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Finite element simulation for the effect of loading rate on visco-hyperelastic characterisation of soft materials by spherical nanoindentation

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Abstract: Nanoindentation test performed by atomic force microscopy is highly recommended for the characterisation of soft materials at nanoscale. The assumption proposed in the characterisation is that the material is pure elastic with no viscosity. However, this assumption does not represent the real characteristics of soft materials such as bio tissues or cells. Therefore, a parametric finite element simulation of nanoindentation by spherical tip was carried out to investigate the response of cells with different constitutive laws (elastic, hyperelastic and visco-hyperelastic). The investigation of the loading rate effect on the characterisation of cell mechanical properties was performed for different size of spherical tips. The selected dimensions of spherical tips cover commercially available products. The viscosity effects are insensitive to the varied dimensions of spherical tip in this study. A limit loading rate was found above which viscous effect has to be considered to correctly determine the mechanical properties. The method in this work can be implemented to propose a criterion for the threshold of loading rate when viscosity effect can be neglected for soft material characterisation.

1. Introduction

The conventional methods for the characterisation of mechanical properties of macroscopic materials are uniaxial tensile test or compression test [1, 2]. In recent years of research, the focus primarily moved to the characterisation of biomaterials including tympanic membrane, brain tissue, cornea, etc [3-5]. Cells are the basic element of these biomaterials, their mechanical properties change with the internal functional state or external environmental stimuli. When a cell exhibit an abnormal status, its normal function can be disabled by the disease arises [6]. The relationship between the mechanical irregularities and status of disease is still ambiguous. Consequently, it is necessary to clarify the role of mechanics at the single cell level to better understand these relations. The characterisation of mechanical properties of single cell, either experimental or computational and both are able to provide an approach to interpreting the pathological phenomenon.

Research in the area of cell mechanics attracts efforts yielded a variety of experimental techniques such as micropipette aspiration [7], micro needles [8], optical tweezers [9], magnetic tweezers [10] and atomic force microscopy [11]. Among these techniques for cell mechanical properties characterisation, atomic force microscopy is one of the most suitable techniques due to its ability to detect the local regions of the cell at high resolution in nanoscale, while other techniques can only obtain the mechanical properties of a large amount of materials. Atomic force microscopy is also commonly used for the measurement of surface topography.

When AFM is used as a nanoindenter to carry out the characterisation of the mechanical properties, the indentation depth in nanoscale is monitored with forces applied in real time. Theses applied forces are generated by a cantilever with a tip in variable geometries. The deflection of the cantilever is related to the interaction between the probe and the sample. The cantilever deflections are monitored by a laser beam, which is reflected from the end of cantilever to a position sensitive detector (PSD). Nanoindenation test carried out by AFM is commonly used to characterize soft materials with low elastic modulus [12]. Also, this technique can be operated in liquid environment, which is a physiological condition for cell test [13]. For the analysis of the results of nanoindentation, materials are commonly treated with linear elastic properties, which means they are homogeneous and isotropic. However, cells are always non-homogeneous due to their complex internal structure, which make the characterisation of mechanical properties of cell is very difficult [14].

The elastic modulus of the tested sample was determined by the force-displacement curve obtained by nanoindentation test. Different analytical models can be implemented to obtain the elastic modulus [15]. One of the most commonly used models is based on the Hertz theory of elastic contact with no adhesion [16]. The limitation of the Hertz theory was investigated by Long et al. that the Hertz theory overestimated Young's modulus. Necessary correction was implemented to counteract this overestimation [17]. Hyperelastic model was used by Lin et al to investigate the nanoindentation of sample with different strain energy to compare the results obtained based on Hertz theory [18]. It was found that the results obtained by the Hertz theory were acceptable when the indentation depth and indenter radius is below a certain value. Ding et al. implemented Neo-Hookean model to describe the hyperelastic behavior of the cell by finite element simulation [19]. Moreover, Ladjal et al. carried out the nanoindentation test on mouse stem cell and compared the results with Hertz theory and finite element simulation [20]. Viscous properties were also considered in other works, Liu et al., proposed a viscoelastic finite element method (FEM) model, which accounted for both cell elasticity and viscoelasticity for cell AFM nanoindentation [21]. An approach of experimental AFM nanoindentation and FEM modelling was proposed by Florea et al, viscohyperelastic model was employed in their study for parameter analysis of

fluid flow through the cell membrane [22]. The geometry of AFM tip is one of the most important factors in the proper characterisation of mechanical properties of cell [23]. Valero et al characterized the mechanical properties of biological materials by spherical tips using FEM simulation [24].

Based on the above documented works, the relationship among viscosity response, the size of spherical tip and loading rate involved in the characterisation of mechanical properties of soft matter are still unclear. Under a slow indentation rate, the viscosity response is inconspicuous, therefore a hyperelastic model can be used instead of visco-hyperelastic model under this circumstance. The aim of the current study is to investigate the threshold of loading rate, under which the results obtained from the hyperelastic model is acceptable compared to visco-hyperelastic model. The effect of radius dimensions of spherical tip is also considered. In this paper, a parametric finite element study of the cell nanoindentation is performed with different size of spherical tips. Three different material models (elastic, hyperelastic, viscohyperelastic) are selected for the investigation of the cell response to the same perturbation when a small indentation is applied. The selection of radius of spherical tip is corresponded to the commercially available products. Six different loading rates have been simulated for each tip geometry for the comparison of hyperelastic and viscohyperelastic model.

2. Methods and models

2.1. Simulation hypothesis

The nanoindentation test on the cell is simulated by using a parametric finite element model in FEA software ABAQUS (Version 6.14). This model is a 2D axisymmetric model instead of 3D model, the direction of indention is the same as the vertical axis. The spherical tip is modelled as a rigid part (there is no change or deformation for the tip in the entire process of indentation) due to the indenter is much stiffer than the sample.

In the parametric study, the radius of spherical tips are 1, 1.5, 2.5, 5, 7.5 and 10 µm, which are corresponding to the available commercial indenters. Cell specimen is set as an incompressible sample as the size of 100 µm in radius and 100µm in thickness. The assembly model is shown in Fig. 1. The vertical displacement of the tip is 0.5µm, therefore the indentation depth only accounts for 0.5 percent of the specimen thickness. Substrate effect may dominant in the indentation process if the indentation depth is larger than 10 percent of specimen thickness [25]. The specimen is large enough that boundary and substrate effect will not be considered in this study.

The bottom surface of the specimen is set as a fixed boundary condition to mimic the attachment of the cell to the petri dish in experiments. The axis symmetric edge is applied with horizontal displacement only. The interaction between tip and specimen is hard contact, no friction and no thermal interaction is considered during the indentation process. The effect of adhesion on the determination of material properties may become significant under the condition that the indentation depth accounted for half of the sample thickness based on the literature [26]. Therefore, effect of adhesion can be neglected due to shallow indentation depth in this study.
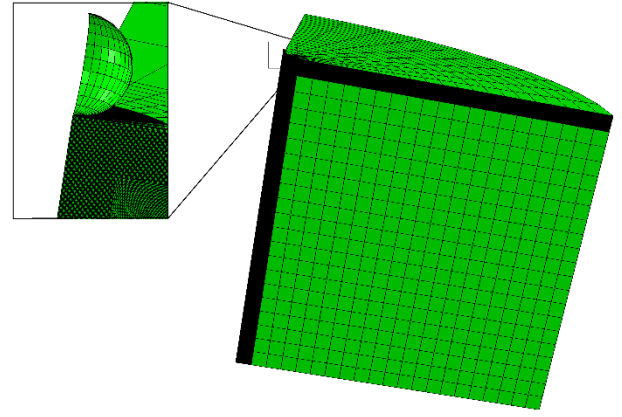

Fig. 1. *2D axisymmetric finite element model used to analysis the spherical indentation*

The Hybrid CAX4H element is selected to simulate the cell specimen. The contact area has a finer mesh compared to other region of the specimens in order to obtain an accurate simulation of the indentation process. The convergence simulation of finite element analysis was carried out and the average size in proximity of the contact region is 0.5 nm. The nonlinear effects of larger deformation and displacements are considered in this finite element analysis.

Various indenter geometries can be used for nanoindentation experiments, including pyramid, conical, flat and spherical tips. Each type has its own advantages and disadvantages. Cell indentation with a flat end tip is also known as cytoindentation [27]. Normally, the size of indentation tip is smaller than the cell. The benefit of this type of tip is that the contact area is constant with less affected by thermal drift and creep. Also a flat end tip is suitable for very soft and fragile cell. The drawback is that the detection of contact point is difficult. The spatial resolution is relatively small compared to other types of tips. Pyramid and conical tip yield small contact area compared with flat and spherical tip. Those sharp tips are particularly used to probe fine feature. The disadvantage is that the sharp edge may damage cell membranes, therefore, it is not suitable for probing living cells. For spherical indenter, the non-linear deformation behavior is smaller than other geometries, such as pyramid or conical tips [28]. Also, spherical tip is ideal for soft and fragile cell, therefore, spherical tip has been selected in this study. The typical radius of the probe is between 1 and 10 µm.

2.2. Constitutive models in finite element analysis

The classical Hertz contact model is commonly used to determine linear elastic properties of the material by nanoindentation test. The application of Hertz contact theory has to be established by some hypotheses. The sample should be homogeneous, isotropic with linear elastic properties. This theory is valid under the circumstance of a small range of deformation to avoid geometric nonlinearities. Also, tested sample should be large enough to avoid the boundary effect. Based on Hertz theory, contact force generated during the indentation can be worked out as the function of mechanical properties of the sample and indentation depth. Another impact factor is the geometry of the indenter, for spherical, pyramid, conical, flat cylindrical tips, each has its own impact parameter. In this study, spherical tip is selected

as the indenter, therefore the correlation among the applied force, indentation depth and tip radius is defined as follows:

$$F = \frac{4ER^{0.5}\delta^{1.5}}{3(1-\upsilon^2)} \quad (1)$$

Where E and υ are elastic modulus and Poisson's ratio of the sample, respectively. δ is indentation depth, R is the radius of spherical tip.

In addition, Hertz contact model can be used to derive the elastic modulus of the material based on the force and displacement curve obtained from experiment as follows:

$$E = \frac{3(1-\upsilon^2)}{4FR^{0.5}\delta^{1.5}} \quad (2)$$

Three different material models are implemented in this study. Firstly, a linear elastic model is selected due to it is considered by Hertz contact model. When the applied force unloaded, elastic materials recover their original shape. For linear elastic material, the deformations are proportional to the load that induces them. Two parameters can be used to describe the mechanical behavior, including elastic modulus and Poisson's ratio. An elastic modulus 5 KPa was chosen for the cell specimen with Poisson's ratio $\upsilon = 0.5$ (assumed to be impressible).

When the indentation force applied for elastic materials, it is known that biological materials exhibit nonlinear behavior under large deformations. Therefore, the isotropic hyperelastic with equivalent elastic properties to elastic model is implemented for better characterisation of the mechanical properties of cells. The indented sample was modelled as a purely hyperelastic material following a Neo-Hookean constitutive law. During nanoindentation process, the equivalent material parameters stiffness modulus (μ) and compressibility modulus (κ) that must be given for hyperelastic model based on the value of elastic modulus E and Poisson's ratio υ .

The strain energy density function of an isotropic material based on Neo-Hookean model is expressed as follows:

$$U = C_{10}(I_1 - 3) + \frac{1}{D_1}(J^{el} - 1)^2 \quad (3)$$

where U is strain energy density per volume unit and I is first strain invariant. C_{10} and D_1 are material parameters that depend on temperature, which are given input to ABAQUS. Those two parameters can be defined as a function of shear modulus (μ) and compressibility modulus (κ):

$$C_{10} = \frac{\mu}{2} \quad (4)$$

$$D_1 = \frac{2}{\kappa} \quad (5)$$

The relationship between shear modulus μ , compressibility modulus κ and elastic modulus E , Poisson's ratio υ are shown as below:

$$\mu = \frac{E}{2(1+\upsilon)} \quad (6)$$

$$\kappa = \frac{E}{3(1-2\upsilon)} \quad (7)$$

For the purely hyperelastic model, the values of C_{10} and D_1 can be calculated and input to ABAQUS software for simulation study.

In order to describe the viscoelastic behavior of indented sample, a visco-hyperelastic model was implemented in the simulation, where the hyperelastic part of the mechanical response was described by Neo-Hookean model. The viscous behavior was modelled by N-term Prony series expansion of the dimensionless relaxation modulus. Therefore, effective relaxation modulus is under the function below:

$$U_r = \mu \cdot [1 - \sum_{k=1}^{N} g_k \cdot (1 - e^{-t/\tau_k})] \quad (8)$$

where μ is shear modulus, g_k is the kth Prony constant ($k=1,2,..,N$), τ_k is the corresponding relaxation time constant, respectively. The Prony expansion is dominated by the first term in the series, therefore the number of term N is 1 in this study.

In order to investigate the effect of indentation rates on the visco-hyperelastic response, different loading rates were performed (0.1, 0.5, 1, 2, 5, and 10 um/s) for each geometric configuration of indenter. The duration of indentation process t is corresponding to the indentation depth divided by loading rate. For visco-hyperelastic parameters, C_{10} and D_1 are same as in the purely hyperelastic model, the viscosity parameter given are: g_1 =0.9681, τ_1 =0.01s.

For each tip geometry configuration under different loading rate, the reaction forces $F_{viscohyperelastic}$ and $F_{hyperelastic}$ can be obtained by ABAQUS simulation. Differences in these two values were defined as

$$\varepsilon = \left| \frac{F_{viscohyperelastic} - F_{hyperelastic}}{F_{hyperelastic}} \right| \times 100\% \quad (9)$$

This different ε shows the effect of viscosity on materials behavior related to tip geometry and loading rates for each tip size and indentation rate. If the value of difference is smaller than 25%, it is acceptable that the hyperelastic model is consistent with a visco-hyperelastic model in the determination of material properties. The limit threshold of 25% is two time as the statistic distribution of force-indentation data obtained in other researchers' work in AFM testing on cell [11]

3. Results

Mises stress distribution in testing sample is sensitive to the size of indenter. When a small indentation depth (0.5um) is applied to material with the same properties, smaller spherical tip induces higher stress (Fig. 2). For linear elastic material indentation, spherical tip in 1 um radius induces the maximum stress in 1.814 KPa. The value for spherical tip in 5 um radius and 10 radius are 0.747 KPa and 0.528 KPa, respectively. While the larger spherical tip exhibits large stress area compared to smaller tip. It means that the stress of indented sample becomes more localized for smaller tips.
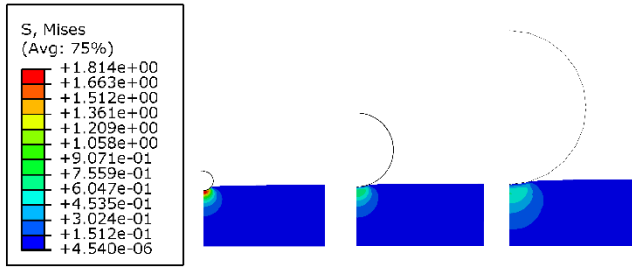
Fig. 2. *Mises stress distributions for linear elastic model under same indention depth: radius dimension, 1um (left), 5um (centre), 10um (right)*

The indentation of three different materials (elastic, hyperelastic, viscohyperelastic) with same tip were carried out by using 2D model. The displacement and contact area are the same among different models. The indentation depth is 0.5um and loading rate is 2um/s. The stress distribution for a spherical tip with 1um in radius is shown in Fig. 3 that stress distribution in linear elastic is similar to hyperelastic model, while the stress in viscohyperelastic model is larger than the other two models. It is found that the highest stress locates under the contact zone between the tip and sample (not at the surface of the sample).
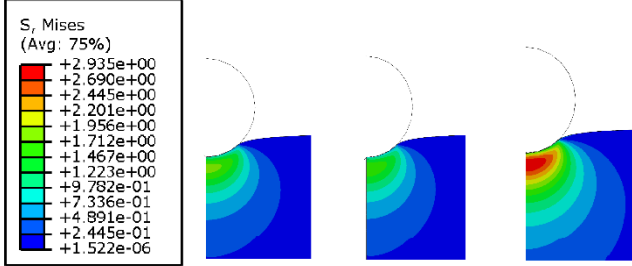

Fig. 3. *Mises stress distribution when using spherical tip with 1um in radius for three models: linear elastic (left), hyperelastic (centre), visco-hyperelastic (right)*

A linear elastic material and an isotropic hyperelastic material in Neo-Hookean model with equivalent properties were simulated to analysis its mechanical behaviors. In addition, the reaction force generated during the indentation process can be obtained by FEA simulation, which can be compared to theoretical Hertz theory. The tips radius is 1μm, and loading rate is 2um/s. It is shown in Fig. 4 that for small indentation depth (0-0.1um), the reaction force computed from the elastic and the hyperelastic model are very closed to theoretical result obtained by Hertz model. When the penetration depth increasing, the Hertz contact model overestimates the indentation force for the elastic and hyperelastic model.
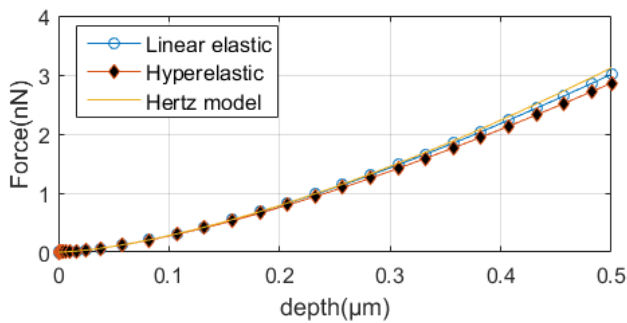

Fig. 4. *Reaction force calculated for a 0.5um depth indentation based on Hertz model and FEA simulation for elastic and hyperelastic sample*

The indentation force generated during indentation with different radius was investigated under the loading rate of 2um/s. It is as expected that in the same indentation depth, larger tip induces a higher indentation force (Fig. 5). For example, in linear elastic material, spherical tip in 1 um radius induces 3.01 nN and 5 um radius tip induces 7.23 nN. The maximum force value 10.4 nN was generated by the largest tip 10 um in radius in this simulation. The same regulation was also found in the other two material models (hyperelastic and visco-hyperelastic).

**Fig. 5.** *Force-indentation curves obtained by FEA for*

(a)

(b)

(c)
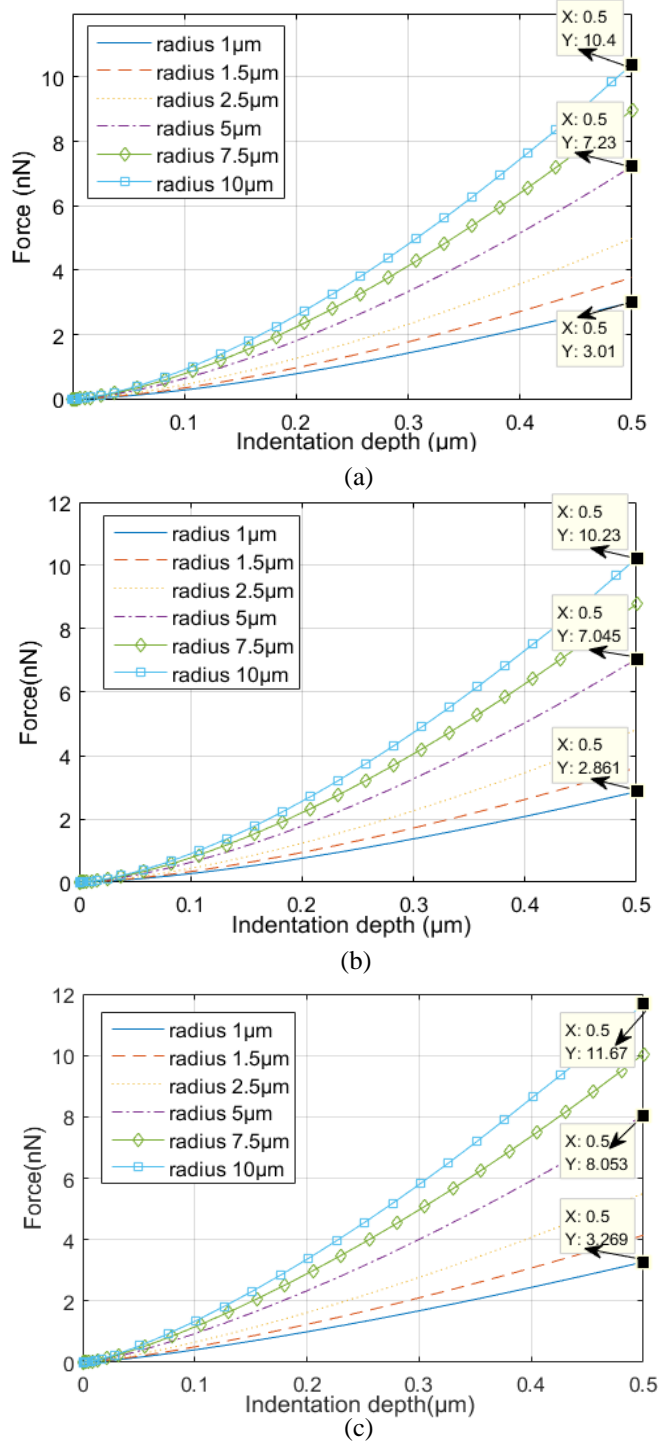
different spherical tips

(a) elastic model, **(b)** hyperelastic model, **(c)** visco-hyperelasitc model

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There is no effect on the results when changing the loading rate for the material with linear elastic and hyperelastic models. In the simulation of materials with viscohyperelastic model, the indentation forces depend on the loading rate. For all the geometry of spherical tips, indentation forces increased with the higher loading rate, according to Fig. 6 and Fig. 7. The loading rate of indentation ranges between 0.1 um/s and 10um/s. Similar regulation was found that the larger size of tip induces a higher force.
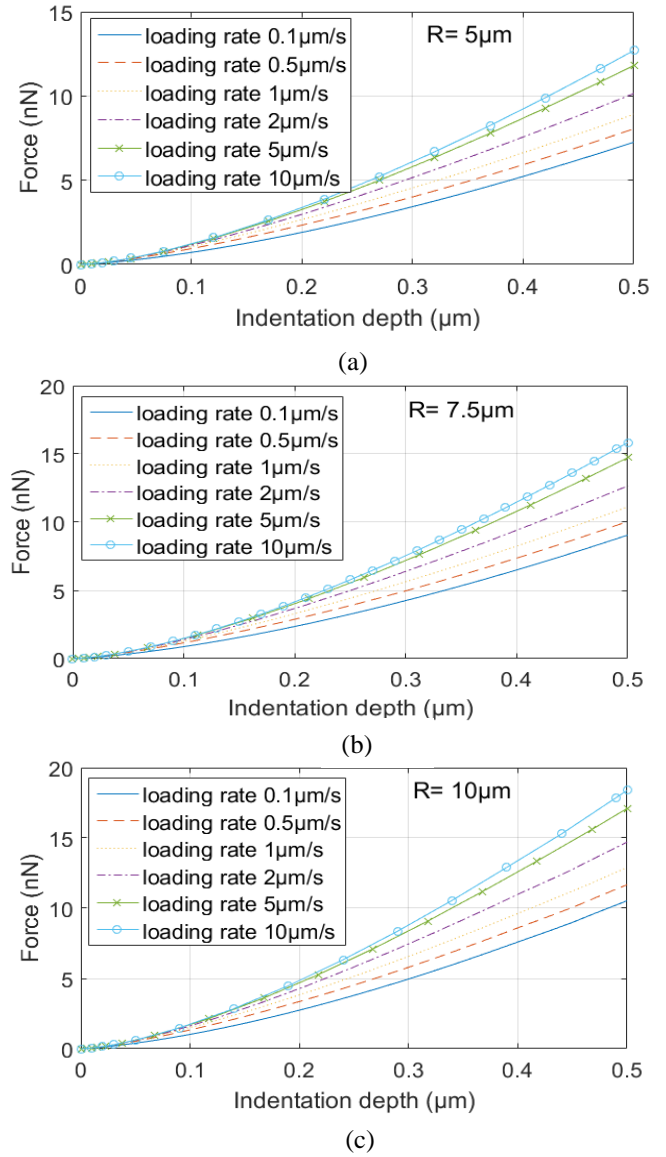
Fig. 6. Force-indentation depth curve obtained by FEA for
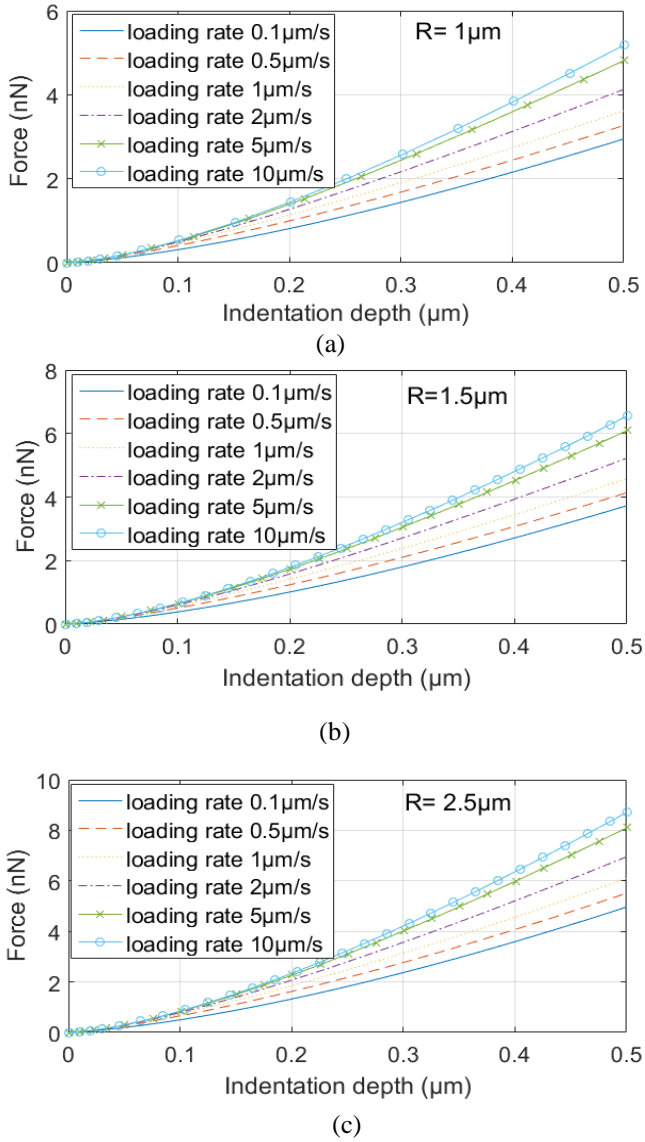
(a)

(b)

(c)

different spherical tips under different loading rate (0.1-10um/s) when implemented viscohyperelastic model:
(***a***) 1 um, (**b**) 1.5 um, (**c**) 2.5um

In order to investigate the effect of viscosity on the behavior of materials, the difference of reaction force generated by the visco-hyperelastic model and the purely hyperelastic model was calculated based on Equation 9. It is shown in Fig. 8 that higher loading rate results in larger differences in two models. It can also be found that the error is insensitive to the tip size between 1 and 10 µm in radius. The limitation indentation threshold rate for the spherical tips is 0.95µm/s according to the illustration in Fig. 8 (ε <25%), which means if the indentation rate is lower than 0.95µm/s,

the visco-hyperelastic model can be replaced by a purely hyperelastic model.

Fig. 7. Force-indentation depth curve obtained by FEA for

(a)

(b)

(c)

different spherical tips under different loading rate (0.1-10µm/s) when implemented viscohyperelastic model:
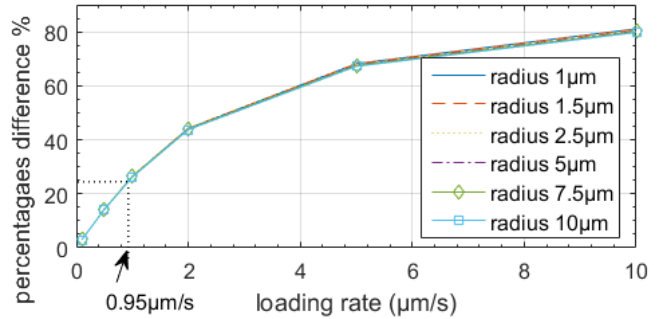(***a***) 5 µm, (**b**) 7.5 µm, (**c**) 10 µm

Fig. 8. *Percentage difference between the indentation forces computed with viscohyperelastic model and hyperelastic model for different loading rate and tip radius*

4. Discussions

Mechanical behavior of bio cell and tissue was widely investigated in recent years by using nanoindentation method

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[11-16]. There is no standard mechanical model available to characterize the mechanical properties of living cell due to its complex physiological composition. Furthermore, the material model selection is one of the key factors that has to be considered for the characterisation of mechanical properties of cells. In order to obtain the elastic modulus of sample, the general method is implementing the Hertz model. However, it is observed that the Hertz model is invalid for large deformation in non-linear regime. It is only valid in a small range in the linear regime. The same conclusion was also found in other researchers' experimental work in which cell indentation exhibits linear deformation only in a shallow indentation [27]. According to Fig. 3, differences in Mises stress distributions have been observed among elastic, hyperelastic and viscohyperelastic model when using the same tip with same indentation depth. The stress distribution of elastic and hyperelastic model is similar, while the value obtained by viscohyperelastic model is higher. The reason is rigidification of the materials near the contact zone of viscohyperelastic model, which prevents the material from being deformed under this loading rate.

The FEA model simulated the contact between spherical tip and the sample. The tip radius was varied to account for the indentation by tips with different size. For each tip geometry, the sample was modelled as a hyperelastic model and a viscohyperelastic model with varied indentation rates between 100 nm/s to 10000 nm/s. This model can be used to estimate the threshold of the indentation rate in which the viscous effects can be neglected to describe the material behavior. The reaction force computed by ABAQUS increased with tip radius increased from 1um to 10um under the same indentation depth (Fig. 5). This can be explained that at a given indentation depth, using a larger tip induces a larger amount of material to be deformed during the indentation process. Therefore, a higher indentation force is required to push the indenter into the sample in the given penetration depth.

With the indentation rate increasing, the reaction force also increasing for each tip geometry, it appears that the higher indentation rate, the more viscous effect. This result is consistent with experimental evidence on the viscoelastic behavior of biopolymers [28]. As a matter of fact, the elastic part of biopolymer depends on its structure of the network and the deformation capability of polymer chains from one to another, the viscous part equivalents to the stiffness of the spring-dashpot system used to model the viscous behavior of the material. In low velocity, the polymer reaction force is dominated by the elastic term. With the velocity increasing, the reaction force goes up by the contribution of viscous until the reaction force climbs up at a plateau value.

This approach can be used to estimate the errors in the determination of the material properties when the viscous effect is neglected. For different size of spherical tips, the percentage difference ε between reaction force computed by ABAQUS for the visco-hyperelastic model and hyperelastic model became more significant in higher loading rate (Fig. 8). When the loading rate is 0.5 µm/s, the results obtained from the two models are very close to each other. As the loading rate increases, the domination of viscous results in a significant difference between two models. It was also interesting to find that the percentage difference ε is

insensitive to the dimension of spherical tip, if the radius is between 1 and 10 µm.

Commercial AFM tips available on the market include conical tips with two opening tips, therefore, it is necessary to develop a 3D FEA model to simulate the real morphology of the probe indented with the sample. This issue will be considered in future work. This model was built on the hypothesis that the contact between probe and sample is Hertz contact with no adhesion considered. It is argued that the conventional conical tip is not a suitable selection to probe the soft cell due to the sharp tip may damage the surface of the sample. The damage occurs due to the local pressure is higher than the critical value. For this reason, the researchers selected the spherical colloidal probes for nanoindentation test. The solution of this problem is carried out pre-test to ensure the pressure value is much lower than the critical value of damage before the nanoindentation experiments. In reality, some degree of tip-cell adhesion is unavoidable in the characterisation of cell mechanical properties by AFM nanoindentation. Therefore, the tip-cell contact model should be replaced by Johnson – Kendall – Roberts (JKR) model [29] or Derjaguin – Muller – Toporov (DMT) [30] model instead for future investigation.

5. Conclusions

In this study, a parametric study of AFM nanoindentation test of the cell by spherical tip was conducted by computation modelling. It is observed that the Hertz model is only valid for shallow indentation depth of nanoindentation test of the cell. Therefore, a new FEM model was developed in this work to account for both hyperelastic and viscohyperelastic properties. The hyperelastic property was described by Neo-Hookean model and viscosity was described by a series of Prony expansions of shear modulus. The relationship among the loading rate, size of spherical tip and viscosity was investigated by this parametric FE model analysis. Viscosity effects appear to be more significant at higher loading rate. The threshold of loading rate is 0.95 µm/s when both hyperelastic and viscohyperelastic models can be implemented to describe the materials. It is also found that the viscosity effects are insensitive to the size of spherical tip. The method developed in this work is general and can be used in other kind of soft materials. In addition, this approach exhibit a great potential ability in the characterisation of interfacial properties, e.g. adhesion between tip-cell or cell-cell.

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