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Osmotic Loading of Spherical Gels: A Biomimetic Study of Hindered Transport in the Cell Protoplasm

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Abstract

Osmotic loading of cells has been used to investigate their physicochemical properties as well as their biosynthetic activities. The classical Kedem-Katchalsky framework for analyzing cell response to osmotic loading, which models the cell as a fluid-filled membrane, does not generally account for the possibility of partial volume recovery in response to loading with a permeating osmolyte, as observed in some experiments. The cell may be more accurately represented as a hydrated gel surrounded by a semi-permeable membrane, with the gel and membrane potentially exhibiting different properties. To help assess whether this more elaborate model of the cell is justified, this study investigates the response of spherical gels to osmotic loading, both from experiments and theory. The spherical gel is described using the framework of mixture theory. In the experimental component of the study alginate is used as the model gel, and is osmotically loaded with dextran solutions of various concentrations and molecular weight, to verify the predictions from the theoretical analysis. Results show that the mixture framework can accurately predict the transient and equilibrium response of alginate gels to osmotic loading with dextran solutions. It is found that the partition coefficient of dextran in alginate regulates the equilibrium volume response and can explain partial volume recovery based on passive transport mechanisms. The validation of this theoretical framework facilitates future investigations of the role of the protoplasm in the response of cells to osmotic loading.

Introduction

Osmotic loading of cells has been used to investigate their physicochemical properties as well as their biosynthetic activities. The classical theoretical framework for analyzing volumetric changes in cells in response to osmotic loads is irreversible thermodynamics [1–3]. In this framework the cell is usually modeled as a fluid-filled membrane [4–8] and is often described as a perfect osmometer. Generally, the cell membrane is either permeable or non-permeable to the osmolyte. For a permeating osmolyte the cell volume change in response to osmotic loading is transient, returning to its original value after sufficient time has elapsed [4]. For a non-permeating osmolyte however, the volume change is sustained and this mode of loading serves as the basis for demonstrating that cells obey the Boyle-van't Hoff relation. It is interesting that this framework does not generally account for the possibility of partial volume recovery in response to loading with a permeating osmolyte, as observed in some experiments [9].

In our recent theoretical study [10], it was demonstrated that the framework of mixture theory [11–18] can be used to generalize the classical Kedem-Katchalsky model for osmotic loading

of cells [4,5]. Our model similarly described the cell as a fluid-filled membrane. However, by accounting for the possibility that the partition coefficient of the permeating osmolyte between the protoplasm (cytoplasm, cytoskeleton, and all enclosed organelles) and external solution is less than unity, this mixture model was able to predict partial volume recovery.

A partition coefficient less than unity implies that the protoplasm behaves as a hydrated gel which limits the solubility of the permeating osmolyte. Such a gel would potentially have different transport properties (water permeability, solute diffusivity) and mechanical properties (elastic moduli) than the external environment. Thus the cell may be more accurately represented as a hydrated gel surrounded by a semi-permeable membrane, with the gel and membrane potentially exhibiting different properties. Such increased complexity in the modeling of a cell would be justified if the gel-like behavior of the protoplasm were to significantly influence the cell's response to osmotic loading.

To help assess whether this more elaborate model of the cell is justified, we propose to first investigate the response of spherical gels to osmotic loading, both from experiments and theory. The objective is to determine experimentally how a spherical gel responds to osmotic loading and whether this behavior is predictable from theory. In this study, the spherical gel is described using the same mixture theory framework used in our earlier approach for modeling a fluid-filled spherical membrane. In the experimental component of the study alginate is used as the model gel, and is osmotically loaded with dextran solutions of various concentrations and molecular weight, to verify the predictions from the theoretical analysis. A validation of the theoretical framework will facilitate future investigations of the role of the protoplasm in the response of cells to osmotic loading.

Theoretical Analysis

Governing Equations

In our recent study on solute transport in dynamically loaded gels [19], the governing equations of mixture theory [12,16–18] were reduced to the special case of intrinsically incompressible, neutrally charged solid, solvent and solute phases, where the solution is assumed to be ideal (solute activity coefficients and osmotic coefficients of unity), and the solid phase is linear isotropic elastic. The resulting equations are summarized here:

$$\mathbf{w} \equiv \phi^w (\mathbf{v}^w - \mathbf{v}^s) = -\tilde{k} \left[\text{grad } p - R\theta \sum_{\alpha \neq s, w} \left(1 - \frac{D^\alpha}{D_o^\alpha} \right) \text{grad } c^\alpha \right] \quad (1)$$

$$\mathbf{j}^\alpha \equiv \phi^w c^\alpha (\mathbf{v}^\alpha - \mathbf{v}^s) = -\phi^w D^\alpha \text{grad } c^\alpha + \frac{D^\alpha}{D_o^\alpha} c^\alpha \mathbf{w}, \alpha \neq s, w \quad (2)$$

$$\frac{\partial(\phi^w c^\alpha)}{\partial t} + \text{div}(\mathbf{j}^\alpha + \phi^w c^\alpha \mathbf{v}^s) = 0, \alpha \neq s, w \quad (3)$$

$$\text{div}(\mathbf{v}^s + \mathbf{w}) = 0, \quad (4)$$

$$-\text{grad } p + (\lambda_s + \mu_s) \text{grad}(\text{div } \mathbf{u}) + \mu_s \nabla^2 \mathbf{u} = \mathbf{0}. \quad (5)$$

where

$$\tilde{k} = \frac{1}{\frac{1}{k} + \frac{R\theta}{\phi^w} \sum_{\alpha \neq s, w} \left(1 - \frac{D^\alpha}{D_0^\alpha}\right) \frac{c^\alpha}{D_0^\alpha}}. \quad (6)$$

These are generalized equations for diffusion-convection problems in deformable porous media, where the solution is dilute. In these expressions, ϕ^w is the solid matrix porosity, which depends on the matrix dilatation according to

$$\phi^w = \phi_r^w + (1 - \phi_r^w) \text{div } \mathbf{u} \quad (7)$$

where ϕ_r^w is the porosity in the reference configuration of zero deformation. c^α is the solute concentration on a solvent volume basis (and $\phi^w c^\alpha$ is the solute concentration on a mixture volume basis); $\mathbf{u}$ is the solid matrix displacement and $\mathbf{v}^s = D^s \mathbf{u} / Dt$ is the solid matrix velocity; $\mathbf{w} = \phi^w (\mathbf{v}^w - \mathbf{v}^s)$ is the volumetric flux of solvent relative to the solid matrix ($\mathbf{v}^w$ = solvent velocity); $\mathbf{j}^\alpha = \phi^w c^\alpha (\mathbf{v}^\alpha - \mathbf{v}^s)$ is the molar flux of solute α relative to the solid matrix ($\mathbf{v}^\alpha$ = solute velocity); p is the fluid (solvent) pressure; R is the universal gas constant and θ is the absolute temperature. The material properties of the mixture (the gel in this model) are given by the elastic moduli λ_s, μ_s (Lamé constants of a linear elastic isotropic solid matrix) and the hydraulic permeability of the gel to pure solvent, k ; $\tilde{k}$ is the hydraulic permeability of the gel to the solution (solvent + solutes); D^α is the solute diffusivity in the gel and D_0^α is its diffusivity in pure solvent. In general, due to steric exclusion effects and tortuosity, D^α is smaller than D_0^α . Note that Eq. (1) is a generalized form of Darcy's law, derived from the momentum equation for the solvent; Eq.(2) is a generalized form of Fick's first law of diffusion, derived from the momentum equation for each solute; Eq.(3), which is a generalized form of Fick's second law of diffusion, is the balance of mass equation for each solute; Eq.(4) is the balance of mass for the mixture; and Eq.(5) is the balance of momentum for the mixture. Given appropriate boundary conditions, the coupled nonlinear partial differential equations in Eqs.(1)–(5) can be solved for $p, \mathbf{u}$ (or equivalently $\mathbf{v}^s$), $\mathbf{w}$, c^α , and $\mathbf{j}^\alpha$ ($\alpha \neq s, w$).

Spherical Gel Model

In this analysis a homogeneous spherical gel of radius a is subjected to osmotic loading from an external bath. For simplicity, only one solute species is considered so that superscripted α 's are dropped. The problem is analyzed using a spherical coordinate system with its origin at the center of the gel. Considering a spherically symmetric analysis, the non-zero dependent variables in this problem are $p = p(r, t)$, $u = u(r, t)$, $w = w(r, t)$, $c = c(r, t)$ and $j(r, t)$, where r is the radial coordinate, t is time, and u , w , and j are the radial components of $\mathbf{u}$, $\mathbf{w}$ and $\mathbf{j}$, respectively. It is assumed that the external bath (which contains the same solvent and solute species as the gel, but no solid matrix) is well mixed. Thus its solute concentration can be imposed as a boundary condition. Eqs.(1)–(5) are first expressed in component form in this spherical coordinate system; then the component form of Eq.(4) can be integrated analytically to yield $w = -v^s$ (where $v^s \approx \partial u / \partial t$), making use of the boundary condition $w(0, t) = v^s(0, t) = 0$.

Substituting this result into the component form of Eqs.(3) and (5) produces a coupled set of nonlinear partial differential equations in the unknowns c and u ,

$$\frac{\partial(\phi^w c)}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \left[-\phi^w D \frac{\partial c}{\partial r} + \phi^w c \left(1 - \frac{D}{\phi^w D_0} \right) \frac{\partial u}{\partial t} \right] \right) = 0, \quad (8)$$

$$H_A \frac{\partial}{\partial r} \left(\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u) \right) - \frac{1}{k} \left[1 + \frac{k R \theta}{\phi^w} \left(1 - \frac{D}{D_0} \right) \frac{c}{D_0} \right] \frac{\partial u}{\partial t} - R \theta \left(1 - \frac{D}{D_0} \right) \frac{\partial c}{\partial r} = 0, \quad (9)$$

where $H_A = \lambda_s + 2\mu_s$ is the aggregate modulus of the solid phase. In addition, from Eqs.(1)–(2) and (7),

$$j = -\phi^w D \frac{\partial c}{\partial r} - \frac{D}{D_0} c \frac{\partial u}{\partial t}, \quad (10)$$

$$\frac{\partial p}{\partial r} = \frac{1}{k} \frac{\partial u}{\partial t} + R \theta \left(1 - \frac{D}{D_0} \right) \left[\frac{c}{\phi^w D_0} \frac{\partial u}{\partial t} + \frac{\partial c}{\partial r} \right], \quad (11)$$

$$\phi^w = \phi_r^w + (1 - \phi_r^w) \left(\frac{\partial u}{\partial r} + \frac{2}{r} u \right). \quad (12)$$

The boundary conditions required to solve Eqs.(8)–(9) are derived from the condition of zero displacement and zero flux at the origin, which produces

$$u(0, t) = 0, \quad \left. \frac{\partial c}{\partial r} \right|_{r=0} = 0, \quad (13)$$

and jump interface conditions required to enforce continuity of mass, total traction, and chemical potentials of permeating species at the interface with the external solution, $r = a$ (see [19]),

$$c(a, t) = \kappa c^*(t), \quad (14)$$

$$H_A \left[\left. \frac{\partial u}{\partial r} \right|_{r=a} + \frac{2\nu}{1-\nu} \frac{u(a, t)}{a} \right] = -R \theta (1 - \kappa) c^*(t), \quad (15)$$

where $c^*(t)$ is the concentration of permeable solute in the external bath and κ is its partition coefficient between the inside and outside of the cell under equilibrium conditions [20,21]; ν

is Poisson's ratio for the solid matrix (where $\lambda_s/H_A = \nu/(1-\nu)$). To get p , Eq.(11) should be integrated subject to the boundary condition

$$p(a, t) = p^* - R\theta(1 - \kappa)c^*(t) \quad (16)$$

derived from the continuity of solvent chemical potential, where p^* is the ambient pressure in the external bath. The cell volume is given by

$$V(t) = \frac{4\pi a^3}{3} \left[1 + \frac{u(a, t)}{a} \right]^3 \approx \frac{4\pi a^3}{3} \left[1 + 3 \frac{u(a, t)}{a} \right], \quad (17)$$

recognizing that $u/a = 1$ in a small strain analysis.

To determine equilibrium conditions, such as the initial condition or the equilibrium response to a step osmotic load, the governing equations in Eqs.(8)–(9) are solved with $\partial/\partial t = 0$, yielding

$$u(r, t \rightarrow \infty) = u_{eq}(r) = \xi r, \quad c(r, t \rightarrow \infty) = c_{eq}, \quad (18)$$

where ξ and c_{eq} are constants. From the boundary conditions of Eqs.(14) and (15),

$$\xi = - \frac{R\theta(1 - \kappa)c_{eq}^*}{3K}, \quad c_{eq} = \kappa c_{eq}^*, \quad (19)$$

where c_{eq}^* is the prescribed external concentration of solute under equilibrium conditions and $K = H_A(1+\nu)/3(1-\nu)$ is the bulk modulus of the gel solid matrix. Recognizing that the initial condition has the same form and substituting this result into Eq.(17) yields

$$\frac{V(\infty)}{V(0)} = \frac{V_{eq}}{V_r} \approx \frac{1 - R\theta(1 - \kappa)c_{eq}^*/K}{1 - R\theta(1 - \kappa)c_r^*/K} \quad (20)$$

where V_{eq} is the volume at equilibrium and V_r is the volume in the reference state (initial volume), when the external concentration is c_r^* . Note that this analysis is valid for both hyper-osmotic loading ($c_{eq}^* > c_r^*$) and hypo-osmotic loading ($c_{eq}^* < c_r^*$), although the experimental component of the current study focuses on the former. The above equation shows that the normalized volume response at equilibrium varies linearly with the non-dimensional parameter $R\theta(1 - \kappa)c_{eq}^*/K$ and it becomes evident from this relation that the partition coefficient κ and bulk modulus K cannot be assessed independently from the equilibrium response to osmotic loading. In practice, additional experiments would be required to measure at least one of these parameters using a different testing configuration.

Special Case: No Solute Transport Into the Gel—The general equations for this problem are nonlinear and cannot be solved in closed-form. However, in the special case when

the solute cannot permeate into the gel, ($\kappa = 0$, $c(r, t) = 0$), the only remaining governing equation is the reduced form of Eq.(9),

$$\frac{\partial}{\partial r} \left(\frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u) \right) - \frac{1}{H_A k} \frac{\partial u}{\partial t} = 0 \quad (21)$$

with boundary conditions

$$u(0, t) = 0 \text{ and } H_A \left[\frac{\partial u}{\partial r} \right]_{r=a} + \frac{2\nu}{1-\nu} \frac{u(a, t)}{a} = -R\theta c^*(t), \quad (22)$$

and initial condition

$$u(r, 0) = -\frac{R\theta c_r^*}{3K} r. \quad (23)$$

This is a classical diffusion problem in spherical coordinates (diffusion of the interstitial water through the porous matrix where the diffusivity is $H_A k$), with mixed boundary conditions, whose solution can be obtained in closed form. For example, if the external solute concentration is changed in a stepwise fashion from the reference state c_r^* to c_{eq}^* according to

$$c^*(t) = c_r^* [1 - H(t)] + c_{eq}^* H(t) \quad (24)$$

where $H(t)$ is the Heaviside step function, then

$$\frac{u(r, t)}{a} = -\frac{R\theta}{3K} \times \left[c_{eq}^* \frac{r}{a} + 4(c_{eq}^* - c_r^*) \sum_{n=1}^{\infty} \frac{3\alpha_n \cos \alpha_n + (\alpha_n^2 - 3) \sin \alpha_n}{2(\alpha_n^2 - 1 + \cos 2\alpha_n) + \alpha_n \sin 2\alpha_n} j_1 \left(\alpha_n \frac{r}{a} \right) \exp \left(-\alpha_n^2 \frac{H_A k}{a^2} t \right) \right], \quad (25)$$

where $j_1(x)$ is the spherical Bessel function of the first kind, of order one,

$$j_1(x) = \frac{1}{x} \left(\frac{\sin x}{x} - \cos x \right), \quad (26)$$

and α_n is the n -th root of the transcendental equation

$$2\alpha \cos \alpha + (\alpha^2 - 2) \sin \alpha + \frac{2\nu}{1-\nu} (\sin \alpha - \alpha \cos \alpha) = 0. \quad (27)$$

For this special case, Eq.(19) produces

$$\frac{V(\infty)}{V(0)} = \frac{V_{eq}}{V_r} \approx \frac{1 - R\theta c_{eq}^*/K}{1 - R\theta c_r^*/K}. \quad (28)$$

This result suggests that the equilibrium response to osmotic loading with a non-permeating solute can yield a measure of the bulk modulus $K = H_A (1+\nu)/3(1-\nu)$, for example from experimental measurements of V_{eq}/V_r , but cannot distinguish between H_A and ν . The transient response is regulated by the time constant $H_A k/a^2$, which appears in the exponential term of Eq.(25). Assuming that K is obtained from the equilibrium response of Eq.(28), an independent measure of ν would still be required to evaluate H_A , so that the permeability k can be deduced from the transient response.

Numerical Scheme—A finite difference scheme was employed to solve the system of non-linear partial differential equations in Eqs.(8)–(9). Second-order discretization was used for the spatial coordinate (41 uniform radial increments in the range $0 \leq r \leq a$), along with first-order backward difference in time, producing a set of non-linear difference equations. These equations were solved iteratively at each time step, using the solution from the previous time step as an initial guess. Numerical convergence of this implicit scheme was achieved unconditionally in all cases. Numerical accuracy was verified for the special theoretical solution of Eq.(25). Curve-fitting of experimental data was performed using an optimization algorithm based on the quasi-Newton method with finite difference gradients (BCONF subroutine, IMSL Math Library, Visual Numerics, San Ramon, CA).

Materials and Methods

Alginate Gel Preparation

Alginate is comprised of two sugars, guluronic and mannuric. It is commonly used in drug delivery, cell encapsulation systems and tissue engineering. Alginate gels in this study were produced from medium viscosity alginic acid (sodium salt from *Macrocystis Pyrifera*, Sigma, St. Louis, MO). Sodium alginate solution was prepared in a 1% (w/v) concentration and gelled in 100mM CaCl_2 (Amend dihydrate M.W.= 147g/mol).

Spherical alginate beads were prepared through an extrusion technique. Droplets of sodium alginate were extruded from a 30 gage syringe needle into a 100mL filled beaker of CaCl_2 . In an effort to control geometry and reduce the occurrence of beads with short tails or streamline profiles, droplets were released from an optimal height of 10cm. By tapping the syringe against the beaker rim, droplets could be released prematurely, allowing for some control over the bead size (radius = 0.616 ± 0.038 mm, $n=27$). In order to minimize the effects of shrinkage from continuous gelling, the beads were allowed to gel at room temperature for approximately 24 hours before being tested.

Alginate samples were also prepared as disks for use in compression, permeation, and partition coefficient testing. Sodium alginate solution was pipetted between glass slides fixed 2mm apart which were then immersed in a bath of CaCl_2 . The slides were lined with filter paper to allow CaCl_2 to penetrate the alginate slab on multiple surfaces. After an 80 minute gelling period, disks were punched out of the alginate slab with a biopsy punch and then returned to a 100mM CaCl_2 bath for approximately 24 hours.

Osmotic Loading of Alginate Beads

Alginate beads were individually placed in a bath of 100mM CaCl_2 , supplemented with dextran (4, 5, 6, or 7mM of 40kDa dextran, or 3mM of 75kDa dextran) (Sigma, dextran from

Leuconostoc Messenteroides), contained in a 9 cm vacuum plasma treated tissue culture dish. While in solution, the bead was viewed under an Olympus CK40 microscope (4× objective) which focused on the bead's mid-plane as indicated by a sharply defined perimeter. Digital images were taken at regular time intervals with a Sony ExwaveHAD camera and a computer-based data acquisition system (640×640 pixels, 3.3375 μm/pixel, Metaview version 4.1). At each time point the bead's cross-sectional pixel area was subsequently determined from image analysis and, under the assumption of a spherical geometry, used to calculate its volume.

In order to avoid the effects of an unstirred boundary layer surrounding the bead, the solution was mixed throughout the trial with a Manostat Varistaltic pump (Barnant, IL). Fluid was circulated from one end of the dish to the other at a rate of 45 μL/sec (Figure 1). In order to prevent bead motion during imaging, the fluid level was slightly adjusted until all but the topmost portion of the bead was submerged. Preliminary studies showed insignificant behavioral variations from a completely submerged bead. The dish was also covered throughout the trial to avoid solvent evaporation.

Compressive Modulus of Alginate Disks

The compressive modulus of 1% alginate was measured with a custom unconfined compression device [22]. The dimensions of each disk ($\text{Ø}6.52 \times 1.65$ mm, $n=5$) was measured with a digital caliper before testing. Four successive stress relaxation tests were run in increments of 5% strain of the original sample thickness. Displacements were controlled by a stepper micrometer and applied at a rate of 1.5 μm/s. The normal reaction force was measured with a load cell for a relaxation period of 30 minutes (250g range, Sensotec, Columbus, OH). The load was considered sufficiently equilibrated when it yielded an average decay rate of 1% per minute [23]. Each sample's compressive modulus was computed from the slope of the linear regression through the equilibrium stress-strain response. Assuming that Poisson's ratio is $\nu = 0$, this measured modulus is equivalent to H_A .

Permeability of Alginate Disks

The permeability of 1% alginate disks to each of the 40kDa dextran testing solutions was measured with a custom-made permeation device similar to that described by Weiss et al. [24] ($n=3$ disks per dextran concentration). After an hour of incubation in the dextran testing solution, disks ($\text{Ø}13.0 \times 1.45$ mm) were placed in the permeation device and subjected to an upstream pressure of 1.34 ± 0.18 kPa. The pressure was applied with a column of fluid (~14 cm) consisting of PBS + 100mM CaCl_2 , and concentrations of 40kDa dextran varying from 0 to 7 mM. The downstream flux was simultaneously measured by stereoscopic imaging of fluid progression through a transparent tube (1.5 mm I.D.) and was found to decrease from 0.55 ± 0.20 μm/s for 0 mM dextran to 0.024 ± 0.008 μm/s for 7 mM dextran. The gel hydraulic permeability was subsequently determined through Darcy's Law. Tests were run until equilibrium and increased in duration (4–48 hrs) with increasing dextran concentration.

Volume Fraction of Alginate Disks

Disks were produced in order to verify the porosity of the 1% alginate gels used in the study.

The volume fraction of water was determined through the equation $\phi_r^w = 1 - W^{dry}/W^{wet}$. Each sample's wet weight (W^{wet}) was determined at 24 hours of gelation and the dry weight (W^{dry}) was measured after 48 hours of freeze drying (Labconco Freezone vacuum chamber).

Partition Coefficient of Dextran in Alginate

The partition coefficient of 70 kDa dextran was measured in 1% alginate gel. Alginate disks gelled for 24 hours were placed in a bath of .5mg/mL (7 μM) 70 kDa dextran (Texas Red) mixed in 100mM CaCl_2 . The disks remained in the bathing solution for 48 hours while being softly

agitated with an orbital shaker. After removal, their dimensions were measured ($\varnothing 3.23 \times 1.24$ mm, $n=6$) and they were placed in 200 μL of CaCl_2 . While in solution, disks were then pulverized and stored at 4°C for over a week to ensure full dissipation of dextran solute from the disks. Samples of the bathing solution were also stored. The solute concentration was measured using a fluorescent plate reader (SpectraFluor Plus, Tecan, Research Triangle Park, NC) and a solute-specific standard curve. The partition coefficient was expressed as the ratio of the solute concentration inside the gel to that of the bathing solution.

Dextran Solution Viscosity

Measurements of the 40kDa dextran solutions used in this study were made with an Ubbelohde glass capillary viscometer in order to determine the significance of viscous variations among different dextran concentrations mixed in 100mM CaCl_2 . Three different solution samples were used for each concentration. Pure 100mM CaCl_2 was also tested and marked as a dextran concentration of zero. Solution densities were also measured and used to convert kinematic viscosity to absolute viscosity.

Statistical Analyses

One-way analysis of variance was used to determine differences in the values of k , D_0 , D and κ in the five groups tested in this study (3 mM 75 kDa, and 4 mM, 5 mM, 6 mM and 7 mM 40 kDa dextran), with $\alpha = 0.05$ and statistical significance set at $p \leq 0.05$. Tukey's post-hoc test was used to detect differences in the means.

Results

For all dextran concentrations, the volume response of the osmotically loaded alginate beads exhibited the same qualitative response: The volume showed a relatively rapid decrease upon osmotic loading, followed by a relatively slower recovery until reaching an equilibrium value (Figure 2). The equilibrium volume was smaller than the initial volume in all cases. With increasing dextran concentration, the volume decreased further.

For each osmotically-loaded alginate bead, the experimental response of V/V_0 was curve-fitted with the theoretical response obtained from the numerical solution of Eqs.(8), (9) and (17). The following material properties were obtained from direct measurements on alginate disks, which yielded an aggregate modulus $H_A = 5.9 \pm 0.9$ kPa ($n=5$) and porosity $\phi_r^w = 0.956 \pm 0.002$ ($n=4$). (The difference between the measured value of the water content ϕ_r^w and its nominal value of 0.99 is most likely due to binding of the polarized water molecules to the charged alginate matrix.) The curve-fitting procedure yielded the alginate permeability k , the dextran diffusivities in free solution and in alginate, D_0 and D , and the partition coefficient of dextran in alginate, κ . For the 40 kDa dextran solutions, due to the nonlinear nature of the governing equations the curvefits generally converged to two optimal solutions, which differed only in the value of the permeability k , while D_0 , D and κ remained essentially unchanged. One of these solutions yielded values of k in the range of $1.4\text{--}2.9 \times 10^{-14}$ $\text{m}^4/\text{N.s}$ ($k \sim 1.3\text{--}2.0 \times 10^{-14}$ $\text{m}^4/\text{N.s}$) for all concentrations of dextran and for both molecular weights, whereas the other solution yielded a permeability which decreased from 1.8×10^{-12} $\text{m}^4/\text{N.s}$ to 2.7×10^{-14} $\text{m}^4/\text{N.s}$ with increasing 40 kDa dextran concentrations ($k \sim 2.9 \times 10^{-13}$ $\text{m}^4/\text{N.s}$ to 1.9×10^{-14} $\text{m}^4/\text{N.s}$). Direct permeation measurements were used to guide the selection of the latter set as the correct solution (Figure 3). A statistical comparison of the curve-fitted k to the measured value showed no difference for all concentrations ($p > 0.8$) except at 4 mM ($p = 0.04$). In contrast, for the 75 kDa dextran the curve-fits yielded a unique value for k . In addition, the measured permeability for 0 mM kDa was $k = 6.4 \pm 1.6 \times 10^{-13}$ $\text{m}^4/\text{N.s}$.

A regression analysis between the curve-fits and experimental responses of alginate beads to osmotic loading yielded a coefficient of determination $R^2 = 0.993 \pm 0.011$, with representative cases presented in Figure 4.

The diffusivities were consistently higher in free solution (D_0) than inside the gel (D), and both parameters showed significant dependence on solution concentration and molecular weight ($p < 0.0001$) (Figure 5). Specifically, the diffusivity generally decreased with increasing 40 kDa dextran concentration, and the diffusivity of 3 mM 75 kDa dextran was smaller than that of 4 mM and 5 mM 40 kDa dextran.

The partition coefficient of dextran in alginate was very close to unity and decreased nonlinearly with increasing concentration ($p < 0.0001$) (Figure 6a). The partition coefficient of 3 mM 75 kDa dextran ($\kappa = 0.950 \pm 0.011$) was also smaller than that of 40 kDa dextran at all tested concentrations. For comparison purposes the direct measurement of the partition coefficient of 70 kDa dextran at a concentration of 7 μ M was $\kappa = 0.992 \pm 0.100$. When plotted against the dilatation at equilibrium, $\text{tr } \mathbf{E} \approx 1 - V_{eq}/V_r$, κ exhibited a nearly linear behavior, indicating that the partition coefficient decreases linearly with decreasing gel volume (Figure 6b). The viscosity of 40 kDa dextran solutions showed a significant dependence on concentration, increasing nonlinearly from $\eta = 9.2 \pm 0.1$ mPa.s at 4 mM to $\eta = 27.8 \pm 0.8$ mPa.s at 7 mM (Figure 7).

Discussion

The objective of this study was to determine experimentally how a spherical gel responds to osmotic loading and whether this behavior is predictable from mixture theory. The model gel used in the experiments was alginate and the osmolyte was dextran. The theoretical model used the framework of mixture theory, encompassing specific enhancements proposed in our recent study [10], such as the incorporation of a partition factor κ in the governing equations, and an explicit distinction between the solute diffusivity in the gel (D) and in free solution (D_0). Though alginate is a negatively-charged gel, the fact that dextran is a neutrally-charged solute implies that the theoretical analysis can capture the salient response to osmotic loading without accounting for charge effects.

The most evident outcome of this study is that the volume response of alginate gels to osmotic loading was described very faithfully from theory, as evidenced by the representative curve-fits shown in Figure 4 and the near-unit values of R^2 for these fits. This successful curve-fitting serves as a necessary step toward the validation of the theoretical framework. The fact that the theory is able to faithfully reproduce the observed volume decrease and partial recovery suggests that it does indeed capture the salient physicochemical characteristics of the observed phenomena. Some further evidence in support of the theory may be derived from the analysis of the material parameters extracted from the curve-fitting procedure. For example, the permeability of alginate to 40 kDa dextran solutions was observed to decrease with increasing concentration, in very good agreement with direct permeability measurements on alginate disks (Figure 3); a similar behavior was found for the diffusivities of 40 kDa dextran in free solution and in alginate (Figure 5). These results may be qualitatively explained by the concomitant increase in solution viscosity with concentration (Figure 7), and the inverse relation between solute diffusivity and solution viscosity predicated by the Stokes-Einstein equation. The observation that the diffusivities of 75 kDa dextran at 3 mM are generally smaller than those of 40 kDa at 4 mM and higher concentrations is consistent with the expectation that the diffusivities of higher molecular weight species are generally smaller than those of lower molecular weights, when measured at comparable concentrations. The order of magnitude of the dextran diffusion coefficient in 1% alginate ($D \sim 2.5 \times 10^{-10}$ m²/s) and in free solution ($D_0 \sim 3.5 \times 10^{-10}$ m²/s) are also in the expected range for molecules in solution ($\sim 10^{-11}$ –

$10^{-9} \text{ m}^2/\text{s}$), and compare well with measurements of dextran diffusion in 2% alginate as reported by Leddy et al. [25] ($D \sim 3.5 \times 10^{-10} \text{ m}^2/\text{s}$ for 23 μM 40 kDa dextran, and $D \sim 1.5 \times 10^{-10} \text{ m}^2/\text{s}$ for 32 μM 70 kDa dextran). The diffusivities of the current study were obtained at significantly higher concentrations, and therefore the comparison should be viewed with caution; nevertheless, these results suggest that the material parameters obtained from curve-fitting are reasonable estimates. This conclusion is further strengthened by the direct comparison of permeability values shown in Figure 3.

The partition coefficient of dextran in alginate, as determined from curve-fitting, was observed to decrease with increasing concentration and molecular weight (Figure 6a). This result is consistent with the fact that the partition coefficient represents the steric volume exclusion of dextran molecules within the pores of alginate. The distribution of pore sizes in a porous structure is usually variable, and it is expected that some pores will be too small to fit a molecule of a certain size. Thus the partition coefficient is expected to decrease with increasing molecular weight. As the concentration of molecules increases the gel reduces further in volume, providing less pore space for the dextran molecules; this is consistent with the observation that κ varies linearly with gel dilatation (Figure 6b). This trend is also consistent with the observation that direct measurements of κ for 70 kDa dextran at the very low concentration of 7 μM ($\kappa \sim 0.99$) yielded a higher value than the curve-fitted value at 3 mM of 75 kDa dextran ($\kappa \sim 0.95$). (The technique for measuring κ directly using fluorescent tagging precludes the use of concentrations in the millimolar range because of fluorescence saturation, which explains the reason for reporting direct measurements at a micromolar concentration only.) As in the case of diffusivities, the observation that the curve-fitted κ is comparable to direct measurements provides further support that the curve-fitted parameters represent meaningful physical measures.

The decrease of the permeability with increasing solution concentration can be explained from two mechanisms: First, higher dextran concentrations result in greater gel shrinkage, which causes a reduction in permeability. Second, higher dextran concentrations result in higher solution viscosity (Figure 7), which also causes a reduction in permeability. A plot of k versus gel dilatation shows an exponential decrease with increasing compressive dilatation (Figure 8).

Combined together, these findings provide strong support for the hypothesis that the mixture framework embodied in Eqs.(1)–(6) is valid for the study of osmotic loading of gels. From this theoretical framework the observed volume response can be explained as follows: Upon adding dextran to the external bathing solution the chemical potential of the solvent (water) in the bath immediately drops below that of the water in the center of the gel; a narrow boundary layer in the chemical potential forms within the gel, near the surface, to maintain continuity of chemical potential between inside and outside as required by the interface boundary conditions. The gradient in water chemical potential in this boundary layer drives water out of the gel, toward the goal of equalizing the chemical potential everywhere inside and outside. This outflow of interstitial water leads to the volume decrease observed in the initial response to osmotic loading.

The resulting compressive stress in the gel matrix helps to resist the osmotic pressure difference produced by the dextran concentration imbalance (lower pressure and concentration inside than outside). As time progresses however, the dextran slowly diffuses into the alginate gel, driven by its concentration gradient, thereby decreasing the chemical potential of water inside the gel toward the value in the external bath. As a result, the osmotic pressure difference decreases back toward zero, and the elasticity of the gel drives a volume recovery with a reversal in the fluid flow, from outside to inside. Since the partition coefficient of dextran in alginate is less than unity, the internal concentration equilibrates to a value smaller than that in the external

bath. Consequently, a small osmotic pressure difference persists at steady state, which is responsible for the incomplete volume recovery. This is evident from Eq.(20), which expresses the relative decrease in equilibrium volume in terms of the ratio of the osmotic pressure difference resulting from $\kappa < 1$, $R\theta(1 - \kappa)c_{eq}^*$, and the bulk modulus of the gel matrix, K .

An interesting observation is that the osmotic pressure difference immediately upon loading, $R\theta c_{eq}^*$ (ranging from 7.4 kPa for 3 mM dextran to 17.3 kPa for 7 mM dextran), is larger than the bulk modulus of alginate ($K \sim 5.9$ kPa). If dextran could not diffuse into alginate ($D = 0, \kappa = 0$), this osmotic pressure difference would shrivel the alginate gel into a very small volume (in which case finite strain theory would be required to describe the volume response). Since dextran does diffuse through alginate, the volume shrinkage reverses itself as explained above, though the higher osmotic pressure difference applied at the higher concentrations manifests itself by a larger initial decrease in volume (Figure 2a).

Cell Modeling

It is interesting that the volume response of alginate gels to osmotic loading with dextran is qualitatively similar to the response of cells osmotically loaded with a permeating solute; this is apparent, for example, in the study of osmotic loading of chondrocytes with 1400 mM glycerol by Xu et al. [26] (Figure 9) or the study of osmotic loading of kidney cells with 100 mM NaCl by Lucio et al. [9]. However this analogy does not imply that cells may be suitably modeled as spherical gels, without incorporating a semi-permeable membrane in the model. Indeed, a similar type of volume response is also predicted by theoretical models of a fluid-filled semi-permeable membrane [4,6,27].

A distinction between the two types of models is that the fluid-filled spherical membrane may contain solutes which cannot permeate out of the cell; consequently, as the cell volume changes in response to osmotic loading the internal concentration of non-permeating solute varies accordingly, counter-acting the osmotic pressure difference generated by the osmolyte. Thus the equilibrium volume response is dictated by a combination of the internal and external concentrations of permeating and non-permeating solutes, as well as membrane tension.

In the spherical gel model, since there is no membrane at the surface, solutes may not be constrained to remain inside the gel. The equilibrium volume response is only a function of the osmolyte concentration outside, the partition coefficient, and the gel bulk modulus. The only resistance to volume change in response to osmotic loading comes from the stiffness of the gel [see Eqs.(20) and (28)]. Because cells have a very low bulk modulus (e.g., on the order of 1 kPa for chondrocytes [28]), the application of osmotic loads on the order of 1000 mM (as in the study of Xu et al. [26]) would simply crush the cells, were it not for the presence of non-permeating solutes inside. Consequently, a cell model consisting only of a spherical gel would be inappropriate.

However, the partition coefficient of the osmolyte in the protoplasm relative to the external bath has a significant influence on the equilibrium volume response. As shown for alginate in Figure 2, even a very small deviation of κ from unity can produce a significant reduction in the equilibrium volume response. We conclude that steric volume exclusion of solutes in the porous matrix of the gel, which is a passive mechanism, can explain incomplete volume recovery. The similarly incomplete volume recovery observed with cells, when loaded with specific osmolytes (Figure 9), may also be a direct consequence of the gel-like nature of the protoplasm and steric volume exclusion effects, and need not be attributed to active regulatory mechanisms (particularly when experiments are conducted at room temperature).

The current consensus is that the transport of water and osmolytes into or out of a cell is limited primarily by the cell membrane permeability, which is determined from curve-fitting the

volume response to the fluid-filled membrane model equations. However, as suggested from the results with alginate gels in this study, the diffusivity of osmolytes and permeability of water in the protoplasm may also regulate the transient volume response.

Consequently, the findings of this study support the hypothesis that modeling cells as gels surrounded by a semi-permeable membrane may provide a more accurate description of the observed volume response to osmotic loading. This more elaborate model may yield new insights into the physicochemical mechanisms regulating the cell response, possibly influencing the interpretation of observed biological responses to osmotic loading. The explicit formulation of this membrane-surrounded gel model will be presented in a future study.

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Figure 1.
Schematic of testing apparatus for osmotic loading of alginate beads.

Figure 2.

Normalized volume response of osmotically-loaded alginate beads: (a) Means and standard deviations for osmotic loading with 40 kDa dextran (4, 5, 6 and 7 mM). (b) Mean and standard deviation for osmotic loading with 75 kDa dextran (3 mM).

Figure 3.

Permeability of alginate to dextran solutions ($\tilde{k}$), obtained from curve-fitting of the volume response of alginate beads to osmotic loading with dextran solutions, and from direct permeation measurements on alginate disks. Within each set, numbers above bars indicate the concentrations against which differences were statistically significant ($p < 0.0001$).

Figure 4.

Representative theoretical curvefits (solid curves) of experimental results (symbols) shown for each dextran concentration and molecular weight (symbols).

Figure 5.

Diffusivity of dextran in free solution (D_0) and in alginate (D), obtained from curve-fitting of the volume response of alginate beads to osmotic loading with dextran solutions. Numbers above bars indicate the concentrations against which differences were statistically significant ($p < 0.005$).

Figure 6.

Partition coefficient of dextran in alginate (κ), obtained from curve-fitting of the volume response of alginate beads to osmotic loading with dextran solutions: (a) As a function of dextran concentration and molecular weight (numbers above bars indicate the concentrations against which differences were statistically significant, $p < 0.0001$); (b) as a function of gel dilatation at equilibrium (40 kDa dextran only).

Figure 7.
Viscosity η of 40 kDa dextran solutions of various concentrations, measured using a glass capillary viscometer.

Figure 8. Hydraulic permeability (k) of alginate to 40 kDa dextran solutions of various concentrations, as a function of alginate gel dilatation at equilibrium ($\text{tr } \mathbf{E} \approx 1 - V_{eq}/V_r$).

Figure 9.

Volume response of bovine articular cartilage chondrocytes to osmotic loading with 1.4 M glycerol at 21°C. Adapted from Figure 7 of Xu et al. [26], with permission.