A linearized formulation of triphasic mixture theory for articular cartilage, and its application to indentation analysis

Xin L. Lu, Leo Q. Wan, X. Edward Guo, Van C. Mow*

Department of Biomedical Engineering, Columbia University, 351 Engineering Terrace 500 West 120th Street, New York, NY 10027, USA

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The negative charges on proteoglycans significantly affect the mechanical behaviors of articular cartilage. Mixture theories, such as the triphasic theory, can describe quantitatively how this charged nature contributes to the mechano-electrochemical behaviors of such tissue. However, the mathematical complexity of the theory has hindered its application to complicated loading profiles, e.g., indentation or other multi-dimensional configurations. In this study, the governing equations of triphasic mixture theory for soft tissue were linearized and dramatically simplified by using a regular perturbation method and the use of two potential functions. We showed that this new formulation can be used for any axisymmetric problem, such as confined or unconfined compressions, hydraulic perfusion, and indentation. A finite difference numerical program was further developed to calculate the deformational, electrical, and flow behaviors inside the articular cartilage under indentation. The calculated tissue response was highly consistent with the data from indentation experiments (our own and those reported in the literature). It was found that the charged nature of proteoglycans can increase the apparent stiffness of the solid matrix and lessen the viscous effect introduced by fluid flow. The effects of geometric and physical properties of indenter tip, cartilage thickness, and that of the electrochemical properties of cartilage on the resulting deformation and fluid pressure fields across the tissue were also investigated and presented. These results have implications for studying chondrocyte mechanotransduction in different cartilage zones and for tissue engineering designs or in vivo cartilage repair.

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1. Introduction

Indentation test is one of the most frequently used methods for studying the biomechanical behaviors of articular cartilage (Sokoloff, 1966, Mow et al., 1989, Bae et al., 2004, Lu et al., 2004, Sweigart and Athanasiou, 2005). It has been used to determine the material properties of cartilage tissue in situ on the bone. The attachment of the cartilage to the bone avoids specimen preparation complications or other disruptive (e.g., scission of native collagen structure) specimen preparation procedures (Athanasiou et al., 1991, Mow et al., 2005, Lu et al., 2009). In contrast with the ease of performing an indentation experiment, however, the mathematics of analyzing the indentation test on a cartilage–bone sample is extremely difficult due to its complex boundary conditions and the multi-dimensional deformations in the tissue layer (Hayes et al., 1972, Mak et al., 1987, Mow et al., 2005). The mathematical solution for the indentation problem using linear elasticity to model a cartilage layer was developed by Hayes et al. (1972), and the solution based on the biphasic constitutive model for cartilage (Mow et al., 1980) was later performed by Mak et al. for a frictionless, rigid, free-draining indenter tip (Mak et al., 1987), and by Spilker et al. with a finite element method for a frictional, rigid, porous indenter tip (Spilker et al., 1992), respectively.

Despite the prevalence of biphasic theory and its wide acceptance in recent years, cartilage is not just a homogeneous mixture of an elastic solid and water. The negative charges fixed on the proteoglycans, a major component of the solid matrix, introduce an osmotic pressure within the tissue that generates significant influence on the stress, strain, fluid pressure, and electrical fields within the cartilage (Donnan, 1924, Maroudas, 1976, Lai et al., 1991). The density of the charges fixed on the proteoglycans is commonly known as the fixed charge density (FCD), and this FCD is responsible for a broad spectrum of observed mechano-electrochemical (MEC) phenomena (Frank and Grodzinsky, 1987, Lai et al., 2000). To account for these osmotic effects, a tertiary mixture theory (i.e., the triphasic theory) was developed for articular cartilage (Lai et al., 1991, Gu et al., 1998). It provides a comprehensive description of cartilage MEC behaviors, and it has been shown that the triphasic constitutive laws can be used to accurately calculate the FCD of a cartilage tissue sample by using indentation testing data (Lu et al., 2007). The mathematical
complexity of the theory, however, has hindered its adaptation for analyzing the indentation experiment.

This study will show that, by using a regular perturbation method and potential functions similar to those commonly employed in 3D elasticity theory (Neuber, 1934), the triphasic governing equations can be significantly simplified to four coupled linear partial differential equations (PDEs). A mathematical solution for an indentation test, incorporating the electrolyte concentration in the external bathing solution, was developed based on this new triphasic formulation. The effects of FCD on the indentation responses were determined numerically and compared with the experimental data. The deformational and flow behaviors of articular cartilage, such as MEC fields, were parametrically studied using various material properties defined in the triphasic constitutive equations (Lai et al., 1991). PG contents, indenter tips, and aspect ratios (ratio of the indenter diameter to the tissue thickness).

2. Materials and methods

2.1. Potential representations of the triphasic governing equations

In this study, articular cartilage was treated as a triphasic material which consists of a charged incompressible, porous-permeable solid phase, an incompressible interstitial fluid phase (water), and two solute species (monovalent cation and anion) representing the third phase. The details of triphasic theory can be found in Lai et al. (1991) and Gu et al. (1998). The cartilage tissue was further assumed to be homogeneous and isotropic, the porous-permeable extracellular solid matrix to be linearly elastic and to be under infinitesimal deformation. The strain-dependent permeability effect and tension-compression nonlinearity of solid matrix were also ignored in this formulation so that we can focus on the effects of FCD on the tissue’s mechanical behaviors. By using a regular perturbation method, the governing equations of the triphasic theory explicitly related to the FCD can be simplified into two parabolic PDEs (Lu et al., 2007) (see online supplementary materials for mathematical details).

\[
\frac{\partial e}{\partial t} = A_1 \nabla^2 e - A_2 \nabla^2 \psi, 
\]

(1)

\[
\frac{\partial \psi}{\partial t} = A_4 \nabla^2 \psi - A_5 \nabla^2 e, 
\]

(2)

where \( t \) is the time; \( A_i \) are coefficients which depend on FCD (i=1,2,4,5); \( e \) the elastic dilatation of solid matrix; \( \psi \) a dimensionless variable related with the ionic concentration. In cartilage indentation testing, the tissue is ideally loaded perpendicular to the end surface of a cylindrical or spherical indenter tip. Thus this is an axial-symmetric problem. In biphasic theory, the governing equations can be shown to be identically satisfied by expressing the fluid pressure, the radial and axial components of the displacement of the solid matrix as functions of two potential functions, \( \phi \) and \( \psi \) (Mak et al., 1987).

\[
u_r' = \frac{\partial \phi}{\partial r} + 2 \frac{\partial \psi}{\partial r},
\]

(3)

\[
u_z' = \frac{\partial \phi}{\partial z} + 2 \frac{\partial \psi}{\partial z},
\]

(4)

\[p = 2 \mu \left( \frac{\partial \phi}{\partial r} + \frac{\partial \psi}{\partial r} \right),
\]

(5)

The theoretical triphasic prediction of cartilage creep response under a constant loading (Heaviside loading) was compared with data from an indentation test (Fig. 2). This mathematical prediction was obtained by using the above triphasic formulation for the indentation creep test. The input values of intrinsic Young’s modulus and Poisson’s ratio were determined by curve-fitting the indentation data of samples bathed in a hypertonic solution with the biphasic indentation solution (Mow, Gibbs et al., 1989, Lu et al., 2004). The hypertonic solution was used to remove the Donnan osmotic pressure

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\text{the boundary of the triphasic mixture is defined by the boundary of the solid matrix, and the interstitial free water and ions can freely exchange with those in the external solution; (3) the solid matrix is bonded to the rigid calcified cartilage/subchondral bone layer that is impervious to fluid phase and ion species; (4) the indenter tip is rigid and its interface with cartilage surface is frictionless; (5) the porous indenter tip is free-draining to both fluid and ionic phases; the latter condition is expressed by the electrochemical potentials at the indenter–cartilage interface to be equal to those in the external solution; (6) at the interface of impervious indenter tip and cartilage, all phases, including solid, fluid, and ions, have zero velocity in the axial direction of indenter tip. These six basic assumptions are widely accepted in literature (Mak et al., 1987, Spikler, 1992, Julkunen et al., 2007). Based on these assumptions, the indentation boundary conditions can be expressed with the four variables in the new governing equations (see online complementary materials). Due to the existence of FCD, cartilage indentation is in a swollen state at physiological condition (Maroudas, 1976). In present study, this free-swollen state will be chosen as reference state for theoretical simulations, and the perturbation on all physical parameters is based on this reference state (Flahiff et al., 2002, Bae et al., 2006, Yao and Gu, 2007).

2.3. Numerical methods

A finite difference program was developed to solve Eqs. (1), (2) and (6), (7) for the indentation configuration as shown in Fig. 1. The numerical method of lines (Schiesser, 1991) was employed and central-difference approximation was chosen for the spatial derivatives. The numerical integration over time scale was performed by a custom-modified commercial solver (MATLAB R13, The MathWorks, Inc., Natick, MA) based on backward differentiation formulas (i.e., Gear’s method). In simulation, the following baseline parameters were used: temperature=293 k, external solution concentration \( \psi^* = 0.15 \text{ M} \), indenter tip radius \( a = 1.05 \text{ mm} \), cartilage thickness \( h = 1.471 \text{ mm} \), water content \( \phi^* = 0.74 \text{, intrinsic aggregate modulus (defined as that modulus without the osmotic effect introduced by fixed charge density) } H_0 = 0.24 \text{ MPa, Poisson’s ratio } \mu = 0.15 \text{, permeability } k = 1.5 \times 10^{-15} \text{ m}^2/\text{N} \cdot \text{s}, \text{ FCD}=0.15 \text{ mEq/ml, } D^* = 0.5 \times 10^{-9} \text{ m}^2/\text{s}, \text{ and } D^* = 0.8 \times 10^{-9} \text{ m}^2/\text{s}. \text{ In numerical analysis, these parameters were necessary to calculate the five coefficients } A_{1-5} \text{ in governing equations, Eqs. (1) and (2), and to transform the results ( } \phi, \psi, e, \text{ and } \gamma ) \text{ back into original physical parameters (e.g., stress and strain) (see supplementary materials).}

3. Results

3.1. Results for creep test

The theoretical triphasic prediction of cartilage creep response under a constant loading (Heaviside loading) was compared with data from an indentation test (Fig. 2). This mathematical prediction was obtained by using the above triphasic formulation for the indentation creep test. The input values of intrinsic Young’s modulus and Poisson’s ratio were determined by curve-fitting the indentation data of samples bathed in a hypertonic solution with the biphasic indentation solution (Mow, Gibbs et al., 1989, Lu et al., 2004). The hypertonic solution was used to remove the Donnan osmotic pressure

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\]

Fig. 1. A schematic diagram of indentation test showing a charged-hydrated cartilage attached to a rigid bone block indented by a circular, rigid, and frictionless indenter tip. The indenter tip may be free-draining or impervious. Due to the symmetry with respect to } \( \text{r}=0 \text{, a half part of the sample was numerically analyzed.}

\[
\text{r}
\]

\[
\text{Subchondral Bone}
\]

\[
\text{Articular Cartilage}
\]

\[
\text{Indenter Tip}
\]
inside the tissue. According to previous experimental study, strains in the deformed configuration are small relative to the hypertonic configuration, as well as to the isotonic state of the tissue (Narmoneva et al., 2001, Flahiff et al., 2002, Lu et al., 2007). For verification, the FCD value was obtained using biochemical GAG assay (Lu et al., 2004). By comparison with the triphasic simulation curve, Fig. 2 shows an accurate theoretical prediction.

To study the effect of FCD on the creep response of cartilage, five creep tests were simulated using the same mechanical properties and loading conditions but with different FCD values (Fig. 3). The differences between equilibrium values illustrate the effect of FCD on tissue's apparent stiffness. Higher fixed charge density results in a stiffer tissue. This is consistent with the indentation creep data shown in Fig. 2, where the creep displacement is smaller for higher FCD values.

The reaction force from articular cartilage surface during displacement control tests (i.e., stress-relaxation experiments) were calculated for cartilage with different FCD values. Both porous free-draining and impervious indenter tips were employed for these calculations. Figure 4 shows the reaction force as a function of time for different FCD values.

The transient history of (A) axial strain ($E_{zz}$) in the loading direction, (B) solid matrix dilatation, and (C) fluid pressure are calculated at three different depths across the cartilage during stress-relaxation tests. Figure 5 illustrates the changes in these variables as a function of time and depth for different FCD values.

### Figures

**Fig. 2.** A typical indentation creep data of cartilage bathed in a physiological environment (0.15 M PBS) and the triphasic theoretical prediction at the same condition using measured material coefficients and FCD.

**Fig. 3.** A group of indentation creep curves of articular cartilage with various FCD values for numerical simulation. The loading profile and all the mechanical properties are kept identical.

**Fig. 4.** Reaction force from articular cartilage surface during displacement control tests (i.e., stress-relaxation experiments) were calculated for cartilage with different FCD values. Both porous free-draining and impervious indenter tips were employed for these calculations.

**Fig. 5.** The transient history of (A) axial strain ($E_{zz}$) in the loading direction, (B) solid matrix dilatation, and (C) fluid pressure. All variables are calculated at three different depths across the cartilage during stress-relaxation tests.
density increases the apparent stiffness of the tissue, which generates a smaller equilibrium deformation. Another important phenomenon revealed in Fig. 3 is that tissues with higher FCD reach equilibrium much faster. The characteristic time for the creep curve from 0.01 mEq/ml FCD is 263 s while the one for 0.1 mEq/ml FCD is 50 s. Therefore, the FCD affects not only the apparent stiffness of cartilage but also the flow-dependent transient responses (i.e., governed by tissue permeability) of the tissue under mechanical loading.

3.2. Results for stress relaxation test

The reaction force on indenter tip from the cartilage with various FCDs (0.05 versus 0.20 mEq/ml) and different indenter tip permeabilities i.e., porous free draining versus solid impervious, in stress relaxation tests are shown in Fig. 4. The tissue is subjected to a displacement $\varepsilon_0 = 10\%$ of its initial thickness (i.e., average strain) with a ramp time $t_0 = 400$ s. This loading protocol was previously adopted in the literature to study the mechanical
behaviors of cartilage (Mak et al., 1987, Spilker et al., 1992). Tissue with higher FCD showed a more distinct stiffening effect, consistent with the finding in indentation creep behaviors (Fig. 3) and those reported in previous experimental studies (Holmes et al., 1985, Frank and Grodzinsky, 1987, Lai et al., 1991). The impervious indenter tip (Fig. 4) generated higher reaction force than that of the porous free-draining tip before reaching the same equilibrium value. Fig. 5 showed the transient histories of three important physical parameters during the stress relaxation test: axial strain $E_{zz}$, dilatation of the solid matrix (tissue volume change), and fluid pressure, at three different depths across the tissue. The axial strain at the surface $(z/h=0)$, middle zone $(z/h=0.5)$, and deep zone $(z/h=1)$ showed significant differences during the relaxation phase. There is a recoil effect for $E_{zz}$ in both the surface and the middle zones (Fig. 5B). Due to the extremely low permeability of the solid matrix, the compression in $z$ direction generated high hydraulic pressure (Fig. 5C), and fluid drag forces inside the solid matrix further forced the matrix to expand in the radial direction. This mechanism endows the tissue a nearly incompressible character in the initial loading phase which is confirmed by the small volume change at the end of loading phase (Fig. 5B). As the fluid is being squeezed out, the solid matrix slowly recoiled back along the radial direction, and this process caused a redistribution of axial strain along the cartilage depth (Fig. 5A). These results indicated that, for the in situ situation, the middle zone cartilage is compressed the most in the loading direction (Fig. 5A), but has the lowest volume change (Fig. 5B). The largest tissue compressive dilatation occurs in the superficial zone (Fig. 5B), and the deep zone has the lowest axial strain. The fluid pressure across the tissue thickness direction is relatively uniform (Fig. 5C).

Fig. 6 shows the effect of indenter tip porosity on cartilage mechanical behaviors at three different depths under indentation test. Three major mechanical parameters, axial strain and axial stress in $z$ direction and the fluid pressure, are plotted versus radial position at time $t_0=400$ s, the end of the ramp phase of compression. First, for all three components, severe gradients exist at cartilage surface due to the sharp edge of cylindrical indenter tip. With increasing depth, smoother distributions are observed for all three variables. Second, significant differences exist in the magnitudes of these three variables for impervious and porous indenters in superficial zone, while such difference vanishes at the deeper zones. Third, the value of all variables diminishes to zero rapidly outside the neighborhood of the indenter tip. All these three phenomena can be simply yet thoroughly explained by St. Venant's principle in elasticity.

In cartilage indentation test, the aspect ratio $\kappa$ is an important parameter in experimental design (Bae et al., 2006). This number is defined as the ratio between indenter radius $a$ and cartilage thickness $h$. Fig. 7 shows the effect of aspect ratio on three major mechanical responses along tissue depth with the tissue thickness kept constant while the aspect ratio varies from 0.5 to 5. While the aspect ratio affects the radial strain distribution most significantly, it has less effect on the axial strain (Fig. 7A and B). Fig. 7B shows that the highest axial strain under indenter has similar magnitudes, although high aspect ratio generates an axial

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**Fig. 8.** Strain fields inside cartilage tissue under indentation tests at the peak-loading time point ($t_0=400$ s) and the final steady state ($t=12,000$ s).
Fig. 9. Effects of indenter tips on fluid pressure field inside cartilage tissue at peak-loading time point (t₀ = 400 s): (A) impervious indenter tip and (B) porous free-draining indenter tip. The pressure fields at equilibrium (C) for both setups are identical and similar with the pattern of the dilatation field of solid matrix (D).

4. Discussion

The governing equations of triphasic mixture theory were linearized and simplified by using a regular perturbation method and by the use of two potential functions (Eqs. (3)–(5)). This new formulation is applicable for any axisymmetric loading case, such as confined or unconfined compressions, hydraulic perfusion, and indentation. In the present study, numerical solutions for both creep and stress-relaxation tests were developed for indentation analysis. The results showed that the mechanical behaviors of indented cartilage, both the transient and equilibrium responses, are significantly affected by the proteoglycan endowed charged nature of solid matrix. The effect of geometric and physical properties of the indenter tip, cartilage thickness, and chemical properties of cartilage on the resulting mechanical fields across the tissues were investigated and presented. The results may provide valuable implications for studying chondrocyte mechanotransduction in different cartilage zones and designing appropriate loading profiles for tissue regeneration.

Certain theoretical limitations should be emphasized regarding the present study. The solid phase of tissue is simplified as a homogeneous, isotropic, linearly elastic material undergoing infinitesimal strain. In reality, the cartilage solid matrix is an inhomogeneous, anisotropic, nonlinear viscoelastic material, with a well-known nonlinear strain dependent permeability (Huang et al., 2005, Mow et al., 2005, Yao and Gu, 2007), and tension compression nonlinearity (Huang et al., 2005). The strain level found in this study also indicates a need for considering a finite deformation model, especially for the analysis around the stress concentration points. Despite the above limitations, applications of the linearized triphasic formulation to axisymmetric problems undergoing infinitesimal deformation is considerably simple. The major conclusions about cartilage indentation tests reported in the present study, as with all previously reported indentation analyses, appear to be valid and provide additional and valuable insights for cartilage indentation behavior.

Conflict of interest statement

The authors and collaborators have no conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jbimech.2009.10.026.

References


