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SPATIOTEMPORAL INTRACELLULAR DEFORMATION OF CELLS DURING FREEZING-INDUCED CELL-FLUID-MATRIX INTERACTIONS

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INTRODUCTION

Freezing of biomaterials is emerging as one of the key biotechnologies in cell/tissue engineering, medicine and biology. Its applications include – 1) preservation of cell/tissue engineering products, 2) quality control of biospecimens cryopreserved in tissue banks and repositories, and 3) synthesis procedures of biomaterials such as decellularization of native tissues to create acellular (i.e., cell-free) complex three-dimensional extracellular matrices (ECMs). Traditionally, research efforts have focused on determining optimal freeze/thaw (F/T) protocols with chemical additives, so called cryoprotective agents, for a given cell/tissue-type by comparing the outcomes of F/T protocols, which are mainly gauged by cell viability. Although cell viability is the major constituent, it has recently been recognized that other features beyond viability are also critical to the functionality of biomaterials, including the microstructure of the ECM, the status of cell-matrix adhesion, and the cytoskeletal structure and organization [1, 2, 3].

Many biological tissues and biomaterials can be approximated as hierarchical porous structures saturated with fluids. The ECM has a fibrous collagen porous structure filled with interstitial fluid, and the cytoplasm has a fibrous cytoskeleton porous structure filled with cytosol [4, 5]. The ECM is connected to the cytoplasm through a complex of proteins on the cell membrane, which is called the "cell-matrix adhesion complex" (CMAC). Thus, in order to successfully freeze/thaw complex biomaterials, we hypothesize that the biphasic (i.e., porous solid and fluid) cytoplasm plays a critical role in freezing-induced biophysical processes. This hypothesis is postulated based on a nanoscale fluid-structure interaction mechanism in the cytoplasm, and hierarchical fluid-structure interactions in the intra-/extra-cellular spaces, which are connected via the CMACs.

In the present study, we developed an experimental technique to measure the spatiotemporal intracellular deformation of cells during freezing-induced cell-fluid-matrix interactions. The measurement results are discussed to establish the mechanistic understanding of freezing-induced biophysical processes around cells within the ECM.

MATERIALS AND METHODS

Cell Culture and Fluorescent Bead Internalization in Cells

Human dermal fibroblasts were cultured and harvested as described previously [3]. The harvested cells were labeled by internalizing 520 nm fluorescent beads (Thermo Scientific) according to the protocol developed previously [6].

Engineered Tissues

Engineered tissues (ETs) were prepared after modification of the method described in Ref. [3]. Briefly, collagen gel was polymerized inside custom-made containers. After polymerization, fibroblasts were seeded on or within the collagen gel. After cells were attached, the ETs were cultured in medium for approximately 24 hours.

Freezing and Imaging

ETs were directionally frozen on a cryostage (Linkam, MDS 600). The cryostage was cooled in a way to mimic the temperature history in ETs as obtained during macroscopic freezing [3]. While freezing, the ETs were imaged using a fluorescence microscope (Olympus BX51) with a high-speed CMOS camera (Hispec I, Fastec imaging) at 40X magnification and in 10 msec interval (Figure 1(a)).

Image Postprocessing and Data Analysis

The captured images were postprocessed using ImageJ, and the locations of beads (i.e. X (t) and Y (t) positions for each bead) were tracked by Metamorph. Subsequently, the 2D tracking data were smoothed using a Robust Locally Weighted Scatter Plot Smoothing method [7]. Using this smoothed data, cells were discretized into

triangular elements (Figure 1(b)) using the Delaunay algorithm [8]. The spatiotemporal deformation of each triangular element was quantified using the large deformation analysis method [9]. Through the analysis the substrate rotation and translation were nullified, and only the local intracellular deformation was captured. From the initial and deformed configurations of the triangles, the deformation gradient tensor and Left Cauchy Green tensor were evaluated. The eigenvalues of the Left Cauchy Green tensor gave the 2D stretch ratios λ_1 and λ_2. The local area-based first invariant given by $I_1 = \lambda_1^2 + \lambda_2^2$ was computed to characterize element deformation. I_1 is a measurement of the combined expansion and compression of an element. In Figure 1(c) the procedure is explained.

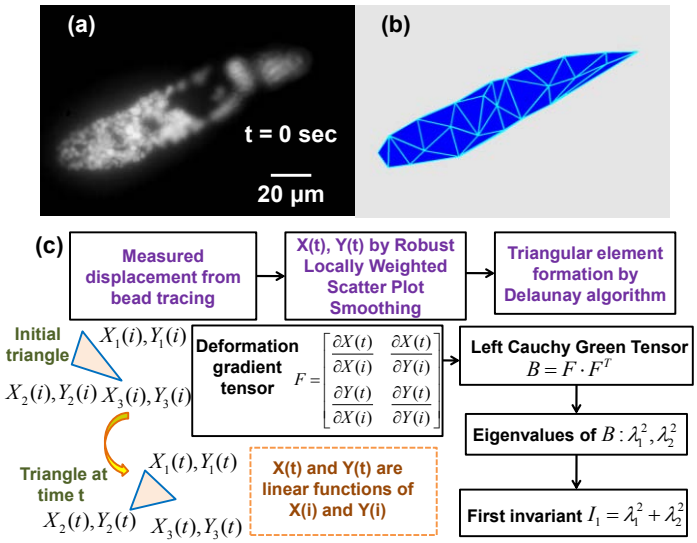

Figure 1: (a) Fluorescent bead-labeled cell attached on ET, (b) Discretized cell with triangular mesh elements, (c) Summary of the large deformation analysis method.

RESULTS AND DISCUSSION

In Figure 2, a cell is shown undergoing deformation during freezing. At t = 2 sec, the freeze-front first touches the left side of the cell and propagates towards the right at subsequent times. The triangular elements deform as the freeze-front approaches and passes. Although the movement of the freeze-front is 1D planar, the cell experiences very complex and spatiotemporally varying displacement resulted from translation, rotation and local deformation.

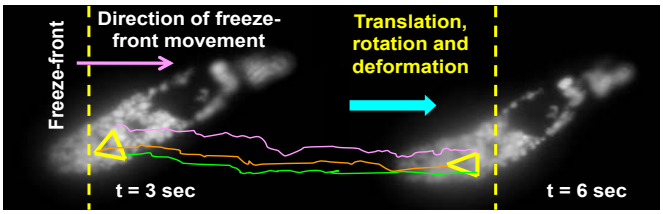

Figure 2: A triangular element is shown to deform, translate and rotate. The dotted line is the freeze-front location.

Figure 3 shows the spatiotemporal deformation in the cell. The element highlighted in (a) undergoes a local deformation to (b). At 5.6 sec, just before freeze-front touches the element, I_1 = 1.05 (<2, the region is compressed) and at 6.05 sec (just after freeze-front passes the element) I_1 = 3.69 (>2, the region is expanded). At 8.3 sec, further element expansion is observed with I_1 = 6.01. In (c) and (d) this compression and expansion are shown at the same time steps as contour plots of I_1. These plots quantify the intracellular deformation at any spatial location at given time points. Figure 3(e) shows the history of I_1 in the given element. Such a sudden deformation change characterized by an almost 500% increase in the value of I_1 in a time frame of 2.3 sec may lead to rupture of the CMAC in that region. From the previous study [3], ECM deformation is shown to have a much lower value (dilatation rate = 0.01 sec^{-1}) for the same freezing condition. The present results suggest that the maximum intracellular strain rate is 0.43 sec^{-1} so that the CMAC is under significant mechanical loading and possible rupture which implies loss of functionality. In addition, sudden jerk movements around cell-matrix adhesion points were also noted (data not shown), which are thought to be associated with the rupture of the CMAC. Further research is still ongoing to investigate the effects of different cytoskeleton structures during the process, and also to simultaneously measure the intra- and extra-cellular deformation under a hierarchical poroelastic framework.

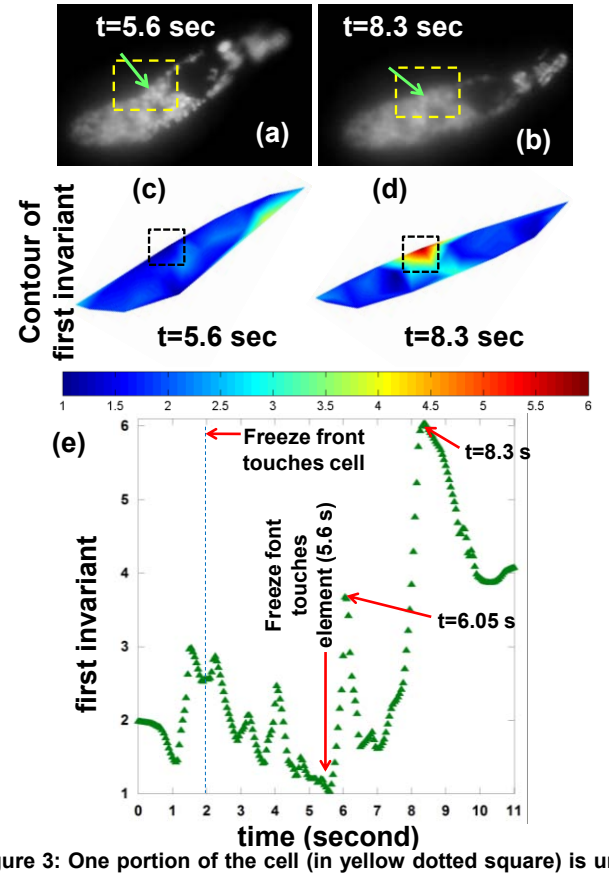

Figure 3: One portion of the cell (in yellow dotted square) is under compression (a) and under expansion at (b). In (c) and (d) the element is shown in black dotted squares as part of the spatial invariant contour at the same time points. (e) shows the temporal invariant history of the triangular element.

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