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Developing AFM Techniques for Testing PEG Hydrogels

Hannah L. Cebull
The University of Akron, hlc31@zips.uakron.edu

Jessica Stukel
University of Akron Main Campus

Rebecca Willits
University of Akron Main Campus

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DEVELOPING AFM TECHNIQUES FOR TESTING PEG HYDROGELS

H. Cebull[1], J. Stukel[1], R.K. Willits[1]
(1) Biomedical Engineering Department, University of Akron
Akron, OH, USA

1. INTRODUCTION

Poly(ethylene glycol) (PEG) hydrogels, provide a broad range of applications in tissue engineering primarily because their mechanical properties can be highly tuned to resemble that of natural tissue [4,14]. Of the mechanical properties, the elastic modulus of hydrogels directly impacts cellular behavior including proliferation and morphology [15]. It is critical to fully characterize this property not only on the bulk scale, but at the micro and nanoscales because the measurement scale is in the order of cell interaction [17]. Nanoindentation is a useful tool for mechanical property and topography characterization because it offers non-destructive methods using extremely light loads and small displacements to the surface of the sample [9]. Using nanoindentation to acquire mechanical properties for soft materials can pose significant challenges because there is not a well-adapted method for fragile materials [5]. Thus, there is need for developing nanoindentation strategies for imaging soft materials, particularly hydrogels [3,9].

This study demonstrated the feasibility of using atomic force microscopy (AFM) to characterize PEG hydrogels, which could be easily translated to other soft materials. In many previous studies, AFM nanoindentation has been used to determine elastic moduli of hydrogels through a minimal amount of testing points [4,17]. However, developing a method which spatially maps the elastic moduli to represent the surface would further ensure the consistency of the hydrogel throughout [13]. This study combined high resolution imaging of PEG hydrogel topography with elastic moduli maps based on force-displacement curves to create a novel technique for the characterization of soft materials. Varying concentrations of PEG-DA, previously verified, were used to fabricate the hydrogels and the results were statistically compared to rheological testing conducted in our lab [14]. This comparison was used to support the accuracy and feasibility of the nanoindentation technique developed.

2. METHODS

2.1 Fabrication of PEG hydrogels

PEG hydrogels (n=3) were made with varying concentrations of PEG-DA (4, 5, 7, 9% w/v), 0.5% Irgacure 2959, and PBS based on a process reported previously [14]. The solution was vortexed for 30 seconds to obtain a well-mixed solution, then pipetted into silicone molds between two glass plates for an even surface. Following crosslinking under UV light (30-60 min), clear, uniform, and circular PEG samples were obtained reproducibly and submerged in a PBS solution until the testing occurred.

2.2 AFM-nanoindentation

The AFM Novascan, ESPM 3D was used in this study. For all testing and imaging conducted, the DNP-S probe (Bruker Nano Inc.), which is designed for soft materials, was chosen. Cantilever "B" was utilized throughout, with a pyramidal tip, 0.12 N m^{-1} spring constant, and 16-28 kHz resonant frequency, based on specifications provided by the manufacturer. This resonant frequency was verified on the Novascan software before testing.

As mentioned previously, there are challenges associated with testing soft materials; one specific difficulty is testing under liquid [6]. Therefore, this study designed a method of approach that mitigated some of the issues with the liquid approach. Each PEG hydrogel sample was tested on a glass coverslip

submerged under deionized water. Before submersion, the AFM performed a dry approach as it reduced the error of liquid interaction with the laser signal and detection before reaching the hydrogel surface. The hydrogel was then submerged and the tip was re-engaged to the surface. This method ensured that the tip was properly engaged because of the dry approach successfully bringing the tip close to the surface.

2.3 Imaging and determining elastic moduli

Hydrogel surfaces were imaged in non-contact mode to produce the highest quality topographies and prevent damage and movement of the sample and the tip [3,6]. The nanoindentation test is illustrated in Fig 1. The tip begins away from the surface of the hydrogel, then it is pressed into the surface with a recorded cantilever deflection. This response along with the force used are measured through the analysis of the loading and unloading curves generated, to be used for measuring mechanical properties and creating topography images [7].
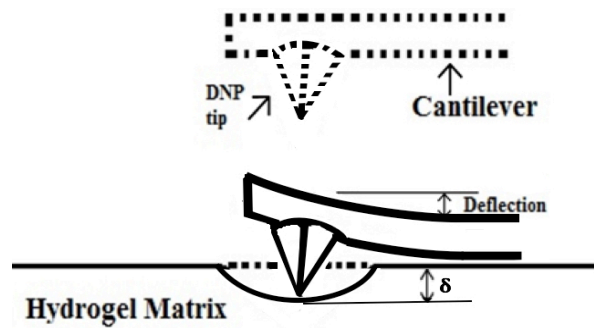

Figure 1. Tip and cantilever schematic: indentation of a hydrogel by a pyramidal DNP probe.

The elastic moduli mapping was conducted in contact mode over a ~1000x1000μm area in 500μm intervals (9 locations) on each hydrogel. Each location was tested three times and averaged to reduce error. The tip was withdrawn between each location and moved using x and y positioning knobs. A small map, 20x20μm area in 5μm intervals (25 locations) was also created on one PEG hydrogel for comparison of localized stiffness.

This was a precise location, using the software to control the tip location. Before conducting the force curves, the probe sensitivity was measured on each hydrogel. For calculating the elastic modulus of each test, the Sneddon mechanical model was used based on the pyramidal tip. This tip is more appropriate for flat surfaces like the samples used in this work [8]. However, since the pyramidal shape does not have a specific half cone angle, the probe manufacturer suggested using an angle two degrees less than the average of all other angles yielding 17°. Because the hydrogel obeys rubber elasticity, the Poisson's ratio was assumed to be 0.5 [1]. Based on these variables and inputs, the elastic moduli were determined by measuring the linear portion of the load-versus-displacement graphs. The relationship between the loading force F and the indentation δ is modeled in the given Sneddon equation using the half cone angle α and the assumed Poisson's ratio v:

$$F = \left(\frac{2}{\pi}\right)\left[\frac{E}{(1 - v^2)}\right]\delta^2 \tan(\alpha) \qquad (1)$$

2.4 Statistical analysis

The mean elastic modulus of a PEG hydrogel was determined from its force mapping values. Data from each group were expressed as the mean ± one standard deviation. The means were analyzed by two-way ANOVA test between AFM and oscillatory shear rheometry methods. The rheometer results were converted from shear to elastic modulus under the assumption that the hydrogels are approximately isotropic [16]. Tukey's HSD test then further compared 4, 5, 7, and 9% PEG hydrogels individually. A p-value <0.05 was considered statistically significant. In addition, the variance between hydrogel fabrication was tested by mapping and averaging the elastic moduli of three 5% PEG hydrogels. They were analyzed for significant differences using a one-sample t test, comparing the values to the mean calculated from the rheological data.

3. RESULTS

3.1 AFM imaging

The imaging conducted on the hydrogel samples was used to observe the topography variations. This imaging obtained revealed a uniform and flat surface with a roughness of approximately ± 494nm. Fig 2 shows a 20µm scan of a 5% PEG hydrogel with the topography variations ranging from 0 to 500nm. This high-resolution imaging provides further information about the type of cellular environment hydrogels create.
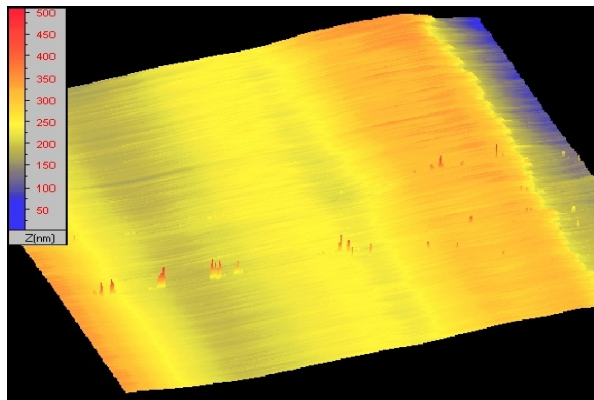

Figure 2. AFM scan of 20µm area of 5% PEG hydrogel sample.

3.2 Statistical analysis and verification

Identical elastic moduli procedures were performed for each 4, 5, 7, and 9% hydrogels with respective average stiffnesses of with respective average stiffness of 1.89 ± 0.17 kPa, 4.76 ± 2.32 kPa, 14.80 ± 0.47 kPa, and 40.86 ± 5.23 kPa. These values were compared to the rheological data previously collected shown in Fig 3. The two-way ANOVA found significant differences between the hydrogels of varying PEG amounts, demonstrating the increasing elastic moduli as the amount of PEG increases. However, no significant differences were discovered between rheological and atomic force microscopy methods, which yielded a *p*-value of 0.58. In addition, the Tukey HSD tests showed no significant differences between methods for each individual PEG amount.

The variation of hydrogel fabrication, calculated using a one-sample t test comparing the mean of each 5% PEG hydrogel elastic modulus to the mean elastic modulus provided from rheological data, is shown in Fig 4. Significant differences were found in two of the hydrogel fabrications, most likely due to human error. Though these significant differences were noted, the overall mean shown in Fig 3 still aligns with the rheological data.
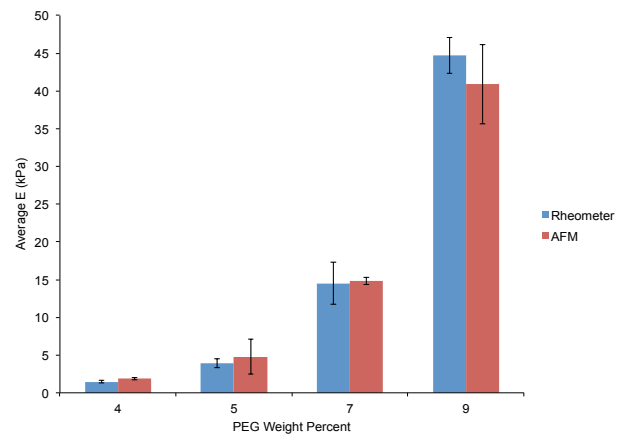

Figure 3. Mean ± standard deviation comparison between rheological and AFM results.
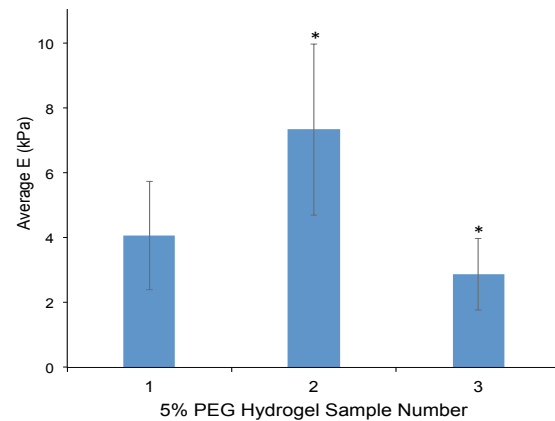

Figure 4. Elastic moduli of 5% PEG hydrogels. Data are presented as mean ± standard deviation for 27 measurements of each hydrogel. * represents significant difference from mean of rheological testing (p<0.05).

4. DISCUSSION

4.1 Sources of error

It must be noted that the values reported in this study are subject to sources of error due to the nature and process of nanoindentation. The significant sources of error include:

1. **Lack of accuracy in spring constant value:** The spring constant used in calculating the elastic moduli was provided through the manufacturer. However, the values provided from manufactures have a wide tolerance because of the difficulty in manufacturing accuracy of the cantilever geometry and thickness [11]. The range provided for the DNP tip used was .06 to .24 $N\ m^{-1}$, where the normal value of .12 was used. This factor may have resulted in a lack of accuracy in measuring the elastic moduli. To increase the confidence in the results of future studies, it would be important to verify the spring constant using a method such as measuring the thermal fluctuation of the cantilever [2].

2. **Accuracy of elastic moduli and tip geometry:** The calculation of the elastic moduli relied on the half cone angle, using the Sneddon mechanical model. Because of the pyramidal shape of the tip used, the half cone angle will inherently have errors. Using equation 1, it was calculated that altering the half cone angle by one degree causes a $\pm$ 0.6-6 change in the elastic moduli values. In addition, the calculations assume that the tip geometry also is not altered resulting from indentation testing. However, previous experiments have shown that the tip may become blunt or altered from the sample [12]. These two factors may also contribute to the inaccuracy of the elastic moduli calculations.

3. **Improper surface detection and interaction:** Testing in a hydrated system can cause non-specific force interaction leading to improper surface detection [4]. Previous studies conducted have shown that there is a problem of zero-displacement determination where a nanoindentation test can occur below or above the hydrogel surface, causing a large difference in elastic moduli [10]. Through the protocol designed in this experiment, the risk of improper surface detection was decreased; however, it could not be completely mitigated since hydrogels require liquid testing. Therefore, there remains a small level of risk, creating the possibility for error or inaccuracy in the force curves obtained.

4.2 Implications of findings

Creating a viable method for determining elastic moduli of hydrogels also provides a translational protocol for various soft samples, which is important because of the various challenges presented with testing of soft samples [17]. Bulk scale measurements provide some information about mechanical properties of the sample, but lack in characterization at the micro and nanoscale levels. This method is especially important for understanding anisotropic samples' range of elastic moduli; it provides the ability to separate mechanical behavior [4]. The hydrogel samples studied in this experiment, were further verified as isotropic due to the insignificant differences between individual elastic modulus values and the mean of each hydrogel. Future testing of hydrogels could include cells to study the effect on the mechanical behavior at the nano and bulk scales. In addition, methods to increase accuracy of elastic moduli calculation parameters are needed, including a model that better fits the tip geometry. With the increased elastic moduli accuracy and the protocol designed in this study for improving the nanoindentation for hydrogels, there are new possibilities of highly tuning the mechanical behavior to modulate desired cell responses.

REFERENCES

[1] Baldwin, F. P., Ivory, J. E., & Anthony, R. L. (1955). Experimental examination of the statistical theory of rubber elasticity. low extension studies. *Journal of Applied Physics, 26*(6), 750-756.

[2] Butt, H., Cappella, B., & Kappl, M. (2005). Force measurements with the atomic force microscope: Technique, interpretation and applications. *Surface Science Reports, 59*, 1-152.

[3] Chih-Wen Yang and Ing-Shouh Hwang and Yen Fu Chen and Chia Seng Chang and Din Ping Tsai. (2007). Imaging of soft matter with tapping-mode atomic force microscopy and non-contact-mode atomic force microscopy. *Nanotechnology, 18*(8), 084009.

[4] Drira, Z., & Yadavalli, V. K. (2013). Nanomechanical measurements of polyethylene glycol hydrogels using atomic force microscopy. *Journal of the Mechanical Behavior of Biomedical Materials, 18*, 20-28.

[5] Ebenstein, D. M., & Pruitt, L. A. (2004). Nanoindentation of soft hydrated materials for application to vascular tissues. *Journal of Biomedical Materials Research.Part A, 69*(2), 222-232.

[6] Hansma, P. K., Cleveland, J. P., Radmacher, M., Walters, D. A., Hillner, P. E., Bezanilla, M., et al. (1994). Tapping mode atomic force microscopy in liquids. *Applied Physics Letters, 64*(13), 1738-1740.

[7] Haque, F. (2003; 2003). Application of nanoindentation development of biomedical to materials. *Surface Engineering, 19*(4), 255-268.

[8] Heuberger, M., Dietler, G., & Schlapbach, L. (1996). Elastic deformations of tip and sample during atomic force microscope measurements. *Journal of Vacuum Science & Technology B, 14*(2), 1250-1254.

[9] Hu, Y., You, J., Auguste, D. T., Suo, Z., & Vlassak, J. J. (2012). Indentation: A simple, nondestructive method for characterizing the mechanical and transport properties of pH-sensitive hydrogels.*27*(1), 152-160.

[10] Kaufman, J. D., Miller, G. J., Morgan, E. F., & Klapperich, C. M. (2008). Time-dependent mechanical characterization of poly(2-hydroxyethyl methacrylate) hydrogels using nanoindentation and unconfined compression. *Journal of Materials Research, 23*(5), 1472-1481.

[11] Levy, R., & Maaloum, M. (2002). Measuring the spring constant of atomic force microscope cantilevers: Thermal fluctuations and other methods. *Nanotechnology, 13*(1), 33.

[12] McConney, M. E., Singamaneni, S., & Tsukruk, V. V. (2010). Probing soft matter with the atomic force microscopies: Imaging and force spectroscopy. *Polymer Reviews, 50*(3), 235-286.

[13] Pharr, G. M., & Oliver, W. C. (1992). Measurement of thin film mechanical properties using nanoindentation.*17*(7), 28-33.

[14] Scott, R., Marquardt, L., & Willits, R. K. (2010). Characterization of poly(ethylene glycol) gels with added collagen for neural tissue engineering. *Journal of Biomedical Materials Research.Part A, 93*(3), 817-823.

[15] Yeung, T., Georges, P. C., Flanagan, L. A., Marg, B., Ortiz, M., Funaki, M., et al. (2005). Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motility and the Cytoskeleton, 60*(1), 24-34.

[16] Zhou, W., Stukel, J. M., Cebull, H. L., & Willits, R. K. (2016). Tuning the mechanical properties of poly(ethylene glycol) microgel-based scaffolds to increase 3D schwann cell proliferation. *Macromolecular Bioscience, 16*(4), 535-544.

[17] Zhu, Y., Dong, Z., Wejinya, U. C., Jin, S., & Ye, K. Determination of mechanical properties of soft tissue scaffolds by atomic force microscopy nanoindentation. *Journal of Biomechanics, 44*(13), 2356-2361.