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A Triphasic Orthotropic Laminate Model for Cartilage Curling Behavior: Fixed Charge Density vs. Mechanical Properties Inhomogeneity

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Abstract

Osmotic pressure and associated residual stresses play important roles in cartilage development and biomechanical function. The curling behavior of articular cartilage was believed to be the combination of results from the osmotic pressure derived from fixed negative charges on proteoglycans and the structural and compositional and material property inhomogeneities within the tissue. In the present study, the *in vitro* swelling and curling behaviors of thin strips of cartilage were analyzed with a new structural model using the triphasic mixture theory with a collagen-proteoglycan solid matrix composed of a three-layered laminate with each layer possessing a distinct set of orthotropic properties. A conewise linear elastic matrix was also incorporated to account for the well-known tension-compression nonlinearity of the tissue. This model can account, for the first time, for the swelling-induced curvatures found in published experimental results on excised cartilage samples. The results suggest that for a charged hydrated soft tissue, such as articular cartilage, the balance of proteoglycan swelling and the collagen restraining within the solid matrix is the origin of the *in situ* residual stress, and that the layered collagen ultrastructure, *e.g.*, relatively dense and with high stiffness at the articular surface, play the dominate role in determining curling behaviors of such tissues.

Keywords

Triphasic Theory; Cartilage; Curling; Inhomogeneity; Fixed Charge Density

1. Introduction

Osmotic pressure is critical for the development of biological tissues and the maintenance of normal functions. In the early development of limb, mesenchymal cells at the distal tip proliferate and secrete extracellular matrix (ECM) including collagen and hyaluronic acid (HA), while cells away from the tip secrete hyaluronidase (HAase) [1,2]. It is the negatively charged HA that keeps limb bud swollen and maintains spacing between cells at the tip, while HAase degrades the HA by cleaving glycosidic bonds, decreases osmotic pressure at the proximal side, and leads to tissue deswelling and chondrogenic condensation [3]. In normal tissue function, osmotic pressure is generated from the hydration of proteoglycans and acts on collagen fibers as tensile forces. The pre-stress (also called 'residual stress') in ECM provides effective mechanical support and shields chondrocytes from excessive compressive loads [4]. Recently, the extraction of proteoglycans has been shown to benefit the elevation of collagen

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contents in engineered constructs, one of greatest challenges in cartilage tissue engineering [5,6]. Therefore, the understanding of the mechanical role of proteoglycans, and associated osmotic pressure, becomes important to elucidate cartilage development, biomechanical function, and cellular responses to proteoglycan loss in cartilage diseases or tissue engineering.

In this study, we focused on an interesting phenomenon, cartilage curling behavior, which was found decades ago in a simple experiment that mature cartilage, upon removal from the underlying subchondral bone, warped toward to articular surface [7]. However, it was not until late 1990's that cartilage curling was specifically studied analytically and quantitatively. Interestingly, the curvature of rectangular full-thickness cartilage strips (1.7 mm width $\times$ 8 mm length) at *steady state* was found to increase monotonically with the decreasing saline concentration (from 0.0015 M to 2.0 M) in the bathing solution [8]. The curling behavior of cartilage is the synergistic result of the osmotic pressure as the motive force and the structural and compositional inhomogeneity as the structural limiting factors [8,9]. As mentioned, the osmotic pressure derives from the fixed charge density (FCD) on glycosaminoglycans (GAGs), which are the side chains of proteoglycans [9,10]. The variation of external saline concentration changes osmotic pressure inside the tissue as it would result from different levels of FCD. The negative charges on GAGs attract more cations than anions into the tissue and thus introducing an imbalance of total ion concentration at the interface of cartilage and the interstitial fluid. The resulting Donnan osmotic pressure due to colligative action of these mobile ions forms the major part of osmotic pressure inside cartilage, with other minor contributions from configuration entropy and mixing entropy of proteoglycan molecules [11–13]. The osmotic pressure causes tissue swelling, which is restricted by the stiffness of the collagen dominates the tensile behavior of the solid extracellular matrix. However, without the inhomogeneity and anisotropy of the tissue, swelling would only be uniform and isotropic and thus the curling behavior would be absent. Indeed, articular cartilage has a layer-wise inhomogeneity of chemical contents, with maximal collagen content in the superficial zone, and higher proteoglycan content in the middle and deep zones [4]. Scanning electron microscopy studies also indicated the collagen fibrils have preferential orientations in the superficial zone (tangential to articular surface) and the deep zone (perpendicular to tide mark at the calcified cartilage), (Fig. 1) [14,15]. It is well known that these compositional and ultrastructural features of cartilage lead to the inhomogeneity and anisotropy in mechanical properties, which manifest as the commonly observed bending of the excised articular cartilage specimens toward the articular surface.

Various mathematical descriptions have been proposed for cartilage swelling [8,16–19], but, to date, there are no biomechanical models which have successfully related this swelling induced curling behavior to mechano-chemical properties of articular cartilage. Setton *et al.* qualitatively attributed the curling behavior to the layer-wise inhomogeneity of the swelling pressure of proteoglycans with a homogeneous, linearly elastic model for the solid matrix [8, 20], but their prediction of the maximal curvature of cartilage strips occurred in 0.3 M saline was contradictory to their later experimental findings [21]. The cartilage specimen was also modeled as a transversely isotropic elastic body with finite element method [22], but an *ad hoc* swelling pressure distribution across thickness was assumed. In this study, the primary objective was to develop a quantitative model to explain the swelling-induced curling behavior of thin excised articular cartilage specimens based on known cartilage chemical contents and material properties. Specifically, a multi-layer triphasic orthotropic model was developed with the classical lamination theory and deformation calculated analytically for thin strips of cartilage specimens in various saline concentrations; based on this simple laminar model, these analytical predictions were compared with previous experimental results. In addition, the contributions of various well-documented intrinsic physical and mechanical properties on the curling behavior of articular cartilage were used for the first time in this model to predict its curling behavior. With this simple mathematical model, it is now possible to calculate the

curling behavior of such tissues with accuracy (as measured by curvature), and hence help to demystify residual stresses, curling behavior, and load-supporting mechanisms of cartilage tissue.

2. Methods

2.1 Statement of the Problem

A rectangular cartilage strip (length $a \times$ width $b \times$ thickness h , Fig. 1) alters its curvature when immersed in saline solutions with different concentrations c^* (e.g., 0.015M, 0.05M, 0.15M, 0.5M, 2M NaCl). The goal of this study is to mathematically model the curling behavior of articular cartilage and to predict the variation of curvature with ion concentrations. An assumption we make for this model is that at the hypertonic state ($c^* \rightarrow \infty$), the swelling effects of the FCD are negligible [11,17,23,24], and that there are no residual stresses and strains inside the solid matrix and that the entire tissue is initially flat [20,21]. Please note that the linearized formulations used in this study also apply to initially slight curved samples. The calculated curvatures, together with other physical parameters after swelling, will be relative to those at this hypertonic reference state (HRS).

2.2 Mathematical formulations for cartilage curling

The triphasic theory [25,26] is used in conjunction with a classical lamination theory [27] to model the curling behavior of thin cartilage strips. To simplify this problem, the cartilage strip is considered to consist of three discrete layers: superficial layer (SL), middle layer (ML), and deep layer (DL). Material properties and chemical parameters are assumed to vary between layers, but to be homogenous within each layer (Fig. 1).

In the triphasic theory, articular cartilage is treated as a mixture of three phases: solid matrix, interstitial water, and mobile ions (sodium and chloride). For each layer, based on the continuity of electrochemical potentials of mobile ions, the total tissue stress (σ) at the steady state consists of elastic stresses inside the solid matrix (σ_s) and the osmotic pressure (p) as shown below:

$$\sigma = -pI + \sigma_s \quad \text{with} \quad p = \pi - \Pi e \quad (1)$$

where $\pi = \phi RT(c^k - 2c^*)$ and $\Pi = \phi RT(c_r^F)^2/(\phi_r^w c^k)$ [28–31]. Here ϕ is the osmotic coefficient, R is the universal gas constant, T is the absolute temperature, c^k is the total ion concentration, e is the solid matrix dilatation, and ϕ_r^w and c_r^F are the porosity and FCD at HRS, respectively.

As the cartilage strip is very thin compared to the specimen length (empirically when length/thickness > 4), under this geometric condition, the classical lamination theory can be adopted to describe the matrix deformation kinematics [27]. In this theory, the laminate (the entire tissue) is presumed to be bonded perfectly between layers, and the displacements are continuous across each lamina (layer) so that no lamina can slip relative to each other (Fig. 2). Since the laminate is thin, the assumption of pure bending also can be employed. This implies that the shear strains in the planes perpendicular to the middle surface are zero, i.e., $\varepsilon_{xz} = \varepsilon_{yz} = 0$. The in-plane shear strain ε_{xy} should also be zero in the current coordinate system. The normal strains in x direction ($\varepsilon_x = \partial u / \partial x$) and in y direction ($\varepsilon_y = \partial v / \partial y$) of any horizontal plane can be expressed as (Fig. 2):

$$\varepsilon_x = \varepsilon_{x0} + z \kappa_{x0} \quad \text{and} \quad \varepsilon_y = \varepsilon_{y0} + z \kappa_{y0}, \quad (2)$$

where z is the vertical distance measured normal from the mid-plane (i.e., half-thickness position where $z = 0$) of the laminate, ε_{x0} ($= \partial u_0 / \partial x$) and ε_{y0} ($= \partial v_0 / \partial y$) are the horizontal strains

at the mid-plane, and $\kappa_{x0} (= \partial^2 w_0 / \partial x^2)$ and $\kappa_{y0} (= \partial^2 w_0 / \partial y^2)$ are the linearized definition of curvatures. Here u_0 , v_0 and w_0 represent the displacements of the mid-plane in x, y and z direction, respectively.

Since there are no shear strains in the primary coordinate directions (*i.e.*, $\varepsilon_{xy} = \varepsilon_{xz} = \varepsilon_{yz} = 0$), the constitutive equation (Eq 1) between total normal stresses and strains for each layer can be written as:

$$\begin{bmatrix} \sigma_x \\ \sigma_y \\ \sigma_z \end{bmatrix} = \begin{bmatrix} c_{xx} & c_{xy} & c_{xz} \\ c_{xy} & c_{yy} & c_{yz} \\ c_{xz} & c_{yz} & c_{zz} \end{bmatrix} \begin{bmatrix} \varepsilon_x \\ \varepsilon_y \\ \varepsilon_z \end{bmatrix} + \Pi e \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} - \pi \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix}. \quad (3)$$

The elements of $[c_{ij}]$ are the intrinsic orthotropic stiffnesses of the solid matrix which include effects of the fiber reinforcement oriented in different directions within the layers (Table 1). Based on collagen fibril orientation, an isotropic model is used for the ML and transversely isotropic model for the DL. The SL has much larger tensile modulus than that in the ML, and it is smaller in the direction perpendicular to the split lines than that in the parallel direction [32,33]. Therefore, an orthotropic model was used for the SL. In addition, due to the well-known tension-compression nonlinearity [34], the stiffness in the predominant direction of collagen fibers depends on the sign of the strain (*i.e.*, tension or compression) experienced by the local collagen fibrils-proteoglycan solid matrix and modeled with the conewise linear elasticity [35] (see Table 1).

Considering the strip to be very thin, we can assume that the laminate is in a state of plane stress with zero total vertical stress within the tissue (*i.e.*, $\sigma_z = 0$) and the resultant force and bending moment over the entire later surface must be zero (*i.e.*, $\bar{F} = 0$ and $\bar{M} = 0$). Under these conditions, the mid-plane strains in x and y directions (ε_{x0} and ε_{y0}) and curvatures in x and y directions (κ_{x0} and κ_{y0}) can be calculated [27].

3. Results

Figures 3 and 4 represent variations of surface stretch ($\lambda = I + \varepsilon$) and curvatures in the directions both parallel (x direction) κ_x and perpendicular (y direction) κ_y to split lines as a function of the external ion concentrations. It can be seen that the tissue at the bottom of the sample is under tension (*i.e.*, convex side with stretch $\lambda > 1$, while at the top (concave side) the strain will be very close to zero, and even slightly negative (*i.e.*, in compression with $\lambda \leq 1$). The curvature increases with the decrease of external saline concentration, and is always larger in the split line direction (x). In Fig. 4 also inserts the images of curved cartilage samples (adapted from Setton et al, 1995) in 0.15M and 2M saline to demonstrate the change of tissue curvature caused by the variation of osmotic pressure.

There has always been the question of whether collagen and/or proteoglycan stratification produce more curling. To answer this question, two cases were theoretically modeled. In the first case, we assumed that the FCD to be uniform, and has a value of 0.218 mEq/mL, which is the average value for the base case. It is found that there was almost no predicted change both in x and y directions in the curling response of the tissue (Fig. 5). In the second case, we assumed the surface to be absent. The average curvature $\bar{\kappa}$ in physiological saline solution (0.15 M), which is defined as $(\kappa_x + \kappa_y)/2$, will decrease from 0.055 mm^{-1} to 0.03 mm^{-1} (Fig. 5).

4. Discussion

The objective of this study was to develop a mechano-electrochemical model (*i.e.*, using the *triphasic theory*) to describe the swelling and curling behaviors of thin cartilage strips under

osmotic loading. For simplicity, the full thickness cartilage strips have been modeled as a thin laminate composed of three fibril-enforced layers with different chemical contents and mechanical properties. Based on the classical lamination theory, simple mathematic solutions have been obtained for the variation of curvatures of the freely swelling cartilage strips in different external saline solutions.

The predicted stretch and curling are comparable to experimental data reported previously by Setton *et al* [21]. Just as shown in their study, the in-plane strains (ϵ_x and ϵ_y) at the articular surface is predicted to be very close to zero for the direction parallel to the split lines (the predominant collagen fiber direction at the surface) as well as for the direction perpendicular to the split lines. The experimental results also show that the strain changes about 0.07–0.11 in the deep zone of the sample strip when the ion concentration varies from 2M to 0.15M, which implies that the overall curvatures changes about 0.035–0.055 mm⁻¹ for a 2mm thick sample. Our values for stretch change (~0.1) and curvature change (~0.05 mm⁻¹) are in these ranges. Considering that the typical material parameters adopted in this study are based on reported experimental data of chemical contents and mechanical properties in previous literatures, we believe that our simple laminate model can indeed capture the actual mechanism of swelling and curling behavior of articular cartilage and therefore be used to predict mechano-electrochemical events happening inside the tissue during osmotic loading. The direct comparison of model prediction and experiments on basis of the individual sample requires sophisticated and careful measurements of chemical and mechanical gradients and may be tackled at a later date.

The accuracy of our model in predicting previous published experimental data gives us confidence that the predicted *in situ* residual stresses and strains should be very close to the actual values one could obtain experimentally. The in-plane strains are continuous and linear, which is a direct consequence from the pure bending assumption as described before. It agrees with the experimental finding that the lateral edge is almost always perpendicular to the surface and bottom of articular strip as shown in the Figure 1 of Setton *et al*'s paper [21]. This is also the reason that the shear stress inside the tissue was ignored and the classical lamination theory was chosen instead of the first-order approximation of the shear-deformed laminated plate theory; this provides an *à posteriori* check for our model assumptions [36]. However, note part of the residual stresses could have already been released when the cartilage strips are excised from the subchondral bones. Therefore, the residual stresses could be larger in the intact cartilage, especially in the superficial zone.

Our numerical results show that there is no significant change in curvature when the stratified variation of FCD is replaced by a uniform distribution of average FCD throughout articular cartilage while the curvature decreases to about one half when the stiffer superficial zone (see Fig. 5) is removed. In previous reported experiments, although the maximum curvature found near the sample mid-length had no significant changes after the SL removal [21], the overall curvature change at physiological condition ($c^* = 0.15\text{M}$) relative to the hypertonic condition ($c^* = 2\text{M}$) did decrease after the SL removal. Physically, this significant curvature change is a result of the substantial lateral stiffness of the superficial layer, which essentially prevents horizontal strains at the top of the sample and thus forcing the neutral plane (where the strain ϵ_x or ϵ_y is zero) to be situated close to the upper surface. The combination of high tensile stiffness and the location of the superficial layer near the articular surface thus appears to play a key role in the curling behavior of articular cartilage and controlling the *in situ* stress; this may indeed be a physiological protective mechanism for exposing normal articular cartilage to high stresses, even though the loading stress is rather high.

In conclusion, the three-layer triphasic orthotropic laminate model has been successfully developed to describe the curling behaviors of thin strips of articular cartilage including the

prediction of tissue strains and bending curvatures. Our results indicate that for a charged hydrated soft tissue the balance of proteoglycan swelling pressure and the collagen restraining stresses within the solid matrix determines tissue residual stress and that the layered collagen ultrastructure and the resulting mechanical properties stratification appear to dominate over FCD inhomogeneity in determining curling behaviors of articular cartilage. By taking into account of the mechanical contributions from both proteoglycans and collagen fibrils, our model provides advantages in understanding cartilage development and loading-support mechanisms of such charged-hydrated-fiber reinforced solid matrix and providing design guidance for better strategies for cartilage repair and regeneration.

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Figure 1.
The layer structure of articular cartilage strip with its dimensions and the predominant orientation of collagen fibrils

Figure 2.

Geometry of laminate deformation. Based on the classic lamination theory, the line (*e.g.*, AD) initially perpendicular to the mid-plane remains a straight line and remains perpendicular to mid-plane after deformation. Here u , v , w are displacements in x , y and z direction, respectively, and u_0 , v_0 , w_0 are displacements for the mid-plane. N_x and N_y are resultant forces, and M_x and M_y are resultant bending moments.

Figure 3.

Variation of stretch ($\lambda = dx/dX$) of cartilage strip in different external ion concentrations (0.015M, 0.05M, 0.15M, 0.5 M, and 2M). The stretch is defined as the ratio of the length after deformation (dx) and the original length (dX).

Figure 4. Variation of curvature of cartilage strip in different ion concentrations for the base case parameters listed in Table 1.

Figure 5.

Variation of average curvature $\bar{\kappa} = (\kappa_x + \kappa_y)/2$ with external ion concentration for the case with uniform fixed charge density (FCD) distribution and the case with the absence of superficial layer (SL) (also shown the results for the base case).

Table 1

Depth-dependent composition, anisotropy, and intrinsic mechanical properties of articular cartilage based on previous literatures [4,32,33]. Please note that in stiffness matrix $[c]$ for cartilage solid matrix (see Eq 3 & Fig 1), values outside/inside parenthesis represent the stiffnesses of local collagen fibrils under tension/compression.

Positions	Superficial Zone	Middle Zone	Deep Zone
Thickness (mm)	0.3	1.2	0.5
Water Content	0.7	0.8	0.6
FCD (mEq/mL)	0.12	0.25	0.2
Collagen Fibril Orientation	x	xyz	z
Stiffness Matrix $[c]$ (MPa)	$\begin{bmatrix} 10.8(3.8) & 0.2 & 0.2 \\ 0.2 & 3.8 & 0.2 \\ 0.2 & 0.2 & 1 \end{bmatrix}$	$\begin{bmatrix} 1 & 0.2 & 0.2 \\ 0.2 & 1 & 0.2 \\ 0.2 & 0.2 & 1 \end{bmatrix}$	$\begin{bmatrix} 0.4 & 0.1 & 0.1 \\ 0.1 & 0.4 & 0.1 \\ 0.1 & 0.1 & 3.3(1.2) \end{bmatrix}$
Symmetry Axes	Orthotropic symmetry with x, y and z axes	Isotropic	Transversely isotropic with z axis