บทความวิชาการ

อิทธิพลของความหยุ่นหนืดที่มีต่อเทคนิคนาโนอินเดนเทชั่น และการประยุกต์ใช้ในการวิเคราะห์เนื้อเยื่อกระดูก

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บทคัดย่อ

นาโนอินเดนเทชั่นเป็นเทคนิคใหม่ที่มีประสิทธิภาพสูงในการวัดสมบัติเชิงกลระดับนาโนของวัสดุตัวอย่างซึ่งมีขนาดเล็ก ในบทความนี้ได้อธิบายหลักการพื้นฐานของเทคนิคนาโนอินเดนเทชั่น โดยเน้นถึงอิทธิพลของสภาพหยุ่นหนืดที่มีผลต่อการทดสอบ และอธิบายถึงผลของอัตราการเปลี่ยนแปลงความเครียดสภาวะหรือกระบวนการการเตรียมสารตัวอย่างที่มีต่อการวัดค่าความแข็งและมอดุลัสยืดหยุ่นของวัสดุตัวอย่าง รวมทั้งได้กล่าวถึงการประยุกต์ใช้เทคนิคนาโนอินเดนเทชั่นในการวิเคราะห์เนื้อเยื่อกระดูก

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Effect of Viscoelasticity on Nanoindentation Measurements: An Application to Bone Tissues

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ABSTRACT

Nanoindentation is a powerful technique to determine the mechanical properties of materials at small volumes. This article reviews the current understanding of nanoindentation testing, in particular under viscoelastic effect. The effect of strain rate and the condition or process of the sample preparation on measurements of hardness and elastic modulus has been discussed. A special focus on its applications to bone tissue is also addressed.

Keywords: nanoindentation, strain rate, viscoelasticity, plasticity

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Introduction

Indentation technique has been commonly used to probe mechanical properties of materials. It is a simple method that involves pressing a harder material whose properties are known into contact with a material of interest whose mechanical properties such as hardness and elastic modulus are unknown. In 1822 the initial quantified test, so-called Mohs' hardness scale, started with materials that can make a permanent scratch on another were ranked harder material with diamond assigned the maximum value of 10 on the scale. Then, the Brinell test was established using spherical steel ball as an indenter to measure the plastic properties of materials. Soon after its introduction, the Brinell test was quickly adopted as an industrial test method, followed by a range of standard hardness tests, which have subsequently been developed using indenters with different geometries (e.g. Vickers, Berkovich, Knoop, and Rockwell tests). The particular attraction of this technique is its unique simplicity as a nondestructive test method. Nanoindentation is an indentation in which the length scale of the penetration depth is measured in nanometers (10^{-9} m) rather than microns (10^{-6} m) or millimeters (10^{-3} m), which are commonly used in conventional hardness and microhardness testing. Conventional indentation testing uses optical imaging to evaluate the contact area of the residual impression in order to calculate the hardness. In nanoindentation tests the size of the residual impression is on the order of submicrons or even less, sometimes on the scale of the very first surface molecular layers, which clearly are too small to be measured directly with sufficient accuracy. Nevertheless, this limitation has been overcome with the "compliance method", which is now commonly applied to a wide range of materials ranging from metals, ceramics, polymers and composites [1-3]. The unique feature of this method is that it does not depend on the direct measurement of the residual impression. The hardness (H) and elastic modulus (E) can be obtained from the analysis of the load-displacement data recorded simultaneously during the entire loading and unloading process performed upon the specimen surface. Associated with the development of the instrumented nanoindentation system, which allow the precise control of either load or displacement during indentation testing, this indentation can be accurately made using forces as small as a few micronewtons over depths in the nanometer range. Thus came the term "nanoindentation". Together with an improved understanding of indentation mechanisms and advances in analysis methodology such as that of Doerner and Nix [4] and Oliver and Pharr [5], instrumented nanoindentation has become a powerful tool for investigating mechanical properties at micron-scale material volumes. Its application areas include microelectromechanical systems (MEMS), optoelectronics, thin films, and coatings [2, 6, 7]. Table 1 summarizes the comparative information between nanoindentation, microhardness, and macroscopic mechanical test, which in case of bone tissue is, for example, the three-point bending test.

Table 1 Comparison of macroscopic mechanical test (three-point bending test), microhardness, and nanoindentation test

Feature	Macroscopic mechanical test (Three-point bending)	Microhardness	Nanoindentation
Test method	Destructive	Nondestructive	Nondestructive
Load range	10^6 - 10^9 mN	10^2 - 10^5 mN	10^{-2} - 10^2 mN
Scale of penetration	–	Microns (10^{-6} m) or Millimeters (10^{-3} m)	Nanometers (10^{-9} m)
Specimen size	Centimeter scale (10^{-2} m)^3	Centimeter scale (10^{-2} m)^3	Micrometer scale (10^{-6} m)^3
Specimen characteristics	Only homogeneous	Only homogeneous	Homogeneous and Heterogeneous
Material response	Elastic / plastic deformation	Only plastic deformation	Elastic - plastic deformation or Viscoelastic deformation
Mechanical property represented	Bulk average property	Bulk average property	Highly localized or gradient property
Mechanical properties provided	Flexural strength and elastic modulus	Hardness and fracture toughness	Highly localized hardness and elastic modulus and fracture toughness
Mechanical property value	Single value per experiment	Single value per experiment	Hardness as function of depth (real-time load-displacement data)

Recently, there has also been a growing interest in probing biological materials including bone tissues [8-11]. As biological materials, the inherent hierarchical nature of bones exhibit substantial variations in composition and morphology at length scales ranging from nanometers to millimeters, resulting in complicated mechanical properties. Viscoelasticity is one of these properties. Bone is well-known to be viscoelastic in both macroscopic and microstructural levels [8, 12, 13]. Nanoindentation technique has been used to determine the mechanical properties of bone at microstructural level [14-17]. The review by Lewis [18] has extensively summarized literature reports on the use of nanoindentation to determine elastic modulus and hardness of bone and teeth tissues. When nanoindentation testing is performed upon bone tissues, additional difficulties are caused by the complex viscoelastic response that is typical of such materials [12-13]. Bones have highly strain and strain rate-dependent properties and behave differently when the indentations are made under different imposed contact conditions; for instance, the geometry of the contact, the penetration depth (i.e. the strain), and the loading rate (or strain rate) [16].

Previously, most studies of mechanical properties of bone tissues were conducted at the macrostructural level with conventional mechanical testing such as bending, tensile, and compression tests. In compression testing, bone was found to be stiffer and stronger with an increasing strain rate [19]. Carter and Hayes [20-21] showed that elastic modulus were approximately proportional to strain rate showing a strain rate sensitivity index of 0.06. Also, the results were suggested that this relationship was true for all bones in the skeleton. However, macroscopic techniques can provide useful information on the bulk average mechanical properties of bone tissues, but the local or gradient information due to their hierarchal structures cannot be obtained. Moreover, due to the limitation of sample preparation, availability of appropriate testing methods [14], and the complexity of bone itself, to date very few studies of viscoelastic properties of bones at the microstructural level has been reported.

Measurement of hardness and elastic modulus

The two mechanical properties measured most frequently using nanoindentation techniques are the hardness and the elastic modulus. As mentioned previously, the conventional hardness tests use imaging technique to determine the residual contact area, and hence the hardness of the specimen. Therefore, this method gives only the plastic response of materials. It does not provide elastic and viscoelastic-plastic properties of materials. In addition, for nanohardness measurement the error from measuring the shallow contact area with traditional optical microscopy can be quite large. Therefore, the compliance method, which provides the load-displacement curve over a complete loading cycle, has become a better alternative. Numbers of studies have been conducted using this method on various materials; for instance, ceramic systems [3] and organic polymers [1, 22]. In this method, a small indentation is made as the indentation load (P) and displacement (h) are continuously recorded during one complete cycle of loading and unloading. Figure 1 shows a typical set of load-displacement data, which defines some of the experimental parameters needed to determine hardness and modulus. According to Figure 1, the key parameters are the peak load (P_{max}), the displacement at peak load (h_{max}), and the residual displacement after an indenter removal (h_f). The intercept of the tangent line drawn from the initial part of the unloading curve, which describes the elastic deformation, and the displacement axis. The slope of this line represents the contact stiffness (S), $S = dP / dh.$ And h_c is considered as being the actual value of the material displacement, which occurs mainly, but not exclusively from plastic deformation behavior. Today's perhaps the most widely used testing methodology for nanoindentation is that of Oliver and Pharr [23]. The method starts from fitting the unloading curve to the power-law relation:

$$P = B(h - h_f)^m \quad (1)$$

Where P is the indentation load, h is the displacement, B and m are empirically determined fitting parameters, and h_f is the final displacement. The unloading stiffness (S) is then obtained by:

$$S = \frac{dP}{dh}(h = h_{max}) = mB(h_{max} - h_f)^{m-1} \quad (2)$$

Also, the contact depth (h_c) can be estimated from the load-displacement curve data:

$$h_c = h_{max} - \varepsilon\left(\frac{P_{max}}{S}\right) \quad (3)$$

Where ε is an indenter geometry dependent constant. Empirical studies have shown that $\varepsilon \cong 0.75$ for a Berkovich indenter [24].

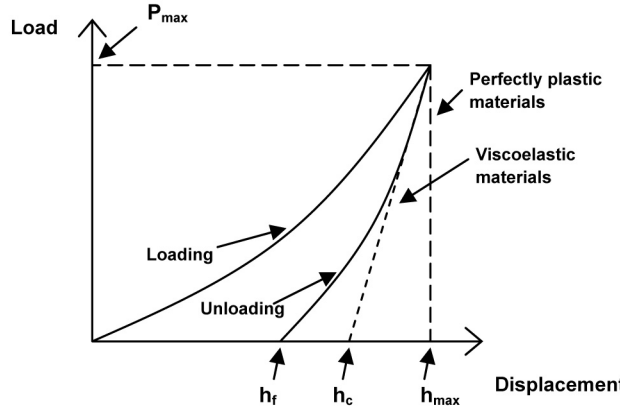

Figure 1 Typical indentation load-displacement curve defining key experimental quantities.

Thus, the projected contact area (A), which is obtained by evaluating an empirically determined indenter area function at the contact depth (h_c); i.e., $A = f(h_c)$

Therefore, the hardness (H) is as follows:

$$H = \frac{P_{max}}{A} \quad (4)$$

And the reduced contact elastic modulus (E_r) is:

$$E_r = \frac{1}{\beta}\frac{\sqrt{\pi}}{2}\frac{S}{\sqrt{A}} \tag{5}$$

E_r has the following relationship with the specimen modulus:

$$\frac{1}{E_r} = \frac{(1-\upsilon^2)}{E} + \frac{(1-\upsilon_i^2)}{E_i} \tag{6}$$

Where E and υ are the Young's modulus and Poisson's ratio for the specimen, and E_i and υ_i are the same quantities for the indenter.

Nearly all nanoindentation investigations on bones reported to date employed the Oliver and Pharr analysis to obtain mechanical properties. This methodology, which is based on the assumption that the material responses purely elastically during unloading. In other words, this analysis is basically for the purpose of measuring hardness and modulus of solids without taking any viscous component in their mechanical properties into account. This has become the limitation for probing mechanical properties of viscoelastic materials, which could exhibit creep or time-dependent behavior under load. Since bone is viscoelastic in nature, this viscoelastic effect during unloading could induce the errors in the estimation of the contact stiffness and the contact area, resulting in the error of the hardness and modulus value. The procedure of adding the holding time period before unloading, as illustrated in Figure 2, and increasing the unloading rate has been suggested to effectively remove viscoelastic effects during unloading [14, 23]. And, hence the accuracy of the measured elastic modulus and hardness can be improved.

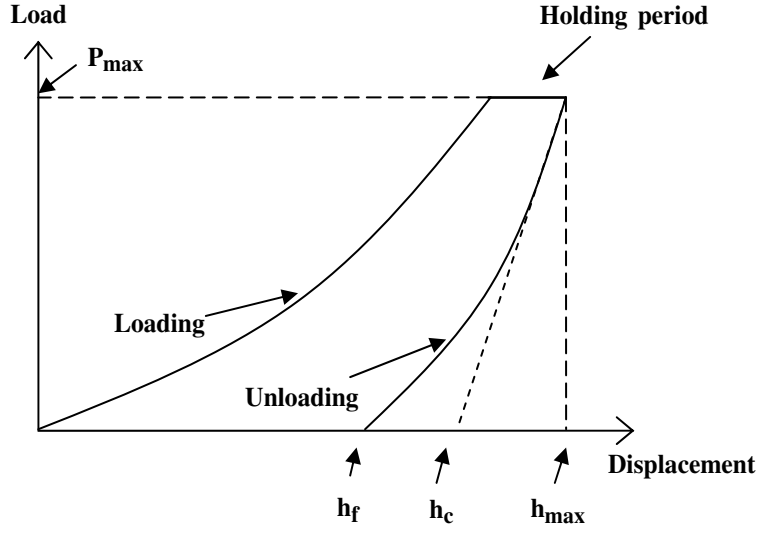

Figure 2 Indentation load-displacement curve with addition of holding period before unloading

Effect of an indentation strain rate

Viscoelasticity describes the time-dependent mechanical characteristics of materials. Bone is viscoelastic material, which means that the stress developed within bone depends on a strain rate; i.e., the rate at which the bone specimen is strained. As a strain rate increases, bone specimen appears stiffer and stronger, with smaller deformation.

The indentation strain rate ($\dot{\varepsilon}$) is defined as the imposed rate of deformation during indentation. Generally, the strain rate is correlated with the displacement rate or the loading rate of the indenter tip over the softer surface. Typically, the strain rate acts nominally in a direction perpendicular to the surface and can be defined as

$$\dot{\varepsilon} = k \left(\frac{\dot{h}}{h} \right) \quad (7)$$

Where h is the instantaneous displacement, $\dot{h}$ is the nominal displacement rate, and k is a material constant, usually equal to 1.

According to the expanding cavity model [24], in an indentation test, the deformed volume of material under the indenter is continuously expanding into the surrounding undeformed material. The plastic strains gradually decrease until the total strain matches the purely elastic strains at some radius (C), where is the elastic-plastic boundary, as presented in Figure 3.

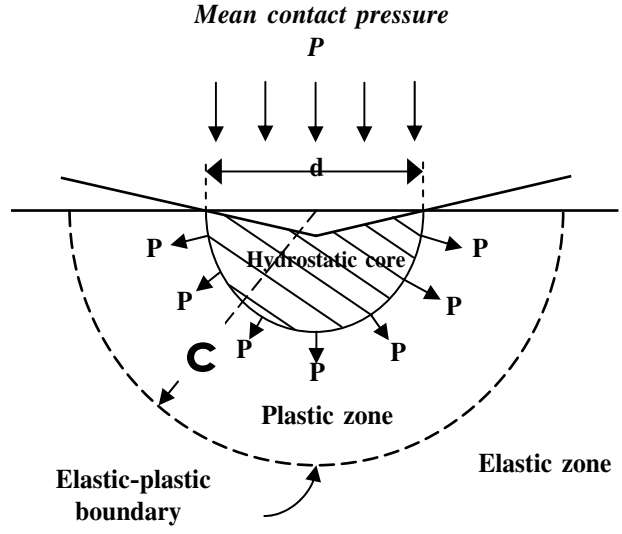

Figure 3 Indentation in an ideal elastic-plastic solid treating the process as resembling the expansion of a hemispherical core.

Since the radius of the elastic-plastic boundary (C) is related to the radius of the indentation, the ratio between the instantaneous change in contact area and the instantaneous contact area $(\frac{dA}{dt} / A)$ is the most appropriate definition for the strain rate as it is a direct measurement of the progression of the elastic-plastic boundary into the material [16]. For Berkovich indenter, which is a geometrically similar indenter, the ratio between nominal displacement rate of the indenter and the instantaneous displacement $(\frac{dh}{dt} / h)$ is simply related to $\frac{dA}{dt} / A$. The strain rate is obtained by extracting the data of the displacement and the corresponding time from each indent and then curve fitted to the displacement-time function at the unloading portion. At any given time point, the strain rate is calculated by taking the derivative of the function and dividing the derivative by the displacement at that point of time. Therefore, it is worth noted that each indentation strain rate is calculated at the same data point where the contact stiffness (S) is determined.

Fan and Rho [16] have investigated the viscoelastic and time-dependent plastic effects on the nanoindentation measurement of osteonal lamella in a human cortical bone. Their result showed that the elastic modulus was proportional to strain rate raised to the power of 0.059, which is surprisingly consistent to those of conventional test results, which were found to range from 0.057 to 0.061 [19, 21, 25]. Therefore, this result was suggested that the strain rate as determined by conventional tests may be proportional to the indentation strain rate [26].

Apart from the fact that a power relationship was found between the elastic modulus and the indentation strain rate, and thus the evaluation of the viscoelastic properties of bone can be obtained. The changes in strain rate are often considered to be a signal that triggers many biological activities of bone remodeling [27]. Therefore, a better understanding of the viscoelastic properties of bone in microstructural level is important in support of understanding many clinical and research problems.

Applications to Bone tissue

Conventionally, the mechanical properties of bone tissues have been derived from mechanical tests at the level of the whole bone or bone sample by bending, compression, tension, or torsion tests. The direct results of those tests reflect both the material properties of the bone matrix and the morphology of the tissue. Therefore, the geometric and technical assumptions needed for extracting the role of bone geometry may no longer be suitable. To minimize the effect of these assumptions, mechanical tests on small volumes of bone tissues with well-defined geometry are required so as to capture the heterogeneity in tissue properties. Nanoindentation is an emerging technology that can overcome this technical limitation by probing mechanical responses at high resolutions of approximately 0.2 nm [7].

Therefore, it can be used to determine the properties of small microstructural features such as individual osteons and trabeculae [15, 28]. In addition, since the mechanical properties are size-dependent, the necessity of mechanical assessment at the level of the material becomes further apparent. For instance, the bulk elastic modulus of trabecular bone not only vary by order of magnitude (0.02-5 GPa), but also that bulk trabecular moduli are much smaller than those of cortical bone (15 GPa) [10]. Therefore, bulk mechanical properties are found to be dependent on the amount and architecture of the bone present as well as on the material properties of the trabeculae themselves.

Due to its high resolution, nanoindentation is used to compare the stiffness of adjacent lamellae, or the properties of newly added bone relative to pre-existing bone [29]. In addition, the determination of the modulus variation on individuals and anatomical sites has been successfully conducted. Moreover, there is also an investigation on variations in degree of mineralization in articular calcified cartilage and subchondrol bone [30]. Therefore, nanoindentation is a mature tool, which is capable of determining bone's mechanical properties on extremely small volumes of tissue. Associated with imaging technique and existing mechanical models, nanoindentation technique can provide an insight into establishment of the link between the mechanical properties from the level of the tissue to that of the organ.

Effect of sample preparation

In typical nanoindentation testing, specimen surface finished (mirror finished) is required so as to minimize the effect of surface irregularities or surface roughness. In cortical bone, the surface finished can be relatively easily achieved by serial polishing. While, when dealing with more porous bone tissue such as trabecular bone, it could become more technically difficult. Normally, an embedding protocol is required. Therefore, it is important to evaluate the effect of embedding and dehydration with subsequent rehydration.

It is well established that the mechanical properties of bone exhibit notable changes after dehydration [14, 31, 32]. Hoffler et al [32] have found that for fresh frozen cadaveric cortical bone specimen, elastic modulus and hardness of dry specimens were significantly greater than those of wet and subsequently rehydrated specimens. In addition, dehydrating process increased the elastic modulus in osteonal tissue and interstitial lamellae by 15.4% and 9.7%, respectively. In this case, it was indicated that dehydration contracted the individual fibrils in the tissue [14]. Therefore, it was apparent that the process and condition of the sample preparation has significant impact on nanoindentation measurement.

Perspective

There are several advantages for applying nanoindentation technique to bone tissue. This technique is capable of determining the mechanical properties at extremely small scales. It can be used to characterize these complex structures of bone tissue. Also, it is useful when the volume of bone specimen is too small for larger scale analyses. The most striking feature of bone tissue is its hierarchical structure. The different hierarchical, structural elements contribute distinct characteristic to mechanical properties at the whole bone level. Therefore, it is essential to understand the mechanical properties of its component phases, and the structural relationship between them and the various levels of hierarchical structural organization in order to understand the mechanical properties of bone tissue [33]. This useful information could lead to recognition of a functional deficit and a search for the responsible characteristics. Therefore, early diagnoses, a guide for clinical therapy and prevention can be provided.

Conclusion

This article provides basic information on nanoindentation testing under the influence of viscoelasticity of bone material, which is viscoelastic in nature. Since the Oliver and Pharr methodology is based on the assumption that the material behaves purely elastically during unloading, the account for viscoelasticity effect is needed to be carefully taken for the measurements of hardness and elastic modulus during indentation. In addition, the effect of strain rate and the process or condition of the sample preparation has been discussed in terms of the applications to bone tissue.

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