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Chondrocyte Nuclear Response to Osmotic Loading

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I. INTRODUCTION

Cartilage compression results in changes in the shape, volume as well as hydrostatic and osmotic pressure of chondrocytes in situ [1, 2]. For example, changes in the cellular osmotic environment have been shown to modulate chondrocyte biosynthesis and gene expression, however, the mechanosensing mechanisms mediating these responses are relatively unknown [3-8]. Nuclear shape and size changes resulting from cell deformation have been suggested to alter cell functions [9-12], and as such we recently performed a study that reported that chondrocytes and their nuclei respond to osmotic loading with alterations in their size [13]. In the current study, we focus on the potential role of the actin cytoskeleton in mediating the transmission of osmotic loading-induced cell size changes to the nucleus.

II. MATERIALS AND METHODS

A. Cell Culture:

Primary chondrocytes were harvested from calf CMC joints via enzymatic digestion and plated at high density. Prior to experiments, chondrocytes were released from the tissue culture dish with trypsin and plated into the microfluidic channel for 45 minutes. The cells were then either loaded with the nucleic acid stain, SYTO13 (Molecular Probes) at 5μM for 45 minutes, or treated with 20 μM cytochalasin D in PBS (Sigma) for 3 hours, then treated with SYTO13 as previously described [13].

B. Microfluidic Device:

As previously described [8], a pressure-driven, low-shear, laminar flow was introduced to a microfluidic channel to apply step osmotic loading to cultured cells. Prior to experiments, the reservoirs were rinsed and 50 μL of the isotonic medium and 30 μL of the experimental medium were added to the input wells and the device was placed on an Olympus IX-70 inverted fluorescence microscope using a 40X objective.

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C. Osmotic Loading:

Hyper- and hypotonic media were made by adding sucrose or ultrapure deionized water (ddH_2O) to Hank's balanced salt solution (HBSS, Sigma) to attain osmolality of 110 and 510 mOsm, respectively. Osmolarity of the culture medium (DMEM, Cellgro) as well as the isotonic HBSS was 310 mOsm, as confirmed by an osmometer (Advanced Instruments). At the onset of experiments, another 50 μL of the experimental medium was added to one of the input reservoirs to initiate the experimental flow. Fluorescence and DIC images of the cells were captured with MetaFluor (UIC) at 0.2 Hz.

D. Image and Data Analysis:

Fluorescence images acquired at 488 nm for nuclear size were analyzed using a custom segmentation and cell tracking program as described previously . An edge detection program based on algorithms described by Alexopoulos et al [14] was used to analyze cell volume (computed assuming a spherical cell). Using Origin (Microcal), the exponential function: $\Delta V/V_o=V_{eq}*(1-\exp^{-t/\tau})$ was used to fit the time-varying volume response, where $\Delta V/V_o$ represents volume change normalized with initial volume (V_o) and V_{eq} corresponds to the estimated final normalized volume and τ indicates the time constant for change. Statistics was performed by Statistica (StatSoft) using repeated measures ANOVA (α=0.05).

III. RESULTS

The pre-osmotic loading cell volume was significantly higher after treatment with cytochalasin, while the nuclear volume did not change with treatment (Figure 2). The relative equilibrium volume change was significantly less for nuclei than for cells under hypotonic loading with HBSS, while there was no difference between cell and nuclei under hypertonic loading in HBSS (Figure 1,3). Cytochalasin had no effect on equilibrium volume change in response to osmotic loading, for cells or nuclei. The time constant did not vary between the cell and nucleus for either hyper- or hypotonic loading. Upon treatment with cytochalasin, the time constant of the cell became significantly higher for both loading conditions, while the time constant of the nuclei became significantly lower.

IV. DISCUSSION

The nucleus is classically described as a porous, fluid-filled sack that allows the free movement of water, ions and small molecules through the nuclear pore complexes , which have been shown to have a effective diameter of 5 nm

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(Figure 4A) [15]. The chondrocyte nucleus has been measured to be 3-4 times stiffer than the cytoplasm [16]. Since osmolytes are assumed to transport freely across the nuclear membrane, we anticipated that the nucleus would not undergo volume changes in response to applied osmotic loading of the cell, since there would not exist a concentration gradient of solutes between the cytoplasm and nucleus, required to drive water flux. However, as noted above, clearly the nucleus size is altered under osmotic loading.

Maniotis et al. has suggested a mechanical role of cytoskeletal attachments in controlling nuclear size, represented by Figure 4B. They used a micropipette to pull on cytoskeletal elements, leading to nuclear deformation [17]. To test whether the f-actin cytoskeleton plays a role in nuclear size change under osmotic loading, by pulling or pushing on the nucleus as the cell membrane shrinks or expands, we treated cells with cytochalasin D, an actin polymerization inhibitor. Nuclear volume was found to change in response to osmotic loading of the cell, either under hyper- or hypotonic loading. This result suggests that mechanical transmission of cell size alterations via the cytoskeleton is not the principal cause for the observed nuclear size change, though we have not yet explored the role of other candidate cytoskeletal elements such as microtubules.

To explain our results, we offer an alternative model that refines the classical Kedem-Katchalsky description of the cell response to osmotic loading, by taking into account the electrical charge of molecules residing in the cytoplasm and nucleus (Figure 4C). The classical Kedem-Katchalsky model predicts that upon osmotic challenge, water will move across the cellular membrane in an attempt to balance the difference in osmolarity inside and outside of the cell, but does not account for the effects of electrical charge of the osmolyte nor that of intracellular ions and molecules. We extend this model to incorporate the effect of charges in the intranuclear and cytoplasmic spaces, refining it to allow for mobile counterions distributed in each compartment (nuclear or cytoplasmic) to maintain electroneutrality. We consider the presence of charged macromolecules in the intranuclear or cytoplasmic regions, which cannot cross the nuclear membrane, that give rise to an effective fixed charge density (FCD) in each compartment. With changes to the water content of the cytoplasmic compartment that accompany cell shrinking or swelling to an extracellular osmotic load, alterations of the effective cytoplasmic FCD would ensue and lead to an intracellular redistribution of the mobile counterions (between the two compartments) that would provide a driving force for fluid flux and nucleus size change [18]. This model would be consistent with our observations that nucleus volume changes with osmotic loading of the cell, and that this volume change is not dependent on interactions with the actin cytoskeleton.

Studies by our group have shown that hyper- and hypotonic loading can lead to changes in cell signaling pathways and levels of gene transcription [19, 20]. The mechanoregulatory mechanisms that mediate these biological responses remain to be elucidated. We propose here that the development of intranuclear osmotic gradients may play a role in mechanotransduction of cellular osmotic loading to the nucleus. We are continuing our efforts that combine experiments and theoretical analysis [21] to gain further insights to the relationship between cell and nuclear size changes in response to osmotic loading and to more directly assess our concept of intranuclear osmotic gradients.

V. References

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VI. FIGURES

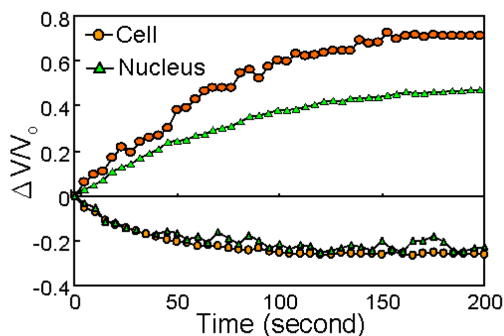

Figure 1. Relative cell and nucleus volume change to hypo-osmotic (top) and hyperosmotic loading (bottom).

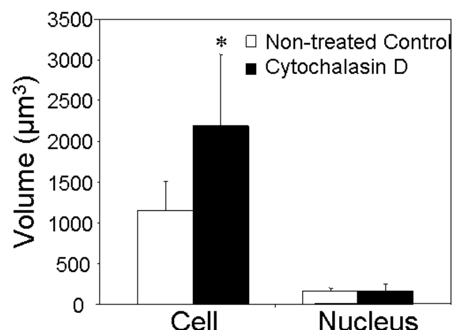

Figure 2. Average initial volume of cells and nuclei with and without cytochalasin treatment (*p<0.05, n=15-21 cells).

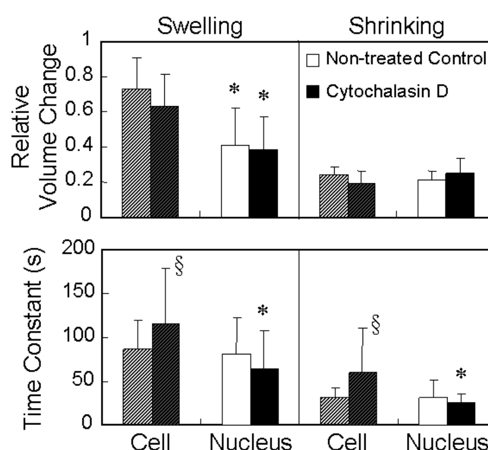

Figure 3. Relative equilibrium volume change and time constant for cells and nuclei under hyper- and hypoosmotic loading, with and without cytochalasin D treatment (*p<0.05 vs. cell, §p<0.05 vs. HBSS control, n=15-21 cells).

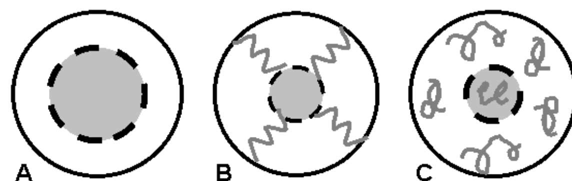

Figure 4. A) Nucleus as a porous sack; B) Nucleus from A with cytoskeletal connections; C) Nucleus from A with charged impermeable macromolecules in intranuclear and cytoplasmic compartments.