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A Noncontacting Method for Material Property Determination for Articular Cartilage from Osmotic Loading

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ABSTRACT Articular cartilage is one of several biological tissues in which swelling effects are important in tissue mechanics and function, and may serve as an indicator of degenerative joint disease. This work presents a new approach to quantify swelling effects in articular cartilage, as well as to determine the material properties of cartilage from a simple free-swelling test. Samples of nondegenerate and degenerate human patellar cartilage were subjected to osmotic loading by equilibrating the tissue in solutions of varying osmolarity. The resulting swelling-induced strains were measured using a noncontacting optical method. A theoretical formulation of articular cartilage in a free-swelling configuration was developed based on an inhomogeneous, triphasic mechano-chemical model. Optimization of the model predictions to the experimental data was performed to determine two parameters descriptive of material stiffness at the surface and deeper cartilage layers, and a third parameter descriptive of thickness of the cartilage surface layer. These parameters were used to determine the thickness-averaged uniaxial modulus of cartilage, H_A. The obtained values for H_A were similar to those for the tensile modulus of human cartilage reported in the literature. Degeneration resulted in an increase in thickness of the region of "apparent cartilage softening," and a decrease in the value for uniaxial modulus at this layer. These findings provide important evidence that collagen matrix disruption starts at the articular surface and progresses into the deeper layers with continued degeneration. These results suggest that the method provides a means to quantify the severity and depth of degenerative changes in articular cartilage. This method may also be used to determine material properties of cartilage in small joints in which conventional testing methods are difficult to apply.

INTRODUCTION

Swelling effects are known to be important in articular cartilage mechanics and function (Maroudas et al., 1986; Mow and Ratcliffe, 1997) and may even serve as an indicator of degenerative joint disease (Mankin and Brandt, 1992; Maroudas, 1975; Maroudas et al., 1986). Articular cartilage consists of two major phases: a fluid phase (60–80% of the tissue wet weight) and a solid phase, with <5% of the total tissue volume occupied by cartilage cells (chondrocytes). The fluid phase is largely water with a physiological concentration of electrolytes (mostly Na^+ and Cl^- ions) and other small solutes (Maroudas, 1979). The solid phase consists of both collageneous and noncollageneous proteins and large proteoglycan molecules immobilized within the collagen fibril network. At physiological conditions, the proteoglycan molecules have a large negative charge resulting in a highly hydrophilic nature for the cartilage solid matrix (Ogston, 1970). The unique composition of cartilage, i.e., a hydrated collagen network with embedded negatively charged proteoglycans, results in a high interstitial swelling pressure (Maroudas and Bannon, 1981; Urban et al., 1979) which is balanced by tensile forces generated in the collagen matrix (Lai et al., 1991; Maroudas, 1976; Maroudas et al., 1986). Swelling pressure plays a major role in the mechanical function of cartilage, supporting compressive loads and maintaining tissue hydration (Maroudas et al., 1986; Mow and Ratcliffe, 1997). Swelling pressure in cartilage is mostly determined by the Donnan osmotic effect (Kovach, 1995; Urban et al., 1979), with a small fraction of the total swelling pressure at physiological conditions arising from a charge-independent origin (Ehrlich et al., 1998). Thus, the amount of proteoglycans in the tissue largely determines the magnitude of the net swelling pressure in cartilage.

The amount of tissue swelling, however, also depends on the properties and structure of the collagen matrix. The matrix is highly nonuniform, with both proteoglycan concentration and collagen fiber orientation varying with depth from the articular surface (Aspden and Hukins, 1981; Clark, 1991; Venn and Maroudas, 1977). Experimental studies of cartilage hydration (Maroudas, 1976; Maroudas and Venn, 1977; Maroudas et al., 1986) demonstrate that healthy human cartilage does not swell to an appreciable extent when excised from the bone and equilibrated in physiological saline. With osteoarthritis, however, compositional and structural changes may occur which affect cartilage-swelling properties and mechanical function. Such changes include cartilage fibrillation, decreases in proteoglycan concentration, altered proteoglycan and collagen composition and molecular structure, and alterations in collagen cross-linking (Mankin and Brandt, 1992; Maroudas et al., 1986). As a result, osteoarthritis affects cartilage swelling behavior with evidence that the volume of fibrillated and osteoar-

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thritic (OA) cartilage samples increases as much as 50% upon removal from the bone and immersion in physiological saline (Maroudas, 1976; Maroudas and Venn, 1977; Maroudas et al., 1986). These effects are believed to be related to a "weakening" of the cartilage fibrillar network with OA, resulting in greater matrix expansion in OA cartilage, as compared with normal tissue (Ehrlich et al., 1998; Maroudas, 1976). Indeed, a recent study (Bank et al., 2000) has shown that increases in tissue swelling may relate to an increased amount of damaged collagen molecules, providing an additional support for this mechanism.

Traditionally, two types of experiments have been used to study swelling behavior of cartilage, including measurements of volumetric swelling and dimensional swelling of cartilage. Volumetric swelling of human cartilage ex situ has been extensively studied by Basser et al. (1998), Ehrlich et al. (1998), Maroudas (1976), Maroudas and Venn (1977), and Maroudas et al. (1986). In these experiments, samples of cartilage were removed from the bone, and their wet weight was measured immediately after excision, and after equilibration in NaCl or polyethylene glycol solutions of varying concentrations. The gain in water weight of the sample was then reported as a measure of cartilage swelling. In studies of dimensional swelling of cartilage, the changes in sample dimensions and geometry, rather than weight, were measured in different swelling configurations (Myers et al., 1984; Maroudas et al., 1986; Setton et al., 1998). The major limitation of these methods is that the experiments were done on cartilage that was removed from the bone, and therefore, the observed swelling behavior may not reflect cartilage swelling in situ. Also, these methods did not allow for quantitative determination of the material properties of the cartilage solid matrix.

Recently, we have developed a new method to measure the depth-dependent distribution of swelling-induced strain fields in canine and human articular cartilage while attached to the subchondral bone (Narmoneva et al., 1999a, b). The results showed a nonuniform swelling strain distribution with compressive strains near the bone and tensile strains in the middle and surface zones of cartilage, which were not observed previously (Narmoneva et al., 1999b). A homogeneous triphasic model (Lai et al., 1991; Setton et al., 1995) was used to predict swelling-induced strains in this cartilage layer, with some evidence of an ability to match experimental trends. The important implication of these results is that measurements of swelling-induced strains in cartilage can potentially be used to determine cartilage material properties. Indeed, estimates of a cartilage uniaxial tensile modulus, H_A, of 27 MPa were made by matching experimentally measured strain values to model predictions using a preliminary formulation of the free-swelling problem. The advantages of this method over the conventional testing methods used to determine cartilage material properties (e.g., tensile, compressive, and shear testing) include testing in a configuration representative of that in situ, and an absence of direct contact between the sample and experimental apparatus (e.g., grips or platens). Complete material property determination necessitates both model advances that incorporate material heterogeneity and experimental advances to record nonuniform strain and swelling pressure profiles.

The goal of this study, therefore, was to develop a noncontacting chemical loading method to precisely quantify material properties of cartilage still attached to the subchondral bone. This method is based on the principle of equivalence of mechanical and "chemical" loading (e.g., free equilibration in osmotically active media) as first suggested by Maroudas and Bannon (1981), with a complete theoretical analysis given recently by Lai et al. (1998). According to this principle, osmotic loading and mechanical loading (e.g., attributable to applied tractions or displacements) can result in equivalent deformation states, provided that the osmotic pressure is equal to the applied mechanical pressure and the shear stresses during mechanical loading are zero. The implication of this principle is that osmotic loading can be used with the appropriate theoretical model to determine the material properties of articular cartilage, parameters of great importance in characterizing cartilage function. The primary objective of the present work is to combine an experimental method to measure swelling-induced strains with a new, inhomogeneous theoretical model to determine the uniaxial modulus of human articular cartilage. In preliminary work, we observed that the strain pattern in OA human cartilage was distinctly different from that in normal cartilage and could not be described by the homogeneous triphasic model of cartilage swelling (Narmoneva et al., 1999a). Thus, the second goal of this study is to characterize changes in swelling behavior and material properties of human articular cartilage with degeneration, using the newly developed method and semiquantitative histological analysis. To model the nonuniform properties of the cartilage solid matrix, an inhomogeneous model with a dependence on three geometric and material parameters of cartilage stiffness is proposed, which is based on a triphasic mechanochemical theory for cartilage swelling (Lai et al., 1991; Setton et al., 1995). This model was able to predict a highly nonuniform distribution of swelling strains observed experimentally. It also provided the parameters to obtain information about changes in magnitude and spatial variation in the cartilage uniaxial modulus with osteoarthritis. In general, the approach described here can be used to model osmotic loading and to estimate material properties of other soft tissues in which swelling effects play an important role in tissue mechanics.

MATERIALS AND METHODS

Sample preparation

Patellae were harvested from knees of human cadavers. The cadaveric specimens (63 ± 16 y.o., $n = 11$, one patella from each cadaver) were

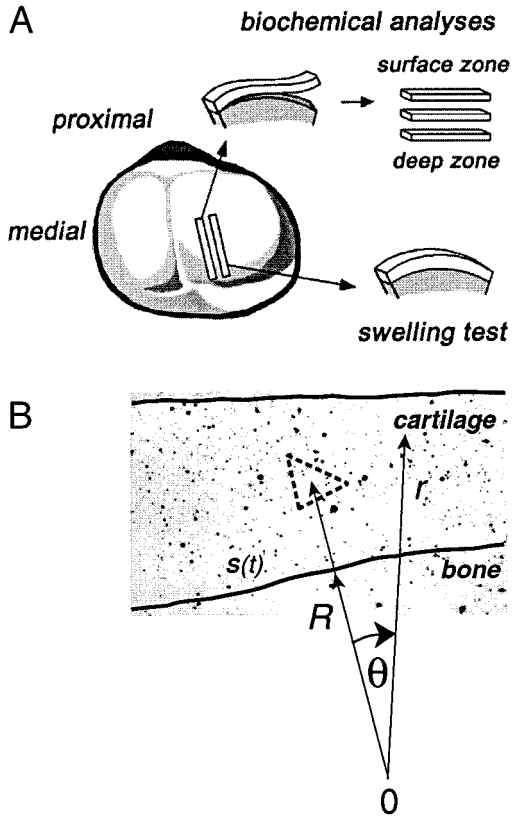

FIGURE 1 (*a*) Representative sample selection for a right patella. (*b*) Image of a representative human cartilage-bone sample with surface markers and a triad. *Solid lines* represent cubic spline fits of the cartilage surface and cartilage-bone interface. Cartilage-bone interface is represented by a parametric curve $s(t)$, which was used to define a local polar coordinate system $(\hat{r},\theta)$ for each triad, where $\hat{r} = (r-R_l)/h_l$, h_l is the local cartilage thickness, and R_l is the local radius of bone curvature.

obtained from the Fresh Human Anatomy Laboratory at Duke University, which is run through a gifts program. Patellae were wrapped in wet gauze soaked with phosphate-buffered saline solution, and stored at −20° until testing. On the day of testing, they were thawed at room temperature for 3–4 h. Two parallel slices of cartilage-bone (1.5 mm thickness) were taken transverse to the cartilage-bone interface at the lateral facet of each patella parallel to the direction of split lines (Fig. 1 *a*) using a low-speed diamond-wheel rotating saw (Narmoneva et al., 1999b). One slice was used in the swelling experiment, and the adjacent slice was used to determine cartilage biochemical composition. The patellae were stored and later used in a related study of the tensile properties of human cartilage (Elliott et al., 1999).

Swelling experiment and image analysis

Free-swelling tests were performed on the planar cartilage-bone samples as described previously (Narmoneva et al., 1999b). Briefly, black enamel markers (20–40 μm diameter) were placed on the planar surface of the cartilage-bone slice with an airbrush (Model 200, Badger Air-Brush Co., Franklin Park, IL). As has been demonstrated previously (Eisenberg and Grodzinsky, 1985; Narmoneva et al., 1999b), ion-induced swelling effects in cartilage are negligible at hypertonic ion concentration, because of shielding of negatively charged proteoglycans. Therefore, in this study, a reference configuration was chosen as 2 M NaCl. Samples were successively equilibrated for 4 h each in NaCl solutions of varying concentrations, c^* (c^* = 2.0, 0.15, 0.015 M NaCl). The 0.15 M NaCl test bath was taken to represent a physiological saline concentration. Preliminary studies showed that this equilibration period results in minimal (<3%) loss of proteoglycans.

An image was taken of the central part of the planar cartilage-bone sample surface with a high-resolution digital camera and computer-based data acquisition system (1340 × 1037 pixels, 2.4 μm/pixel, Kodak MegaPlus 1.4, Eastman Kodak, Rochester, NY). Image analysis was performed to calculate the two-dimensional components of the Lagrangian strain (E_{ij}) as functions of position within the cartilage layer for all NaCl concentrations. The analysis was performed on a graphics workstation (Indigo XZ2, SGI, Inc., Mountain View, CA) using a custom-written computer code based on image analysis software (PV-WAVE, Visual Numerics, Inc., Houston, TX). For each sample, two reference markers were selected on the subchondral bone on the reference image and identified in each subsequent image. A Cartesian coordinate system was defined relative to these markers. This coordinate system was used to calculate the centroid coordinates of all remaining surface markers for this sample at each concentration. Because the bone does not swell, this reference coordinate system does not change from image to image with changes in the external NaCl concentration and, thus, allows tracking the marker positions in the cartilage layer as it swells.

The cartilage surface and cartilage-bone interface were defined on the reference image using a cubic spline fit through points visually selected within the image domain by a user. The spline fit for the cartilage-bone interface was then used as a parametric curve $s(t)$ to calculate the origin, O, and the radius of curvature, R_i, defining a local polar coordinate system (r, θ) at each point (see Fig. 1 *b*). Marker triads were defined on the sample surface. The position of a triad centroid within the cartilage layer was then described using two parameters, $(\hat{r}_i, t_i)$, where t_i represents the position of a triad along the cartilage-bone interface, $\hat{r}_i = (r_i - R_i)/h_i$ is the radial position of the centroid measured from the cartilage bone interface, and h_i is the local cartilage thickness at $t = t_i$. Two-dimensional components of Lagrangian strain E_{ij} ($i, j = r, \theta$) were calculated for each triad from images recorded at 0.15 and 0.015 M NaCl with respect to the hypertonic reference state, as functions of $(\hat{r}, t)$. For the protocol as given, the overall uncertainty in measuring E_{rr}, $E_{r\theta}$, and $E_{\theta\theta}$ was ± 0.008 strain.

The theoretical model used in this study also required two geometric parameters, the regional (or average) thickness of the cartilage layer (h) and the regional radius of the bone curvature (R), to be determined for each sample to predict the distribution of swelling-induced strains in the cartilage layer. These parameters were calculated from the reference image as follows. Points (n = 30–50) were selected along the interface line, and the local cartilage thickness h_i was calculated at each point as a distance between the cartilage-bone interface and the cartilage surface perpendicular to the cartilage-bone interface. The regional thickness, h, was then calculated as $h = (\Sigma h_i)/n$. The regional radius of the bone curvature, R, was calculated by fitting a circle to the cartilage-bone interface data points.

Biochemical analyses

The reference water volume fraction (ϕ_0^W) and proteoglycan-associated negative fixed charge density (c_0^F) were required for estimation of the swelling pressure within articular cartilage (Lai et al., 1991; Narmoneva et al., 1999b) under conditions of varying osmolality as studied here. Biochemical assays of glycosaminoglycan content, as a measure of negative fixed charge density, and water content were performed for cartilage adjacent to the sites of swelling tests (Fig. 1 *a*) for calculation of c_0^F and ϕ_0^W. Full-thickness cartilage (2–4 mm thickness) was excised from the bone and microtomed to obtain eight slices. To determine a tissue wet-weight independent of swelling effects, slices were allowed to equilibrate in the reference test bath (2 M NaCl) for ~40 min and then weighed. Then the slices were soaked in physiological saline for 40 min to remove excess salt, and lyophilized to obtain the sample dry weight. Values for the water

volume fraction were calculated from the tissue wet weight, tissue dry weight after lyophilization, and values for intrinsic water density and solid density of bovine articular cartilage (Gu et al., 1996), as described previously (Narmoneva et al., 1999b). Fixed charge density, c_0^F, in articular cartilage was calculated from measures of the glycosaminoglycan content determined with a modified 1,9 dimethyl-methylene blue dye-binding assay (Narmoneva et al., 1999b). Values for c_0^F were calculated on a water volume as well as a dry-weight basis at the reference configuration (2 M NaCl). The results for c_0^F and ϕ_0^W for eight slices were numerically fit to a cubic polynomial in $\hat{r}$ to interpolate $c_0^F(\hat{r})$ and $\phi_0^W(\hat{r})$ at each position in the cartilage layer.

Theoretical model

In this study, a triphasic mechanochemical theory (Lai et al., 1991) was used to predict the magnitude and distribution of swelling strains in cartilage. Cartilage was modeled as a mixture of a linear, isotropic, incompressible collagen-proteoglycan solid matrix and an incompressible fluid consisting of water and NaCl ions (Setton et al., 1995). In that model, the cartilage solid matrix was assumed to have homogeneous material properties, i.e., uniform uniaxial modulus, H_A, and Poisson's ratio, ν_s. The preliminary studies (Narmoneva et al., 1999a) showed that the homogeneous material model could not describe highly nonuniform swelling effects observed in OA human cartilage. Therefore, to allow for model predictions of nonuniform swelling effects in this study, the triphasic model for cartilage swelling (Lai et al., 1991; Setton et al., 1995) was extended to include inhomogeneous material properties through the cartilage thickness.

In this analysis, cartilage was represented by a cylindrical convex layer of a triphasic material rigidly attached to the subchondral bone. To model the free-swelling experiment, this layer was assumed to be equilibrated against an external bath of water and NaCl ions (0.15 and 0.015 M NaCl), and swelling-induced strains were predicted relative to the hypertonic reference configuration (2 M NaCl). The model predicts that the total stress for the mixture under this configuration would consist of two components (Lai et al., 1991), the interstitial fluid pressure (p) and the elastic stress component which depends on the material properties of the cartilage solid matrix (represented by Lame coefficients, λ_s and μ_s, for a linear isotropic material),

$$\sigma = -p\mathrm{I} + \lambda_s \mathrm{tr}(\epsilon)\mathrm{I} + 2\mu_s\epsilon, \tag{1}$$

where ϵ is the infinitesimal strain tensor $\epsilon_{ij} = 1/2[\partial u_i/\partial x_j + \partial u_j/\partial x_i] \cdot (i, j = \hat{r}, \theta)$, $\vec{u}(x_i)$ is a displacement field. In the present analysis, the entropic contribution to the swelling pressure is neglected, and it is therefore assumed that all swelling effects arise from a Donnan effect, i.e., the electrostatic interactions between negatively charged proteoglycans and Na^+ and Cl^- ions. Then, in the absence of an externally applied hydrostatic pressure, the fluid pressure p represents the Donnan osmotic pressure. A linearized constitutive expression for p can be obtained from the boundary conditions for equivalent chemical potentials of water and NaCl ions across the free surface (Lai et al., 1991),

$$p \sim \mathrm{RT}\left(\left[(c_0^F)^2 + (2c^*)^2\right]^{1/2} - 2c^* - \frac{(c_0^F)^2}{\phi_0^W\left[(c_0^F)^2 + (2c^*)^2\right]^{1/2}}\, tr(\epsilon)\right), \tag{2}$$

where c^* is the NaCl concentration in the external bath, and c_0^F and ϕ_0^W are the fixed charge density and water volume fraction of cartilage measured at the reference configuration, respectively.

To model cartilage-bone samples, a cylindrically symmetric representation (r, θ, z) was used (Fig. 2), with the radial coordinate (r) defined as perpendicular, the circumferential coordinate (θ) defined as tangential to the cartilage-bone interface, and the axial coordinate (z) as parallel to the long axis of the cylinder. Out-of-plane swelling was assumed to be negligible, i.e., the z-component of the displacement vector, $\vec{u}$, was assumed to be zero ($u_z = 0$). For this configuration, the tangential component of the displacement field, u_θ, is zero, and there is no shear strain ($\epsilon_{r\theta} = 0$). The free-swelling problem is subject to the laws of conservation of mass and balance of linear momentum (Lai et al., 1991; Setton et al., 1995). The governing equation is the balance of linear momentum (Eq. 3), and the boundary conditions are that of zero displacement at the bone and zero stress traction at the free surface (Eqs. 4, 5):

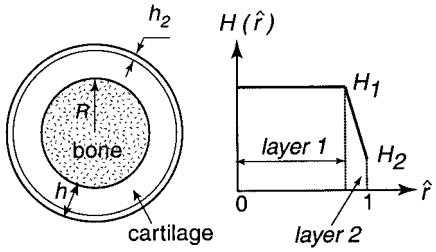

FIGURE 2 Schematic diagram of the two-layer model of a cartilage-bone sample. Cartilage is modeled as a two-layer structure, where the first layer (attached to the bone) is assumed to have a homogeneous uniaxial modulus ($H_A = H_1$), and the uniaxial modulus for the second layer is allowed to vary from H_1 to a maximum or minimum (H_2) at the articular surface ($\hat{r} = 1$), as shown here. R is the radius of bone curvature, and h_2 is the thickness of the surface layer.

$$\frac{\partial \sigma_{rr}}{\partial r} + \frac{1}{r}(\sigma_{rr} - \sigma_{\theta\theta}) = 0, \tag{3}$$

$$u_r|_{\hat{r}=0} = 0, \tag{4}$$

$$\sigma_{ij} n_j|_{\hat{r}=1} = 0. \tag{5}$$

With the constitutive Eq. 1, the governing Eq. 3 reduces to a second-order linear differential equation in terms of the displacement field, $u_r(\hat{r})$, where $\hat{r}$ is a normalized radial position defined as $\hat{r} = (r-R)/h$ (h is cartilage thickness, R is radius of bone curvature).

The solution to this problem exhibits a dependence on compositional (c^*, c_0^F, ϕ_0^W) and geometric parameters (R, h) that were directly measured, as well as material properties of the cartilage solid matrix which can be described by Lame coefficients (λ_s and μ_s) or by Poisson's ratio ($\nu_s = \lambda_s/[2(\lambda_s+\mu_s)]$) and uniaxial modulus ($H_A = \lambda_s + 2\mu_s$). Model predictions were relatively insensitive to values for the Poisson's ratio, ν_s, for these sample geometries, so that a constant value of $\nu_s = 0.05$ was used as representative of human cartilage (Athanasiou et al., 1991). Thus, the problem reduces to a dependence on one parameter, the uniaxial modulus H_A.

To provide for an inhomogeneity in the cartilage uniaxial modulus with thickness, the cartilage sample was modeled as two concentric cylindrical layers of a triphasic material. Layer 1, attached to the subchondral bone, was assumed to have spatially varying values for $c_0^F(\hat{r})$ and $\phi_0^W(\hat{r})$ as measured experimentally, and homogeneous material properties defined by the uniaxial modulus, H_1, and Poisson's ratio, ν_s. Layer 2 was similarly modeled with spatially varying values for $c_0^F(\hat{r})$ and $\phi_0^W(\hat{r})$ and a constant value for ν_s. However, the uniaxial modulus was allowed to vary linearly from H_1 at the interface with layer 1 to a minimum or maximum at the articular surface, H_2 (Fig. 2). The thickness of layer 2, h_2, is the depth over which the matrix modulus was allowed to vary. Thus, the radial component of swelling strain (E_{rr}) was predicted to depend on position in the cartilage layer $\hat{r}$, test bath concentration (c^*), sample geometry (bone radius of

curvature, R, cartilage thickness, h), physicochemical parameters (ϕ_0^W, c_0^F), material properties (H_1, H_2, ν_s) and model parameter h_2. All model parameters were directly measured or held constant except for H_1, H_2, and h_2. The numerical solution for cartilage swelling strains was obtained for each sample using a finite difference approximation of Eq. 3 (Mathematica, Wolfram Research, Inc., Champaign, IL, and custom-written code). Model predictions were matched to experimental measures of $E_{rr}(\hat{r})$ by least-squares optimization to obtain three model parameters, H_1, H_2, and h_2. Because the compressive strain near the bone could not be predicted by the model, only data for positive swelling strains were used. The radial positions corresponding to negative values of swelling strain were identified on both swelling images, and the maximum of these positions, $\hat{r}^*$, was defined as the cutoff for positive swelling strains. A thickness-averaged modulus, H_A, was calculated for each sample by integrating $H(\hat{r})$ over the entire cartilage layer, h, assuming that cartilage for $\hat{r} < \hat{r}^*$ is of stiffness corresponding to the modulus H_1.

Semiquantitative histomorphometry

After swelling tests were completed, cartilage-bone samples were fixed in 4% paraformaldehyde, decalcified, and embedded in paraffin. Cartilage-bone sections (5 μm thick) were prepared from each sample and stained with hematoxylin and eosin or toluidine blue. Stained sections were graded using a modified grading scheme (Mankin and Brandt, 1992) which included gross assessment of the cartilage surface (0–4), fibrillation (0–8), chondrocyte cloning in the superficial zone (0–3), microcracks in the calcified cartilage (0–2), and loss of proteoglycan staining (0–6), where the minimum score in each category corresponds to normal cartilage and the maximum score is associated with severe degeneration. The thicknesses of the calcified cartilage and subchondral bone were measured from digitized images of the hematoxylin- and eosin-stained sections as an average of 10 equidistant measurements. Loss of proteoglycan staining was assessed using the toluidine blue sections.

Because it was not known a priori how to weight the measured variables to obtain a quantitative measure of cartilage degeneration, the histology data were analyzed using Factor Analysis (Statistica, StatSoft, Inc., Tulsa, OK). The main purpose of this analysis was to extract the factors (principal components) that were responsible for most of the variation in the histomorphometric data, and thus, to reduce the description of cartilage degeneration to a set of independent variables.

Statistical analysis identified three factors that together accounted for 81% of the total variation in the histomorphometric data. Factor 1 (36% of variance) was weighted primarily by measures of microcracks, the thickness of the calcified cartilage, and gross surface appearance. Factor 2 (28% of variance) was weighted by measures of cartilage changes including loss of proteoglycan staining, extent of surface fibrillation, and cell cloning in the surface zone. Factor 3 (17% of variance) was weighted primarily by morphometric features of cartilage and bone, including the thicknesses of the cartilage layer and the subchondral bone.

A sum of all parameters in the grading scheme was also calculated for each sample. This sum represents the Mankin score, which is often used as a measure of cartilage degeneration (Mankin and Brandt, 1992). Factors 1 and 3 did not correlate with Mankin score ($P > 0.05$, analysis of variance [ANOVA]). However, there was a significant correlation between factor 2 and Mankin score ($P < 0.001$). Therefore, factor 2 was used to represent a quantitative measure of cartilage degeneration. For the purposes of data classification, nondegenerate samples were grouped as those with positive values of factor 2 ($n = 6$), and degenerated (OA) samples were grouped as those with negative values of factor 2 ($n = 5$).

Statistical analyses

One-factor ANOVA was performed to test for the difference between the model and biochemical parameters for nondegenerated and degenerated groups. A linear regression analysis was performed to test for correlations between model parameters (H_1, H_2, h_2), thickness-averaged uniaxial modulus (H_A), thickness-averaged biochemical parameters (ϕ_0^W, c_0^F), and the histological factor 2. All tests were performed at a significance level of 0.05.

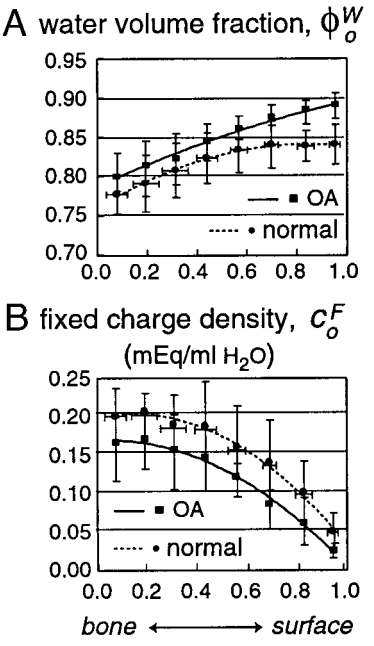

FIGURE 3 Cartilage water volume fraction (*a*) and fixed charge density (*b*) as functions of radial position ($\hat{r}$), determined at the reference zero-swelling configuration for nondegenerated samples ($n = 6$) and degenerated samples ($n = 5$)(mean ± SD). Data for each sample were fitted using a cubic polynomial (*solid and dashed lines*), and these fits were then used to represent specimen-specific biochemical properties as functions of position in cartilage layer.

RESULTS

Biochemical analyses

Both reference volume fraction (ϕ_0^W) and reference fixed charge density (c_0^F) were found to vary with radial position in the cartilage layer (Fig. 3). Thus, c_0^F had a relatively high magnitude in the deep and middle zones, and decreased 4-fold at the articular surface. Water content followed an opposite trend, i.e., it was lower near the bone and higher at the surface. These trends are consistent with results presented previously (Venn and Maroudas, 1977), where water content (water weight per sample wet-weight) and fixed charge density (moles of charge per tissue wet-weight) were measured in full-thickness articular cartilage after equilibration in 0.15 M NaCl and subsequent removal from the bone. In that work, the authors also reported a significant inverse correlation between fixed charge density per wet weight and water content for degenerated, but not normal cartilage. For our samples, we also observed a significant inverse correlation between thickness-averaged values for ϕ_0^W and C_0^F

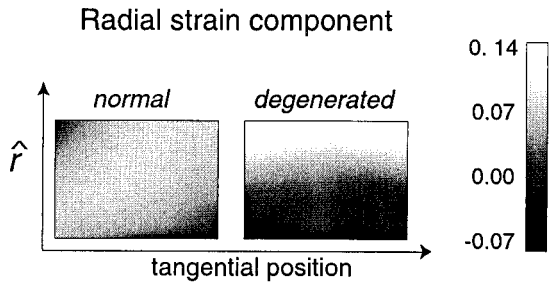

FIGURE 4 Radial component of swelling-induced strain as function of position within cartilage layer ($c^* = 0.015$ M NaCl). For all samples, the radial strain component did not vary with tangential position. For normal and mildly degenerated cartilage, swelling strains were small in magnitude and relatively uniform (*left*). However, the radial strain component was highly nonuniform in the thickness direction for degenerated cartilage samples, with dramatic increases in magnitude at the articular surface (*right*).

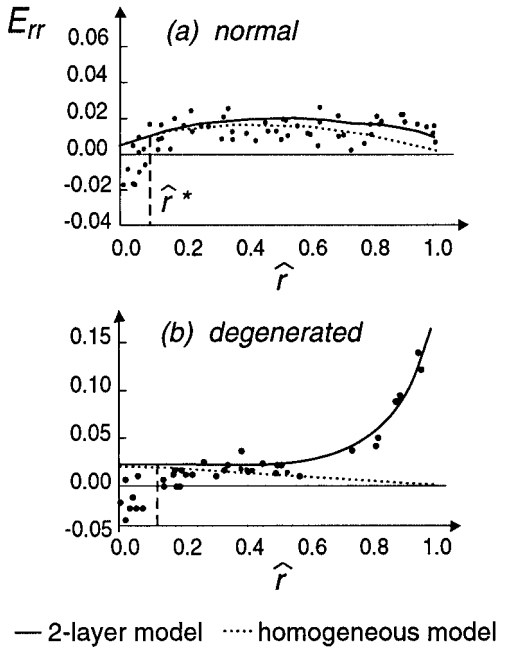

FIGURE 5 Experimental data (●) and model predictions (*solid* and *dotted lines*) for radial component of swelling-induced strain as function of position within the cartilage layer for typical samples ($c^* = 0.015$ M NaCl). For model predictions, only data points with $\hat{r} > \hat{r}^*$ were used (the position where $\hat{r} = \hat{r}^*$ is denoted by *dashed line*). The inhomogeneous two-layer model was able to predict strain distribution patterns for both normal and degenerated cartilage (*solid lines*). Model parameters: (*a*) $H_1 = 23.6$ MPa, $H_2 = 0.09$ MPa, $h_2 = 0.34$; (*b*)$H_1 = 34.7$ MPa, $H_2 = 0.01$ MPa, $h_2 = 0.6$.

(water volume basis) ($R^2 = 0.75$, $P < 0.05$), but not between ϕ_0^W and C_0^F on a dry weight basis ($P > 0.1$, data not shown). Values for the water volume fraction for degenerated samples were higher than those for nondegenerated samples, especially near the articular surface. The values for fixed charge density, however, were lower for the degenerated group, than for nondegenerated samples. For the thickness-averaged values of biochemical parameters, there was a significant increase in ϕ_0^W for the degenerated group relative to nondegenerated samples ($P < 0.05$, ANOVA), whereas the difference in thickness-averaged C_0^F between the two groups was not significant ($P > 0.1$, ANOVA).

Swelling-induced strains

Similar to our previous results for canine cartilage (Narmoneva et al., 1999b), only the radial strain component, E_{rr}, was significantly different from zero, and both tangential and shear strain components had magnitudes less than the experimental error (±0.008 strain). Also similar to observations for canine cartilage, compressive strains were observed in the deep zone near the cartilage-bone interface. The origin of these compressive strains is not clear, although nonuniform structure of the cartilage solid matrix, as well as documented differences in tensile and compressive properties of cartilage may be the factors involved (Narmoneva et al., 1999b). Two distinctive patterns of cartilage swelling in the radial direction were observed (Fig. 4). For nondegenerated and mildly degenerated samples (nondegenerated group, $n = 6$), radial swelling strains were small in magnitude (<5%) and uniform throughout the thickness. These low values for swelling strain have been measured previously for canine cartilage (Narmoneva et al., 1999b), and are consistent with the observation that nondegenerate human cartilage does not swell to an appreciable extent (Maroudas et al., 1986). However, for samples with moderate and severe degeneration (degenerated group, n = 5), large increases in swelling strains were observed at the articular surface, with strain magnitudes as high as 15% for some samples. For all samples, the radial strain component did not vary with tangential position along the cartilage-bone interface.

Uniaxial modulus predictions

Because the theoretical model could not predict compressive strains, only data for $\hat{r} > \hat{r}^*$ (*dashed line* in Fig. 5) were used in the minimization procedure to obtain the parameters H_1, H_2, and h_2. The two-layer material model was found to describe patterns of nonuniform swelling for both nondegenerated and degenerated cartilage (Fig. 5). For comparison, the predictions of the homogeneous model (with uniaxial modulus, H_A, constant throughout the thickness) are also shown. For both normal ($0 <$ factor $2 \leq 1.84$) and highly degenerated ($-1.32 \geq$ factor $2 < 0$) cartilage samples, the modulus in the surface layer was lower than that near the bone, with values for H_2 as much as two orders of magnitude smaller than the modulus in the deep layer, H_1. For all samples, the thickness of the surface layer where the modulus was reduced, varied between 26 and 70% of the

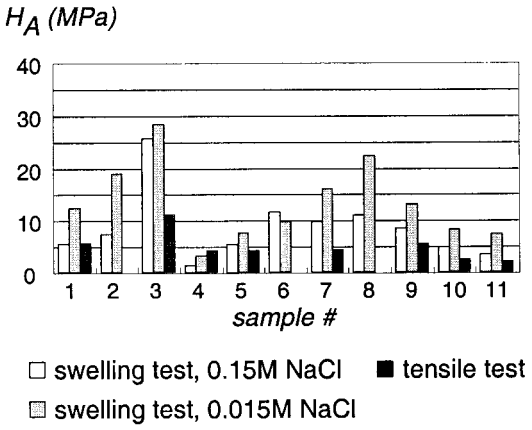

FIGURE 6 Thickness-averaged values for the uniaxial modulus determined from the swelling data at 0.15 and 0.015 M NaCl ($n = 11$). Values for the tensile modulus measured in uniaxial testing of site-matched samples are shown for comparison ($n = 8$) (Elliott et al., 1999).

total cartilage thickness. Average values for the model parameters for nondegenerated samples were $H_1 = 19.5 \pm 8.3$ MPa, $H_2 = 2.9 \pm 4.0$ MPa, and $h_2 = 0.43 \pm 0.17$ (0.015 M NaCl), and $H_1 = 12.4 \pm 9.3$ MPa, $H_2 = 1.7 \pm 2.3$ MPa, and $h_2 = 0.40 \pm 0.11$ (0.15 M NaCl) (mean $\pm$ SD, n = 6). For degenerated samples, the average values were $H_1 = 14.5 \pm 10.2$ MPa, $H_2 = 0.14 \pm 0.18$ MPa, and $h_2 = 0.61 \pm 0.11$ (0.015 M NaCl), and $H_1 = 9.3 \pm 6.9$ MPa, $H_2 = 0.08 \pm 0.11$ MPa, and $h_2 = 0.63 \pm 0.17$ (0.15 M NaCl) (mean $\pm$ SD, n = 5). Because there was a wide range of values (almost two orders of magnitude difference) for the values of model parameter H_2, the relative error in this parameter (rather than an absolute one) was kept constant during the minimization procedure. Therefore, it was possible to use a logarithm of H_2, instead of H_2, in the statistical analyses. The differences in the model parameters h_2 and $\log(H_2)$ between degenerated and nondegenerated groups were significant ($P < 0.05$, ANOVA), whereas the difference in the model parameter H_1 between the two groups was not significant ($P > 0.1$, ANOVA).

Thickness-averaged estimates for H_A based on strain data measured in physiological and hypotonic saline solutions are shown in Fig. 6, with an average for the normal samples of 10.3 ± 7.4 MPa (0.15 M NaCl) and 16.0 ± 7.1 MPa (0.015 M NaCl) (mean $\pm$ SD, n = 6), and the values for the degenerated samples of 6.4 ± 4.6 MPa (0.15 M NaCl) and 10.1 ± 7.1 MPa (0.015 M NaCl) (mean $\pm$ SD, n = 5). Values for H_A determined from the triphasic model and experimental data were similar to site-matched values for the tensile modulus, E (Fig. 6, Elliott et al., 1999), as reflected by significant correlation between H_A and E ($E = 0.36\ H_A$, $R^2 = 0.66$, $P < 0.01$ for 0.015 M NaCl, and $E = 0.46\ H_A$, $R^2 = 0.51$, $P < 0.05$ for 0.15 M NaCl). The values for H_A were also similar to values previously reported for the tensile (and not compressive) modulus of human cartilage (Akizuki et al., 1986; Kempson, 1979). The values for uniaxial modulus, H_A, estimated at hypotonic solution (0.015 M NaCl) were consistently higher than those at physiological saline (0.15 M NaCl), with the difference ranging from 6 to 35%.

Correlation of biochemical and material properties with histological factor

Linear regression analysis demonstrated significant correlations between thickness-averaged measures of C_0^F and ϕ_0^W and histological factor 2 (Fig. 7 *a*, *b*). In general agreement with trends reported in the literature for OA cartilage (Maroudas and Venn, 1977; Maroudas et al., 1986), cartilage water content was observed to increase, and fixed charge density was observed to decrease with degeneration. However, no correlation was detected between fixed charge density on a dry-weight basis and the histological factor 2. This result is in agreement with previous studies, which showed that there is little or no change in fixed charge density per dry weight with cartilage degeneration (Bank et al., 2000; Basser et al., 1998; Maroudas and Venn, 1977).

There was evidence of changes in model parameters, h_2 and H_2, with cartilage degeneration. Thus, h_2 (the thickness of the surface layer) was found to significantly increase with degeneration (Fig. 7 *c*). Also, uniaxial modulus at the surface, H_2, significantly decreased with degeneration, as detected by a negative correlation with factor 2 (Fig. 7 *d*). However, no significant correlation was found between H_1 and factor 2.

DISCUSSION

This work presents a new approach to study cartilage swelling on the bone and to determine the material properties of normal and degenerated articular cartilage using a noncontacting osmotic loading method. It was found that degeneration is associated with a highly nonuniform distribution of swelling-induced strains in the cartilage layer, as compared with normal cartilage. A new theoretical model was developed that was able to describe swelling patterns for both nondegenerated and degenerated cartilage. This model includes an explicit dependence on three geometric and material parameters that can be used to determine a uniaxial modulus, H_A, for cartilage. The values for H_A determined from the model predictions and experimental data agreed well with previously measured values for the tensile modulus of human cartilage (Akizuki et al., 1986; Kempson, 1979), demonstrating the potential of this noncontacting method to measure material properties of cartilage while intact and on the bone.

The results of this study showed that, for both normal and degenerated cartilage, there exists a region of a reduced uniaxial modulus near the articular surface. The physical

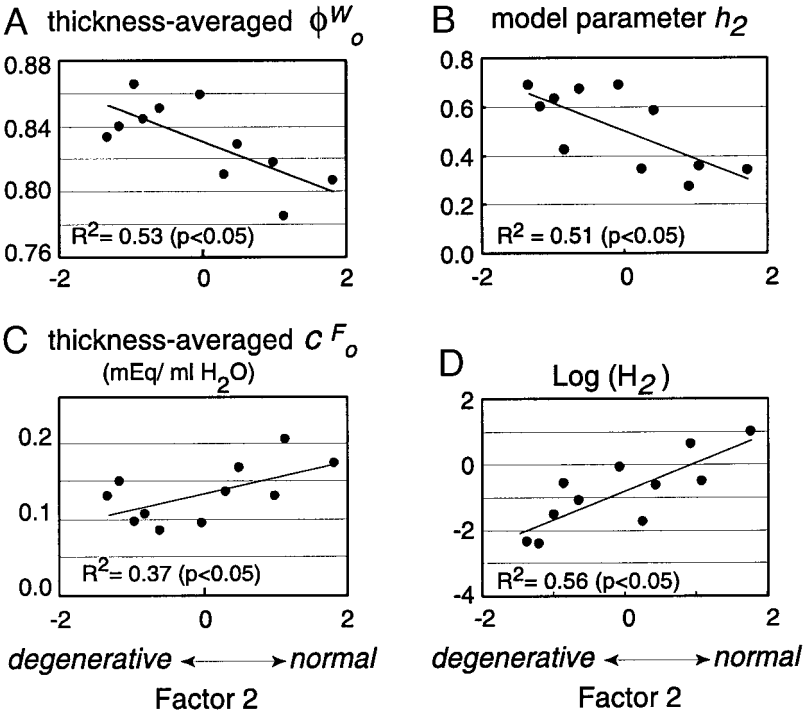

FIGURE 7 Significant correlations were observed between factor 2 and the thickness-averaged water volume fraction (*a*) and fixed charge density (*b*) measured at the reference configuration, as well as between factor 2 and the thickness of the surface layer (*c*), and between factor 2 and the model parameter H_2 (*d*).

explanation for this finding can be found in the nonuniform and anisotropic structure of the collagen matrix in the cartilage layer. Indeed, in the deep zone near the bone, collagen fibers are perpendicular to the bone (Aspden and Hukins, 1981; Clark, 1990), and matrix stiffness is greatest in the direction of the fibers, i.e., in the radial direction. Farther from the bone, however, fibers lose their preferred orientation and gradually become parallel to the surface, i.e., perpendicular to the radial direction. Therefore, matrix stiffness of the surface layer is highest in the *tangential* direction, or direction aligned with the collagen fibers. In the radial direction, the matrix stiffness at the surface layer may be decreased compared with the stiffness in the deep layer, which is in agreement with the model predictions in this study. This conclusion is also supported by findings that values for the thickness-averaged H_A (which are dominated by the modulus values in the deep-middle zones) are similar to values for the tensile moduli of cartilage measured in simple tension at the articular surface, but not deep zone (Elliott et al., 1999) (Fig. 6).

Increases in cartilage volumetric swelling, measured as water weight gain, have been reported as an early sign of osteoarthritis (Mankin and Brandt, 1992; Maroudas et al., 1986). The results of this study show that degeneration significantly alters the magnitude and distribution of swelling-induced strains in the cartilage layer. Swelling strains were small and relatively uniform in normal and mildly degenerated cartilage. With the progression of degeneration (as characterized by changes in factor 2 values from -2 to 2), the magnitude of swelling strains increased remarkably (more than 5-fold) at the articular surface. These results represent the first data available for the change in spatial distribution of swelling strains in human cartilage with osteoarthritis, and are in qualitative agreement with previous observations for increases in volumetric swelling of OA human cartilage, as compared with normal cartilage (Maroudas and Venn, 1977; Maroudas et al., 1986).

The results of this study indicate that cartilage degeneration involves changes in both compositional parameters and structure of the cartilage matrix. Thus, cartilage water volume fraction (ϕ_0^W) significantly increased, and negative fixed charge density per water volume (c_0^F) significantly decreased with degeneration, as detected by correlations with factor 2, which was weighted primarily by changes in matrix staining, surface fibrillation, and chondrocyte cloning (Fig. 7, *a* and *b*). However, there was no correlation between fixed charge density on a dry weight basis and factor 2. This result suggests that decreases in c_0^F are not attributable to a change in the absolute amount of proteoglycans in the tissue, but are rather attributable to an increase in tissue water content with degeneration. Similar findings have been reported previously by Maroudas

(1976), Maroudas and Venn (1977), and Maroudas et al. (1986). They proposed that an increase in water content of degenerative cartilage may be directly related to a weakening of the collagen matrix and disruption of the balance between the high interstitial swelling pressure and restraining forces of the collagen network. This suggestion has been recently confirmed by Basser et al. (1998), by using the principle of balance of forces in cartilage (Maroudas and Bannon, 1981) to determine tensile forces within the collagen network of articular cartilage samples ex situ. It was shown that degenerated cartilage deforms more than normal tissue, suggesting a loss of collagen network integrity and a reduction in the tensile stiffness of OA cartilage samples. The results of the present work corroborate these findings and provide new information on spatially varying structural changes in the cartilage solid matrix with osteoarthritis. Thus, degeneration resulted in a decrease in the value for uniaxial modulus at the surface (model parameter H_2, Fig. 7 *d*), whereas no changes were found in values for uniaxial modulus in the deep-middle zones (model parameter H_1). Furthermore, it was found that the thickness of the surface layer (h_2), which represents the depth of the region of "apparent softening," increased with degeneration, as detected by a negative correlation with factor 2 (Fig. 7 *c*). These findings give evidence that collagen matrix disruption starts at the articular surface and progresses into deeper layers, as the degenerative process progresses. Importantly, the model presented here provides the material parameter, H_2, and the structural parameter, h_2, to quantify the magnitude and depth of these degenerative changes, respectively.

There are several assumptions used here which limit the utility of the model for describing the material behavior of articular cartilage. One limitation is the assumption of material isotropy for the cartilage solid matrix. There is significant evidence for cartilage anisotropy in tension (Roth and Mow, 1980; Woo et al., 1976, 1979), including the results of a related study of simple direct tensile testing (Elliott et al., in preparation), as discussed. Therefore, incorporation of material anisotropy may be considered as an important step to further extend this model. Another model assumption is that of linear material behavior for cartilage. It is known that cartilage can behave nonlinearly in tension (Elliott et al., 1999; Kempson, 1979; Roth and Mow, 1980; Woo et al., 1976, 1979). This fact could potentially provide an explanation for the difference between values for H_A determined in the physiological solution, and those in the hypotonic solution. For example, solid matrix nonlinearity can result in a "stiffening" effect and greater values for H_A determined at 0.015 M NaCl, than those determined at 0.15 M NaCl, because of larger strain values measured at 0.015 M NaCl. However, a ~20% increase in H_A was observed here even for normal samples, for which both the strain magnitudes and the difference between strain magnitudes in two solutions were very small. Therefore, based on a typical stress-strain curve for human cartilage (Elliott et al., 1999; Kempson, 1979), it seems unlikely that cartilage nonlinearity had a significant effect on the estimated values for H_A in this study.

In this study, the contribution of entropic effects into the total swelling pressure in cartilage was neglected, and only changes in the Donnan osmotic pressure between the reference (hypertonic) and test configurations (physiological or hypotonic) were considered. Our preliminary estimates using a model for swelling pressure that incorporated entropic effects gave rise to little change in the determined material properties (<5% in the thickness-averaged modulus, H_A) (Narmoneva, 2000). This result is not unexpected, because the magnitude of the entropic component of swelling pressure in cartilage does not directly depend on NaCl concentration, c^* (Kovach, 1995; Narmoneva, 2000), whereas the Donnan component has a nonlinear dependence on c^* and is therefore the primary driving force for cartilage to swell between the reference and physiological or hypotonic configurations.

The difference in swelling pressure between the reference configuration (2 M NaCl) and physiological (0.15 M NaCl), or hypotonic (0.015 M NaCl) configurations was estimated using the ideal Donnan model. To describe electrostatic interactions in cartilage, the ideal Donnan model assumes a constant electrostatic potential in the tissue and neglects local spatial variations in this potential. Recently, an alternative approach (Poisson-Boltzmann [PB] model) has been developed by several research groups (Basser and Grodzinsky, 1993; Buschmann and Grodzinsky, 1995; Ehrlich et al., 1998), which takes into account variations in electrostatic potential in cartilage at molecular scale. It has been shown that the ideal Donnan model can accurately predict the charge-dependent component of the swelling pressure in cartilage at low ionic strengths, and that for concentrations of $c^* \leq 0.025$ M NaCl, the two models are in a good agreement (Basser and Grodzinsky, 1993; Buschmann and Grodzinsky, 1995). Therefore, the values for H_A determined at hypotonic configuration (0.015 M NaCl) would be similar regardless whether the Donnan or the PB model is used. For higher ionic strengths, however, including physiological concentrations (0.15 M NaCl), the Donnan model results in larger values for the swelling pressure than both the values predicted by the PB model and those measured experimentally (Buschmann and Grodzinsky, 1995; Ehrlich et al., 1998). Because both models predict similar values for pressure at low c^*, the discrepancy in model predictions only increases with c^*. In the present study, it is the difference between the swelling pressure at the reference (hypertonic) state and the physiological configuration that is used to determine H_A at 0.15 M NaCl. Because of a highly nonlinear dependence of pressure on c^*, the difference in swelling pressure between hypertonic and physiological states is actually less for the Donnan model, compared with the PB model. Thus, the ideal Donnan model underestimates the swelling pressure which gives rise to dimensional swelling

in our model. Therefore, the values for H_A determined at physiological configuration (0.15 M NaCl) would be smaller for the ideal Donnan model than for the PB model, and thus, smaller than the values for H_A determined at hypotonic configuration, as is the case reported in this study. Potentially, the swelling pressure in cartilage can be predicted using a non-ideal Donnan model, which is obtained if one introduces non-ideal values for the activity and osmotic coefficients of cartilage to the expression for the swelling pressure. As has been shown by Buschmann and Grodzinsky (1995), these activity coefficients can be calculated using the PB model and used as a corrective term in the non-ideal model. The data necessary to implement this modification are not available, however, so that the magnitude of the ideal assumption may not be adequately estimated.

In summary, the results obtained here demonstrate that the osmotic loading technique can be applied to determine the material properties of cartilage using the experimentally measured values for swelling-induced strains. Together with inhomogeneous model, this method may be useful for quantifying the extent of damage to the cartilage extracellular matrix. Because this method is noncontacting, it may be used to measure the material properties of cartilage in small animal models of osteoarthritis (Leddy et al., 2000), where other testing methods are difficult to apply.

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