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Cavitation Rheology for Soft Materials

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To guide the development of tissue scaffolds and the characterization of naturally heterogeneous biological tissues, we have developed a method to determine the local modulus at an arbitrary point within a soft material. The method involves growing a cavity at the tip of a syringe needle and monitoring the pressure of the cavity at the onset of a mechanical instability. This critical pressure is directly related to the local modulus of the material. The results focus on the network development of poly(lactide)–poly(ethylene oxide)–poly(lactide) triblock copolymer and poly(vinyl alcohol) hydrogels. These materials serve as model materials for tissue scaffolds and soft biological tissues. This new method not only provides an easy, efficient, and economical method to guide the design and characterization of soft materials, but it also provides quantitative data of the local mechanical properties in naturally heterogeneous materials.

1. Introduction

The measurement of mechanical properties in soft materials, such as hydrogels, is important for numerous applications ranging from tissue engineering to food science.[1–4] Although numerous techniques are available for measuring the mechanical modulus of a material (Table 1), most methods are either difficult to implement for soft materials or do not provide local measurement at any arbitrary point. Here, we present a novel and easy method by which to determine the modulus on length scales similar to biological cells ($\sim$10–50 μm) at specific points within a material. The simplicity and sensitivity of this technique not only enhance its potential impact, but also present numerous questions about the material physics related to this novel approach.

The method involves inducing a single cavity within a soft gel material and quantifying a critical pressure of mechanical instability. The pressure of the instability is directly related to the modulus of the material. As this method measures the modulus local to the site of cavitation, heterogeneities within a material can easily be probed. This capability is particularly important for monitoring the heterogeneities within a scaffold structure or local mechanical property changes within a native structure. For example, the technique introduced here can be used to probe biological tissues with integrated layered structures, such as within the mitral valve[5] or the eye,[6] as well as their property changes with the onset of disease. To demonstrate the technique, we focus on the characterization of two model synthetic hydrogel materials: 1) triblock copolymer of poly(lactide) [PLA] and poly(ethylene oxide) [PEO]; and 2) poly(vinyl alcohol) [PVA]. In the following sections, we detail this novel technique and discuss its use in measuring the mechanical properties of these hydrogel systems.

2. Experimental

2.1 Cavitation rheology technique (CRT)

Cavitation rheology involves quantifying the pressure dynamics of a growing bubble, or cavity, within a soft solid or fluid. Cavity introduction is controlled and monitored with a custom-built, semi-automated instrument that consists of a syringe pump (New Era Syringe Pump NE1000), pressure sensor (Omega Engineering PX26-001GV), syringe needle (Fisher Scientific 91033), microscope (Edmunds Optics), and personal computer to record the pressure and microscope images of cavity growth as a function of time. A schematic of the instrument is shown in Fig. 1. The combined cost of the syringe, the needle, the pressure sensor, and tubing is approximately $150, demonstrating the cost advantage of the technique. The pressure and image acquisition are controlled by a custom-written program within National Instruments LabView software. All experiments were performed at a syringe pump rate of 6.7 $\mu L\ s^{-1}$.

The test begins with the controlled lowering of the syringe needle into the sample. An initial pressure signal is recorded. The air in the syringe is compressed *via* the syringe pump beginning at time, t, arbitrarily defined as $t = 0$. The pressure within the system is measured throughout the experiment. Although images of the growing cavity are not necessary to determine the modulus for most materials, images are collected and can provide further insight into material properties.

2.2 Cone and plate rheology

Cone and plate shear rheometry (TA Instruments rheometer) was used to measure the shear moduli of the hydrogels for comparison with the moduli values measured with CRT. A 2°, 40 mm acrylic cone was used for all samples. Stress controlled frequency sweeps (0.1–2.0 Pa depending on the sample) were performed in a range of 0.01–100 Hz. Although not rigorously true for all hydrogels, we assume that our materials are isotropic and incompressible. This assumption is used to

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Table 1 Description of current techniques available to measure the mechanical properties of soft tissues

Advantages	Disadvantages
Macroscopic length scales: shear rheometry	
Modulus can be measured	Heterogeneities cannot be probed
Gel point is easily determined	Requires a large amount of material
Naturally determines an "average" response	Expensive set-up, requires training
Mesoscopic length scales: contact mechanical test (AFM)	
Modulus can be measured	Can only probe surface properties
Length scales of cell can be probed	Expensive set-up, requires training
Microscopic length scales: microbead rheology.[14,15]	
Modulus can be measured producing values similar to traditional rheology	Generally used for softer materials, <100 Pa
Smaller length scales can be probed	Requires training

transform shear-moduli data to linear elastic moduli for comparison with the CRT data.

3. Materials

3.1. PLA–PEO–PLA triblock copolymer hydrogels

Linear triblock copolymers of PLA–PEO–PLA (PPP) were synthesized as previously described in the literature,[7] but using solution polymerization (refluxing in toluene) instead of bulk polymerization. Using this technique, the chain lengths are controlled and the PDI is generally less than 1.1. For the experiments discussed here, the molecular weight of the PEO mid-block was 8800 g mol^{-1} and the end-block molecular weights were 2700 g mol^{-1} for a total chain molecular weight of approximately 14 200 g mol^{-1}. Gels were prepared by slow addition of the dried polymer to DI water followed by stirring and heating. Crystallinity was inhibited in the end-block domains by using poly(DL-lactide).

3.2 PVA hydrogels

The PVA hydrogel was prepared in a manner similar to Wang[8] where the polymer (M_w 13 000–23 000 g mol^{-1}, 99.9% hydrolysis, Aldrich) was heated in a mixed solvent of dimethyl sulfoxide and water in a 40 : 60 ratio with 2% boric acid as a cross-linking agent at 90 °C. After the polymer was fully dissolved, the sample was cooled to room temperature to form a gel. Unless stated, hydrogels of 10% PVA were used. Samples were stored in airtight containers between experiments to prevent solvent loss.

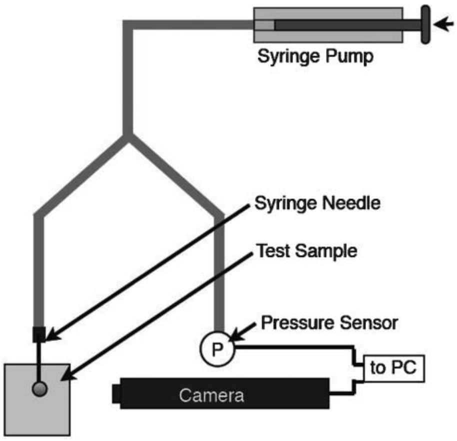

Fig. 1 Diagram of experimental setup.

4. Results and discussion

4.1 Cavitation in a hydrogel

Fig. 2 illustrates a typical pressure history for a CRT experiment in a 15% PPP hydrogel system. At time $t = 0$, the syringe pump begins compressing the volume of air, V, in the syringe system. V is defined as:

$$V = V_0 - \mu t + V_c \qquad (1)$$

where V_0 is the volume of the system at $t = 0$, μ is the pump compression rate, and V_c is the volume of the cavity induced at the syringe needle tip. Assuming that air is immiscible with the hydrogel and the number of air molecules remains fixed, the pressure, P, as a function of time, t, can be described by:

$$\frac{P}{P_0} = \left(1 - \frac{\mu t}{V_0} + \frac{V_c(t)}{V_0}\right)^{-1} - 1 \qquad (2)$$

where P_0 is the initial pressure in the system recorded for $t < 0$. For $V_c/V_0 \ll 1$, eqn (2) predicts that P increases linearly with time, as observed in Fig. 2a. In fact, multiple cycles can be run for $P < P_c$ verifying the linearity of the measurement for an elastic material, as seen in the inset of Fig. 2a. At a critical pressure, P_c, the positive slope in the pressure history changes instantaneously. This change is related to the sudden dominant increase in the rate of cavity growth relative to μ.

As seen in the images of Fig. 2c, the cavity at the syringe tip has very little growth until the critical pressure is reached. Within 200 ms, the cavity volume increases significantly. This sudden increase in the cavity size marks the onset of an elastic instability and is clearly observed in the sudden increase of the cavity-extension ratio in Fig. 2b.

4.2 Mechanics of pressure instability in soft matrices

The introduction of a bubble, or cavity, into a Newtonian liquid follows the simple relationship of $P = 2\gamma/r$, which relates the pressure, P, of the cavity to its size, r, and the surface energy, γ, of the liquid. For the growth of a cavity in an elastic network, the pressure is balanced by both the surface energy and elastic restoring energy of the surrounding material. The elastic restoring energy is conventionally described by the strain-energy function, W, that relates the amount of elastic energy to the amount of strain imposed in a given material.

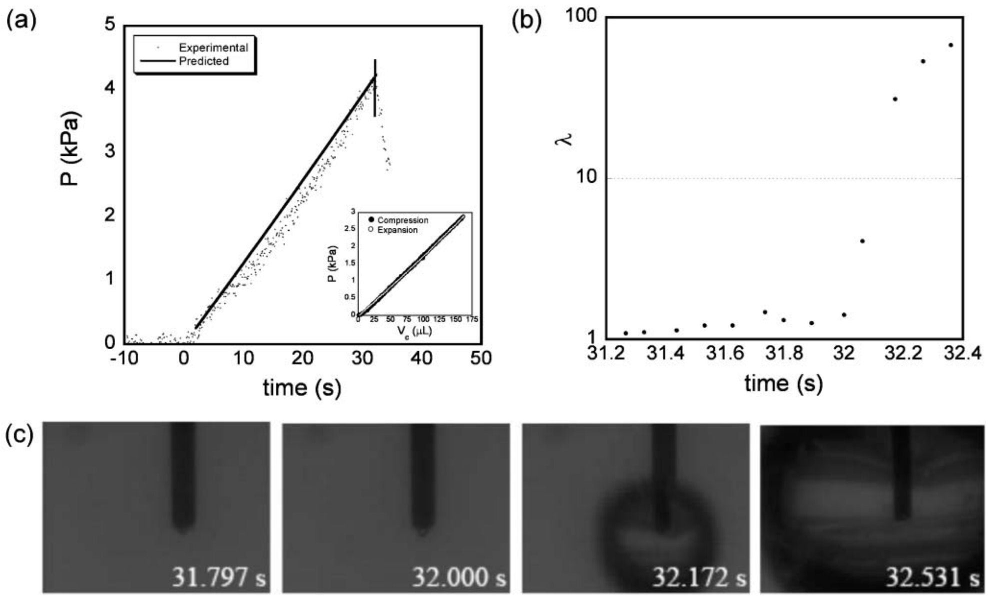

Fig. 2 The cavitation process as the pressure within the system increases with time. (a) Pressure in the system *versus* time, both experimental and predicted. Inset shows pressure at tip of syringe *versus* compressed volume for cyclic loading at $P < P_c$ to demonstrate linearity of the system. (b) Extension ratio of the cavity with time. (c) Images of the cavitation event. The outer diameter of the syringe needle is 210 μm.

For hydrogel materials, a simple strain-energy function is the neo-Hookean relation:

$$W = (E/6)(2\lambda^2 + \lambda^{-4} - 3) \tag{3}$$

where E is an elastic coefficient that is equal to the linear modulus at small strains, and λ is the extension ratio of the cavity radius, $\lambda = r_c/r_{c_0}$ From this strain energy function, the pressure-growth relationship for a spherical void with internal pressure, P, can be determined:[9,10]

$$\frac{P_1}{E} = \frac{P - {}^{2\gamma}\!/_{r}}{E} = \frac{5}{6} - \frac{2}{3\lambda} - \frac{1}{6\lambda^4} \tag{4}$$

where P_1 is the elastic pressure contribution, and λ for our measurements is taken as the extension ratio of the cavity surface area $\lambda = (A_c/A_{c_0})^{1/2}$ Eqn (4) is written for an incompressible material (*i.e.* Poisson's ratio = 0.5), but equivalent expressions for general elastic materials are listed in many references.[9–13] According to eqn (4), the extension ratio approaches infinity as P_1 reaches a critical value of $5E/6$ (Fig. 2).

Although cavity growth in eqn (4) is monotonically dependent upon pressure, the conditions of the CRT experiments provide a clear definition of a critical pressure. Due to the conservation of air in the CRT experiment and controlled volume conditions for pressurization, the onset of "infinite" extension in the cavity causes a definitive change in the P *vs.* t curve. This behavior is described quantitatively by using eqn (4) to determine V_c and substituting into eqn (2). Using an iterative numerical solution, we can predict this cavitation behavior for the CRT experiment. As the model predicts, the critical, or maximum, pressure (P_c) is equal to $5E/6$ for materials described by the neo-Hookean strain-energy model (Fig. 2a). Therefore, the modulus of the elastic network can be determined as $E = 6P_c/5$. This critical pressure is only sensitive to the volume of material roughly described by r_s^3 (where r_s is the inner radius of syringe). Therefore, the CRT modulus measurement provides information on the local network structure. Note that the exact strain-energy function for a given material may alter the exact value of the proportionality constant between E and P_c, but this constant ranges in general between 0.5 and 2.0.[11] Therefore, CRT provides sufficient accuracy combined with the advantages of ease, efficiency, and spatial resolution for use in the development of soft materials, such as tissue scaffolds. Although cavitation has been observed in materials with moduli as large as 10^6 Pa,[9] different deformation mechanisms such as yielding or fracture will most likely occur prior to cavitation in more rigid materials.

4.3 Cavitation in PPP hydrogels

To demonstrate this technique, we measure the elastic moduli using the CRT for three PPP hydrogels of polymer mass fractions 10, 15, and 20 wt % and compare these values to measurements from classical shear rheology. For both techniques, Fig. 3 shows that the modulus increases with polymer concentration. This trend is expected due to the increase in cross-link density as the number of polymer chains per unit volume increases. More importantly, the modulus values from the CRT are similar compared to the values measured by classical cone and plate rheology. This observation verifies that the critical pressure of instability is closely related to the elastic modulus of the hydrogel.

At lower polymer concentrations, the CRT measures a higher modulus value. This deviation is due to the convolution of the surface-tension properties. According to eqn 4, the material-defined length scale ($2\gamma/E$) must be smaller than the inner radius (r) of the syringe for the pressure-instability

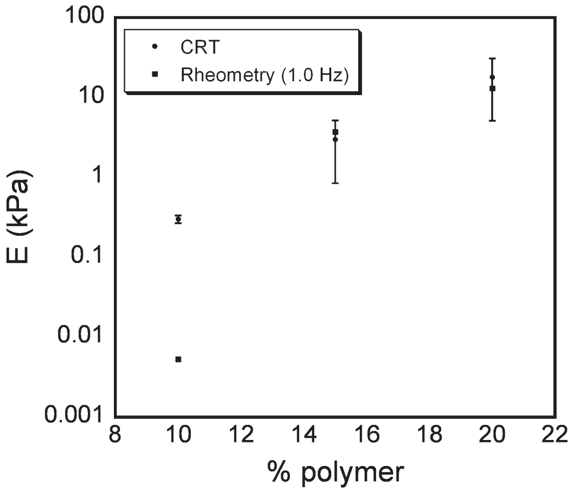

Fig. 3 Modulus *versus* percent polymer (PPP) in sample as determined by both CRT and classical rheology. For these calculations, γ was found to be 0.067 N m^{-1}.

to be sensitive to the modulus of the network. When $2\gamma/Er$ approaches or exceeds a value of 1, then surface tension will dominate the resistance to cavity growth. For the 10% PPP hydrogel, the surface-tension term is equal to 13.4 as compared to a value of 0.39 for the 15% hydrogel and 0.23 for the 20% hydrogel.

The exact value of the CRT moduli is dependent on the apparent heterogeneities of the network. These heterogeneities give rise to distributions of moduli, which are reflected in the error bars in Fig. 3. Each data point is an average of 6–10 measurements taken at different points within the sample. At lower polymer concentrations, it can be seen that the standard deviation of the measurements is small compared to samples of higher concentration. In fact, for the 20% PPP sample, heterogeneities were visually observed as dense polymer conglomerates at elevated temperatures. This mechanical heterogeneity is not detectable from macroscopic shear rheology techniques, but may have significant impact on tissue development in an engineered scaffold.

4.4 Cavitation in PVA: measurement of heterogeneities

To emphasize a primary advantage of CRT to characterize heterogeneities throughout the bulk of a soft material, we used CRT to quantify the elastic modulus as a function of location in a PVA gradient hydrogel. The PVA gradient hydrogels were fabricated as shown in the inset of Fig. 4, where 3 mL of a 12% PVA solution were put in a vial with a subsequent 3 mL of a 10% PVA solution put on top of the initial solution. With time, limited diffusion occurs across the two concentrations resulting in the formation of a natural gradient. Diffusion is arrested by the onset of the gel network. CRT measurements were then taken at different heights within the samples. It is clear from the graph in Fig. 4 that the modulus values increase as the gradient goes from lower to higher PVA concentration. Related to the same mechanism observed in the PPP hydrogel data in Fig. 3, this increase in modulus with increasing polymer concentration arises from an increase in cross-link density within the system.

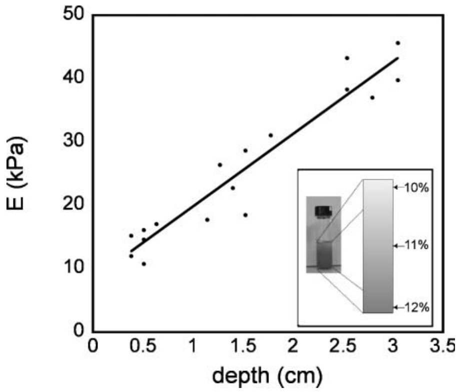

Fig. 4 Modulus as a function of depth for a concentration-gradient sample in a PVA hydrogel. Inset shows the development of the gradient in the samples.

4.5 Cavitation in PVA hydrogels: gel point determination

CRT can also be used to quantify the dynamics associated with gelation, or the onset of the hydrogel network. To demonstrate this, we measure the critical pressure of cavitation as a function of process time, t_p, in PVA hydrogels. The time $t_p = 0$ is defined as the time at which the polymer solution is made. Therefore, at short times, the pressure associated with the cavity instability relates to the resistance provided by the surface energy of a viscous fluid. As time proceeds, the PVA molecules form semi-crystalline domains, which serve as physical cross-links to form a polymer network. As $t \rightarrow \infty$, the formation of semi-crystalline regions will be balanced by the osmotic stresses of the water content and the elasticity of the chains between physical cross-links; therefore, the critical pressure will plateau. This transition of P_c is shown in Fig. 5. Although the exact relationship between P_c and the relevant material properties for a viscoelastic fluid (*e.g.* η', η'', or G', G'') are not currently known, the inflection point observed in Fig. 5 can be assumed to be approximately equal

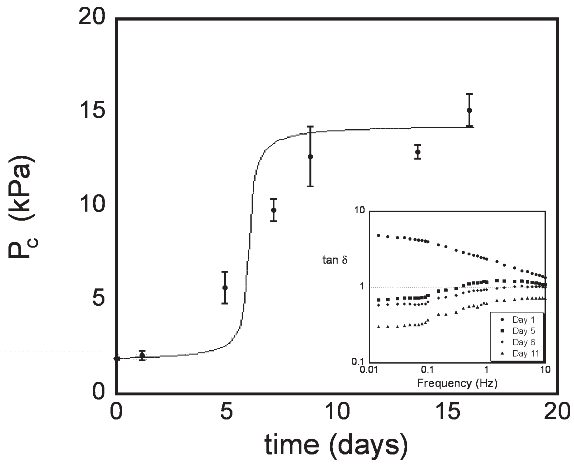

Fig. 5 Gelation time of PVA hydrogel determined by CRT compared with shear rheometry (frequency of 1.5 Hz) where the liquid-like to solid-like transition can be observed at tan $\delta = 1$. The inflection point of the increase in P_c reflects the gel transition in the CRT experiments. The solid line through the CRT data is for illustrative purposes.

to the onset of a gel network. This assumption is verified through shear-rheometry measurements of PVA hydrogels as a function of time after mixing (Fig. 5). Accordingly, the transition in tan δ can be used to track the change from liquid-like to solid-like behavior of a material. This transition is observed when tan δ is equal to 1 over all frequencies. This transition in tan δ curves was seen between 5 and 6 days, confirming that found with CRT.

Similar to data for the PPP hydrogel, the final moduli values of the PVA hydrogel are similar for both CRT and shear rheometry. The modulus measured from bulk rheology after 260 hours was approximately 2 kPa compared to a modulus of 5 kPa determined by the CRT. As discussed above, this discrepancy is most likely associated with differences in the exact nature of the strain-energy function. In fact, based on the PVA gelation process it is highly probable that many uncross-linked polymer chains still exist, thus causing an unknown viscoelastic contribution to P_c. These contributions will be explored in future research.

Conclusions

In summary, unstable growth of an internal void in an elastic network will occur if the pressure in the void approaches the elastic moduli of the surrounding region. Using a syringe needle to prescribe an internally pressurized void within a soft material, we can record the onset pressure for unstable growth and indirectly quantify the elastic modulus of the soft material. This technique, called cavitation rheology testing (CRT) provides a novel alternative for determining the mechanical properties of soft gel materials. We have used this technique to characterize two model hydrogels. These measurements were compared to moduli measured using conventional shear rheometry. Due to the technique's unique ability to determine the modulus at points local to the tip of the syringe, CRT can study such phenomenon as heterogeneities within a gel system or the presence of a gradient, making it a useful tool in studying layer-by-layer structures that are commonly formed in biological tissue. We anticipate that these advantages will greatly enhance the development of scaffolds for tissue engineering and the characterization of complex biological tissues.

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