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Computational simulation of static/cyclic cell stimulations to investigate mechanical modulation of an individual mesenchymal stem cell using confocal microscopy

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ABSTRACT

It has been found that cells react to mechanical stimuli, while the type and magnitude of these cells are different in various physiological and pathological conditions. These stimuli may affect cell behaviors via mechanotransduction mechanisms. The aim of this study is to evaluate mechanical responses of a mesenchymal stem cell (MSC) to a pressure loading using finite elements method (FEM) to clarify procedures of MSC mechanotransduction. The model is constructed based on an experimental set up in which statics and cyclic compressive loads are implemented on a model constructed from a confocal microscopy 3D image of a stem cell. Both of the applied compressive loads are considered in the physiological loading regimes. Moreover, a viscohyperelastic material model was assumed for the cell through which the finite elements simulation anticipates cell behavior based on strain and stress distributions in its components. As a result, high strain and stress values were captured from the viscohyperelastic model because of fluidic behavior of cytosol when compared with the obtained results through the hyperelastic models. It can be concluded that the generated strain produced by cyclic pressure is almost 8% higher than that caused by the static load and the von Mises stress distribution is significantly increased to about 150 kPa through the cyclic loading. In total, the results does not only trace the efficacy of an individual 3D model of MSC using biomechanical experiments of cell modulation, but these results provide knowledge in interpretations from cell geometry. The current study was performed to determine a realistic aspect of cell behavior.

1. Introduction

One of the main objectives of cell mechanics studies is investigating a fundamental cellular process from the macroscopic to microscopic scale. Cells respond to the applied forces in various temporal and spatial scales, such as proliferation, differentiation, alterations in their shape and regulation of gene expression [1,2]. Cell levels modeling was developed to study maturing adipocytes using acquired geometrical properties and analyzing increased effective stresses with cell maturation [3]. Attempts are on-going to detect the effects of mechanical loads on cell deformations using mechanotransduction, such as plasma membrane strain [4,5]. Also, a 3D computational soft matter model which can show that the cell can sense substrate elasticity was developed [6]. Previous investigations reported that in vitro loadings initiated mechanotransduction in different types of stimuli and in a controlled cell culture medium [7].

Since the nucleus plays an important role in the mechanical behavior of the cell, it has been modeled as an incompressible elastic material and the results revealed interactions between the deformed nucleus and cell adhesion [8].

Knowledge of cellular changes in response to alterations in cellular components is largely based on empirical studies which investigate how these changes would damage cells and tissues. Hence, researchers have used finite elements method (FEM) to study this issue and developed two and three-dimensional models using such approaches [9]. Simulations such as that conducted by Gefen [10] have modeled mouse fibroblast cells in three dimensions and evaluated mechanical properties of the cells [10]. In another study, skeletal muscle cells were analyzed in an undifferentiated state with a 3D geometry and large cellular deformations in response to compressive and tensile loads were examined [11]. Elucidation of biomechanical factors is essential, because they affect cellular responses. Mechanotransduction mechanism can provide a novel guideline for mechanical modulation of cells based on cell-specific studies (for example regeneration, bone homeostasis, and cell therapies for osteoarthritis) [12,13].

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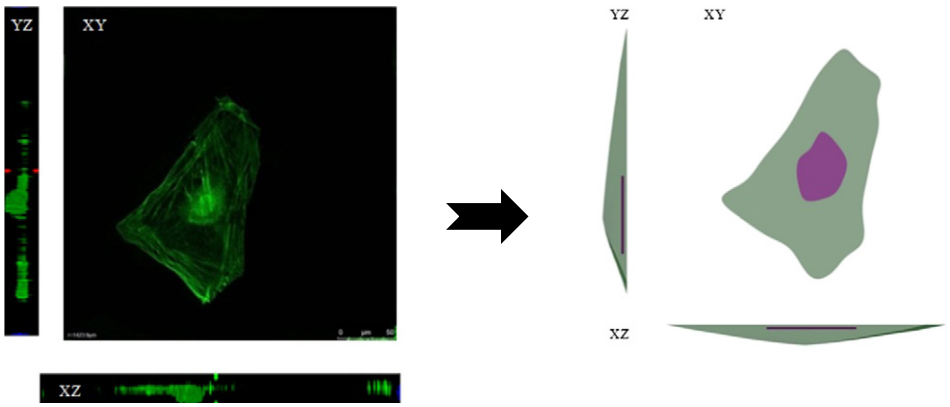

Fig. 1. Preparation and staining the cells with FITC Phalloidin; (A) principle and simplified optics of a confocal microscope; (B) three-dimensional image obtained by confocal microscopy. Scale bar: 50 μm.

Stem cells can be used in cell and gene therapy for various types of diseases, because of their self-renewal and multi-lineage differentiation capabilities, and therefore, are considered as a suitable cell source for preparing an alternative tissue in regenerative medicine [14]. By developing a perspective in mechanical and architectural features, recent studies on cellular mechanical properties show that bone cells, cartilage cells, and fat cells exhibit different properties as compared to primitive mesenchymal stem cells (MSCs). While most stiff cells have the potential to differentiate into bone cells. Cells with higher viscosity have the potential to differentiate into chondrocytes and softer cells tend to differentiate into fat cells [15]. Another research by Guilak et al. [16] describes the mechanical regulation of a cell component (nucleus) and its behavior based on mechanotransduction mechanisms. In this regard, behavior of stem cells under platen compression was investigated using computational methods. The results showed that the cell can sense its substrate stiffness which is strongly related to the cell behaviors such as cell adhesion and migration [17].

Morphology of cells in general and MSCs in particular, make it possible to examine the effect of cell geometry on its fate and in particular differentiation [18,19]. Our previous study demonstrated stem cell responses to fluid shear stress based on a fluid structure interaction (FSI) simulation analyzing cell deformations. Tolerable levels of shear stress were exerted on a MSC which show intracellular stress and strain values as a response to oscillatory flow [20].

Standing on legs is a simple example of applied compressive loading on bone cells and cartilage. This investigation seeks to evaluate the mechanical behavior of the cell as a result of external or internal compressive loads. This analysis is so important to quantify the phenomena of regulating MSCs functions through mechanotransduction and in pressure ulcers and tissue injury researches such as investigations about cell compressive stimulations conducted by Gefen [10]. Also, by virtue of new tools to aid stem cell therapy, stem cell was considered as an attractive choice to model cellular response based on 3D confocal imaging. In this research, the simulation is based on applying forces in the range which they occur within the body. Then, mechanophysiological conditions, such as what happens in articular cartilage, required for chondroinduction of MSCs are simulated through a compressive loading regime. The main objective is to analyze the deformed cell so as to determine the stress distribution within the cell and its internal components. A major novelty of this investigation is the incorporation of viscohyperelastic behavior for the realistic stem cell geometry. Furthermore, comparison between static and cyclic loads seems to be essential to design in vitro tests. A practical approach for such a study is to employ FEM analysis for generated stresses within the cell and its components due to the application of a compressive load over time. Thereafter, cell components stress distribution and deformation gradients and other desired parameters in large deformations were analyzed.

Table 1
Detailed sizes of the mesenchymal stem cell.

Geometrical properties	Geometrical size
Cell height (μm)	16
Maximum width of the cell (μm)	65
Cell area (μm^2)	1767/16
Area of the cell membrane (μm^2)	0.2997
Cell volume (μm^3)	6.9385
Surface area of the cell nucleus (μm^2)	24486
Volume of the cell nucleus (μm^3)	2.31283

2. Materials and methods

2.1. Cell culture and specifying 3D dimensions of the cell for confocal microscopy imaging

Mesenchymal stem cells were provided by the National Cell Bank of Pasteur Institute of Iran and were used as undifferentiated cells which kept in vitro in Dulbecco's Modified Eagle's medium (DMEM; with 4.5 g/l glucose Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich) and 1% Penicillin/Streptomycin (10,000 μ/ml penicillin and 10 mg/ml streptomycin). The cells in the second passage were trypsinized and prepared for the subsequent stages of the procedure. The smallest adjustable focal volume is of great importance when using confocal microscopy (Zeiss Bio-Rad MRC 1024 model). Here, fluorescent staining of cells (actin filaments) was used to identify the cell morphology and cells were fixed for permeability staining. One day before staining, 12,000 cells were plucked off and partially trypsinized and grown upon the coverslip which is coated with collagen type I on a round cover glass. After 24 h of transfection, the cells are ready for staining. For the staining, initially, the cell culture medium was evacuated and the cells were washed several times with PBS. Then, they were covered with formaldehyde 3.7% in PBS for 3 to 5 min and after that the cells were fixed. After discharging the culture medium on the cells, they are washed several times with PBS. In the duration of 10 min, the cells were treated in Triton X-100 0.1% in PBS. This substance causes permeability of cell membranes until the color enters into the cells easily. The cells were washed several times with PBS and then dissolved Phalloidin was placed in PBS and the cells were incubated for 45 min. Over time, these cells were washed several times with PBS. Then, they get ready for imaging by confocal microscopy. In confocal microscopy, a laser light was irradiated along the Z-axis to scan the cell surface area and the resulting data were converted to an image. Then, three-dimensional images were made using microscopic sections of different cell depths (Fig. 1A). Maximum height and width of the cell,