Correlation of Thin and Bulk Hydrogel Mechanics to Measure the Mechanical Properties of Soft Biomaterials

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Abstract

Bacterial biofilms are complex structures of bacteria that can form on nearly every exposed surface[1]. While biofilms negatively impact many aspects of industry and are associated with nearly 80% of infections in humans, humans wouldn't be able to survive without them[2,3]. Understanding the prevention and growth of biofilms has been a topic in research for years. Recent research has begun to quantify mechanical properties of biofilms and their responses to mechanical stress, however there are limited techniques available to accurately characterize these properties[4]. This study investigates macro and micro scale characterization of thin and bulk gel layers using gelatin, PDMS and polyacrylamide to model the mechanical properties of bacterial biofilms. The methods and results of this study can be used to characterize the mechanical properties of bacterial biofilms grown in a variety of conditions.

Background and Motivation

Hydrogel Mechanics

Poroelasticity:

- Time and area dependent stress for a given displacement due to diffusion (Ɗ) limited migration of incompressible solvent[5-7]
- As a result, much higher initial force F_0 vs. equilibrium Force F_∞

$$F_0 \approx \frac{16}{3} R^{1/2} d^{3/2} G \quad (1)^5$$

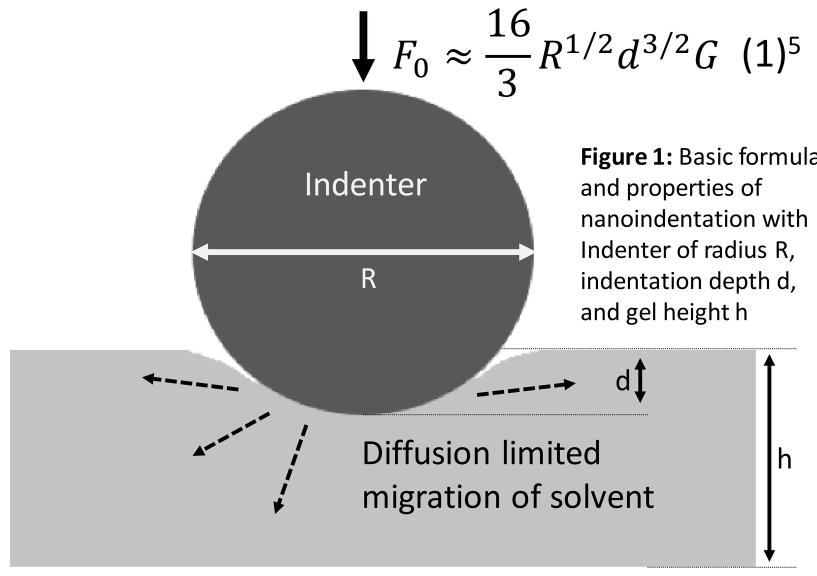
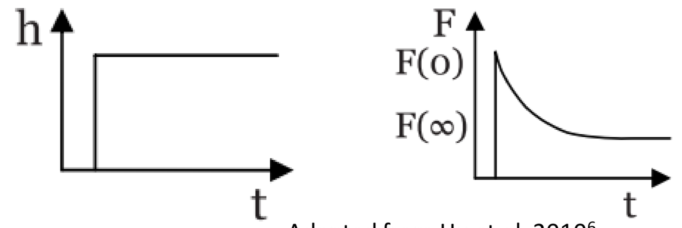


Figure 1: Basic formula and properties of nanoindentation with Indenter of radius R, indentation depth d, and gel height h



Adapted from Hu et al. 2010[6]

Viscoelasticity:

- Time dependent reduction in stress for a constant displacement due to molecular movement[7]
- Polymer chains "store" elastic energy, but the movement of molecules leads to energy being "lost" to heat.
- E'/G' → Storage Modulus
- E"/G"→ Loss Modulus
- G* = G' + iG''

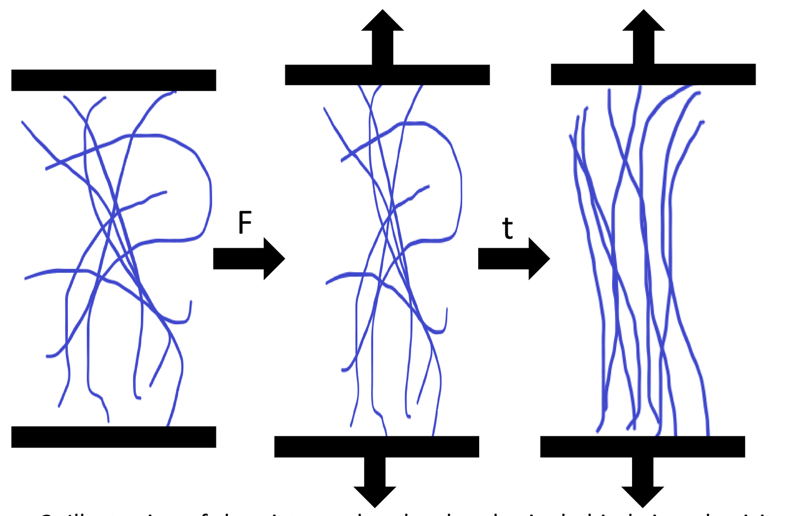
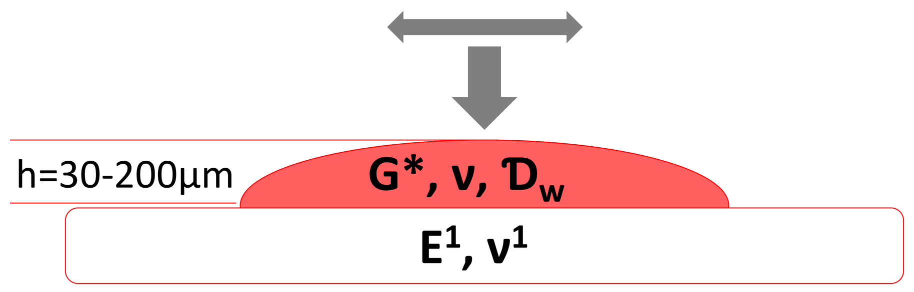


Figure 2: Illustration of chemistry and molecular physics behind viscoelasticity. **(Left):** Resting state of polymer chains intertwined. **(Center):** Immediately after force is applied the chains deform somewhat. (**Right):** After time the chains become detangled and relax some stress

Problem:

Hard to test mechanical properties of thin films accurately because of substrate effects:

Mechanical testing of thin film

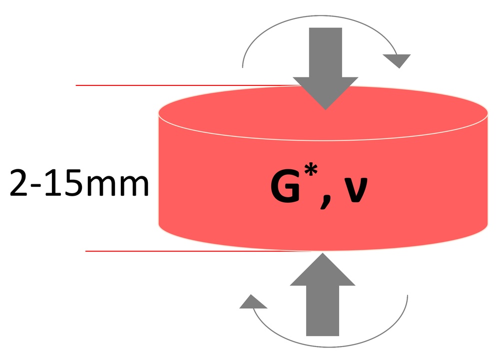


- Typical size of a biofilm
- Very low forces (µN-mN)
- Substrate effects
- Nanoindentation and AFM

$$F_0 \approx 16/3 * R^{1/2}\,\delta^{3/2} G * f(\sqrt{Rh}/d) \quad (2)^8$$

$$F_\infty \approx \frac{8R^{1/2}\delta^{3/2}G}{3(1-\nu)} * f(\sqrt{Rh}/d) \quad (3)^8$$

Mechanical testing of bulk specimen



- Much larger sample
- Larger forces (1-100N)
- Minimal substrate effects
- Indentation, rheometer, bulk compression

Figure 3: Properties and general testing strategy in thin film and bulk material. **(Left)** From indentation and shear, one can get the shear modulus, Poisson's ratio and diffusivity of water. **(Right)** From compression and rotational shear one can get shear modulus, and Poisson's Ratio.

Hypothesis:

We can develop methods for testing mechanical properties of a thin hydrogel and verify results based on properties of bulk hydrogel. These results will provide a verified method for determining mechanical properties of soft biomaterials

Results

Bulk Gel (Gelatin):

Nonlinearity: 1st Order Ogden Model

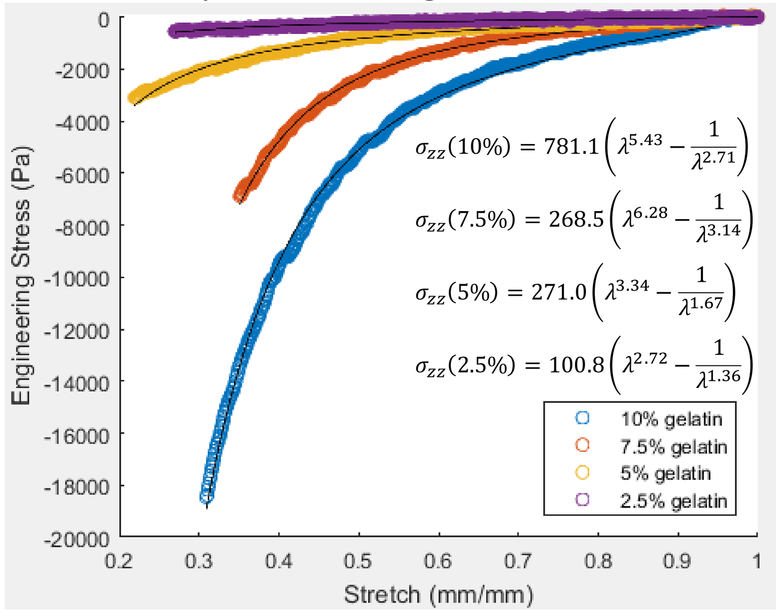


Figure 5: Stress plotted against stretch showing the strain hardening properties of gelatin at various concentrations. All data is fit with the 1st order Ogden model. Equation for fit is shown on the graph.

Conc.	μ_1 (kPa)	α_1	G (kPa)
10%	4.68 (.52)	2.46 (.04)	11.51 (.52)
7.5%	4.21 (3.64)	2.83 (.45)	11.91 (3.6)
5%	.67 (.42)	3.37 (.58)	2.25 (.71)

Table 1: Average μ_1 and α_1 for Gelatin of varying concentrations. General trend seemed to be decreasing μ_1 with increasing α_1. Standard deviation in parentheses, n=2.

Poro-viscoelasticity: Minute scale

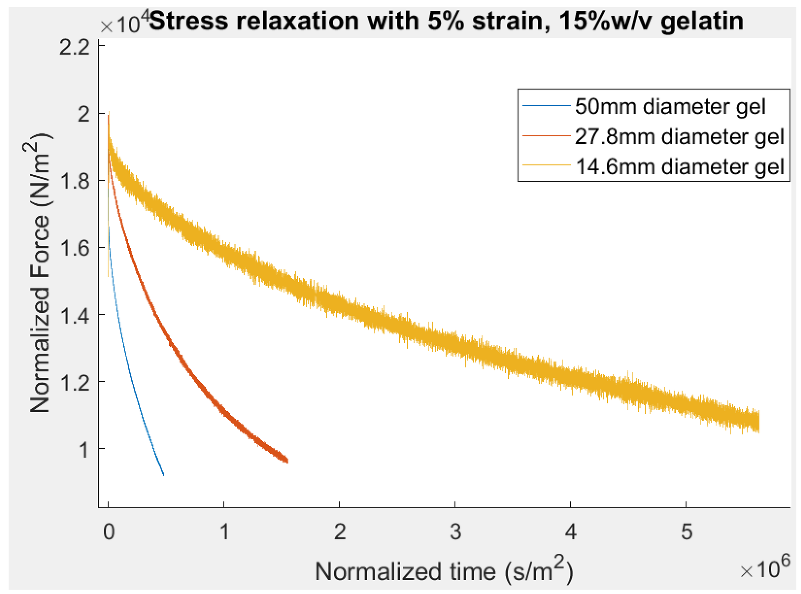


Figure 6: Normalized Force plotted against Normalized time (0-5min). Separation of the curves at this time scale indicates that the viscoelastic properties of the matrix dominated over the poroelastic properties.

Nanoindentation (Gelatin):

Force relaxation:

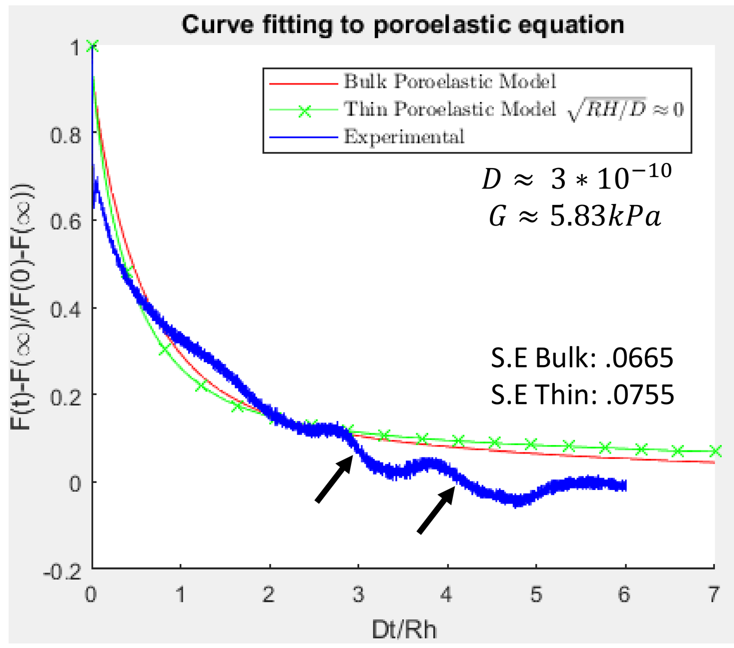


Figure 7: Fitted force relaxation for a poroelastic gel, however the gel was not thin. The data was fit to two models, either the model presented in introduction[8], or a bulk poroelastic model[6]. The function was plotted, and Dt/Rh was calculated using various values of D until the fit was sufficient. Possible fractures indicated by arrows.

Displacement Rate Dependence:

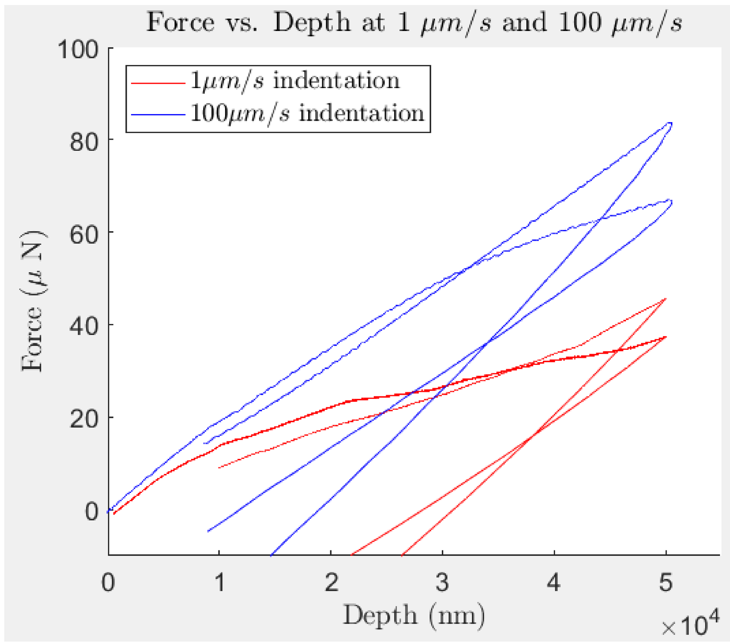


Figure 8: Force vs. Depth at two strain rates. Again samples from different locations (same color) showed very similar mechanical properties. Secondly, the increased apparent stiffness at the faster strain rate (blue) indicates poro-viscoelastic properties, but it is not possible to decouple them with this method.

Methods

Bulk Gel:

- Varying stiffness gels cast into 14-50mm diameter molds
- Compressed and rotationally sheared between two parallel plates.
- Stress/strain: can find G through eq. 4

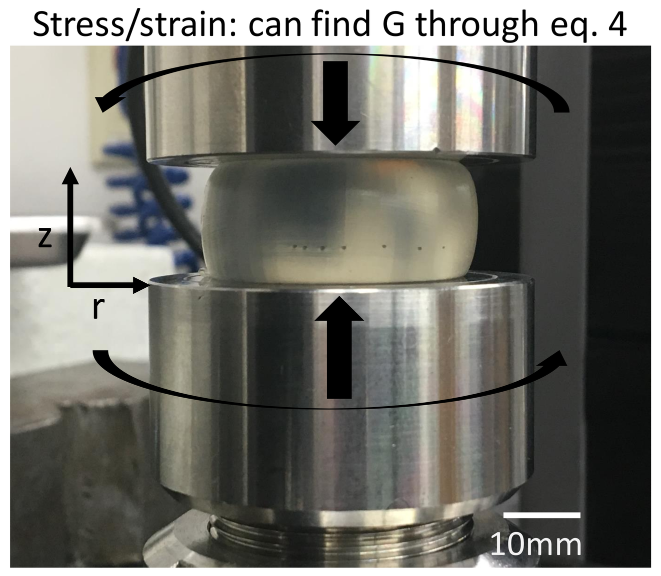


Figure 4 (above): Setup of bulk gel testing, the plates can either move along or rotate about the z-axis, 10mm Scale bar. **Figure 5 (right):** Image of surface of gel after nanoindentations, these spots show possible fractures. Scale bar shown is 50µm.

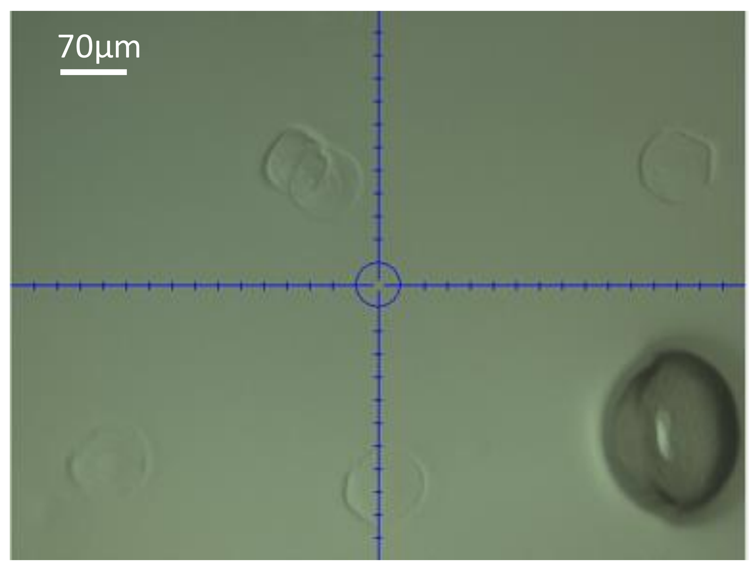
$$\sigma_{zz} = \sum_n \mu_n \left(\lambda^{\alpha_n} - \frac{1}{\lambda^{\frac{1}{2}\alpha_n}}\right), G = \sum_n \mu_n \alpha_n \quad (4)^9$$

$$G' = \frac{\tau_0}{\gamma_0}\cos(\delta) \; (5)^7 \qquad G'' = \frac{\tau_0}{\gamma_0}\cos(\delta) \; (6)^7$$

Thin Film:

1. Coat petri dish with thin gel
2. Indent 10-30µm, record force and time
3. Define τ and x to be dimensionless time and displacement: $\tau \equiv \frac{Dt}{Rh}, x \equiv \frac{\sqrt{R\delta}}{h}$
4. Plot dimensionless force with respect to these variables and use curve fitting with n = 2 to find α, β, and γ.

$$\frac{F(t) - F_\infty}{F_0 - F_\infty} = g(\tau, x) \approx \sum_n \alpha_n(x) \exp(-\beta_n \tau^{\gamma_n}) \quad (7)^8$$



Discussion of Results

Presentation of Property	Bulk Gel	Nanoindentation	Explanation
Hyperelasticity	✓	✗	▪ Difficult to identify hyperelasticity on the nanoscale due to fractures, and indenter and instrument limits.
Poro-viscoelasticity	✗/✓	✓	▪ Expect to see poro-viscoelasticity at any length scale ▪ Time scale length vs. poroelasticity: is poroelasticity present? ▪ Strain rate dependence observed as expected.

Poroelasticity:

- Poroelastic model fits fairly well, but still unsure whether poroelasticity is dominant.
- Diffusion very small, possibly supports poroelasticity
- Need to do stress relaxation at greater time scale (hours)

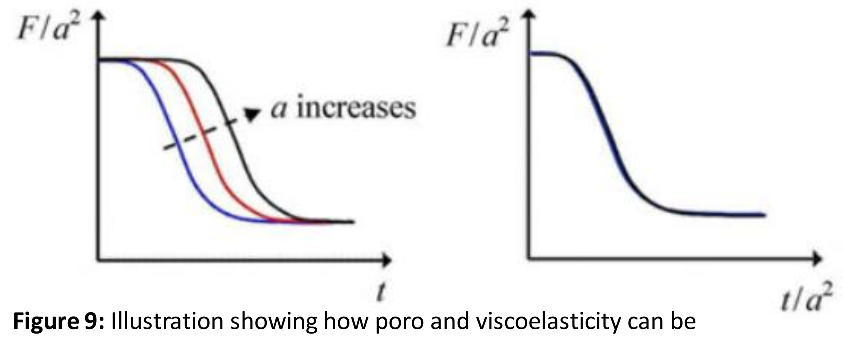


Figure 9: Illustration showing how poro and viscoelasticity can be separated. Poroelastic effects show normalized force vs. time as distinct curves, but normalizing time to the length scale collapses the curves into one. Adapted from Wang et al. 2014[10]

Bulk vs. Nanoscale:

- Shear modulus on same order of magnitude on nano and macro scale (5.83kPa, n=1, vs. 9.98kPa, n=2)
- Viscoelasticity grossly seen on both length scales
- Hyperelasticity not seen, maybe too small of length scale to be seen

Limitations:

- Possible fracture due to drying: Gelatin hydrogel has water evaporate from the surface and so the surface tends to be more brittle than the bulk.
- Not thin indentation: thick (1-2mm) gel was used for indentation.
- Greater implications of drying: Surface characteristics completely different than bulk possibly

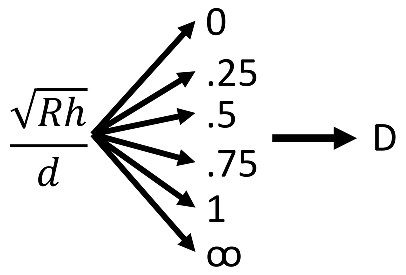
Conclusion and Future Work

Conclusions:

- Shear moduli fairly close at each scale, non-quantified properties qualitatively similar. More work needed to draw strong conclusions.
- Hard to conclude about similarity of properties due to drying and not using thin indentation.

Future Work:

- Replicate Diffusivity at different values of $\sqrt{Rh}/d$, at different values, should get same diffusivity:
- Use crosslinked gel, immerse in solvent
- Eventually use to measure bulk properties of biomaterials



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