Published in final edited form as:
J Biomech. 2021 May 07; 120: 110343. doi:10.1016/j.jbiomech.2021.110343.

VISCOELASTIC AND EQUILIBRIUM SHEAR PROPERTIES OF HUMAN MENISCUS: RELATIONSHIPS WITH TISSUE STRUCTURE AND COMPOSITION

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Abstract

The meniscus is crucial in maintaining the knee function and protecting the joint from secondary pathologies, including osteoarthritis. Although most of the mechanical properties of human menisci have been characterized, to our knowledge, its dynamic shear properties have never been reported. Moreover, little is known about meniscal shear properties in relation to tissue structure and composition. This is crucial to understand mechanisms of meniscal injury, as well as, in regenerative medicine, for the design and development of tissue engineered scaffolds mimicking the native tissue. Hence, the objective of this study was to characterize the dynamic and equilibrium shear properties of human meniscus in relation to its anisotropy and composition.

Specimens were prepared from the axial and the circumferential anatomical planes of medial and lateral menisci. Frequency sweeps and stress relaxation tests yielded storage (G') and loss moduli (G''), and equilibrium shear modulus (G). Correlations of moduli with water, glycosaminoglycans (GAGs), and collagen content were investigated.

The meniscus exhibited viscoelastic behavior. Dynamic shear properties were related to tissue composition: negative correlations were found between G', G'' and G, and meniscal water

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Author Contributions Statement: Research design was conducted by CN, GF, FA, TMB, MB, FT and ARJ; data acquisition and analysis was carried out by CN and FT; data interpretation involved CN, FT, GF, TMB and ARJ; all the authors were involved in drafting and revising the manuscript, as well as reading and approving its final version.

content; positive correlations were found for G’ and G’’ with GAG and collagen (only in circumferential samples). Circumferential samples, with collagen fibers orthogonal to the shear plane, exhibited superior dynamic mechanical properties, with G’ ~70 kPa and G’’ ~10kPa, compared to those of the axial plane ~15 kPa and ~1kPa, respectively. Fiber orientation did not affect the values of G, which ranged from ~50 to ~100kPa.

Keywords

fibrocartilage; storage modulus; loss modulus; frequency sweep; stress relaxation collagen; glycosaminoglycans

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INTRODUCTION

The menisci play a crucial role in the functioning of the knee joint, bearing 45–75% of the total load on the knee, as well as maintaining congruency and lubrication in the joint (Shrive et al., 1978). Nevertheless, meniscal tearing is the second most prevalent sports-related injury, and it has been strongly associated with anterior cruciate ligament tears (Hagino et al., 2015; Joseph et al., 2013) and early onset osteoarthritis (OA) (Joseph et al., 2013; Paxton et al., 2011; Pena et al., 2005; Stärke et al., 2009; Tengrootenhuysen et al., 2011; Zantop et al., 2006). Although the etiology of meniscal tears is not completely understood yet, results from biomechanical and orthopedic studies suggest that shear forces experienced in the knee are highly correlated with meniscal injury (Greis et al., 2002; Pena et al., 2005; Stärke et al., 2009; Zantop et al., 2006). Therefore, understanding meniscal shear properties is crucial to elucidate the mechanisms of tissue injury. Such knowledge is critical for developing strategies to treat and repair the damaged tissue: when designing engineered tissue constructs, biomimicry of tissue mechanical properties is essential for biomechanical stability at the site of implantation, cellular engraftment and differentiation, and new extracellular matrix (ECM) deposition. Moreover, quantitative information on shear properties is critical for developing computational models of the meniscus, which can be employed to understand and predict tissue behavior *in vivo*, and also to derive novel treatment approaches.

As fibrocartilaginous tissues, meniscal structure and biochemical composition contribute to tissue load bearing capabilities. The meniscal ECM is primarily composed of collagen type-1 and type-2, and proteoglycans (Fox et al., 2012; Pereira et al., 2013). The outmost layer of the meniscal ECM comprises unaligned collagen fibers, similar to hyaline cartilage, which aids in joint lubrication. Progressing deeper into the tissue, bundles of collagen fibers are oriented radially and circumferentially. This structural arrangement of fibers is thought to optimize the tissue’s compressive and tensile load bearing properties (Athanasiou and Sanchez-Adams, 2009; Fox et al., 2012; Pereira et al., 2013). Specifically, it has been shown that the meniscus exhibits anisotropic mechanical behavior, which is largely due to the presence of circumferential fibers. In fact, compression, tension, and shear tests have shown that the meniscus is stiffer in the circumferential direction, when compared to the axial or the radial direction (Abraham et al., 2011; Athanasiou and Sanchez-Adams, 2009; Chia and Hull, 2008; Coluccino et al., 2017; Fischenich et al., 2017; Fithian et al., 1990; Fox et al., 2012; Gabrion et al., 2005; Leslie et al., 2000; Nguyen and Levenston, 2012; Peloquin et al., 2016; Pereira et al., 2013; Proctor et al., 1989; Skaggs et al., 1994; Spilker et al., 1992; Sweigart et al., 2004; Tissakht and Ahmed, 1995). While previous studies tested tensile, compressive and equilibrium shear properties of human meniscus (Bursac et al., 2009; Chia and Hull, 2008; Fischenich et al., 2017; Leslie et al., 2000; Sweigart et al., 2004; Tissakht and Ahmed, 1995), to date, dynamic shear properties have only been investigated in animal tissue (Anderson et al., 1991; Nguyen and Levenston, 2012; Zhu et al., 1994).

Others have studied the role of meniscal ECM components, such as water and glycosaminoglycans (GAGs), on tissue compressive properties, and suggested these two components play a major role in the capability of the tissue to withstand load and dissipate mechanical stress(Bursac et al., 2009; Nguyen and Levenston, 2012; Pereira et al., 2013; Sanchez-Adams et al., 2011). Nguyen and Levenston studied the influence of osmotic swelling, indirectly related to water and GAG content, on the shear properties of bovine meniscus (Nguyen and Levenston, 2012). To date, there is no quantitative description of how the major components of its ECM (e.g., water, GAGs and collagen) affect the shear properties of the human meniscus. Accordingly, our objective was to characterize the dynamic and equilibrium shear properties of human meniscus in relation to its anisotropy and composition. We hypothesized that the unique arrangement of collagen fibers (i.e., anisotropic structure) and their relative abundance within the tissue (i.e., composition) would significantly affect the viscoelastic and equilibrium shear properties of the meniscus. Accordingly, we measured the storage (G') and loss moduli (G''), and the equilibrium shear modulus (G) in axial and circumferential oriented human meniscus specimens. We also sought to correlate these with water, GAG and collagen contents.

METHODS

Specimen Preparation:

Samples were prepared from twelve frozen human menisci (6 medial and 6 lateral, n = 6) obtained from ten donors (6 females and 4 males) of age 64.1±5.1 y.o. after autopsy. Menisci were visually inspected to determine their degeneration grade, which was deemed normal to moderately degenerated. All the samples were harvested from the central region of the meniscus. A schematic of specimen preparation and grouping for experiments is reported in Figure 1a. More specifically, tissue wedges were dissected from the midbody and punched with a corneal trephine (d=5mm) to obtain cylindrical cores with axial and circumferential orientations. A compresstome (VF-210-0Z, Precisionary Instruments, Inc., Natick, MA) was used to cut the specimens to ~1mm height. All specimens were obtained from the deep region of the tissue, far from the superficial or lamellar portions. For each meniscus, 3 samples per tissue orientation (axial or circumferential) were prepared: one sample for measurements of tissue composition, another for determining dynamic shear properties, and the final for measuring equilibrium shear properties. Additional samples were also prepared for preliminary tests to determine the linear viscoelastic range of shear strain, see below. Specimens were preserved and tested in a protease inhibited (Complete Tablets, Roche, Basel, SWI) 1X phosphate buffered saline (PBS) solution (Sigma Aldrich, St. Louis, MO).

Measurement of Shear Properties:

Shear properties were measured with a rheometer (AR-G2, TA Instruments, New Castle, DE) fitted with an 8 mm sandblasted flat steel rotational plate geometry and a 40mm sandblasted flat fixed steel plate. To ensure no slip conditions between the specimen and plates, 150 grit sandpaper was adhered onto both plates. To ensure contact between the sample and plates, the rotational plate was lowered until reaching a threshold normal force of 0.2N. After initial contact was made, the sample was completely immersed in a protease inhibited 1X PBS kept at 37°C, see Figure 1b.

To test shear properties within the linear viscoelastic range (LVR), preliminary strain sweep tests were conducted. The results indicated that the complex modulus (G^*) is independent of the shear (γ) magnitude up to $\gamma \approx 2\%$ for axial specimens and $\gamma \approx 0.5\%$ for circumferential specimens, see Figure 2. Accordingly, dynamic and equilibrium shear tests were carried out at $\gamma = 0.25\%$ for both axial and circumferential samples.

To characterize dynamic shear properties, frequency sweep tests, ranging from 0.05 Hz to 10 Hz, were conducted to determine the storage modulus (G'), the loss modulus (G''), the phase angle (δ) and the associated G^*. Frequency sweeps were performed at three levels of compression: 5%, 10% and 20% of the initial sample height. Based on a published experimental protocol (Zhu et al., 1994) and our own observations, after each compression, specimens were allowed to equilibrate for 30 minutes before testing.

To determine shear equilibrium properties, shear stress relaxation tests were performed at a shear strain of 0.25% for 2 hours to yield the equilibrium shear modulus (G). The tests were performed at three levels of compression: 5%, 10% and 20% of the initial height of the sample. Based on a published experimental protocol (Zhu et al., 1994) and our own observations, after each compression, samples were allowed to equilibrate for 30 minutes before testing.

All experimental data were collected and post-processed via TA Rheology Advantage™ Data Analysis software v5.5.24.

Determination of Tissue Composition:

Water, GAG and collagen content of menisci specimens was measured. Using an analytical balance (Model ML104, Mettler Toledo, Columbus, OH), specimens were weighed immediately after preparation (W_{wet}) and after lyophilization over night (W_{dry}). The water content was measured as:

$$\varphi^{water} = \frac{W_{wet} - W_{dry}}{W_{wet}}\% . \quad (1)$$

Subsequently, The GAG content was measured using a 1,9-dimethylmethylene blue (DMMB) (Polysciences Inc., Warrington, PA) binding assay(Burton-Wurster et al., 2003). Following lyophilization, tissues were digested using papain solution (250μg/ml). Once digested, tissues were mixed with DMMB and the absorbance was measured using a multi-mode microplate reader (Molecular Devices SpectraMax M2 Series, Sunnyvale, CA) at

525nm wavelength. Collagen was measured using a commercially available collagen assay kit (Sigma Aldrich, St. Louis, MO). The GAG and collagen contents were defined as:

$$\varphi^{GAG} = \frac{W_{GAG}}{W_{dry}}\%, \tag{2.i}$$

$$\varphi^{collagen} = \frac{W_{collagen}}{W_{dry}}\%, \tag{2.ii}$$

Statistical Analysis:

All data were reported as mean ± standard deviation. Data normality was assessed via Anderson-Darling test. Data from dynamic shear tests were normally distributed. Therefore, when investigating the dynamic shear properties, one-way ANOVA tests were performed to determine whether, for each type of meniscus (medial or lateral), for each sample orientation (axial or circumferential) and level of compression (5%, 10% or 20%), the magnitude of frequency affected the values of G^*, δ, G' and G''. Also, for each frequency investigated, one-way ANOVA tests were conducted on axial medial, axial lateral, circumferential medial and circumferential lateral samples, separately, to determine whether compression affected the magnitude of the dynamic shear moduli and phase angle. Finally, for each frequency and level of compression investigated, 2-sample t-tests were carried out to determine whether tissue orientation (axial vs. circumferential) or tissue type (medial vs. lateral) affected the magnitude of G^*, δ, G' and G''.

The distributions of G were also normally distributed. Accordingly, one-way ANOVA tests were conducted to determine whether compression affected the magnitude of G in axial lateral, axial medial, circumferential lateral, and circumferential medial samples, separately. Also, for each level of compression, 2-sample t-tests tests were performed to determine effects of tissue type (medial vs. lateral) and orientation (axial vs. circumferential) on the magnitude of G.

Regression analyses were conducted to determine empirical relations, if any, between φ^{water}, φ^{GAG} or $\varphi^{collagen}$, and G', G'' and G. Grubbs' test was used to identify outliers, if any. For all the tests performed, the level of significance was set to α=0.05.

RESULTS

The mean values of G^* and δ, for all the shear frequencies and levels of compression investigated are reported in Figure 3. The values of G^* varied from ~20 to ~100 kPa in axial samples, and from ~100 to 250 kPa in circumferential samples, respectively. For all the cases investigated, shear frequency did not significantly affect the magnitude of the complex modulus ($p>0.05$), although data suggest a positive trend of G^* with frequency. There was a statistically significant difference between axial lateral and circumferential lateral groups ($p<0.05$) at both 10% and 20% compression. Additional statistical differences were found between axial medial and axial lateral groups ($p<0.05$) when the level of compression was 10% and 20%. The majority of mean values of δ fell between 5° and 25°, see Figure 3d–e.

No statistically significant relationship with frequency, tissue type (lateral vs. medial), fiber orientation (axial vs. circumferential) or compression was found ($p>0.05$).

Neither the shear frequency or the level of compression significantly affected the values of G' and G''. Therefore, in investigating the effect of tissue type (lateral vs. medial) or orientation (axial vs. circumferential), data collected at all shear frequencies and levels of compression were pooled together. The values of G' of the circumferential lateral and medial samples were significantly larger than those relative to the axial lateral and medial specimens ($p<0.05$), see Figure 4a. The values of G'' were the highest in the circumferential medial samples. It was also found that circumferential lateral samples exhibited significantly larger values of G'' when compared to axial lateral ones ($p<0.05$). No significant differences were found between axial medial and lateral samples or between axial medial and circumferential lateral samples ($p>0.05$), see Figure 4b.

The mean values of G ranged from ~25 to 250 kPa, see Table 1. No statistically significant differences were found in G values between medial and lateral menisci, as well as across different levels of compression or tissue orientation ($p>0.05$).

Tissue composition measurements are summarized in Table 2. Water accounted for ~72% of the total mass, while GAG and collagen accounted for ~7 and ~91% of the dry weight, respectively. The values of G', G'' and G were negatively related to φ^{water}, with the strongest correlations observed in circumferential lateral specimens for G' (R^2=88.8%) and G'' (R^2=84.3%), and axial lateral specimen for G (R^2=70.7%). Weaker positive correlations were observed between both G' and G'', and φ^{GAG}, see Table 3. Statistically significant positive regressions between G' and G'' with $\varphi^{collagen}$ were found in circumferential samples, with the strongest correlations observed in lateral menisci for both G' (R^2=88.4%) and G'' (R^2=74.46%). No significant correlation was found in axial samples. Finally, for all the cases investigated, the values of G did not correlate with the collagen content.

DISCUSSION

This study investigated viscoelastic and equilibrium shear properties of human meniscal tissue. We hypothesized that the unique arrangement of collagen fibers (i.e., anisotropic structure) and their relative abundance within the tissue (i.e. composition) would significantly affect the viscoelastic and equilibrium shear properties of the meniscus. To the best of our knowledge, this is the first contribution reporting measurements of dynamic shear moduli in human meniscus and their relationships with tissue composition. Interestingly, most of our results are comparable to similar studies conducted on other animal tissues (Abraham et al., 2011; Anderson et al., 1991; Sweigart et al., 2004; Travascio et al., 2020; Zhu et al., 1994).

Previous studies measured the viscoelastic shear properties of animal menisci at different values of shear strain (Abraham et al., 2011; Anderson et al., 1991; Zhu et al., 1994). It has been shown that the elastic response of bovine meniscal tissue is non-linear when the shear strain exceeds 13% (Abraham et al., 2011). Aimed at providing results independent of the extent of applied shear, we conducted a preliminary strain sweep analysis and found that threshold values of γ preserving the LVR were 2% for axial specimens and 0.5% for circumferential specimens, see Figure 3. Therefore, all experiments were conducted at γ=0.25%.

As in previous studies (Travascio et al., 2020; Zhu et al., 1994), the dynamic shear properties were investigated for oscillation frequencies within a physiologically relevant range of 0.05 to 10 Hz. Also, all tests were performed at different levels of compression, ranging from 5 to 20%. It has been shown that such magnitudes of compressive strain are likely to occur *in vivo* (Chia and Hull, 2008; Yang et al., 2010). Our findings suggest that the magnitude of G^* increases with increasing oscillation frequency, see Figure 3a–c. This is in agreement with similar tests conducted on porcine, bovine and equine tissue (Anderson et al., 1991; Travascio et al., 2020; Zhu et al., 1994), and illustrates the viscoelastic behavior of the human meniscal tissue. The magnitudes of G^* were larger, although comparable, to those found in porcine and bovine menisci (Travascio et al., 2020; Zhu et al., 1994), but less than half of the values reported for equine meniscus (Anderson et al., 1991). Such discrepancy may be because equine menisci were tested within a frequency range almost two orders of magnitude larger than that used in the current study. As previously observed (Anderson et al., 1991; Travascio et al., 2020; Zhu et al., 1994), the extent of compression did not significantly affect meniscal shear properties; in contrast, tissue type and sample orientation affected tissue shear stiffness: in axially oriented samples, G^* of medial menisci was significantly larger than that of lateral ones; also, in lateral meniscal specimens, G^* of circumferential samples was significantly larger than that in axial samples.

Similar to animal tissue (Anderson et al., 1991; Travascio et al., 2020; Zhu et al., 1994), the phase angle δ of the human meniscus did not change with frequency, compression, tissue type or fiber orientation, see Figure 3d–e. Most importantly, the mean values of δ were included between 5° and 25°, denoting a prevalence of the elastic component as opposed to the viscous one in viscoelastic behavior. This finding is corroborated by the data reported in Figure 4, showing G' being almost an order of magnitude larger than G''. Also, the effect of fiber orientation (axial vs. circumferential) on the magnitude of G' and G'' was significant, in agreement with what was found for G^*.

The mean G measured in this study ranged from ~25 to ~250 kPa, see Table 1. Such values are within the same order of magnitude reported in other studies on human tissue (Sweigart et al., 2004). In addition, similar to that reported for bovine menisci (Zhu et al., 1994), G did not depend on compression, tissue type or fiber orientation.

Tissue composition was also investigated to identify potential relationships with measured mechanical properties. The average φ^{water}, φ^{GAG}, and $\varphi^{collagen}$ were within the ranges typically observed for human meniscus (Herwig et al., 1984; Makris et al., 2011), see Table 2. Both G' and G'' were found to be negatively related to φ^{water}, and positively related to φ^{GAG}, with the strongest correlations found in lateral specimens, see Table 3. Correlations with $\varphi^{collagen}$ were also found, but only in circumferential specimens. This finding suggests that collagen fibers may play a role in both storing and dissipating elastic energy during instantaneous shear deformation. Meniscal degeneration has been associated with the onset and progression of knee OA (Englund et al., 2009). It is known that, during the process of degeneration, the GAG and collagen contents of the meniscus decrease, and water mass fraction increases (Adams et al., 1983; Herwig et al., 1984). According to the empirical relations found in this study, the increase of water and reduction of GAG and collagen content observed during degeneration would increase tissue compliance and reduce its capability of dissipating mechanical energy, thereby affecting meniscal load bearing function and congruency of the knee joint. Finally, it was found that, in lateral specimens, G was negatively correlated to φ^{water}, further suggesting that an increase of water content due to degeneration would result in a deterioration of the shear properties of the tissue.

Taken together, the results of this study indicate that, at least within the range of experimental parameters explored here, the dynamic and equilibrium properties of the human meniscus do not depend on the extent of tissue compression. This was expected considering that, during a pure shear test, the mechanical behavior of the meniscus is solely determined by its solid phase, mainly composed of proteoglycans and collagen (Makris et al., 2011). Upon compression, water exudes from the samples, while the solid phase content (per dry weight) does not change. Accordingly, if no microstructural changes (e.g. buckling of collagen fibers) occur upon compression, G^*, δ and G should not vary with the extent of compressive strain. Our findings also corroborate the notion that mechanical properties of the meniscus depend on fiber orientation within the sample: circumferential specimens exhibit superior shear properties compared to their axial counterparts (Abraham et al., 2011; Anderson et al., 1991; Travascio et al., 2020; Zhu et al., 1994). A mechanistic interpretation of this anisotropic behavior has been proposed: in circumferential samples, collagen fibers are mainly oriented perpendicular to the shear plane, so that, during shear, they stretch and contribute to the overall shear stiffness of the tissue; in axial specimens, collagen fibers are mainly distributed in the shear plane, hence, when shear load is applied, they are sheared relative to each other not contributing to the strengthening of the sample. Such sliding motion occurring in the axial plane may lead to separation of bundles of collagen fibers and, eventually, tear formation (Zhu et al., 1994). This mechanistic interpretation is also supported by the empirical relationships among dynamic moduli and tissue composition found in this study. The positive correlations of G' and G'' with $\varphi^{collagen}$ observed in circumferential specimens (Table 3) indicates that as the collagen content increases, more fibers are recruited during shear; a lack of similar correlation in axial specimens was expected since the circumferential fibers are not engaged in shear deformations in the axial plane. The results of the present study also suggest that tissue strength due to collagen fibers observed in the circumferential samples has a time-dependent viscoelastic nature: when the shear deformation is held for a long time interval, like in a stress-relaxation test, the stiffness of the circumferential specimens is not different from that of the axial ones, see the G values reported in Table 1.

Some limitations are noted. This study did not include radial specimens, whose fiber organization is similar to that of axial specimens with the addition of collagen fibers oriented in the radial direction. However, experiments on bovine menisci suggest that the contribution of radial fibers to the overall shear stiffness of the tissue is negligible since both G^* and G of radial specimens were not statistically different from those of axial specimens (Zhu et al., 1994). We harvested samples from the core of the midbody of the meniscus. A previous study showed that the shear modulus of the anterior region of the meniscus is significantly larger than those of the central and posterior regions (Sweigart et al., 2004). It should be noted that in previous experiments, shear properties were measured on the outer lamellar surface of the meniscus, whose structure is characterized by randomly oriented collagen fibers, as opposed to the highly fiber-aligned core of the tissue (Athanasiou and Sanchez-Adams, 2009). Further studies are required to determine if shear properties of the core of the meniscus are region dependent. Also, meniscus specimens came from older individuals which may have some degree of degeneration (deemed only moderate is some cases by visual inspection). However, since this study correlated findings directly with compositional measurements, the results presented here do provide valuable insight into structure-function relations for the meniscus. Finally, the limited sample size used in this study might have prevented us from detecting potential additional statistical differences in the parameters measured across the sample groups tested. This would be particularly true for the measurements of G, whose values for circumferential samples were characterized by large standard deviations. Although the magnitudes and trends of G we found are in agreement to those reported in (Zhu et al.1994), future studies with larger statistical power should further ascertain whether meniscal shear equilibrium properties are anisotropic or not.

In conclusion, results of this study provide new knowledge on dynamic and equilibrium shear behavior of the human meniscus and their relationship with tissue anisotropy and composition. Such information may increase our understanding of the etiology of meniscal tears. Moreover, these findings can be leveraged to design tissue-engineered scaffolds that can better recapitulate the mechanical behavior of native tissue, and/or to develop a robust computational model to predict meniscus mechanical behavior *in vivo*.

ACKNOWLEDGMENTS

The project described was supported by Grant Number 1R01AR073222 from the NIH (NIAMS). The authors wish to thank Dr. Loren Latta and Abeer Albarghouthi for their assistance in tissue harvesting, and Dr. Lukas Jaworski for his help in measuring tissue composition.

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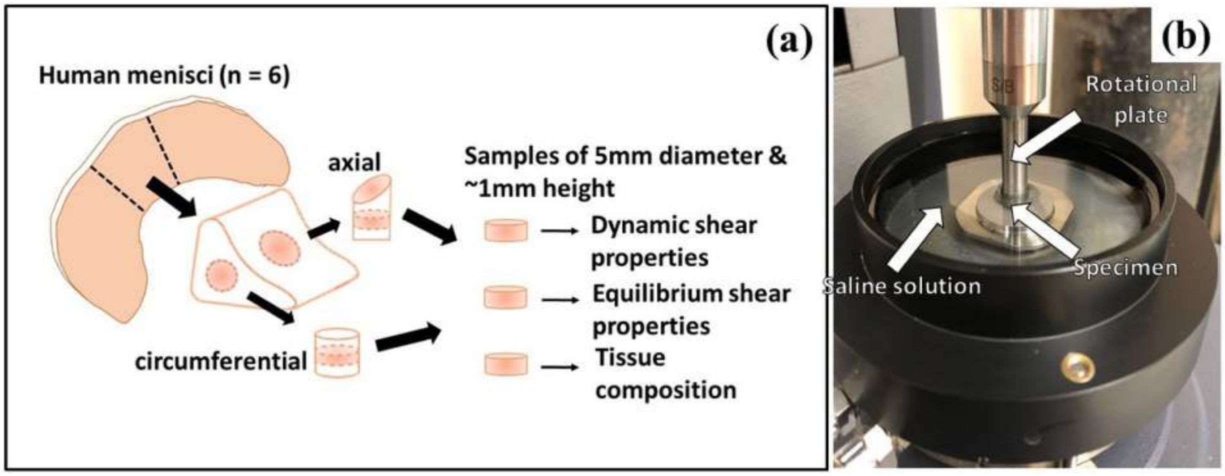

Figure 1.

a) Schematic of specimen preparation. A total of 3 specimens in the axial direction and 3 others in the circumferential direction were prepared for measurements of dynamic shear properties, equilibrium shear properties and tissue composition. b) Experimental setup: the rotating plate, the location of the specimen and the saline bath solution are shown

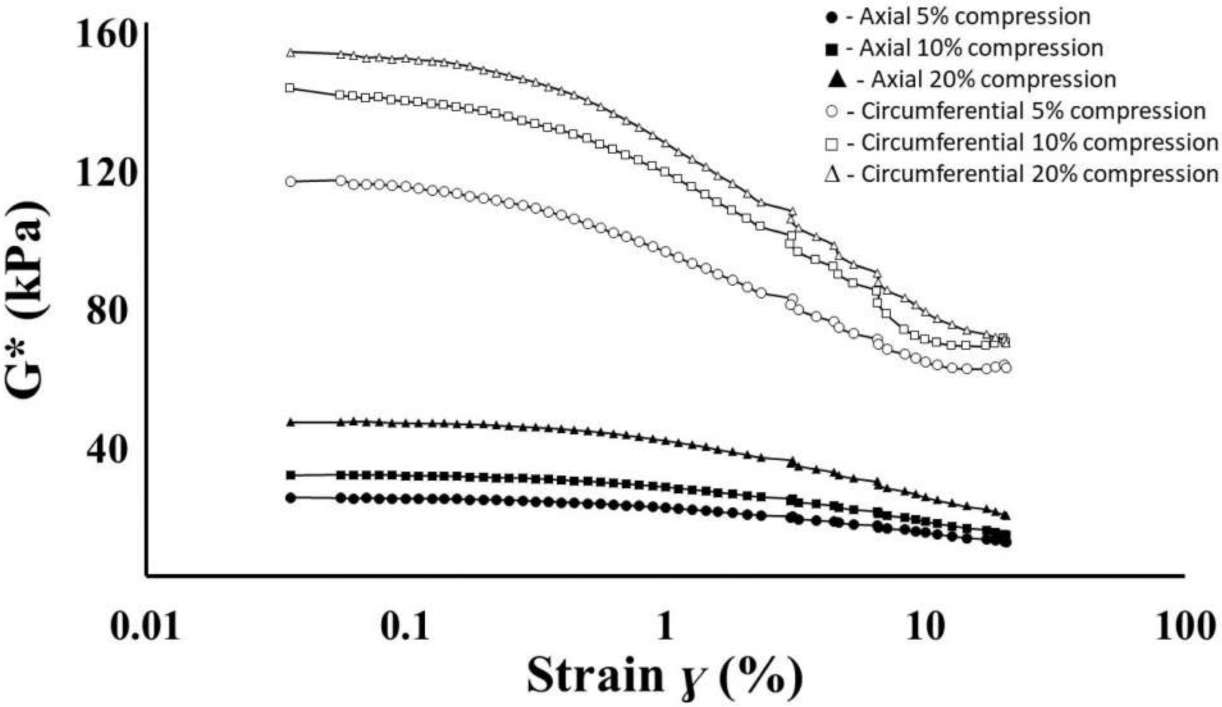

Figure 2.

Representative strain sweep diagram for an axial and a circumferential sample of the same meniscus. Data are reported for circumferential and axial samples at 5%, 10% and 20% level of compression.

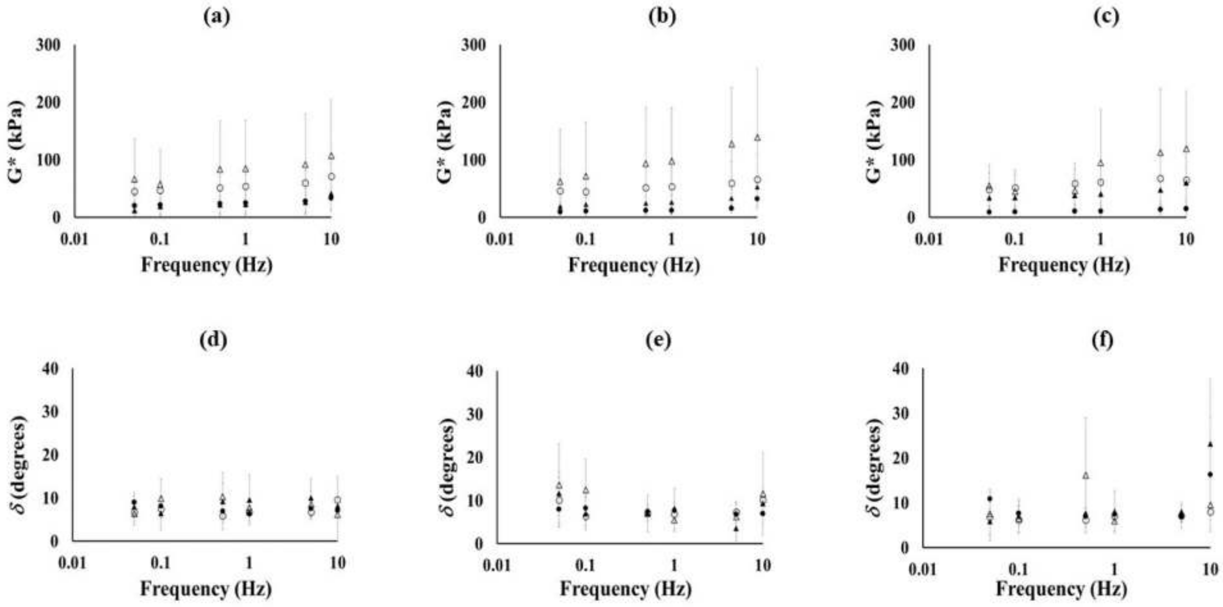

Figure 3.

Mean values of *G** and *δ* of axial lateral, axial medial, circumferential lateral and circumferential medial meniscal samples at various levels of compression: a) *G** at 5% compression; b) *G** at 10% compression; c) *G** at 20% compression; d) *δ* at 5% compression; e) *δ* at 10% compression; f) *δ* at 20% compression.

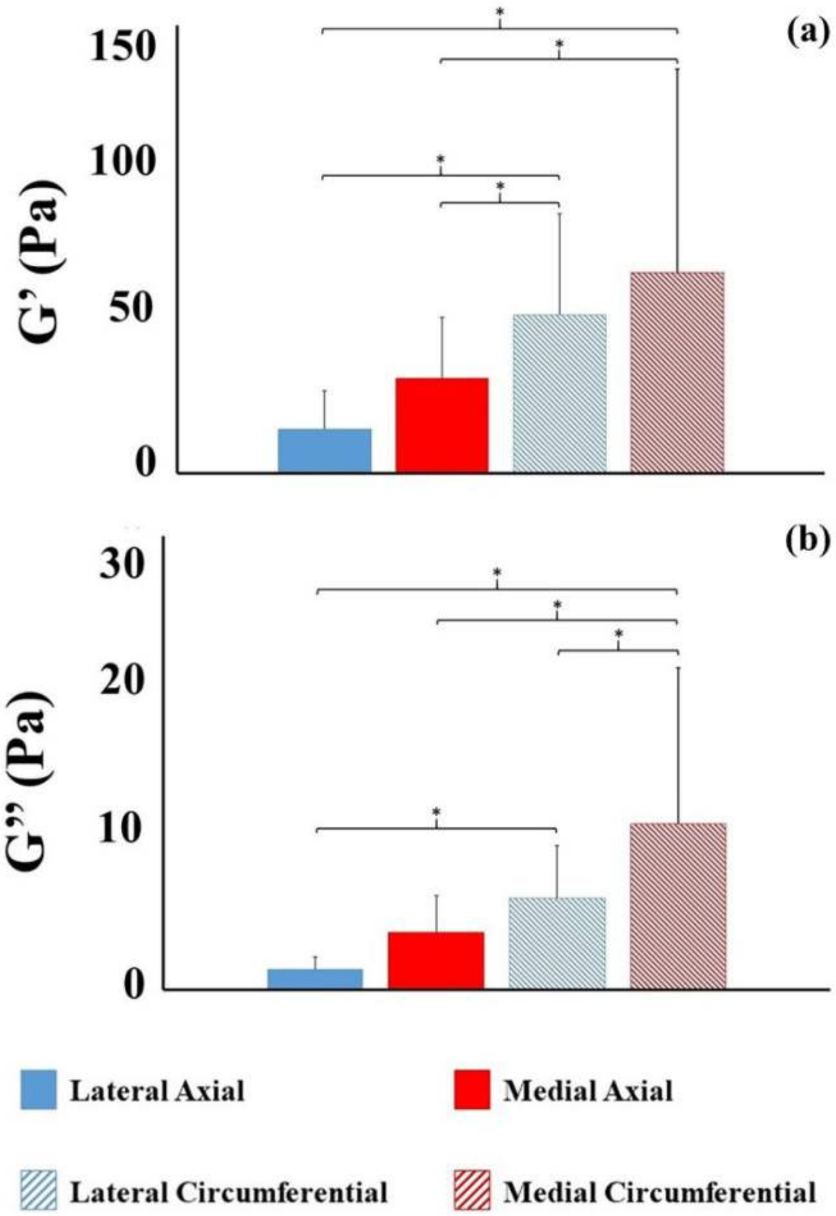

Figure 4.

Mean values of storage and loss moduli: a) *G*' values; b) *G*'' values. For all the data reported in the figure, (*) indicates significant statistical difference ($p < 0.05$).

Table 1.

Equilibrium shear modulus *G* of human lateral and medial meniscus (n = 6). Data are reported in kPa (mean ± standard deviation) for lateral and medial meniscal samples at various levels of compression and for different collagen fiber alignment (axial vs. circumferential) in the specimens.

Tissue type	Fiber Orientation	Level of Compression		
		5%	10%	20%
Lateral	**Axial**	52.79 ± 27.32	41.8 ± 23.42	58.52 ± 30.16
	Circumferential	71.42 ± 30.59	103.17 ± 148.48	100.35 ± 102.14
Medial	**Axial**	45.16 ± 23.44	53.29 ± 20.89	69.78 ± 30.16
	Circumferential	78.34 ± 78.59	54.29 ± 50.12	84.74 ± 90.6

Table 2.

Mass fraction of water, GAGs and collagen in human menisci samples. Both GAG and collagen fractions are calculated with respect to tissue dry weight. Data are reported as mean ± standard deviation.

φ^{water}	φ^{GAG}	$\varphi^{collagen}$
72 ± 6%	6.8 ± 2.6%	91.2 ± 2.1%

Table 3.

Relationships among shear moduli *G’, G”* and *G* and the mass fractions of water, GAGs and collagen in medial and lateral human menisci samples. The reported p-values and R^2 values were determined via simple linear regression with level of significance $\alpha = 0.05$.

		φ^{water}		φ^{GAG}		$\varphi^{collagen}$	
	Sample orientation	**P-value**	**R^2**	**P-value**	**R^2**	**P-value**	**R^2**
G’	*Axial Lateral*	0.0001	43.05%	0.19	3.4%	0.094	8.5%
	Circumferential Lateral	0.0001	88.8%	0.002	49.84%	0.0001	88.4%
	Axial Medial	0.0001	32%	0.15	3.4%	0.872	0.05%
	Circumferential Medial	0.0001	38.1%	0.004	14.3%	0.0001	78.31%
G”	*Axial Lateral*	0.007	16.7%	0.0001	57.2%	0.195	5.54%
	Circumferential Lateral	0.0001	84.3%	0.0001	29%	0.0001	74.46%
	Axial Medial	0.0001	50.4%	0.0001	28.8%	0.977	0.17%
	Circumferential Medial	0.68	0.29%	0.45	1.2%	0.0001	61.78%
G	*Axial Lateral*	0.005	70.7%	0.097	10.3%	0.338	9.21%
	Circumferential Lateral	0.018	36%	0.098	19.7%	0.122	48.9%
	Axial Medial	0.912	0.16%	0.249	10.1%	0.669	1.3%
	Circumferential Medial	0.285	11.3%	0.027	28.7%	0.332	9.43%