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Research Paper

Microscale consolidation analysis of relaxation behavior of single living chondrocytes subjected to varying strain-rates

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ABSTRACT

Besides the elastic stiffness, the relaxation behavior of single living cells is also of interest of various researchers when studying cell mechanics. It is hypothesized that the relaxation response of the cells is governed by both intrinsic viscoelasticity of the solid phase and fluid-solid interactions mechanisms. There are a number of mechanical models have been developed to investigate the relaxation behavior of single cells. However, there is lack of model enable to accurately capture both of the mechanisms. Therefore, in this study, the porohyperelastic (PHE) model, which is an extension of the consolidation theory, combined with inverse Finite Element Analysis (FEA) technique was used at the first time to investigate the relaxation response of living chondrocytes. This model was also utilized to study the dependence of relaxation behavior of the cells on strain-rates. The stress–relaxation experiments under the various strain-rates were conducted with the Atomic Force Microscopy (AFM). The results have demonstrated that the PHE model could effectively capture the stress–relaxation behavior of the living chondrocytes, especially at intermediate to high strain-rates. Although this model gave some errors at lower strain-rates, its performance was acceptable. Therefore, the PHE model is properly a promising model for single cell mechanics studies. Moreover, it has been found that the hydraulic permeability of living chondrocytes reduced with decreasing of strain-rates. It might be due to the intracellular fluid volume fraction and the fluid pore pressure gradients of chondrocytes were higher when higher strain-rates applied.

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1. Introduction

Living cells are the basic structural units existing in all known living organisms. They perform several functions and metabolism activities within organs and tissue. It is well known that cells are sensitive to variation in their mechanical and physiological environments. Therefore, studying the mechanical properties and behavior of individual living cells can enhance knowledge of and insight into the role of mechanical forces in supporting tissue regeneration or degeneration, leading to new therapies and treatment. In the literature, the mechanical deformation and relaxation behavior of single cells have been studied widely since it is believed that these properties play an important role in biophysical and biological responses (Guilak 2000; Costa 2004; Nguyen et al., 2014). Understanding these mechanical properties of single cells would provide an insight into not only cell physiology and pathology but also how cell physically interact with its extracellular matrix as well as how its properties influence the mechanotransduction process.

It is hypothesized that the relaxation response subjected to mechanical loading of single cells, which is governed by both intrinsic viscoelasticity of solid phase and fluid-solid interaction mechanisms (Guilak and Mow, 2000; Baaijens et al., 2005; Trickey et al., 2006), is similar to that of fluid-filled tissues (Setton et al., 1993). There are several mechanical models, among which the viscoelastic is probably one of the most common models, have been developed to characterize the relaxation response of single living cells (Cheng et al., 2010; Darling et al., 2006; Guilak et al., 2002). This model has been improved, leading to the thin-layer viscoelastic model in order to consider the effects of the sample thickness (Darling et al., 2007, 2008). However, the viscoelastic models assume the cells to be solid-like materials, whereas they consist of both solid and fluid constituents. As a result, the biphasic (Guilak and Mow 2000; Trickey et al., 2006; Alexopoulos et al., 2005) and poroelastic (Moeendarbary et al., 2013; Zhou et al., 2013; Chan et al., 2012) models have been proposed and utilized to study the relaxation behavior of single living cells. Nevertheless, the purely biphasic and poroelastic models can only capture the flow-dependent (fluid-solid interactions) response of the cells (Baaijens et al., 2005; Chan et al., 2012; Hu et al., 2011). Therefore, a more suitable model, which can consider both solid–solid and fluid–solid mechanisms of cells responses, is necessary. This is a complex issue that would require computational modeling which in turn would require mathematical idealization.

The mechanical properties and responses of tissues and cells subjected to varying rates of loading have been widely studied (Moo et al., 2012; Oloyede et al., 1992; Ewers et al., 2001; Kurz et al., 2001; Quinn et al., 2001; Radin et al., 1970; Nguyen and Gu 2014), wherein has been observed that the response of a tissue can be transformed from the fluid-dominated to a purely elastic behavior at very high to impact rates of loading (Oloyede et al., 1992; Oloyede and Broom, 1993). In a previous study, we reported that the elastic stiffness of single living chondrocyte was dependent on strain-rates and that the porohyperelastic (PHE) model (i.e. an extension of the poroelastic or consolidation theory) was able to capture this strain-rate dependent behavior (Nguyen and Gu, 2014; Nguyen et al., 2014). This paper extends this previous work and examines whether or not the PHE model can be applied to the strain-rate dependent relaxation behavior of chondrocytes.

There are a number of experimental techniques developed to characterize and study the viscoelastic behavior of living cells such as micropipette aspiration, cytoindentation, Atomic Force Microscopy (AFM), etc. (Darling et al., 2006; Sato et al., 1990; Shin and Athanasiou, 1999). Among these techniques, AFM is an advanced method that is capable of high resolution imaging and mechanical properties probing of tissues, cells and artificial surfaces both qualitatively and quantitatively (Touhami et al., 2003; Rico et al., 2005; Zhang and Zhang, 2007; Lin et al., 2007; Kuznetsova et al., 2007; Faria et al., 2008; Yusuf et al., 2012). This facility utilizes a tip of microscopic dimension, which is attached to a very flexible cantilever, to indent the material/sample. The deflection of the cantilever is used to measure the applied force in order to obtain the force-indentation (F–δ) curve (Darling et al., 2006; Faria et al., 2008; Ladjal et al., 2009). This powerful tool is increasingly applied in the study of cell responses to external stimuli and is therefore used in this study.

In order to obtain this insight, AFM stress–relaxation experiments at varying rates of loading were conducted on living chondrocytes. Inverse FEA was conducted with the implementation of the PHE model to extract the material properties under these loading and boundary conditions based on the assumption that this model can adequately capture the strain-rate dependent response. The results were then compared to those of the thin-layer viscoelastic model (Darling et al., 2007), which is one of the most common models for single cells biomechanics, in order to investigate the application of the PHE model for relaxation behavior simulation.

2. Materials and method

2.1. *Sample preparation and AFM set-up*

The primary chondrocytes, which were given from Institute of Health and Biomedical Innovation (IHBI), QUT, Brisbane, Australia, 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) and seeded onto poly-D-lysine (PDL, Sigma-Aldrich) coated cultured petri dish for 1–2 h. The PDL surface was to maintain a strong attachment with a round morphology that demonstrates that they are healthy. All biomechanical testing were conducted at room temperature, and the cells were taken at Passage 2.

The AFM system used was a Nanosurf FlexAFM (Nanosurf AG, Switzerland). A colloidal probe SHOCONG-SiO_2-A-5 (App-Nano) cantilever was used in the experiment. The colloidal probe had a diameter of 5 μm and its spring constant was