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Three dimensional multi-scale modelling and analysis of cell damage in cell-encapsulated alginate constructs

Karen Chang Yan [a], Kalyani Nair [b], Wei Sun [b,*]

[a] Mechanical Engineering Department, The College of New Jersey, Ewing, New Jersey, USA
[b] Department of Mechanical Engineering and Mechanics, Drexel University, Philadelphia, Pennsylvania, USA

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ABSTRACT

One of the major challenges in scaffold guided regenerative therapies is identifying the essential cues such as mechanical forces that induce cellular responses to form functional tissue. Developing multi-scale modelling methods would facilitate in predicting responses of encapsulated cells for controlling and maintaining the cell phenotype in an engineered tissue construct, when mechanical loads are applied. The objective of this study is to develop a 3D multi-scale numerical model for analyzing the stresses and deformations of the cell when the tissue construct is subjected to macro-scale mechanical loads and to predict load-induced cell damage. Specifically, this methodology characterizes the macro-scale structural behavior of the scaffold, and quantifies 3D stresses and deformations of the cells at the micro-scale and at a cellular level, wherein individual cell components are incorporated. Assuming that cells have inherent ability to sustain a critical load without damage, a damage criterion is established and a stochastic simulation is employed to predict the percentage cell viability within the tissue constructs. Bio-printed cell-alginate tissue constructs were tested with 1%, 5% and 10% compression strain applied and the cell viability were characterized experimentally as $23.2 \pm 16.8\%$, $9.0 \pm 5.4\%$ and $4.6 \pm 2.1\%$. Using the developed method, the corresponding micro-environments of the cells were analyzed, the mean critical compressive strain was determined as 0.5%, and the cell viability was predicted as 26.6 ± 7.0, 13.3 ± 4.5, and 10.1 ± 2.8. The predicted results capture the trend of the damage observed from the experimental study.

1. Introduction

Tissues in the body undergo continuous growth and remodelling in response to environmental cues including biomechanical forces. It is observed that bone changes its shape, density, and stiffness with changing mechanical loads (Turner and Pavalko, 1998; Mullender et al., 2004); blood vessels are capable of adaptive change in response to varying shear stresses and pressures (Owens, 1996; Williams, 1998). Abnormal mechanical loading conditions can alter cellular function and change the extracellular matrix (ECM), eventually lead to tissue or organ pathologies such as osteoporosis, osteoarthritis, tendinopathy, and atherosclerosis (Chicurel et al., 1998; Grodzinsky et al., 2000; Ireland et al., 2001; Ross, 1986). Hence, for applications from studies on disease progression to tissue engineering, it is essential to understand how cells sense external forces and how mechanical signals are translated into the cascade of biochemical events (Ingber, 1997).

While current technologies enable researchers to observe responses of cells, sub-cellular components, and biomolecules under mechanical stimuli through high precision probes and imaging techniques, computational models have also been developed to interpret the experimental observations (Vaziri and Gopinath, 2008). Since a wide range of temporal and spatial scales are involved, computational models often incorporate multiple scales with a starting point close to either end of the spectrum: the single-molecule level or tissue level. Buechler related the response of collagen fibrils to its distinctive structure and amino acid composition (Buehler, 2006). Coughlin and Stamenovic addressed the nature of the force-transmitting filaments in the cytoskeleton (Coughlin and Stamenovic, 2003). Multi-scale finite element approaches have been developed to predict the local cell deformations in tissue constructs under macroscopic loads (Breuls et al., 2002), and to characterize the interactions between the chondrocyte and the ECM (Guilak and Mow, 2000). However, a full characterization needs collective information at different length scales. In particular, when a tissue construct is subjected to mechanical loads, multi-scale models can provide accurate stress and strain information at the cell level from cell–tissue interactions; the results can aid in predicting force transmission to the sub-cellular components, which can be

* Corresponding author. Tel.: +1 215 895 5810; fax: +1 215 895 2094.
E-mail address: sunwei@drexel.edu (W. Sun).

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used for developing models addressing mechanical force-induced biochemical events. Nevertheless, challenges lie in coupling between the length scales. For tissue engineering applications, mechanical forces are involved in fabricating cell-encapsulated tissue constructs, stimulating cell/tissue growth and answers to the following questions are critical: how external loads are transferred within the tissue construct, how the cells sense these loads and translate into the sequence of events affecting cell behaviours, and what are the limits of mechanical loads that cells can sustain without compromising their normal functionality? Computational models would provide the information in the micro-environment of encapsulated cells and further aid in maintaining and controlling the cell phenotype to form functional tissue in an engineered construct.

The objective of this research is to develop a three dimensional multi-scale modelling approach for the analysis of cell damage in bio-printed tissue constructs. Finite element analyses (FEA) at macro, multi-cellular, and single-cell level provide the stress and deformations in the micro-environment of encapsulated cells due to macroscopic loads. Furthermore, it is hypothesized that the mechanically induced deformation can lead to cell injury and/or death if it exceeds a certain limit, and such a limit can be considered as a random variable due to variations in terms of cell responses. A damage theory is formulated to link the cell viability to the applied macro-forces; a stochastic simulation is developed to predict the resulting cell viability within the construct. Specifically, cell damage in encapsulated alginate constructs under compression was analyzed using the developed method. To measure mechanical-load induced cell damage, cell viability was determined via both experimental characterization and theoretical predictions; cell-to-cell variations including the mechanical properties and sizes were examined as contributing factors for large variations in the experimental results.

2. Experimental characterization

Alginate/endothelial cell tissue constructs were printed and tested under uniaxial compression. Endothelial cells were chosen because of its usefulness in angiogenesis and cardiovascular engineering (Bader et al., 1998). A proprietary cell-printing system was used for printing 3D tissue constructs (Khalil and Sun, 2007). The system was optimized to minimize cell damage (Chang et al., 2008; Nair et al., 2009). Rat adrenal medulla endothelial cells (RAMEC) (ATCC, MA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) and then mixed with a 1.5% (w/v) alginate solution with the cell concentration as 1×10^6 cells/ml; the mixture was printed and cross linked with 0.5% $CaCl_2$ (Nair et al., 2009). Samples were 9 mm in thickness and 4 mm in radius. Compressive strains of 1%, 5% and 10% were applied using a 4442 Instron mechanical tester. Three samples from each set were analyzed with apoptotic assay immediately after compression testing. Additional printed samples were used as controls.

Annexin V staining kit (Biovision, Mountainview, CA) was used for quantifying live, injured and dead cells. When used in conjunction with dye measuring membrane integrity, early apoptotic cells (annexin-V positive only) can be distinguished from late apoptotic/necrotic cells (annexin-V and membrane integrity measuring dye positive). Briefly, each sample was treated with 300 μl of EDTA for dissolution of alginate; 500 μl of 1X Binding Buffer, 5 μl of Annexin V-FITC and 5 μl of propidium iodide (PI). The cell suspension samples were viewed under a fluorescence microscope, and corresponding images were analyzed following the process described by Shounan et al. (1998). Percentages of live, injured and dead cells were determined.

3. Three dimensional multi-scale modelling

The multi-scale FEA has been used to establish linkage between the microscopic behavior and the macroscopic phenomena for heterogeneous materials (Smit et al., 1999; Kouznetsova et al., 2001; Wang and Yan, 2005). It may be utilized to determine the cell responses to the external load and understand the effects of the parameters at the different scales. Fig. 1 depicts schematic of the methodology. At the macro-level, the tissue construct is modelled as a homogeneous material. With detailed microstructures restored to a region of interest, a meso-level analysis incorporating multiple cells is performed to determine the local deformation and stresses within the region. Utilizing the local deformation and stress field, a single cell is then analyzed to determine responses at sub-cellular level. A single-cell damage criterion is developed to predict cell viable state, and a linkage between the individual cell state and the cell viability observed experimentally is established via a stochastic simulation. Specifics at each level are detailed below.

3.1. Macro-level analysis

3D FE models were developed to simulate the compression tests. In our previous study, mechanical behavior of tissue constructs were characterized experimentally and the results suggest that the Ogeden model produces a much closer approximation to the test data comparing with the Neo Hookean and Mooney–Rivlin models (Nair et al., 2008). In the Ogden model (Boyce and Arruda, 2000; ABAQUS, 2002), the strain energy

Table 1
Material constants for the Ogden polynomial with $n=4$.

i	1	2	3	4
μ_i	−0.50	0.23	0.65	−0.37
α_i	8.71	9.37	7.83	7.66

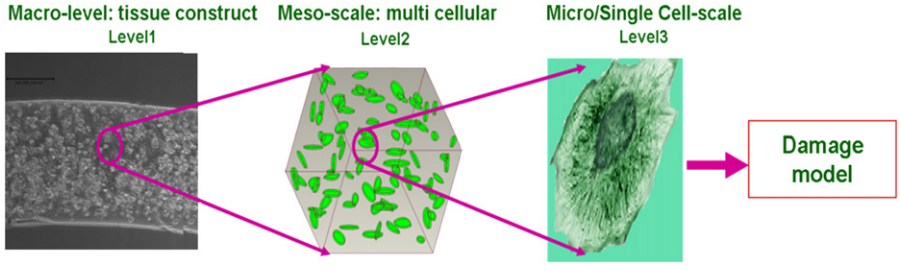

Fig. 1. Schematic of overall methodology for analysis of single cell damage.