Mesoindentation of Polyacrylamide: Effects of Sample Thickness, Tip Size, Tip Geometry, and Load on Young's Modulus

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Statement of Purpose: The purpose of this study was to determine if polyacrylamide (pAA) is a suitable calibration material for microindenters used for indenting soft hydrated substrates (water content > 90%, Young's Modulus (E) < 100 kPa). Further, it was sought to determine the effect of tip geometry, tip size, sample thickness, and maximum applied load on the measured modulus of pAA in a load controlled test.

Materials: Eight batches of pAA (8% acrylamide and 0.2% bis-acrylamide (crosslinker) were synthesized from different starting solutions in separate polypropylene test tubes. Samples (n= 4, 5mm thick) from each batch were randomly cut using a razor blade. Thin samples (100 um, 200 um and 400 um thick; n= 4/ thickness) were synthesized on glass coverslips according to methods specified by Pelham and Wang [1]. Each sample was indented at a minimum of 4 random locations.

Mechanical testing and analysis: Samples were indented using a custom built mesoindenter[2], operating in load control mode. To study the effects of tip size, tip geometry, and sample thickness, the maximum applied load was 8 µN. To study the effect of maximum applied load, the load was varied between 8 µN and 18 µN. The two tip geometries were spherical (radii 142 µm, 174 µm, and 325 µm) and flat cylindrical tips (radii 90 µm, 120 µm, and 345 µm). Samples were submerged under deionized water during the entire course of testing. Four samples from each batch were indented using varying tip sizes and geometry. One tip of each geometry was used for indenting thin samples. The tip, attached to a cantilever, was driven into the sample using a motor until the predefined load was reached (loading cycle) and then retracted until it came off the surface (unloading cycle). The sample displacement and cantilever deflection were continuously monitored during the entire test and a resulting load-displacement curve was obtained. A custom written routine in Matlab was then used to filter and analyze the data. Young's modulus was calculated using Hertzian contact mechanics for thick samples and a correction factor was applied for thin samples to account for substrate effects[3]. One way and two -way ANOVA statistical analysis and Tukey-Kramer post-hoc tests were performed using SAS (version 9.1). A p-value of < 0.05 was considered to be significant.

Results: As shown in Figure 1, no variation amongst batches or within samples was observed in the indentation of pAA using spherical tips. There was an effect of tip radius on the measured modulus with the largest tip (325 µm radius) giving a significantly lower modulus than the 174 µm tip. No differences in modulus were seen between the 174 and 142 µm radius tips. For cylindrical tip based indentation (CTBI), no batch to batch or sample to sample variation was seen. However, tip radius and maximum load effects were significant: the largest tip showed the lowest modulus in comparison to both smaller tips; and a higher load resulted in a significantly higher modulus. Thin sample testing was feasible with both spherical and cylindrical tips and the use of the correction factor scaled down the modulus obtained for thin samples using Hertzian approaches to that of thick samples for spherical tips. However, this was not the case in CTBI. Figure 2 shows the effect of sample thickness on measured modulus. A spherical tip of 174 µm radius was used. No effect of sample thickness was observed for samples with thickness ≥ 200 µm (correction formula applied), but samples with 100 µm thickness showed a higher modulus than all other groups.

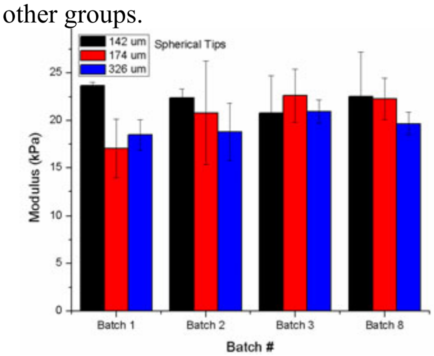

Figure 1.Variation of modulus amongst different batches of pAA (8% AA and 0.2% crosslinker) across different tip sizes. No sample to sample or batch to batch variation was seen within a tip radius. Significant effects of tip radius on modulus were seen with the largest tip showing a lower modulus.

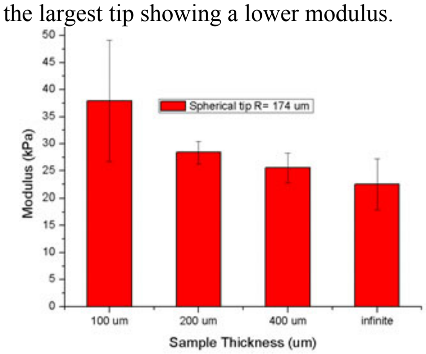

Figure 2. Variation of modulus with sample thickness. 100 µm thick samples showed a significantly higher modulus than other groups ($p < 0.02$). No other differences between groups were seen.

Conclusions: pAA was shown to be a useful calibration material based on the invariability between samples and batches. Spherical tip based indentation showed little variation in modulus with load, contrary to CTBI. Based on the variation in modulus with load and area for cylindrical tips, displacement controlled tests for cylindrical tips are recommended. Samples as thin as 200 µm could be successfully indented and substrate effects were evident in samples 100 µm thick. Future work will focus on using tips from both geometries which are similar in size. Effects of tip size, geometry and sample thickness were evident and must be taken into account while comparing across results from other sources.

References: 1) Pelham and Wang, Proc. Natl. Acad. Sci., 1997, 13661. 2) Saxena et al. JBMR A., 2008, in print 3) Dimitriadis et al. Biophysical Journal 82; 2002 (2798-2810)