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The Consolidation Behavior of Silk Hydrogels

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Abstract

Hydrogels have mechanical properties and structural features that are similar to load bearing soft tissues including intervertebral disc and articular cartilage, and can be implanted for tissue restoration or for local release of therapeutic factors. To help predict their performance, mechanical characterization and mathematical modeling are available methods for use in tissue engineering and drug delivery settings. In this study, confined compression creep tests were performed on silk hydrogels, over a range of concentrations, to examine the phenomenological behavior of the gels under a physiological loading scenario. Based on the observed behavior, we show that the time-dependent response can be explained by a consolidation mechanism, and modeled using Biot's poroelasticity theory. Two observations are in strong support of this modeling framework, namely, the excellent numerical agreement between increasing load step creep data and the linear Terzaghi theory, and the similar values obtained from numerical simulations and direct measurements of the permeability coefficient. The higher concentration gels (8% and 12% w/v) clearly show a strain-stiffening response to creep loading with increasing loads, while the lower concentration gel (4% w/v) does not. A nonlinear elastic constitutive formulation is employed to account for the stiffening. Furthermore, an empirical formulation is used to represent the deformation-dependent permeability.

Keywords

Silk Hydrogel; Poroelasticity Models; Protein Polymer Mechanics

1. Introduction

The goal of tissue engineering is to design clinically-relevant implantable materials for the eventual replacement of diseased or damaged tissues. To ensure the viability of devices as potential replacements for musculoskeletal or craniofacial tissue, suitable biomechanical properties of a biomaterial implant or cell-seeded scaffold must be demonstrated prior to clinical investigation (Butler et al., 2008). Tissue engineers use metrics established by the field of biomechanics (Mow and Huiskes, 2005) to contextualize and evaluate the results of testing, analysis, and constitutive modeling of biomaterials. Models chosen typically range from purely phenomenological to micromechanical that incorporate knowledge of their structure and behavior unique to the material chosen. The results of such studies can then be applied in further

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design refinement or towards functional tissue engineering design paradigms (Guilak, 2003; Nerurkar et al., 2008), where the material may undergo similar loading conditions during tissue culture *in vitro* (Hung et al., 2004). While acclimating to loads either *in vitro* or *in vivo*, the material will be remodeled and may concurrently transduce external mechanical forces to growing cells (Kluge et al., 2008), in which cell phenotype and function are tightly linked to material stiffness (Engler et al., 2006) and permeability (O'Brien et al., 2007). Therefore, establishing physiologically-relevant test data and constitutive formulations regarding material behavior, prior to investigating the influence of culture conditions or the actual implant environment, is essential.

Biomaterials derived from the reprocessed silk fibroin protein have been heavily investigated for use in functional tissue engineering and drug delivery applications (Hofmann et al., 2006; Vepari and Kaplan, 2007; Wenk et al., 2008). A novel method to rapidly form silk-based hydrogels was recently reported using ultrasonication to accelerate the kinetics associated with the gelation process (Wang et al., 2008). Based on this platform, a method to entrap human cells within these rapidly-forming gels was also demonstrated. Such a hydrogel system could therefore offer the potential for tissue augmentation and repair strategies based on injecting repair mediators along with the pre-gelation solution or implanting appropriately-shaped post-gelation constructs to meet functional requirements. In testing the latter approach, subcutaneous implantation of the gels in a mouse model demonstrated a mild acute inflammation to the post-sonication silk gel, followed by a slow remodeling of the gel structure lasting on the order of weeks to months (Etienne et al., 2009). However, these studies did not address the potential of the gel systems to restore physiological load-bearing function *in situ* for more demanding implant environments nor the effects that load may have on the resultant structure of the gel.

Since the morphology of silk gels and the viability and ingrowth potential of entrapped cells within the gels are highly dependent on initial protein concentration (Wang et al., 2008; Kim et al., 2004), a range of silk solution concentrations must be mechanically evaluated. Hydrogel systems are often assessed using rheological testing, however these tests do not capture the deformation state typically encountered *in situ*. Many highly hydrated tissues such as skin, cartilage, and intervertebral disc (IVD) see significant compressive loads in daily use; therefore, the confined compressive properties of silk gels are of interest for filler repair of these tissues. The experimental results of such studies can then be used to validate constitutive formulations (Mow and Huiskes, 2005). Incorporating the fluid-solid ratio of the silk gels into the mathematical formulation will account for the nonlinear interaction of permeability and mechanical deformation.

As is the case with highly hydrated biological tissues, compressive loads applied to fluid-saturated porous materials such as hydrogels will cause fluid extrudation, compaction of the solid phase, and thus a phenomenon known as *consolidation* (Biot, 1941). Although applied more extensively in geomechanics, consolidation testing has found recent use in biological contexts. *In vivo*, the gradual loss of water from certain tissues acts as a hydraulic lubricant and cushion to reduce friction and soften the impact of sudden loading in the joint or tissue cavity. The process of consolidation, applied to a one-dimensional confined compression loading scenario, has been demonstrated for several tissues including hyaline cartilage (Oloyede and Broom, 1994; Oloyede et al., 2004), intervertebral disc (Martinez et al., 1997), and brain tissue (Franceschini et al., 2006). Moreover, the general theory of poroelasticity, explaining the elastic deformation of fluid-saturated solids, is the foundation for consolidation theory, and has been broadly used to describe fluid-flow dominated phenomena in biological tissues (Yang and Taber, 1991; Mow et al., 1980).

Morselized cancellous bone (MCB) grafting (a water-fat biphasic mixture), used to fill irregular shaped voids caused by bone loss, has been evaluated for rates of consolidation in simulated implant environments (Voor et al., 2000). The rate of primary consolidation of MCB and the corresponding total strain shows a large compositional-dependency (water content), suggesting that successful surgical outcomes may be dependent on pre-screening of graft material content in anticipation of some permanent deformation. Similarly, a fully hydrated hydrogel will restore function by optimizing its intrinsic stiffness and permeability, but must be evaluated for volumetric changes due to applied loads. Understanding these parameters as a function of material processing environment are useful design inputs.

Our hypothesis is that silk hydrogels respond to load by a consolidation mechanism similar to saturated poroelastic solids, see Scanning Electron Micrograph in Fig. 1. We conducted a series of confined creep tests using a custom-designed odeometer to assess the time-dependent deformation response of gels to incrementally-increasing step loads. Specifically, the effects of applied loads on the rate of consolidation and the associated changes in volume were evaluated. The material response to sequential load increments was accurately described using the linear-poroelastic theory (Holtz and Kovacs, 1981). In addition, a direct permeability measurement was performed to validate the theoretical prediction. The boundary conditions imposed on cylindrical specimens allow uniaxial creep deformation and generate a nearly hydrostatic state of stress. A nonlinear constitutive formulation is used to describe the pressure-volume relation at equilibrium, i.e. when all excess pore water pressure has been dissipated. To quantify the influence of the solid volume content of the gel on the *pressure-volume response* and on the *permeability coefficient*, we investigated a range of silk concentrations. The results are then compared to similar studies found in the literature for various musculoskeletal tissues and their hydrogel replacements.

2. Methods

2.1. Silk Fibroin Solutions

Silk fibroin aqueous stock solutions were prepared as previously described (Sofia et al., 2001). Briefly, cocoons of *B. mori* were boiled for 40 minutes in an aqueous solution of 0.02M Na_2CO_3 (Sigma), and then rinsed thoroughly with pure water. After drying, the extracted silk fibroin was dissolved in 9.3M LiBr (Sigma) solution at 60°C for 4 hours, then dialyzed against distilled water to remove the Li^+ salt. The solution was centrifuged to remove impurities, and the final weight concentration of silk fibroin aqueous solution was approximately 8% (w/v). The ratio of solids weight to solution volume, used herein to define the wt% of the silk solution and final solidified gel, is initially determined prior to sonicating by sampling a small volume of solution and weighing the residual solid after drying in an oven at 60°C.

2.2. Sonication to Induce Gelation

Rapid silk gelation protocols have been described elsewhere (Wang et al., 2008), but a brief description of the sample preparation method will be discussed herein. A 12% (w/v) silk solution was prepared by dialyzing the stock solution (8%) against 15% (w/v) PEG (10,000 g/mol, Sigma) solution at room temperature for 8 hours, while 4% (w/v) silk solutions were prepared by diluting the purified stock solution with de-ionized water. Silk solutions at 4%, 8%, and 12% concentration were added to glass flasks, and autoclaved for 20 minutes at 121°C. The solutions were supplemented with Dulbeccos Modified Eagle's Medium (DMEM) (Invitrogen) powder and Na_2HCO_3 (Sigma) powder. Sonication parameters were tailored for each different concentration to achieve a solution-to-gel transition within 2 hours.

For each concentration, a 10mL volume of silk solution was sonicated for 15 seconds. Immediately thereafter, the entire volume was poured into a custom rapid-prototyped dish

(ABS, Stratysys, Inc.) measuring 35.5mm inner diameter, see Fig. 1. Thereafter, the solution in the container was covered and allowed to form a gel while being visually monitored in a 37°C incubator. Once the solution turned opaque and small beads of water condensed onto the gel surface, the reaction was assumed complete and the solid gel was removed from the container by breaking the sidewalls and bottom support and the sample submerged into DMEM solution (Invitrogen) for at least 30 minutes prior to testing to ensure complete hydration. Generally, the sample would reach approximately 10mm in height, giving an aspect ratio of 3:1 as recommended for consolidation testing (Franceschini et al., 2006; Terzaghi et al., 1996).

2.3. Creep Test Apparatus

All creep deformation studies were conducted in a confined consolidation testing device, often referred to as an oedometer (Franceschini, et al., 2006) but dimensionally modified for sample size considerations. As shown in Fig. 2, the specimen chamber (made of stainless steel, inner diameter = 35.5mm, depth = 40mm) radially confined the test specimen. Drainage from the top and bottom faces of the specimen was accomplished by a dual-filter system: single sheets of filter paper (Grade 595, Whatman) were applied on opposing sides of the gel to catch loose gel particulates, while loading was transferred through rigid porous stones made of sintered aluminum oxide (Geocomp Corp., Boxborough, MA) that rested behind the filter paper. The chamber was surrounded by a bath of .1M Phosphate Buffered Saline (PBS, Sigma) to keep the sample hydrated through drainage holes in the specimen chamber and Teflon loading piston. The loading piston was then fastened to an axially-applied hanging dead weight assembly. As shown in Fig. 2, the linkage connecting dead weights was suspended below the testing platform. The choice of a dead weight system ensured a constant force condition for the duration of testing and eliminated load cell feedback and control considerations. The loading piston displacement was measured using a LVDT (LD610-10, Omega) connected to a National Instruments model 6229 DAQ board with a 32-bit A/D converter. The entire assembly was placed in an environment held at $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for the duration of testing.

2.4. Creep Loading Protocol and Data Reduction

Consolidation testing was performed similarly to published methods (Franceschini et al., 2006) with additional details provided below. To establish accurate measurements of the sample height for mechanical testing, calibrated LVDT readings were taken immediately with the application of tare loading. For each creep test, the minimum primary load (2.915N, due to the linkage and piston assembly weight) was applied and allowed to equilibrate for 12 hours to seat the sample, then additional load steps were sequentially applied at 24-hour intervals. The sequence and magnitude of subsequent load steps (see Table 1) were selected to achieve a reduction in gel height at each step of approximately 5 – 10%. Preliminary testing showed that 24 hours were sufficient to surpass an inflection point that signifies the end of primary consolidation (see Section 2.5). In order to adequately capture the response in different phases of the same load step, the sampling rate was automatically adjusted using LabVIEW software: 10Hz during the first 10 seconds, 1Hz for the next 200 seconds, and 0.2Hz for the remaining 24 hours.

2.5. Consolidation Data Analysis

Fig. 3 contains a representative graph of the gel height change over time in response to 3 sequential load steps. Immediately after the load is applied a rapid change in height will occur, followed by a long-duration plateau. To analyze the *individual* load step behavior over time (0–24 hours), the displacement of the gel's top surface was plotted vs. log-time to visualize subtle transitions that occur in an exponentially-decaying system typical of creep loading.

Without directly measuring internal pressure dissipation during consolidation, the transition from primary consolidation to secondary compression must be determined (Terzaghi et al., 1996). The *end of primary consolidation* is defined as the point of complete dissipation of excess pore water pressure. The Casagrande method was used to locate this point as shown in the bottom of Fig. 3. Briefly, two linear sections of the experimental displacement vs. log-time data plots were approximated by log-linear regression. The intersection of these lines defined the end of primary consolidation and the corresponding time t_p . For a uniaxial confined compression test of a cylindrical specimen, the consolidation ratio U is the fraction of the overall height change measured at time t (and location z). The progression of U was evaluated for each load step, starting at zero and slowly approaching unity at the end of primary consolidation (Taylor, 1948). Since the consolidation ratio is different at different locations across the thickness of the gel, an *average* consolidation ratio is assumed for the value of U .

2.6. Consolidation Modeling

Consolidation theory will predict the amount and time rate of settlement of fluid-saturated engineering systems, which in the present case refers to the slow compressibility of silk gel under a constant load. Various boundary-value problems have been numerically solved to address specific test conditions used in geotechnical contexts. For the derivation and solution of Terzaghi's one-dimensional consolidation theory, the reader is directed to (Taylor, 1948) and (Holtz and Kovacs, 1981). However, a brief overview will be provided for familiarity with notation and conventions.

We consider the silk gel to be a solid composed of a network of physical crosslinks, whose pore-like architecture (see SEM image in Fig. 1) is completely fluid-saturated. We also assume that compressive loads will cause extrusion of unbound water in a time-dependent fashion. Therefore, the load applied to the top surface is carried by both the fluid portion and solid skeleton of the gel. This leads to the additive decomposition of the total stress σ into the pore water pressure p and the effective stress acting on the gel *skeleton* σ' in the form

$$\sigma = p + \sigma' \quad (1)$$

In general, consolidation of porous materials causes the pore water pressure p to vary both with respect to time and position throughout the specimen.

Consider a cylindrical specimen with circular cross-sectional area, which is not allowed to drain and deform in the radial direction, but is allowed to drain freely on the top and bottom surfaces. The consolidation phenomenon reduces to the dependence on the axial (vertical) coordinate z and time t . Let the specimen be in a state of equilibrium where all applied load is carried by the effective stress σ'_0 in the solid skeleton and all pore water pressure has been completely dissipated, see Fig. 4. Application of a load increment induces an instantaneous increase of the effective stress σ' and pore pressure p in the specimen. Due to the drainage boundary conditions and symmetry requirements, the pore pressure on the top and bottom surfaces is zero and achieves its maximum value at the mid-height of the specimen. In Fig. 4 the vertical axis represents the total height of the specimen $2H$ and the horizontal axis the additive decomposition of the total stress σ .

The change of pore water pressure as a function of time and location is given by

$$c_v \frac{\partial^2 p}{\partial z^2} = \frac{\partial p}{\partial t}, \quad (2)$$

where c_v denotes the consolidation coefficient. It can be expressed as

$$c_v = kE, \quad (3)$$

where k is Darcy's permeability coefficient and E is the *constrained modulus* in uniaxial confined compression. For the problem here considered, the value of k will be determined by two alternative methods and the results compared. In the first method, the magnitude of k is determined indirectly from the value of c_v by applying the solution of Eq. (2) to the available test data. The magnitude of the confined modulus E is given by the slope of the stress-deformation response recorded after all excess pore water is dissipated. In the second method, for the 8% gel only, the value of k is determined directly by permeability testing.

In order to solve Eq. (2), boundary and initial conditions must be specified. For the double-drained test case here considered, the boundary conditions are

$$p = \begin{cases} 0 & \text{if } z=0 \\ 0 & \text{if } z=2H. \end{cases}$$

For the initial condition at $t = 0$ we assume the initial excess pore water pressure, denoted p_0 , to be equal to the total stress increment, i.e. $p_0 = \Delta\sigma$.

Following the method suggested by Terzaghi and Taylor (Taylor, 1948), we expand the pore pressure p as a time-decaying Fourier series. The solution to Eq. (2) can then be written as

$$p(z, t) = \sum_{n=1}^{\infty} \left(\frac{1}{H} \int_{z=0}^{z=2H} p_0 \sin \frac{n\pi z}{2H} dz \right) \sin \frac{n\pi z}{2H} \exp \left(-\frac{c_v t n^2 \pi^2}{4H^2} \right), \quad (4)$$

which provides the pressure p at the axial location z at time t . For a constant p_0 the integral can be solved analytically, which allows Eq. (4) to be rewritten as

$$p(z, t) = \Delta\sigma \sum_{n=0}^{\infty} \frac{4}{(2n+1)\pi} \sin \left(\frac{(2n+1)\pi z}{2H} \right) \exp \left(-\frac{(2n+1)^2 \pi^2 T}{4} \right), \quad (5)$$

where we replaced p_0 by $\Delta\sigma$ as specified by the initial condition and where we introduced the dimensionless time factor T

$$T = \frac{c_v t}{H^2}. \quad (6)$$

For the test condition considered and for materials in which the induced deformation depends linearly on the effective stress, the consolidation ratio can be written as

$$U(z, t) = \frac{\sigma'(z, t) - \sigma'_0}{\Delta\sigma'} = \frac{p_0 - p(z, t)}{p_0} = 1 - \frac{p(z, t)}{p_0}, \quad (7)$$

where $\sigma'(z, t)$ is the effective stress at location z and time t , σ'_0 is the effective stress at $t = 0$, and $\Delta\sigma'$ is the increment of effective stress (equivalent to the total stress increment $\Delta\sigma$) after all excess pore water pressure has been dissipated. In addition, we use the property that for a constant applied load the change in effective stress is interchangeable with pore pressure as shown by Eq. (1).

In order to evaluate the experimental data, it is convenient to define an *average* consolidation ratio which depends on time only and is denoted $U(t)$, as defined in (Taylor, 1948). It has the form

$$U(t) = \frac{1}{2H} \int_0^{2H} U(z, t) dz = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2 \pi^2 T}{4}\right). \quad (8)$$

2.7. Compressibility Analysis and Modeling

The cylindrical specimens were laterally constrained and subject to a constant applied pressure P to its top surface. The change in sample height was recorded at the end of the 24-hour creep test for each load increment using the top mounted LVDT, see Fig. 2. The creep deformation is uniaxial and therefore the associated change of volume, denoted by J , is the ratio of the sample height in the current configuration to the height in the undeformed configuration. In the undeformed configuration we therefore have $J \equiv 1$. It can be shown that this loading configuration generates a nearly hydrostatic state of stress in the material of magnitude P . For a compressible material at the end of primary consolidation when all excess pore water (pressure) is dissipated, the pressure-volume relation is accurately described by

$$P = \sigma' = \frac{\alpha}{\beta} (J^{\beta-1} - J^{-1}), \quad (9)$$

where α is a stiffness term and β a dimensionless material parameter (Ogden, 1972). Eq. (9) was used as comparison with the experimental data and its derivative with respect to J to determine the *constrained modulus* E for a given applied load.

An additional analysis was performed in an attempt to correlate the permeability coefficient k with the *initial* gel concentration and with the *current* solid-fluid ratio in the material. Following previous methods to define the deformation-dependent permeability of hydrated gels (Gu et al., 2003), the water volume fraction φ^w can be related to the volumetric change J by

$$\varphi^w = \frac{\varphi_0^w + J - 1}{J}, \quad (10)$$

where φ_0^w is the volume fraction of water in the undeformed configuration. The empirical formulation relating the permeability k to the change in volume J is given by

$$k = k_0 \left(\frac{\varphi_0^w + J - 1}{\varphi_0^w} \right)^n, \quad (11)$$

where k_0 is the permeability in the load free reference configuration and n is a material parameter. The values of k_0 and n are determined by fitting this model to the available k vs. J data for different gel concentrations.

2.8. Permeability Test Apparatus

Direct permeability studies were conducted on a custom-designed constant head pressure testing equipment, modeled after those reported in previous studies (Gentleman et al., 2004; O'Brien et al., 2007; Spain et al., 1998). The vertical column of water was raised and supported above a ball valve to expose the testing chamber to a constant pressure when opened. To create water-tight samples, large-diameter gels were sectioned into four quadrants, then punched into plugs using a stainless steel punch. This procedure has been used in the past to prepare biological samples (Accadbled et al., 2008). The punches were designed to have external thread features, allowing them to be screwed into the specimen holder, creating a water-tight seal. We assumed negligible head pressure change during the course of the experiment due to the large inner diameter of the water column (100mm). The permeability k can be calculated from Darcy's Law (Taylor, 1948) as follows

$$k = \frac{l}{\gamma_w h A} \frac{dQ}{dt}, \quad (12)$$

where the volume flow rate dQ/dt was measured gravimetrically (model S40103 scale, 100g capacity, Fisher Scientific) for 1 hour; A denotes the inner punch cross sectional area ($=111.22\text{mm}^2$); h the height of the saline column ($=2\text{m}$) and γ_w the specific weight of PBS ($=9.8 \times 10^3 \text{ N/m}^3$). The sample heights l were measured by compressing the virgin gels between two impermeable stainless steel platens and recording the height at a 1N preload. Darcy's Law in Eq. (12) assumes steady and laminar flow as it passes through the pore network architecture of the hydrogel since the fluid velocity is so small. The results of the direct permeability testing of the 8% silk gel (average of $n=3$ tests) are used as comparison to numerical model predictions.

2.9. Statistical Analysis of Data

To fit Eq. (8) to the experimental data for each load step, a sum of squares minimization procedure was used to determine the values of c_v using MatLab software (Version 7.4, MathWorks™, Natick, MA). The goodness of fit is reported as R^2 for each curve. Three gels ($n=3$) were tested for each concentrations (4%, 8% and 12%), resulting in 9 tests lasting 4 days each, and the data from each load step were analyzed separately. Averaged values of constrained modulus E were used to approximate the permeability coefficient k using Eq. (3). Using $n=3$ samples per concentration, model fit parameters were averaged and standard deviations calculated. A one-way analysis of variance (ANOVA) was conducted using SPSS™ software (SPSS Inc., Chicago IL) to assess differences between parameters (namely c_v and k) defined at each load step within each gel concentration. A Student Newman-Keuls (SNK) post-hoc analysis was performed and differences were considered significant when $p < 0.05$ and very significant when $p < 0.01$.

3. Results

3.1. Experimental Data

A representative data plot of a 12% gel undergoing a series of consolidation load steps and the individual response to the second load step of 25N are shown in Fig. 3, top and bottom respectively. The end of primary consolidation was seen in every test across every silk gel concentration, typically within 10 minutes for each step (see representative curves in Figs. 5–7). The inflection points were most noticeable in higher concentrations (8% and 12%), but were sometimes difficult to define for the 4% gels, specifically at the third load step which showed a more gradual decay profile. In this case, we assumed a 10-minute pore water extinction time and used this value for t_p in the numerical evaluation, which still resulted in an excellent fit (see Fig. 5).

The results of the applied pressure, which at the end of primary consolidation is equivalent to the effective stress σ' in the sample, are plotted as a function of the volumetric change J in Fig. 8. Each gel concentration shows a unique overall compressibility profile. While the 12% and 8% gels show an exponential-type strain-stiffening effect (nearly doubling in constrained modulus with each step), the 4% gels did not show this trend, and instead maintained a proportional decrease in volume with increasing load for the conditions pursued herein. The average value of hydraulic permeability k measured directly by permeability testing is shown in Table 3. Direct permeability measurements were difficult to perform on 4% gels, which seemed to crack or break in the permeability test chamber, and the 12% gels which were prohibitively stiff to fit snugly inside the permeability chamber adaptor.

3.2. Numerical Results

The second column of Table 2 shows the effective stress immediately before each load increment and after 24 hours, when all excess pore pressure has been dissipated. The values of J corresponding to the midpoint of each stress interval were used to determine the values of constrained modulus E using the derivative of Eq. (11). For convenience we used the same approach for the 4% gel as well.

Using the Terzaghi theory to approximate experimental data for each concentration and each load step determines the values of c_v . These are shown in the fourth column of Table 2. Increases of E and decreases in c_v led to a decrease in the values of the permeability k , see right column of Table 2. The numerical fit of the measured average consolidation ratio U is very good for all load steps across all gel concentrations, as evidenced by the average R^2 values $\geq .92$. One-way ANOVA was conducted to assess the impact of increasing load on the numerical values of the consolidation coefficient c_v and permeability coefficient k . All the values of c_v decreased with increasing applied load for all gel concentrations investigated, but there was only a statistically significant difference at the $p < .05$ level for c_v measured in the 8% gel case. Post-hoc comparisons revealed a significant difference between steps 1 and 3 of the 8% gel case. Likewise, all the predicted permeability values decreased with increasing load, and did so highly significantly at the $p < .01$ level for the 4% and 8% gels. Specific differences identified by post-hoc analysis are indicated in Table 2, and were found between step 1 and other steps of the 4% gel case and between steps 1 and 3 of the 8% gel.

The data points in Fig. 8 of the applied pressure (effective stress σ') vs. volumetric change J are averaged for each concentration and used to determine the material parameters in Eq. (9). We determined the best fit by keeping the value $\beta = 7$ constant for each gel concentration, and using a linear correlation between the material stiffness α and the gel concentrations. In particular, the values of α were equal to 8.1kPa, 40kPa, and 84kPa respectively for the 4%, 8% and 12% gels. The numerical results are compared to the experimental data in the lower image

of Fig. 8. The numerical simulation of the 8% and 12% gels is very accurate ($R^2=.995$ and $.999$, respectively) and poor for the 4% case ($R^2=.756$), which is not surprising.

Finally, Eq. (11) was used to determine the change of k as a function of the current volume fraction of water ϕ^w . The water volume fractions ϕ_0^w in the undeformed configuration were determined by dividing the silk concentration by the silk density (1.355g/mL, see Rajkhowa et al. (2000)) and are listed in Table 3. The numerical results, shown in Fig. 9, are excellent ($R^2 > .98$). Table 3 shows that the values of the material parameters k_0 and n are unique for each concentration and differ from the values reported for agarose gels and articular cartilage (Gu et al., 2003).

4. Discussion

The total amount and rate of consolidation over time are based on material features such as the ratio of the fluid-solid content, physical features of the underlying solid skeleton such as stiffness and permeability, and the level of applied loads (Terzaghi et al., 1996). One objective of this study was to determine the relative influence of these factors on the compressibility and permeability of the hydrogels. The compressibility of the gels, as shown by the pressure-volume response in Fig. 8, indicates a large concentration dependency, which is not a surprising result given the high water content. Likewise, the nonlinear correlation between the permeability coefficient and water content, see Fig. 9, suggest major structural and thus functional changes within the gel. These changes may furthermore alter biological properties including mass transfer rates via diffusion and ingrowth potential of cells.

We found a near linear stress-strain behavior for the 4% silk samples, whereas for the 8% and 12% gels a stiffening effect occurs. Our results of previous cell culture suggest microstructural differences between the three conditions as well: cell survival after encapsulation within these gels was highly dependent on concentration, the 4% gels greatly outperform higher concentration (Wang et al., 2008). The same large aggregates of silk that block nutrient transport throughout the porous network in 8% and 12% gels may also be responsible for the observed strain-stiffening.

Previous studies showed a nonlinear dependence of the permeability on the volume fraction of solid and fluid within a biphasic medium (Holmes and Mow, 1990; Johnson and Deen, 1996). The increase in the solid-fluid ratio will in general increase fluid tortuosity through the solid and induce a decrease in permeability (Mow and Huijskes, 2005). For this reason, we chose to estimate the permeability coefficient k at the midpoint of each load step. At each midpoint, the volume fraction of water ϕ_w was also determined using Eq. (10) and the corresponding value of permeability determined using Eq. (11). These results, shown in Fig. 9, confirm a nonlinear relation. We did not find a unique set of parameters that would match all the experimental data, which differs from the findings reported by (Gu et al., 2003). In particular, the predicted value of the permeability coefficient k_0 was higher for the 8% gel compared to 4% gel, which is counter to our expectations. Based on this result, and the generally poor fit of the confined compressibility of 4% gels, we suggest that the modeling proposed here is likely not appropriate for concentrations below 8%.

There are no published data to support the universal application of any biphasic or poroelastic model capable to capture the time-dependent behavior of natural or synthetic hydrogels across multiple load scenarios, despite a variety of attempts. Both biphasic and poroelastic formulations appear valid and essentially identical for fluid-saturated compression problems (Mow, 1987). Consolidation testing and modeling was chosen herein as it offers a clear physical approach, which is based solely on Darcy's permeability equation. The experimental data,

namely equilibrium stress-strain relations and permeability coefficients, can also be used for numerical simulations using nonlinear finite element methods (Simon, 1992).

Comparing the consolidation behavior of silk gels to other hydrogel systems or biological tissues is difficult due to lack of experimental data and the large dependence of the result on the sample geometry, boundary conditions, and test types. Comparisons of the confined compression creep behavior of silk gels to other materials, either human tissue or implant device, can be seen in Table 4. For example, most recent efforts in characterizing hydrogel biomaterials for tissue engineering have used a biphasic model, such as agarose (Benkherourou et al., 1999; Johnson and Deen, 1996; Mauck et al., 2000; Normand et al., 2000), alginate (Mauck et al., 2000), hyaluronic acid (Cloyd et al., 2007), fibrin (Noailly et al., 2008), and gelatin (Awad et al., 2004), where time-dependent properties were assessed using either confined or unconfined testing. Some data attempting to validate predicted permeability by direct experimentation, similar to that pursued herein, are also presented in Table 4. The large disparity between experimentation and prediction is clearly evident and warrants further exploration for an improved understanding of these discrepancies.

It has been observed that purified silk fibroin fibers are viscoelastic, nonlinear and strain-rate-dependent materials (Perez-Rigueiro et al., 2000), which would suggest that, aside from the fluid-solid interactions, the underlying protein skeleton will cause the gel system to respond in a time-dependent manner. As a result, one limitation of the current study is the reliance on an approximate method to define the end of primary consolidation, on which the parameter c_v is dependent. Previous attempts to monitor excess fluid pressure dissipation simultaneously during testing using polyacrylic acid hydrogels (Broom and Oloyede, 1998) and agarose hydrogels (Mauck et al., 2000) revealed final pressure extinction times ranging from approximately 10–180 minutes and 6 minutes, respectively, which is in a range commensurate with our findings (see graphs in Figs. 5–7 and Table 4). However, since the strength of the polymer network and its permeability make comparisons somewhat difficult, direct measurements of excess pore water dissipation should be investigated in the future.

Additionally, although not shown by the consolidation curves, the end of primary consolidation does not lead to a plateau behavior; instead, the silk gel continues to slowly deform during *secondary compression*, see Fig. 3, which is presumably due to the viscoelastic behavior. A viscoelastic model, which captures the time-dependent response of the protein skeleton is currently being investigated, see (Franceschini et al., 2006) for a more detailed discussion on viscoelasticity.

5. Conclusion

To the best of our knowledge, this study represents the first attempt to apply a consolidation model to regenerated silk-based biomaterials. The model is based on methods and analysis originally developed to describe the behavior of geomaterials. It is flexible enough such that determining a single material parameter (the consolidation coefficient) is enough to obtain an excellent agreement between experimental and numerical data. Furthermore, a unique pressure-volume equilibrium curve was established for each silk concentration, providing a structure-property relation that will allow for tailored design to meet specific tissue metrics. Although the values recorded by direct experimentation are well below those reported in the literature for major musculoskeletal tissues, the system of testing and analysis provides a platform for analyzing hydrogel biomaterials for further design improvements.

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References

- Accadbled F, Ambard D, de Gauzy JS, Swider P. A measurement technique to evaluate the macroscopic permeability of the vertebral end-plate. *Med Eng Phys* 2008;30 (1):116–122. [PubMed: 17446114]
- Ateshian GA, Warden WH, Kim JJ, Grelsamer RP, Mow VC. Finite deformation biphasic material properties of bovine articular cartilage from confined compression experiments. *J Biomech* 1997;30 (11–12):1157–1164. [PubMed: 9456384]
- Awad HA, Wickham MQ, Leddy HA, Gimble JM, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. *Biomaterials* 2004;25 (16):3211–3222. [PubMed: 14980416]
- Benkherourou M, Rochas C, Tracqui P, Tranqui L, Gumery PY. Standardization of a method for characterizing low-concentration biogels: elastic properties of low-concentration agarose gels. *J Biomech Eng* 1999;121 (2):184–187. [PubMed: 10211452]
- Best BA, Guilak F, Setton LA, Zhu W, Saed-Nejad F, Ratcliffe A, Weidenbaum M, Mow VC. Compressive mechanical properties of the human annulus fibrosus and their relationship to biochemical composition. *Spine* 1994;19 (2):212–21. [PubMed: 8153833]
- Biot MA. General theory of three-dimensional consolidation. *J Appl Phys* 1941;12 (2):155–164.
- Broom ND, Oloyede A. The importance of physicochemical swelling in cartilage illustrated with a model hydrogel system. *Biomaterials* 1998;19 (13):1179–1188. [PubMed: 9720901]
- Butler D, Lewis J, Frank C, Banes A, Caplan A, De Deyne P, Dowling M, Fleming B, Glowacki J, Guldberg R, Johnstone B, Kaplan D, Levenston M, Lotz J, Lu E, Lumelsky N, Mao J, Mauck R, McDevitt C, LCM, Murray M, Ratcliffe A, Spindler K, Tashman S, Wagner C, Weisberg E, Williams C, Zhang R. Evaluation criteria for musculoskeletal and craniofacial tissue engineering constructs: a conference report. *Tissue Eng Part A* 2008;14 (12):2089–2104. [PubMed: 19093294]
- Cloyd JM, Malhotra NR, Weng L, Chen W, Mauck RL, Elliott DM. Material properties in unconfined compression of human nucleus pulposus, injectable hyaluronic acid-based hydrogels and tissue engineering scaffolds. *Eur Spine J* 2007;16 (11):1892–1898. [PubMed: 17661094]
- Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 2006;126 (4):677–689. [PubMed: 16923388]
- Etienne O, Schneider A, Kluge JA, Bellemin-Lapponnaz C, Polidori C, Leisk GG, Kaplan DL, Garlick JA, Egles C. Soft tissue augmentation using silk gels: an in vitro and in vivo study. *J Periodontol* 2009;80 (11):1852–8. [PubMed: 19905955]
- Franceschini G, Bigoni D, Regitnig P, Holzapfel GA. Brain tissue deforms similarly to filled elastomers and follows consolidation theory. *J Mech Phys Solids* 2006;54 (12):2592–2620.
- Gentleman E, Nauman EA, Dee KC, Livesay GA. Short collagen fibers provide control of contraction and permeability in fibroblast-seeded collagen gels. *Tissue Eng* 2004;10 (3–4):421–427. [PubMed: 15165459]
- Gu WY, Mao XG, Foster RJ, Weidenbaum M, Mow VC, Rawlins BA. The anisotropic hydraulic permeability of human lumbar annulus fibrosus. influence of age, degeneration, direction, and water content. *Spine* 1999;24 (23):2449–55. [PubMed: 10626306]
- Gu WY, Yao H, Huang CY, Cheung HS. New insight into deformation-dependent hydraulic permeability of gels and cartilage, and dynamic behavior of agarose gels in confined compression. *J Biomech Eng* 2003;36 (4):593–598.
- Guilak F. *Functional tissue engineering*. Springer; New York: 2003.
- Heneghan P, Riches PE. Determination of the strain-dependent hydraulic permeability of the compressed bovine nucleus pulposus. *J Biomech* 2008;41 (4):903–6. [PubMed: 18164714]

- Hofmann S, Foo CT, Rossetti F, Textor M, Vunjak-Novakovic G, Kaplan DL, Merkle HP, Meinel L. Silk fibroin as an organic polymer for controlled drug delivery. *J Control Release* 2006;111 (1–2): 219–227. [PubMed: 16458987]
- Holmes MH, Mow VC. The nonlinear characteristics of soft gels and hydrated connective tissues in ultrafiltration. *J Biomech* 1990;23 (11):1145–1156. [PubMed: 2277049]
- Holtz, RD.; Kovacs, WD. An introduction to geotechnical engineering. Prentice-Hall; Englewood Cliffs, N.J: 1981.
- Hung CT, Mauck RL, Wang CC, Lima EG, Ateshian GA. A paradigm for functional tissue engineering of articular cartilage via applied physiologic deformational loading. *Ann Biomed Eng* 2004;32 (1): 35–49. [PubMed: 14964720]
- Johannessen W, Elliott DM. Effects of degeneration on the biphasic material properties of human nucleus pulposus in confined compression. *Spine* 2005;30 (24):E724–9. [PubMed: 16371889]
- Johnson EM, Deen WM. Hydraulic permeability of agarose gels. *AIChE J* 1996;42 (5):1220–1224.
- Kim UJ, Park J, Li C, Jin HJ, Valluzzi R, Kaplan DL. Structure and properties of silk hydrogels. *Biomacromolecules* 2004;5 (3):786–792. [PubMed: 15132662]
- Kluge, JA.; Leisk, GG.; Kaplan, DL. Mechanical determinants of tissue development. In: Atala, A.; Lanza, R.; Thomson, J.; Nerem, R., editors. *Principles of Regenerative Medicine*. 1. Academic Press; Boston: 2008. p. 480–497.
- Laude D, Odlum K, Rudnicki S, Bachrach N. A novel injectable collagen matrix: in vitro characterization and in vivo evaluation. *J Biomech Eng* 2000;122 (3):231–235. [PubMed: 10923290]
- Martinez JB, Oloyede VO, Broom ND. Biomechanics of load-bearing of the intervertebral disc: an experimental and finite element model. *Med Eng Phys* 1997;19 (2):145–156. [PubMed: 9203149]
- Mauck RL, Soltz MA, Wang CC, Wong DD, Chao PH, Valhmu WB, Hung CT, Ateshian GA. Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. *J Biomech Eng* 2000;122 (3):252–260. [PubMed: 10923293]
- Mow VC. Reply to harrigan's letter: Cartilage is poroelastic but not biphasic. *Journal of Biomechanics* 1987;20 (8):828–829.
- Mow, VC.; Huiskes, R., editors. *Basic Orthopaedic Biomechanics and Mechano-Biology*. 3. Lippincott Williams & Wilkins; Philadelphia: 2005.
- Mow VC, Kuei SC, Lai WM, Armstrong CG. Biphasic creep and stress relaxation of articular cartilage in compression: Theory and experiments. *J Biomech Eng* 1980;102 (1):73–84. [PubMed: 7382457]
- Nerurkar NL, Mauck RL, Elliott DM. Issls prize winner: Integrating theoretical and experimental methods for functional tissue engineering of the annulus fibrosus. *Spine* 2008;33 (25):2691–2701. [PubMed: 19018251]
- Noailly J, Van Oosterwyck H, Wilson W, Quinn TM, Ito K. A poro-viscoelastic description of fibrin gels. *J Biomech* 2008;41 (15):3265–3269. [PubMed: 18930461]
- Normand V, Lootens DL, Amici E, Plucknett KP, Aymard P. New insight into agarose gel mechanical properties. *Biomacromolecules* 2000;1 (4):730–738. [PubMed: 11710204]
- O'Brien FJ, Harley BA, Waller MA, Yannas IV, Gibson LJ, Prendergast PJ. The effect of pore size on permeability and cell attachment in collagen scaffolds for tissue engineering. *Technol Health Care* 2007;15 (1):3–17. [PubMed: 17264409]
- Ogden RW. Large deformation isotropic elasticity: On the correlation of theory and experiment for compressible rubberlike solids. *Proc R Soc Lond A* 1972;328:567–583.
- Oloyede A, Broom ND. The generalized consolidation of articular cartilage: an investigation of its near-physiological response to static load. *Connect Tissue Res* 1994;31 (1):75–86. [PubMed: 15609624]
- Oloyede A, Gudimetla P, Crawford R, Hills BA. Consolidation responses of delipidized articular cartilage. *Clin Biomech (Bristol, Avon)* 2004;19 (5):534–542.
- Perez-Rigueiro J, Viney C, Llorca J, Elices M. Mechanical properties of single-brin silkworm silk. *J Appl Polym Sci* 2000;75 (10):1270–1277.
- Rajkhowa R, Gupta VB, Kothari VK. Tensile stress-strain and recovery behavior of indian silk fibers and their structural dependence. *Journal of Applied Polymer Science* 2000;77 (11):2418–2429.
- Simon BR. Multiphase poroelastic finite element models for soft tissue structures. *Appl Mech Rev* 1992;45 (6):191–218.

- Sofia S, McCarthy MB, Gronowicz G, Kaplan DL. Functionalized silk-based biomaterials for bone formation. *J Biomed Mater Res* 2001;54 (1):139–148. [PubMed: 11077413]
- Spain TL, Agrawal CM, Athanasiou KA. New technique to extend the useful life of a biodegradable cartilage implant. *Tissue Eng* 1998;4 (4):343–352. [PubMed: 9916167]
- Taylor, DW. *Fundamentals of Soil Mechanics*. Prentice-Hall, Inc; Englewood Cliffs: 1948.
- Terzaghi, K.; Peck, RB.; Mesri, G. *Soil mechanics in engineering practice*. 3. Wiley; New York: 1996.
- Vepari C, Kaplan DL. Silk as a biomaterial. *Prog Polym Sci* 2007;32 (8–9):991. [PubMed: 19543442]
- Voor MJ, Nawab A, Malkani AL, Ullrich CR. Mechanical properties of compacted morselized cancellous bone graft using one-dimensional consolidation testing. *J Biomech* 2000;33 (12):1683–1688. [PubMed: 11006393]
- Wang X, Kluge JA, Leisk GG, Kaplan DL. Sonication-induced gelation of silk fibroin for cell encapsulation. *Biomaterials* 2008;29 (8):1054–1064. [PubMed: 18031805]
- Wenk E, Wandrey AJ, Merkle HP, Meinel L. Silk fibroin spheres as a platform for controlled drug delivery. *J Control Release* 2008;132 (1):26–34. [PubMed: 18761384]
- Yang M, Taber LA. The possible role of poroelasticity in the apparent viscoelastic behavior of passive cardiac muscle. *J Biomech* 1991;24 (7):587–597. [PubMed: 1880142]

Figure 1.

Process to form sonication-induced hydrogels for compression testing. A volume of silk solution and DMEM powder is sonicated for 30 seconds and then added to a cup made from ABS plastic. The side walls of the mold are subsequently broken and gel removed (left). The

Scanning Electron Micrograph shows the microstructure of an 8% gel solution that has been flash-frozen and freeze-dried prior to imaging (right).

Figure 2. Confined compression testing apparatus schematic (left) and details of the specimen chamber (right). The scale bars at bottom show the size of the entire apparatus and zoomed-in chamber.

Figure 3.

The upper graph shows the change in sample height of a 12% gel for preload and increasing load steps of 10N, 25N, and 40N. The lower graph shows the use of the Casagrade method to estimate the end of primary consolidation. The normalized displacement and two best-fit regression lines (using Step 2 data) is plotted vs. logtime. The intersection of these two lines defines the inflection point t_p corresponding to the end of primary consolidation and transition into secondary compression.

Figure 4.

Stress decomposition into pore pressure and effective stress within a cylindrical specimen with initial height $2H$. The curved lines are isochrones indicating the spatial distribution of pore water pressure p at times $(t_1, t_2...t_\infty)$. At time $t = 0$, the current effective stress is indicated by σ'_0 , the increment in applied load by $\Delta\sigma$. Fluid flows through an infinitesimal element dz at a rate dQ in response to the local pressure gradient (here shown positive downward due to its location below the gel mid-height). Figure adapted from (Holtz and Kovacs, 1981).

Figure 5.

A representative experimental response (dotted line) and numerical fit (solid line) of 4% silk gel under 5N, 7.5N and 10N sequential consolidation load steps, from the top to the bottom.

Figure 6.

A representative experimental response (dotted line) and numerical fit (solid line) of 8% silk gel under 7.5N, 15N and 25N sequential consolidation load steps, reading from the top to the bottom.

Figure 7.

A representative experimental response (dotted line) and numerical fit (solid line) of 12% silk gel under 10N, 25N and 40N sequential consolidation load steps, reading from the top to the bottom.

Figure 8.

The confined compressibility of 4% (◆), 8% (○), and 12% (■) hydrogels shown as average and standard deviation for $n=3$ samples (top). The corresponding numerical results using Eq. (9) with $\beta = 7$ and $\alpha = 8.1, 40$ and 84 kPa are shown below.

Figure 9.

Data of permeability values k vs. volume fraction of water ϕ^w for 4% (◆), 8% (○), and 12% (■) gels. There is excellent agreement between data and numerical results using Eq. (11). Values of model parameters are listed in Table 3. Permeability values k_0 for the load free configuration are depicted for each gel solution by ★.

Table 1

Loading steps for creep analysis.

Silk Conc.	Tare [N]	Step 1: Load [N]	Step 2: Load [N]	Step 3: Load [N]
4%	2.915	5	7.5	10
8%	2.915	7.5	15	25
12%	2.915	10	25	40

Table 2

Load step creep test data and model fit values.

Silk. Conc.	Effective Stress σ' [kPa] for Step 1–3	Constrained Modulus E [kPa] fit by Eq. (9)	Coeff. Consol. c_v [mm ² /min] (mean $\pm$ SD, n=3)	R^2	Predicted Perm. k [$\times 10^{-14}$ m ⁴ /Ns] (mean $\pm$ SD, n=3)
4%	2.9 – 5.0	34.17	1.80 $\pm$ 0.11	.985	87.79 $\pm$ 5.51**
	5.0 – 7.6	43.72	0.84 $\pm$ 0.53	.969	32.02 $\pm$ 20.23
	7.6 – 10.1	74.30	0.66 $\pm$ 0.37	.992	14.93 $\pm$ 8.18
8%	2.9 – 7.6	95.56	9.40 $\pm$ 3.54*	.961	163.88 $\pm$ 61.80**
	7.6 – 15.1	159.33	5.15 $\pm$ 1.95	.922	53.83 $\pm$ 20.35
	15.1 – 25.2	249.30	3.38 $\pm$ 0.08	.972	22.62 $\pm$ 0.54
12%	2.9 – 10.1	154.80	5.45 $\pm$ 2.35	.931	58.65 $\pm$ 25.34
	10.1 – 25.2	263.34	4.90 $\pm$ 3.30	.966	31.03 $\pm$ 20.91
	25.2 – 40.4	430.26	2.88 $\pm$ 1.76	.946	11.16 $\pm$ 6.82

* Significant differences at the $p < .05$ level.

** Significant differences at the $p < .01$ level.

Table 3

Numerical permeability data.

	4%	8%	12%
φ_0^w	0.9705	0.941	0.9114
k_0	3.53×10^{-12}	9.78×10^{-12}	1.35×10^{-12}
n	11.97	18.72	10.06

Values of the material parameters φ_0^w , k_0 and n used in Eqs. (10) and (11) to determine the porosity k as a function of the current volume fraction of water φ^w for the 4%, 8% and 12% gel solutions. Units of the porosity k_0 are $[m^4/Ns]$.

Table 4

Permeability and confined compression test data for human soft tissues and hydrated gels.

Material	Peak Stress Investigated [kPa]	Time Pore [*] Water Loss [min]	Measured Permeability k [$\times 10^{-14} \text{m}^4/\text{Ns}$]	Predicted Permeability k [$\times 10^{-14} \text{m}^4/\text{Ns}$]	References
Silk Gel (8% w/v)	25.2	4.03	184	164	This Study
Agarose Gel (2% w/v)	≈ 8	≈ 7	220	1.0	(Mauck et al., 2000)
Injectable Collagen Gel	15.6	≈ 15	1.35	1.31	(Laude et al., 2000)
Articular Cartilage	1250	≈ 300	0.141	0.220–0.270	(Oloyede and Broom, 1994; Gu et al., 1999; Ateshian et al., 1997)
Nucleus Pulposus	3000	≈ 3000	0.032–0.208	0.029–0.091	(Martinez et al., 1997; Johannessen and Elliott, 2005; Heneghan and Riches, 2008)
Annulus Fibrosus	110	≈ 250	0.115–0.193	0.022–0.027	(Best et al., 1994; Gu et al., 1999)

* Estimates only, based on published graphical data or explicitly-stated values.