DEFORMATION OF THE NUCLEUS BY CELL SPREADING: ANALYSIS OF THE HYPOTHESIS OF DIRECT MECHANOTRANSDUCTION IN ENDOTHELIAL CELLS

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INTRODUCTION

External stress is known to be transduced into a variety of biochemical signals near the surface of the cell membrane that ultimately alter gene expression. Studies of cellular spreading on micropatterned substrates have shown that the area of spreading is critical in determining the cell's fate [1]. The demonstration in [2] that cells possess connections between integrins, the cytoskeleton, and the nucleus is important from a physiological standpoint, since this provides a possible direct line of force transfer between the surface of the cell and the nucleus. An interesting hypothesis is whether or not external force is directly transferred through the cytoskeleton to the nucleus, causing nuclear deformations, and possibly resulting in modulation of gene expression (*direct mechanontransduction*).

Recently, several groups have begun to characterize the physical properties of the cell nucleus. In [3], micropipet aspiration was used to determine the viscoelastic response of isolated chondrocyte nuclei. Using a microplate compression technique, more quantitative estimates for the nuclei of endothelial cells were estimated in [4].

In order to begin to test the hypothesis of direct mechanotransduction, we look at the relation between a surface force and nuclear deformation. We consider the transition of an endothelial cell from a spread state to a rounded state. This provides us with a reference state and deformed state for the nucleus.

Here, we present the results of the measurements of changes in the relative area and length of the main axes of 2-D cross-sections of the nuclei of endothelial cells. We combined our 2-D experimental results with a theoretical model and estimated characteristics of the 3-D strain field in the nucleus. We are also pursuing the role of the components of the cytoskeleton in the active and passive modes of mechanotransduction from the adhesion site to the nucleus.

EXPERIMENTAL METHODS

For this study, we used bovine pulmonary aortic endothelial cells (BPAEC) between passages 13-15. These cells were cultured using Gibco low glucose DMEM/ 10% calf serum/ 1% antibiotic. Cells were grown to confluency, and passed into 35mm tissue-treated plastic dishes. The cells were stained with Hoechst 33258 nucleic acid stain prior to experiments. All cells were imaged with a Nikon TE-200 microscope equipped with a mercury arclamp for fluorescence and IPLab v. 3.070 acquisition software. Images were processed on a PC using Adobe Photoshop v. 5.0 and analyzed using MatLab v. 5.3.

MEASUREMENTS

We are currently in the process of obtaining three-dimensional, quantitatively-reliable images of the cell nuclei. In the meantime, we selected the projected two-dimensional area change between the two states as our deformation parameter.

In order to determine if the 2-dimensional projected area of the nucleus exhibited a significant change when the cell transitioned from the spread to rounded configurations, we added a trypsin/PBS solution to the media of spread cells in order to cause the cells to round up. We computed the projected area of the nucleus from the cell spread to rounded states. Representative images of the endothelial cells and nuclei in spread and rounded states are presented in Figures 1 and 2, respectively.

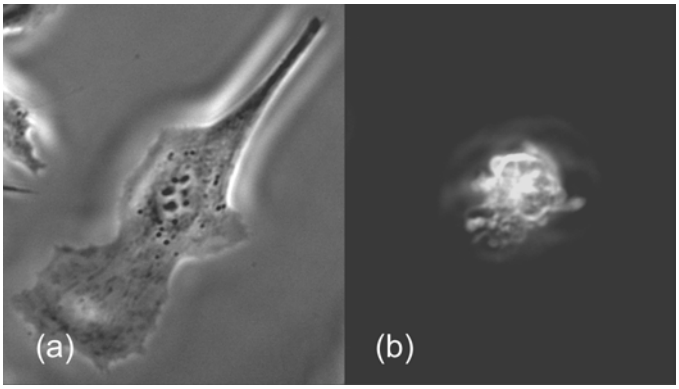

Figure 1. Endothelial Cells in the: (a) spread configuration; and, (b) rounded after trypsinization.

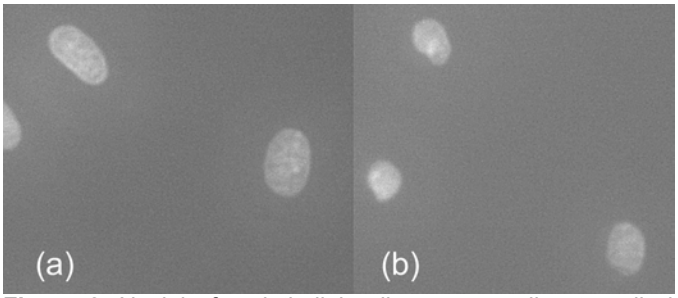

Figure 2. Nuclei of endothelial cells corresponding to cells in the: (a) spread configuration; and, (b) rounded after trypsinization.

For n=41 cells, the mean and standard deviation of the ratio of the area of the nucleus in the rounded to the spread states were 0.52 and 0.08, respectively.

We also were able to extract changes in the lengths of the long and short axes of the cross-sections in cells that did not experience significant rotation in the plane (n=12). The mean and standard deviation for the relative length changes of the long axis were 0.69 and 0.06, respectively, and those for the short axis were 0.69 and 0.04, respectively.

The relative area change is equal to the product of the relative length changes of the long and short axes, assuming an elliptical geometry. However, the shape of the nucleus is not a perfect ellipse, and error due to slight rotations is present. Nevertheless, our estimates of the stretches and relative area change are in close agreement.

CHARACTERIZATION OF THE STRAIN IN THE DEFORMED NUCLEUS

We use the measured parameters of the 2-D deformation of the imaged cross-section to characterize the 3-D strain field in the nucleus. The transport across the membrane caused by the deformation of the nucleus is probably negligible to change the volume of the nucleus. Thus, we assume that the material of the nucleus is incompressible. Based on our observations of rotations, we will use a model of a tri-axial state to estimate the strain characteristics in the nucleus.

In the coordinate system with axes x_1 and x_2 along the long and short axes of the elliptical cross-section of the nucleus (Fig. 2) and axis x_3 normal to the plane of the cross-section, we interpret the measured relative changes in the lengths of the main axes of the cross-section as the principal stretches λ_1 and λ_2. The assumption of incompressibility results in the expression

$$\lambda_3 = \lambda_1^{-1}\lambda_2^{-1} \tag{1}$$

that characterizes the deformation of the nucleus in the direction normal to the adhesion area. The table below shows the principal stretches and strains in the deformed nucleus.

i	1	2	3
λ_i	0.69	0.69	2.10
ε_{ii}	-26%	-26%	170%

Table 1. Mean values of the principle stretches and strains of the deformed nucleus.

It is also of interest to estimate the density of the energy W stored in the nucleus as a result of its deformation. Assuming the model of an incompressible neo-Hookean material [5] we obtain

$$W = 0.5\mu(\lambda_1^2 + \lambda_2^2 + \lambda_1^{-2}\lambda_2^{-2} - 3) \tag{2}$$

where μ is the shear modulus of the material. Taking into account the estimate of Young's modulus obtained in [4] and the relationship between shear modulus and Young's modulus in incompressible materials, we can estimate the shear modulus as

$$\mu \approx 1670 \text{ Pa} \tag{3}$$

By substituting the shear modulus and the mean values of the principal stretches (Table 1), we obtain

$$W \approx 1960 \text{ Pa} \tag{4}$$

DISCUSSION

We found significant deformation of the cell nucleus when the cell went from a spread state to a rounded state. The corresponding strains and the strain energy density are high. The nucleus is compressed in both directions in the image cross-section (the cross-sectional area is obviously not preserved). To preserve its volume, the nucleus elongates in the normal direction. The major effect of the cytoskeleton on the nucleus is probably in the plane of the cell, and the deformation normal to the plane is of a reactive nature.

An important question is what kind of forces the cytoskeleton generates to deform the nucleus. There are two options: the forces are passive and they are caused by changes in the "boundary conditions" at the adhesion site. In this case the level of these forces is determined by the passive elastic properties of the cytoskeleton. An alternative scenario is that the cytoskeleton generates additional active forces via actin/myosin interaction or (and) polymerization/depolymerization.

To understand the actual mechanism of the force generation by the cytoskeleton, we explore two avenues. First, we dissolve the major components of the cytoskeleton and analyze changes in the deformation of the nucleus. Second, we use the previously estimated stiffness of the cytoskeleton to check the produced energy against the energy stored in the nucleus, whose density we estimated here. The characterization of the deformation of the nucleus as a result of changes in the cell's state can help in developing advanced models of the cytoskeleton of the endothelial cell.

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