Characterizing Silicone and Polyacrylamide Gel Substrates for Mechanobiology Studies Using a Widefield Fluorescence Microscope

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Abstract

Epithelial and other cells in soft tissues adhere to a microenvironment whose stiffness typically falls in kilopascal range. Flexible substrates such as polyacrylamide and silicone gels have proven to be excellent biomimetic substrates for cell culture. To characterize the Young's moduli of isotropic linear elastic substrates, we present here a simple method that only employs a widefield fluorescence microscope for the actual stiffness testing. Use of suitable indentors and methods to restrict fluorescent marker beads to the substrate top surface enable this method.

Background

Several methods have been used to measure the stiffness of flexible substrates, including atomic force microscopy, macroscopic deformation of whole samples upon stretching, rheology and indentation using spheres and spherically tipped microindentors. While each technique has its own advantages and disadvantages, indentation with a sphere is an especially simple yet fairly accurate method that only requires access to a widefield fluorescence microscope. Recently, confocal microscopy has also been used for an elegant characterization of the indentor method [1]. Here, we show that a widefield microscope may be sufficient, provided a suitable protocol is used to confine fluorescent beads to the top surface or to a thin slice in the top region of the substrate.

Methods

Polyacrylamide (PAA) gels were prepared on a coverslip by polymerizing acrylamide and bis-acrylamide solutions in water with polymerizing chemical agents for 45 minutes. A thin top layer of PAA solution with fluorescent beads was then polymerized on the previously formed polymerized gel.

Soft silicone was prepared in a petri dish by mixing the base and curing agent at a 1:1 ratio followed by curing at 70 degrees C for half an hour. Fluorescent beads of 0.44 micron diameter were coupled to the silicone top surface using deep UV treatment followed by EDC/NHS chemistry. A millimeter-scale sphere was used to intend the top surface (by gravity), fluorescent beads on the indented silicone surface were imaged using a fluorescent microscope and the resultant images were analyzed to calculate the Young's Modulus of the silicone substrate as specified in the results section.

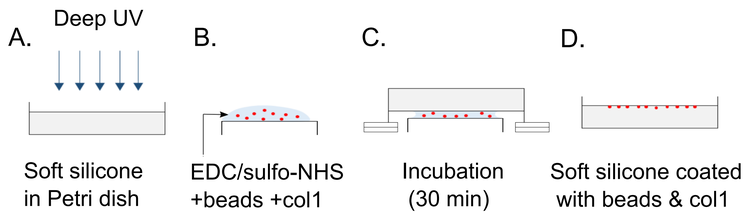

Figure 1: Schematic depiction of the procedure for coupling fluorescent microbeads to the top surface of soft silicone. (A) Soft silicone that has been cured is exposed to deep UV light for 5 min. (B) A mixture of EDC, sulfo-NHS, beads, and collagen I in water is pipetted down onto a piece of parafilm placed on top of a lid of smaller diameter. (C) The soft silicone sample is inverted on this mixture so that it is in contact with the liquid but not with the top surface of the smaller lid underneath. Two glass slides on either side, under the Petri dish, act as spacers. (D) After washing the sample with PBS, the soft silicone surface coated with fluorescent microbeads is ready for stiffness measurement.

Results

Fluorescent image of the microbeads in the top surface of the silicone as taken with a widefield microscope is shown in Fig. 4. Z-stack of the regions under the indentor and away from the indentor are shown as well. For the 1:1 soft silicone, the Young's modulus we obtained was 7.2 ± 2.4 kPa. We also showed that decreasing the silicone mix ratio yielded higher stiffness (37.6 ± 3.9 kPa for 4:7 and 64.1 ± 6.9 kPa for 1:2) To calculate the Young's Modulus, we used a modified [2] Hertz equation:

$$E = c\frac{\left[3\left(1-\nu^2\right)F\right]}{4R^{0.5}\delta^{1.5}}$$

Where:
c = a correction factor that modifies the Hertz model expression that follows it;
v = Poisson's ratio of the silicone gel (taken as 0.5 as for incompressible materials);
F = the indentation force;
R = the indentor radius; and
δ = the indentation depth

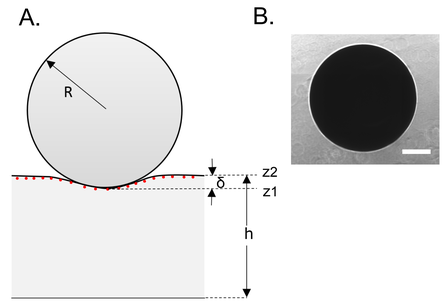

Figure 3: Schematic illustration of sphere indentation of the soft silicone surface. (A) This schematic depiction shows a spherical indentor on the surface of a soft silicone sample. Various parameters of interest are indicated. (B) This panel shows an image of a 1 mm indentor (on a soft silicone sample) obtained via phase imaging. The scale bar indicates 250 μm.

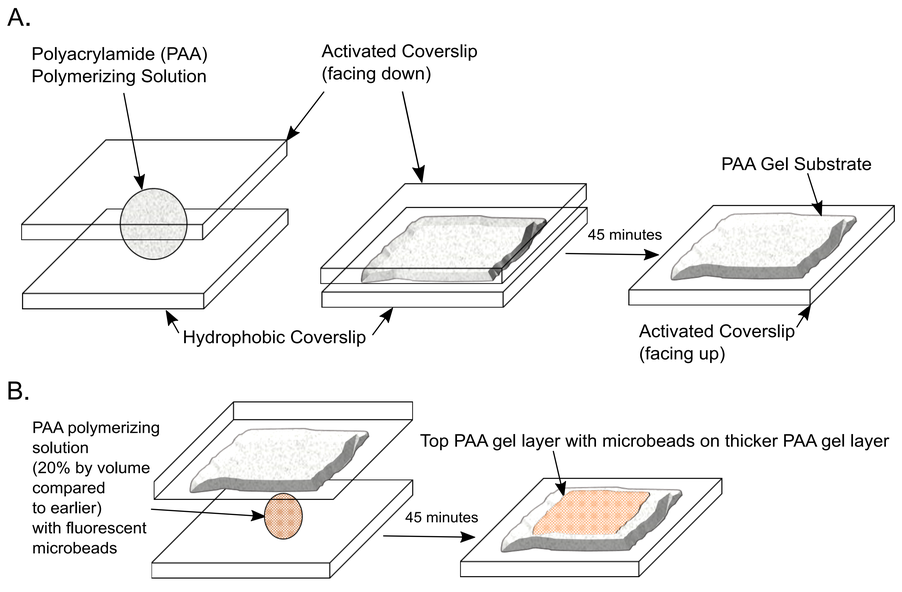

Figure 2: Schematic depiction of the procedure for the preparation of PAA gel substrate and coupling of fluorescent microbeads to the top surface. (A) Polyacrylamide gel preparation using polymerizing mixture: A drop of the polymerizing solution is placed on a hydrophobic coverslip and sandwiched with an activated coverslip on the top. After 45 minutes of polymerization, PAA gel is obtained. (B) Using 20% of the volume for the first layer, but with fluorescent microbeads, a thinner PAA layer is polymerized on the top of the thicker PAA gel layer below.

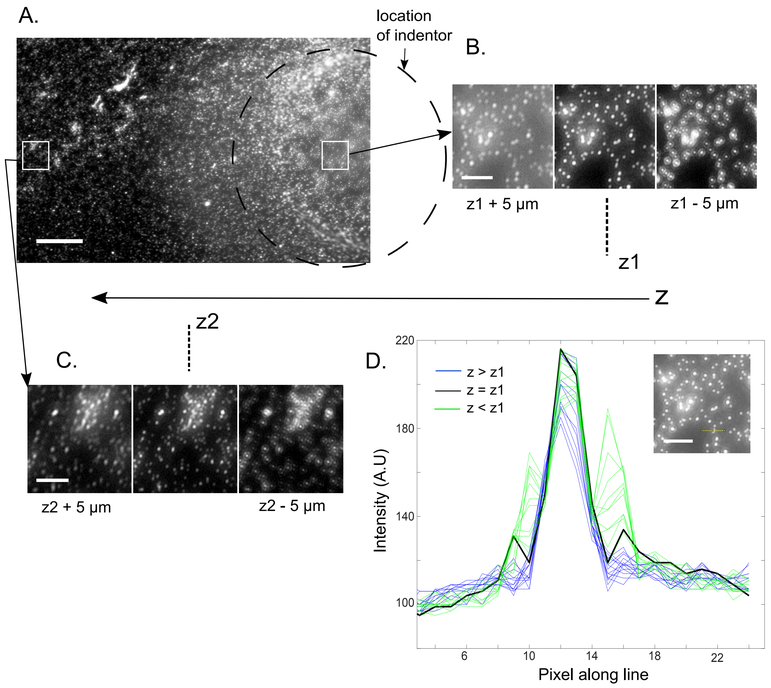

Figure 4: Bead image acquisition and determination of the in-focus image. (A) This fluorescence image shows microbeads on the top surface of the soft silicone sample and the desired x-y location of its frame relative to the indentor (dotted line). The scale bar indicates 150 μm. Panels B and C show z-stack fluorescence images of regions on the soft silicone surface (B) under the indentor and (C) away from the indentor (boxed regions in the top image). The indicators z1 and z2 correspond to the z-values at which the region under the indentor and the region away from the indentor are in focus, respectively. The scale bars indicate 20 μm. The monochrome images shown are those obtained in the red channel since nominally pink microbeads were used whose excitation and emission profiles fit the red channel. (D) This panel shows an intensity line scan across a micro-bead (shown in the inset image with a yellow line across it) as the focus is varied in z-increments of 0.5 μm. The focus (z-value) corresponding to the in-focus image can be objectively chosen based on the z-value corresponding to the line scan with the highest maximum intensity. The scale bar in the inset indicates 20 μm. Repeat of such scans for several nearby beads can be used to obtain an improved estimate of the in-focus z value.

Conclusions

We have presented a relatively simple method to couple fluorescent beads to the soft silicone top surface (or a PAA gel top layer) and measure its Young's modulus using an indentor, from data taken with a widefield fluorescence microscope. Common availability of this equipment makes this method widely accessible.

References

1. Lee, D. et al., Langmuir.31(35), 9684-9693 (2015)
2. Dimitriadis. E.K. et al., Biophysical Journal, 2798-2810 (2002)

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