Cartilage Strain Distributions Are Different Under the Same Load in the Central and Peripheral Tibial Plateau Regions

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There is increasing evidence that the regional spatial variations in the biological and mechanical properties of articular cartilage are an important consideration in the pathogenesis of knee osteoarthritis (OA) following kinematic changes at the knee due to joint destabilizing events (such as an anterior cruciate ligament (ACL) injury). Thus, given the sensitivity of chondrocytes to the mechanical environment, understanding the internal mechanical strains in knee articular cartilage under macroscopic loads is an important element in understanding knee OA. The purpose of this study was to test the hypothesis that cartilage from the central and peripheral regions of the tibial plateau has different internal strain distributions under the same applied load. The internal matrix strain distribution for each specimen was measured on osteochondral blocks from the tibial plateau of mature ovine stifle joints. Each specimen was loaded cyclically for 20 min, after which the specimen was cryofixed in its deformed position and freeze fractured. The internal matrix was viewed in a scanning electron microscope (SEM) and internal strains were measured by quantifying the deformation of the collagen fiber network. The peak surface tensile strain, maximum principal strain, and maximum shear strain were compared between the regions. The results demonstrated significantly different internal mechanical strain distributions between the central and peripheral regions of tibial plateau articular cartilage under both the same applied load and same applied nominal strain. These differences in the above strain measures were due to differences in the deformation patterns of the collagen network between the central and peripheral regions. Taken together with previous studies demonstrating differences in the biochemical response of chondrocytes from the central and peripheral regions of the tibial plateau to mechanical load, the differences in collagen network deformation observed in this study help to provide a fundamental basis for understanding the association between altered knee joint kinematics and premature knee OA. [DOI: 10.1115/1.4031849]

Introduction

The mechanical loads applied to knee articular cartilage during ambulation play an important role in the maintenance and degeneration of cartilage health by influencing the expression levels of matrix proteins and degenerative enzymes through chondrocyte cell deformation, nucleus deformation, and membrane stretching [1–6]. In addition, it has been shown [7] in an in vitro study that chondrocytes from the central regions of the tibial plateau express different levels of messenger ribonucleic acid (mRNA) for structural proteins (type II collagen and aggrecan) compared to chondrocytes from the peripheral regions of the tibial plateau under equivalent mechanical loads. Similarly, substantial heterogeneity has been reported [8] in the elastic properties of cartilage explants taken from different regions of the tibial plateau and in vivo studies [9,10] demonstrated that individual variations in the location of thickest cartilage on the femur were associated with knee kinematics during walking. Based on the above evidence, and given both the effect of mechanical loads on chondrocytes and the non-uniform mechanical load distribution within the knee [11], the above variations in cartilage structure, mechanical properties, and

biology within the knee joint may represent a conditioning of cartilage to repetitive local ambulatory loads.

The regional conditioning of cartilage to its local mechanical loading has been suggested [12] as a potential explanation for the increased risk of developing knee OA following kinematic changes at the knee, which are common to some of the primary risk factors for OA [13]. The clinical evidence for this sensitivity is most clearly apparent with the incidence of premature knee OA developed following joint destabilizing events, such as ACL and meniscus injury [12,14]. Such kinematic changes can shift the distribution of macroscopic joint loads within the knee, potentially leading to an increase in contact pressure in the peripheral region of the plateau, as has been shown previously [11]. Furthermore, the sensitivity of cartilage health to kinematic changes is supported by a number of studies that demonstrated that regional variations in articular cartilage content and structure within the knee joint correlate with regions of functional load bearing [15–23]. The studies suggest that local regions within the tibial plateau have adapted to specific mechanical loading environments. Therefore, understanding the internal cartilage tissue strains, and ultimately the chondrocyte loading, in each region under macroscopic joint loading is important for both understanding the mechanical sensitivity of cartilage health to shifts in applied load.

While several studies have analyzed the nominal (thru-thickness) strain behavior of both cartilage in the joint [24] and in confined and unconfined compression [17], few studies have analyzed the

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internal deformation patterns of cartilage. Quantitative measurements of internal cartilage deformation have been performed on osteochondral plugs in confined compression [25], unconfined compression [26], and shear [27–30]. Additional studies have qualitatively described the internal deformation patterns in bulk specimens or intact joints [31–37]. These studies demonstrated substantial differences in the internal deformation distribution in bulk specimens/intact joints compared to osteochondral plugs. However, regional variations in internal cartilage deformation have not been quantified. Understanding and quantifying the internal deformation in local regions of knee articular cartilage to macroscopic surface loads is an important element in understanding the mechanical stimuli applied to the cartilage matrix and, correspondingly, the pathways to cartilage degeneration during OA. Therefore, the purpose of this study was to test the hypothesis that cartilage from the central and peripheral regions of the tibial plateau has different internal strain distribution patterns under the same applied load and same applied nominal strain by quantifying the tissue deformation in bulk specimens of tibial plateau articular cartilage under compressive joint loading applied to local regions of the plateau.

Materials and Methods

Specimen Preparation and Loading. Osteochondral blocks were harvested from the medial compartment of the tibial plateau of fresh, mature (physes closed) ovine stifle joints. The blocks were 1.75 cm × 1.3 cm in the transverse plane and contained approximately 6 mm of subchondral bone. The blocks were harvested from either the central or the peripheral region of the plateau (Fig. 1). After harvesting, the subchondral bone of each specimen was scored in the medial–lateral direction through approximately 50% of the bone thickness (to facilitate freeze fracturing after loading). Care was taken to not let the specimens dry out during preparation by regularly misting the cartilage with an isotonic saline solution (phosphate-buffered saline, pH 7.2, Invitrogen, Carlsbad, CA).

The specimens were loaded using an MTS Mini-Bionix II (MTS, Minneapolis, MN) with an 8 mm diameter, hemispherical, nonpermeable indenter while submersed in saline. Following the application of a 1 N tare load to establish contact, the central and peripheral region specimens were loaded for 20 min at 1 Hz (sinusoidal waveform) with a force that cycled between the tare load and a peak load of 14.5 N. This peak load corresponded to an average applied contact pressure of approximately 1 MPa (calculated as peak load over the contact area, which was obtained from imaging of the deformed cartilage). The load frequency of 1 Hz was taken to be comparable to loading rates experienced by the cartilage in vivo during walking. Subsequently, in order to

compare specimens under the same applied nominal strain, a second set of central region specimens was loaded cyclically for 20 min at 1 Hz to a peak load of 27 N, which was found to consistently yield a similar nominal (thru-thickness) strain as was observed with 14.5 N in the peripheral region. The above combination of osteochondral specimen size and indenter diameter was selected to isolate the central and peripheral regions during loading, while applying contact stresses in the physiologic range [11]. For each region and loading condition combination analyzed, five specimens were tested. To ensure the cartilage was not overloaded during the testing, several specimens were analyzed after being allowed to recover in free-swelling conditions for 30 min following the cyclic loading.

At the end of the cyclic loading, the specimens were cryofixed by immersion in an isopentane slush at -160°C with the indenter still in contact with the specimen [34]. After cryofixation, the specimens were transferred to liquid nitrogen and freeze fractured [38] along the scored bone through the region of indentation. The cartilage matrix of each specimen was then viewed using a Hitachi S-3400N (Hitachi, Tokyo, Japan) variable pressure SEM at an acceleration voltage of 5 kV. The SEM contained a cold stage that maintained the specimens below -35°C during viewing. Only specimens that fractured within the center 25% of the total indentation width were used for analysis.

Calculation of Internal Cartilage Strain Distribution. The internal matrix strain distribution for each specimen was determined by quantifying the deformation of the collagen network. The orientation of the deformed radial collagen fibers was traced in each SEM image from the subchondral bone to the articular surface (Fig. 2). These traced fibers were then combined into a single panorama using the scale and position information recorded by the SEM, yielding the deformed position of the cartilage matrix.

In order to calculate the deformation and strain distributions in the cartilage, an initial (undeformed) position for the collagen network was estimated from the undeformed cartilage outside of the indentation region. For peripheral region specimens, the initial angle of the collagen fibers relative to the subchondral bone was assumed to vary linearly between the fiber angles in the undeformed cartilage to the left and right of the indentation region (Fig. 3). In the central region, the initial fiber orientation was estimated using a bilinear function with linear sections spanning

Fig. 1 Location of peripheral (dotted) and (dashed) specimen harvest location and freeze fracture plane (long dashed)

Fig. 2 Deformed matrix orientation traced on SEM image. Deformed cartilage can be viewed under the arrow, which was directly loaded by the indenter. Images across the entire specimen were obtained and combined to form a single panorama using the position data from the SEM.

Fig. 3 Comparison of estimated initial collagen orientation (solid lines) and deformed collagen orientation (X-marked lines) for (a) peripheral and (b) central region specimens

between each end of the indented region and the center of the indented region. The collagen orientation angle relative to the subchondral bone in the center of the loaded region was empirically associated with the cartilage thickness according to Eq. (1),

$$\theta_{\text{center}} = 28.65 \cdot \text{thickness}_{\text{center}} + 0.2 \quad (1)$$

where θ_{center} is in degrees and $\text{thickness}_{\text{center}}$ is in millimeters. Eq. (1) was determined by analyzing the fiber orientation of unloaded specimens. The bilinear distribution of the collagen

fibers in the central region specimens was due to the variation in the subchondral bone profile, which was relatively flat near the peripheral region, but was curved near the tibial spine (Fig. 3).

The above technique of estimating the initial collagen fiber orientation in the central and peripheral regions was separately validated on three specimens from each region, which were cry-oxified, but not loaded. For each validation specimen, the collagen network orientations at the edges of the specimen were used to predict the orientation values in the portion of the specimen where the indenter would have contacted the cartilage. These predicted values were compared to the true network orientation distribution measured in the SEM. The precision of the algorithm was determined by calculating the average absolute tangential strain error at the surface (where the error would be maximum).

Once the initial and final collagen network position fields were determined, the displacement and strain fields were calculated at all points in the cartilage. Except at a small region directly under the indenter, where the collagen fibers were typically too disrupted or compacted to discern any matrix orientation, the two-dimensional displacement field was calculated directly in MATLAB (Mathworks, Natick, MA) as the difference in the final and initial collagen network position fields. To calculate displacement field in the small region under the indenter, the initial position of the collagen fibers was mapped into a finite-element mesh in ABAQUS v6.5 (Simulia, Pawtucket, RI). The known displacements calculated in MATLAB were applied as boundary conditions across the cross section of the cartilage, and under the indenter, boundary conditions were also applied to the articular surface to mimic the experimental observations for each specimen (Fig. 4). The cartilage was modeled as linear elastic with a Young's modulus of 6 MPa [39] and Poisson ratio of 0.2 [40]. However, since the region where the finite-element nodes were not constrained was small and therefore highly constrained by the surrounding tissue, a sensitivity analysis to the Young's modulus demonstrated that the material properties used had little effect on the calculated strain results. Outside of the unconstrained region, the material properties used in the analysis did not affect the calculated strains since displacement boundary conditions were applied at all nodes.

Fig. 4 Representative strain distributions calculated from the displacement fields for (a) the peripheral region and (b) the central region

Fig. 5 Bending deformation pattern in the central region in (a) full thickness view and (b) higher magnification image near surface

The nonlinear Green–Lagrange strain distribution for each specimen was calculated from the displacement field under plane strain. The strain tangential to the surface, the peak maximum principal strain, and the maximum shear strain within the tissue were extracted to compare between the central and peripheral regions. These strain measures were chosen since they have been implicated in causing chondrocyte metabolism detrimental to cartilage [41,42]. Representative images of the tangential strain distribution in central and peripheral specimens are shown in Fig. 4.

Statistical Analysis. In addition to the initial position assumption precision, the reproducibility precision was calculated using the coefficient of variation ($CV = \text{standard deviation} / \text{mean} \times 100\%$). This was calculated by analyzing the same specimen on four different days. The comparison between regions was done using an analysis of variance with a Tukey post-hoc analysis for pairwise comparisons at a significance of 0.05.

Results

Central and Peripheral Region Strain Distributions. The overall pattern of deformation differed between the central and peripheral region specimens. In the central region, the deformed network curved through the thickness of the tissue (Fig. 5(a)) with the tightest bend typically near the articular surface (Fig. 5(b)). In the peripheral region, however, the collagen network remained straight (Fig. 6(a)) and displaced laterally out from under the indenter (Fig. 6(b)).

Fig. 6 Peripheral low-load specimen under load. (a) Full thickness profile and (b) zoom-in at deep/transitional zone interface. No bending occurred during loading.

Table 1 Internal strain measures for the central and peripheral region specimens under the same applied load (mean $\pm$ standard deviation, mm/mm)

Strain measure	Central region	Peripheral region
Tangential strain	0.31 ± 0.079	0.17 ± 0.082
Maximum principal strain	0.39 ± 0.11	0.20 ± 0.071
Maximum shear strain	0.59 ± 0.11	0.52 ± 0.12

Table 2 Internal strain measures for the central and peripheral region specimens under the same applied nominal strain (mean $\pm$ standard deviation, mm/mm)

Strain measure	Central region	Peripheral region
Tangential strain	0.33 ± 0.15	0.17 ± 0.082
Maximum principal strain	0.43 ± 0.12	0.20 ± 0.071
Maximum shear strain	0.67 ± 0.15	0.52 ± 0.12

The observed differences in matrix deformation resulted in significant differences in the internal strain distributions between the central and peripheral regions (Tables 1 and 2 and Fig. 7). Under the same applied load (Table 1), the tangential strain and

Fig. 7 Comparison of strain components under same applied load (a) and same applied nominal strain (b). The central region tended to have higher strains under same load and same nominal strain. The dashed lines on each bar show the average precision error associated with the initial position estimation for each region.

maximum principal strain were significantly higher in the central region compared to the peripheral region, while under the same applied nominal strain (Table 2), all three strain measures were significantly higher in the central region compared to the peripheral region. In addition, there was a significant difference in the depth of the peak maximum principal strain between the central and peripheral regions under the same applied nominal load, with the peak maximum principal strain occurring at a depth of 25% of the total tissue thickness in the peripheral region, but at a depth of 2% in the central region. In both the central and peripheral regions, the peak tensile strain tangential to the surface and the highest compressive strain through the thickness of the tissue (vertically) occurred at the articular surface. In addition, in all specimens, the strains tangential to the surface were tensile in the region of loading, but compressive at the edges of the loading. In addition, as shown in Figs. 2 and 3, the osteochondral blocks were large enough to ensure that regions of undeformed cartilage were present outside of the loaded regions to minimize any potential edge effects from cut cartilage surfaces.

Analysis of Initial Position Algorithm, Reproducibility, and Comparison to MTS Data. For the reproducibility analysis, the coefficient of variation for the tangential strain was 2%, for the maximum principal strain was 2%, and for maximum shear strain was 3%, demonstrating good reproducibility for the analysis technique. From the validation of the initial position algorithm, the average absolute strain error at the surface was 3% in the peripheral region and 5% in the central region for the unloaded specimens, which are well below both the measured strains for each region and the differences in strains between regions

(Fig. 7). The maximum nominal displacements calculated from the SEM images were similar to the overall MTS displacement for all specimens, with no statistical difference between the MTS displacement and the calculated displacement for any test configuration. In addition, there was no evidence of macroscopic breakdown following the loading (fissures), and following a 30-min unloaded recovery period, SEM imaging of three specimens demonstrated that the cartilage returned to its full thickness, as indicated by a smooth surface and the collagen fibers in the loaded region running nominally straight through the thickness of the cartilage. Correspondingly, no matrix damage was observed on these specimens that were allowed to recover.

Discussion

The results of this study demonstrated that under both the same applied load and same applied nominal strain, the internal mechanical strain distribution is significantly different between the central and peripheral regions of tibial plateau articular cartilage. The variables quantified in this study focused on the strain components supported by the collagen network (tension and shear). Therefore, the differences in strain between the regions are likely due to differences in the collagen network structure. These regional differences in internal mechanical environment under the same applied load and nominal strain suggest a sensitivity of cartilage to shifts in the location of applied joint load and support the hypothesis that altered gait kinematics may be a mechanism to cartilage degeneration [13].

In addition to quantifying differences in the tissue strain response in different regions of the plateau, the results of this study provide some new insights in understanding internal cartilage deformation in bulk tibial plateau specimens (as opposed to osteochondral plugs) under cyclic loading. Thambyah and Broom [32] loaded osteochondral blocks in static equilibrium and calculated the average tangential surface strain spanning from the center of the loading to unloaded tissue, which they found to be tensile. The results of the current study, however, demonstrated that this strain is highly nonuniform with tensile strains under the indenter and compressive strains near the edges of the indenter. Schinagl et al. [25] and Wang et al. [26] loaded osteochondral blocks statically in confined and unconfined compression, respectively, and found a nonlinear strain distribution through the thickness of the tissue, with the highest compressive strains at the articular surface, but did not find the lateral bending that was observed in this study. The strain distributions from this study also had the highest compressive strains near the surface, which is in agreement with these previous studies. However, studies loading intact joints under both static and cyclic loading [34,43] consistently observe a bending deformation pattern of the cartilage tissue, as was seen in this study, suggesting that cartilage deforms differently through the thickness of the tissue in smaller osteochondral plugs compared to bulk specimens. Therefore, the results of the current study provide internal strain data for this more physiologically relevant deformation pattern.

The internal deformation pattern within the cartilage is due to a complex interaction between the fluid and solid components. The results of this study provide some initial insights into the mechanisms of cartilage deformation and highlight the fluid/solid interactions. The strain tangential to the surface in all specimens was tensile under the region of loading and compressive at the load edges. This type of deformation is consistent with an incompressible material and suggests that the fluid is being pressurized, bearing load, and influencing the deformation of the solid matrix. The bending deformation exhibited by the matrix, however, suggests the presence of shear stresses within the cartilage, which must be supported by the solid matrix. Therefore, the solid matrix also plays an important role in the response of cartilage to compression, and movement of the matrix will likely influence the ability of the fluid to pressurize locally. Together, the deformation patterns observed under cyclic loading confirm the importance of

both the fluid to support compression and the collagen to withstand shear.

The major finding of this study is the difference in internal strain distribution between the central and peripheral regions under the same applied load and same applied nominal strain. This suggests a potential pathway to degeneration following altered knee kinematics [13], which likely shift the regions of high load from the central region to the peripheral region [11,14,44]. Previous studies have demonstrated differences in biomechanical properties between regions [8,17,45], but did not analyze the internal strain fields in isolated regions of cartilage. Given the influence of chondrocyte metabolism on cartilage health, understanding the mechanical environment within the cartilage is important in understanding the mechanisms leading to cartilage degeneration. The deeper peak maximum principal strain in the peripheral region also corresponds well with clinical studies demonstrating that the initial degeneration in the peripheral region occurs near the transitional/deep zone interface [20]. In the central region, however, initial degeneration occurs at the articular surface (fibrillation) [20], which is again consistent with the locations of peak tensile strain occurring in this study. The variation in internal mechanical strain distribution between regions supports the hypothesis that altered loading may initiate cartilage degeneration through either mechanical overload or altered chondrocyte metabolism [12,14]. In addition, as shown in Fig. 4, the central region often exhibited an asymmetric indentation from the indenter compared to the symmetric indentation observed in the peripheral region. This difference was a result of the central region cartilage curving upward toward the tibial spine relative to the indenter (which was oriented orthogonal to the overall tibial plateau). The curvature of the central region further highlights its potential sensitivity to changes in gait altering the loads and corresponding strains within the cartilage.

Previous studies using scanning electron microscopy have raised concerns over tissue deformation during specimen preparation, such as during chemical fixation, dehydration, and drying, all of which introduce artifacts due to specimen shrinkage [46]. The use of a cold stage SEM in this study allowed for specimens to be viewed in their fully hydrated, unfixed state. Prior to testing, ice crystal artifact from the cold stage was a concern since the stage maintains the specimens below -35°C (above the recrystallization temperature of approximately -70°C). However, the presence of ice crystals was not observed during any testing, which is believed to be due to the rapid sublimation of any ice near the observed surface as the chamber vacuum pressure was lowered since the specimens were initially at -196°C (liquid nitrogen boiling temperature) when they were placed into the SEM chamber.

In conclusion, the results of this study support the hypothesis that altered kinematics may initiate cartilage degeneration through a significant change in internal mechanical load following a local increase in load. These results help provide a fundamental basis for altered joint kinematics to initiate OA and will be important for clinical treatments of OA, which attempt to replace degraded cartilage or alter loads at the knee through bracing or modified footwear.

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