Toward a Constitutive Law of Cartilage: A Polymer Physics Perspective

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Summary: To describe load bearing and lubrication of cartilage requires treating its collagen network and proteoglycan (PG) phases separately in a constitutive law of the tissue. We propose a framework for developing such an empirical constitutive law that treats the cartilage extracellular matrix (ECM) as a composite medium, with a PG phase that exerts a swelling pressure, and a collagen network phase that restrains it. We compare and contrast this model to a biomechanical constitutive law that aggregates the collagen and PG phases into a single "solid-like" elastic tissue matrix, and show that aggregation obscures essential differences in the physical-chemical properties of the collagen and PG constituents as well as their distinct biological roles within cartilage's ECM. We also relate moduli in the aggregate constitutive model to quantities measured in an osmotic stress titration experiment.

Keywords: aggregate; cartilage; collagen; composite; constitutive; ECM; osmotic; pressure; proteoglycan; swelling

Introduction

Cartilage is a remarkable material—able to lubricate joints and bear static and dynamic loads often throughout one's lifetime [1]. One feature that distinguishes cartilage from common engineering materials that perform lubricating and load-bearing functions is that its network consists entirely of "squishy" constituents, primarily Type II collagen and proteoglycans (PGs), along with water and mobile ions.

Understanding the physical basis of the functional (material) properties of cartilage is fundamental to tissue biophysics. In principle, this knowledge could be embodied in a constitutive law of cartilage, which relates the local stress developed within an infinitesimal block of tissue to physical-chemical variables that characterize the local state of the tissue's components. This constitutive relationship, in turn, could be used in conjunction with laws of momentum, mass, and charge conservation (with appropriate boundary and initial conditions) to predict the distributions of stress and strain within the tissue under various environmental conditions. The successful completion of this modeling

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program could help us understand better how cartilage microstructure, composition and material properties affect its behavior under static and dynamic joint loading.

The development of such a physically based constitutive law of cartilage is also essential for understanding the effects of endogenous and exogenous changes in tissue constituents on the functional properties of the aggregate tissue. Endogenous changes can occur normally in development or aging, or in degeneration and disease (like osteoarthritis) whereas exogenous changes can occur following the addition of a biochemical agent such as collagenase or trypsin, or potentially by external genetic manipulation. Moreover, we would like to understand differences in functional properties between tissues of different species (such as bovine and human), as well as between tissues from same species or even from different regions within the same joint. In tissue engineering, one would like to design extracellular matrix (ECM) with tailored mechano-chemical properties that ensure compatibility with the surrounding tissue and its ultimate integration.

An impediment to achieving these objectives, however, is that while static and dynamic loading processes and joint lubrication occur at the macroscopic or tissue length scale, the interactions that give rise to these processes occur at molecular, mesoscopic, and microscopic length scales. A challenge in tissue biophysics is to formulate a *macroscopic* constitutive law of cartilage in terms of variables and parameters that embody relevant physical and chemical processes and properties of cartilage's constituents at these finer length scales. To date, this goal has not been achieved.

As an interim pragmatic step, we propose an approach for developing an empirical constitutive law of cartilage. This is done by considering the three salient contributions to the total tissue stress: the retraction stress developed by the three-dimensional collagen network, the swelling stress developed by the PGs trapped within it, and the hydrodynamic stress developed by the pore fluid. Here, equations of state for the collagen network and PG phases are obtained using experimental data [2]. We compare and contrast this constitutive modeling approach with one promulgated in the biomechanics literature, which aggregates the collagen and PG phases into a single "solid-like" elastic tissue phase [3]. We relate moduli in the biomechanical constitutive model to quantities measured in an osmotic stress titration experiment.

Methods

The experimental data used here is given in Basser et al. [2], in which cartilage specimens were compressed osmotically by successively equilibrating them in polyethylene glycol

(PEG) solutions having different, known osmotic pressures. Using this "stress titration" data, along with a simple mathematical model, it is possible to obtain empirical equations of state (i.e., "pressure vs. volume" curves) for both the collagen network and PG phases individually. These can be used in constructing a constitutive law of the cartilage ECM.

To construct an empirical constitutive law, we use four equations. These are 1) the conservation of mass of tissue water and tissue constituents (PG and collagen), 2) the conservation of fixed charge, 3) an equation of state for the PGs (relating its concentration to the osmotic pressure), and 4) an equation of state for the collagen phase (relating its hydration to the osmotic pressure). These four equations relate four dependent variables that characterize the equilibrium state of cartilage [4]. We can solve these equations simultaneously for their equilibrium state variables as done in [2].

We can represent these data graphically using a convenient four-quadrant scheme as shown in Figure 1.

Data represented schematically in quadrant III are obtained from solution experiments obtained using extracted proteoglycans as described in Urban et al. [5] and Ehrlich et al. [6]. The $[FCD]_{eff}$ is the fixed charge density of the PGs (in mEq per gm of *extra*-fibrillar water). This represents the molar concentration of charges based upon the water volume in the compartment accessible to the PGs [4].

The equation of the conservation of charge is represented by the relation in quadrant IV, which requires that no fixed charge is lost or gained during compression or swelling of the tissue. This results in an inverse relationship between the $[FCD]_{eff}$ and the mass or volume of extra-fibrillar water in the tissue.

The equation of conservation of mass of the tissue constituents is shown in quadrant I, which just states that the total wet weight of the tissue must be the sum of the weight of the water in the extrafibrillar space, the water in the intrafibrillar space, and the dry weight of the polymeric components of the tissue. (Intrafibrillar water is the water that is collagen fibril associated water and the extrafibrillar water is the water associated with the PG and other constituents.)

The relationship shown in quadrant II illustrates empirical findings of Maroudas et al. [7] that the collagen fiber hydration, defined as the mass of intrafibrillar water, $m_{IF_{H2O}}$, divided by the measured mass of dry collagen, m_c, is a function of the net applied pressure acting on the collagen fibrils, which in our experiment is Π_{PG}.

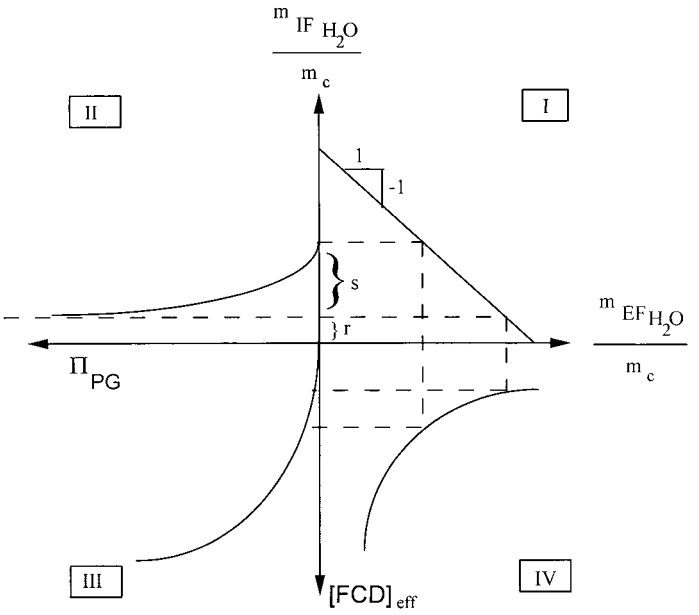

Figure 1. A four-quadrant model of cartilage tissue behavior and its graphical solution calculated from data obtained from a 90-year old normal human hip [2]. The first quadrant (I) contains the equation of conservation of mass. The fourth quadrant (IV) contains the equation of conservation of charge. The third quadrant (III) contains the empirical relationship between the effective fixed charge density, FCD_{eff}, and the total equilibrium osmotic pressure, Π_{PG}, exerted by the PGs. The second quadrant (II) contains the empirical equilibrium relationship between the applied stress acting on collagen network (which in this experiment is Π_{PG}) and the hydration of the collagen fibrils. Parameters r and s as represent the asympototic value and intercept of the curve. A stable solution results when a closed rectangular orbit evolves as shown above. Equilibrium values of the four dependent variables are also shown at the intersection points of the stable closed trajectory and the four coordinate axes. While these simultaneous equations are solved numerically, it is also instructive to see how a stable equilibrium solution evolves graphically. This is done simply by choosing a point on one of the curves and move clockwise or counter-clockwise parallel and perpendicular to the coordinate axes until a stable orbit is achieved.

The four equations displayed above completely specify the four dependent variables at mechanochemical equilibrium, once all experimental parameters for each specimen have been determined experimentally.

We solve these equations simultaneously using the Newton-Raphson method but it is instructive to see how the numerical equilibrium can be achieved graphically, as described in the caption to Figure 1.

Once the equilibrium value of Π_{PG} is determined, we can use the requirement that the tissue is in mechanical equilibrium to calculate the collagen network tensile stress, P_c from Π_{PEG}. Specifically, $\Pi_{PEG} = \Pi_{PG} - P_c$. Typical results are shown in Figure 2 from applying this approach for the stress titration experiment described above.

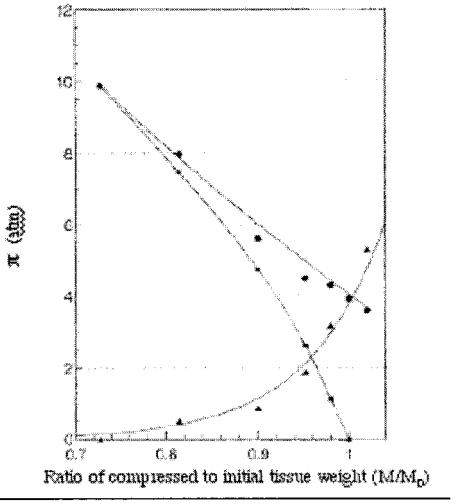

Figure 2. "Balance of forces'' concept illustrated in normal adult cartilage specimen (obtained with permission from [2]). The applied osmotic stress, π_{PEG} (shown as) equals the osmotic pressure of the cartilage proteoglycans, π_{PG} (shown as) less the collagen tensile stress, P_c (shown as). Each is plotted against the tissue's "wet weight" under compression, M, normalized by the tissue's "wet weight" under no load, M_0. Age of donor: 55 years.

Discussion

One notable feature shown in Figure 2, is the highly non-linear P_c vs M/M_0 curve of the collagen network. In particular, the collagen network has a non-zero resting or unstressed tissue wet weight (not shown) below which the network is effectively buckled, and above which, the collagen network resists any increase in tissue volume (or dilatation) at an increasing rate. This is indicated by the positive curvature or increasing slope of the P_c vs M/M_0 curve with increasing tissue volume as shown in Figure 2[1]. The Π_{PG} vs M/M_0 curve in Figure 2 exhibits a non-linear relationship with a negative slope. As tissue volume

increases, PGs become more dilute and their osmotic pressure decreases. Qualitatively similar behavior is observed in Boyle's law of an ideal gas.

Modern biomechanical models of cartilage began with McCutchen [8], who likened cartilage to a poro-elastic medium (like some soils, clays and rocks) that deform elastically while expressing fluid when loaded. This viewpoint was formalized and codified in a number of subsequent studies in which Biot's theory of poro-elasticity [9,10] was applied to describe cartilage's static and dynamic behavior [3]. Subsequently, mixture models [11] were proposed as more ionic phases were introduced to account for various observed electrokinetic effects [12], however, the "tissue matrix" in these models was consistently treated as a single elastic solid phase.

McCutchen also contributed significantly to the development of the biophysical picture of the cartilage matrix. He wrote, "Articular cartilage is, in effect, a gel dotted with cells, and permeated by a network of collagen fibers ... that ... are present in order to withstand tensile stresses imposed by the mechanical loads experienced in their service" [13]. Ogston [14] extended this picture of the cartilage ECM, proposing that cartilage's collagen network resists the osmotic swelling pressure exerted by proteoglycans. Moreover, he posited that the balance of forces between these two phases gives rise to the tissue's overall mechanical stiffness and load bearing ability. This idea was further developed and advanced by Maroudas and co-workers [15,16] and is embodied in the mathematical model and experimental framework described above.

The simplest way to illustrate the shortcomings of the biomechanical picture of cartilage is to consider a "free swelling" experiment in which there are no external loads or stresses applied on a cartilage tissue specimen. In the biomechanical model, the total tissue stress is given by [3]:

$$\tau_{ij}(c) = 2G_A(c)\varepsilon_{ij} + (\lambda_A(c)\varepsilon_{kk} - \beta_A(c))\delta_{ij} - P\delta_{ij} \qquad (1)$$

where τ_{ij} is the total stress tensor, ε_{ij} is the aggregate *tissue* strain tensor, ε_{kk} is the aggregate *tissue* dilatation, $G_A(c)$ is the aggregate shear modulus of the tissue, $\lambda_A(c)$ is the aggregate Lamé constant of the tissue, $\beta_A(c)$ is the aggregate chemical stress (analogous to

[1] Qualitatively similar behavior is observed (in one dimension) when stretching a nylon stocking. It too has a non-zero unstressed length, and then becomes increasingly stiff as more tensile force is applied

a thermal stress), P is the hydrostatic pressure, δ_{ij} is the identity tensor, and c is the concentration.

In mechanical equilibrium, when the tissue is kept at a constant ambient pressure, we have $P = 0$. In normal physiological saline, we can set $\beta_A(c) = 0$, which is an appropriate reference value. Also, since the applied stress on the cartilage tissue is zero, $\tau_{ij}(c) = 0$, which implies that $\varepsilon_{ij}(c) = 0$. Thus, the aggregate model of cartilage tells us that in normal saline, the unloaded tissue is both stress free and strain free. We see above in Figure 2 that this "no-stress condition" for the aggregate tissue corresponds to the case in which the tissue is unloaded, i.e., when $M/M_0 = 1$. While the tissues as a whole may be unloaded, there are significant internal stresses developed within it. We see above that at $M/M_0 = 1$, the collagen network tensile stress is approximately 4 atm., which is exactly balanced by the osmotic swelling pressure of 4 atm. exerted by the PGs! This is approximately the gas pressure developed in a bicycle tire to keep it inflated.

We can also relate various moduli in our two-component model of the cartilage matrix under isotropic loading to biomechanical models that aggregate the cartilage matrix into a single phase. In equilibrium isotropic loading, we have $P = 0$; and in normal physiological saline, again we can set $\beta(c) = 0$. We can add up the stress applied, τ_{ij}, on each face of incremental tissue block as follows[2]:

$$\tau_{ij}\,\delta_{ij} = \Delta\Pi_{PEG}\,\delta_{ij}\,\delta_{ij} = 3\,\Delta\Pi_{PEG} \qquad (2)$$

The right hand side of the equation becomes

$$\left(2G_A(c)\varepsilon_{ij} + \left(\lambda_A(c)\varepsilon_{kk} - \beta_A(c)\right)\delta_{ij}\right)\delta_{ij} = \left(2G_A(c) + 3\lambda_A(c)\right)\varepsilon_{kk} \qquad (3)$$

We can now obtain an expression for the isotropic compressibility:

$$\frac{\Delta\Pi_{PEG}}{\varepsilon_{kk}(c)} = \frac{2G_A(c) + 3\lambda_A(c)}{3} = K_A(c) \qquad (4),$$

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[2] Below we use the Einstein convention, in which the recurrence of any index means one should sum over that index.

and recognize this ratio equals the "aggregate" bulk modulus, $K_A(c)$.

Since in an equilibrium experiment in which the tissue is isotropically loaded by a small applied pressure, $\Delta\Pi_{PEG}$, the tissue dilatation, $\varepsilon_{kk}(c)$, can be approximated by

$$\varepsilon_{kk} \approx \frac{V^{eq'} - V^{eq}}{V^{eq}} \tag{5}$$

where V^{eq} is the volume of tissue in the unloaded equilibrium state, and $V^{eq'}$ is the volume of tissue in equilibrium subject to the isotropic load, $\Delta\Pi_{PEG}$. It is not difficult to show that the change in the applied isotropic stress on the cartilage network divided by its fractional change in equilibrium volume can be approximated by:

$$\frac{\Delta\Pi_{PEG}}{\frac{V^{eq'} - V^{eq}}{V^{eq}}} \approx V^{eq}\left(\left.\frac{d\Pi_{PG}}{dV}\right|_{V=V^{eq}} - \left.\frac{dP_c}{dV}\right|_{V=V^{eq}} \right) \tag{6}$$

From this expression, we see that the ability of the cartilage network to resist isotropic compression arises from the PG's osmotic compressibility AND that of the collagen network's. Moreover, combining the three equations above, we see that

$$K_A(c) \approx V^{eq}\left(\left.\frac{d\Pi_{PG}}{dV}\right|_{V=V^{eq}} - \left.\frac{dP_c}{dV}\right|_{V=V^{eq}} \right) \tag{7}$$

Therefore, we have related the aggregate bulk modulus of the tissue to the individual pressure vs. volume curves of the PG and collagen network phases.

Because P_c vs V and Π_{PG} vs V are both monotonic functions, the stability of mechanical equilibrium is assured. The system always returns to its equilibrium state after a perturbation from it. This is crucially important, because cartilage, like man-made composites, such as reinforced concrete, would be poor structural materials if they could assume a multiplicity of equilibrium configurations under the same static loading conditions. Such an elastic instability could lead to a catastrophic structural failure. Many non-electrolyte and polyelectrolyte gels [17] that exhibit multiple stable equilibrium states can change volume discontinuously at thermodynamic equilibrium. If cartilage exhibited

such behavior, then, for the same Π_{PG} there might be several stable (or unstable) equilibrium tissue volumes, likely leading to a mechanical failure of the tissue. However, one fundamental difference between the architecture of cartilage and that of a simple cross-linked polyelectrolyte gel is that in cartilage, the PG constituent that produces the osmotic swelling pressure and the collagen network that resists it are distinct, whereas in simple cross-linked polyelectrolyte gel the charged groups causing the swelling and the cross-links producing the elastic restraint are all part of the same polymer. By separating these two phases, Nature may have precluded the possibility that cartilage exhibits these catastrophic critical phenomenon (at least in the physiological regimes we have studied). One could also speculate that this is why organisms do not make cartilage using only cross-linked, long-chain polyelectrolyte gels (such as hyaluronic acid) even though they could be synthesized at a lower metabolic cost.

Besides drawing distinctions between cartilage and polyelectrolyte gels, it is also important to distinguish it from a sponge or a clay. Following McCutchen's seminal suggestion that in dynamic loading, cartilage deforms like a sponge or saturated soil [8], subsequent biomechanical models of cartilage behavior described its material properties using constitutive laws that are identical to those used to describe rocks and soils [18]. Cartilage was modelled like a clay, consisting of a "pore fluid" phase and a single "elastic network" phase. As our study demonstrates, to understand even the simple equilibrium behavior of cartilage requires a more biologically realistic model of the ECM.

Regrettably , aggregation of the distinct cartilage matrix phases into a single phase has lead to the widespread use of the equilibrium (unstressed) state of the tissue specimen as the reference state from which the tissue's strain is measured, and from which the tissue's stress is calculated. As we have shown, when the cartilage tissue matrix is in its "unstrained" (unloaded equilibrium) state, i.e., $\varepsilon_{ij} = 0$, the collagen matrix is swollen to about 115% of its rest volume and is supporting a tensile stress of 4 atm (0.4 MN/m^2)!

Moreover, our previous data obtained from normal, osteoarthritic, and enzyme depleted cartilage samples, show that biological changes associated with degeneration or degradation significantly affect the equilibrium tensile stress and PG osmotic pressure, as well as the degree of deformation of the collagen network in the unloaded equilibrium state [2]. When we try to make comparisons between different tissues, we should make them with their collagen networks in the same state of deformation. Otherwise, comparisons among tissues obfuscate rather than clarify differences in mechanical properties.

By separating individual contributions of the collagen network and PG phases, we are for the first time able to predict behavior that cannot presently be gleaned from existing aggregation models. For example, we can now determine a) collagen's unstressed volume or hydration, b) its deformation with respect to its unstressed state c) the network tensile stress and the PG osmotic pressure when the tissue is unloaded in equilibrium, d) the network tensile stress and the PG osmotic pressure when the tissue is isotropically loaded in equilibrium; and e) the compressibilities of the collagen network or PG at equilibrium and their dependence upon tissue volume.

Since the collagen network is largely uncharged at physiological pH, its properties are largely independent of perturbations of ionic strength around physiological conditions. Although they are may not directly be affected by charge, the collagen network state will be influenced by changes in ionic strength because it has a dramatic effect on the osmotic swelling pressure exerted by the GAGs on the collagen network (primarily by affecting their mutual electrostatic repulsion, and secondarily, entropic interactions). In general, aggregation masks effects of any physical-chemical or biochemical process that affects collagen and GAGs selectively or differentially. This includes changes in temperature, pH, solvent composition, and ionic strength, enzymatic digestion, etc.

Since the "biphasic" and subsequent "triphasic" models aggregate the contributions of the collagen network and GAGs into a single "solid-like" tissue. Thus, they cannot be used retrospectively to predict the individual states of stress or strain of these constituents. For example, the triphasic theory does not allow us to predict how inflated or distended the collagen network is with respect to its own unstressed or resting state for a particular set of environmental conditions, or even what the collagen's unstressed configuration is. It cannot tell us how much osmotic pressure the GAGs exert on the collagen network at equilibrium. It cannot tell us what the individual compressibilities of the collagen network or GAGs molecules are at equilibrium.

Conclusions

Although not analytically derived, the new empirical constitutive law represents a departure from existing continuum models of cartilage behavior. Moreover, the mechanical, electrical and chemical consequences of changes in their properties can also be examined both qualitatively and quantitatively.

Model assessment is also simple to implement either graphically or numerically. This simplicity is achieved, in part, by combining conservation laws (e.g., conservation of

momentum and charge) that must hold, with phenomenological constitutive laws of each polymeric phase that we measure. Our use of this empirical approach, however, is a pragmatic requirement as existing theoretically derived constitutive laws do not predict experimental findings adequately. In particular, there is no adequate mathematical model of electrostatic and entropic interactions between PGs to replace our empirical relationship between osmotic pressure and fixed charge density. There is also no adequate model of the non-linear behavior of the collagen network that we observed in our free-swelling experiments to replace our constitutive relationship between collagen network pressure, P_c and tissue volume, V. We would strongly encourage more physics-based research directed to deriving the form of these empirical constitutive laws from basic principles.

This constitutive model expresses formally what the biochemical and physiological communities have known intuitively for decades, but was not incorporated into mathematical models of cartilage behavior -- that in cartilage, at a sub-microscopic scale, PGs produce the osmotic swelling pressure and the collagen network resists it. The subtle Yin-Yang balance between these two physically and chemically distinct constituents of cartilage, with distinct physiological functions, and distinct roles in the etiology of extracellular matrix pathologies, is essential to our understanding of cartilage behavior. While in equilibrium free-swelling, the importance of the contributions of each constituent is immediately apparent, in other biologically relevant loading regimens, such as those occurring during locomotion, the proper characterization of cartilage dynamics will also require treating the PGs and collagen as separate phases.

In our model, the contributions of the PG phase that causes the tissue to swell, and the collagen network that resists swelling, are treated separately. This simplification requires further examination. While the PG and collagen constituents of cartilage have distinct physico-chemical properties, as well as perform distinct physiological and biochemical functions which aggregation into a single "solid-like" phase obscures, we must still test for and allow for the possibility that they interact beyond the simple fact that they both "compete" for the same water molecules and ions.

A challenge remains to develop an analytical continuum model that can describe the dynamic behavior of cartilage under loading (such as consolidation and lubrication) while still treating the collagen and PG phases separately (albeit interacting). Any description of cartilage dynamics in the limit of equilibrium free swelling, however, should be comparable to what we have reported here.

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