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Asymmetrical Strain Distributions and Neutral Axis Location of Cartilage in Flexure

Gregory M. Williams, M.S.¹, Kenneth R. Gratz, Ph.D.¹, and Robert L. Sah, Sc.D.M.D.^{1,2}

¹ Department of Bioengineering, University of California, San Diego, 9500 Gilman Dr., Mail Code 0412, La Jolla, CA 92093, USA

² Whitaker Institute of Biomedical Engineering, University of California, San Diego, 9500 Gilman Dr., Mail Code 0412, La Jolla, CA 92093, USA, Tel.: 858-534-0821, Fax: 858-822-1614, E-mail: rsah@ucsd.edu

Abstract

Flexural deformations have been used for the biomechanical characterization of native and engineered cartilage and as a mechanical stimulus to induce alteration of cartilage shape during *in vitro* culture. Flexure is also a physiologically relevant mode of deformation for various cartilaginous structures such as the ears and nose, but a kinematic description of cartilage in flexure is lacking even for simple deformations. The hypothesis of this study was that tension-compression (T-C) nonlinearity of cartilage will result in asymmetrical strain distributions during bending, while a material with similar behavior in tension and compression, such as alginate, will have a more symmetrical distribution of strains. Strips of calf articular cartilage and alginate were tested under uniform circular bending, and strains were determined by a micromechanical analysis of images acquired by epifluorescence microscopy. This experimental analysis was interpreted in the context of a model of small-deflection, pure bending of thin, homogeneous beams of a bimodular elastic material. The results supported the hypothesis and showed that marked asymmetry existed in cartilage flexural strains where the location of the neutral axis was significantly different than the midline and closer to the tensile surface. In contrast, alginate samples had a centrally-located neutral axis. These experimental results were supported by the model indicating that the bimodular simplification of cartilage properties is a useful first approximation of T-C nonlinearity in these tests. The neutral axis location in cartilage samples was not influenced by testing orientation (towards or away from superficial-most tissue) or magnitude of flexure. These findings characterize the kinematics of cartilage at equilibrium during simple bending and indicate that T-C nonlinearity is an important determinant of the flexural strain distributions in the tested tissue.

Keywords

cartilage; biomechanics; flexure; strain; tension-compression nonlinearity

Correspondence to: Robert L. Sah.

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Introduction

Mechanical loading of cartilage explants can regulate chondrocyte metabolism and direct growth and remodeling (Guilak, et al., 1997). Specific mechanical stimuli may be used to manipulate cartilage properties including matrix composition, mechanical properties, and shape. For instance, bending of immature articular cartilage during *in vitro* culture can induce marked changes in tissue shape (Williams, et al., 2007). Identification of the mechanobiological processes underlying these shape changes may be facilitated by a biomechanical characterization of cartilage subjected to such flexure. This technique of shaping cartilaginous tissues may have applications towards creating grafts of specific shapes for joint repair or craniofacial reconstruction and towards understanding the effects of physical forces on the developing morphologies of cartilage structures.

Flexure is a physiologically relevant mode of deformation for many types of cartilage including those located within the nose, ears, and ribs. To better understand the biomechanical functions of these tissues, tests such as three-point or curved-beam bending have been used to characterize nasal septal, auricular, costal, and tracheal cartilages, with moduli reported in the range of 4–9 MPa (Farhadi, et al., 2006, Grellmann, et al., 2006, Lambert, et al., 1991, Roy, et al., 2004). These properties may also serve as useful benchmarks for assessing engineered cartilage being developed for therapeutic purposes (Farhadi, et al., 2006, Roy, et al., 2004). While bending may produce tensile, compressive, and shear deformations, previous studies of cartilage flexural properties have not experimentally quantified strain distributions within the tissue during bending.

The complex mechanical properties of cartilage increase the difficulty of predicting deformation behavior when the tissue is subjected to bending. In particular, articular cartilage has a well characterized, nonlinear, equilibrium stress-strain response that is stiffer in tension (Akizuki, et al., 1986, Williamson, et al., 2003) than in compression (Mow, et al., 1980, Williamson, et al., 2001). The transition between these two regimes occurs smoothly, and this behavior of cartilage has been termed tension-compression (T-C) nonlinearity (Chahine, et al., 2004, Laasanen, et al., 2003, Soltz and Ateshian, 2000). Additional reports of nonlinear behavior include slight softening with increasing compressive strain (15–30%) in immature bovine cartilage and stiffening with increasing tensile strain (2–10%) in more mature bovine cartilage (Charlebois, et al., 2004, Ficklin, et al., 2007). These properties may result in stress and strain distributions within cartilage during flexure that significantly deviate from those predicted by fundamental, linearly elastic beam theory. An experimental approach for measuring intra-cartilage strains may provide insight into the biomechanics of cartilage in flexure.

Methods for measuring intra-cartilage strains have combined microscopy with point-tracking of fluorescently labeled chondrocyte nuclei (Schinagl, et al., 1997) or digital image correlation (Wang, et al., 2002). These techniques have been useful for determining spatially varying strain distributions arising from depth-dependent inhomogeneity of material properties in articular cartilage under compression (Schinagl, et al., 1997, Wang, et al., 2002). Complex strain patterns within cartilage near an indentation probe or an articular defect have been assessed through similar methods (Bae, et al., 2006, Gratz, et al., 2008). The application of these techniques to cartilage in flexure could produce a detailed depiction of the intra-tissue mechanical environment in this deformation state.

The hypothesis of this study was that T-C nonlinearity, such as that attributed to cartilage, will result in asymmetrical strain distributions during bending, while a material with similar behavior in tension and compression, such as alginate, will have reduced asymmetry. In

addition, effects of potential cartilage inhomogeneity and magnitude of flexure were examined by varying the tissue orientation and bending radius of curvature.

Materials and Methods

Cartilage Sample Preparation

Cartilage blocks were harvested from the patellofemoral grooves of 1–3 week old bovines obtained fresh from an abattoir. Slices of middle zone cartilage (~0.5–1.5 mm from the articular surface) were obtained using a vibrating microtome (Vibratome, St. Louis, MO) and were cut into strips measuring $\sim 1 \times 2 \times 10 \text{ mm}^3$ (H $\times$ W $\times$ L). As a critical dimension in determining flexural strains, the thickness was measured at three locations along the span of each cartilage sample and averaged. Each sample was found to be uniformly thick ($\pm 0.02 \text{ mm}$), and the average thickness of all samples was $1.00 \pm 0.04 \text{ mm}$ (mean $\pm$ SD). Cartilage strips were stored for up to 48 hours in Dulbecco's Modified Eagle's Medium at 4°C. Prior to mechanical testing, cartilage was immersed for 20 min in 1 ml of phosphate buffered saline (PBS) containing 20 $\mu\text{g/ml}$ propidium iodide to stain chondrocyte nuclei and then washed twice for 10 min each in PBS.

The effect of cartilage orientation relative to the direction of bending (towards or away from the superficial-most side) was assessed in four pairs of adjacent cartilage strips (n=8) from 2 animals. Upon finding no significant effect of orientation as indicated in the results, these data were pooled with those from an additional animal (n=12 strips total). The effect of the bending radius of curvature was investigated using cartilage strips (n=7) from 2 animals.

Alginate Sample Preparation

For comparative analysis, alginate was primarily chosen for having similar tensile and compressive moduli, homogeneity at a microscale, and the ability to include fluorescent fiducial markers. A 2% solution of alginate (Keltone LVCR, Kelco, Chicago, IL) was prepared in 0.9% saline and passed through a 0.22 μm polyethersulfone filter (Millipore, Billerica, MA). Fluorescent microspheres (Bangs Laboratories, Fishers, IN) with 7.32 μm mean diameter were suspended in the solution at 4×10^6 beads/ml. Alginate was gelled into slabs using a custom mold that allowed Ca^{++} diffusion, as described previously (Williams, et al., 2005), and cut into strips ($\sim 1 \times 2 \times 10 \text{ mm}^3$; H $\times$ W $\times$ L) for flexure or disks ($\varnothing 5 \times 1 \text{ mm}$) for unconfined compression. Each alginate strip was uniformly thick ($\pm 0.04 \text{ mm}$), and the average of all strips was $1.05 \pm 0.09 \text{ mm}$ (mean $\pm$ SD). Alginate samples were equilibrated in Dulbecco's PBS (D-PBS) containing calcium and magnesium (Invitrogen, Carlsbad, CA) for at least 24 hours prior to mechanical testing to stabilize the mechanical properties of the polymerized hydrogel (Leroux, et al., 1999).

Mechanical Test in Flexure

In the first set of experiments, bending deformations were applied to cartilage and alginate strips in a configuration consistent with that used previously for the *in vitro* reshaping of cartilage (Williams, et al., 2007). A custom, microscope-mounted testing device consisted of a chamber containing a cylindrical, self-aligning loading post and a sample support, which were displaced relative to one another by a hand-controlled micrometer. Displacement rates were similar among tests ($\sim 0.25 \text{ mm/s}$) but not explicitly controlled, since only equilibrium states were being studied. Specimens were supported over a 7 mm span and bent flush around the loading post ($\varnothing 4.75 \text{ mm}$) to achieve a uniform circular deformation (Fig. 1A). The chamber allowed visualization by epifluorescence microscopy of the sample surface in the plane of bending relative to the x- and z-axes (length and depth; also anterior-posterior and superficial-deep in cartilage samples). Digital images were acquired in the unloaded reference state and the equilibrium deformed state following stress relaxation (1 hr for alginate; 2 hrs for cartilage;

based on pilot studies) (Fig. 1B). Throughout the test, cartilage and alginate samples were immersed in PBS and D-PBS, respectively, at room temperature (22–24°C).

In the second set of experiments, cartilage strips were bent around a series of loading posts of decreasing diameter, Ø 15.53 mm (large), 7.92 mm (medium), and 4.75 mm (small). Following each stress relaxation and imaging step, samples were briefly unloaded to switch loading posts and then deformed around the next smaller post.

Micromechanical Strain Analysis

Strain analysis was performed with a custom-written Matlab algorithm (The Mathworks, Natick, MA) using a combination of discrete point tracking and digital image correlation as described in detail elsewhere (Gratz, et al., 2008). Briefly, a region of interest (ROI) was manually chosen in the center of each sample, spanning the full thickness and ~0.67 mm in length (Fig. 1B). Fluorescent nuclei and beads were localized, and a subset of these, spaced at ~46 µm, was tracked between the reference and deformed states by maximizing the normalized cross-correlation. A uniform square mesh of points, also with 46 µm spacing in the reference state, was selected in the ROI starting one unit mesh length from the concave surface ($z=0$). Spacing parameters were chosen to provide sufficient resolution of data points within the ROI and to help maximize correlation. The location of each mesh point in the deformed state was determined by a local affine mapping of tracked nuclei or beads within 93 µm. Displacements of mesh points were used to calculate displacement gradients and Lagrangian strains. For each test, strain profiles (E_{xx} , E_{zz} , and E_{xz}) through the thickness of the sample were determined at an x -coordinate where $\Sigma|E_{xz}|$ was a minimum.

Model of Pure Beam Bending of a Bimodular Material

According to Euler-Bernoulli beam theory, small-deflection, pure bending (i.e. uniform bending moment) of a thin, homogeneous beam results in a uniform circular deformation, a uniaxial state of stress in the longitudinal direction (x -axis), and a linear profile of longitudinal normal strain (E_{xx}) from compression to tension (Beer, et al., 2006). The flexure test used here imposed a uniform circular deformation on samples to approximate pure beam bending. A simple analytical model of pure bending was employed to illustrate the consequences of material T-C nonlinearity on the neutral axis location (depth where $E_{xx}=0$) and to predict the location based on published or measured values of cartilage and alginate mechanical properties.

As an approximation, constitutive relationships of cartilage and alginate were simplified to those of bimodular elastic materials, possessing a different modulus in tension (+), E_+ , than that in compression (–), E_- . The neutral axis location in an Euler-Bernoulli beam composed of a bimodular material subjected to pure bending has been previously derived as:

$$d_{NA} = \frac{\sqrt{E_+}}{\sqrt{E_+} + \sqrt{E_-}} d \quad (1)$$

where d_{NA} is the distance from the concave surface to the neutral axis and d is the beam thickness (Jones, 1976). For cartilage samples, E_+ and E_- are equilibrium tensile and unconfined compressive moduli reported for bovine calf tissue with similar age, depth, and orientation as used here (Asanbaeva, et al., 2008, Ficklin, et al., 2007). Likewise, E_+ for 2% alginate of a similar preparation was obtained from the literature (Williams, et al., 2005), while E_- was measured by equilibrium unconfined compression testing for lack of a suitable reference. The values for these parameters are shown in Table 1.

Data Analysis and Statistics

The effect of cartilage orientation on E_{xx} was determined using repeated measures ANOVA with orientation as a between-subjects factor and depth as a within-subjects factor. Planned comparisons were made at each depth between orientations using paired t-tests. The neutral axis, defined as the z -coordinate where $E_{xx}=0$, was determined by linear regression of E_{xx} on z for each sample. The neutral axis location was compared to the sample midline (half the thickness) by paired t-tests. Linear regressions of E_{zz} on z in the tensile and compressive regions were performed for cartilage. Assuming uniaxial stress, apparent Poisson's ratios in tension (ν_{+xz}) and compression (ν_{-xz}) were calculated as the negative ratio of the slope of E_{zz} (dE_{zz}/dz) to the slope of E_{xx} (dE_{xx}/dz) and compared by paired t-tests. Dilatation was calculated as $(1+E_{xx})(1+E_{yy})(1+E_{zz})$, where E_{yy} was estimated as stated in the results.

Effects of loading post size on neutral axis location and dE_{xx}/dz were determined using repeated measures ANOVA with post size as a within-subjects factor and post-hoc comparisons with Bonferroni correction. The slope, dE_{xx}/dz , was also linearly regressed on the inverse of the bending radius of curvature, and the slope of this regression was compared to the predicted value of 1 by t-test, with adjustment of the standard error for repeated measures (Donner, 1984).

Results are presented as means $\pm$ SEM, unless noted otherwise. Coefficients of determination (r^2) are reported for regressions. For all comparisons, a significance level, α , of 0.05 was used.

Results

Flexural Strains in Cartilage Versus Alginate

The first set of tests exposed similarities and differences in the flexural mechanics of cartilage and alginate. Representative strain maps (E_{xx} , E_{zz} , E_{xz}) within the ROI and superimposed on the reference image reveal general patterns for cartilage and alginate samples (Fig. 2). For both specimen types, the longitudinal normal strain, E_{xx} demonstrated a gradient from compression on the concave side to tension on the convex side. An opposing gradient was produced in the transverse normal strain, E_{zz} , exhibiting a Poisson's effect. Shear strains, E_{xz} , were small throughout, as expected for materials in pure bending, and were generally less than the error (approximately $\pm 1\%$) of this experimental technique (Gratz, et al., 2008).

Quantification and comparison of strain patterns was achieved by analyzing profiles through the thickness (along the z -axis). Bending of cartilage towards or away from the superficial-most tissue was not found to be a statistically significant factor in determining the profile of E_{xx} ($p=0.25$). Planned comparisons further revealed that at all depths there was no significant difference between the two orientations. Consequently, data were pooled along with those from an additional animal. Together, the averaged cartilage strain profiles reveal a marked asymmetry of strains during flexure (Fig. 3A). E_{xx} varied nearly linearly with z ($r^2>0.97$) from approximately -20% to 7% . The neutral axis location was determined to be 0.75 ± 0.02 mm from the concave surface, which was significantly different than the midline (0.50 ± 0.01 mm, $p<0.001$). In contrast, alginate specimens demonstrated a highly symmetrical strain state during flexure (Fig. 3B). E_{xx} was also highly linear ($r^2>0.97$), but varied from approximately -15% on the concave side to 14% on the convex side. The alginate neutral axis was located at 0.53 ± 0.02 mm and was not different than the midline (0.52 ± 0.02 mm, $p=0.48$).

Further analysis of cartilage revealed strong linearity of E_{zz} both on the tensile and compressive sides of the tissue ($r^2=0.91\pm 0.03$ and $r^2=0.95\pm 0.01$, respectively). The apparent Poisson's ratio in tension was greater than that in compression ($\nu_{+xz}=0.78\pm 0.11$ vs. $\nu_{-xz}=0.32\pm 0.03$, $p<0.01$). Estimating dilatation in the bent cartilage samples was accomplished by inferring the behavior in the y -direction. The normal strain, E_{yy} , was calculated from E_{xx} by assuming $\nu_{-xy}=0.14$ as

previously published (Ficklin, et al., 2007) and $v_{+xy}=v_{+xz}=0.78$, as measured in this study. Dilatation varied through the thickness with values approaching zero near the neutral axis and negative throughout the remaining tissue (Fig. 4). The volume loss increased with distance from the neutral axis to reach a maximum of -12% at the concave (compressive) surface and -4% at the convex (tensile) surface.

Effects of Magnitude of Bending on Cartilage Flexural Strains

A relationship between the magnitude of bending and the cartilage flexural strains was determined by varying the size of the loading post. Decreasing the loading post size induced greater strains in the cartilage and increased the slope of the longitudinal strain profile (dE_{xx}/dz) with each being greater than the previous ($p<0.001$) (Fig. 5). However, the neutral axis location was not significantly affected, being 0.75 ± 0.01 , 0.77 ± 0.01 , and 0.77 ± 0.01 mm with the large, medium and small posts, respectively. The strain distributions induced by the small loading post were consistent with those found in the previous set of tests, even though these cartilage samples were preconditioned by two cycles of increasing flexural deformation.

The slope, dE_{xx}/dz , and the radius of curvature were further examined by linear regression ($r^2=0.88$, Fig. 6). Here, the bending radius of curvature was defined as the radius of the loading post plus the distance to the neutral axis, and then plotted as the inverse. The slope of this regression (0.93 ± 0.04) was not significantly different than unity, the value predicted by Euler-Bernoulli beam theory for materials in pure bending (Beer, et al., 2006).

Bimodular Model Predictions

Mean values of E_+ and E_- for calf articular cartilage and alginate (Table 1) were substituted into Equation (1) to predict the neutral axis location in samples with the same average thicknesses as those used in the experiment. The bimodular model predicted that the cartilage neutral axis would be 0.71 mm from the concave surface (Table 1). On the other hand, the alginate neutral axis was predicted to be more centrally-located at 0.61 mm.

Discussion

The results of the micromechanical strain analysis support the hypothesis that tension-compression nonlinearity plays an important role in determining the flexural biomechanics of cartilage. The observed asymmetry in the longitudinal normal strain, E_{xx} , with the neutral axis being closer to the tensile surface, is consistent with the known material properties of cartilage (i.e. stiffer in tension than compression) and the necessary force balance within each sample. In contrast to the strain asymmetry of cartilage, alginate, a hydrogel with similar tensile and compressive moduli, exhibited a nearly symmetrical strain state.

Further support for the effects of T-C nonlinearity was provided by modeling cartilage and alginate as bimodular elastic materials in pure bending. The use of a model of pure beam bending was justified by several features consistent with the experimental flexure test, including uniform circular deformation, linear longitudinal strains, minimal shear strains, and a relationship between the bending radius of curvature and the slope of the longitudinal strain profile equal to ~ 1 . The neutral axis locations predicted by the model were similar to those measured experimentally, suggesting that the bimodular simplification of T-C nonlinearity is a useful first approximation for the materials in these tests.

Articular cartilage exhibits depth-dependent inhomogeneity of tensile and compressive properties which could influence flexural mechanics depending on the sample preparation and orientation of bending (Kempson, et al., 1968, Schinagl, et al., 1997). In this study, the superficial tissue including the articular surface (~ 0.5 mm) was removed from the cartilage to

aid in preparation of uniformly flat strips and also to avoid a region where highly varying properties exist (Asanbaeva, 2006, Klein, et al., 2007). Significant effects of inhomogeneity on flexural strains were excluded by testing paired samples in opposing orientations. However, the potential effects of inhomogeneity on bending mechanics may be an important consideration for other sources or preparations of cartilaginous tissues.

Testing cartilage in flexure enables simultaneous observation of mechanical behavior over a wide range of tensile and compressive strains. Quantification of the apparent Poisson's ratio, ν_{xz} , of cartilage in both compression and tension was possible in a single test. The measured value of ν_{-xz} (0.32 ± 0.03) is similar to values ($0.22-0.25 \pm 0.05$) previously obtained from unconfined compression of calf cartilage in the anterior-posterior and medial-lateral directions (Ficklin, et al., 2007, Wang, et al., 2003). Likewise, the measured ν_{+xz} (0.78 ± 0.11) is also consistent with published values of Poisson's ratios in tension ($\sim 0.5-2$) for bovine and human articular cartilage (Chang, et al., 1999, Charlebois, et al., 2004, Elliott, et al., 2002, Woo, et al., 1979). Calculation of these Poisson's ratios assumes uniaxial loading in the ROI without significant contact pressure from the loading post, which would otherwise act to decrease E_{zz} , near the concave surface, and correspondingly decrease ν_{-xz} . Since neither a shallowing of the profile of E_{zz} near the concave surface nor a comparably low ν_{-xz} was observed, the assumption of negligible contact pressure appears justified.

The range of tensile and compressive strains imposed on the samples can also be controlled during bending by varying the size of the loading post. Thus, modulating the post size could provide information about nonlinear stress-strain behavior, with a shift in the neutral axis location indicating a relative change in the compressive and tensile moduli over the difference in the ranges of strain. There were no significant differences between the cartilage neutral axis locations using the three loading post sizes to support previous reports of compressive stress-softening. The bending experiments may not have been sufficiently sensitive to detect subtle shifts of the neutral axis produced by such phenomenon, which may be small in magnitude (Ficklin, et al., 2007) or limited to a narrow range of compressive strains ($\sim 0-5\%$) (Chahine, et al., 2004). However, additional flexure tests, particularly if done with multiple deformation states and in other planes (e.g. $x-y$, $y-z$), may have further utility in validating constitutive models of nonlinear and anisotropic cartilage behavior.

The techniques developed in this study may have applications in research involving other types of cartilage and soft tissues. For instance, quantification of the failure strain in flexure of engineered cartilage may be a useful functional measure for therapies targeting ear and nose reconstruction where bending is expected. Previous investigation of flexural failure in engineered auricular cartilage has been limited to marking lines on the tissue surface and qualitatively observing displacements (Jian-Wei, et al., 2005). By tracking ink microdots sprayed on the surface of arterial segments, a process similar to that presented here albeit at a coarser resolution, flexural strains have been measured to determine the mechanical properties of arterial wall layers (Yu, et al., 1993). Flexural strains have also been examined in comparisons of native and bioprosthetic aortic valve leaflets by analysis of banding patterns produced by polarized light microscopy (Vesely and Boughner, 1989). Previous validation of the strain analysis algorithm used in the current study found maximum errors of $\sim 1\%$ strain for large deformations (Gratz, et al., 2008), making it an attractive technique for these types of applications.

The results of this study help elucidate the equilibrium distribution of strain within cartilage during simple flexure. Since the deformation applied during these tests mimicked that used previously for inducing alterations in cartilage explant shape, the findings are directly relevant to understanding the reshaping process (Williams, et al., 2007). Specifically, these results identify regions of tissue predominantly loaded in tension or compression and nonuniform

volumetric changes which may differentially mediate cartilage shape change during *in vitro* culture. The characterization of the biomechanical state of these explants may facilitate further examination of the mechanisms of cartilage reshaping as this technology is developed for bioengineering shaped chondral tissues.

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Figure 1.

(A) Illustration of the mechanical testing setup for applying bending deformations of uniform curvature and (B) experimental images of cartilage in unloaded and deformed configurations. Central region of interest (ROI) is indicated by white dashed boxes.

Figure 2. Lagrangian strain maps within the ROI of representative cartilage and alginate specimens bent around the small diameter loading post. Maps are superimposed on the reference images.

Figure 3.

Strain profiles through the thickness of (A) cartilage and (B) alginate specimens from the concave surface ($z=0$) to the convex surface ($z\approx 1$). Location of the neutral axes, where $E_{xx}=0$, and sample midlines are indicated by dotted and dashed lines, respectively. Mean $\pm$ SEM; $n=12$ for cartilage and $n=9$ for alginate.

Figure 4. Estimated dilatation profile through the thickness of cartilage samples. Assumptions included $\nu_{-xy} = 0.14$ (Ficklin, et al., 2007), and $\nu_{+xy} = \nu_{+xz} = 0.78$, as measured in this study. Mean $\pm$ SEM; n=12.

Figure 5. Effect of loading post size (and consequently radius of curvature) on longitudinal strain profiles (E_{xx}) of cartilage specimens. Mean $\pm$ SEM; $n=7$.

Figure 6. Relationship between the inverse of the radius of curvature at the neutral axis and the slope of the longitudinal strain profile (dE_{xx}/dz) for cartilage specimens.

Table 1

Predicted neutral axis locations for cartilage and alginate modeled as bimodular elastic materials in pure bending versus the experimentally measured locations. Mechanical property input parameters (equilibrium tensile moduli, E_+ , and unconfined compression moduli, E_-) were measured or obtained from (Asanbaeva, et al., 2008, Ficklin, et al., 2007, Williams, et al., 2005). Mean $\pm$ SEM.

Material	Tensile Modulus, E_+	Compressive Modulus, E_-	Modeled Neutral Axis	Measured Neutral Axis
cartilage	3.0 ± 1.0 MPa	0.5 ± 0.2 MPa	0.71 mm	0.75 ± 0.02 mm
alginate	4.1 ± 0.4 kPa	1.9 ± 0.1 kPa	0.61 mm	0.53 ± 0.02 mm