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Strain-rate Dependent Nonlinear Tensile Properties of the Superficial Zone of Articular Cartilage

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ABSTRACT

Aim of the study: The tensile properties of articular cartilage play an important role in the compressive behavior and integrity of the tissue. The stress-strain relationship of cartilage in compression was observed previously to depend on the strain-rate. This strain-rate dependence has been thought to originate mainly from fluid pressurization. However, it was not clear to what extent the tensile properties of cartilage contribute to the strain-rate dependence in compressive behavior of cartilage. The aim of the present study was to quantify the strain-rate dependent stress-strain relationship and hysteresis of articular cartilage in tension.

Methods: Uniaxial tensile tests were performed to examine the strain-rate dependent nonlinear tensile properties of the superficial zone of bovine knee cartilage. Tensile specimens were oriented in the fiber direction indicated by the India ink method. Seven strain-rates were used in the measurement ranging from 0.1%/s to 80%/s, which corresponded to nearly static to impact joint loadings.

Results: The experimental data showed substantial strain-rate and strain-magnitude dependent load response: for a given strain-magnitude, the tensile stress could vary by a factor of 1.95 while the modulus by a factor of 1.58 with strain-rate; for a given strain-rate, the modulus at 15% strain could be over 4 times the initial modulus at no strain. The energy loss in cartilage tension upon unloading exhibited a complex variation with the strain-rate.

Conclusion: The strain-rate dependence of cartilage in tension observed from the present study is relatively weaker than that in compression observed previously, but is considerable to contribute to the strain-rate dependent load response in compression.

Keywords: Bovine knee cartilage; Collagen viscoelasticity; Hysteresis; Strain-rate dependent property; Tensile test

1. INTRODUCTION

The tensile properties of articular cartilage are essential for the tissue integrity and normal mechanical function of the joint, although cartilage bears compressive external loadings in the joint [1]. This is because the tissue experiences lateral expansion, or tension in the direction tangential to the articular surface, when cartilage is compressed in the thickness direction. The lateral expansion is caused mainly by Poisson's effect during nearly static slow compression and mainly by fluid pressurization in the tissue during fast compression. The Poisson's ratio is small ($<$ 0.2) at equilibrium and at very slow compression [2], but the effective Poisson's ratio can be increased up to its maximum (0.5) at a fast compression when a large fluid pressure produces a large lateral expansion [3]. The collagen fibers in the lateral direction resist the expansion. Therefore, the tensile properties of the tissue contributed by the tangential fibers play an important role in the compressive stiffness of cartilage. For example, the nonlinear load response of cartilage in compression is highly associated with the nonlinear properties of collagen network in the tissue [4]. The tensile properties were associated with cartilage health [5-7] and particularly important for supporting fast loading [8]. Therefore, it is necessary to understand the tensile properties of cartilage in order to understand its mechanical response to compressive loadings, and further to understand the mechanical function of the joints.

The tensile properties of both articular cartilage and self-assembled collagen fibers have been investigated [9-11]. The effect of aging was observed [12-13], and the effective Poisson's ratio was also measured [14]. The tensile properties were usually obtained from uniaxial tensile tests, although the tensile modulus obtained from biaxial tensile tests was greater than that from uniaxial tensile tests [15]. Similar to compression, cartilage in tensile testing also exhibited time-dependent mechanical behavior or viscoelastic response [13,16]. Under significant tension in the fiber direction, the collagen network bears most of the loading: the fluid pressure is minimal due to the small thickness and free surface boundary condition of the specimen; and the tensile resistance of the nonfibrillar matrix (proteoglycans) is secondary to that of the fibers [12]. Therefore, the time-dependent behavior observed from tensile testing should be primarily attributed to the collagen network [16,17]. This collagen viscoelasticity can be best understood with tensile tests because it is coupled with the fluid pressure when the tissue is in compression or indentation.

Articular cartilage exhibits strain-rate dependent mechanical behavior, e.g. a greater stress is produced if a strain is applied at a greater strain rate. The tissue also experiences hysteresis associated with energy loss during unloading [18]: the stress-strain curve obtained from a loading and unloading cycle forms a loop called the hysteresis loop that has an opening at zero stress, i.e. the strain does not go back to zero immediately after the stress has vanished (Fig. 1). However, the strain-rate dependence of the tissue response was less examined in tension than in compression [19-21]. Only one previous study explored the tensile properties of cartilage using the strain-rate as a variable. The modulus obtained at 70%/s was one order of magnitude greater than that at 20 and 50%/s, while the moduli obtained at 20 and 50%/s were essentially the same [22]. Another study investigated the dynamic modulus of immature cartilage at different frequencies and small strains (<0.6%) where the strain-rate was not a control variable. The tensile modulus at 10 Hz was found to be 2.3 times the equilibrium modulus [23]. Similarly, the tensile stress in anterior cruciate ligament was found to increase by a factor of 3 within the strain-rates from 0.1%/s to 40%/s [24], while a weak strain-rate dependence was found in the tensile properties of sheep disc annulus fibrosus [25].

The objective of the present study was to examine the strain-rate dependent stress-strain relationship and hysteresis of articular cartilage in tension using uniaxial tensile testing of bovine cartilage. We investigated the strain-rate dependent tensile response of articular cartilage in a full range of physiological strain-rates and strain-magnitudes. Three nominal strains, 3, 8 and 15%, were applied at a constant strain-rate from 0.1%/s to 80%/s.

2. METHODS

Bovine knee cartilage was used because the large size of tissue makes it possible to extract good-sized samples from the surface with little surface curvature. Two stifle joints were used to identify the split-line patterns of the joints using the India ink method [9,15,26]. This method involved piercing cartilage surface with a fine needle stained with India ink. The needle point perforations caused the propagation of the ink in the small splits, which are believed to point to the directions of collagen alignment. The split-lines were recorded for later use to identify the collagen fiber directions so the tensile specimens could be cut with the axis oriented in the fiber direction. These two joints were not used for extracting specimens because the tissue surface was disrupted by the needle punctures.

Thirty-seven dumbbell-shaped specimens were extracted from the femoral condyles and grooves of 12 fresh bovine stifle joints acquired from a local butcher in ~24h after slaughter (age 16-24 months; site differences were not studied; failed specimens were not included). The specimens were prepared on the same day and tested in 2 days after the joint was obtained. First, a cylinder of cartilage and bone with diameter of 12.7mm was extracted using a drill with a diamond core bit. Before the cylinder was removed from the joint, a small cut stained with India ink was used to mark the fiber direction using the split-line pattern obtained previously as a reference. The cut was carefully chosen to be outside of the region where the final specimen was to be obtained. Second, a cartilage disc of target thickness of 250μm was sliced from the superficial zone of the cylinder with a rotary microtome (Leica RM2125 RTS). Finally, a dumbbell-shaped specimen was cut from the disc using a plastic template of the dumbbell shape and a razor blade. The thickness of the sample was 271±48 μm (mean ± standard deviation) and its width of the middle part was 6.23±0.18 mm (*n*=37). The dumbbell shape was chosen following several tensile tests of cartilage from the literature [9,10,22,23]. Tissues from deeper layers were not studied because their tensile stiffness is less considerable.

Tensile tests were performed with a Bose ElectroForce® 3200 (Bose Corporation, Minnesota, USA) that can run at high frequency and was able to capture the expected range of loads in our study. A 45 N load cell (force transducer) was used in the experiments which provided good accuracy and resolution for the range of expected forces. Two clamps were used to grip the samples to load on the tester: one was mounted on the load cell and the other on the actuator. Cartilage samples were very thin and slippery. Precise gripping was essential to prevent slippage and yet preserve tissue integrity. Small pieces of 1500 grit sandpaper were used to increase friction between the clamp and specimen based on previous experience [27]. A very thin layer of glue was also used between the sandpapers and the cartilage samples to minimize potential slippage. The tissue hydration was maintained by spraying phosphate-buffered saline on the specimens. A waiting time of 5 minutes was given to allow the recovery of tissue hydration between two loading cycles.

The following loading protocol was used on each specimen. Three nominal strains at 3%, 8% and 15% were used sequentially, and each was applied, respectively, at seven strain-rates of 0.1, 1, 10, 25, 40, 50 and 80%/s in the order from low to high strain-rates. The nominal strain, $\bar{\varepsilon}$, is shown in percentage to distinguish from the logarithmic strain,

$$\varepsilon = \ln(1+\bar{\varepsilon}) \tag{1}$$

Each test consisted of both loading and unloading phases under a constant strain-rate. This loading protocol was designed to consider the effects of both strain-magnitude and strain-rate on the stress and hysteresis. Before a sample was tested under the aforementioned loading protocol, a tare load of 0.1 N was applied to ensure the tissue was properly loaded in tension and was then preconditioned for 30 cycles under sinusoidal loading at 1Hz. Repeatable results were observed at the end of preconditioning. In order to make sure the specimen remained firmly fixed on the grips and the tissue was not structurally damaged after each targeted loading and unloading, a preconditioning was always performed prior to each targeted test, even when the same specimen was preconditioned earlier for a test at a different strain. The strain amplitude for the preconditioning was chosen to be the same as the strain to be applied in the targeted test indicated in the loading protocol.

The tests were closely monitored for slipping at the grips. First, the grip positions were marked so any obvious slipping could be noted. Second, preconditioning further tested the adequacy of griping. Third, the loading and displacement curves were further examined to identify slipping. Moreover, the specimen was carefully inspected visually for potential damage after each loading cycle. The data were excluded from analysis when tissue slipping or damage was found. Thirty-seven specimens were successfully tested.

The data acquisition was done using the Wintest® software provided with the Bose tester. The raw data included the time and force recorded for each specimen under a particular strain and strain-rate condition. Programs written in MATLAB v7.12 (MathWorks Inc., Natick, MA, USA) were then used to determine the stress-strain relationship, peak stress and hysteresis area.

3. RESULTS

The nominal stress is shown as a function of the stretch ratio, λ, for a given strain-rate (Fig. 2). For clarity of the figure, only 4 curves are shown. The stretch ratio is the ratio of the stretched versus original lengths of the specimen ($\lambda = 1 + \bar{\varepsilon}$). The strain-rate dependence was more obvious at higher stretch ratio. The ratio of stresses obtained at 80%/s and 0.1%/s was 1.95 for the case of

15% strain, as compared to 1.60 for the case of 8% strain. The stress-stretch relationship was fitted by the following equation

$$\sigma = \frac{1}{2} E_0 \lambda \left(\lambda^2 - 1 \right) + \hat{E} \lambda \left(\lambda^2 - 1 \right)^2 \tag{2}$$

The material properties, E_0 and $\hat{E}$, changed with strain-rate as shown in the caption of Fig. 2. Noting that the strain ε is related to the stretch ratio by $\varepsilon = \ln \lambda$ and $d\lambda = \lambda d\varepsilon$, the tangential modulus of the tissue is obtained as follows

$$\frac{d\sigma}{d\varepsilon} = \frac{d\lambda}{d\varepsilon} \frac{d\sigma}{d\lambda} = \lambda \left[\frac{1}{2} E_0 (3\lambda^2 - 1) + \hat{E} (\lambda^2 - 1)(5\lambda^2 - 1) \right] \tag{3}$$

which is equal to E_0 at no strain ($\lambda = 1$). Therefore, E_0 can be considered as the initial modulus at no strain, or the modulus when the strain approaches to zero. This modulus then increases with stretch ($\lambda > 1$).

The statistical results consistently show that the stress increased with both strain and strain-rate and that the stress was more sensitive to the strain-rate when the strain-rate was not too high (Fig. 3). However, the stress still increased significantly for the case of 15% strain when the strain-rate increased from 50%/s to 80%/s.

The elastic modulus increased with strain-rate rapidly at low strain-rates but almost reached a plateau at 25%/s (Fig. 4). In mathematical terms, it slowly approached to an asymptote afterwards. This is the transient modulus defined as $d\sigma/d\varepsilon$, also referred to as apparent modulus, which differentiates from the modulus measured at equilibrium. The transient modulus is a function of loading history and so is the transient stress and strain.

The hysteresis loops were plotted for the test case of 15% strain (Fig. 5). The area of the loop represents the energy loss during the unloading process. For the purpose of comparison, the energy loss was normalized to the total energy at the end of the loading phase in each test to show the relative energy loss (Fig. 6). The relative energy loss was more dependent on the strain-rate than strain magnitude. The loss reduced to the lowest at moderate strain-rates (around 10%/s). In addition, the energy dissipation at 40%/s and higher strain-rates was greater than that at 0.1%/s for all 3 strains (Fig. 6).

4. DISCUSSION

Our tensile experiments on articular cartilage showed substantial strain-rate dependence of the load response of the tissue on a full range of strain-rates at physiologically reasonable deformation that has not been fully examined previously. Furthermore, the strain-rate dependence was nonlinear and augmented at greater strain-magnitudes, among the 3 strains considered (Fig. 3). The strain-rate dependence was less considerable at 3% strain. This may explain why the quasi-linear viscoelastic theory approximated the tensile tests of biological tissues with small deformation at acceptable accuracy, but failed to describe tensile tests with large deformation [28].

The strain-rate dependence of cartilage in tension revealed in the present study was much lower than that in compression reported previously. The ratio of stresses obtained at high and low-rate tension was less than 2, while it was substantially over 10 in compression [19]. The strain-rate dependence of the tissue in tension was most likely due to the intrinsic properties of the collagen network, because the proteoglycan matrix of the tissue is insufficient to resist tension. In fact, no significant difference in the tensile stiffness was found between normal and proteoglycan extracted specimens stretched at a small constant rate [29]. However, the proteoglycan matrix influenced the creep response when a load was suddenly applied to produce a strain up to 50% [29], which is out of the range considered in the present study. This strain-rate dependence in tension could also partially contribute to the strain-rate dependence of articular cartilage in compression, because the tensile stiffness in the tangential direction influences the compressive stiffness in the normal direction. The strain-rate dependence in compression was previously shown to be dominated by fluid pressurization [30]. The present study indicated that the strain-rate dependence in compression is enhanced by the strain-rate dependence in tension. In addition, a weaker strain-rate dependence in tension than in compression agrees with previous results that showed a weaker transient load response in tension than in compression [27]. It is also compatible with the dynamic modulus obtained as a function of frequency that varied by a factor of 2.3 in tension but 24 in unconfined compression [23].

The nonlinear stress-stretch relationships were found for all strain-rates considered. Previously, the nonlinearity was established at low strain-rates (~1%/s) only [9,10]. By further examining the properties (E_0 and $\hat{E}$) obtained from the curve fit, the nonlinearity is observed to become weaker at higher strain-rates (Fig. 2). This variation of nonlinearity with strain-rate was different from that of cartilage in compressive testing. For example, cartilage in low strain-rate tensile testing also exhibited nonlinear stress-strain relationships, in contrast with linear stress-strain relationships at nearly static compressive testing shown in experiments [19] and explained in modeling [4].

The relative energy loss in tensile behavior during unloading demonstrated by the hysteresis was strongly strain-rate dependent (Figs. 5 & 6). It only slightly depended on the strain magnitude (Fig. 6). This trend of energy loss may be explained by a damping mechanism in the tissue. This damping mechanism caused the energy loss to be nonlinear with the lowest relative energy loss at a moderate strain-rate. As the tensile loading in the tensile tests was predominantly supported by the fibers, the damping mechanism was likely provided by the fiber network, including its interaction with the fluid and proteoglycans. The hysteresis testing on single collagen fibers also showed a similar change of hysteresis with strain-rate [31]. Therefore, the aforementioned results highlight the role of collagen network in the hysteresis of cartilage.

The energy loss in tension exhibited a complex nonlinear pattern. Fiber nonlinearity is often explained by an uncrimping process: collagen fibers are naturally in a wavy configuration when they are not loaded; they are gradually recruited to resist loading when they are straightened with increased tension [32]. This uncrimping process results in higher stiffness of the structure at larger stretch. This stiffening of cartilage with larger stretch was previously observed at equilibrium [33]. The present results indicate strain-rate dependent stiffening of collagen network: the stiffening was boosted at higher strain-rate loading. However, the more stiffening during loading, the greater decrease in stress during unloading. The larger difference in loading and unloading stresses at a higher strain-rate caused a larger relative energy loss during unloading. Different loading and unloading nonlinear behaviors was also observed in unconfined compression testing of cartilage [34]. In addition, studies on other molecules such as titin showed an elevated hysteresis when loading was applied over a specified stretch [35], indicating a threshold in the mechanism of hysteresis.

The range of strain-rates used in the present study (up to 80%/s) was sufficient to reveal a full variation of transient tensile modulus; there was no need to use greater strain-rates beyond this range because the asymptote of strain-rate dependent modulus was reached (Fig. 4). In fact, the modulus did not increase much when the strain-rate increased from 25%/s to 80%/s (Fig. 4). The transient stress at 15% strain showed a larger increase with strain-rate (Fig. 3) because the stress (= $\int E d\varepsilon$) amplifies the increase in E at larger strains (E is a nonlinear function of ε). That is why a significant increase in the stress is still seen after the strain-rate increased from 25%/s to 80%/s (Fig. 3).

The strain or loading-rate dependent tensile properties of articular cartilage were only documented in 2 studies in the literature. In one study, bovine knee cartilage was stretched at a constant strain-rate (1, 20, 50 or 70%/s) until failure so the strain reached up to 50% [22]. However, we are not able to explain the results of that study on why there was no significant increase in the transient modulus with increasing strain-rate from 20 to 50%/s, but one order increase with increasing strain-rate from 50 to 70%/s [22]. We found a continuous increase in the modulus (before reaching an asymptote) with strain-rate at a smaller scale in contrast with a sudden jump of one order of magnitude in that study. We noticed that only 2 bovine knee joints were used in that study and tissues were stored at -18°C until the day before testing [22], while our specimens were extracted from 12 bovine stifle joints and tested without freezing. In addition, only femoral cartilage was used in the present study, while tibial cartilage was also included in the previous study [22]. On the other hand, it was reasonable to limit the strain to 15% in our study because the tissue strength was not studied here. Although test conditions were different, our results were somehow in agreement with what was obtained from immature bovine shoulder cartilage, where the tensile modulus at 10 Hz was found to be 2.3 times the equilibrium modulus [23]. The exact equilibrium response was not tested in the present study, but the ratio of stresses obtained at 80%/s and 0.1%/s was up to 1.95 and the ratio of moduli was up to 1.58 depending on the strain-magnitude. Our results showed a significant weaker rate-dependence, considering up to 15% strain examined in our study and 0.6% strain in the reference study (~7 MPa compared to 0.262 MPa in stress) [23]. The difference in rate-dependence might be due to the different variables considered: a constant strain-rate was used in all tests in the present study, while a sinusoidal loading (variable strain-rate) was used in the reference study. It is noted that the ratio of dynamic moduli obtained at 10Hz and 0.001Hz was 1.8 [23]. On the other hand, the magnitude of modulus obtained in the present study was smaller than what was found in one study [22] but larger than what was reported in other studies [13,23].

The gauge-to-gauge measure of strain was used in this study, which must have provided average tensile strain of the specimens with acceptable accuracy (maximum strain was 15%; no necking). This strain measurement was used in tensile testing of articular cartilage in a few studies [27, 33], while optical techniques were employed in other studies to measure the strain in the center of the specimen [9, 10]. The optical measurement can provide the results for the central portion of the specimen that is not influenced by the end conditions. However, it can be difficult to obtain clear images when fluid or bathing solution is present [33], and when the tests are performed under high frequencies [23]. Therefore, we used the gauge-to-gauge measure of strain to obtain the average stress-strain relationship that should be the same qualitatively as that obtained by a more accurate measurement.

A standard uniaxial tensile test was performed using dumbbell-shaped explants as done by several research groups [9,10,23]. This method is convenient in testing and simple in data interpretation. The mechanical testing environment, however, does not represent a physiological loading condition. For example, cartilage often bears compressive loadings in the thickness direction but experiences tensile deformation in all directions perpendicular to the thickness direction (other than in one direction only). Furthermore, tissue harvesting from a joint would somewhat compromise the integrity of the collagen network and thus lower the tensile stiffness of the specimen. A more realistic tensile test would be biaxial tensile testing that indeed revealed a greater tensile modulus than that obtained from a uniaxial test, which was most significant in the toe region [15]. After taking this biaxial effect into consideration, the present results can be used to refine the constitutive laws of cartilage. Complex loading conditions can then be effectively modeled computationally.

The strain-rate dependent nonlinear tensile behavior observed in this study is believed to be attributed primarily to the collagen network due to its role in the tensile load support in the specimen. The exact mechanism of the strain-rate dependence, however, could not be confirmed by the present study. It was possibly influenced by the interplay of fluid, proteoglycans and fiber recruitment in articular cartilage. Collagen-proteoglycan interactions have been observed experimentally [36-38] and are believed to influence collagen reorganization and alignment in tension and thus play a role in the load bearing of the tissue [29,39]. Also, the change in fluid content during tension affects the electrochemical envioronment, and, consequently the viscoelasticity of the tissue [40-41]. However, further discussions on the mechanism are beyond the scope of the present study.

In conclusion, the tensile properties of bovine articular cartilage were found to be substantially nonlinear and strain-rate dependent. For a given tensile strain, the tensile stress increased substantially with strain-rate. However, the strain-rate dependence was much weaker in tension than in compression, which indicates that the strain-rate dependence in compression is mainly modulated by the fluid pressurization in the tissue with a minor contribution from the strain-rate dependence of the tensile properties. The tensile nonlinearity was also strain-rate dependent, but different from the compressive nonlinearity on the strain-rate. The energy loss in cartilage tension was highly associated with the strain-rate and nonlinear mechanism. The results obtained from the present study may facilitate understanding the mechanical functions of the joint. In particular, the strain-rate dependent nonlinear tensile response of articular cartilage should contribute substantially to the compressive behavior of the joint and protect the joint from excessive loadings during a variety of physical activities, as a greater tensile stiffness is generated at a faster loading. The strain-rate dependence may also have implications in cartilage homeostasis as cartilage biosynthesis was found to be associated with loading magnitude and frequency [42].

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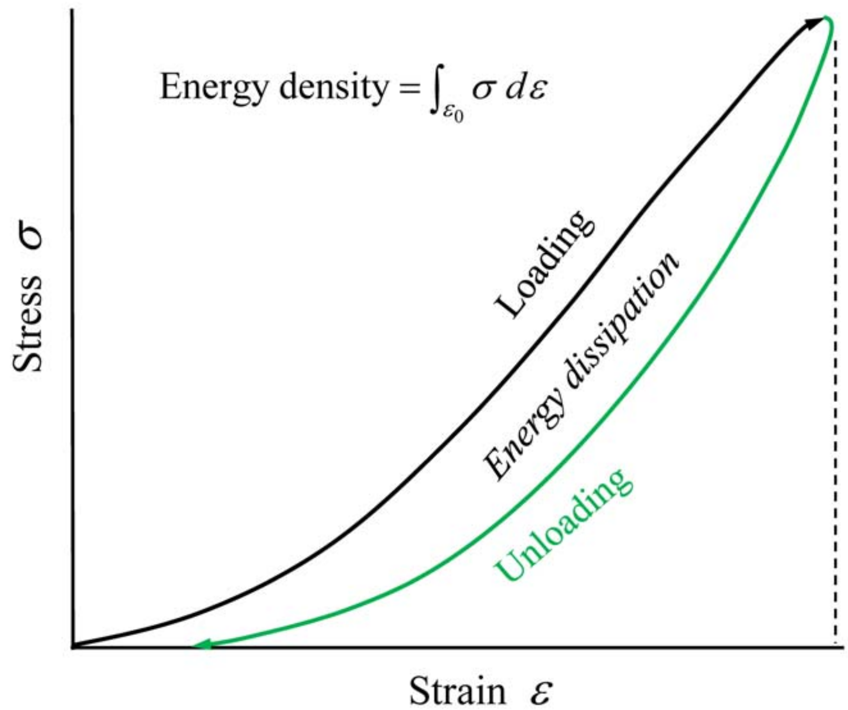

Figure 1. Schematic representation of the hysteresis in biological tissues (hysteresis loop). The path of unloading (green curve) does not overlap the path of loading (black curve) due to energy loss in damping. The energy density at the end of the loading phase is equal to the area bounded by the loading curve, horizontal axis and the dashed vertical line. The area bounded by the hysteresis loop corresponds to the density of energy dissipation.

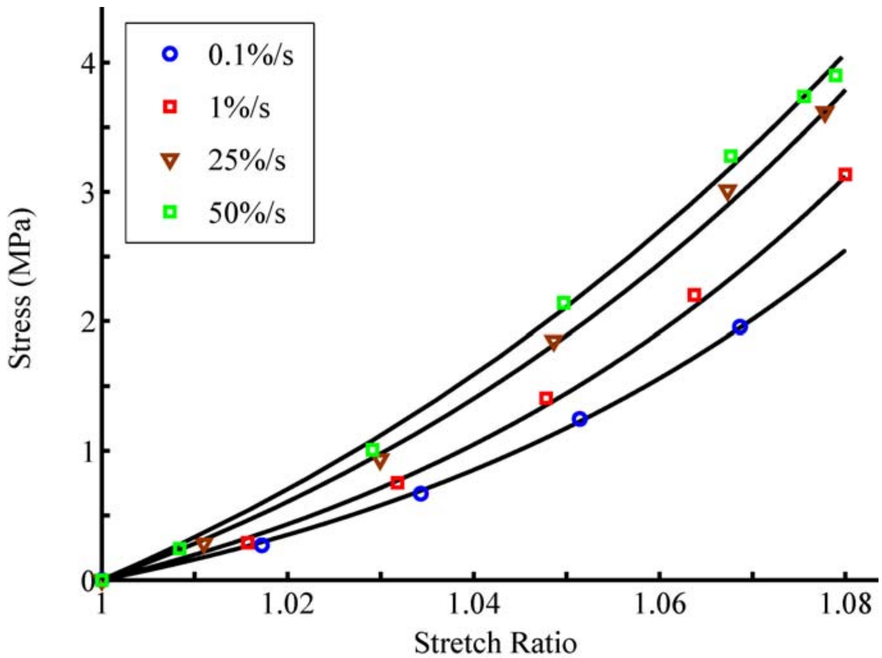

Figure 2. Stress-stretch relationship of articular cartilage for different strain rates. The measured data were fitted using Eq. (2) with (E_0, $\hat{E}$) to be (12.26, 47.48), (12.42, 67.00), (17.92, 80.37), and (25.82, 60.12) MPa, respectively, for the strain rates of 0.1, 1, 25 and 50%/s.

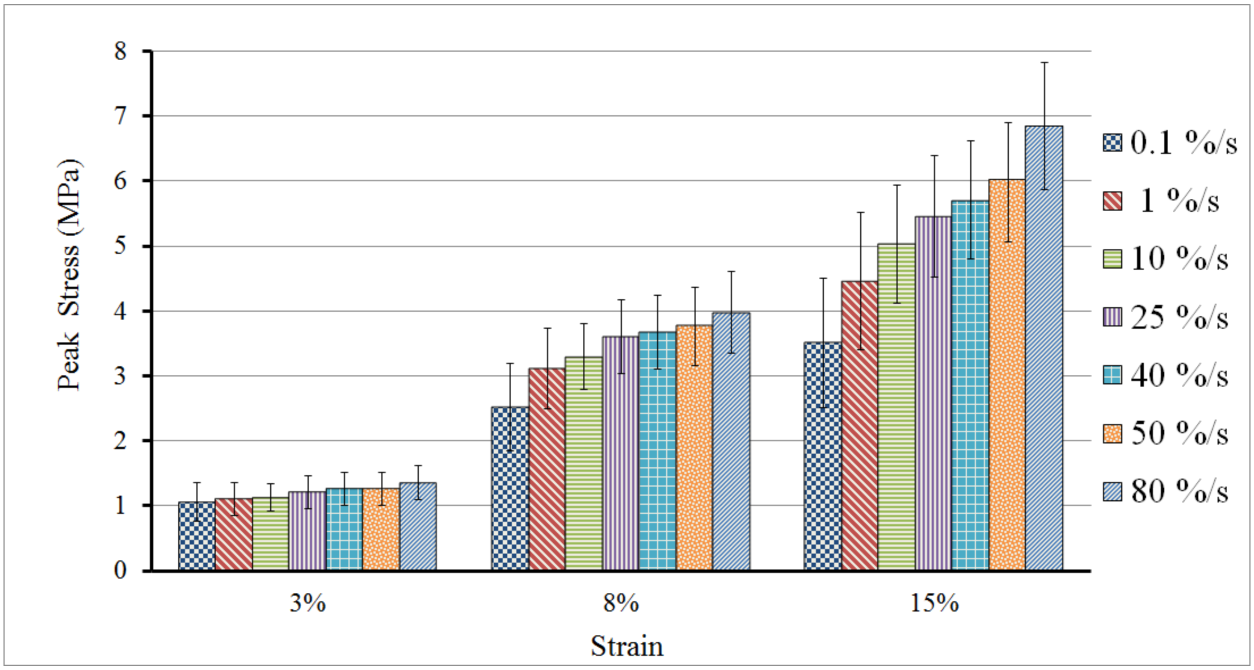

Figure 3. The tensile stress obtained at the end of the loading phase in each tensile test of articular cartilage under a given strain applied at a constant strain rate (n=37). The stress increased monotonically with strain and strain rate.

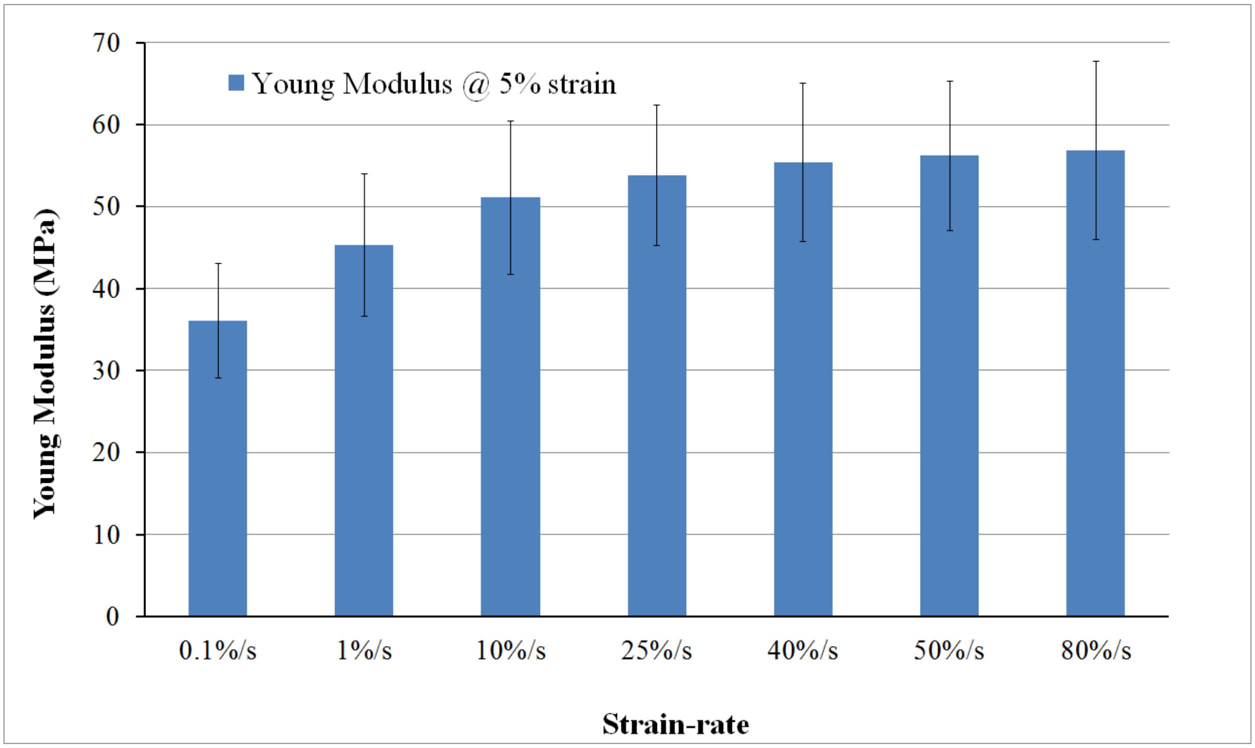

Figure 4. Elastic modulus at 5% strain for strain-rates 0.1-80%/s (n=37). This transient modulus is defined as the tangent of the stress-strain curve, i.e. $d\sigma / d\varepsilon$. The asymptote is sufficiently shown with the range of strain-rates considered.

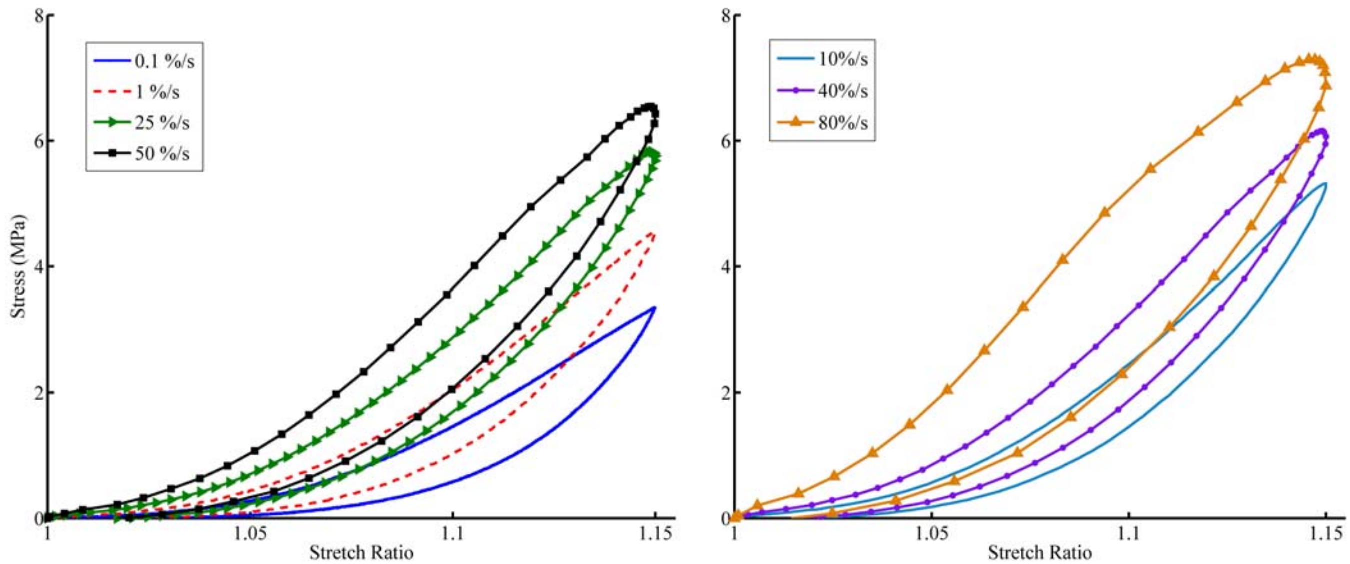

Figure 5. The strain-rate dependent hysteresis of articular cartilage stretched up to 15% strain.

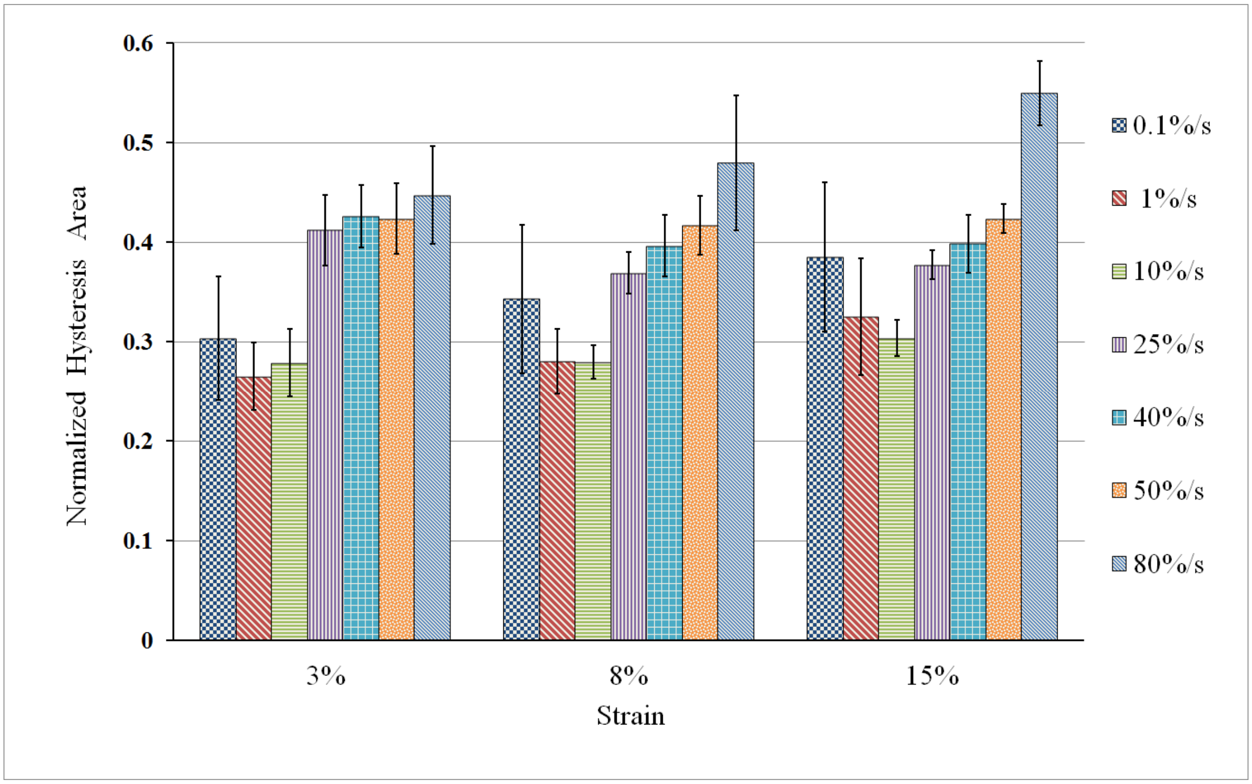

Figure 6. The normalized hysteresis area for various strains and strain-rates used in the tensile tests of articular cartilage (n=37).