Applications of indentation in biology and biomedical domain

Relevant for: Biomedicine, nanoindentation

Characterization of mechanical properties of biomedical and biological materials is an important part of their development. For many biomaterials, mechanical properties have to be studied very locally or in relatively small areas. Furthermore, the pre-clinical research is very often done on small animal models such as rats or mice. The measurement techniques must therefore be sufficiently local so that even such small samples can be tested. The nanoindentation technique, introduced to biomedicine in the last years, is especially suitable for this type of characterization. This application report shows some applications of nanoindentation for testing of bone, teeth and contact lenses.

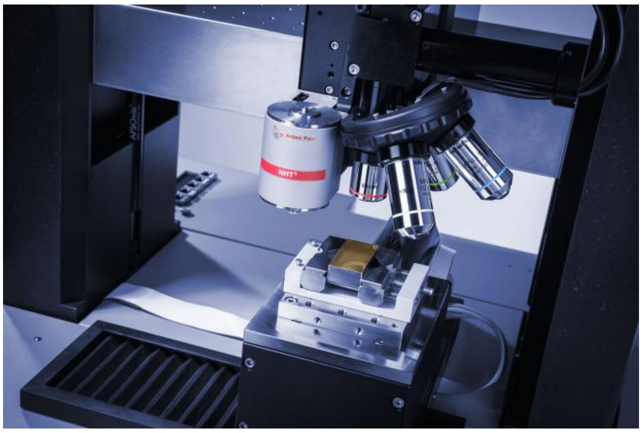

Figure 1 - The Anton Paar Nanoindentation Tester NHT^3 on the STeP 4 platform.

1 Introduction

In the last several decades, characterization of the mechanical properties of biomaterials and other biomedical or biological materials has become an important phase in their development. Researchers and engineers were interested in knowing the mechanical properties of both biological materials (soft and hard tissues, bones, tendons, cartilage, teeth, etc.) and artificial (man-made) biomaterials (implants, stents, resolvable sutures, scaffolds, etc.). Knowing the mechanical properties of biological materials such as tissues and organs is necessary for development of new materials and tissues in the human body as well as for assessment of the effects of different medical treatments.

In many of these applications, the mechanical properties have to be studied very locally, within relatively small regions. Furthermore, the pre-clinical research is very often done on small animal models such as rats or mice. The measurement techniques must therefore be sufficiently local so that even such small samples can be easily tested.

This application report summarizes the use of instrumented indentation for characterization of biomaterials, bio-inspired materials and biological materials. Other application reports will focus on scratch testing (adhesion and scratch resistance) and tribology testing (coefficient of friction and wear.

1.1 Nanoindentation

The nanoindentation technique has been used in the biomedical domain for about two decades. Several researchers used this method to study the effects of osteoarthritis or different nutrition regimes on the mechanical properties of the bone [1–3]. The nanoindentation technique is very useful mainly because of the fact that it offers local characterization of different regions of bone compared to bulk tensile or compressive testing which characterizes the entire structure. The local character of indentation is extremely important in studying the effects of medical treatments or lesions because these treatments often result in very local changes of stiffness of the biological material. The following examples demonstrate the use of instrumented indentation in various biomedical domains.

2 Effect of medical treatment of bovine bones on their mechanical properties

The ability to determine the effects of medical treatment requires a good knowledge of the properties of a healthy bone structure. Therefore, together with the tests on treated bones, similar tests on healthy bone have to be performed. Furthermore, the tests parameters should be such that the volume of the material involved in the indentation response is always the same (or at least very similar) and should represent the relevant structural unit where the effects of the treatment can be observed. In many cases the

Anton Paar Microindentation Tester (MHT^3) can be used: with its large force range (between ~30 mN and 10 N) it can test different volumes of the biomaterial. In other cases when even smaller indentations are required, use of the Anton Paar Nanoindentation Tester (NHT^3) is recommended. Figure 2 shows an example of indentation curves recorded on bovine bone samples using the MHT^3. The bovine epiphysis (rounded end of a long bone at its joint with adjacent bone) was subject to three different treatments. Microindentation experiments with 400 mN maximum load were done in several areas on each bone with each area having a different treatment. The values of elastic modulus and hardness obtained from the indentation data showed clearly the differences between the treatments and so the efficiency of the treatment could easily be evaluated (Figure 3).

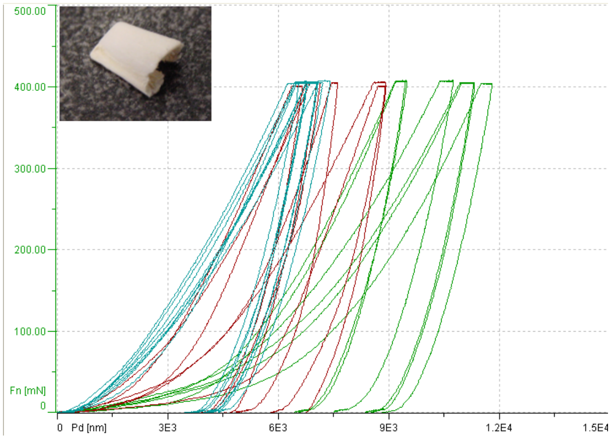

Figure 2 – Typical load vs. penetration depth curves - three bone samples with different treatments were tested.

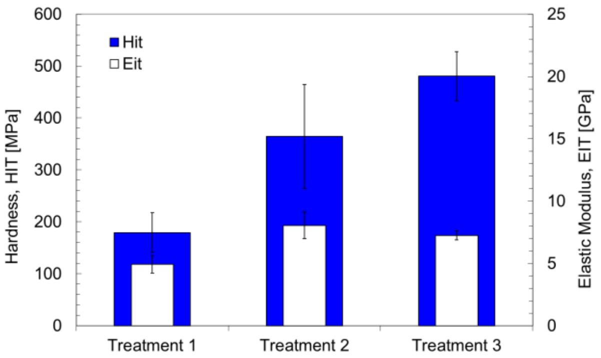

Figure 3 - Comparison of hardness and elastic modulus between the three types of bone treatment.

3 Evolution of mechanical properties of treated tooth enamel

Tooth enamel is another material that has been studied by nanoindentation [4,5]. The nanoindentation technique is indeed one of the most adapted methods for mechanical testing of such small samples. An interesting study concerning the effects of chemical and biological treatment on mechanical properties of tooth enamel has recently been performed using the Anton Paar Table Top Nanoindentation Tester (TTX-NHT^3). The treatments of tooth enamel (Figure 4) are expected to influence not only the surface properties but also the material below the treated surface. This is because of the morphological and chemical changes that are induced in the enamel whose thickness is between 1 mm and 2 mm. These treatments usually modify the enamel composition which results in modification of mechanical properties of the tooth below the surface (from the depth of a few micrometers down to a few hundred micrometers – depending on the treatment applied).

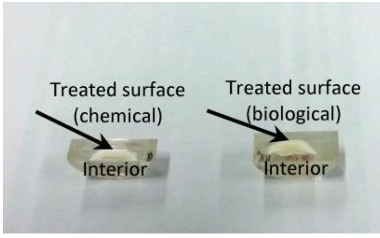

Figure 4 - Two tooth enamel samples with treated surfaces characterized by nanoindentation.

Analyzing the evolution of mechanical properties below the treated surface is of great interest in the quantification of the effects of teeth treatments, e.g. in the domain of caries (cavities) prevention or for the minimally invasive treatment of early caries lesions.

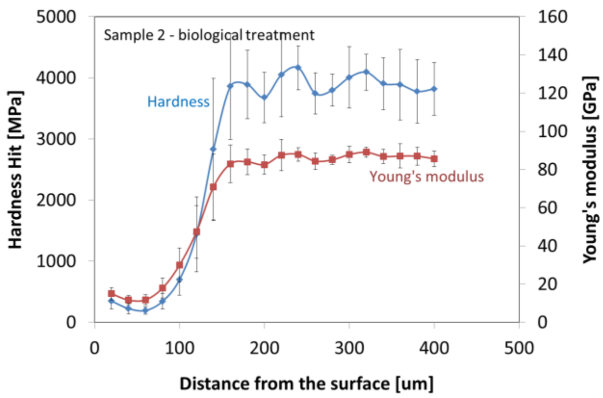

Figure 5 – Evolution of hardness and elastic modulus values with distance from the surface on biologically treated tooth enamel.

The example in Figure 5 illustrates significant changes in elastic modulus and hardness after production of an artificial caries lesion by biological treatment in bovine tooth enamel. The indentation measurements were performed in several parallel lines on the cross-sectioned bovine tooth, from the surface to a depth of approximately 400 µm. The changes in hardness and elastic modulus reveal a strong decrease in both hardness and elastic modulus towards the surface of the tooth after the enamel was biologically treated. The decrease in hardness and elastic modulus was less marked on enamel that was treated chemically – but the interior mechanical properties of the enamel were similar for both samples (Figure 6). The nanoindentation technique and namely the robust Anton Paar TTX-NHT[3] nanoindenter therefore allows for reliable and fast identification of the effects of various treatments on mechanical properties of tooth enamel.

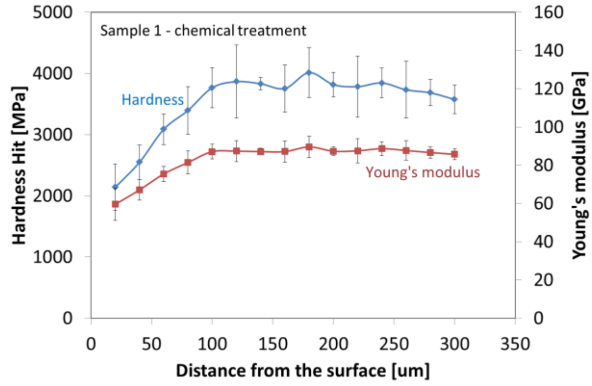

Figure 6 – Evolution of hardness and elastic modulus values with distance from the surface on chemically treated tooth enamel.

4 Effect of scaffold on regeneration of cartilage of animal femur with lesion

Although nanoindentation of hard biomaterials or biological materials represents a great part of local mechanical testing, there is a growing number of applications where much softer (bio)materials have to be measured [6]. These soft materials can have elastic moduli well below 100 MPa and often must be maintained in fluid. In addition, their surfaces can be uneven and cannot be prepared by standard methods such as cutting or polishing. A typical example of such soft material is articular cartilage. Extensive research has recently been carried out to study the effects of various types of scaffolds on cartilage regeneration. These scaffolds are used to accelerate the reconstitution of cartilage namely in case of injury by promoting the tissue ingrowth. Some studies of the mechanical properties of scaffolds and healthy cartilage have already been performed [7]. Only few results concerning the progressive integration of these scaffolds and the evolution of their mechanical properties are however available.

A recent study on goat femur cartilage with lesions was performed using the Anton Paar Bioindenter. The lesions in femur cartilage (whose diameter was ~2.5 mm) were filled with scaffolds and the animals carried on living for another five months. After this period, the animals were sacrificed and the whole femur joint was extracted. Nanoindentation experiments were done on the regenerating cartilage with scaffolds in the lesions but also on the healthy (intact) cartilage. All indentations were done with a spherical indenter with 0.5 mm radius and maximum force of 1 mN (healthy cartilage) or 0.1 mN (regenerating cartilage) to reach approximately the same depth in both regions (Figure 7 and Figure 8).

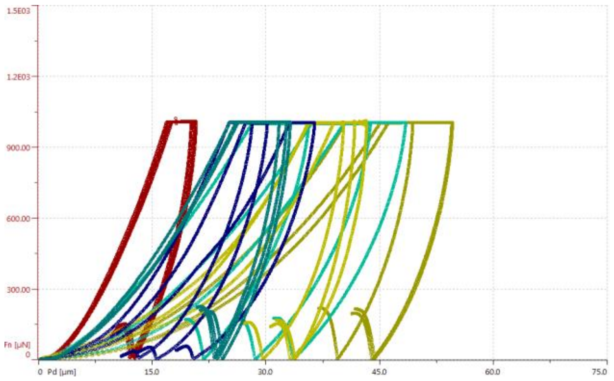

Figure 7 – Indentation load-displacement curves from healthy cartilage (1 mN load).

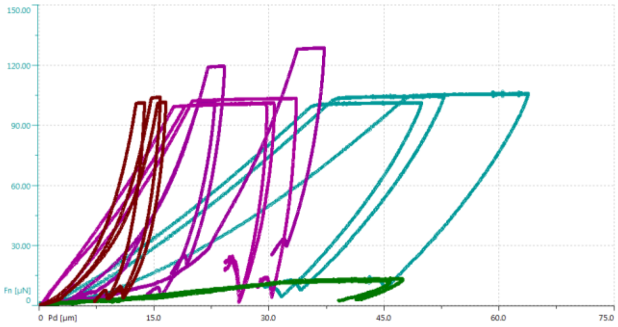

Figure 8 – Indentation load-displacement curves from regenerating cartilage with scaffolds (0.1 mN load).

The tests performed using the Bioindenter showed differences in the elastic modulus between the healthy and the regenerated cartilage: the elastic modulus (calculated using the Hertz model for contact of sphere with a flat) of the healthy cartilage was approximately seven times higher than that of the regenerating cartilage. In extreme cases the ratio between the elastic modulus of the healthy cartilage and the regenerating cartilage was 300 but in some cases this ratio could as low as 2 (Figure 9).

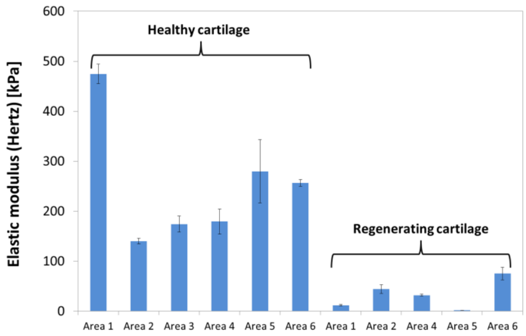

Figure 9 - Elastic modulus of healthy and regenerating cartilage of a goat femur.

These results show the slow regeneration of the cartilage even with the presence of scaffolds: five months after the lesion the regenerating cartilage was still at only ~10 % of its initial stiffness. The use of the indentation technique is very beneficial for fast and efficient estimation of the degree of cartilage regeneration; it is expected that fully regenerated cartilage should have mechanical properties very close to the mechanical properties of the healthy cartilage (~0.5 MPa for goat's femur cartilage).

5 Mechanical characterization of contact lenses

Soft contact lenses are used by many people in everyday life because of their simple application and low cost. Their stiffness (represented by elastic modulus) and eventually creep of different contact lenses can vary significantly depending on the type of material used. The choice of material is influenced either by optical properties, wearing comfort or duration of use of the lens. The stiffness of contact lenses can be measured locally using the Bioindenter, which is compatible with testing in liquids. Usually, a spherical indenter is used to avoid excessive stresses in the contact lens. The lens has to be permanently immersed in liquid so a special sample holder for contact lenses was developed. Figure 10 shows the Bioindenter measurement setup with the contact lens in the special sample holder. A spherical indenter with 0.5 mm radius and maximum force of 0.02 mN with 30 s hold was used. The maximum indentation depth was between 4 µm and 10 µm depending on the type of contact lens.

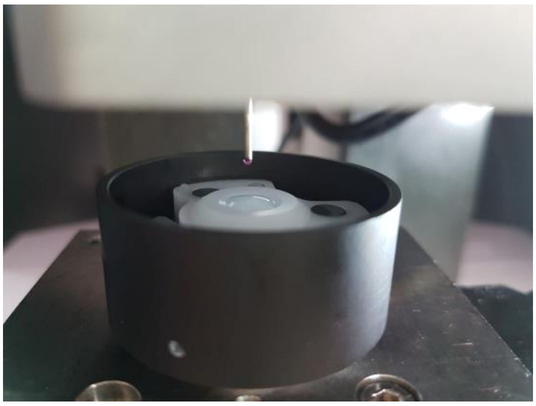

Figure 10 - Bioindenter setup for indenting contact lens in a specially developed sample holder. To better show the setup, no liquid was used.

Several indentations were performed in the center of two types of contact lenses (L1 and L2). Figure 11 shows typical indentation (load-displacement) curves obtained on these two types of lenses. Clearly, the L2 lens was stiffer than the L1 lens (E_{IT} ~106 kPa compared to ~40 kPa for the L1 lens). The data was analyzed using the ISO 14577 standard but also using the Hertz fit on the loading portion of the load-displacement curve. The Hertz fit gave E_{IT} values approximately two times lower than the ISO 14577 fit. This is probably due to the fact that the ISO 14577 fit is more influenced by the creep due to poroelastic behavior, which is reflected more in the unloading part than in the loading part. Indentation tests can also be used to determine creep properties of contact lenses – either via the C_{IT} creep value (as defined by the ISO 14577 standard) or by using creep fit method [8]. Both these of analysis as well as the Hertz fit are available in the Anton Paar Indentation software.

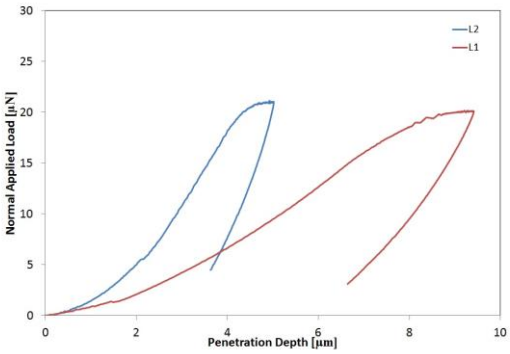

Figure 11 - Typical load vs. indentation curves for the L1 and L2 contact lenses.

6 Conclusions

Instrumented indentation is one of the new techniques for local characterization of mechanical properties of biomedical and biological materials. The advances in the instrumentation and testing methodology now allow for the testing of hardness and elastic modulus of both hard and soft biomaterials and biological materials. Nanoindentation testers are suitable instruments for local mechanical analysis of many types of materials; dry or immersed in liquid, hard or soft.

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7 References

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