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Determining equilibrium osmolarity in poly(ethylene glycol)/chondrotin sulfate gels mimicking articular cartilage

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HIGHLIGHTS

- A comprehensive model of a multi-phase, multi-species polyelectrolyte gel model is proposed.
- Model features include: elasticity via covalent cross-links in the polymer network, microscopic nearest-neighbor interaction between the various polymer/solvent species, electrostatic and osmotic pressure.
- *In vivo* studies are conducted and the equilibrium electrochemical composition are compared with the model output.
- It is generally seen that swelling is aided in the case of a highly charged gel with low cross-link fractions or the ones immersed in a bath with low solute concentration or a high pH.
- A de-swelled state of the gel is preferred at high cross-link fraction and higher average charge per monomer.

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ABSTRACT

We present an experimentally guided, multi-phase, multi-species polyelectrolyte gel model to make qualitative predictions on the equilibrium electro-chemical properties of articular cartilage. The mixture theory consists of two different types of polymers: poly(ethylene gylcol) (PEG), chondrotin sulfate (ChS), water (acting as solvent) and several different ions: H^+, Na^+, Cl^-. The polymer chains have covalent cross-links whose effect on the swelling kinetics is modeled via Doi rubber elasticity theory. Numerical studies on equilibrium polymer volume fraction and net osmolarity (difference in the solute concentration across the gel) show a complex interplay between ionic bath concentrations, pH, cross-link fraction and the average charge per monomer. Generally speaking, swelling is aided due to a higher average charge per monomer (or a higher particle fraction of ChS, the charged component of the polymer), low solute concentration in the bath, a high pH or a low cross-link fraction. A peculiar case arises at higher values of cross-link fraction, where it is observed that increasing the average charge per monomer leads to gel deswelling.

1. Introduction

Articular cartilage is a polyelectrolyte biogel that forms a thin tissue layer lining the articulating ends of all diarthrodial joints in the body and contribute to the lubrication mechanisms in the joint (Mow and Ratcliffe, 1997). The water phase of cartilage constitutes on average from 60 to 80% of the total weight for normal tissue while the dominant structural components of the solid matrix are the collagen molecules and proteoglycans (PGs) (Mow et al., 1993). The collagen fibrils are densely packed polymer strands with a high resistance against fluid flow (Armstrong and Mow, 1982), which helps in retaining the shape of the cartilage when compressed (Maroudas, 1979). PGs are macromolecules consisting of a protein core to which are attached short chains of negatively charged glycosaminoglycans (GAGs) (Setton et al., 1993). The primary GAGs associated with PGs in cartilage are chondroitin 4-sulfate, chondroitin 6-sulfate and keratan sulfate (Maroudas, 1979).

An increase in cartilage tissue hydration, governed by the density and the nature of fixed charges on the PGs as well as the density of the mobile counter ions in the interstitial fluid are the earliest signs of articular cartilage degeneration during

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osteoarthritis (Armstrong and Mow, 1982; Maroudas, 1979). For this reason the development of mathematical models and numerical methods to quantify cartilage swelling have been of great interest for many years. Each PG-associated negative charge on the polymer chain (known as the fixed charge), requires a mobile counter-ion (e.g., Na^+) dissolved within the interstitial fluid in the cartilage gel to maintain its electro-neutrality (Maroudas et al., 1991). This effect gives rise to an imbalance of mobile ions across the gel interface. The excess of mobile ions colligatively yields a swelling pressure, known as the osmotic pressure (Maroudas, 1968), while the swelling pressure associated with the fixed charges is known as the Donnan pressure (Donnan, 1924). Changes in this internal swelling pressure, arising from altered ion concentrations of the external bath, or changes in the fixed charges result in changes in tissue dimensions and hydration. However, this swelling pressure is balanced by tensile forces generated in the collagen network (Muir et al., 1970). This effect is due to the presence of covalent cross-links within the gel matrix.

Mathematical models are essential for understanding systems with many interacting components (e.g. the collagen network, proteoglycans, interstitial fluid and ionic solutions present in cartilage), since the complexity of the interactions taking place overwhelms the ability of the human intuition to make accurate predictions of their behavior. Early work in this direction began with the classical work of Flory (1953), Flory and Rehner (1943a, 1943b) and Katchalsky and Michaeli (1955) (see also Doi and Edwards, 1986; Flory, 1976) to understand the swelling and deswelling of hydrogels. In this theory, the free energy is used to make predictions about the thermodynamical equilibrium configurations of polymer gels and their dependence on environmental parameters such as temperature or solvent ion concentrations. An important problem is to understand how the kinetics (and not simply the equilibria) of swelling and deswelling is governed. An early answer was given by Tanaka and Filmore (1979) and Tanaka et al. (1980) who developed a kinetic theory of swelling gels viewing a gel as a linear elastic solid immersed in a viscous fluid. Subsequent studies relaxed the assumption of linear elasticity by defining the force on the gel to be the functional derivative of the free energy function for the polymer mesh (Durning and Morman, 1993; Maskawa et al., 1999; Onuki, 1989; Sekimoto et al., 1989; Yamaue et al., 2000). However, most of these works neglected the fluid flow that must accompany swelling. Wang et al. (1997) added fluid flow by application of two-phase flow theory but considered only small polymer volume fractions and small gradients in the volume fraction. Durning and Morman (1993) also used continuity equations to describe the flow of solvent and solution in the gel, but used a diffusion approximation with a constant diffusion coefficient to determine the fluid motion. Similarly, mathematical models and simulations of polyelectrolyte gels have been developed by other groups, in an effort to examine the effect of fixed charge density via Donnan potential (English et al., 1996; Wolgemuth et al., 2004; Hou et al., 1989; Lai et al., 1991; Gu et al., 1998), Nernst-Planck motion of ions (Setton et al., 1993; Dhanarajan et al., 2002) and van't Hoff osmotic pressure (Lanir, 1996), in cartilage. These efforts include using the Poisson–Boltzmann equation and Biot's theory of poroelasticity (De et al., 2002), as well as phenomenological modifications of Flory–Higgins theory to capture some effects of multi-ionic cations (Kokufuta, 2005; Hirotsu, 1991; Zribi et al., 2006). However, these current state of the art models do not couple the binding/unbinding of the ions to the network micro-structure (Calderer et al., 2013). The National Institute of Health (NIH) states that these binding/unbinding events are critical to describing the local ion environment inside cartilage, the environment that cells ‘see’, and the bulk mechanical properties of the tissue and/or polymer (Health, United States, 2006).

In this regard, the innovative approach of this work is to create the next generation, multi-phase, mechanistic ionic gel-swelling model which mimics the equilibrium, free swelling/deswelling environment within the articular cartilage and which captures all of the fundamental scientific elements mentioned above. Using this model we will outline how to extract the information from the macro-scale data of gel-swelling (derived via in vivo experiments) and help explain some micro-scale effects (e.g., details on polymer cross-linked structure). To calibrate the outcome of this model, in vitro set-ups of cross-linked copolymerized poly(ethylene glycol) (PEG) and chondrotin sulfate (ChS) gels were developed (Bryant and Anseth, 2003; Bryant et al., 2004; Villanueva et al., 2010). PEG was chosen since it can be functionalized to enable cross-linking to form a 3-D matrix, a system that promotes the deposition of proteoglycans and collagen molecules and emulates the mechanical strength, load bearing capabilities and resilience of cartilage tissue (Bryant and Anseth, 2003). The ChS component serves two purposes: to mimic the biochemical environment of cartilage (since it is the main component of proteoglycans) and to introduce fixed negative charges into the network (Bryant et al., 2004). In the next section, we present the details of this model, including the chemical potentials (Section 2.1) and the chemistry of the binding reaction at quasi-equilibrium conditions (Section 2.2). Further, we provide the details of the in vitro gel-swelling experiments (Section 3) and the methods to estimate the model parameters (Section 4). The results pertaining to the equilibrium configuration of these ionic gels under different electro-chemical stimuli are presented in Section 5. We conclude with a brief discussion of the implication of these results and the focus of our future directions.

2. Multi-species, multi-phase cartilage-gel model

We view the PEG-gel with negatively charged ChS strands in ionic solution, as a multi-component material, including solvent particles, polymer and particles of several ionic species. The polymer is assumed to be made up of two types of monomers the uncharged units (PEG segment of the polymer) and those that are the charged ones (ChS segment of the polymer). Only the charged units participate in the binding reactions, carry a double negative charge and are denoted as M^{2-}. The positively charged ions in the solvent are Hydrogen (H^+) and Sodium (Na^+). The negatively charged ions could include Hydronium (OH^-) and Chloride (Cl^-). Because the negatively charged ions are assumed to be not involved in any binding reactions with the gel, acting only as counterions to positive charges, we identify these ions by the name Chloride. The binding reactions of the positively charged ions with the monomers are

$$\begin{aligned}
&(a)\ M^{2-} + H^+ \underset{k_{-h}}{\overset{k_h}{\rightleftharpoons}} MH^-,\\
&(b)\ M^{2-} + Na^+ \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} MNa^-,\\
&(c)\ MH^- + H^+ \underset{k_{-h2}}{\overset{k_{h2}}{\rightleftharpoons}} MH_2,\\
&(d)\ MNa^- + Na^+ \underset{k_{-n2}}{\overset{k_{n2}}{\rightleftharpoons}} MNa_2,\\
&(e)\ MH^- + Na^+ \underset{k_{-hn}}{\overset{k_{hn}}{\rightleftharpoons}} MHNa. \qquad (1)
\end{aligned}$$

We assume that all the binding sites/charge sites are identical and the binding affinities for the different ions are different. The species M^{2-}, MH^-, MNa^-, MH_2, MNa_2 and MHNa are different monomer species of the charged type, all of which move with the same polymer velocity. The ion species are freely diffusible, but because they are ions, their movement is restricted by the requirement to maintain electroneutrality. Finally, because a small amount of water dissociates into hydrogen and hydronium, we are