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Mechanical Properties of Hydrated Acoustically Sensitive Alginate-Based Microcapsules Confined in a Microfluidic Device as a Function of Size and Composition

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Abstract

Understanding the mechanical properties of alginate-based microcapsules according to size and chemical composition allows researchers to zero in on the treatment and methods required to engineer optimized implantable alginate-based artificial cells for chemotherapy. Cross-linked medium viscosity alginate capsules ranging from 1.1% (w/v)-1.8% (w/v) in composition and 200 µm-1200 µm in size, encapsulating ultrasound contrast agents and blue dextran were compressed within a 40 µm high polydimethylsiloxane microfluidic device and subsequently examined using 2D microscopy for strain deformation aimed at the calculation of poisson ratios and volume loss post-compression. Results indicate a decrease in Poisson ratio as a function of alginate concentration, with statistically significant increases in Poisson ratios and percent volume loss as a function of size and composition. For an average of 120 s observation time post compression, in light of the volume loss correlated to the number of cross-links as a function of capsule size and alginate concentration, a strong case for the dominance of poroelasticity vs. viscoelasticity can be made. While there was a decrease in mean Poisson ratio as a function of concentration, at 1.8% (w/v) the mean strain value converged to 0.5, the theoretical ideal isotropic value associated with soft biological tissue.

Keywords: Alginate; Acoustically sensitive microcapsules; Microfluidic device; Poisson ratio; Concentration polarization

Introduction

For the past 30 years, biocompatible hydrogels namely agarose, alginate, chitosan, collagen, fibrin and hyaluronic acid have been extensively used in drug delivery, tissue engineering and regenerative medicine due to their biocompatibility, viscoelastic characteristics, and ease of fabrication into specific shapes and sizes namely microcapsules, microfibers and patches [1-4]. Amongst, the emerging applications are engineering of micro-environments for eukaryotic cell differentiation [5-7] and implantation for ultrasound-mediated localized drug delivery [8,9]. In tissue engineering the challenge is to replicate the native tissues' mechanical and viscoelastic properties [10]. In drug delivery, the challenge is the simultaneous optimization of mechanical strength dictated by implantation site and rate of diffusion governed in turn by size and membrane permeability [11].

Biocompatible hydrogels can be modeled as viscoelastic materials that exhibit rubber like characteristics [12-15]. Because of their highly water-swollen nature, hydrogels might lose water if they are deformed, causing changes in the mechanical properties of the gel. The deformation of these elastomeric gels is time-dependent, resulting from concurrent molecular processes. The stress–strain relationship obtained from conventional testing therefore reflects a combination of the intrinsic mechanical properties of the matrix itself and its permeability [16,17]. For a given gel, measured properties will depend on liquid flows, and therefore upon the size of the particle as well as the time scale of the observations [18,19]. Concurrent time-dependent viscoelasticity and poroelasticity have been observed in many materials. In living tissues and cells, viscoelasticity results from the conformational change of macromolecules and poroelasticity results from the migration small molecules [20,21]. Values of the viscoelastic relaxation time \( \tau \) and the material-specific length \( (D\tau)^{1/2} \) where \( D \) is the solvent diffusivity vary greatly among different materials. For a given polymer-solvent pair, the values of \( \tau \) and \( (D\tau)^{1/2} \) also depend on the crosslink density of the polymer network, the concentration of the solvent; multiple times are also possible for different molecular processes of relaxation [14].

Several systems have been reported in the literature for testing mechanical properties of hydrogels like microcapsules ranging from a hundreds of µm to mm range or porous slabs [19,22-33]. Some setups use indenters [19,22-27] and others use parallel plates [16,22,25,27] to compress a single microcapsule or multiple microcapsules [28]. More recent work involves the use of a MEMS force sensor [33] or texture analyzers [29-31]. In general, all mechanical testing was done on single microcapsules with the size ranging from a few microns to the millimeter scale. Various types of capsules were tested – most common being alginate, alginate chitosan, chitosan-genipin and melamine formaldehyde filled with a liquid. Effects of the encapsulated liquid on mechanical strength were not addressed. Membrane thickness was also a parameter that was studied [28,31]. It was found that a capsule with increased membrane thickness was able to sustain more force before bursting. In terms of the mechanical properties measured,

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bursting force [19,24,26,29,31,32] and/or pressure [23,24,32] was most commonly measured. Also Young's modulus [17, 23,28,30,33], rupture strength [29], deformability [20,29,31,32] and percentage strain at burst [31,32] were other properties measured by these systems. Additional research has been conducted on cross-linked alginites and cell clusters [31,32] were other properties measured by these systems. Additional research has been conducted on cross-linked alginites and cell clusters. The time-dependent processes should occur under either the condition $t\sim \tau_v$ for viscoelastic relaxation or the condition $t\sim L^2/D$ for poroelastic relaxation [34-41].

The specific aim of this research is to map compression effects on Acoustically Sensitive Microcapsules (ASMs) of different size and membrane composition in an aqueous environment using a custom-designed microfluidic set-up. Results obtained from the stress test recorded using 2D microscopy, will be used for a twofold characterization subjected to statistical analysis. Variations in capsule strain expressed in terms of Poisson ratio, and, volume compression expressed in terms of percentage volume loss will serve as indicators of viscoelastic and poroelastic deformation, respectively. Based on the findings an ideal set of size and alginate composition be identified for future ultra-sound based drug release. Shown in Figure 1a is a micrograph of ASMs encapsulating blue dextran as a MW marker and Ultrasound Contrast Agents (UCAs). Shown in Figure 1b is a localized ASM membrane burst post ultrasound treatment.

### Materials and Methods

#### Polydimethylsiloxane microfluidics device fabrication

Polydimethylsiloxane (PDMS) microfluidics devices were created using the procedures for rapid prototyping of PDMS microfluidic systems [9,42]. These steps included: (1) spin-coating a single layer of SU-8 (SU-8 2035, Micro-Chem Corp., Newton, MA) onto a silicon wafer, (2) creating a photo-pattern via UV radiation exposure, (3) developing photosensitive and leaving a master, (4) vapor-treating the surface with tridecafluorooctyltrichlorosilane and vacuum-casting the PDMS solution, (5) curing the PDMS layer at 150°C and then releasing it from the SU-8 master, and (6) plasma-treating and bonding the PDMS microfluidics device to a glass slide.

A 500 µm thick PDMS microfluidics device was made. The procedure included mixing 5 g of elastomer base to 1 g of curing agent (Sylgard 184 Silicone elastomer kit, Dow Corning Corp., Midland, Michigan, USA). The solution was stirred thoroughly for two minutes and placed into a vacuum chamber. The vacuum chamber was sealed and the vacuum turned on using a switch valve between the vacuum pump and vacuum chamber, air was quickly released every one to two minutes. Cycling between vacuum pressure and atmospheric pressure during the degassing process aided in the removal of air bubbles that formed in the solution during the elastomer base and curing agent mixing process. Once the air bubbles were removed, the vacuum was turned off and the degassed solution was removed from the vacuum chamber and poured onto the SU-8 master.

#### Microcapsule fabrication

Microcapsules were fabricated using the mechanism of ionotropic gelation of medium viscosity alginate (A2033, Sigma Aldrich, St. Louis, MO, USA) by means of atomization [43]. The following 3 alginate concentrations 1.1 % (w/v) (G1), 1.5% (w/v) (G2) and 1.8% (w/v) (G3) were used in this study where a sodium-alginate solution was jetted into a 1.5% CaCl2 bath. This concentration range was chosen to encompass the wider 2%- 3% (w/v) for reported pre-sterilized alginate concentration range used in cell microencapsulation [1]. The cross-linking time was set to 1hr. The air ($F_a$) and liquid ($F_l$) flow rates were adjusted to accommodate the hydrogel viscosity and surface tension for producing a target range of 200 µm-1500 µm microcapsules at each alginate concentration. The atomizer needle assembly was a concentric 24 G needle surrounded by a 16 G needle, through which the sodium alginate and air flow. The calcified sodium-alginate beads were then washed with 0.9% (w/v) NaCl twice. For each run in ASM production, 5 mL of solution was required; comprising of 2.75 mL of alginate solution and 2.25 mL of blue dextran solution to which 0.3 mL of UCAs (Targesar P, Targeson, San Diego, USA) was added. The ASMs were kept in solution and stored in a 2-8°C refrigerator for a maximum of 24 hours prior to use.

#### Stress test and image capture

A single microcapsule was isolated at a time and examined under the microscope according to the scheme outlined in Figures 2a-2c. A small amount of saline was placed in the microfluidics device with the microscope according to the scheme outlined in Figures 2a-2c.
the microcapsule via a pipette. The solution reduced microcapsule shrinkage and maintained the equilibrium swollen state. To apply compression onto the constrained microcapsule, a 1mm thick glass slide was gently placed on top of the microfluidics device. Doing so trapped the microcapsule within microfluidics device, flattening the microcapsule into a cylindrical disk of a height equal to the depth of the microfluidics device (40 µm). The assembly was then placed on 2D light microscope stage. Capsule diameter prior and post compression in the X and Y directions was measured using a Nikon transmission microscope/camera (Nikon Eclipse Ti-S) equipped with an Andor Technology Interline CCD camera. The microcapsules were examined using 40X magnification using the image analysis software (NIS-Elements v3.2.2). Diameters were measured at five random locations around the perimeter of the capsules and the average findings were recorded. It was assumed that the microcapsule height post-compression was equal to the height of the microfluidic device. The average time between the post-compression and size recording steps was 120 s.

Image Analysis

Based on the diameter lengths, the strain in both X and Y axes of the ASM were calculated using Equation 1

\[ \varepsilon_{x/y} = \frac{D_{\text{final}} - D_{\text{initial}}}{D_{\text{initial}}} \]  

(1)

Where \( \varepsilon \) is strain, \( D_{\text{initial}} \) is the initial ASM diameter before compression, and \( D_{\text{final}} \) is the final microcapsule diameter after compression.

The strain in the z direction (\( \varepsilon_z \)) was calculated by using the averaged non-compressed diameter value in the X and Y directions as initial diameter and the height of the microfluidic device as the final diameter.

An average Poisson ratio (\( v_{\text{mean}} \)) was calculated by averaging the diameters in the X and Y directions using Equation 2.

\[ v_{\text{mean}} = \frac{\varepsilon_{x/y}}{\varepsilon_z} \]  

(2)

The percent change in volume (\%V) was also estimated by assuming a spherical shape of the microcapsules before deformation (\( V_{\text{initial}} \)) and a cylindrical ASM shape after compression (\( V_{\text{final}} \)). An average initial and compressed diameter was calculated by averaging the diameters in the X and Y directions. For the compressed volume, the area calculated based on the averaged volume was multiplied by the height of the microfluidic device.

Statistical Analysis

For each alginate concentration (G1-G3) capsules were divided according to their size into two categories, the 200 µm-500 µm (S) and the 500 µm above size range (B) in order to secure a minimum sample size 10 in each sub-population. Normality testing was conducted for the six sub-populations on Poisson ratios and percent volume compression prior to conducting a 1-sided student t-test at the 90% CI. Statistical analysis was conducted using MATLAB 2013.

Results

Filtering capsules for sphericity

Capsules were filtered for sphericity in order to deconvolute the effect of initial shape from compression. The inclusion criterion for the ratio of the axes (X/Y) was set between 0.8 and 1.25. The limits of this criterion were established based on results of screening trials where strain outlier values and capsule burst were associated with oblique capsules. Up to 20% burst was observed in the absence of filtering. Shown in Figure 3 are the pooled results of strain in the Y direction (\( \varepsilon_y \)) by size as a function of alginate concentration. As indicated by the coefficients of determination (\( R^2 \geq 0 \)) approaching zero, the artifacts of morphology can be decoupled from compression effects.

Effect of capsule size on the average Poisson ratio and Volume compression

Shown in Figure 4 is a sample probability plot of the average Poisson ratio (\( v_{\text{mean}} \)) for the population subgroup G1S. As indicated by the plot linearity, the capsules have been sampled from a normally distributed sub-population hence parametric analysis was applied to the data without any mathematical transformation [45]. All other sub-populations (G1B, G2S, G2B, G3S, G3B) were also subjected to Normality testing with positive results, validating the use of parametric analysis.

Results of the t-test are summarized in Table 1 as well the number of capsules analyzed per subpopulation. As indicated by the p-values (≤ 0.1), the average value of both metrics, was significantly higher for the 500 µm and higher category, hence the Null-hypothesis was accepted. The Null hypotheses were in this case that the Poisson ratio and percent volume compression were higher for the larger capsules than the smaller ones.

Although a larger surface area was constrained in contact with the microfluidic device for the B capsules than for the S subpopulation, the apex of the contact area being 40 µm the height of the channel, the capsules became stiffer with size. This finding is contradicted by tensile deformation measurements conducted on alginate ranging from 1%-3% (w/v) in concentration and several hundred micron capsules using micro-indentation [9]. With respect to volume loss due to compression leading to a net efflux of solvent, the number of cross-links in the semi-
Figure 3: Capsule sphericity vs. strain as function of alginate concentration; (a) 1.1% (w/v), (b) 1.5% (w/v), (c) 1.8% (w/v).

Figure 4: Half-normal probability plot for the ≤ 500 µm capsules comprised of 1.1% alginate (Subpopulation G1S).

Table 1: Significance testing for the Poisson ratio and Percentage of Volume Compression at the 90% CI.

| Sample | G1S | G1B | G2S | G2B | G3S | G3B |
|--------|-----|-----|-----|-----|-----|-----|
| Mean(υ<sub>mean</sub>) | 0.60 | 0.69 | 0.55 | 0.61 | 0.44 | 0.54 |
| Variance(υ<sup>2</sup>)<sub>mean</sub>) | 0.05 | 0.07 | 0.03 | 0.04 | 0.03 | 0.02 |
| N | 32 | 26 | 22 | 42 | 10 | 32 |
| P(T<=t) one-tail | 0.097 | 0.101 | 0.041 |

| Sample | G1S | G1B | G2S | G2B | G3S | G3B |
|--------|-----|-----|-----|-----|-----|-----|
| Mean (%V) | 51.5 | 75.3 | 70.9 | 79.8 | 72.2 | 84.2 |
| Variance (%V) | 218.8 | 36.6 | 103.5 | 54.7 | 30.2 | 18.0 |
| N | 32 | 26 | 22 | 42 | 10 | 32 |
| P(T<=t) one-tail | 1.33E-10 | 8.31E-05 | 3.45E-09 |

Figure 5: Correlation plots between the strain in the Y and X directions as a function of alginate concentration; (a) 1.1% (w/v), (b) 1.5% (w/v) and (c) 1.8% (w/v).
porous capsules fabricated by ionotropic gelation is proportional to the volume of the spherical structure.

**Effect of alginate concentration on the Poisson ratios and Volume Compression**

The correlations plots for the strain in the Y and X directions as a function of alginate content are presented in Figures 5a-5c. As shown by the progression of the coefficient of determination values (0.093, 0.503, 0.727) and slopes (0.282, 0.656, 0.853), the association between the magnitude of strain deformation in each direction became stronger with increasing alginate concentrations, inferring transition towards isotropic behavior.

Shown in Figure 6 was the monotonous decrease of the average Poisson ratio ($\nu_{\text{mean}}$) as a function of alginate concentration. At 1.8% (w/v) the mean metric value converged to 0.5 the theoretical ideal isotropic value associated with soft biological tissue [44]. Across literature an increase in the modulus of elasticity and/or an increase in strain as a function of alginate concentration are reported across multiple strain measurement techniques characterized by a wide range of measurement noise [9,28,46-48]. Theoretically a decrease in the average Poisson ratio should be correlated to a decrease in strain [44]. Discrepancies between the current findings and cited literature are two-fold: 1) In the absence of measurements in the Z direction it has been assumed that the default height of the capsule post-compression is constant at 40 µm and thus an inherent error in the strain calculation was introduced, 2) Although a monotonous decrease was observed in Figure 6, experimental noise was predominant as reflected in the overlapping standard deviations. Estimating neither the modulus nor the elasticity limit was conducted in this work. It required estimating the stress on each capsule by the microfluidic device, subjecting capsules of identical size and composition to compression using multiple channel depths which is not trivial and requires separate simulations and measurements.

As for the percent volume compression, as shown in the Box plot in Figure 7 the distribution range decreased as the alginate concentration was increased and no outliers were observed at the highest concentration. A p-value of $1.4 \times 10^{-15}$ at the 95% CI is obtained for the ANOVA conducted on microcapsules comprised of the 3 concentration groups. This analysis coupled with the examination of Figure 6 indicated an increase in volume loss as a function of alginate concentration.
concentration. By analogy to the effect of capsule size on volume compression, it could safely be assumed that for the same cross-linker concentration, the higher the alginate concentration, the higher the number of cross-linked pores through which solvent loss occurred.

Discussion

Evidence of Poroelasticity

In light of the volume loss correlated to the number of cross-links as a function of capsule size (contact area) and alginate concentration, and, noisy strain measurements a strong case for the dominance of poroelasticity over viscoelasticity can be made [34-36]. The reported effective diffusivity of magnitude of the solvent diffusivity. The reported small molecule concentration, the higher the alginate concentration, the higher the compression, it could safely be assumed that for the same cross-linker concentration. By analogy to the effect of capsule size on volume compression, it could safely be assumed that for the same cross-linker concentration, the higher the alginate concentration, the higher the number of cross-linked pores through which solvent loss occurred.

Concentration Polarization

A marked study between the present work and other strain measurement efforts is the entrapment of blue dextran in the capsules. In this study, strain deformation results of hydrated alginate-based Microcapsules Confined in a Microfluidic Device as a Function of Size and Composition. J Biotechnol Biomater 3: 161. doi:10.4172/2155-952X.1000161

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