CSM Instruments
Advanced Mechanical Surface Testing

APPLICATIONS BULLETIN

Nanoindentation approach to mechanical testing of extremely soft materials

//// Introduction

The characterization of the mechanical properties of tissues and other biological materials is becoming an important topic in today's biomedical research. As biomedical engineering science has advanced, new materials for replacement of human tissues have been developed. To properly tailor such new tissues, one needs to know their biocompatibility, resistance to inflammations and lifetime – but also their mechanical properties. Therefore, a method that allows assessment of mechanical properties of these new materials is now needed. This method would also be helpful in another way – it is known that ill or infected cells and tissues change their stiffness with respect to healthy ones. A suitable method for measurement of mechanical properties of tissues could therefore quickly determine whether the tissue is healthy or ill.

However, tissues are a challenging class of materials as they are composed of very complicated structures. At the same time, they are usually much softer than commonly tested materials – metallic materials have typical elastic moduli in the range of ~100 GPa while elastic moduli of soft biological tissues vary between 5 kPa and 1000 kPa. Of course there are stiffer and harder biological tissues such as dentine but testing of these materials is quite similar to testing of common engineering materials and does not present a substantially challenging task. A method that quickly determines the mechanical properties of such soft materials is therefore of a great interest.

//// Instrumented indentation as a tool for testing soft materials

So far the measurement of mechanical properties of biological tissues has been difficult to perform and the output was rather complicated [1]. Instrumented indentation, on the other hand, offers quite a simple method for measurement of mechanical properties of many types of materials and does not require complicated preparation of samples. Instrumented indentation systems such as the CSM Instruments Ultra Nanoindentation Tester (UNHT) or a Table Top NHT system (see Fig. 1) are relatively compact and can therefore be easily installed in medical research centers. These instruments offer simple and comprehensible output, which is a great advantage over many biological methods. Furthermore, the measurements results can be understood by relatively inexperienced user.

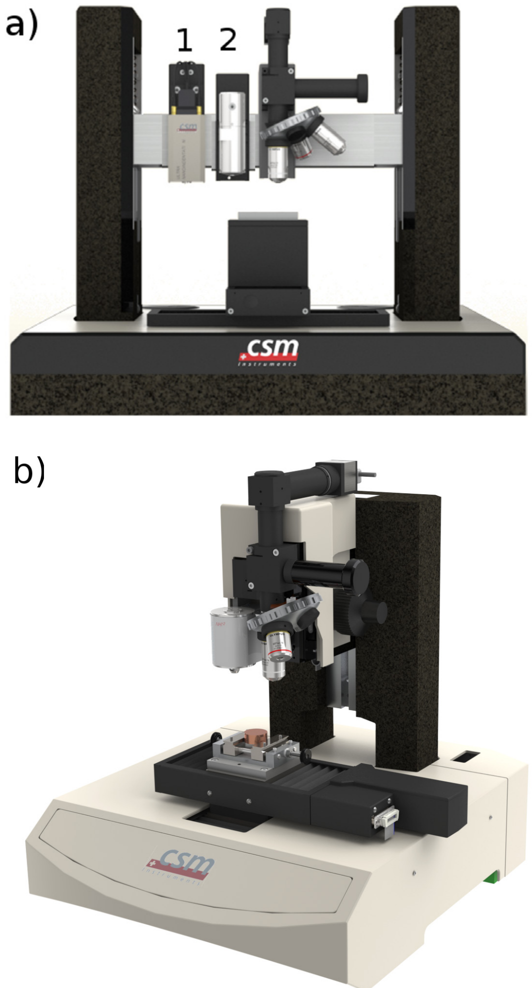

Fig. 1 – Ultra Nanoindentation Tester (1) on a Compact Platform (a) and Table Top Nano Indentation Tester (b). Both instruments are equipped with optical microscope. Optionally an AFM (2) can be mounted next to the UNHT head on the Compact Platform.

The instrumented indentation technique has been used for more than 20 years and is now well established for various types of materials. Currently available instruments feature sufficient force and displacement resolution so that depths down to several nanometers and loads down to a few tens of micronewtons are well controlled. On the other hand, the instruments can easily attain displacements up to several tens of micrometers and loads up to hundreds of millinewtons.

The design of the CSM Instruments indentation systems (and particularly that of the UNHT) ensures excellent stability of both load and displacement signals. The unique active surface referencing principle along with the use of the most advanced electronics and materials almost entirely eliminates the problem of thermal drift and frame compliance (See Ref. [2] for more details and features of the UNHT system). Thanks to its principle using two independent force and displacement sensors, the UNHT system can also easily detect adhesion effects that are commonly observed on extremely soft materials.

The question covered here is whether current nanoindentation systems are also suitable for indentation of biological tissues and various types of soft gels. During an ongoing joint project between CSM Instruments and ETHZ in Zürich, considerable challenges had to be faced when developing the indentation methodology on this special class of materials. To name the most important ones, the instrument should:

- be able to apply very low loads (~ µN),
- measure large depths (~ µm) at low loads,
- have excellent stability of normal force,
- be able to record displacement and normal force signals before and after the indentation process itself.

One additional issue was to find out which indenter geometry is the most suitable for indenting such soft materials.

//// Experimental framework

The idea of using instrumented indentation on extremely soft materials was quite new and there was no common methodology available at that time. It was therefore decided to begin with indentation on soft elastomers where considerable experience has already been gained. A flowchart of the experimental work is shown in Fig. 2.

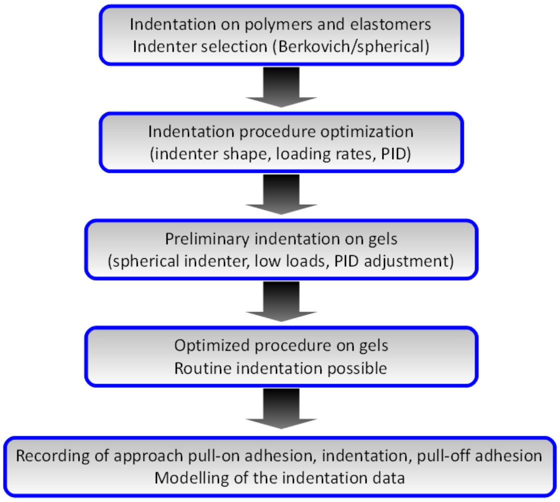

Fig. 2 – Flowchart of the experimental work for development of a dedicated methodology for indentation of extremely soft materials.

//// Indentation on elastomers

Three types of Vishay photoelastic sheets ranging from hard (PS-4A, E = 3 GPa) down to relatively soft (PS-6C, E = 0.7 GPa) were used for the indentation experiments on elastomers.

These materials covered a broad range of mechanical properties and were therefore considered as a suitable approach to the indentation of even softer gels and tissues. The measurements were performed with the CSM Instruments UNHT system using both Berkovich and spherical indenters in load controlled mode. The indentations were made at several loading rates with a 120 second hold at the maximum load. The purpose of these experiments was to optimize the indentation parameters such as the radius of the indenter, the applied loads and loading rates and also the feedback control parameters (PID).

The typical indentation force-displacement curves for the three tested Vishay elastomers are shown in Fig. 3. They clearly illustrate large differences in stiffness for the three samples: although the maximum load was in all cases set to 500 µN, the maximum indentation depth reached 11200 nm in the case of the PS-6C sample but only 312 nm in the case of the PS-8A sample.

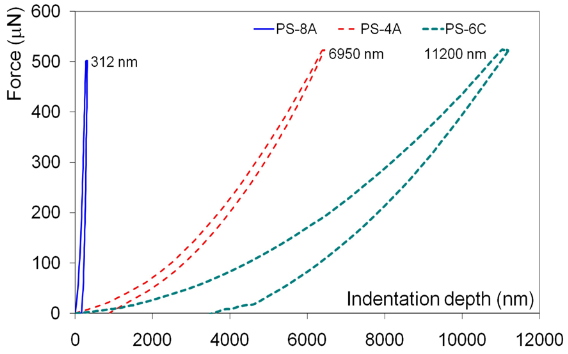

Fig. 3 – Typical load-displacement plots for the three types of Vishay photoelastic sheets.

The elastic modulus calculated by Oliver & Pharr (O&P) approach for the hardest elastomers was in very good agreement with the manufacturer's data while for the softer elastomers the elastic modulus was overestimated. This was due to the strong visco-plastic behavior of the elastomers, which resulted in higher apparent values of elastic modulus for the softer samples. Ongoing work is now focused on application of a proper model for calculation of visco-elastic properties.

//// Indentation of gels

Once the optimal parameters (indenter type, indenter approach speed, loading rate and maximum load) for indentation of soft elastomers were identified, the experimental work was focused on indentation of gels. The measurements were performed on three types of commercially available decorative gels, distinguished by color (white, yellow and blue), that had similar properties to gels used in biological tissue replacement. In addition, the results of indentation on Ecoflex 0030 hydrogel (elastic modulus ~29 kPa) were used for modeling of its mechanical properties. Indentation on the gels was performed with a spherical indenter of 100 µm radius. The maximum load used in measurements was set to 20 µN with a 120 s hold at this maximum load.

The preliminary experiments on gels served for optimization of the feedback control parameters (PID) and establishment of the optimal indentation procedure. Although the UNHT system feedback control is optimized for a very broad range of materials, it had to be adapted for extremely soft materials. The PID parameters are crucial for proper control of the force and displacement once the indentation has been started. Improper control parameters can lead to severe oscillations and damage of the instrument. Therefore the optimization of the PID parameters for indentation on soft gels was an indispensable step in gel characterization by instrumented indentation. Once the set of optimized parameters had been determined at CSM Instruments, the user will not have to change these except for some extreme testing conditions. After this important stage, the indentations could be routinely performed and the indentation procedure itself could be fine tuned. After a large number of experiments we found that using spherical indenters with radii in the range ~100 μm gave the following typical parameters for indentation on a soft gel:

- maximum load of ~20 μN,
- maximum indentation depth ~20 μm,
- pull-on and pull-off adhesion ~30 μN,
- total indenter travel ~35 μm (required to record all phases of the indentation process).

Therefore an instrument capable of controlling such low loads while measuring large displacements was a crucial factor in this study.

//// Optimization of the indentation procedure on gels

During the spherical indentations the phenomenon of adhesion became immediately apparent. This effect was observed during indenter approach (pull-on adhesion) and during indenter retraction (pull-off adhesion). This was due to much larger contact area of the spherical indenter in comparison with a Berkovich indenter. Also other phenomena such as extremely low elastic modulus of the soft gels, which enabled mutual approach of the sample surface and the indenter where playing a key role in the adhesion effects. The indentation procedure had to be adjusted in order to record all indentation data: (1) the indenter approach in the air, (2) the pull-on adhesion, (3) the indentation itself, (4) the pull-off adhesion and removal of the indenter – see Fig. 4.

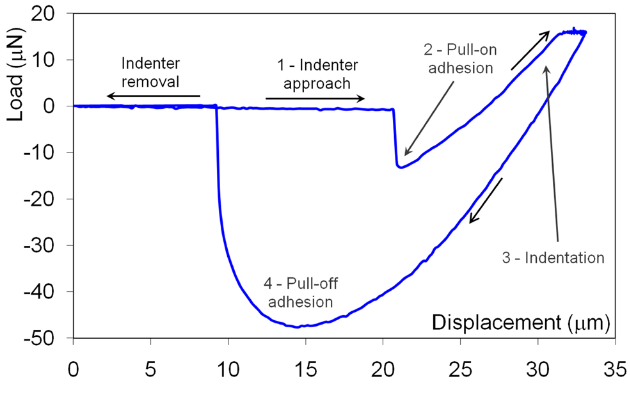

Fig. 4 – Typical indentation response of a gel. The four phases of the load-displacement curve are shown (R=100 μm indenter on blue decorative gel).

This optimization was necessary for correct determination of the contact point which was indispensable for computer modeling.

The spherical indentation on the softest elastomers showed that an indenter with radius of 100 μm was most suitable for the gel measurements. Smaller radii indenters would lead to unacceptably large depths while larger radii indenters would result in high adhesion forces.

//// Adhesion effect and contact point determination

One of the main complications with the indentation of gels (also expected in the indentation of other soft biological tissues) was the adhesion effect [3,4].

The indentation procedure had to be adjusted so that the recording of the force and displacement signals would start well before contact with the sample surface. Normally, the retraction of the indenter (distance above the surface of the sample where the recording of the normal force and displacement begins) is set so that the whole indentation process is reasonably fast. However, in the case of materials exhibiting adhesion effects this retraction distance has to be set quite large in order to accommodate both the movement of the indenter in the air and the pull-on adhesion effect. At the same time, the indenter retraction distance has to be kept small enough in order to be able to observe and record the pull-off adhesion, which appears after the unloading phase. The approach speed during the indenter approach to the surface also has to be increased as the distance to be travelled is much larger than in the case of common materials.

Figure 4 shows a typical record of the normal force: during the indenter approach the normal force is zero as the indenter moves in the air only. The recording of the force and displacement signals had to be kept sufficiently long so that the pull-off adhesion was observed up to the point where the indenter was again in the air (Indenter removal portion of the curve in Fig. 4, normal force returns to zero).

The fact that all the indentation data including the pull-on and pull-off adhesion are recorded gives the user the liberty of deciding where the contact point will be defined – since there is no common agreement on the definition of the contact point in case of materials exhibiting adhesion effects.

The whole indentation process on gel can take up to 5 minutes – a negligible thermal drift of the instrument is therefore indispensable. It was also obvious that even when using a 100 μm radius indenter the adhesion forces (~15 μN for pull-on adhesion and ~48 μN for pull-off adhesion) were similar or even higher than those applied during the indentation itself. The adhesion effect therefore has to be taken into account and has to be included in the analysis of the indentation data.

The pull-on adhesion also complicated the determination of the contact point, i.e. the point where the indenter first comes into contact with the sample surface. Due to the shape of the force-displacement curve, this point can be defined in several ways. In our approach, the contact point was set in a similar way as in a standard indentation, i.e. the point where the force starts to increase. This is shown schematically in Fig. 5.

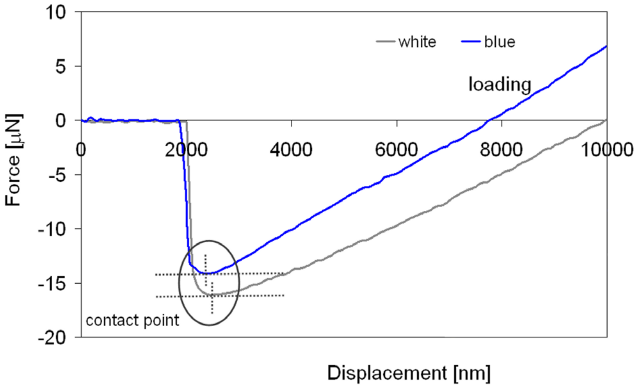

Fig. 5 – Determination of the contact point as the point of inflexion on the force curve during pull-on adhesion.

Typical force-displacement curves from indentation experiments on the three decorative gels together with the PS-6C elastomers are compared in Fig. 6. Clearly there were differences between all four tested materials with the PS-6C elastomer being much stiffer than the three decorative gels. In the first approximation, this difference was also confirmed by the values of elastic modulus calculated by the O&P model.

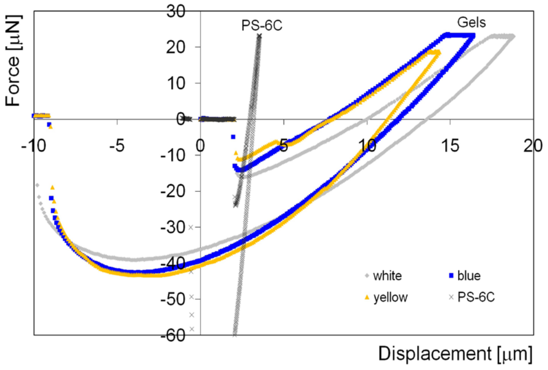

Fig. 6 – Comparison of force-displacement plots for the three gels (blue, yellow, white) and the softest PS-6C elastomer.

//// Modelling

Although there are several models for interpreting the indentation data, there is a lack of existing models dedicated to soft and extremely soft materials. Furthermore, the problem of adhesion must be properly understood and incorporated in the model. In most cases concerning modeling of adhesion the theory of Johnson, Kendall & Roberts (JKR) is used for calculation of adhesion forces. The application of this approach is somewhat complicated as the contact area is varying during the indentation. Nevertheless, modeling by Finite Element Analysis (FEA) using the JKR theory was done with promising results. The modeling is now being optimized towards extracting the mechanical properties of the gels from the indentation data.

//// Conclusions

Instrumented indentation has proved to be a powerful method for the measurement of extremely soft gels with properties and structure similar to biological tissues. The utility of the method stems from the ability to nondestructively measure small samples while yielding indentation data that can be analyzed to extract the mechanical properties of the sample. A great advantage of the CSM Instruments Ultra Nanoindentation Tester is that the system can be used for such measurements without any hardware modifications. Thanks to its unique design and features such as absence of thermal drift and excellent force stability, the UNHT system is a fast and easy way for testing of extremely soft materials. All this makes the indentation method very attractive for testing in biomedical research facilities and hospitals.

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//// References

1. Anseth K. S, Bowman C. N, Brannon-Peppas L: Mechanical properties of hydrogels and their experimental determination, Biomaterials 1996, Vo. 17 No. 17, p. 1647-1657.

2. Nohava J, Randall, N. X, Conté N.: Novel ultra nanoindentation method with extremely low thermal drift: Principle and experimental results, J. Mater. Res., Vol. 24, No. 3, Mar 2009, p. 873-882.

3. Ebenstein, D. M, Pruitt, L. A.: Nanoindentation of biological materials, Nanotoday, August 2006, Vol. 1, No. 3, p. 26-33.

4. Handbook of nanoindentation with biological applications, M. L. Oyen (Ed.) Pan Stanford Publishing Pte. Ltd. Singapore, 2011.

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