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Eliminating the surface location from soft matter contact mechanics measurements

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ABSTRACT

The material properties of soft materials can be measured with rheometers and tensile testing instruments whenever there exist few limitations on sample volume, fixturing and general sample preparation, where samples often need to be prepared specifically to work with the hardware of a given instrument. By contrast, indentation methods are well suited for measuring material properties when sample preparation and geometry are highly constrained, as is the case with living cells, confluent cell layers, tissue samples, hydrogel coatings or soft objects with defined shapes like contact lenses. For example, indentation can be performed directly on cells grown in a Petri dish, without modifying typical cell culture protocols or materials. However, the low elastic modulus of these soft materials make it extremely difficult to determine when an indentation instrument first makes contact with a sample, which is critically important to know if material properties are to be determined with confidence. Here, we present an analysis method that eliminates the need to identify when an instrument makes contact with a sample. The method recasts the traditional force–displacement models of contact mechanics in terms of the first derivative of applied normal force with respect to indenter position, which automatically removes the unknown point of contact. This approach enables the selection of appropriate theoretical models for a given data-set and allows the measurement of sample material properties with the only fitting parameter being the elastic modulus.

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Soft matter; contact mechanics; indentation; elastic modulus; hydrogel

Introduction

Nano- and microindentation measurements have long been used to characterise the elastic modulus and stiffness of hard materials like ceramics and metals [1–4]. While several mechanical models have been developed to capture the elastic loading and unloading behaviour of a spherical indenter in contact with a substrate, within a limited range of forces they can often be reduced to simple power–law relationships, given by $F = Kd^P$, where F is the force required to indent a sample by an amount d; K contains material constants, and the exponent, P, is controlled by the system geometry [5–9]. Recently, these indentation experiments and models have been extended to the study of soft matter, where materials have elastic moduli between 6 and 10 orders of magnitude lower than typical hard materials [10–14]. While significant work has been conducted to characterise the material properties of hydrogels [15], cells [16,17] and cell layers [18,19], a major limitation in most measurements is the difficulty of accurately determining the zero-displacement location, d_0, of a sample surface; great care is often taken to determine d_0 by optical means, *in situ* [11,20,21]. Very often, imaging the contact between a spherical indenter and a sample is not possible, so d_0 must be allowed to vary freely in curve-fitting procedures, drastically decreasing confidence in determining P and generating large errors in fitted elastic moduli [22,23]. Moreover, allowing d_0 to be an unconstrained fitting parameter can artificially produce good agreement between an inappropriate theoretical model and experimental data. An indentation method for determining the elastic modulus and exponent, P, that does not rely on knowing d_0 would facilitate appropriate model choice and would markedly increase the confidence in determining the material properties of soft materials through indentation measurements.

Here, we present a method that does not rely on knowing the zero-displacement surface location, d_0, for fitting power–law force–displacement models to experimental microindentation data. To demonstrate the robustness of our analysis method compared to a more traditional curve-fitting approach, we simulate indentation data using two commonly used contact mechanics models, and perform curve fits. Using traditional fitting where d_0 is allowed to vary freely, we can achieve excellent fits even when the wrong model is intentionally chosen to fit

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a simulated dataset (R^2 between 0.99 and 0.995), yet we determine moduli that deviate from the known values by up to 300%. By contrast, using the method described below, we also achieve excellent fits (R^2 between 0.95 and 0.99), determining moduli that are within about 1% of the known value. We also demonstrate how the appropriate choice of model is unambiguous. Finally, we test our analysis method in real experiments by performing indentation measurements on polyacrylamide (pAAm) hydrogel samples with varying elastic moduli and thickness, finding moduli comparable to those determined from bulk testing methods.

Results

To investigate how uncertainty in d_0 enables the application of inappropriate theoretical models to experimental data and leads to the determination of erroneous material constants, we simulate indentation data of pAAm hydrogels based on the experiment illustrated in Figure 1 (see Materials and Methods), and intentionally fit the wrong model to the simulated data. In these fits, we employ the Levenberg–Marquardt algorithm [24], carefully choosing initial parameters near the global minimum of error before initiating iterations. To simulate indentation of a thick hydrogel, we use the Hertz contact model [6], given by

$$F = \frac{4}{3}E^{*}R^{\frac{1}{2}}(u - d_0)^{\frac{3}{2}} \equiv K_H d^{\frac{3}{2}},$$

where F is the applied normal force, E^* is the effective modulus, R is the radius of curvature of the hemispherical indenter tip, u is the arbitrary position of the indenter tip and d_0 is the location of the un-indented sample surface in the indenter's arbitrary coordinate system. Here, when $u = d_0$, the indenter makes contact with the sample and the true indentation depth, $d = u - d_0$, equals zero.

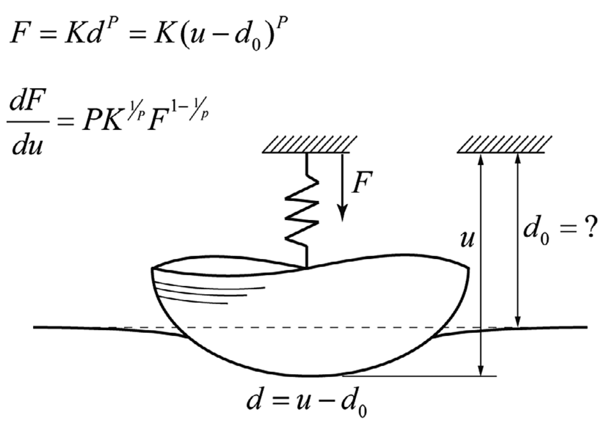

Figure 1. Indentation measurements are performed by bringing a borosilicate glass hemisphere into contact with a pAAm hydrogel substrate and measuring the force–displacement (F–u) relationship. By taking the derivative of the force with respect to displacement, the elastic modulus can be determined independently of d_0 by fitting a power law to the F'–F data.

Similarly, to simulate a thin hydrogel, we use the Winkler model [7], given by

$$F = \pi E^{*}\frac{R}{h}(u - d_0)^2 \equiv K_W d^2,$$

where the parameters are the same as in the Hertz model apart from h, which is the slab thickness. For these simulations of thin slab hydrogels $h = 100$ μm; h is assumed infinite for the thick slab case. The uncertainty in knowing h and R can be accounted for in determining a confidence interval for E^*; here, we focus on the case where uncertainties in h and R are negligible, determining confidence intervals for E^* directly from curve fits. In simulations of both thick and thin hydrogels, we use an indenter radius of $R = 1$ mm and a modulus of $E^* = 10$ kPa. In generating initial model data, we set $d_0 = 0$ but we allow d_0 to vary freely in fitting the data. To generate data that resembles real experimental data, we add ±5 μN of white noise to the simulated force, which is a typical level observed using our piezo-driven, quasi-static transducer indentation system (see Methods and Materials).

Fitting the Winkler model to simulated data generated by the Hertz model (Figure 2(a)), we find a modulus of 0.79 ± 0.01 kPa with an R^2 value of 0.997 (± corresponds to 95% confidence interval from non-linear least squares fitting). This modulus is less than one tenth the known modulus. Similarly, fitting the Hertz model to simulated data generated by the Winkler model (Figure 2(b)), we find a modulus of 39.9 ± 1.2 kPa with an R^2 value of 0.99, nearly four times the known modulus. In both cases, the best fit d_0 is relatively small: −28.4 ± 1.2 μm for the Hertz data and 2.8 μm ± 0.3 μm for the Winkler data were found. In practice, the experimentalist might dismiss these small offsets as having negligible effects on the results. To test one example where d_0 is simulated to be large, corresponding to a measurement in which the instrument is not 'zeroed' anywhere near the contacting configuration, data are generated using the Winkler model with $d_0 = 100$ μm (Figure 2(c)). We find $E^* = 36.2 \pm 1.8$ kPa and $d_0 = 102.0 \pm 0.4$ μm with an value of 0.99. These results demonstrate the significant pitfalls of using uncontrolled fitting approaches to determine material properties from indentation experiments. Moreover, unconstrained fits to each data-set using the *correct* corresponding model, allowing all parameters to vary, results in R^2 of 0.995 (both models), extremely close to those found when fitting the wrong model. We note here that allowing P to vary freely in either model results in E^* having unphysical units which will vary from fit to fit, muddying comparisons of E^* between different fits and different fitting methods. Of course, good fits are achieved with *a priori* knowledge of the appropriate model by fixing P to its known value, yielding R^2 of 1 for Hertz data and 0.992 for Winkler data; the correct E^* is determined to within 3% for the Winkler data and less than 1% for the Hertz data. It is not surprising that

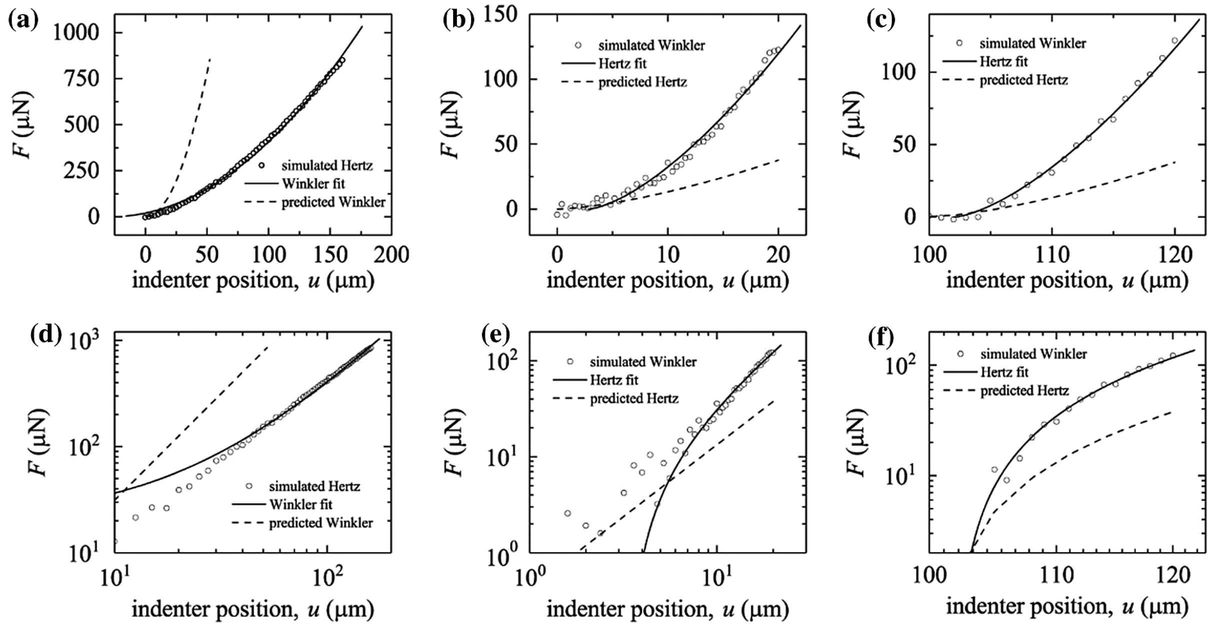

Figure 2. Simulated indentation data generated by the Hertz model (a,d), Winkler model (b,e) and Winkler model with a large d_0 value (c,f) are intentionally fit with the wrong theoretical model (points: simulated data; solid lines: fits of wrong model; dashed lines: prediction of wrong model using correct modulus). We re-plot the data and fits on a log–log scale to show how shifts in power laws produce curved line shapes (d: Hertz; e: Winkler; f: Winkler with 100 μm shift). In curve fitting with d_0 and E^* as free parameters, fits with high R^2 values yield large errors in E^*.

model data is well fit by the right model; more surprising is that the wrong model fits very well too. Finally, one can see that using the known E^* in combination with the wrong model produces F–u curves that strongly diverge from the simulated data (Figure 2, dashed lines).

To improve the process of fitting experimental indentation data by any simple physical model that relates F and d through a power law, we developed a strategy that eliminates the need to know d_0. By differentiating the F–u power law that includes the unknown offset, d_0, a simple relationship between $F' = \mathrm{d}F/\mathrm{d}u$ and F is given by

$$F' = K_G F^n,$$

where $K_G = PK^{1/P}$ is a generalised coefficient and $n = (P-1)/P$. Here, K can be K_H, K_W or the coefficient of any F–u power–law relationship. With this procedure, the number of independent fitting parameters is reduced to two; if the appropriate model is known, n can be fixed and the only fitting parameter is the effective modulus, E^*. For the Hertz model, $p = 3/2$ and $n = 1/3$; for the Winkler model, $p = 2$ and $n = 1/2$.

To implement this analysis method, experimental force data must be differentiated with respect to indenter position. Random noise in indentation measurements creates challenges in differentiating experimental data; instantaneous jumps between data points, even if small, produce derivatives that dominantly measure noise behaviour rather than the rate of change of force with displacement. To overcome this challenge, we bin the raw F–u data points using the estimated noise statistics to choose a bin size. For the simulated data described above, we choose force bins of 10 μN, computing the mean force and the corresponding mean indenter position within each bin. These averaged data are then differentiated discretely by taking the difference in sequential mean forces and dividing by the difference in sequential mean indenter positions. The resulting F'–F data are smooth enough to allow fitting theoretical F'–F curves (Figure 3(a)–(c)). Unconstrained fitting of the simulated data, where n (or equivalently, P) freely varies demonstrates the ability to choose the appropriate model for a given data-set; $p = 1.5 \pm 0.02$ for simulated data produced by the Hertz model; $p = 2.1 \pm 0.18$ and 2.18 ± 0.14 for simulated data produced by the Winkler model. Thus, fixing $p = 3/2$ for the Hertz model data, we determine $E^* = 9.94 \pm 0.05$ kPa, very close to the known 10 kPa value. Likewise, fixing $p = 2$ for the Winkler model data, we determine $E^* = 10.0 \pm 0.3$ kPa and 9.95 ± 0.25 kPa. We note that even in the extreme case where $d_0 = 100$ μm, the error in E^* is about 1%. Applying these fitting results to the models in F–u space produces curves that lay on top of the unprocessed simulated data (Figure 3(d)–(f)).

To verify that this analysis method can be applied to experimental data, we create hydrogel samples of differing thickness and elastic modulus and test their properties with a Hysitron BioSoft indentation instrument equipped with an $R = 1$ mm hemispherical borosilicate glass indenter tip (see Materials and Methods). To reduce the effects of instrumental noise on the derivative of experimental data, we choose a force bin size larger than the amplitude of random force fluctuations

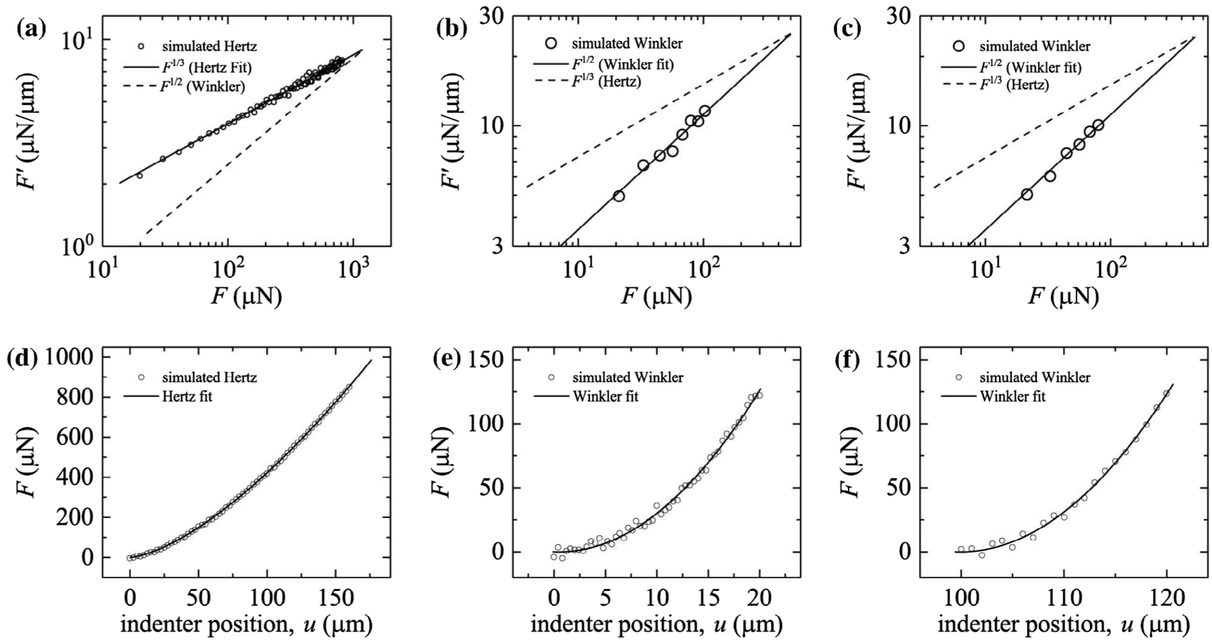

Figure 3. Simulated indentation data generated by the Hertz model (a), Winkler model (b) and Winkler model with a large d_0 value (c) are differentiated with respect to u, producing F'–F curves, which eliminates the unknown contact position, d_0. The data (symbols) are well fit by the corresponding model (solid lines) and follow scaling laws that can be seen to differ from the competing models (dashed lines). After fitting in F'–F space, the model curves lay on top of the original data in F–u space (d,e,f).

reported by the unloaded instrument. Additionally, the bin size must be small enough to maintain an adequate number of points to facilitate curve fitting. We find that 1-mm thick gels exhibit a mechanical response predicted by the Hertz theory; F' is proportional to $F^{1/3}$ as expected, corresponding to $p = 3/2$ (Figure 4(a)). Fitting the Hertz model to data collected from a 5% pAAm gel and a 7.5% pAAm gel, we determine $E^* = 5.7 \pm 0.1$ kPa and $E^* = 23.6 \pm 0.2$ kPa, respectively. Similarly, we find that 100-μm thick gels exhibit a mechanical response predicted by the Winkler model; F' is proportional to $F^{1/2}$ as expected, corresponding to $p = 2$ (Figure 4(b)). Fitting the Winkler model to data collected from the 5% pAAm and 7.5% pAAm gels, we determine $E^* = 3.9 \pm 0.1$ kPa and $E^* = 19.8 \pm 0.7$ kPa, respectively, close to the values obtained from measurements on thicker samples made at the same composition. These moduli are also comparable with measurements made on a rheometer, where we find $E^* = 4.4$ kPa for the 5% pAAm gel and $E^* = 14.4$ kPa for the 7.5% pAAm (see Materials and Methods). In our experience, it is infrequent to find close agreement between moduli determined by rheology and indentation testing, so we consider these results promising. Finally, examining the data without fitting, it can be observed visually that slabs of different thickness, made from the same material, exhibit different F'–F scaling laws (Figure 4(c) and (d)).

Discussion

The method described in this manuscript for analysing force–displacement indentation data facilitates the reliable characterisation of soft materials using simple theoretical models of contact mechanics. This method is especially useful when the point of contact is difficult to determine empirically or an appropriate theoretical model is not known. Many different types of robust indentation instruments with precise motion control and low-noise sensing have been developed for decades, yet the experimentalist still faces significant data analysis challenges [25,26]. The simulations and analyses described here illustrate these challenges; with traditional fitting methods, erroneous results can be found even when raw data follow nearly perfect trends and good fits are achieved. Accordingly, the quality of a model fit to experimental data is not a sufficient *post hoc* standard for justifying the use of a particular theoretical model. We hope that the approach described here will help researchers to analyse indentation data without significant limitations on instrumentation or analysis software packages. Moving forward, this method can be improved by eliminating the need to perform least-squares fitting; once an appropriate theoretical model is chosen with confidence, the data can be directly processed and E^* can be determined through statistical analysis by computing simple means and standard deviations. However, to establish confidence with such a process, this endeavour requires more careful accounting for sources of uncertainty and propagating them through the various calculation steps. In future work, we plan to systematically investigate how random noise, sample material properties and the unknown location of the sample surface each contribute to uncertainties in characterising materials using the method presented here.

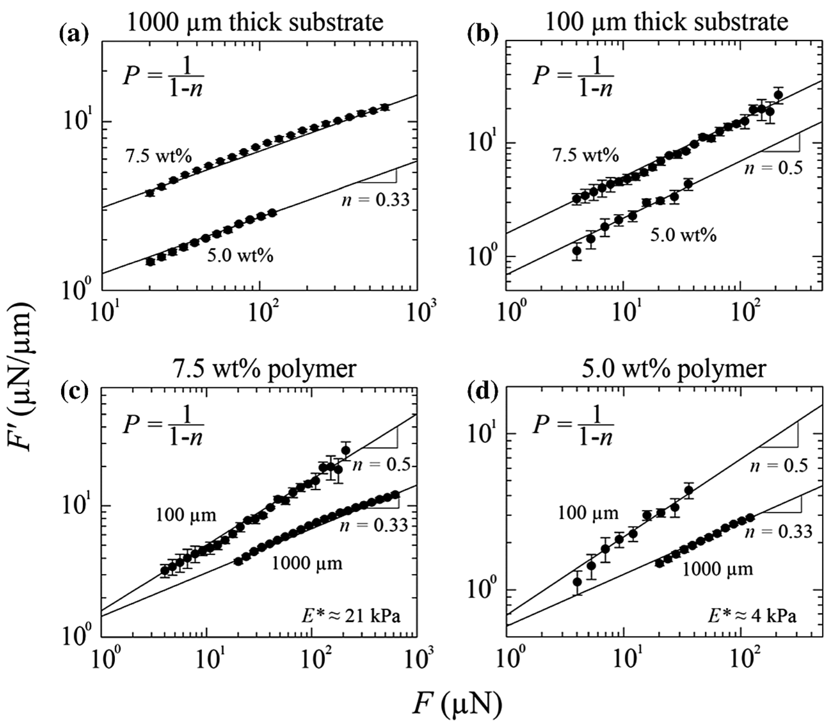

Figure 4. Indentation measurements are performed on pAAm substrates with varying composition (5.0 and 7.5 wt-% pAAm) and thickness (100 and 1000 µm). (a, b) Power law fits of the F'–F data for the 1000-µm-thick samples find $P = 1.5$, as predicted by Hertz contact theory, whereas 100-µm-thick samples find $P = 2$, characteristic of Winkler contact theory. The separation in data-sets reflects the different moduli of the 5 and 7.5 % pAAm samples. (c,d) Differences in these scaling laws are noticeable when comparing gels with the same moduli but different thicknesses. Measured elastic moduli are consistent across substrate thicknesses and are comparable with rheological measurements. Data points are mean values from multiple indentation measurements with error bars corresponding to ± one standard deviation.

Methods and materials

Hydrogel Preparation

Polyacrylamide (pAAm) hydrogel samples are made with different polymer concentrations and sample thicknesses. 5% (w/w) and 7.5% (w/w) pAAm sheets are prepared using the protocols from Urueña et al. [27] and allowed to equilibrate in ultrapure water (18.2 MΩ) for 24 hours. The samples thickness is controlled during polymerisation by casting the hydrogel between two 22 × 22 mm^2 glass cover slips separated using 100- and 1000-µm thick spacers. The top coverslip is removed before indentation either spontaneously through the swelling process or by gentle mechanical agitation.

Indentation

Indentation occurs near the centre of the hydrogel sample to minimise any possible edge effects. Estimates for the maximum contact width ($2a$) for the 100- and 1000-µm samples is 465 and 565 µm, respectively which are 10 times below the typical sample diameter. The hydrogel sample is submerged in ultrapure water and indented with a piezo-driven, quasi-static transducer indentation system (Hysitron BioSoft) with a hemispherical probe following a typical loading and unloading curve. The probe, which is also submerged in water, begins at some unknown distance from the surface, d_0, approaches the surface monotonically, touches the surface and indents at a constant piezo displacement rate (10 nm/s for 100-µm specimens and 1 µm/s for 1000-µm specimens). Thinner samples (Winkler governed indentations) are sensitive to indentation speeds and great care must be taken when choosing an appropriate indentation velocity otherwise a force response much stronger than expected ($p > 2$) will be observed, likely dominated by the resistance to fluid flow through the thin slab. The system loads and unloads to a specified z-displacement (20 and 80 µm) in a displacement-controlled configuration. Force response and position is measured simultaneously by the quasi-static transducer at a rate of 125 Hz.

The indentation probe is a hemispherical borosilicate glass lens (R = 1 mm) attached to the indentation system. The probe is plasma cleaned and coated with F-127 Pluronic between each experiment set to reduce adhesion between probe and surface.

Rheology

The 5 and 7.5% pAAm hydrogel samples are cast as 1-mm thin slabs between two roughened parallel plates on a Malvern Kinexus rheometer. The hydrogel precursor solutions are allowed to gel for 40 minutes before commencing rheological testing. Once samples have fully gelled we measure the elastic shear moduli, G', by applying 1% strain at an oscillatory frequency of 1 Hz.

Young's modulus can be found from the shear moduli. Ultimately, Young's modulus is related to the effective modulus, $E = 2G'(1 + \nu)$. Here, we estimate Poisson's ratio, $E^* = \frac{E}{1-\nu}$, to be 0.5, based on previous measurements of the same type of hydrogel [14].

Disclosure statement

No potential conflict of interest was reported by the authors.

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Marcus Garcia is a graduate student at the University of Florida. In his research, he focuses on methods of material characterization of soft matter and collective motion in epithelial cell layers.

Kyle D. Schulze is a postdoc at the University of Florida. His research interests include soft matter mechanics, tribology, and surface science.

Christopher S. O'Bryan is a graduate student at the University of Florida. His research interests include new methods for soft matter manufacturing and investigating cell mechanics in 3D.

Tapomoy Bhattacharjee is a graduate student at the University of Florida. His research is focused on microgel yielding, manufacturing soft structures of cells and investigating cell migration through 3D microgel growth medium.

W. Gregory Sawyer is a professor in the Department of Mechanical & Aerospace Engineering at the University of Florida. His research interests include tribology of soft and hard materials.

Thomas E. Angelini is an associate professor in the Department of Mechanical & Aerospace Engineering at the University of Florida. His research interests include soft matter physics, biophysics and tribology at soft interfaces.

References

[1] Carpick RW, Salmeron M. Scratching the surface: fundamental investigations of tribology with atomic force microscopy. Chem Rev. 1997;97:1163–1194.

[2] Fischer-Cripps AC. Critical review of analysis and interpretation of nanoindentation test data. Surf Coat Technol. 2006;200:4153–4165.

[3] Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J Mater Res. 1992;7:1564–1583.

[4] Tabor D. Surface forces and surface interactions. J Colloid Interface Sci. 1977;58:2–13.

[5] Derjaguin BV, Muller VM, Toporov YP. Effect of contact deformations on the adhesion of particles. J Colloid Interface Sci. 1975;53:314–326.

[6] Hertz H. On the contact of elastic solids. J Reine Angew Math. 1881;92:156–171.

[7] Johnson K. Contact mechanics. Cambridge [Cambridgeshire]. New York (NY): Cambridge University Press. xi; 1985. p. 104–106.

[8] Johnson KL, Kendall K, Roberts AD. Surface energy and the contact of elastic solids. Proc. R. Soc. Lond. A. 1971;324:301–313.

[9] Sneddon IN. The relation between load and penetration in the axisymmetric boussinesq problem for a punch of arbitrary profile. Int J Eng Sci. 1965;3:47–57.

[10] Chan EP, Hu Y, Johnson PM, et al. Spherical indentation testing of poroelastic relaxations in thin hydrogel layers. Soft Matter. 2012;8:1492–1498.

[11] Erath J, Schmidt S, Fery A. Characterization of adhesion phenomena and contact of surfaces by soft colloidal probe AFM. Soft Matter. 2010;6:1432–1437.

[12] Hu Y, Zhao X, Vlassak JJ, et al. Using indentation to characterize the poroelasticity of gels. Appl Phys Lett. 2010;96:121904.

[13] Nalam PC, Gosvami NN, Caporizzo MA, et al. Nano-rheology of hydrogels using direct drive force modulation atomic force microscopy. Soft Matter. 2015;11:8165–8178.

[14] Schulze KD, Hart SM, Marshall S, et al. Polymer osmotic pressure in hydrogel contact mechanics. Biotribology. 2017;11:3–7.

[15] Oyen M. Mechanical characterisation of hydrogel materials. Int Mater Rev. 2014;59:44–59.

[16] Mahaffy R, Shih C, MacKintosh F, et al. Scanning probe-based frequency-dependent microrheology of polymer gels and biological cells. Phys Rev Lett. 2000; 85:880.

[17] Sen S, Subramanian S, Discher DE. Indentation and adhesive probing of a cell membrane with AFM: theoretical model and experiments. Biophys J. 2005;89:3203–3213.

[18] Schulze K, Zehnder S, Urueña J, et al. Elastic modulus and hydraulic permeability of MDCK monolayers. J Biomech. 2017;53:210–213.

[19] Zehnder SM, Wiatt MK, Uruena JM, et al. Multicellular density fluctuations in epithelial monolayers. Phys Rev E. 2015;92:1104.

[20] Chaudhury MK, Whitesides GM. Direct measurement of interfacial interactions between semispherical lenses and flat sheets of poly (dimethylsiloxane) and their chemical derivatives. Langmuir. 1991;7:1013–1025.

[21] Schulze KD, Bennett AI, Marshall S, et al. Real area of contact in a soft transparent interface by particle exclusion microscopy. J Tribol. 2016;138:041404.

[22] Cao Y, Yang D, Soboyejoy W. Nanoindentation method for determining the initial contact and adhesion characteristics of soft polydimethylsiloxane. J Mater Res. 2005;20:2004–2011.

[23] Kaufman JD, Klapperich CM. Surface detection errors cause overestimation of the modulus in nanoindentation on soft materials. J Mech Behav Biomed Mater. 2009;2:312–317.

[24] Marquardt DW. An algorithm for least-squares estimation of nonlinear parameters. J Soc Ind Appl Math. 1963;11:431–441.

[25] Chen X, Dunn AC, Sawyer WG, et al. A biphasic model for micro-indentation of a hydrogel-based contact lens. J Biomech Eng. 2007;129:156–163.

[26] Dunn AC, Urueña JM, Huo Y, et al. Lubricity of surface hydrogel layers. Tribol Lett. 2013;49:371–378.

[27] Urueña JM, Pitenis AA, Nixon RM, et al. Mesh size control of polymer fluctuation lubrication in gemini hydrogels. Biotribology. 2015;1:24–29.