic modulus using

$$E_{0,\infty} = 2G_{0,\infty}^v(1+\nu) \quad (10)$$

Elastic moduli were calculated for all three regions, on both proximal and distal meniscal surfaces. Furthermore, an 'elastic-fraction' was calculated by taking the ratio between steady-state and instantaneous elastic modulus. This term describes material elastic/viscous behavior, with a value equal to 1 signifying a perfectly elastic material, and a value equal to 0 embodying a perfectly viscous material (Oyen, 2011). Material property values from each indent were calculated and then averaged to gain an overall average material property value from each sample to encompass the meniscal surface.

2.3 Statistical Analysis

A three-way Analysis of Variance (ANOVA) was performed to determine differences in material properties (instantaneous and steady-state) among meniscal surface (proximal/distal), anatomical location (medial/lateral) and meniscal region (anterior/central/posterior), for both instantaneous and steady-state moduli. The statistical power analysis revealed a power of 95%, given an expected difference from the calculated material property value was 25%.

3 Results

Nano-indents produced a displacement-time curve with a linear region during ramp loading followed by a horizontally asymptotic curve during the held load (Figure 2), indicative of steady-state behavior. The average indenter contact radius was $53.61 \pm 3.53 \mu$, as the displacement into the meniscal surface was approximately $10 \mu\text{m}$ for each indent (Figure 1).

All meniscal samples, regardless of contacting surface, region or anatomical location, exhibited statistically similar behavioral mechanics, when nano-indented (Figure 3). Variation among individual indents was less than 5% of the overall average material property value for each specimen, indicating the homogeneity among individual indents on each meniscal sample. When comparing steady-state elastic moduli values between anatomical locations, anterior and posterior regions tended to display lower distal surface values on the medial meniscus compared to the lateral, and larger proximal surface values on the medial meniscus compared to the lateral (Figure 3A), however this was not statistically significant. Additionally, instantaneous elastic moduli did not demonstrate any noticeable trends and all surfaces and regions appeared to be very comparable (Figure 3A & B).

When only examining meniscal regions, the anterior ($3.86 \pm 0.30 \text{ MPa}$) and central ($3.56 \pm 0.19 \text{ MPa}$) region displayed the largest and smallest instantaneous elastic modulus, respectively. The opposite was true for the steady-state elastic modulus, as the central region ($1.65 \pm 0.07 \text{ MPa}$) had the largest value and the anterior region ($1.58 \pm 0.08 \text{ MPa}$) was the smallest.

The three-way blocked ANOVA revealed no significant differences for steady-state and instantaneous material property values between meniscal regions, surface or anatomical locations. There were no significant differences in elastic fraction values between meniscal regions, surface, or anatomical locations. All elastic fraction values were between 0.4 and 0.5 (Figure 3C).

4 Discussion

While other studies have investigated the compressive properties of mammalian menisci (Chia, et al., 2008; Joshi, et al., 1995; Li, et al., 2007; Sweigart, et al., 2004), this is first study to nanoindent human meniscal surfaces and considers the three distinct regions (anterior, central and posterior). Nanoindentation was used rather than other compression-based methods (micro-indentation, confined compression, etc.) for both the increased spatial resolution and the aspiration of elucidating single meniscal surface material properties rather than gathering material properties from bulk constituents. While indentation depths and contact areas shown in this study were in the microscale range, the commercially available nanoindenter used had a resolution of 0.01 nm and 50 nN. To ensure consistency in the literature, we refer to the technique used for material characterization as nanoindentation (Ebenstein, et al., 2006). A spherical nanoindentation tip was used to ensure consistent contact area (Oyen, 2005) given the uneven meniscal surface contour. Also, nanoindentation

was utilized to isolate the meniscal superficial layer by only penetrating approximately 10 microns into the specimen surface, whereas the superficial layer is suspected to be 150–200 microns thick (Ling, et al., 2012; Petersen, et al., 1998).

The results from this study indicate there are no significant nano-mechanical differences among meniscal regions, surfaces or anatomical locations. It is very important to note that the proximal and distal meniscal surfaces both behave similarly, as the fluid interaction with the tibial and femoral articular cartilage is crucial for decreasing friction and allowing for proper joint articulation. While the proximal and distal surfaces of the meniscus are often considered important because of their ability to provide a low friction surface on which the joint can articulate, it is unknown how the mechanical properties determined in this study influence this function. The material property similarities may be expected, given the collagen fiber orientation at the meniscal superficial layer is a random fiber network, but with fibrils coplanar to the surface (Aspden, et al., 1981; Petersen, et al., 1998). Additionally, meniscal composition within the superficial layer, other than a collagen fiber network, is not well characterized, and it is known that other meniscal constituents, i.e. proteoglycans, have a direct correlation with mechanical strength and viscosity in compression (Sanchez-Adams, et al., 2011). While no significant differences in elastic fraction were found in the current study, elastic fraction has been shown to help identify connective tissue type and geometry, in addition to aiding in evaluation of mechanical degeneration (Dunn and Silver, 1983). Further investigation of meniscal composition in the superficial zone may help form a better understanding of the structure-function relationship at this crucial interface.

Li et al. conducted a very similar indentation study on mini-pig menisci, using a 500 μm diameter conospherical tip and using a peak indentation load of 10 mN. Although anatomical location, region or surface was not specified, a Young's modulus value was reported to be approximately 1.3 MPa, the same order of magnitude of results shown in the current study (Li, et al., 2007). Other studies have also analyzed the human meniscus to gather regional mechanical properties (compressive and tensile); however, these tests were completed on a micro-scale or greater (Chia, et al., 2008; Fithian, et al., 1990; Joshi, et al., 1995; Sweigart, et al., 2004; Tissakht, et al., 1995; Whipple, et al., 1985). Evaluating the meniscus on a larger scale (micro-indentation, confined compression, etc.), may result in mechanical properties that represent the bulk constituents of the menisci, rather than using small-scale investigations (nano-indenting) to isolate specific features.

Other compression based studies, focusing on the medial meniscus, have shown the meniscus to be weakest in the posterior region (Table 1), possibly elucidating why the majority of meniscal tears occur in the posterior meniscus (Ahn, et al., 2009; Brody, et al., 2006; Choi, et al., 2008; De Smet, et al., 2009; Helms, et al., 1998; Lykissas, et al., 2010; Singson, et al., 1991). These macro-scale test results are approximately an order of magnitude smaller than what is seen here from nanoindentation. This discrepancy could be due to engagement of specific meniscal constituents, rather than bulk material, or differences in test modalities. By limiting surface penetration to approximately 10 μm , only the tightly woven superficial collagen is engaged rather than spatially averaging between the structurally unique superficial and deep zones. The ramifications of this testing indicate that the surface layer is specifically adapted at supporting compressive loading and perhaps pivotal for allowing fluid movement while maintaining basic function. Also, meniscal superficial zone is crucial for fluid flow within the meniscal body (Setton, et al., 1993; Setton, et al., 1993) and each different test may influence the varying fluid flow patterns of the meniscus. It has been shown that the average time it took meniscal surface specimens to reach a steady-state displacement, defined as a change in displacement less than 0.01%, was approximately 57 seconds. To better elucidate on the importance of fluid flow through

meniscal surface canals, this value is compared to a similar indentation study, utilizing a 1.0 mm flat punch indenter, where the amount of time to steady-state behavior was shown to be 1000 seconds (Sweigart, et al., 2004). This large discrepancy in time may demonstrate the importance of meniscal canals for fluid flow patterns and evacuation. Nanoindentation, with a rigid tip, may cause the solid and fluid phases of the meniscal surface to behave differently than when compressed with a 1–2 mm porous platen (Joshi, et al., 1995; Sweigart, et al., 2004), as contact areas are altered, and fluid movement through meniscal canals differ for fluid evacuation (Bird, et al., 1988; Bird, et al., 1987; Neurath, et al., 1992).

When comparing various compression based testing methods for articular cartilage, it can be seen that the same relationship for macro- and nano-scale testing applies for the meniscus as for articular cartilage (Table 2). Unconfined and confined compression based methods, both utilizing larger platens for compression, exhibit nearly order of magnitude smaller values than -indentation type tests. This further affirms the close comparison between articular cartilage and meniscus fibrocartilage.

These material properties on the nano-scale coupled with other larger scale tensile, compressive and shear properties of the meniscus (Chia, et al., 2008; Fithian, et al., 1990) will help accurately model the meniscus for the ultimate goal of creating an effective meniscal replacement that can mimic the native human menisci (Aufderheide, et al., 2007; Aufderheide, et al., 2005; AufderHeide, et al., 2004; Abraham, et al., 2011; Donahue, et al., 2002; Hauch, et al., 2009; Maes, et al., 2006). While a previous study analyzed the regional inhomogeneity of both the proximal and distal meniscal surfaces on the microscale (Sweigart, et al., 2004), this is first study to regionally isolate and compare meniscal surfaces on the nano-scale. It is essential to chronicle the fact that the meniscal surface layer has been shown to be structurally homogenous, and now this has been validated by showing the material properties of the meniscal superficial zone do not vary among regions or contacting surface. It has been shown that there is an order of magnitude difference between meniscal surface material property values when examined on the nano-scale compared to the micro-scale. This discrepancy may prove vital when attempting to recreate human meniscal replacements. Additionally, these data will likely prove to be imperative for a thorough understanding of the how the meniscus interacts with articular cartilage contacting surfaces.

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Figure 1.

A schematic diagram of the complete testing procedure conducted on the human meniscus.

a) Both medial and lateral menisci are trisected into b) anterior, central and posterior regions. c) A cross-section is cut from each region and proximal and distal surfaces are excised, 2mm deep from respective meniscal surface. Included is a nanoindentation schematic displaying relative indenter tip size and depth, along with meniscal constituents within superficial layer. d) Representative sample placed in well, fully hydrated, with indentation conducted normal to specimen surface. Dashed lines represent specimen cutting.

Figure 2. Representative displacement-time creep curve from nanoindentation on meniscal surfaces. Dots represent data points, solid line is the curve fit for the viscoelastic model used in this analysis.

Figure 3.

A. Average steady-state elastic modulus- B. Average instantaneous elastic modulus- C. Average elastic fraction values from nanoindentation conducted on both proximal and distal meniscal surfaces. Error bars represent standard error.

Table 1

Material property comparison among varying compression based mechanical tests on the medial meniscus
(Steady-state modulus (MPa) $\pm$ Standard Deviation)

Regions Studied	Nanoindentation (current study)	Microindentation (Sweigart, et al., 2004)	Unconfined Compression (Chia, et al., 2008)	Confined Compression (Joshi, et al., 1995)
Anterior	1.63 ± 0.10	0.16 ± 0.04	0.14 ± 0.17	NA
Central	1.67 ± 0.13	0.11 ± 0.04	0.080 ± 0.078	NA
Posterior	1.47 ± 0.07	0.10 ± 0.03	0.033 ± 0.046	0.22 ± 0.07

Table 2

Material property comparison among varying compression based mechanical tests on articular cartilage at various scales.

Type of Study	Study	Steady-State Modulus $\pm$ SD (MPa)
Nanoindentation	(Thambyah, et al., 2006)	3.64 ± 1.23
Micro-indentation	(Athanasίου, et al., 1991)	0.54 ± 0.10
Unconfined Compression	(Boschetti, et al., 2004)	0.23 ± 0.15
Confined Compression	(Armstrong, et al., 1984)	0.79 ± 0.36
	(Boschetti, et al., 2004)	0.25 ± 0.10