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Substrate effect on Dynamic Indentation Measurement of Biological Cell Properties

Guoxin Cao and Namas Chandra
Department of Engineering Mechanics, University of Nebraska-Lincoln, Lincoln, NE 68588

ABSTRACT

Viscoelastic mechanical properties of biological cells are commonly measured using atomic force microscope (AFM) dynamic indentation method with spherical tips. Storage and loss modulii of cells are then computed from the indentation force-displacement response under dynamic loading conditions. It is shown in current numerical simulations that those modulii computed based on existing analysis can not reflect the true values due to the substrate effect. This effect can alter the indentation modulus by changing the geometric relations between the indentation displacement and the contact area. Typically, the cell modulii are significantly overestimated in the existing indentation analysis.

INTRODUCTION

It is believed that AFM is one of the fastest, cheapest and most convenient ways to measure the cell properties [1-9]. In this method, the biological cell attached onto the substrate is indented using the spherical indenter with either the force (or the displacement) specified. The indentation response of the cell is obtained by measuring the P-δ relationship of the indenter tip. Using this relationship, the mechanical properties of the cell can be computed. The substrate can significantly affect the cell indentation behavior especially in the thin region of the cell with deep indentation. Typically, the basic mechanism of the substrate effect is considered as the stress stiffen effect since the substrate is orders of magnitude stiffer than the cell [5, 6]. In present study, it is found that the substrate can also change the relationship between the indentation contact area and the indentation displacement in cell indentation. Due to this change, the cell modulus will be significantly overestimated based on the existing indentation analysis. In order to obtain the true cell properties, the true relationship between the contact radius and the indentation displacement is required. In present work, the effect of substrate on the contact area is identified and a new relationship between indentation displacement and contact area is determined based on the numerical simulations.

THEORETICAL BACKGROUND AND COMPUTATIONAL METHODS

The linear elastic quasi-static indentation behavior can be described by Hertz contact theory. For a rigid spherical indenter, the indentation force is given by

$$P = \frac{4}{3}\frac{E}{1-\nu^2}\frac{a^3}{R}, \qquad (1)$$

where a is the contact radius, R the spherical indenter tip radius, ν the Poisson's ratio, E the elastic modulus. The schematic of spherical indentation using AFM is shown in Figure 1. If the strain is within the elastic limit when $\delta << R$, the contact radius is commonly approximated as the Hertz elastic contact radius: $a \approx (R\delta)^{0.5}$, where δ is the indentation displacement.

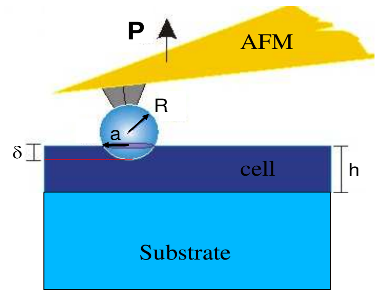

Figure 1 The schematic of dynamic indentation on cell with spherical indenter.

For linear viscoelastic materials, the dynamic modulus is commonly employed

$$E^*(\omega) = E'(\omega) + iE''(\omega). \quad (2)$$

E′ is the storage modulus. E″ is the loss modulus. Both E′and E″are functions of the frequency of applied dynamic load. E′and E″are usually measured using the dynamic indentation, wherein the applied indentation displacement and the resulting indentation force can be expressed as sinusoidal functions :

$$\delta(t) = \delta_0 + \Delta\delta \sin(\omega t) \text{ and } P(t) = P_0 + \Delta P \sin(\omega t + \phi), \quad (3)$$

At a given frequency ω, they can be obtained by

$$\frac{E'}{1-\nu^2} = \frac{1}{2a}\frac{\Delta P}{\Delta\delta}\cos\phi, \quad \frac{E''}{1-\nu^2} = \frac{1}{2a}\frac{\Delta P}{\Delta\delta}\sin\phi. \quad (4)$$

All the above indentation analysis are based on the Hertz elastic contact solution, $a \approx (R\delta)^{0.5}$. However, this relationship might not be true in cell indentation.

In present work, the substrate effect on the indentation contact area of cell is studied using FEM simulations. The cell is simply modeled as a linear viscoelastic material using standard linear solid model. The substrate and indenter tip are assumed as rigid elastic material. The spherical indenter tip is modeled as an 2-D axisymmetric surface. Cell is represented by 25000 4-node axisymmetric elements with reduced integration. All degrees of freedom of nodes on the bottom of the cell are constrained to simulate the condition that the cell is fully adhered to the rigid substrate surface. In order to examine the true substrate effect, the indentation displacement selected in the simulations coincide with the lest experimentally applied value [5, 6]. All FEM simulations are performed using commercial code ABAQUS v.6.8.

CONTACT RADIUS IN CELL INDENTAION

The variation of contact radius (normalized by the indenter tip radius) computed from FEM with the indentation displacement (normalized by the cell thickness) is shown in Figure 2. For the sake of reference, the Hertz contact radius a is shown as the dashed line. It shows that the cell indentation contact radius is not only dependent on the value of δ, R but also sensitive to the cell thickness h: $\tilde{a} = f(R, \delta, h)$. In all cases, the deviation between the numerical solution ($\tilde{a}$) computed from FEM and Hertz solution (a) increases with the increase in δ/h; this deviation also depends on the indenter tip size R. For a smaller R, $\tilde{a}<a$; for a larger R, $\tilde{a}>a$. The deviation between $\tilde{a}$ and a is from -11% (R/h=0.1) to 25% (R/h=3) at δ/h=10% in the current study range. These results are mainly caused by the substrate effect (for a large R) and the indenter tip size effect (for a small R). When R is in the medium (e.g. R=4μm) and h=10μm, $R>>\delta$ can be essentially met and the substrate effect is also weak. There is a good agreement between $\tilde{a}$ and a. which shows that Hertz solution is accurate when $R>>\delta$, without substrate effect.

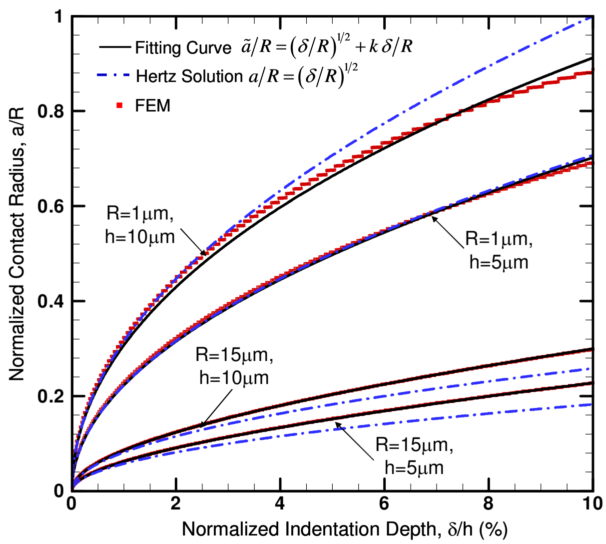

Figure 2 The relationship between normalized contact radius *a/R* and normalized indentation displacement δ/h.

The contact radius calculated from FEM can be approximately fitted as:

$$\tilde{a} = (\delta R)^{0.5} + k\delta, \qquad (5)$$

where the fitting parameter $k = k(R, h)$. For all cases except the one with R=1μm and δ=1μm, k can be fitted as: $k = k_1 + k_2 * R/h$. The fitting parameters k_1 and k_2 are essentially constants and $k_1 \approx$ -0.083, $k_2 \approx 0.48$.

DISCUSSION

The substrate effect can increase the indentation contact area with the same indentation displacement except for extremely small indenter tip size. When the indenter tip penetrates into the top surface of cell, the compress along the cell thickness direction produces the radial stretching due to the Poisson's effect. Both the compression and the stretch cause the true penetration depth, δ_{in}, to be less than the displacement of indenter tip, δ. For example, $\delta \approx 2\delta_{in}$, in the Hertz solution. If a cell is fully adhered onto a substrate, the substrate will constrain the bottom surface of the cell and prevents the motion in both thickness and radial directions. This constraint will reduce the cell stretching in the radial direction. This constraint effect will increase the penetration depth δ_{in} under the same applied δ compared with the case without the substrate. Thus, the contact radius will be larger than the Hertz contact radius due to the substrate effect. The substrate effect on the contact radius increases with a increase of R or decreases with a increase of h.

However, when R is very small, i.e. R=1μm, it is shown that $\tilde{a}<a$, which is caused by the indenter tip size effect. The Hertz's solution of the elastic contact problem is based on the theory of infinitesimal deformation, $\delta << R$. The higher order terms of δ are neglected and the contact radius is approximated as: $a \approx (R\delta)^{0.5}$. However, the cell indentation typically requires a relative small R and a large δ, wherein the omission of higher order terms of δ results in error in the determination of the contact radius. The indenter tip size effect will reduce the contact radius compared with the Hertz solution. For a given R, this effect increases with the increase of δ. Generally, the indenter tip size effect can be reduced with a larger indenter tip size. Since the substrate effect is very weak with a very small indenter tip size, the computed contact radius is less than the Hertz contact radius due to the indenter tip size effect.

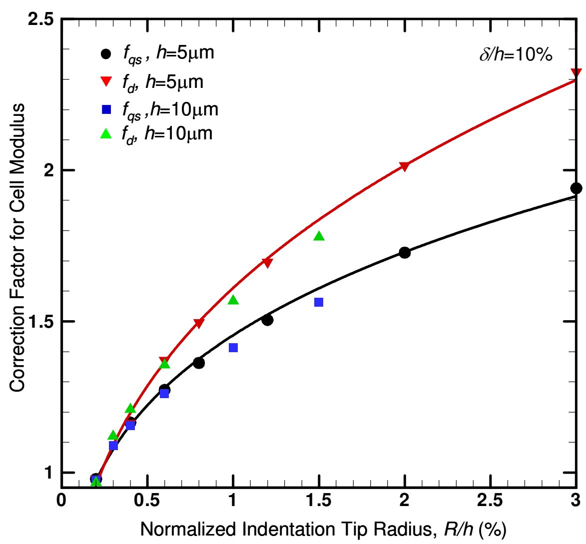

Figure 3 The cell modulus correction factor from the inaccurate contact area

ELASTIC MODULUS IN CELL INDENTAION

Substitution of the computed contact radius $\tilde{a}$ into equation (1) gives

$$P = \frac{4}{3}\frac{E}{1-\nu^2}\frac{\left(\sqrt{R\delta}+k\delta\right)^3}{R}. \quad (6)$$

If $a \approx (R\delta)^{0.5}$ is used, the elastic modulus in quasi-static indentation will be overestimated by a factor $f_{qs} = (\tilde{a}/a)^3$. Figure 3 shows the variation of f_{qs} with R/h for the samples with different R and h at δ/h =10%. It is shown that for a thin cell adhered with a substrate, the indentation modulus can be overestimated even at a small indentation displacement based on the existing quasi-static indentation analysis. In the current study, the elastic modulus can even be overestimated by about over 90% at δ/h=10% in the quasi-static indentation analysis due to the underestimation of contact area.

For viscoelastic materials, the dynamic indentation force can be calculated using the correspondence principle: replacing the time independent constant in equation (6) by the corresponding differential operators of the viscoelastic constitutive model [10]. The resulted indentation force can be described as $P(t)=P_0+P^*(t)$, where the oscillated part is:

$$P^*(t) = \Delta P \sin(\omega t + \phi) = \frac{4}{3\left(1-\nu^2\right)R}\int_0^t E(s)\frac{d\left\{\sqrt{R\delta(t-s)}+k\delta(t-s)\right\}^3}{ds}ds, \quad (7)$$

Therefore,

$$\Delta P = \frac{4}{3}\frac{1}{1-\nu^2}\xi(\delta_0)\left(E'(\omega)^2 + E''(\omega)^2\right)^{\frac{1}{2}}\Delta\delta, \quad (8)$$

$$\frac{E'(\omega)}{1-\nu^2} = \frac{3}{4}\frac{\Delta P}{\Delta\delta}\frac{1}{\xi(\delta_0)}\cos\phi, \quad \frac{E''(\omega)}{1-\nu^2} = \frac{3}{4}\frac{\Delta P}{\Delta\delta}\frac{1}{\xi(\delta_0)}\sin\phi, \quad (9)$$

where

$$\xi(\delta_0) = \sum_{m=1}^{4} n_m \frac{m+2}{2}(k)^{m-1}(R)^{1-\frac{m}{2}}(\delta_0)^{\frac{m}{2}}. \quad (10)$$

It is shown that the dynamic modulus components are not only the functions of the oscillated load frequency ω, but also depend on the direct indentation displacement δ_0 and increase with the increase of δ_0. Equation (4) will overestimate the complex modulus with the factor $f_d = 2\xi(\delta_0)/(3a(\delta_0))$ if the Hertz contact radius is used, but the phase difference ϕ between the indentation force and the indentation displacement is not affected by the contact area correction. Figure 3 also shows that the dynamic indentation analysis provides a even higher overestimation of elastic modulus E than the quasi-static indentation analysis due to the underestimation of contact area. In the current study, the elastic modulus can be overestimated by 130% using the dynamic indentation analysis for the case wherein it is overestimated by 90% in the quasi-static analysis. Therefore, it is very important to introduce the corrected contact radius into the dynamic indentation analysis in order to obtain the accurate dynamic modulus.

CONCLUSIONS

In this study, the evaluation of the cell mechanical properties using spherical indentation measurement is investigated based on numerical simulation (FEM). The results show that the dynamic modulus of cell measured using spherical indentation can be significantly overestimated using the existing indentation analysis. This overestimation is mainly caused by the deviation of indentation contact radius from the Hertz solution. These influences are mainly caused by the substrate effect and the nonlinear geometrical effect. Comparing with the Hertz solution, the substrate can increase the contact radius, but the nonlinear geometrical effect can reduce the contact radius. Since the substrate effect is typically much stronger except for extremely small indenter tip size, typically, the cell modulus is significantly overestimated based on the existing indentation analysis. In order to obtain the true cell modulus, the corrected contact radius needs to be introduced into the existing indentation analysis.

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