available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jmbbm

Technical note

Evaluating the effective shear modulus of the cytoplasm in cultured myoblasts subjected to compression using an inverse finite element method

Noa Slomka[a], *Cees W.J. Oomens*[b], *Amit Gefen*[a,*]

[a] *Department of Biomedical Engineering, Faculty of Engineering, Tel Aviv University, Tel Aviv 69978, Israel*
[b] *Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands*

ARTICLE INFO

Article history:
Received 30 December 2010
Received in revised form
5 April 2011
Accepted 7 April 2011
Published online 19 April 2011

Keywords:
Cellular mechanics
Mechanical properties
Reverse engineering
C2C12 skeletal muscle cells
Confocal microscopy

ABSTRACT

In the present study, we employ our recently developed confocal microscopy-based cell-specific finite element (FE) modeling method, which is suitable for large deformation analyses, to conduct inverse FE analyses aimed at determining the shear modulus of the cytoplasm of cultured skeletal myoblasts, G_{cp}, and its variation across a number of cells. We calibrate these cell-specific models against experimental data describing the force–deformation behavior of the same cell type, which were published by Peeters et al. (2005b) [J. Biomech.]. The G_{cp} calculated for five different myoblasts were contained in the range of 0.8–2.4 kPa, with the median value being 1 kPa, the mean being 1.4 kPa, and the standard deviation being 0.7 kPa. The normalized sum of squared errors resulting from the fit between experimental and calculated force–deformation curves ranged between 0.12–0.73%, and Pearson correlations for all fits were greater than 0.99. Determining the mechanical properties of the cytoplasm through cell-specific FE will now allow calculation of cell stresses using cell-specific FE under various cell loading configurations, in support of experimental work in cellular mechanics.

1. Introduction

Computational modeling is increasingly being used in mechanotransduction studies, to correlate between mechanical loading applied and sensed by cells, and their biochemical and biophysical responses. Loads in cells, and loads that cells are able to sense, are strongly affected by the mechanical properties of the cell components, such as those of the cytoplasm and nucleus, as well as by the mechanical properties of the cell surroundings. The last decade brought about breakthroughs in measurement techniques to determine cell stiffnesses, including atomic force microscopy (e.g. Collinsworth et al., 2002, Darling et al., 2008 and Mathur et al., 2001), micropipette aspiration (e.g. Hochmuth, 2000 and Zhao et al., 2009), cell compression and cytoindentation (e.g. Koay et al., 2003 and Peeters et al., 2005a), magnetic and optical tweezers (e.g. Kamgoué et al., 2007, Laurent et al., 2002) and bead rheometry (e.g. Bausch et al., 1998). These techniques are generally able to provide either hyperelastic or time-dependent viscoelastic cell material properties.

* *Corresponding author.* Tel.: +972 3 640 8093; fax: +972 3 640 5845.
E-mail address: gefen@eng.tau.ac.il (A. Gefen).

1751-6161/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jmbbm.2011.04.006

However, when applied to measurements in intact cells, these methods all measure either the effective cell stiffnesses (i.e. where cells are considered as composite bodies), or the stiffnesses of components - but under the influence of other interacting cellular structures. Additional modeling is therefore typically required to extract mechanical properties of individual cell components (e.g. the cytoplasm or nucleus) from the measured structural properties. An exception to this would be that one of the cell components is tested directly, in isolation, such as in the nucleus stiffness studies of Guilak et al. (2000) and Caille et al. (2002). These two latter studies used mechanical and chemical treatments, respectively, to isolate the nuclei prior to testing, but such treatments are inadequate for isolating the cytoplasm. Hence, determining the mechanical properties of the cytoplasm must involve modeling, such as inverse finite element (FE) analyses.

Inverse FE analyses in cellular mechanics studies attempt to evaluate the stiffnesses of individual cell components from measurements of stiffness of the whole cell obtained using one of the aforementioned techniques (Caille et al., 2002; Gladilin et al., 2007; Gladilin and Eils, 2010; Kim et al., 2011; McGarry, 2009; Ofek et al., 2009). However, cells in these studies were so far modeled as idealized shapes, such as spheres, domes, ellipsoids or the like, which inherently limits the prediction capacities of the modeling with regard to the desired mechanical properties, as well as in regard to variation of the properties in a cell population. In the present study, we employ our recently developed confocal microscopy-based cell-specific FE modeling method, which is suitable for large deformation analyses (Or-Tzadikario and Gefen, 2011; Slomka and Gefen, 2010), to conduct inverse FE analyses aimed at determining the shear modulus of the cytoplasm of cultured skeletal myoblasts, and its variation across a number of cells. We calibrate these cell-specific models against experimental data describing the force–deformation behavior of the same cell type, which was published by Peeters et al. (2005b). Determining the mechanical properties of the cytoplasm through cell-specific FE will allow calculation of cell stresses using cell-specific FE under various cell loading configurations, in support of experimental work in cellular mechanics.

2. Methods

2.1. *Cells and computational configuration*

In this study, we utilized our previously-developed confocal microscopy-based three-dimensional (3D) cell-specific FE modeling methodology for simulating cellular mechanics experiments involving large cell deformations, which was introduced in Slomka and Gefen (2010). We previously demonstrated the versatility of cell-specific FE modeling in studying cells with substantially different geometrical shapes, e.g. myoblasts (Slomka and Gefen, 2010), fibroblast-like pre-adipocytes and mature adipocytes (Or-Tzadikario and Gefen, 2011). Following this methodology, undifferentiated C2C12 cells from a murine cell line (#CRL1722, ATCC, VA, USA) were fixated and their actin filaments were stained with FITC-labeled Phalloidin in order to confocally-demonstrate the cell contours. Cells were allowed to fully attach to the culture plate prior to fixation, hence the modeling described below represents mature cell attachments. Stained cells were then scanned using a Zeiss LSM-510 confocal microscope, using a ×100 magnification lens with a numerical aperture of 1.4 and a pinhole size of 154 μm, at 0.4 μm intervals. The 3D geometry of each cell, including its nucleus, was then reconstructed from these z-stack images, using Solid Modeling software (SolidWorks 2009, SolidWorks, MA, USA). Solid models of 5 different cells, which were randomly selected from the fluorescently-stained C2C12 culture, were created through this process (Fig. 1). The main geometrical characteristics of each cell are reported in Table 1.

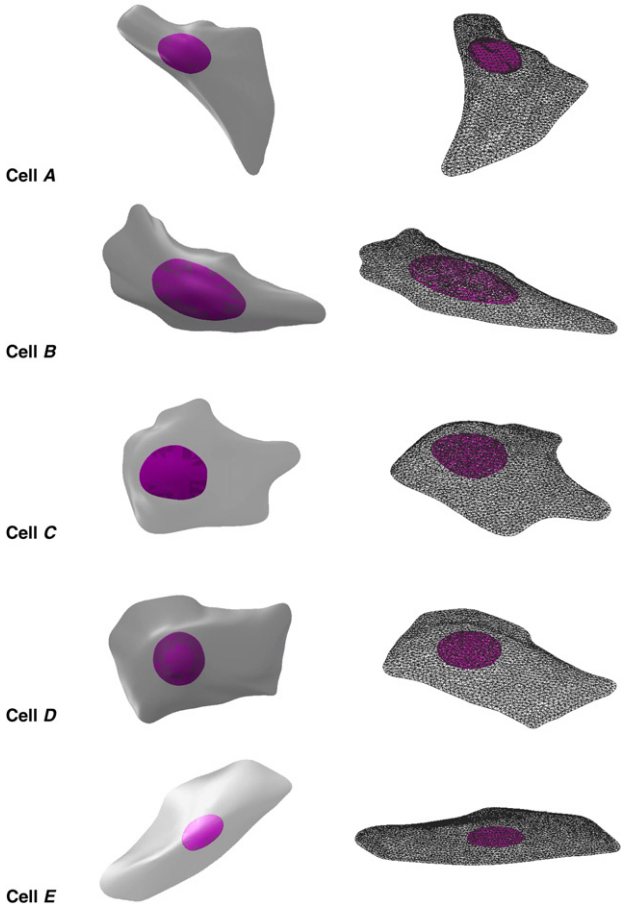

Fig. 1 – The confocal-based cell-specific finite element (FE) models for the five cells studied herein. Top views of the solid models of cells are shown in the left column, and isometric views of the corresponding FE meshes are shown in the right column. The cells are not scaled with respect to each other; cell dimensions are provided in Table 1.

The 3D geometry of each cell was imported to an FE solver (ABAQUS version 6.9FE, SIMULIA, RI, USA). A plasma membrane with thickness of 10 nm (Becker et al., 2003; Chen and Moy, 2000), a culture dish as a foundation and a compressive plate on top, were incorporated into the FE model of each cell as well, for later on simulating the cell compression experiments of Peeters et al. (2005b). The cytoplasm, nucleus and plasma membrane components were