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The effect of compressive deformations on the rate of build-up of oxygen in isolated skeletal muscle cells

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ABSTRACT

In this study we integrated between confocal-based cell-specific finite element (FE) modeling and Virtual Cell (VC) transport simulations in order to determine trends of relationship between externally applied compressive deformations and build-up rates of oxygen in myoblast cells, and to further test how mild culture temperature drops (~3 °C) might affect such trends. Geometries of two different cells were used, and each FE cell model was computationally subjected to large compressive deformations. Build-up of oxygen concentrations within the deformed cell shapes over time were calculated using the VC software. We found that the build-up of oxygen in the cells was slightly but consistently hindered when compressive cell deformations were applied. Temperature drops characteristic to ischemic conditions further hinder the oxygen built-up in cells. In a real-world condition, a combination of the deformation and temperature factors should be anticipated, and their combined effect might substantially impair cell respiration functions.

1. Introduction

Deep tissue injury (DTI) is a severe type of pressure ulcer which occurs internally. A DTI is likely to initiate in skeletal muscle tissues, in vicinity to a bony prominence, and it then spreads in other subdermal tissues [1–6]. Individuals with impaired motor and/or sensory capacities are susceptible to DTI, and may develop serious complications resulting from the DTI, including sepsis, osteomyelitis, myocardial infracts, rental failure and multiple organs dysfunction [4]. Due to the silent nature of progress of DTI in subdermal tissues, and since it only becomes visible when reaching superficial tissues, at which time, purple or black spots appear on the skin, it is critical to understand the condition and its aetiology.

Indeed, several theories were proposed to explain how DTI may occur, and all these theories are related to sustained mechanical loads in muscle tissues. Specifically, the following factors were suggested to contribute to the progressive cell death occurring in DTI: structural damage in cells [3,5], ischemia [6], impaired lymphatic drainage [1,2], reperfusion injury [7,8], and apoptotic response to an initial necrosis [9]. Our group previously showed, using finite element (FE) modeling, that compressive loading in soft tissues, imposed by a bony prominence during weight-bearing, translates to tensile loads at the cellular scale [10,11]. These tensile loads in the cells stretch the plasma membrane (PM) as well as other intracellular structures, e.g. the nucleus [11]. Using mathematical modeling, we further studied a hypothesis that stretches in the PM may locally alter its permeability, and, over time, allow molecules to penetrate or leave a cell, in an uncontrolled manner, which disrupts the homeostasis [10]. Such interference with the cellular homeostasis, caused by bone-compression-driven prolonged PM stretches, may occur on top of any direct mechanical damage effects. However, the theoretical modeling previously developed in this regard [10] was simple in the sense that it only involved a one-dimensional solution to a diffusion through a straight PM, hence, no cell geometry was taken into account. It is unclear how metabolites such as oxygen might diffuse through a curved PM, and more importantly, how the diffusivity of metabolites would change if compressive loads are imposed on the cell, so that its PM is being stretched [10,11].

In order to make a first step towards quantifying potential effects of compressive strains applied to cells on the oxygen flux through their PM, as related to the aetiology of DTI, we used computational modeling here, which was developed mostly by means of the Virtual Cell (VC) software (NRCAM, Connecticut, USA). The modeling considered real planar geometries of skeletal muscle cells – obtained from confocal microscopy – whose deformed shapes at different compression levels were calculated using cell-specific FE [11,12], and were then employed to study the oxygen build-up rates in the deformed cells as function of the cell compression levels. We

Abbreviations: 2D, Two-dimensional; 3D, Three-dimensional; DTI, Deep tissue injury; ECS, Extracellular space; FE, Finite element; GCD, Global cell deformation; MM, Michaelis–Menten; OCR, Oxygen consumption rate; PM, Plasma membrane; ROI, Region of interest; VC, Virtual Cell.

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further studied the effect of temperature changes on the oxygen flux in the deformed cells, since our previous work indicated that in animal models of DTI, tissue temperatures decrease by ~3 °C at ischemic sites [13]. Hence, the effects of cellular deformations and ischemia-related temperature drops were studied here in compressed isolated muscle cells to determine whether these factors might affect the oxygen flux into the deformed cells, independently or together. This is the first computational modeling attempt to look at the effect of deformation in individual isolated cells on intracellular oxygen diffusion, for which we have built the VC simulations into cell-specific FE [11,12]. Accordingly, this work opens a new pathway of expanding cell-specific FE to study multiphysics phenomena, that is, different physical processes which affect each other, at the cell level [14,15]. We chose oxygen as the diffusing molecule not only because it is required for cellular respiration, but also because its transport parameters, including the permeability coefficient, diffusion coefficient, oxygen consumption rate (OCR) and oxygen concentrations in cells and in the extracellular space (ECS), are all well characterized in the literature [16–19]. Thus, our objective was to determine trends of relationship between externally applied compressive deformations and build-up rates of oxygen in the cells, and to further test how might mild temperature drops (~3 °C) affect such trends.

2. Methods

The VC modeling software was used to study oxygen transport in uniaxially compressed cultured cells. Compression of cultured individual cells *in vitro* is conducted in experimental settings such as that described by Peeters and colleagues [20] to study cellular-scale processes and properties that are relevant to DTI such as cell stiffness and strength under compression. In order to simulate these experiments computationally, geometries of two different myoblast cells were acquired from confocal microscopy scans. Each cell model was compressed computationally using FE software (ABAQUS version 6.8, SIMULIA, RI, USA) to simulate the large tensional strains that occur at the free boundaries of the cells during such compression experiments, using the previously developed cell-specific FE modeling methodology [11]. We quantified cell deformations using the global cell deformation (GCD) measure, which is defined as the difference between the undeformed and compressed cell heights over the undeformed cell height, in %. The build-ups of oxygen in the cytosol and nucleus of the deformed cells were studied as function of the GCD. We further explored the effect of a 3 °C temperature reduction on the aforementioned flux of oxygen, on top of the effect of applied compressive deformations. Model compartments and species simulated in each cell included the ECS, PM, cytosol, nucleus and the diffusing oxygen molecules (Fig. 1). A detailed description of each of these modeling aspects follows.

2.1. Geometry

A methodology of confocal microscopy-based three-dimensional (3D) cell specific FE modeling, recently introduced by our group, was used to build the geometry of cells based on confocal images. This methodology is described in detail in Slomka and Gefen [11], and its essential components are summarized here for completeness. Undifferentiated myoblasts from a C2C12 cell line (ATCC, VA, USA) stained with phalloidin were scanned using a confocal microscope. The confocal *z*-stack images were imported into solid modeling software (SolidWorks 2008, MA, USA) in order to build 3D geometrical models of the undeformed cells. The boundaries of the cells and their nuclei were drawn manually for each confocal slide and then lofted to create the 3D models, as

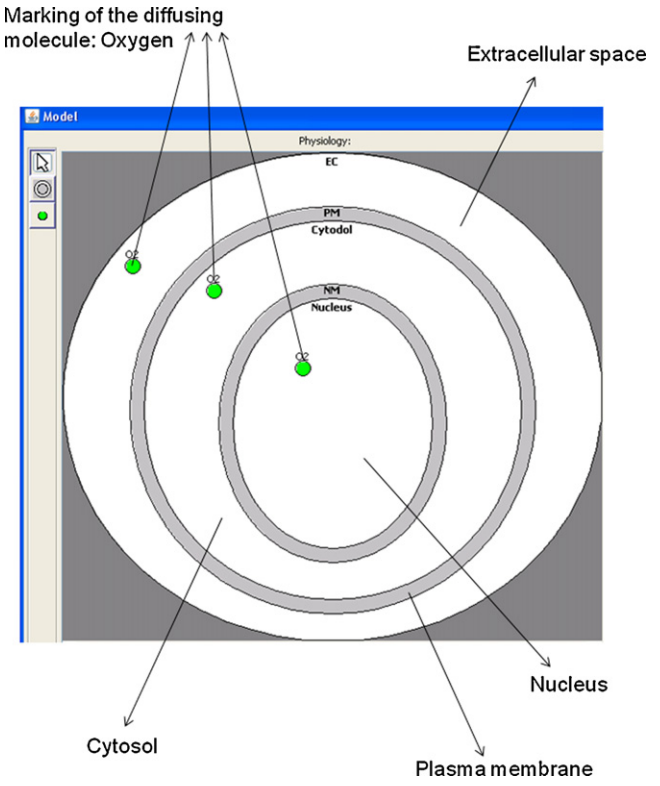

Fig. 1. The schematic model design in the Virtual Cell (VC) software, showing the VC program components used in the present simulations.

Table 1
Geometrical characteristics of the two-dimensional projections of the two cells studied herein (Fig. 2), in their undeformed condition.

Geometrical property	Cell *A*	Cell *B*
Maximal cell width [μm]	53.8	43.3
Maximal nucleus width [μm]	20.6	12.6
Cell perimeter [μm]	161.7	114.4
Nucleus perimeter [μm]	61.7	34.6
Cell surface area [μm^2]	1453.8	912.3
Nucleus surface area [μm^2]	267.3	119.6

described in Slomka and Gefen [11]. A rigid substrate was added beneath the cells and a compressive plate was added on top, in a configuration simulating classic cell compression experiments [20]. The cell models were then imported to ABAQUS, where the compressive platen was lowered to induce GCD levels up to 53% in cell *A* and 43% in cell *B*.[1] From these simulations, we were able to extract 2D projections of the deformed cell shapes, which included the cell nucleus (characteristic dimensions of these 2D projections are provided in Table 1). These projections were obtained for the 30%, 43% and for cell *A*, also for the 53% GCD levels (Fig. 2). Hence, the full 3D cell geometry represented in the FE analyses of the deformed cell shapes was reduced to 2D cell projections, with dimensions specified in Table 1, for the purpose of the oxygen transport studies which are described further. Oxygen diffusion around and within each cell was studied in a 2D region of interest (ROI) with size of 162 μm × 100 μm, containing the cell and surrounding ECS (Fig. 3).

[1] The maximal GCD that can be reached in cell compression simulations using cell-specific FE depends on the cell size and location of the nucleus (Slomka and Gefen 2010), hence maximal GCD differ between the two cells *A* and *B*.