CHONDROCYTE SIZE CHANGES IN RESPONSE TO CYCLIC HYPERTONIC LOADING USING A NOVEL MICROFLUIDIC DEVICE

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INTRODUCTION

Osmotic loading provides a means for modulating cell volume/shape without physical contact with the tissue/cell, providing a valuable tool to investigate cell mechanotransduction and mechanical properties [1-8]. While most researchers focus on the effects of static osmotic loading, we previously demonstrated a novel microfluidic device to study real time cell size changes in response to applied cyclic osmotic loading [9]. In this study, we characterized the transient chondrocyte size response to cyclic osmotic loading at 0.0125 Hz, where cells cultured at 360 mOsm are subjected to interchanging step hypertonic loading of 580 mOsm for 40 seconds and then returned to the isotonic solution of 360 mOsm.

METHODS AND MATERIALS

Cell Culture: Primary chondrocytes were harvested enzymatically from freshly killed calf CMC joints. Cells were plated at high density in 10% FBS supplemented Dulbecco's Modified Eagles's Medium (DMEM) supplemented with essential and non-essential amino acids, TES, BES, HEPES, and pen/strep to maintain their phenotype. For the current studies, first passaged chondrocytes were isolated via trypsinization and loaded with CellTracker Dye (Molecular Probes). Labeled cells were then plated into the microchannels at 1.3×10^6 cells/ml for one hour. Microfluidic Device: Modulated fluid flow was obtained by an alternating fluid packets injection scheme by regulating hydrostatic pressure in the reservoirs [9]. In short, the microchannel chips made with PDMS (Corning) are 300 μm wide and 100 μm deep in a 'Y' shape. The chips were sterilized and assembled with coverslips. A field of view located a distance of 100 μm downstream from the 'Y' was studied (Figure 1). Osmotic Loading: Hypertonic media (580 mOsm) was made by adding sucrose to serum-free DMEM described above (360 mOsm). The experimental medium was loaded with 5 mg/L Texas Red-conjugated-dextran (70 kDa, Molecular Probes) for fluorescence detection. For isotonic loading, one of the isotonic channels was labeled with the same dye. After an hour of cell plating, the wells were rinsed and 50 μL aliquots of medium was added to each input well and put on an Olympus IX-70 inverted epifluorescence microscope. The chip was connected to two syringe pumps (noted by arrows in Figure 1) via collection needles placed in each input well. The syringe pumps were interlinked and programmed to output hydrostatic pressures to each input well. Cyclic osmotic loading was applied at 0.0125 Hz and fluorescent images of cells and medium were taken at 0.3 Hz with MetaFluor (Universal Imaging). The flow rate in the outlet channel is 220 μm/s with a calculated wall shear stress of 1.22×10^{-2} Pa.

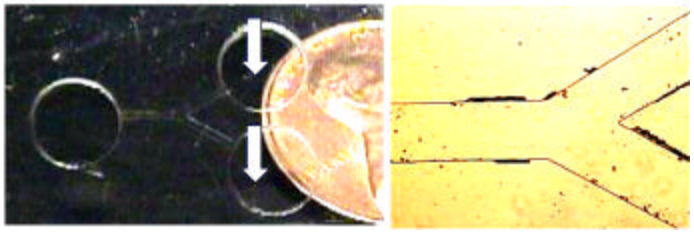

Figure 1. Y-channel microfluidic system.

Image Analysis: Two different methods were used to quantify cell size changes. First, a custom Matlab program was used to automate the measurement of cell sizes as previously described [9]. The image processing algorithm included: smoothing with a Gaussian filter, computation of bimodal histogram, identification of the 'best' gray level threshold to isolate cells and binary segmentation. Images from each time point were segmented and each cell detected in the image was assigned a label. Cells were tracked and individual cell sizes measured in pixel area for each time point. Relative cell sizes were determined by normalizing pixel area of each time point to the original cell pixel area (at t=0). The second technique measured the fluorescence intensity of the cells, which is inversely proportional to the cell area. After background subtraction, a region within the cells was identified and intensities measured for all time points using the 'region measurements' function of MetaMorph (Universal Imaging). Intensities were then normalized to the initial cell intensity at t=0 and inverted. Data Analysis: Cell volume was calculated for individual cells (assuming a spherical geometry) and normalized to its

maximum/minimum volume prior to shrinking/relative swelling. The exponential function $y = 1+A*(1-\exp^{-Bt})$ was used to fit the normalized volume using Origin (Microcal). Student's *t-test* and Pearson's correlation test (Statistica, Statsoft) were performed using $\alpha=0.05$.

RESULTS

Average changes in chondrocyte cell size, as measured in pixel area and inverse intensity, in response to applied cyclic hypertonic (580 mOsm) loading at 0.0125 Hz are shown in Figure 2. Maximum cell shrinking and relative swelling were 17% and 29% from pixel area measurements and 10% and 25% from inverse intensity measurements, respectively. There was a strong positive correlation between the normalized pixel area and inverse intensity r=0.64 (Figure 3, $p<0.05$, n=320). The exponential curve fits for cell shrinking and relative swelling yielded R^2 values ranging from 0.68 to 0.98. To assess differences in the cell shrinking and relative swelling behavior, the normalized cell volume for several cells (designated by each symbol) subjected to hypertonic loading (lower curves) are plotted superimposed with their respective cell size change to re-introduction of isotonic medium (upper curves) (Figure 4). Disparate responses were observed for shrinking and relative swelling Curve-fitting yielded A_{shrink} equals -0.409 ± 0.093 and A_{swell} equals 0.905 ± 0.566. The shrinking response occurred more rapidly, B_{shrink} equals 0.155 ± 0.051 and B_{swell} equals 0.051 ± 0.015 (n=6, p=0.006). Similar results were observed with the inverse intensity measurements (A_{shrink}=-0.236 ± 0.042 and A_{swell}=0.462 ± 0.186; B_{shrink}=0.087 ± 0.034 and B_{swell}=0.043 ± 0.038 (n=7, p=0.042 for B)).

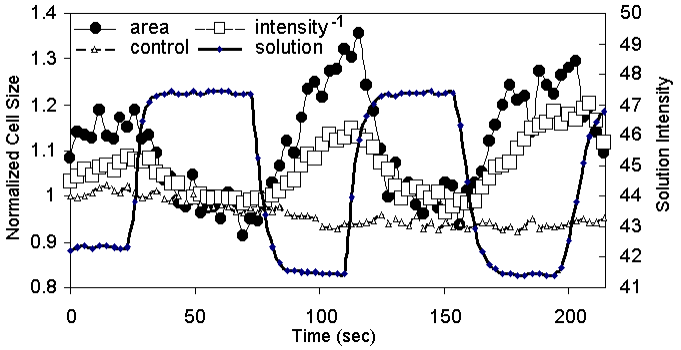

Figure 2. Mean chondrocyte size changes in response to cyclic hypertonic loading. Cell intensity is inversely related to cell area.

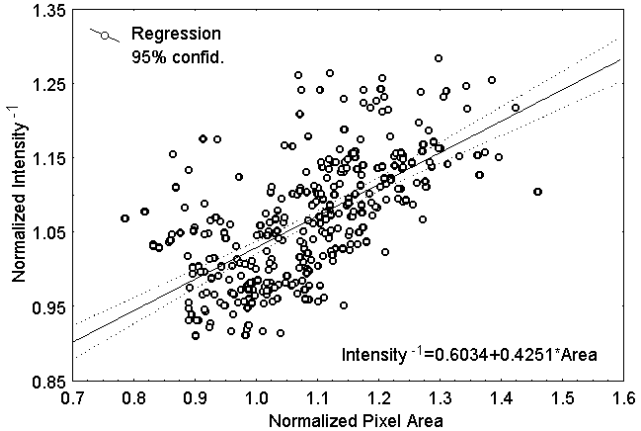

Figure 3. Correlation between normalized area and normalized intensity measurements, r=0.64 ($p<0.05$).

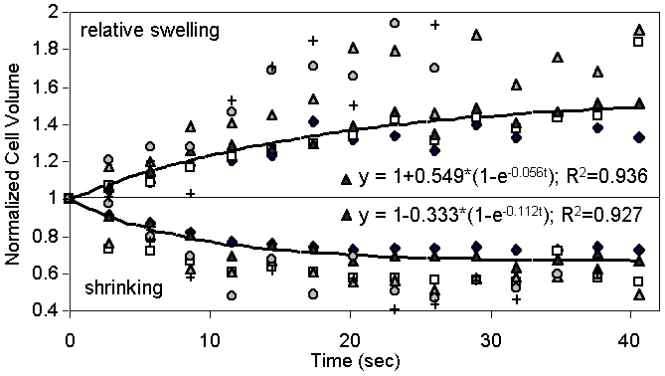

Figure 4. Plot of normalized cell volume versus time for hypertonic and relative hypotonic response of same cells. Representative curve fit for one cell (line, dark triangles).

DISCUSSION

Using a novel microfluidic device, we characterized the real-time chondrocyte cell size response to cyclic hypertonic loading (with alternating step hypertonic and isotonic loading every 40 seconds) and demonstrated that the passive shrinking of the chondrocyte occurs more quickly than the relative swelling response (triggered by isotonic medium) under the conditions of the present study. Interestingly, this finding is similar to cell size changes that we monitored in response to an applied step osmotic loading that was maintained for several hours. In this earlier study, we reported that a step hypertonic load (+Δ225 mOsm) was observed to initiate cell size changes that reached equilibrium faster than those for cells subjected to a step hypotonic (-Δ225 mOsm) change from an isotonic initial medium (300 mOsm) [3]. The physiologic implication of the finding is unclear, and may suggest disparate material properties (e.g., hydraulic permeability and/or stiffness) in chondrocyte swelling and shrinking. Incorporation of multiphasic constitutive modeling with a dynamic input stimulus may permit viscoelastic or other time-dependent behavior of cells to be determined. Since there is a good correlation between cell area and fluorescence intensity, either technique appears to be reasonable for determination of cell size changes. With our novel microfluidic device, new insights to cell mechanotransduction as well as cell material properties are possible.

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