Porohyperelastic Analysis of Single Chondrocyte Using AFM and inverse FEA

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Abstract

The aim of this paper is to determine the creep and relaxation responses of single chondrocytes *in vitro*. Firstly, Atomic Force Microscopy (AFM) was used to obtain the force-indentation curves of single chondrocytes at the strain-rate of 7.05 s^{-1}. This result was then employed in inverse finite element analysis (FEA) using porohyperelastic (PHE) idealization of the cells to determine their mechanical properties. The PHE model results agreed well with AFM experimental data. This PHE model was then utilized to study chondrocyte's creep and relaxation behaviors. The results revealed that the effect of fluid was predominant for cell's mechanical behaviors and that the PHE is a good model for biomechanics studies of chondrocytes.

Keywords: Biomechanics, Chondrocytes, Finite Element Analysis, Porohyperelastic, AFM.

Introduction

Chondrocytes are cytoskeleton (CSK)-rich eukaryotic cells which are the mature cells in cartilage tissues performing a number of functions within the cartilage. The deterioration of the mechanical properties of these cells is believed to be one of the main factors in the development and progression of osteoarthritis (Jones et al. 1997, Trickey, Lee and Guilak 2000). Cellular behaviour in response to external stimuli such as shear stress, fluid flow, osmotic pressure and mechanical loading have been investigated recently (Guilak, Erickson and Ting-Beall 2002, Ofek et al. 2010, Wu and Herzog 2006).

There are several continuum mechanical models that have been developed for the single cell as well as other biological tissues (Lim, Zhou and Quek 2006). One of them is porohyperelastic (PHE) model which can account for the non-linear behavior, fluid-solid interaction and rate-dependent drag effects is potentially a good candidate for investigating the responses of a cell to external loading and other load-induce stimuli. This PHE model considers soft tissues as porous materials consisting of a pore fluid that saturates the tissue and percolates and exudes transiently relative to the deformable porous elastic solid skeleton. Although the PHE model has been widely and effective utilized in biomechanics, e.g. articular cartilage modeling (Oloyede and Broom 1991, Oloyede and Broom 1996), its application in the modeling of the single living cell has been quite limited.

Because of recent advances in nanotechnology, a number of new experimental techniques for characterizing and studying the mechanical behavior of living cells have been developed. One such technique is based on Atomic Force Microscopy (AFM) which is a state-of-the-art experimental facility for high resolution imaging of tissues, cells and artificial surfaces, including probing the mechanical properties of samples both qualitatively and quantitatively (Touhami, Nysten and Dufrene 2003, Rico et al. 2005, Zhang and Zhang 2007, Lin, Dimitriadis and Horkay 2007, Kuznetsova et al. 2007, Faria et al. 2008, Yusuf et al. 2012). Its principle is to indent the material/sample with a tip of microscopic dimension which is attached to a very flexible cantilever and the force is measured from the deflection of the cantilever to obtain the force-indentation (F-δ) curve (Darling, Zauscher and Guilak 2006, Faria et al. 2008, Ladjal et al. 2009). This powerful tool

is increasingly applied in the study of cell responses to external stimuli such as mechanical and chemical loading. This tool is ideal for bridging the research gap in the understanding of microscale responses of biological organisms.

The aim of this study is to utilize the PHE model to explore the creep and relaxation responses of non-living chondrocytes using Atomic Force Microscopy (AFM) and inverse finite element analysis (FEA).

Methodology

1. *Sample preparation and AFM set-up*

The chondrocytes were cultured using Dulbecco's Modified Eagle's Medium (low glucose) (GIBCO, Invitrogen Corporation, Melbourne, Australia) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logen, UT) and 1% penicillin and streptomycin (P/S) (GIBCO, Invitrogen Corporation, Melbourne, Australia). After culturing for a week until the cells were confluent, they were detached using 0.5% Trysin (Sigma-Aldrich). They were seeded onto poly-D-lysine (PDL, Sigma-Aldrich) coated cultured petri dish for 1-2h. Chondrocytes were placed on the PDL surface to form a strong attachment while keeping their morphology round. Then the chondrocytes were fixed using 4% Paraformaldehyde (Sigma-Aldrich) for 20 minutes before changing it to Phosphate Buffered Saline (PBS, Sigma-Aldrich). All the samples were stored at -4^0C until required for experiments. Biomechanical testing was conducted at room temperature. Atomic Force Microscopy (AFM) (NT-MDT SOLVER P47-PRO SPM) was used in this study. A triangular colloidal probe CP-PNPL-BSG-A-5 (NanoAndMore GMBH) cantilever attached to a crystal cantilever holder was used in the experiment. This allows the scanning and force spectroscopy of samples in liquid. The colloidal probe is of diameter 5 μm and its spring constant is 0.08 N/m.

The force-indentation curves of single chondrocytes were first obtained with the AFM. The porohyperelastic (PHE) FEA model of the chondrocyte was then developed and used to determine its mechanical properties by inverse analysis, where the experimental data was used as input. Following this, the model was extended to study the creep and relaxation responses of the cell.

2. *Porohyperelastic (PHE) field theory*

It has been applied in many engineering fields including Soil Mechanics (Sherwood 1993) and Biomechanics (Simon 1992, Meroi, Natali and Schrefler 1999, Nguyen 2005, Olsen and Oloyede 2002); with the theoretical details extensively presented by several authors (Simon 1992, Simon et al. 1996, Simon et al. 1998b, Simon et al. 1998a, Kaufmann 1996). The field equations for the isotropic form of this theory are summarized below:

Conservation of linear momentum:

$$\frac{\partial T_{ij}}{\partial X_j} = 0 \tag{1}$$

Conservation of fluid mass (Darcy's law):

$$\tilde{k}_{ij}\frac{\partial \pi^f}{\partial X_i} = \dot{\tilde{w}}_j \tag{2}$$

Conservation of (incompressible) solid and (incompressible) fluid mass:

$$\frac{\partial \tilde{w}_i}{\partial X_k} + JH_{kl}\dot{E}_{kl} = 0 \tag{3}$$

The constitutive law:

$$\sigma_{ij} = \sigma^e_{ij} + \pi^f \delta_{ij}, \qquad \sigma^e_{ij} = J^{-1}F_{im}S^e_{mn}F_{jn} \tag{4}$$

$$S_{ij} = S^e_{ij} + J\pi^f H_{ij}, \quad H_{ij} = F^{-1}_{im}F^{-1}_{jm}, \quad S^e_{ij} = \frac{\partial W^e}{\partial E_{ij}} \tag{5}$$

where T_{ij}, π^f, $\tilde{k}_{ij}$, $\hat{w}_j$, H_{ij}, S^e_{ij} and W^e are first Piola-Kirchhoff total stress, fluid stress, symmetric permeability tensor, Lagrangian fluid velocity, Finger's strain, second Piola-Kirchhoff stress and effective strain energy density function, respectively. Neo-Hookean strain energy density function shown below would be used in this study (Brown et al. 2009, ABAQUS 1996):

$$W^e = C_1(\bar{I}_1 - 3) + \frac{1}{D_1}(J-1)^2 \tag{6}$$

where J is the volume strain of the material, $\bar{I}_1 = J^{-2/3}I_1$ is the first deviatoric strain invariant, and C_1 and D_1 are material constants.

The hydraulic permeability of the chondrocyte was assumed to be deformation-dependent in this study. The constitutive law of deformation-dependent permeability proposed by (Holmes and Mow 1990) was adopted. In order to adopt this to finite element simulations, the permeability was employed as a function of void ratio which is the ratio of the volume of fluid to the volume of solid component as proposed by (Wu and Herzog 2000):

$$k = k_0\left(\frac{e}{e_0}\right)^{\kappa} exp\left\{\frac{M}{2}\left[\left(\frac{1+e}{1+e_0}\right)^2 - 1\right]\right\} \tag{7}$$

where k_0 is the initial permeability, e_0 is the initial void ratio, and κ and M are non-dimensional material parameters.

Note that void ratio e relates to porosity n e.g. the volume of the matrix occupied by fluid by: $e = n/(1-n)$. The chondrocyte's water content was determined to be around 60% of total volume (Oswald et al. 2008). Thus, the initial void ratio e_0 was calculated to be e_0 = 1.5. The material parameters κ and M have been determined to be 0.0848 and 4.638, respectively in (Holmes 1986), and used in (Wu and Herzog 2000, Holmes and Mow 1990, Moo et al. 2012). Figure 1 presents the strain-dependent permeability used in ABAQUS model in this study.

The volume strain of the cell is given by:

$$J = \frac{dV}{dV_0} = \frac{1+e}{1+e_0} \tag{8}$$

where V and V_0 are deformed and undeformed volume of material, respectively.

3. Chondrocytes diameter

In order to develop a FEA model, there are several important parameters required. One of these is the chondrocytes diameter. It was measured with a Leica Light Microscope M125 (Leica Microsystems). Note that only the round chondrocytes were picked for measurement and the diameter is the average of the horizontal and vertical diameters, leading to 16.99 ± 2.041 µm (n = 50) (Figure 2). This average diameter was used in the FEM modeling of the single chondrocyte.

4. Finite Element Analysis (FEA) model

A finite element analysis (FEA) model of a single chondrocyte was developed (Figure 3) to study its micro-deformation response, using the commercial software ABAQUS version 6.9-1 (ABAQUS Inc., USA). The atomic force microscopy (AFM) nano-indentation experiment was simulated with this model. Because both the chondrocyte and AFM tip are spherical, axisymmetric geometry and element-approximation was assumed, thereby saving computational cost (ABAQUS 1996). The model consists of a chondrocyte cell of diameter 17 µm which was indented with a colloidal probe of diameter 5 µm. At first, the chondrocyte was indented to a maximum strain of around 15% of cell's diameter (corresponding to a displacement of approximately 2.5 µm). The reaction forces were then extracted and compared to the experimental data to determine cell's mechanical

properties which are C_1, D_1 and k_0 in Eq. (6) and (7) using inverse FEA procedure. This model was also used to study the relaxation of chondrocytes by keeping the displacement of the tip constant. Secondly, the force of 27.7 nN was applied on the spherical tip and kept constant to study chondrocyte's creep response. This force value was the maximum force obtained from previous model.

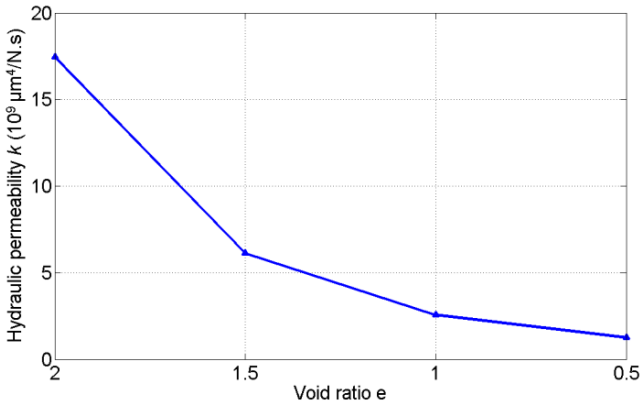

Figure 1 Deformation dependent hydraulic permeability

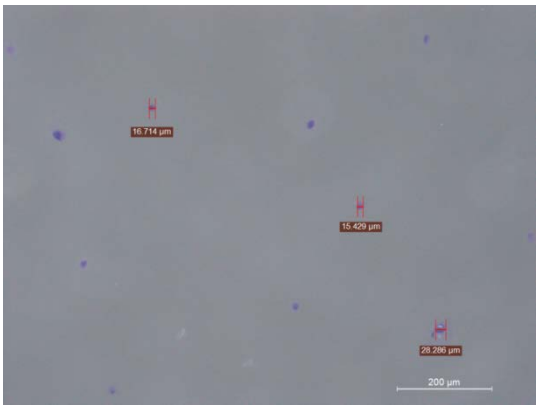

Figure 2 Chondrocytes diameter measurement

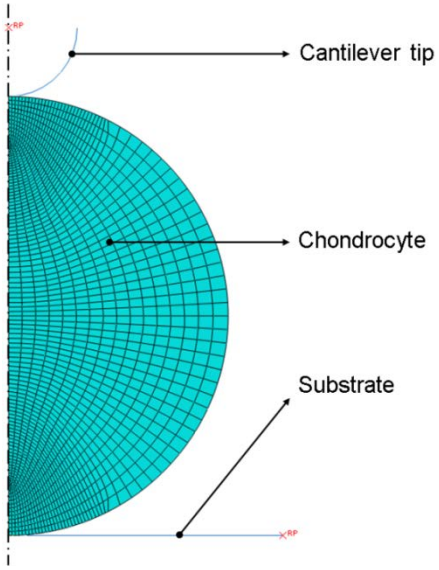

Figure 3 FEM model of single chondrocyte

RESULTS AND DISCUSSION

1. *AFM experiment*

Before conducting force spectroscopy to probe the mechanical properties of chondrocytes, scanning was done to locate the position of single chondrocytes using the contact mode scanning method. Figure 4 presents the height scanned image of chondrocytes. Using this image, the cantilever was adjusted so that the colloidal tip was placed centrally at the top of the chondrocyte to apply a load. Following this, the cell was indented to approximately 15% of its diameter at strain-rate of 7.05 s^{-1}, and the force-indentation curves were recorded.

2. *FEM results*

In the first model, the chondrocyte was indented to 15% strain of cell's diameter. The PHE model using Neo-Hookean strain energy density function was developed and analyzed using inverse FEA

modeling methodology in order to capture the behavior of the cell. The software ABAQUS was utilized with pore fluid/stress axisymmetric elements with the PHE constitutive material law presented earlier. The material parameters which were determined using inverse FEA procedure mentioned above are presented in table 1.

Table 1 PHE model material parameters (Oswald et al. 2008)

C_1	D_1	Initial permeability k_0 (μm^4/N.s)	Initial void ratio e_0
0.00105	3060	6.14×10^9	1.5

Figure 5 presents the AFM experimental data and PHE simulation results. It is observed that the PHE model agreed well with AFM experiment demonstrated that this model can be used to capture cells' mechanical behavior. The relaxation of the cell was then studied by keeping the displacement of spherical tip constant for around 70 s. It can be observed that the PHE can capture the relaxation of chondrocyte very well (Figure 6). Note that the relaxation response was fast because of the effect of pore fluid pressure developed during indentation. This pressure gradient caused the fluid to exude out from the cell fast enough in the relaxation state. The volume strain of the chondrocyte at the end of transient and relaxation states were determined to be 0.969797 and 0.829007, respectively using Eq. (8). It demonstrated that there was loss of fluid during relaxation state. This response will be considered clearer in the next model.

In the second model, the chondrocyte was applied a force of 27.7 nN on cantilever tip instead of displacement in the first model. This force was then kept constant for around 70 s to study the creep response of the cell. When the force was kept constant, the chondrocytes continued to deform until its deformation reached an asymptotic value (Figure 7). This is when the cell is in its equilibrium condition. In order to have a clearer understanding, the von Mises or solid skeleton stress and fluid pore pressure at the closest node to the cantilever tip was shown in Figure 8. It is observed that the Mises or solid stress increased significantly in the transient state and gradually reached its maximum value in the steady state e.g. when the applied force was kept constant. Also, in this steady state, the pore pressure dropped to its asymptotic/limiting low value. It demonstrated that the solid skeleton provided load bearing stiffness in this state where the pore pressure is practically zero.

It is worthy to note that pore fluid pressure reached its maximum value earlier compared to the applied load (Figure 8). It demonstrated that the effect of fluid is superior only in the beginning of the indentation. In brief, in the transient state, all of applied load was taken by the pore fluid pressure. The load was then transferred from fluid to solid skeleton when the pore pressure dropped and all of load was taken by solid skeleton after all. These results reveal the predominant role of the fluid in determining the responses of a chondrocyte to mechanical stimuli.

CONCLUSIONS

Both creep and relaxation responses of single chondrocytes were investigated in this study using AFM and inverse FEA modeling methodology. The results revealed that PHE model can capture these responses very well and that the effect of fluid was predominant for cell's mechanical behaviors. This model can also be improved to account for other behaviors i.e. swelling effect. Thus, PHE is a very good candidate for exploration of mechanical deformation responses of chondrocyte cells.

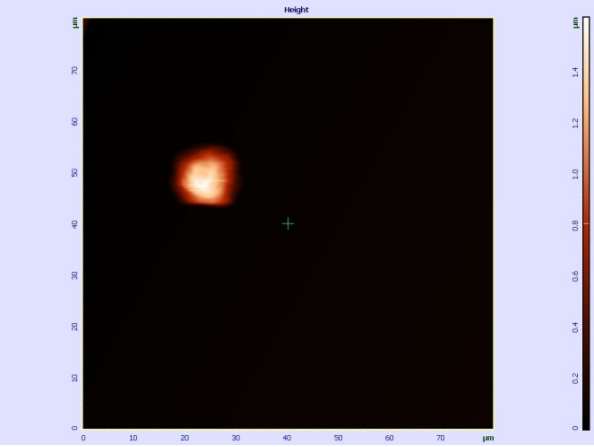

Figure 4 Height image of chondrocyte

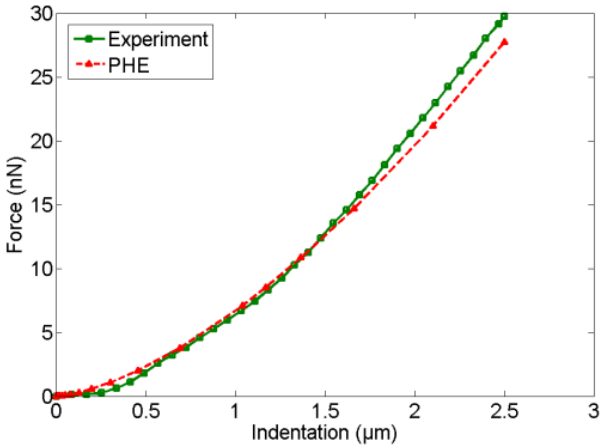

Figure 5 Force-indentation curves of AFM and FEM-PHE model with Neo-Hookean density function

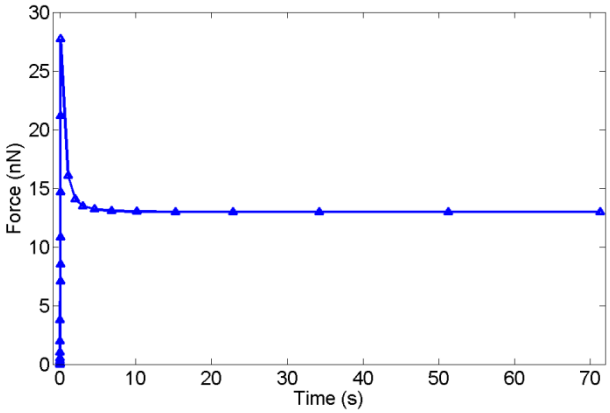

Figure 6 Relaxation behavior of chondrocyte

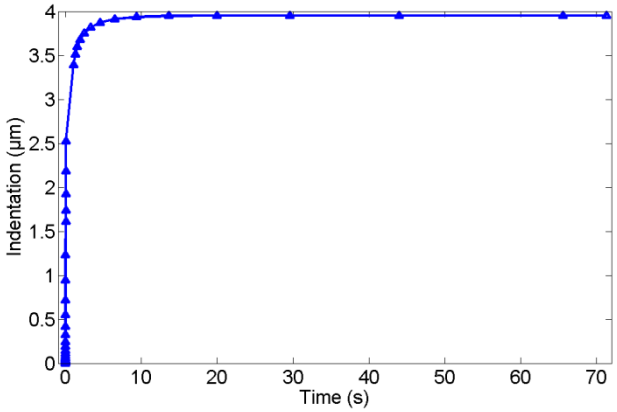

Figure 7 Creep responses of chondrocytes

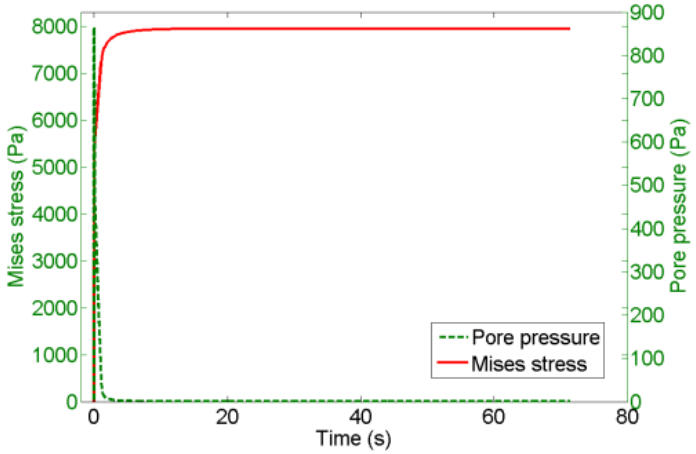

Figure 8 The von Mises or solid skeleton stress and pore pressure versus time curves

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