Spherical nanoindentation of polyacrylamide hydrogels by the Bioindenter

Relevant for: Biomedicine, nanoindentation, Bioindenter

Hydrogels are very compliant materials suitable for tissue engineering in various areas of biological and clinical research. Appropriate and effective application of hydrogels for specific cellular regeneration often requires precise knowledge of their mechanical properties. The present application report focuses on the measurements of mechanical deformation and creep properties of polyacrylamide hydrogels using the Anton Paar Bioindenter. Four concentrations of polyacrylamide gel were tested under four different loading rates to study the mechanical response of the material. This work contributes to the understanding the results of instrumented indentation of extremely compliant materials with respect to their viscoelastic properties.

Figure 1 - An example of hydrogel.

1 Introduction

Hydrogels (Figure 1) are extremely soft materials with high liquid content that have recently been used in various areas of biological and clinical research, e.g. from osteoporosis through tissue regeneration to hemorrhage control. Many hydrogels are considered as potential candidates for replacement or regeneration of many types of tissues or as growth substrates for other soft tissues in human body. Appropriate and effective application of hydrogels for specific cellular regeneration, growth and tissue replacement requires deep knowledge of their mechanical properties [1,2]. For example, the structure and the mechanical properties of the growth substrate can act as a biomechanical modulator of cellular behavior and hence determine the function and quality of the growing cell [3]. It has also been found that the elasticity of the hydrogel substrate can significantly influence the homeostasis of tissues, which is crucial for efficient tissue regeneration. Measurement of elastic – and in general term mechanical – properties of hydrogels used in biomedicine is therefore extremely important.

Figure 2 - The Anton Paar Bioindenter.

This application report presents the results of measurements of mechanical and creep properties of several types of soft polyacrylamide hydrogels using a novel nanoindentation device for bioindentation called Bioindenter.

2 The Anton Paar Bioindenter

The Anton Paar Bioindenter (Figure 2) has been designed for the use in biomechanical domain which often requires different sample handling and testing conditions in comparison to 'traditional' hard materials. This nanoindentation device is based on the successful Ultra Nanoindentation Tester and uses its excellent thermal stability and high resolution in both force and displacement measurements. Normal loads in micronewtons and displacements in tens of micrometers are required because the biological materials are extremely soft and good thermal stability is indispensable for determination of creep properties of these materials. The indentation procedure has been adapted and verified for automated testing in liquids and testing of samples with uneven surface. Such indentation procedure allows not only measurement of elastic properties of biological and biomedical samples but also of their time dependent properties.

For the purpose of this study, polyacrylamide (PAAm) hydrogels have been selected since they are a common growth substrate used in many biological laboratories for cell cultivation or tissue replacement. Their mechanical properties can easily be tailored to obtain Young's modulus ranging from ~10 kPa up to ~200 kPa and they are therefore suitable candidates for demonstration of the bioindentation procedures over a large range of elastic properties.

3 Experimental setup & nanoindentation conditions

Following a preliminary study in [3], four concentrations of polyacrylamide (PAAm) were selected for the nanoindentation experiments: 0.05%, 0.1%, 0.2% and 0.8%. Such range of polyacrylamide hydrogels is considered to have suitable mechanical properties to promote stem cell cultures to differentiate and form specific cell types. The selected concentrations cover a relatively broad range of Young's modulus from ~20 kPa to ~200 kPa (i.e. ten-fold increase) and they exhibit important time-dependent (creep) behavior. The nanoindentation tests were performed with the newly developed Bioindenter device using spherical indenter with 500 µm radius made of ruby. Figure 3 shows the Bioindenter during measurement with the indenter approaching the surface of the PAAm hydrogel immersed in water.

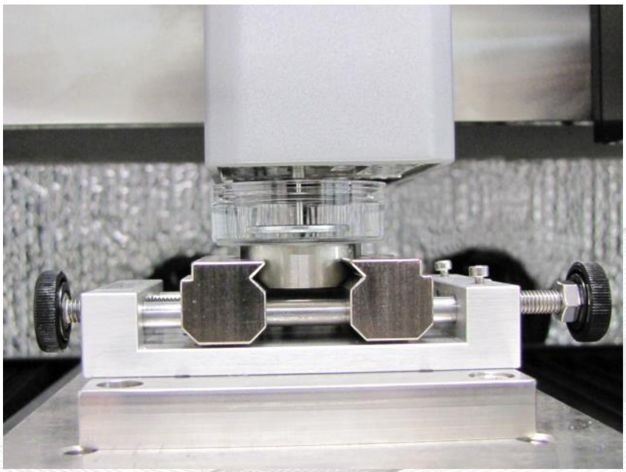

Figure 3 - The Bioindenter setup for measurements of the PAAm hydrogels.

Note that the special long shaft indenter has shaft with only 1 mm diameter to reduce the capillary and buoyancy effects. The sheets of PAAm with approximately 20x20 mm dimensions and ~2 mm thickness were placed in 35 mm diameter Petri dish. As the hydrogel has similar density as water and it would normally float in water, stainless steel washer was used to keep the hydrogel on the bottom of the Petri dish.

All tests were performed at room temperature with the sample completely immersed in water to avoid drying of the sample. The nanoindentation protocol was established so that the time-dependent response of the PAAm hydrogels could also be evaluated: the loading & unloading times were set to 1 s, 10 s, 30 s and 60 s. Hold period of 100 s at the maximum load (F_{max}) of 50 µN was maintained constant in all experiments. The increase of penetration depth was monitored during this long hold period and the time-dependent properties of the individual concentrations could be compared. These nanoindentation conditions resulted in mean contact pressure of ~2 kPa to ~4 kPa, which is acceptable also for many types of biological materials.

The load-displacement data were analyzed using Hertz solution for spherical contact on the loading part (1), where P is the indentation load, E^* is reduced modulus (which can be set equal to Young's modulus of the sample since the indenter can be considered as a non-compressible body), R is the radius of the indenter and h is indentation depth. The unloading part of the indentation curve was analyzed according to the ISO 14577 [4] standard. The indentation creep (C_{IT}) was calculated according to equation (2) where h_i and h_e is the depth at the beginning and the end of the hold period respectively.

$$P = \frac{4}{3} E^* R^{1/2} h^{3/2} \qquad (1)$$

$$C_{it} = \frac{h_e - h_i}{h_i} \qquad (2)$$

4 Results – Elastic modulus

The nanoindentation experiments showed large differences in mechanical properties of the four concentrations of the PAAm hydrogels. Typical load-displacement curves obtained on all four types of PAAm gels with 10 s loading time are shown in Figure 4. The capillary and buoyancy forces on the indenter were found to be negligible and also the adhesion effects were minimal, without any negative effect on the measurement. The determination of contact point was straightforward: the contact point was defined as the point where the normal force started to monotonically increase. Such simple detection of contact point was due to independent true force sensor in the Bioindenter. The Young's modulus as calculated according to Hertz equation for spherical contact (1) on the loading part of the load-displacement curve varied between 122 kPa for the highest 0.8 % PAAm concentration and 37 kPa for the lowest 0.05 % PAAm concentration.

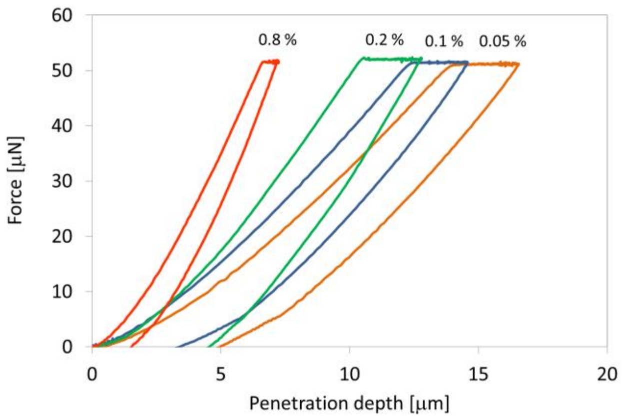

Figure 4 - Typical load-displacement curves obtained on the polyacrylamide gels with the Bioindenter (500 µm radius spherical indenter, 10 s loading time, 100 s hold period).

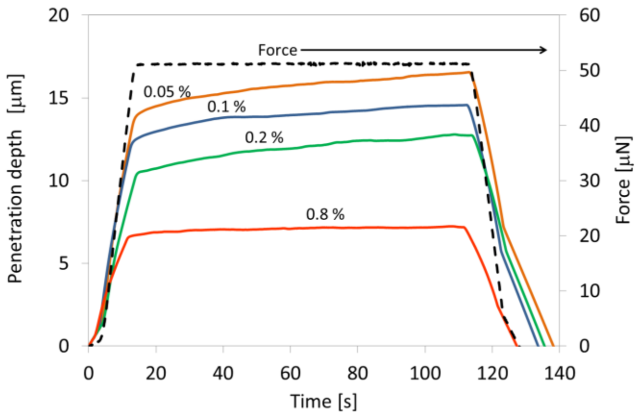

Figure 5 - Typical penetration depth versus time indentation curves (10 seconds loading). This data was used for evaluation of creep. Corresponding force profile (dashed line) is also shown.

4.1 Effect of loading times on Young's modulus

The Young's modulus from the loading curves of individual concentrations of the PAAm gel varied with the duration of the loading time: the shorter the loading time, the higher the Young's modulus. For the 0.8% PAAm and 1 s loading the Young's modulus was 122 kPa while for 60 s loading the Young's modulus of the same material dropped to 75 kPa.

4.2 Creep

During the nanoindentation experiments we observed important creep (penetration depth increase during hold period at constant force F_{max}). The level of creep can be evaluated via the C_{IT} value (2), obtained from the hold period. The creep values varied between ~8% for the 0.8% PAAm concentration and ~20% for the 0.05% PAAm concentration. In all samples shorter loading times lead to greater creep during the hold period. Figure 5 summarizes creep as a function of loading time for all tested concentrations of the PAAm gels. For longer loading times (≥30 seconds), the C_{IT} value stabilized at approximately 10%, irrespective of the PAAm concentration. Although quite long hold period was defined (100 seconds), the penetration depth was still increasing at the end of hold period for lower concentration gels (0.05% and 0.1%) and faster loading (1 s and 10 s). On the other hand, the penetration depth was almost constant at the end of the hold period on samples with higher PAAm concentration (0.2% and 0.8%) and loading times above 30 seconds. Such differences in creep behavior illustrate the importance of characterization of time dependent properties of hydrogels and similar materials containing large amounts of liquid.

4.3 Comparison of Young's modulus values from ISO 14577 and Hertz model

The values of Young's modulus were calculated also according to the ISO 14577 (Oliver&Pharr) standard based on the analysis of the unloading part of the load-displacement curve. This type of analysis is available in most commercial nanoindentation software packages. However, its validity for such soft materials must be confirmed and therefore we performed comparison of Hertz fit on loading with ISO 14577 fit on unloading. Comparison of the Young's modulus values obtained by these two methods is given in Table 1.

Table 1 - Young's modulus values calculated by Hertz and ISO 14577

Young's modulus [kPa]				
Hertz fit to loading				
Loading time	0.05 %	0.1 %	0.2 %	0.8 %
1 s	36.9±1.1	49.7±0.7	62.8±1.2	121.8±2.8
10 s	37.1±0.9	43.9±0.7	55.5±2.4	111.0±3.1
30 s	32.3±0.4	40.1±0.8	49.1±3.6	77.4±4.6
60 s	31.0±0.1	38.5±1.7	45.3±1.4	74.6±2.2
ISO 14577 (Oliver & Pharr) fit to unloading				
Loading time	0.05 %	0.1 %	0.2 %	0.8 %
1 s	24.2±0.5	31.1±0.8	36.7±1.9	74.4±1.9
10 s	22.6±0.3	27.1±0.4	34.5±0.9	77.8±0.9
30 s	22.0±0.4	25.4±0.7	33.3±1.0	65.7±1.0
60 s	21.3±0.6	27.1±0.9	34.1±1.8	65.9±1.8

5 Discussion

5.1 General measurement remarks

The results of the indentation experiments showed that it is possible to perform local characterization of mechanical properties of very soft materials immersed in liquids provided that both reliable measurement protocol and instrument are available. In general, the indentation procedure consists of using very low loads (few tens of µN), use of spherical indenter with large radius, long hold period and complete immersion of the sample in liquid. At the same time, the nanoindentation system should be capable of measuring large displacement because of the extremely low compliance of hydrogels and biological materials. The capillary and adhesion forces, which were initially thought to negatively affect the normal force measurements, were found to be negligible.

5.2 Young's modulus

Although commercially available indentation software often calculates Young's modulus of the tested material from the unloading part of the load-displacement curve, the validity of this approach should be verified. The analysis of the indentation results by Hertzian fit on the loading part showed considerable differences from the Young's modulus calculated from the unloading part: values obtained by Hertz fit on loading were up to ~50 % higher than those calculated by the ISO 14577. However, this discrepancy almost disappeared for stiffer PAAm hydrogels.

5.3 Creep

The tested hydrogels (and also similar types of materials) exhibit pronounced creep behavior. In the case of our PAAm hydrogels the Cit values varied from ~8% up to ~20% and the depth increase during the hold period was up to 4 µm in 100 s. It is therefore crucial to include hold period in the loading protocol and use various loading times in order to characterize the time-dependent properties of the material. The complete analysis of such nanoindentation experiments should therefore include not only standard ISO 14577 analysis but also analysis of the creep data as proposed in literature [5,6].

5.4 Hertz model and ISO 14577 Analysis

The comparison of the results of Hertzian fit on the loading part and ISO 14577 analysis on the unloading part of the indentation curve show that both methods yield similar results for higher PAAm concentrations. This is in agreement also with the creep data, showing that higher PAAm concentrations (stiffer material) have less pronounced creep properties and the standard indentation analysis (Hertz or ISO 14577) can be applied. Also, close agreement of results of both methods shows that in the ISO 14577 method can be applied also on materials deforming predominantly elastically. The calculation of hardness, on the other hand, is quite irrelevant for such materials since most of these materials fully recover after unloading, leaving no residual impression.

6 Conclusions

This application report presents the results of bioindentation experiments on extremely soft polyacrylamide hydrogels. The measurement protocol for such soft materials was successfully applied using the newly developed Bioindenter with spherical indenter with 500 µm radius, maximum load of 50 µN and hold period of 100 seconds. Large range of loading conditions was applied during the experiments and apart from the Young's modulus also creep properties could be determined. In all cases, shorter loading time leads to higher Young's modulus and larger creep. For constant loading time, the Young's modulus increased with increasing concentration of the PAAm hydrogel. The time dependent properties of hydrogels are important when characterizing mechanical properties of these materials and the bioindentation protocol (including suitable instrument) should therefore allow such measurements. The Young's modulus values obtained by Hertzian fit and ISO 14577 norm fit revealed considerable differences in both methods especially for low concentrations of PAAm. For higher PAAm concentrations the agreement between the Hertz model and ISO 14577 norm was surprisingly good, indicating that in the first approximation the ISO 14577 results can be used.

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7 References

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