Supplementary Information for
Nano-rheology of hydrogels using direct drive force modulation atomic probe microscopy

Prathima C. Nalam a, Nitya N. Gosvami a, Matthew Capporizzo b, Russell Composto b and Robert Carpick a,b,*

a Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, USA
b Department of Materials Science and Engineering, University of Pennsylvania, Philadelphia, USA
* Corresponding Author: E-mail: carpick@seas.upenn.edu

Supplementary Information 1:

Fig. S1: In Hertz contact mechanics, the mechanical response of bodies in contact are considered to be infinite under equivalent condition, i.e., probe dimension are larger than the contact size, i.e., \( a < R \), where \( a \) is the contact radius and \( R \) is the probe radius. Indenting soft materials with sharp probes leads to sample deformations larger than the probe radius thus violating the above
assumption. Colloid – attached AFM cantilever with a larger probe radius lowers the contact pressures and the sample deformations resulting in smaller contact areas in comparison to probe radius. Further, the strains and deformations induced by the probe should be held extremely small to deform the contacts in the linear elastic limit, i.e., \( d \ll R \), where \( d \) is the depth of indentation. The Landau and Lifshitz calculations show that soft materials with elastic modulus of \( \sim 10 \) kPa, when indented using blunt AFM probes (pyramidal tips) with radius \( \sim 100 \) nm, results in non-linear deformations already at less than 1 pN of load\(^1\) (strain < 0.2%). Using colloid probes with large radius can overcome this limitation. The figure S1 shows the calculated maximum normal strain for a Hertzian contact calculated from Landau and Lifshitz\(^1\) for a sharp AFM tip with a radius \((R)\) of 100 nm (solid lines) and for a colloid probe of radius 3 µm, similar to the radii of the colloids used in this study (dotted lines) respectively for sample moduli varying from 1-40 kPa. A Poisson’s ratio of 0.45 is used. The maximum occurs at the center of the contact area. The horizontal dashed line represents the 20% of strain above which the material deforms non-linearly, resulting in a non- Hertzian contact.

**Supplementary Information 2:**

**Fig. S2:** (a) \( a/R \) ratios as a function of force (indentation depth) obtained for low and high crosslinked hydrogels estimated from static force-distance curves. (b) Hertz theory of contact for elastic bodies, however, is valid only for low values of \( a/R \) (< 0.1) where \( a \) and \( R \) are the radii of the contact and that of the sphere, respectively. Yoffe\(^2\) has modified and extended the classical
Hertz model to larger value of $a/R$ and estimated the probable error in elastic modulus when Hertz approach was used at higher $a/R$ ratios. Incompressible materials, such as hydrogels (Poisson’s ratio $\nu = 0.4$), shows $E/E_H$ values are closer to 1 with increase $a/R$ (i.e. error $< 1.5 \%$ at $a/R = 0.4$), indicating the validity of Hertz model at higher $a/R (> 10 \%)$ ratios.

Supplementary Information 3:

![Graph showing storage shear modulus as a function of strain amplitude for high (red) and low (blue) crosslinked polyacrylamide hydrogels. The data were acquired using a parallel-plate shear rheometer (plate diameter = 25 mm, oscillating frequency = 1 Hz). A strain amplitude of 0.1 was used to measure the frequency dependence studies as shown in Fig. 3.](image)

**Fig. S3:** The storage shear modulus as a function of strain amplitude for high (red) and low (blue) crosslinked polyacrylamide hydrogels. The data were acquired using a parallel-plate shear rheometer (plate diameter = 25 mm, oscillating frequency = 1 Hz). A strain amplitude of 0.1 was used to measure the frequency dependence studies as shown in Fig. 3.

Supplementary Information 4:
**Fig. S4:** a) Topographical image of a colloid probe attached to an *idrive*™ cantilever. The image is acquired through reverse imaging of a TGT-01 grid (NT-DMT, USA) against the colloid-attached AFM cantilever. b) The section profile along the diameter of the colloid.

**Supplementary Information 5:**

![Diagram](image)

**Fig. S5:** Schematic representation of discrete loading employed for dynamic modulation measurements. The load for dynamic modulation measurements was incremented in steps. The cantilever was approached from out of contact to the applied set force at an indentation speed of 4 µm/s (1 Hz). The speed of approach was held constant at 4 µm/s for all measured depths of indentation and the modulating frequencies. The amplitude and phase signals were recorded during the dwell and later the tip was retracted from the sample. This procedure was repeated at different indentation depths/ loads. This methodology provides several advantages such as the initial creep in the material (1 – 2 s at the beginning of the dwell) can be avoided in the calculations, the time constant between the rate of acquisition and the lock-in signal can be
accurately controlled and the history of hydrogel (conformational changes) from prior indentations can be avoided.

Supplementary Information 6:

![Graph](image)

**Standard Viscoelastic Solid Model:**

\[ Z(t) = \frac{F_o}{k_1} + \frac{F_o}{k_2} \left[ 1 - \exp \left( -t \frac{k_2}{\eta_2} \right) \right] \]

<table>
<thead>
<tr>
<th>Relaxation time (s)</th>
<th>( \tau = \frac{\eta_2}{k_2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Crosslinked Gel</td>
<td>1.0 ± 0.7</td>
</tr>
<tr>
<td>Low Crosslinked Gel</td>
<td>0.35 ± 0.05</td>
</tr>
</tbody>
</table>

**Fig. S6:** Creep of the hydrogels measured at a constant applied force (stress) using colloid-probe AFM on high- and low- crosslinked gels. A standard viscoelastic solid model was fit to the data to obtain the relaxation time for the gels (as given in the table), \( Z(t) \) is the creep deformation and \( F_o \) is the applied static force.

Supplementary Information 7 - Contact mechanical models for adhesive contacts:

Adhesive contacts for elastically deformed materials can be described using either Johnson, Kendall and Robertson (JKR) model or Derjaguin-Müller-Toporov (DMT) model\(^3\). The JKR model is used when the adhesive stresses are strong and act within the contact zone leading to large contact areas in comparison to Hertz (non-adhesive) contact model. This model is more applicable for soft materials with high interfacial surface energies. On the other hand, the DMT model is an extension of the Hertzian model to include adhesive forces and is generally applicable for stiff and low surface energy materials whose contact area does not significantly deviate from the Hertzian contact under adhesive stresses.
A continuous transition between the above regimes was developed by Maugis by applying Dugdale model to describe the interaction potential between the two surfaces. A non-dimensional Tabor parameter ($\mu$) estimates the contact mechanical regime for adhesive contacts. For $\mu > 5$, JKR regime is applicable and for $\mu < 1$ the DMT theory is valid. The value of $\mu$ is defined as:

$$\mu = \left( \frac{16RW_{\text{adh,eff}}}{9K^2z_o^3} \right)^{\frac{1}{3}} \quad (S1)$$

where $W_{\text{adh,eff}}$ is effective work of adhesion between the colloid probe and the hydrogel, $R$ is the effective radius at the contact; $R = \frac{R_1R_2}{R_1 + R_2}$ with $R_1$ and $R_2$ are the radii of the contacting geometries, $K$ is the effective modulus $K = \frac{4}{3} \left( \frac{1-v_1^2}{E_1} + \frac{1-v_2^2}{E_2} \right)^{-1}$ and $z_o$ is the equilibrium separation distance between the surfaces (usually varies between 0.3-0.5 nm).

**Supplementary Information 8:**

Figure S7: The (A) approach (red) and (B) retraction (blue) FD curves obtained for low cross-linked hydrogel respectively. The fits were Hertz (yellow) and JKR (green) models were used for the curves.
Supplementary Information 9: Strain-rate dependence for static FD measurements:

![Graph showing Young's modulus vs. frequency for high and low crosslinked gels.]

Fig. S8: Frequency dependence of Young’s modulus from static FD measurements using AFM, for high- and low- crosslinked hydrogels.

Supplementary Information 10:
**Fig. S9:** The storage stiffness vs. load measured using dynamic modulation for high (circles) and low – (diamond) crosslinked gels at different modulating frequencies are shown. The punch fits (dash lines) using eqn. (9) is shown for all the measured frequencies.

**Supplementary Information 11:**

![Graph 1](image1)

**Fig S10:** Storage stiffness as a function of force measured using static FD and dynamic modulation measurements at a strain rate of 10 Hz for (a) low- and (b) high- crosslinked hydrogels, respectively. At transition frequency ~ 10 Hz, the Hertz fit and the dynamic punch fit overlapped for the measured data. However, beyond 10 Hz, the dynamic punch model showed more appropriate fit to the dynamic modulation data and below 10 Hz the Hertz fit showed a more appropriate fit for static FD curves.

**Supplementary Information 12:**
Fig. S11: Storage modulus as a function of frequency from nano-rheological measurements obtained using two different tips for both low- (red) and high – (blue) crosslinked gels are represented. Note that the nano-rheology moduli presented in Fig. 8 are the average values of the moduli represented here from two different tips.

Supplementary Information 13:
Fig. S12: Comparing loss tangents (tan δ) as a function of frequency obtained from parallel-plate rheometer (closed symbols) and force-modulated AFM measured (open symbols) for low- and high- crosslinked polyacrylamide hydrogels. The tan δ at macro-scales, similar to nano-rheological measurements shows a decrease and then an increase with increase in frequency. However the minimum value is reached much earlier for macro-scale rheology in comparison to the nano-scale rheology. While the true nature for this off-set is not yet well understood but one of the possible reasons can be that dissipative losses are affected by the size on the contacting geometries.

References: