Effects of Flotage on Immersion Indentation Results of Bone Tissue: An Investigation by Finite Element Analysis

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In reality, nanoindentation test is an efficient technique for probing the mechanical properties of biological tissue that soaked in the liquid media to keep the bioactivity. However, the effects of flotage imposed on the indenter will lead to inaccuracy when calculating mechanical properties (for instance, elastic modulus and hardness) by using depth-sensing nanoindentation. In this paper, the effects of flotage on the nanoindentation results of cortical bone were investigated by finite element analysis (FEA) simulation. Comparisons of nanoindentation simulation results of bone samples with and without being soaked in the liquid media were carried out. Conclusions show that the difference of load-displacement curves in the case of soaking sample and without soaking sample conditions varies widely based on the change of indentation depth. In other words, the nanoindentation measurements in liquid media will cause significant error in the calculated Young's modules and hardness due to the flotage. By taking into account the effect of flotage, these errors are particularly important to the accurate biomechanics characterization of biological samples.

1. Introduction

It is now appreciated that nanoindentation technique has been successfully applied to characterize elastic and plastic behaviors of biological tissue such as Young's modulus, hardness, and viscoelasticity, when combined with the in situ observation of electron microscope [1–3]. Recently, Young et al. [4] probed the viscoelasticity of a spider's biological vibration filter by utilizing an atomic force microscopy (AFM) technique, and they investigated the elastic modulus over a temperature range of 15–40°C at various loading frequencies. Yoo et al. [5] applied an indentation technique to characterize viscoelastic properties of small ocular and orbital tissue specimens, and they developed quantitative models to predict the wide range response of the tissue's biomechanics based on the experimental measures. To maintain the freshness and prevent dehydration, all of experimental biological tissues should be soaked in the liquid media during the indentation. However, the method to keep the bioactivity of biological sample is seldom mentioned in the above-mentioned experiments. And this will lead to the inaccurate characterization of mechanical properties. Nevertheless, Selby et al. [6] conducted the indentation experiment of the contact lens soaked in phosphate buffered saline during the test. They investigated the influence of hydrogel film thickness on the measured elastic modulus. Li et al. [7] utilized the indentation method to characterize the elastic and viscoelastic mechanical properties of brain tissues, and they immersed the tissue sample in the artificial cerebrospinal fluid to maintain its bioactivity during the whole experimental process. However, when conducting the nanoindentation test in the liquid, the indenter will inevitably bear the flotage, and this will result in the inaccurate collection of reaction force about the indenter. Specifically, the effects of flotage in the experimental microenvironment were not investigated in detail in their work. Regarding the fact that precise evaluation of mechanical properties of biological tissue in immersion indentation is based on fitting unloading stage of load-displacement curves and that these curves are determined by accurate collection of contact force between...
2. Theory of Traditional Indentation and Immersion Indentation

2.1. Traditional Indentation. It is well known that the method of nanoindentation analysis to determine Young’s modulus \(E\) and hardness \(H\) proposed by Oliver and Pharr is applicable for bulk specimens [8]. The formula of Oliver and Pharr is established based on the following assumptions [9]:

(i) The material of specimen is homogenous during the deformation process.

(ii) The material of specimen undergoes elastic-plastic deformation on the loading process and only elastic recovery on the unloading process.

(iii) The creep and viscoelastic deformation of the material should be negligible.

Obviously, the method of calculating elastic modulus and hardness is different from traditional method, which can be written as follows [8, 9]:

\[
S = \frac{dp}{dh} \quad (1)
\]

\[
h_c = h_{\text{max}} - \frac{\varepsilon \times P_{\text{max}}}{S} \quad (2)
\]

\[
A_c = f (h_c) \quad (3)
\]

\[
H = \frac{P_{\text{max}}}{A_c} \quad (4)
\]

\[
E_r = \frac{\sqrt{\pi}}{2\beta \sqrt{A_c}} \times \frac{S}{S} \quad (5)
\]

\[
\rho = \frac{1 - v_i^2}{E_i} + \frac{1 - v_f^2}{E_f} \quad (6)
\]

In (1) to (6), \(S\) is the contact stiffness by fitting the unloading force-displacement curves [8, 9]. \(P_{\text{max}}\) is the maximum indentation load; \(A_c\) is the projected contact area under the peak indentation depth. \(h_c\) is contact depth that is used to calculate the real contact area and is determined by Sneddon’s equation [8]. \(H\) is the hardness of specimen, \(\beta\) is a correction factor that depends on the geometry of indenter, and \(\varepsilon\) is a geometry constant that equals 0.75 for a Berkovich indenter [10].

Obviously, the relation between \(E\) and \(H\) can be determined by the projected contact area \(A_c\), contact stiffness \(S\), Young’s modulus and Poisson’s ratio of indenter \((E_i\) and \(v_i)\), contact depth \(h_c\), reduced modulus \(E_r\), and indentation load-displacement \((P-h)\) curve. Regarding the traditional indentation testing on a bone specimen, the unloading stage of load-displacement \((P-h)\) curve directly responds to the elastic-plastic deformation behavior and determines the mechanical parameters of materials [11]. According to (1) and (4), \(H\) is directly determined by the ratio of \(P_{\text{max}}\) to \(A_c\), and \(S\) is defined by the slope of the initial unloading stage of \(P-h\) curve (relevant with \(h_{\text{max}}\) and residual depth \(h_f\)) [8]. Furthermore, on the basis of (2) and (3), \(h_c\) is determined by maximum indentation depth \(h_{\text{max}}\) and \(S\) [12], and \(A_c\) is proportional to the square of \(h_c\) [9]. According to (5) and (6), the elastic modulus and hardness of the specimen will be easily obtained on the condition that the contact stiffness \(S\) and the projected contact area \(A_c\) are known.

2.2. Immersion Indentation. A general sketch map of immersion indentation experiment is shown in Figure 1(a). Cortical bone specimen is rigidly fixed on the glass slide that soaked in the liquid media. Assuming that the bone specimen itself will not be influenced by the flotage, only the indenter will bear flotage during the whole experiment process. When conducting the immersion indentation, the indenter moves downward bit by bit, and then the indenter will bear flotage exactly at the time when it touches the liquid level. And this flotage will increase along with the increases of immersion depth \(h\) during the whole immersion state. Therefore, the maximum load \(P_{\text{max}}\) in immersion indentation might be higher than that in traditional indentation due to the inaccurate collection of reaction force on the indenter. Accordingly, the loading and unloading stages of \(P-h\) curve in immersion indentation will both exhibit different extent of deviation from the traditional indentation \(P-h\) curve. Thus, it will be of great significance to be certain of the effect of flotage on these two kinds \(P-h\) curves by a numerical method. Figure 1(b) shows a two-dimensional axisymmetric immersion indentation model, whose Berkovich indenter tip is exactly in touch with the specimen. And \(h\) is the measured immersion depth, \(h_{\text{flo}}\) is the submergence depth of specimen, and \(h_{\text{ind}}\) is the tip height of indenter. The clamping end of the indenter is equivalent to a cylinder and its radius is \(R_{\text{c}}\). The relationship between the submergence depth of specimen \(h_{\text{flo}}\) and the tip height of indenter \(h_{\text{ind}}\) will be clearly clarified here.

Obviously, the measured immersion depth \(h\) can be considered as the sum of the maximum indentation depth \(h_{\text{max}}\) and the submergence depth \(h_{\text{flo}}\), namely, \(h = h_{\text{max}} + h_{\text{flo}}\). Moreover, the flotage applied to the indenter will increase along with the increase of immersion depth \(h\); thus, the measured immersion depth \(h\) can be classified into two different conditions; namely, \(h_{\text{flo}} \leq h_{\text{ind}}\) and \(h_{\text{flo}} > h_{\text{ind}}\).
Indentation testers
Immersion liquid
Glass slide
Container
Specimen
Testbed

(a)

Indenter
Fluid level
Specimen
X
Y
L
L
PG;R
Ra
h_{ind}

(b)

Figure 1: (a) Sketch map of immersion indentation experiment and (b) two-dimensional axisymmetric experiment model of nanoindentation.

Table 1: Geometric parameters of different indenters.

<table>
<thead>
<tr>
<th></th>
<th>Berkovich indenter</th>
<th>Cubic indenter</th>
<th>Spherical indenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_{c}</td>
<td>24.56h_{c}^{-2}</td>
<td>2.5981h_{c}^{-2}</td>
<td>\pi (2Rh_{c} - h_{c}^{-2})</td>
</tr>
<tr>
<td>\varphi</td>
<td>70.32'</td>
<td>42.28'</td>
<td></td>
</tr>
<tr>
<td>\alpha</td>
<td>65.3'</td>
<td>35.2644'</td>
<td></td>
</tr>
</tbody>
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Assuming that the fluid keeps relatively static in the whole indentation process and the liquid media’s surface tension adhered to the indenter can be neglected, thus the indenter will only bear the flotage in liquid media. It is clear that the magnitude of this flotage depends on the geometry parameters of indenters, and Table 1 shows some geometry parameters about three indenters used in this paper [13, 14]. Here, \varphi is the equivalent semicone angle, \alpha is the included angel between indenter’s geometry center line and faced plane, and it will be utilized to calculate the indenters' geometric volume that determines the flotage.

Regarding the Berkovich indenter, when the submergence depth \( h_{\text{flo}} \) is smaller than the tip height of indenter \( h_{\text{ind}} \), namely, \( h_{\text{flo}} \leq h_{\text{ind}} \), the formula of flotage can be written as follows:

\[
P_{\text{flo}} = \sqrt{3} \rho g h_{\text{flo}}^{3} \tan \alpha \quad (h \leq h_{\text{ind}})
\]

\[
P_{\text{flo}} = \sqrt{3} \rho g h_{\text{flo}}^{3} \tan \alpha + \rho g \pi R_{a}^{2} (h - h_{\text{ind}}) \quad (h > h_{\text{ind}}).
\]

Similarly, the flotage applied to other spherical indenter and cubic indenter can be deduced by using trigonometric functions and Archimedes flotage principle. Regarding the spherical indenter, when the submergence depth \( h_{\text{flo}} \) is smaller than the tip height of indenter \( h_{\text{ind}} \), namely, \( h_{\text{flo}} \leq h_{\text{ind}} \), the formula of flotage can be written as

\[
P_{\text{flo}} = \rho g \pi R_{a}^{2} \left( R_{a} - \frac{h}{3} \right) \quad (h \leq h_{\text{ind}}).
\]

\[
P_{\text{flo}} = \frac{2}{3} \rho g \pi R_{a}^{3} + \rho g \pi R_{a}^{2} (h - h_{\text{ind}}) \quad (h > h_{\text{ind}}).
\]

On the other hand, when the condition is \( h_{\text{flo}} > h_{\text{ind}} \), the formula of flotage can be written as

\[
P_{\text{flo}} = \sqrt{3} \rho g h_{\text{ind}}^{3} \tan \alpha + \rho g \pi R_{a}^{2} (h - h_{\text{ind}}) \quad (h > h_{\text{ind}}).
\]

Regarding the cubic indenter, when the submergence depth \( h_{\text{flo}} \) is smaller than the tip height of indenter \( h_{\text{ind}} \), namely, \( h_{\text{flo}} \leq h_{\text{ind}} \), the formula of flotage can be written as

\[
P_{\text{flo}} = \sqrt{3} \rho g h_{\text{flo}}^{3} \tan \alpha + \rho g \pi R_{a}^{2} (h - h_{\text{ind}}) \quad (h \leq h_{\text{ind}})
\]

\[
P_{\text{flo}} = \sqrt{3} \rho g h_{\text{flo}}^{3} \tan \alpha + \rho g \pi R_{a}^{2} (h - h_{\text{ind}}) \quad (h > h_{\text{ind}}).
\]

3. FEA Simulation Results and Discussion

3.1. Simulation Model. In order to investigate the effects of flotage on the results of immersion indentation, finite element analysis (FEA) was carried out by using the commercial
The specimen was meshed with four node bilinear axisymmetric reduced integration elements (CAX4R element type), and the whole mesh size was 0.3 μm. No friction was considered between the indenter and specimen. Due to the large deformations involved in the simulation, adaptive meshing was used. The indenter had only one degree of freedom and was applied with displacement boundary to accomplish the loading and unloading steps. Compared with the sample, the hardness of diamond indenter was 13.56 GPa and percent errors of hardness Error_H are about 120.6%, which are obtained from Berkovich indenter. Meanwhile, the percent errors of hardness Error_H are about 120.6%, 82.7%, and 56.7%, which are obtained from Berkovich indenter, cubic indenter, and spherical indenter, respectively.

Similarly, when the maximum indentation depth increases to 1000 nm, the Error_E values decrease to 17.5%, 4.4%, and 10.9%, while the Error_H values decrease to 5.8%, 5.1%, and 7.2% when using Berkovich, cubic, and spherical indenters, respectively. Finally, the percent errors of elastic modulus for cubic indenter have always been the smallest one when compared with other indenters at the same indentation depth h_max.
Figure 3: Load-displacement curves of immersion indentation and traditional indentation with different indenters, (a) cubic indenter, (b) Berkovich indenter, and (c) spherical indenter.

4. Conclusions

Regarding the immersion indentation whose specimen is soaked in the liquid media, the effects of flotage on the indentation results used to be neglected, and this will cause the properties such as elastic modulus and hardness of the sample to be somehow inaccurate. In this paper, the effects of flotage on the results of cortical bone nanoindentation were investigated by finite element analysis simulation. Conclusions are drawn as follows:

1. The comparison studies in $P-h$ curves of immersion indentation and traditional indentation indicate that the flotage could to some extent affect the envelope area of the $P-h$ curves. When under the same indentation depth, the envelope area of immersion...
indentation will be larger than that of traditional indentation.

(2) From the calculated percent errors of elastic modulus and hardness, it is known that when the maximum indentation depth ranges from 200 nm to 1000 nm, the percent errors of elastic modulus for cubic indenter is the smallest and the Berkovich indenter is the largest one all the time.

(3) With the decrease of maximum indentation depth, the percent errors values of elastic modulus and hardness become larger on the contrary.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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