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An Alternative Method to Characterize the Quasi-Static, Nonlinear Material Properties of Murine Articular Cartilage

With the onset and progression of osteoarthritis (OA), articular cartilage (AC) mechanical properties are altered. These alterations can serve as an objective measure of tissue degradation. Although the mouse is a common and useful animal model for studying OA, it is extremely challenging to measure the mechanical properties of murine AC due to its small size (thickness < 50 μm). In this study, we developed novel and direct approach to independently quantify two quasi-static mechanical properties of mouse AC: the load-dependent (nonlinear) solid matrix Young's modulus (E) and drained Poisson's ratio (ν). The technique involves confocal microscope-based multiaxial strain mapping of compressed, intact murine AC followed by inverse finite element analysis (iFEA) to determine E and ν . Importantly, this approach yields estimates of E and ν that are independent of the initial guesses used for iterative optimization. As a proof of concept, mechanical properties of AC on the medial femoral condyles of wild-type mice were obtained for both trypsin-treated and control specimens. After proteolytic tissue degradation induced through trypsin treatment, a dramatic decrease in E was observed (compared to controls) at each of the three tested loading conditions. A significant decrease in ν due to trypsin digestion was also detected. These data indicate that the method developed in this study may serve as a valuable tool for comparative studies evaluating factors involved in OA pathogenesis using experimentally induced mouse OA models. [DOI: 10.1115/1.4038147]

Keywords: articular cartilage, mechanical properties, inverse finite element analysis, compression, mouse

1 Introduction

Articular cartilage (AC) is a load-bearing tissue that transmits forces across synovial joints. Degradation of AC is a primary feature of osteoarthritis (OA), the leading cause of disability in the U.S. [1]. With the onset and progression of OA, cartilage mechanical properties are altered [2–6], compromising the joint's ability to withstand physiological loads. Many useful and established models of this disease are available in the mouse, where OA is easily induced through genetic and surgical manipulation [7–14]. These models provide a well-controlled research platform to study the progression of AC degeneration in OA. Unfortunately, alterations in the mechanical properties of murine AC are extremely challenging to measure due to its small size (thickness < 50 μm).

Due to its practical difficulty, few studies have characterized the mechanical properties of mouse cartilage. Moreover, most published methods involve nano- or micro-indentation of the articular surface. For example, biphasic material properties were determined through micro-indentation and nonlinear optimization-based finite element modeling (FEM) techniques [15]. In other studies, data gathered from atomic force microscope (AFM)-based indentation tests of murine cartilage were fit to analytical solutions to obtain material properties (permeability, shear modulus, indentation modulus) [16–20]. Nano-indentation, secondary to micro-indentation techniques, was also utilized to characterize the dynamic elastic modulus of intact murine cartilage [19,21]. A

key advantage of these powerful techniques over methods involving testing of excised explants in larger animal models [22–35] is that they are nondestructive. In particular, the tissue's native boundary conditions and intact structure are maintained, allowing for a presumably more accurate measure of its inherent mechanical properties. However, indentation approaches require separate measurements of cartilage thickness from histological sections or other means. Moreover, Poisson's ratio is generally assumed [18–21] or is one of several material properties obtained by fitting two or more parameters at once to a single experimental dataset [15].

Therefore, our objective is to develop an alternative, more direct approach to measure quasi-static, nonlinear material properties in intact murine cartilage, where solid matrix Young's modulus (E) and drained Poisson's ratio (ν) are measured individually in separate experiments such that there is no need to fit multiple parameters to a single mechanical test. The technique involves inverse finite element modeling and confocal microscope-based three-dimensional (3D) thickness mapping of intact specimens subjected to compression. It exploits the thin dimensions of murine cartilage (thickness < 50 μm), allowing for 3D imaging throughout its entire depth using confocal microscopy, which is limited to imaging depths less than 80 μm due to scattering.

In this study, we validate this new method by comparing the material properties of two treatment groups: control and trypsin-treated murine cartilage. Trypsin digests core proteins in glycosaminoglycans (GAGs), thereby effectively removing proteoglycans from the tissue. In large animal models, researchers have measured dramatic GAG loss and decreased AC Young's modulus following treatment with trypsin [36,37] or similar enzymes [38]. We

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hypothesize that trypsin-treated murine cartilage will exhibit a lower Young's modulus compared to the control group, thereby demonstrating the ability of our method to detect mechanical alterations due to proteolytic degradation in articular cartilage.

2 Materials and Methods

Characterization of material properties (solid matrix Young's modulus E and drained Poisson's ratio ν) involves multiple experimental and computational methods. These methods and their sequence are as follows (Fig. 1): (1) visualization of multi-axial, spatially varying cartilage deformation; (2) calculation of ν through implementation of inverse finite element analysis based on experimentally measured lateral strain distribution and relative boundary displacements; and (3) calculation of E through implementation of inverse finite element analysis (iFEA) based on experimentally measured boundary forces and relative boundary displacements (and using the predetermined value of ν).

2.1 Specimen Preparation. Intact distal femurs (cartilage and bone) from 8–10 week-old female BALB/c mice were carefully dissected immediately after sacrifice and stored at -20°C until testing. The bone on distal femur was cut 8 mm above tibio-femoral joint. Specimens were randomly divided into control and trypsin-treated groups ($n=9$ per group). In the trypsin-treated group, after thawing, specimens were incubated for 12 h at 37°C in buffer with trypsin (Cellgro, 0.25% trypsin/2.21 mM EDTA, Manassas, VA). In the control group, after thawing, specimens were immediately prepared for mechanical testing. Preparation for testing consisted of fluorescent staining for 1 h with 5'-DTAF (10 $\mu\text{g}/\text{mL}$) (Sigma-Aldrich, St. Louis, MO), a general extracellular matrix (ECM) stain that binds to amines in proteins, to enable visualization of the ECM, followed by 3 h of wash in phosphate buffer saline (PBS) at 23°C .

2.2 Mechanical Testing. For each mechanical test, femurs hydrated in a PBS bath were placed onto the glass slide of a custom microscope-mounted device and sequentially loaded with 0.1 N, 0.2 N, and 0.3 N on the anterior side (on top of the patella-femoral groove) such that cartilage on the posterior femoral condyles was compressed against the cover glass (Fig. 2). Loading on the anterior femur was applied by a cylindrical weight whose vertical location was set through a pulley system driven by a computer-controlled linear actuator. Note that it was assumed that the bone, which has a Young's modulus ~ 1000 times higher than cartilage, did not deform. The load on the anterior femur was computed to be mg (where m is the mass of the weight and g is the acceleration due to gravity). Cartilage on medial femoral condyle was imaged with a $40\times$ dry lens (LUCPLFLN, NA 0.6) on an Olympus FV1000 laser scanning confocal microscope (Olympus America, Center Valley, PA). Confocal image stacks ($0.317\text{ }\mu\text{m} \times 0.317\text{ }\mu\text{m}/\text{pixel}$) acquired along the z -axis at intervals of $1.64\text{ }\mu\text{m}$ were taken before compression (0 N) and 5 min after each sequential load was applied. Given

Fig. 2 Schematic representation of the experimental setup. Controlled loads (F_{applied}) of 0.1 N, 0.2 N, or 0.3 N were applied on the anterior side of the murine distal femur. Cartilage on the posterior medial femoral condyles was imaged with confocal microscope as it was compressed against the cover glass.

that the gel diffusion time t_{gel} can be approximated as $(1+\nu)(1-2\nu)h^2/E(1-\nu)k$ [39], we estimate that t_{gel} is 6 s in murine articular cartilage assuming thickness $h=38.1\text{ }\mu\text{m}$ (average thickness measured in the current study, see Sec. 2.3), Young's modulus $E=2\text{ MPa}$, permeability $k=1.1\times 10^{-16}\text{ m}^4/\text{N s}$, and Poisson's ratio $\nu=0.2$ based on previous studies [15]. Since t_{gel} is a measure of the timescale for mechanical equilibration [39], the 5 min wait after each application of load should be more than sufficient for the tissue to reach steady-state. All mechanical tests were performed at room temperature.

Since 0.1 N, 0.2 N, and 0.3 N loads on the anterior femur were found to induce tissue strains at the high end of physiological levels, additional experiments were performed with a smaller load (0.025 N) to assess tissue mechanical properties at physiological strain levels ($n=9$ per group). The same mechanical testing protocol was used as described earlier.

2.3 Characterization of Cartilage Deformation. From the acquired 3D confocal stacks obtained across treatment groups (control, trypsin-treated) and loading groups (0 N, 0.1 N, 0.2 N and 0.3 N), spatially varying thickness maps were quantified by tracking the fluorescently stained ECM using custom written MATLAB code (The MathWorks Inc., Natick, MA) (Fig. 3). Specifically, image stacks were preprocessed with a Gaussian blur filter in ImageJ [40] to fill gaps embedded with chondrocytes such that only the ECM was visible. Normalized depth-wise intensity profiles of the preprocessed image stacks (across the z direction (Fig. 3)) were fitted with Gaussian curves of the form $f(x) = Ae^{-(x-\mu)^2/2\sigma^2} + B$, where $f(x)$ are intensity values; x is the location along the cartilage depth; and A , B , μ , and σ are fit parameters. σ is a measure of the spread of the depth-wise intensity profile; hence, we assume that sigma (σ) is proportional to the tissue thickness. This assumption was validated by analyzing the relationship between cartilage thickness (assessed in Image J) and the parameter σ for measurements performed under every loading (0 N–0.3 N) and treatment condition (see Supplemental Material 1, which is available under the “[Supplemental Materials](#)” tab for this paper on the ASME Digital Collection). The strong correlation ($R^2=0.93$) and linear relationship (slope = 4.743) between measured thickness and σ allows for automated quantification of the stretch (λ) as the ratio of σ after loading to σ before loading. Thus, spatially varying tissue deformation was quantified by subtracting thickness maps obtained before and after tissue compression. The *relative boundary displacement* (e.g., the displacement of the cartilage–bone interface relative to the glass slide) was defined as the difference between the average cartilage thickness before and after compression within a radius of $5\text{ }\mu\text{m}$ from

Fig. 1 Flow chart of the experimental approach for independent measurements of drained Poisson's ratio (ν) and solid matrix Young's modulus (E). Characterization of these quasi-static material properties includes computational and experimental methods. Arrows indicate the sequence of steps.

Fig. 3 Representative sagittal cross sections (left) and spatially varying thickness maps (right) obtained from confocal z-stacks of murine cartilage prior to compression (top row) and after application of 0.3 N (bottom row). Thickness maps were used to quantify cartilage deformation.

Fig. 4 Representative finite element model of murine cartilage on the medial femoral condyle. The tissue was modeled as an ideal hemispherical shell governed by a neo-Hookean, hyperelastic constitutive relationship. A rigid wall (flat plate) contact was applied with a prescribed displacement to compress the bottom (outer) surface in the positive z -direction. The top surface represents the cartilage–bone interface, which was constrained from any rotation and translation. The FEM was used to determine drained Poisson’s ratio (ν) and solid matrix Young’s modulus (E) through iFEA.

site of maximal tissue deformation. Cartilage thickness in the unloaded state was assumed to be uniform.

2.4 Finite Element Model Construction. A 3D finite element model of murine articular cartilage on the medial femoral condyle was created using the FEBIO software suite [41] (Fig. 4). The cartilage was approximated as an ideal hemispherical shell with outer radius 1.002 ± 0.111 mm and uniform thickness 38.1 ± 4.87 μ m. The subject-specific thickness was measured as described above (see Sec. 2.3). The subject-specific outer radius was computed based on MATLAB measurements of two-dimensional (2D) cross sections in the sagittal plane at the apex of the condyle (Fig. 3(a)). Specifically, the outer boundary (articular surface) of the cartilage was defined using an edge detection algorithm (Sobel filter) in MATLAB. This boundary was then fit with a circle positioned at the center of the medial condyle. The geometry was discretized with 12,288 eight-node hexahedral elements using FEBio’s internal meshing algorithm [41]. The boundary

conditions constrained the model from any translation and rotation on the top surface (cartilage–bone interface). A rigid wall contact was applied at the outer cartilage surface (bottom surface) with a prescribed displacement in the z -direction, corresponding to the measured relative boundary displacement (see Sec. 2.3). Although articular cartilage is a poroelastic/biphasic material, a hyperelastic, neo-Hookean, isotropic, homogeneous constitutive model was used, simulating the mechanical response of articular cartilage at mechanical equilibrium. Instead of employing a nonlinear constitutive model—which increases the number of fit parameters—tissue nonlinearity was accounted for by characterizing neo-Hookean mechanical properties at three different loads. A convergence study was performed and demonstrated that the change in reaction force due to a prescribed rigid wall boundary displacement stabilized to $\leq 0.12\%$ with increase in the mesh size. In the convergence study, values of drained Poisson’s ratio ($\nu = 0.2$) and solid matrix Young’s modulus (2 MPa) were taken from the literature [15].

2.5 Characterization of Tissue Lateral Strains. A total of 21 circular spots with diameter 7 μ m were photobleached in a cross-like pattern onto the articular surface of the medial condyle after equilibration at 0.3 N loading using a 405 nm laser at 100% power (Fig. 5). The photobleached spot located at the center of the cross was placed at the center of contact, considered to be the origin of the grid. The centroids of the adjacent photobleached spots were spaced approximately 20 μ m apart. After photobleaching, 2D images of the photobleached grid were obtained using at 40 $\times$ magnification on the laser scanning confocal microscope (see Sec. 2.2) with the aperture opened slightly (400 μ m) to allow for a larger depth of field. The cartilage was then unloaded and imaged again after 1 additional minute. Centroids (x and y coordinates) of photobleached spots were determined using a custom MATLAB code that minimized the sum of intensities along the x and y directions inside a square-shaped region of interest containing each circular photobleached spot. The centroids were spatially tracked in 2D confocal micrographs before and after unloading to quantify compression-induced lateral strains on the tissue surface at locations between the photobleached spots. Lateral strains were circumferentially averaged along each direction extending from the origin. The outermost photobleached spots were not considered in the analysis since they moved out of focus (due to surface curvature) when the load was removed. Thus, four averaged lateral strains were calculated per specimen.

2.6 Characterization of Drained Poisson’s Ratio ν . A FEM-based sensitivity study simulating the effect of Poisson’s ratio ranging from 0 to 0.49 (in increments of 0.01) on spatially varying lateral strain distribution was performed in FEBio.

Fig. 5 Representative confocal micrographs of murine AC surface with 21 spots photobleached onto the extracellular matrix (a) 1 min after unloading ($F=0$ N) and (b) 5 min after loading to $F=0.3$ N. These micrographs were used to quantify compression-induced lateral strains between the photobleached spots from the center of compression (the center of the grid). (c) Representative FEM simulation (view from the bottom surface) used to determine drained Poisson's ratio (ν) by matching experimentally and computationally quantified lateral strains. The highlighted nodes in the simulation are along the photobleached grid pattern in the experiments.

Importantly, no effect on nodal displacement—and thus no effect on lateral strain distribution—was found for solid matrix Young's moduli ranging from 0.1 to 21 MPa (see Supplemental Material 2, which is available under the “Supplemental Materials” tab for this paper on the ASME Digital Collection). The lateral strains based on FEM nodal displacements (Fig. 5(c)) were compared to lateral strains measured experimentally at equivalent locations through quantification of the square root of the sum of squared error ($\sqrt{\text{SSE}}$) (Eq. (1))

$$\sqrt{\text{SSE}} = \sqrt{\sum (\text{Lateral strain}_{\text{exp}} - \text{Lateral strain}_{\text{FEA}})^2} \quad (1)$$

The drained Poisson's ratio (ν) was then taken to be the global minimum of the $\sqrt{\text{SSE}}$ curve plotted for the entire range of Poisson ratios (Fig. 6). At the global minimum, the experimental and finite element simulation results are closest.

2.7 Measurement of Boundary Forces. Application of a controlled force on top of the anterior side of the femur results in three distinct specimen-glass contact points: two cartilage-glass contacts on the femoral condyles and one bone-glass contact on the proximal end of the femur. Therefore, the magnitudes of the boundary forces exerted on the two femoral condyles are a fraction of the load applied on top of the patella-femoral groove. The relative boundary force distribution between femoral condyles was characterized through moment balance based on the contact force locations obtained from micrographs (Fig. 7). Specifically, each specimen was placed on and compressed with a 0.3 N load against a sheet covered with black ink. The contact surface of the cylindrical weight applying the load on the anterior side of the femur was also covered with black ink. Thus, after one minute of static loading, ink was transferred to the contact areas on both the

Fig. 6 (a) Comparison of the experimentally measured, spatially varying lateral strains in a representative specimen (circles) to FEM simulation results for $\nu=0$, $\nu=0.21$, $\nu=0.35$, and $\nu=0.49$ (triangles). The closest match between experimental and FEM results was observed for $\nu=0.21$ in this representative dataset. (b) Representative $\sqrt{\text{SSE}}$ curve calculated for a range of simulated drained Poisson's ratios ($\nu=0$ – 0.49 in increments of 0.01). $\sqrt{\text{SSE}}$ is the square root of the sum of squared error between the simulated lateral strains (in the FEM) and the experimentally calculated lateral strains. ν was determined based on the global minima of the curve ($\nu=0.21$ for the representative dataset).

Fig. 7 Representative images of (a) posterior and (b) anterior sides of a murine femur. Prior to imaging, the femur was placed posterior side down onto a sheet of paper with ink. Thus, the contact points on the posterior condyles and on the bone are ink-stained and visible in image (a). Additionally, a flat weight with ink was placed onto the anterior side of the femur, enabling visualization of the weight-femur contact point (i.e., the point of force application) in image (b). (c) Posterior side of the femur with superimposed coordinates of contact forces from the weight (F_{applied}), from the glass onto the femoral condyles (F_2 and F_3) and from the glass onto the bone (F_1). These coordinates are calculated relative to a reference point (ref) point on the tape used to secure the specimen onto the cover glass.

posterior and anterior sides of the specimens. Subsequently, specimens were placed on a cover glass, secured with a tape to avoid specimen translation and rotation, and imaged with a $4\times$ dry lens (NA 0.13) on an Olympus IX-81 inverted microscope under reflective light (to image the bottom of the specimen) and with a stereomicroscope (to image the top of the specimen) (Fig. 7(a) and 7(b)).

From the acquired images, the centroids of the stained contact areas were determined in ImageJ and coordinates relative to a reference point were obtained. The reference point was taken to be an arbitrarily chosen edge of the tape. This reference point was viewable from both perspectives (above and below the specimen). The image obtained from the stereomicroscope was compensated for rotation in order to superimpose the location of the applied force (on top of the specimen) and the locations of the reaction forces from the glass (Fig. 7(c)).

The relative force distribution acting on the two condylar surfaces (F_2 and F_3 , given as a fraction of the force applied on top of

the specimen) was obtained from static equilibrium moment balance around the bone–glass contact point about the x - and y -axes (Fig. 7(c), Eq. (2)) as follows:

$$\begin{bmatrix} F_2 \\ F_3 \end{bmatrix} = \begin{bmatrix} (y_2 - y_1) & (y_3 - y_1) \\ (x_1 - x_2) & (x_1 - x_3) \end{bmatrix}^{-1} \begin{bmatrix} (y_{app} - y_1) \\ (x_1 - x_{app}) \end{bmatrix} \quad (2)$$

where (x_2, y_2) and (x_3, y_3) are the coordinates of the two condylar contact points, the force F_{app} applied on the anterior side of the specimen at location (x_{app}, y_{app}) is equated to unity, and (x_1, y_1) are the coordinates of the contact force acting on the proximal end of the bone. Equilibrium moment balance was applied under the assumption that the point forces acted perpendicular to the glass. The actual medial condyle contact force was calculated by multiplying the relative force (F_2) by the applied load on the anterior side of the femur (0.1, 0.2, or 0.3 N).

2.8 Characterization of Solid Matrix Young's Modulus E . A subject-specific finite element model (see Sec. 2.4) with a predetermined drained Poisson's ratio (see Sec. 2.6) subjected to the experimentally measured relative boundary displacement was used to estimate the solid matrix Young's modulus as a function of applied load on each tested specimen. Specifically, an iterative optimization algorithm (iFEA) implemented in FEBio [41] was used to match the experimentally measured boundary force to the reaction force on the rigid wall contact when the measured displacement (between the unloaded and loaded state) was applied. To more sensitively assess murine cartilage nonlinearity, we also analyzed the *incremental* Young's modulus of this tissue by comparing tissue deformation before and after each loading increment (see Supplemental Material 4, which is available under the "Supplemental Materials" tab for this paper on the ASME Digital Collection).

2.9 Validation Using AFM-Based Nano-Indentation. Atomic force microscopy-based nano-indentation testing was performed to validate the confocal microscope-based method. A total of $n = 3$ specimens (obtained from 11 week-old female BALB/c mice and prepared as described in Sec. 2.1) were glued to a cover glass such that the cartilage on femoral condyles was oriented upward to allow for nano-indentation testing using an AFM. A silicon cantilever beam (nominal spring constant $k \approx 2.8$ N/m) with an attached spherical borosilicate colloidal probe ($d = 5 \mu\text{m}$, NanoAndMore USA, Watsonville, CA) was used to indent the distal femoral condyles. Specimens were immersed in a PBS bath during the experiments. Each indentation was performed at piezo displacement rate of $5 \mu\text{m/s}$ until the indentation depth reached $1 \mu\text{m}$. Indentations were performed at multiple randomly chosen locations on the articular surface, and Young's Modulus (E) was obtained from fitting force-indentation curves to a classical Hertzian contact model with predetermined Poisson's ratio (ν) of 0.25 as measured in our confocal microscope-based experiments (see results).

2.10 Analysis of Uniqueness. A representative FEM was used to analyze output parameter variability by fitting one (E) or two parameters (E and ν) at once based on different initial guesses for iterative optimization. Inverse finite element simulations were implemented to evaluate the uniqueness of output parameters. For all simulations, the initial guess for the solid matrix Young's modulus was 0.1–10 MPa. For one-parameter optimizations, the drained Poisson's ratio was set to its measured value (0.21). For two-parameter optimizations, the initial guess for the drained Poisson's ratio ν ranged from 0 to 0.49.

2.11 Statistical Analysis. Drained Poisson's ratio ν was compared between control and trypsin treated groups using a Student's t -test. Solid matrix Young's modulus was compared across treatment and loading groups using a two-way repeated measures analysis of variance with Sidak and Tukey post hoc analyses,

respectively, in GRAPHPAD PRISM (version 7.01, La Jolla, CA). Boundary forces and displacements were compared across treatment groups by a two-way analysis of variance with Sidak post hoc analysis. The threshold for significance was set to $\alpha = 0.05$.

3 Results

Trypsin-treated murine specimens experienced significantly higher relative boundary displacements on the medial condyle as compared to controls for all applied forces (Fig. 8(a)). Importantly, the force transmitted to the medial condyle was not affected by trypsin treatment at any of the applied loads (Fig. 8(b)). Force-displacement curves were qualitatively consistent with a nonlinear mechanical response (Fig. 8(c)).

Reduced solid matrix Young's modulus E was observed in the trypsin treated group as compared to controls for all loading conditions (0.1 N, 0.2 N, and 0.3 N) (Fig. 9(a)). Furthermore, E increased with applied load in the control group, reflecting the nonlinear mechanical properties of cartilage (Fig. 9(a), Fig. S4). Drained Poisson's ratio ν was also significantly altered by trypsin digestion. Specifically, ν was found to be 0.26 and 0.21 for the control and trypsin-treated groups, respectively (Fig. 9(b)). Importantly, at a lower applied load (0.025 N) leading to lower applied strain (13.8%), the method was still able to detect trypsin-induced changes in cartilage material properties (Figs. 10(a) and 10(b)). Namely, both Young's modulus and Poisson's ratio were significantly reduced by trypsin treatment (6.4 MPa in control specimens versus 1.5 MPa in trypsin-treated specimens, Figs. 10(a) and 10(b)). Moreover, AFM-based nano-indentation experiments yielded comparable values of solid matrix Young's modulus (ranging from 3.3 MPa to 7.6 MPa, Fig. 10(c)) in control specimens, validating the confocal microscope-based approach.

Finally, analysis of the uniqueness of the inverse FEM-based measurements of material properties demonstrated that a one-parameter fit approach with predetermined drained Poisson's ratio yields a unique solution for E ; in contrast, a two-parameter fit approach was highly sensitive to the initial guess for both E and ν (Fig. 11).

4 Discussion

This study successfully developed an alternative method to quantify the nonlinear material properties of murine articular cartilage at equilibrium. This method provides direct measurements of multi-axial tissue deformation, allowing material properties (drained Poisson's ratio ν and solid matrix Young's modulus E) to be determined separately without multiparameter fitting. Specifically, determination of ν and E is decoupled: ν is assessed based on lateral and axial tissue strains and E is found (as a function of load) based on the boundary force versus displacement curve and the predetermined value of ν .

The mouse provides multiple benefits over large animal and human models in studying cartilage mechanics and OA. First, in the mouse, OA can be easily induced through genetic or surgical means [7–14]. Second, this model provides a valuable research platform to study the progression of articular cartilage degeneration in OA. Third, in large animal studies, investigators typically use explants removed from the native joint to perform mechanical tests for characterization of material properties. In contrast, due to its small size, the mouse facilitates nondestructive mechanical evaluation of intact specimens without compromising native boundary conditions. Furthermore, due to the small contact areas generated in compressed, intact mouse specimens (Fig. 3), testing requires application of substantially less load in order to obtain comparable tissue strains to those observed in large animal studies. Finally, murine models provide investigators with the ability to assess the role of specific molecules in cartilage mechanics (i.e., "structure–function relationships") through genetic manipulation.

One possible advantage of the methodology described in this study is that ν and E are independently assessed with separate

Fig. 8 (a) Relative boundary displacements measured for each loading group in control and trypsin-treated specimens. Increased displacements for a given force in trypsin-treated specimens suggest decreased stiffness in comparison with controls. (b) Reaction forces measured on the articular surface of the medial condyle for each loading group in control and trypsin-treated specimens. As expected, the fraction of the applied force transmitted onto the medial condyle was independent of treatment. (c) Force versus displacement for both treatment groups. These curves are qualitatively consistent with a nonlinear mechanical response. Statistical analyses of these data are in (a) and (b). In all figure panels, data are mean + standard deviation. * denotes $p \leq 0.05$ versus control. (d) FEM-predicted behavior of reaction force versus displacement for representative specimens from the control (solid lines) and trypsin-treated (dashed lines) groups comparing every load level to the unloaded state. Incremental analysis comparing every load level to the previous loaded state was also performed (see Supplemental Material 4, which is available under the “Supplemental Materials” tab for this paper on the ASME Digital Collection).

experiments and separate iFEA-based one-parameter optimizations. We found in this study that the alternative approach—computing both ν and E from a single experiment using a two-parameter optimization strategy—fails to converge to a unique solution. Nevertheless, multiparameter fitting appears to be

Fig. 9 (a) Solid matrix Young's modulus E of murine femoral AC in control and trypsin-treated specimens ($n = 9/\text{group}$) estimated for applied loads of 0.1 N, 0.2 N and 0.3 N. In all loading groups, trypsin treatment significantly reduced E . (b) Drained Poisson's ratio ν in control and trypsin-treated specimens. ν was also reduced by trypsin treatment. In both panels, data are mean + standard deviation. Brackets denote $p \leq 0.05$ for pairwise comparisons between loads; * denotes $p \leq 0.05$ versus control.

Fig. 10 (a) Solid matrix Young's modulus E of murine femoral AC in control and trypsin-treated specimens ($n = 9/\text{group}$) estimated for applied load of 0.025 N. Trypsin treatment significantly reduced E . (b) Drained Poisson's ratio ν in control and trypsin-treated specimens ($n = 9/\text{group}$). ν was also reduced by trypsin treatment. (c) AFM-based nano-indentation experiment performed on murine femoral AC in the control group ($n = 3/\text{group}$). AFM- and confocal microscope-based measurements yielded similar values of E . Each data point represents averaged Young's moduli obtained at multiple locations of murine AC per condyle. The data are mean + standard deviation. * denotes $p \leq 0.05$ versus control.

effective when analyzing time-dependent stress relaxation curves instead of force–displacement data [15].

Murine cartilage mechanical properties found in this study are consistent with values reported in the literature [15]. According to

a previous micro-indentation study, drained Poisson's ratio $\nu = 0.2$ in mouse cartilage [15]. Similarly, we found $\nu = 0.25$ and $\nu = 0.19$ for control and trypsin-treated specimens, respectively. However, the authors of this prior study reported $E = 2$ MPa while our values ($E = 6.4$ MPa and $E = 1.5$ MPa in the 0.025 N loading group for control and trypsin-treated specimens, respectively) are different. In the current study, similar values of E were obtained using AFM-based nano-indentation, validating our confocal microscope-based approach (Fig. 10(c)). Thus, the apparent discrepancy between our findings and previous studies is likely explained by the different mouse strain and age (6 month old C57BL/6 in Cao et al. versus 8–10 weeks-old BALB/c in our study). Note that Young's modulus of femoral bone is also substantially lower in C57BL/6 mice as compared to BALB/c mice [42]. In addition, the anatomical location of cartilage also differed between studies (lateral tibial plateau in Cao et al. versus medial femoral condyle in our study).

A similar experimental setup to our own was successfully used to map cell deformations and tissue strains in the superficial zone of rabbit cartilage [43]. However, the authors sought to assess in situ matrix and tissue deformations under a range of loading conditions, not to calculate tissue material properties. Since rabbit AC is thicker (~ 250 μm) than the maximum imaging depth of a conventional confocal microscope, strains in the intermediate and deep zones of the tissue could not be assessed. Thus, while our study demonstrated that confocal microscope-based approaches are effective at elucidating full-thickness material properties in mouse cartilage, this technique can only be applied to the superficial region in larger animal models.

This study served as a proof of principle for our material property characterization method, demonstrating the expected decrease of solid matrix Young's modulus E in trypsin-treated specimens as compared to controls. Trypsin treatment proteolytically degrades articular cartilage, thereby mimicking some features of osteoarthritis. Importantly, it has previously been demonstrated that trypsin digestion reduces GAG content and cartilage Young's modulus [36–38]. A similar phenomenon was observed herein, demonstrating the ability of our method to detect degradation-induced alterations in mouse cartilage material properties. In this study, the impact of overnight trypsin treatment may

include both the effects of (1) specimen degradation due to endogenous protease activity from overnight incubation in buffer; and (2) proteolytic degradation mediated by exogenous trypsin activity. When specimens were incubated overnight in buffer without trypsin, we observed less dramatic changes in mechanical properties (see Supplemental Material 3, which is available under the "Supplemental Materials" tab for this paper on the ASME Digital Collection).

To minimize the number of fit parameters, tissue nonlinearity was accounted for by separately determining the solid matrix Young's modulus E at three different loads. However, one limitation of the proposed study is that biphasic/poroelastic, anisotropic, and heterogeneous material properties were not assessed or accounted for. Nevertheless, although articular cartilage is a poroelastic material, our hyperelastic FE model accurately simulated its equilibrium response. Future studies will expand the proposed method to incorporate measurements of biphasic material properties (e.g., hydraulic permeability) responsible for the transient deformation (creep) of articular cartilage under prescribed loading conditions. While anisotropy and heterogeneity were not included in the constitutive model, our findings still elucidate the nominal behavior of murine cartilage averaged across directions and depth. Moreover, due to the extremely small thickness (< 50 μm) of murine articular cartilage [44,45], zonal heterogeneity is less pronounced in this tissue [46,47]. Furthermore, to our knowledge, no prior investigations have accounted for either anisotropy or heterogeneity in murine articular cartilage due to its small dimensions [15]. More importantly, despite our simplified constitutive model, the experimental methods described in this study successfully differentiated degraded versus nondegraded tissue. Nevertheless, we view this investigation as a first step toward developing methods to assess mechanical properties using more sophisticated models. To this end, our approach can be adapted in future investigations to account for anisotropy and nonlinearity through more sophisticated constitutive models (e.g., fiber-reinforced models with nonspherical fiber distributions). However, since this strategy would involve multiparameter fits of a single data set, it may be challenging to obtain unique convergence for all material properties.

Given the acknowledged limitations of our simplified constitutive model, an alternative approach for comparing the mechanical response of murine cartilage across experimental groups is through their model-independent load–displacement curves. The boundary displacement of a tested specimen of cartilage for a given reaction force is related to its stiffness. Thus, the stiffer, undigested control tissue exhibited smaller boundary displacements than trypsin-treated tissue at all reaction forces (Fig. 8). In the current study, tissue thickness was not affected by trypsin digestion (data not shown). However, in cases where tissue thickness changes (e.g., late-stage OA), it may be more appropriate to investigate load–strain or load–stretch curves (instead of load–displacement curves) as a means of comparing the mechanical response across groups in a manner that does not require defining a constitutive model.

Another limitation of the study is that the current approach can only detect changes near the apex of the posterior femoral condyle (the region where the femoral cartilage contacts the glass plate). Importantly, in the mouse, this is a major load-bearing area of cartilage that physically contacts the tibial plateau and meniscus and is likely to be strongly affected by OA. (Note that the mouse hind limbs are generally in a state of deep flexion.) Moreover, as OA progresses, this disease affects articular cartilage more uniformly. Thus, even if focal changes in material properties occur elsewhere in the early stages of pathology, the region that we assess should eventually exhibit OA-associated alterations. However, quantification of changes in material properties only at specific locations could conceivably inhibit detection of OA in very early stages. A potential solution to this limitation is to grip the femur with an armature attached to a translation/rotation stage. The position of contact between the femoral cartilage and the cover glass can then

Fig. 11 Analysis of the uniqueness of iFEA measurements for (a) solid matrix Young's modulus E and (b) drained Poisson's ratio ν based on one- and two-parameter fits. Every data point represents a solution (output parameter) of the same representative iFEA based on different initial guesses for iterative optimization. Initial guesses for ν in two-parameter fits were 0–0.49, whereas ν for one-parameter fits was set to its measured value (0.21). Initial guesses for the solid matrix Young's modulus E varied between 0.1 and 10 MPa for both approaches. These data indicate that only the one-parameter approach converged to a unique solution for E .

be accurately controlled, allowing for assessment of material properties in different spatial locations using the proposed method.

Although this is an alternative method to quantify the nonlinear material properties of murine articular cartilage at equilibrium, the primary benefit of our technique is that it can be used in laboratories where a confocal microscope is available, but atomic force microscopes and nano-indentation platforms are not accessible. It also offers several potential advantages over other approaches. With our method—in contrast to indentation-based strategies—tissue deformation is directly assessed over a large spatial extent and with a high spatial resolution. Thus, our method may be further adapted in future studies to investigate depth- or scale-dependent changes in murine articular cartilage. Moreover, in OA, cartilage may exhibit areas of erosion or fibrillation. Using indentation-based approaches, it may be difficult to delineate whether these are areas of altered geometry or altered material properties. However, with our method, the tissue is imaged before and after indentation. Thus, geometric irregularities can be assessed and accounted for in analysis.

Determination of mouse cartilage mechanical properties provides an objective measurement that could supplement semiquantitative analyses and help researchers assess articular cartilage degradation due to experimentally induced OA. Thus, the testing platform described in this study could serve as a valuable tool for comparative studies evaluating factors involved in OA pathogenesis or promising treatments for OA.

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