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Flow-induced hardening of endothelial nucleus as an intracellular stress-bearing organelle

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Abstract

The mechanical contribution of nucleus in adherent cells to bearing intracellular stresses remains unclear. In this paper, the effects of fluid shear stress on morphology and elastic properties of endothelial nuclei were investigated. The morphological observation suggested that the nuclei in the cytoplasm were being vertically compressed under static conditions, whereas they were elongated and more compressed with a fluid shear stress of 2 Pa (20 dyn/cm²) onto the cell. The elongated nuclei remained the shape even after they were isolated from the cells. The micropipette aspiration technique on the isolated nuclei revealed that the elastic modulus of elongated nuclei, 0.62 ± 0.15 kPa (n = 13, mean \pm SD), was significantly higher than that of control nuclei, 0.42 ± 0.12 kPa (n = 11), suggesting that the nuclei remodeled their structure due to the shear stress. Based of these results and a transmission electron microscopy, a possibility of the nucleus as an intracellular compression-bearing organelle was proposed, which will impact interpretation of stress distribution in adherent cells.

1. Introduction

Application of shear stress to endothelial cells (ECs) regulates genes responsible for a variety of cellular functions (Davies, 1995; Davies et al., 1999). One probabilistic pathway through which shear stress may be transduced to a gene-regulating signal is a cytoskeletally-mediated deformation of the nucleus (Davies, 1995; Ingber, 1997; Janmey, 1998) since shear stress causes elongation of the nucleus (Flaherty et al., 1972) that encloses the DNA and defines the nuclear compartment in which gene expressions are regulated. Maniotis et al. (1997) demonstrated by using a micropipette loading that the endothelial nucleus and the cell membrane were hard-wired to each other with actin filament network and coordinately deformed together. However, how physiological forces such as shear stress are transmitted to and borne by the nucleus remains elusive.

To elucidate the mechanical contribution of endothelial nucleus to bearing intracellular stresses, the following hypotheses were made in the present study; if a nucleus bore intracellular stresses and accordingly had a pre-existing strain in living cells as other studies proposed (Ingber, 1997), then a morphological or stiffness change of the nucleus is expected to appear when its surrounding mechanical environment was changed. Also, since the stiffness of endothelial cells change in response to shear stress probably due to rearrangements of actin filaments (Sato et al., 1987, 1996, 2000; Charras and Horton, 2002; Ohashi et al., 2002), the mechanical properties of nucleus might also be dependent on its mechanical environment. To test the hypotheses, the endothelial nucleus was isolated from the cells, and the resultant morphological changes were observed with fluorescence microscopy. To further examine force-induced morphological changes, the nuclei isolated from fluid shear stress-imposed ECs were also examined. To investigate the effect of shear stress on the mechanical properties of nucleus, the elastic modulus was measured with the micropipette aspiration technique

before and after the exposure to flow. Morphological observation of the nucleus was also conducted with transmission electron microscopy (TEM).

2. Materials and methods

2.1 Cell culture

ECs isolated from bovine thoracic aortas were cultured with Dulbecco's modified Eagle medium (Invitrogen, NV Leek, The Netherlands) containing 10% heat-inactivated fetal bovine serum (JRH Biosciences, Lenexa, KS), 1% penicillin, and 1% streptomycin in an incubator at 37°C and 5% CO₂/95% air. Cells were seeded on a 35-mm-diameter custom-built glass base culture dish (Asahi Techno Glass, Tokyo, Japan) designed to allow of shear stress experiments. Cells between passages 3-11 were used for all experiments.

2.2 Isolation of nucleus

Nuclei were isolated from the cultured ECs with a chemical treatment previously reported (Katoh et al., 1998). Briefly, the confluent cells were washed with PBS (Dulbecco's phosphate-buffered saline without Ca²⁺ or Mg²⁺, Wako, Osaka, Japan) and treated with an ice-cold low-ionic-strength extraction solution consisting of 2.5 mM triethanolamine (Wako), 1 µg/ml leupeptin (Wako), and 1 µg/ml pepstatin (Wako) in distilled water for 10 min. The cells were then treated with 0.05% NP-40 (Wako) in PBS (hereafter, containing 1 µg/ml leupeptin and 1 µg/ml pepstatin and kept at 4°C) for 1 min. The dorsal side of many cells was broken away, and then the nuclei popped out by shaking the dish gently under a phase-contrast microscope. A small volume (approximately 1 ml) of the supernatants containing the isolated nuclei was aspirated with a pipette and suspended in 50 ml PBS, and the

solution was centrifuged at 400 x g for 10 min at 4°C with two changes of PBS. The pellet was suspended in fresh PBS in a culture glass dish for fluorescence microscopy, a hand-made chamber (described below) for pipette aspiration tests, or a culture plastic dish for TEM.

2.3 Shear stress experiment

Confluent ECs were either kept as static controls or subjected to a laminar shear stress in a rectangular flow channel system composed of a flow chamber, a roller pump, and two reservoirs (Sato et al., 2000). Under the Poiseuille flow and the Newtonian fluid, the two-dimensional Navier-Stokes equation and the equation of continuity in the rectangular channel are integrated to the following form:

$$\tau = 6\mu Q / h^2 b \quad (1)$$

where τ , μ , Q , h , and b are the shear stress at the wall, the fluid viscosity (9.6×10^{-4} Pa·s for the culture medium), the flow rate, the height of channel (0.5 mm), and the depth of channel (12 mm), respectively. Equation (1) gives the mean shear stress to which the cells at the wall are exposed. The flow rate Q was adjusted to give the shear stress of 2 Pa (20 dyn/cm^2), an average level in large arteries, by using the roller pump. The experiments were performed for 24 h at 37°C.

2.4 Fluorescence microscopy

Changes in the nucleus morphology were examined both when ECs were exposed to the shear stress and when nuclei were isolated from the cells. Before observation, the cells were incubated with SYTO 13 (Molecular Probes, Eugene, OR), a marker of nucleic acids, for 15 min at 37°C for staining nuclei. The nuclei still in the cells were first observed by using a digital CCD camera (C4742-95, Hamamatsu

Photonics, Hamamatsu, Japan) on a fluorescence microscope (IX-71, Olympus, Tokyo, Japan). Subsequently, the isolation procedures were conducted while preventing the culture dish from being exposed to light with an aluminum foil to reduce bleaching. The isolated nuclei suspended in PBS at 4°C were then observed. Setting the time nuclei were isolated to be 0 h, the fluorescence images were obtained at 0, 1, 2, 3, 4, 12, and 24 h after the isolation. Identical observation was also performed for shear stress-imposed ECs. The morphology of nuclei was evaluated with the following two parameters, i.e. the area in the horizontal plane (to be observed) and the shape index defined as $4\pi(\text{the area in the horizontal plane}) / (\text{the nucleus perimeter})^2$. The image analysis was performed by using NIH Image software. Here, the shape index represents circularity and is equal to 1 when the nucleus is a circle, whereas it approaches zero for a highly elongated nucleus. Identical experiments were conducted four times. For statistical analysis, 50 cells and nuclei were randomly selected for each condition. Data were expressed as mean \pm SD. Differences were analyzed by the Student's unpaired t-test and considered significant when $p < 0.01$.

2.5 Micropipette aspiration test

The Young's modulus of isolated nuclei was determined with the micropipette aspiration technique similar to those described previously (Sato et al., 1987; Theret et al., 1988). The experimental system was mainly composed of an inverted microscope (IX-70, Olympus) with a 100x oil immersion objective (UplanApo, NA = 1.35, Olympus) equipped with a hydraulic micromanipulator and an analog CCD camera (TMI 650, Toshiba, Tokyo, Japan) connected to a personal computer through a 5x relay lens (Fig. 1A). A micropipette was connected to a reservoir through a silicone tube, all the path of which was filled with PBS, and was placed on the gripper of micromanipulator. The reservoir was

opened to the atmosphere, partly filled with PBS, and placed on a sliding stage. The aspiration pressure was controlled by adjusting the height of reservoir and was measured by a pressure transducer (PGM-05KG, Kyowa Electronic Instruments, Tokyo, Japan) connected to a data recorder through an amplifier (AS1203, NEC, Tokyo, Japan). Micropipettes were made by drawing glass capillary tubes (1 mm in outer diameter, Narishige, Tokyo, Japan) with a glass-electrode puller (PP-83, Narishige) and then cut with a microforge (MF-900, Narishige) to have an inner radius of 2.0-2.5 μm and outer radius of 2.3-3.2 μm . The micropipettes were coated with poly-D-lysine (Sigmacote, Sigma, St Louis, MO), which forms a tight microscopically thin film of silicone on a glass surface, to prevent cellular debris adhesion during tests.

Before experiments, both the control and the shear stress-imposed ECs were incubated with 1 $\mu\text{g/ml}$ cytochalasin D (Sigma), an inhibitor of actin polymerization, in the culture medium at 37°C for 30 min. Fluorescence microscopy confirmed that the cytochalasin D treatment disrupted rhodamine phalloidin-labeled actin-associated structures, which often adhere around the nucleus and may prevent from accurate measurements of the mechanical properties of nucleus itself. The nuclei were then isolated and suspended in the chamber designed to allow of the entry of micropipette from the side (Sato et al., 1987). The experiment was performed at room temperature. The tip of micropipette was touched gently to the surface of a targeted nucleus. A step-wised pressure of 0.1 kPa was applied to the nucleus at 2 min intervals. A portion of the nucleus was drawn into the pipette, and the phase-contrast images were observed and recorded on the computer (Fig. 1B and C). Aspirated length was measured by using NIH Image software. The relation between the negative pressure and the aspirated length was analyzed.

The Young's modulus of isolated nuclei was evaluated according to the previously reported theory

(Theret et al., 1988). Under the assumptions that the nucleus was a homogeneous, isotropic, and incompressible elastic medium with a half-space, the Young's modulus of nucleus E is given by the following form;

$$E = 3a\Delta P\Phi(\eta) / 2\pi L \quad (2)$$

where a , ΔP , $\Phi(\eta)$, and L are the inner radius of pipette, the trans-wall negative pressure, a function associated with the shape of pipette, and the aspirated length inside the pipette, respectively (Fig. 1D).

Here, $\Phi(\eta)$ was fixed to be 2.1. To investigate the effect of shear stress on Young's modulus, the differences were analyzed by the Student's unpaired t-test and considered significant when $p < 0.05$.

2.6 Electron microscopy

Microstructure of the nuclei was observed with TEM. Control, sheared ECs, and their isolated nuclei were fixed with fresh 2.5% glutaraldehyde, 2% paraformaldehyde, and 0.5% tannic acid in 0.1 M sodium cacodylate buffer (pH 7.4) for 24 h at 4°C. Fixed specimens were rinsed for 1 h with 0.1 M sodium cacodylate buffer at 4°C, removed mechanically from the dishes, post-fixed for 2 h with 1% osmium tetroxide in the same buffer at 4°C, dehydrated through a graded series of ethanol (60, 70, 80, 90, and 100%) with two changes of 30 min at each concentration, infiltrated with QY-1 (n-butyl glycidyl ether), embedded in Epon812, and cut with an ultramicrotome (Reichert Jung, Walldorf, Germany). Ultra-thin sections from selected blocks were mounted onto copper grids, stained with aqueous uranyl acetate and lead citrate, and viewed using a TEM (H-7100, Hitachi, Tokyo, Japan) at an accelerating voltage of 75 kV.

3. Results

3.1 Isolation of nucleus

Hereafter, the nuclei isolated from the control and the sheared ECs are termed NECs and NSECs, respectively. The chemical isolation technique yielded apparently whole intact nuclei in phase-contrast (Fig. 2A–D) and fluorescence (Fig. 2E–H) images. An electron micrograph of the isolated nuclei showed typical morphological features of nucleus, e.g. the nuclear membrane with a couple of nuclear pores, chromatins that highly aggregate in the nuclear periphery, and nucleolus that locates around the center part of nucleus (Fig. 3). The ultra-microstructure of the isolated nuclei appeared similar to that of the nuclei in intact cells.

3.2 Morphological analysis

Changes in the morphological parameters of nuclei were obtained from the fluorescence microscopy (Fig. 4, n = 50). The cross-sectional area of NECs increased significantly from $144 \pm 39 \mu\text{m}^2$ (mean \pm SD) to $211 \pm 80 \mu\text{m}^2$ after exposure to the fluid shear stress. The area of both NECs and NSECs decreased significantly to $83 \pm 35 \mu\text{m}^2$ and $101 \pm 40 \mu\text{m}^2$, respectively, immediately after the isolation. The shape index of NECs decreased significantly from 0.89 ± 0.06 to 0.85 ± 0.07 after exposure to shear stress, indicating that the nuclei were elongated as in vivo experiment (Flaherty et al., 1972). The shape index of both NECs and NSECs remained almost constant even after the isolation, indicating that both NECs and NSECs did not become spherical in shape when they were isolated from the cells, and that the isolated NSECs retain a more elongated shape than NECs. At 24 h after the isolation, a significant difference between NECs and NSECs was still found.

3.3 Elastic modulus measurement

A pipette aspiration was performed on the isolated nuclei to measure the Young's modulus. For elongated nuclei, the pipette was placed to the direction perpendicular to the long axis of elongated nuclei (Fig. 1C). When aspirated, the nuclei were deformed in a step-wise and asymptotically reached equilibrium at the time scale of experiment. For unloading of aspiration pressure, nuclei finally returned to their original shape, suggesting that the deformation was elastic. Relationships between normalized displacements defined as the ratio of aspirated displacements to the inner radius of micropipette (L/a) and aspiration pressures (ΔP) were averaged for each 0.1 kPa interval (Fig. 5). We assumed that the plots in the pressure range between 0.0–0.6 kPa to be linear, in which the assumptions in the derivation of Eq. (2) are valid, and obtained the regression line by the least-squares method to calculate the Young's modulus from the Eq (2). The Young's modulus of nucleus increased significantly from 0.42 ± 0.12 kPa ($n = 11$, mean \pm SD) to 0.62 ± 0.15 kPa ($n = 13$) with the fluid shear stress (Fig. 6).

4. Discussion

If the nucleus in ECs bore an intracellular stress, then it amounts that the nucleus underwent a deformation from the zero-stress state, i.e. the nucleus might have pre-existing strain in the cytoplasm. Based on this hypothesis, the cell membrane was removed to isolate nuclei. It was expected that a morphological change of the nucleus associated with the release of pre-existing strain might appear after the nucleus popped out. The fluorescence microscopy showed that the cross-sectional area of nucleus decreased significantly after the isolation, whereas the shape index remained almost unchanged. It is possible that the decrease in the area may be driven by osmotic pressure or surface

tension like the way cells become spherical after detach. However, the result that almost no difference of the shape index appeared after the isolation suggests that the decrease may not be caused predominantly by osmotic pressure or surface tension because those forces would drive the surface area to be minimized, resulting in an increase in the shape index. Alternatively, if the nuclei behave like an elastic material, the decrease in the area indicates that the nuclei in adherent and spread cells had been compressed vertically, then they extended upward after the cell membrane was removed, and therefore the area (in the horizontal plane) decreased. The absence of change in the shape index may be attributable to that the nuclei deformed in an isotropic manner, and therefore the release of vertical compression resulted in a deformation geometrically similar in shape but not a directional deformation. After exposure to the shear stress, the area of nuclei increased, indicating that the compressed nuclei were further compressed. This result might be associated with that provided by atomic force microscopy in which the average height of ECs decreased in response to shear stresses (Barbee et al., 1994; Ohashi et al., 2002). The shear stress induced a decrease in the shape index, suggesting that the nuclei elongated. This is consistent with the *in vivo* experiment by Flaherty et al. (1972) in which nuclei elongated and oriented in the direction of blood flow. Here, note that there is almost no change in the shape index between before and after the isolation, showing that the isolated nuclei kept their elongated shape even after the isolation. A significant difference of the shape index between NECs and NSECs was still observed after 24 h, showing that the isolated elongated-nuclei did not return to the original rounded shape though mechanical loadings onto them had been removed.

At the end of pipette aspiration tests, we did not find perceptible residual strain of the nuclei. Caille et al. (2002) also reported that a short-time-compression onto a chemically isolated nucleus did not result in a residual strain. Accordingly, isolated nuclei behave in an elastic manner, showing that the

above interpretation that nuclei were being compressed elastically is possible, and the residual morphological change was not caused by a plastic behavior. ECs are commonly known to show a shear stress-elicited remodeling (Flaherty, 1972; Davies, 1995), and the elongated shape was maintained even after the cells were mechanically detached from the substrate (Sato et al., 1987, 1996). Our results showed that not only the cell but also its organelle, nucleus, remodeled the structure in response to the shear stress.

We estimated the height of nucleus at each experimental condition under rough assumptions that the nuclei were an ellipsoid, isotropic, and incompressible (see Appendix A for the derivation). Considering the average height of NECs as H , the heights of nucleus in ECs, nucleus in sheared ECs, and NSECs were estimated to be $0.59H$, $0.39H$, and $0.82H$, respectively (Fig. 7). Namely, the height of nuclei in the cells decreased to ~ 0.66 -fold of the initial height after the shear stress application. Barbee et al. (1994) measured the surface topography of EC height with atomic force microscopy and reported that the height of unsheared cells was $3.4 \pm 0.7 \mu\text{m}$ (mean \pm SD). After 24-h exposure to laminar shear stress of 1.2 Pa (12 dyn/cm^2), the cell height was significantly decreased to $1.8 \pm 0.5 \mu\text{m}$, which was equal to 0.53-fold of the initial height. Ohashi et al. (2002) similarly reported that the peak EC height significantly decreased from $2.8 \pm 1.0 \mu\text{m}$ to $1.4 \pm 0.5 \mu\text{m}$ with shear stress of 2.0 Pa (20 dyn/cm^2) for 6 h, which was equal to 0.5-fold of the initial height. The decrease in the nucleus height might be caused by the decrease in whole cell height.

Another finding was that the nucleus hardened with the fluid shear stress. The nucleus may harden to adjust the surrounding mechanical environment because cytoplasmic region is known to increase its stiffness after exposure to shear stress (Sato et al., 1987, 1996, 2000; Charras and Horton, 2002; Ohashi et al., 2002). At present, the mechanism of hardening remains unclear. However, the hardening

might be due to both the deformation and the structural remodeling. The nuclear envelope has closely inter-connected lamin filament network. The lamina network itself is elastic and exhibits a strain-dependent mechanical behavior (Dahl et al., 2004). Since the nucleus is stretched and/or compressed under the shear stress as our observation indicated, the lamina network or enclosed chromosomes might be also stretched, and that may increase the stiffness of whole nuclear structure. Otherwise, the remodeling of nucleus structure may also be the reason of stiffening like the way ECs developed cortical layer after exposure to shear stresses and hardened accordingly (Sato et al., 1987, 1996). We investigated with TEM whether there was a significant change in the thickness of nuclear membrane between control and sheared ECs. No perceptible difference, however, was found in the thickness of nuclear lamina, envelope, and aggregated chromosomes (Fig. 8A, B). The mechanism of stiffening will be the subject of future investigations.

The Young's modulus of the isolated endothelial nuclei, 0.42 kPa, was one order of magnitude lower than that of Caille's result, 8 kPa (2002). In the present study, the aspiration pressure was applied at local regions of the nuclear membrane. In contrast, Caille et al. compressed whole nucleus structure and evaluated the modulus in the range of more than 50% deformation. The difference of measuring method and loaded range may be the reason of difference.

The determinant of cell morphology in the vertical direction has been less discussed despite of the plenty of studies in the horizontal plane (Ingber, 1997). The TEM showed that the nuclei appeared to occupy almost the entire height of spread cells in the middle cross-section (Fig. 8C). The force balance between the nucleus and tension in the cell membrane and/or actin filaments running between apical and basal membrane (Kano et al., 2000) addresses that the nuclei can be passively positioned around the center of adherent cells. In high-power views of middle cross-section of spread ECs, a

number of membrane-bound organelles such as Golgi apparatus, endoplasmic reticulum, and mitochondria were quite often observed to be located between the nucleus and luminal or abluminal membrane as if they were being compressed vertically and pushed mechanically a local part of the nucleus (Fig. 8D). In general, nuclei have been reported to be stiffer than the other cytoplasmic region by measuring force-induced deformations (Maniotis et al., 1997; Guilak et al., 2000; Caille et al., 2002). Membrane-bound organelles in the cytoplasm are connected through cytoskeletons, and the loose connections may be responsible for the large deformation observed in the macro-scale views. However, in the micro-scale views, it appeared as if smaller membrane-bound organelles pushed mechanically a part of the nuclei presumably because the stiffness of the former was higher than the latter for their size. This suggests an alternative hypothesis that membrane-bound organelles can transmit forces over the cytoplasm by its compression although it is commonly considered that what could bear intracellular stresses was only cytoskeletal network based on the view that cytoskeleton is probably the only cellular structure directly linking the cell surface to the nucleus (Ingber, 1997; Janmey, 1998; Davies et al., 2003). The evidence for this hypothesis is still indirect, but it addresses the importance that the local push may affect nucleus functions, like the way tension in cytoskeletons was proposed to physically open the nuclear pores (Janmey, 1998).

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Appendix A

To estimate the order of nuclear deformation, rough assumptions were made as follows. The geometry of nucleus was approximated to be an ellipsoid. The nucleus was also assumed to be continuous, homogeneous, incompressible, and isotropic. When a , b , and c are the radii of the major and the minor axes and half height of NEC, respectively (Fig. 9), the volume (V_i) and the area in the horizontal (x-y) plane (S_i) of the ellipsoid are represented in the form:

$$V_i = 4\pi abc / 3 \quad (\text{A.1})$$

$$S_i = \pi ab \quad (\text{A.2})$$

Similarly, when A , B , and C are the radii of corresponding axes of nucleus in the cell, respectively, the volume (V_c) and the corresponding area (S_c) are represented as follows:

$$V_c = 4\pi ABC / 3 \quad (\text{A.3})$$

$$S_c = \pi AB \quad (\text{A.4})$$

Since the area before isolation is 1.7-fold larger than that after isolation,

$$1.7\pi ab = \pi AB \quad (\text{A.5})$$

Taken Eq. (A1) to (A5) together, the height of nucleus in the cell can be obtained as follows;

$$C = c / 1.7 = 0.59c \quad (\text{A.6})$$

Similarly, the height of nucleus in sheared cell and NSEC are obtained as $0.39c$ and $0.82c$, respectively.

The assumption relating to nuclear shape is based on the electron microscopy, in which many

nuclei have either an ellipsoidal shape or the form an ellipse revolved about its minor axis. The degree of change in volume was investigated for chondrocytes embedded in a gel (Guilak, 1995), which reported that 15% compression caused 9.8% volume changes in chondrocyte nucleus. However, the nucleus volume change for adherent vascular cells caused by physiological stress is poorly understood.

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Captions

Fig. 1. Pipette aspiration test. (A) Schematic diagram of experimental setup. (B, C) An NEC (nucleus isolated from EC) (B) and an NSEC (nucleus isolated from sheared EC) (C) aspirated by micropipette. Negative pressure was applied to the direction vertical to the long axis of elongated shape. Scale bars = 10 μm . (D) Schematic diagram of a nucleus under pipette aspiration.

Fig. 2. Micrographs of endothelial nuclei stained with SYTO 13. (A–D) Phase-contrast images of an NEC (A), an NSEC (B), a nucleus in a cell (C), and a nucleus in a sheared cell (D). (E–H) Fluorescence images of an NEC (E), an NSEC (F), an EC (G), and a sheared EC (H). Scale bar = 5 μm .

Fig. 3. Transmission electron micrographs of endothelial nuclei. (A, B) Both an NEC (A) and a nucleus in a cell (B) contain nucleolus around the center part (open arrowheads) and chromatins that aggregate in the nuclear periphery, either of which is a typical morphological feature of nuclei. (Insets) A couple of nuclear pores (arrows) are observed in nuclear membrane (closed arrows).

Fig. 4. Changes in the area and the shape index of nuclei obtained in the fluorescence microscopy.

Fig. 5. Relationship between the normalized displacement L/a and aspiration pressure ΔP . (A) NECs ($n = 11$). (B) NSECs ($n = 13$). Mean \pm SD. Broken lines were obtained by least-squares regression for all plots.

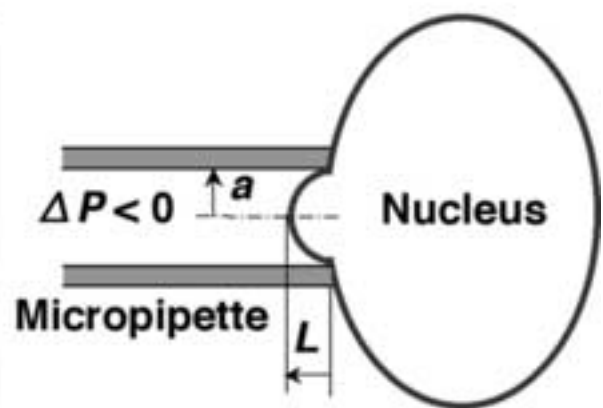
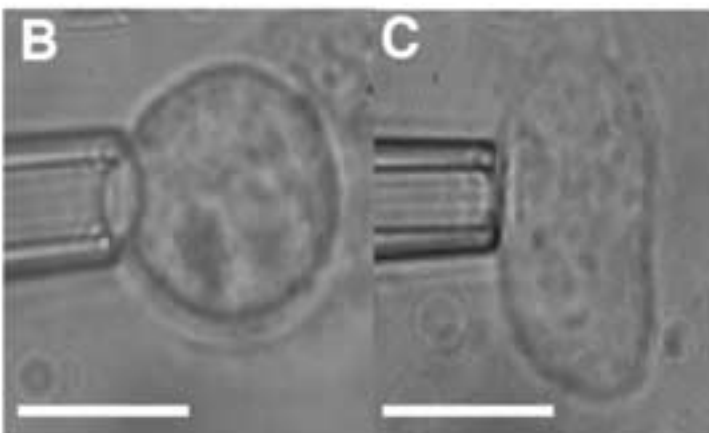
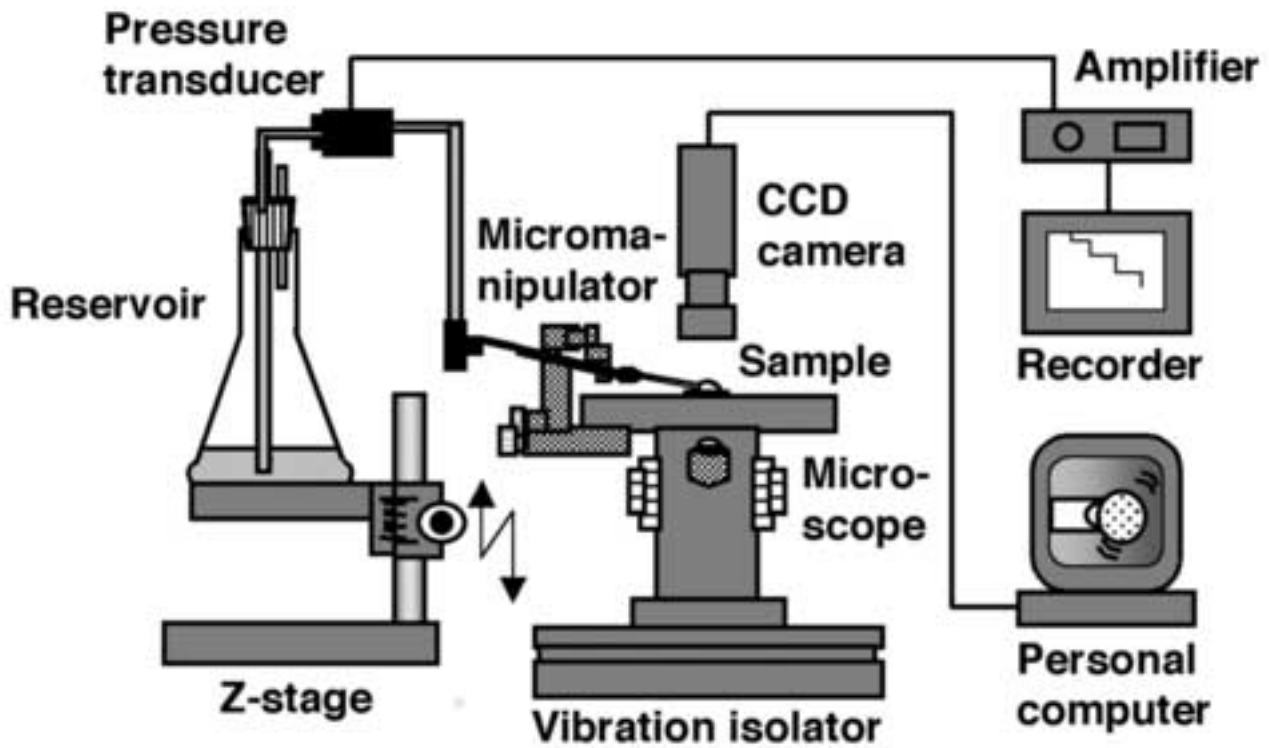
Fig. 6. Young's modulus of isolated nuclei determined by the pipette aspiration test. Mean + SD.

Fig. 7. Height of endothelial nucleus at each experimental condition estimated from the morphological observation. Schematic drawings show the lateral views of the nuclei (and the surrounding cell membrane for clarity). H is the height of nucleus isolated from EC, which is assumed to be the zero-stress state. See the text in detail and Appendix A for the derivation.

Fig. 8. Transmission electron micrographs of ECs. (A, B) Typical images of nucleus envelope of a statically cultured cell (A) and a fluid shear stress-imposed cell (B). (C) A whole cell cut in around the middle cross-section. The profile of spread EC seems to be determined by the size of nucleus. (D) A part of nucleus cut in around the middle cross section. Note that the nucleus seems to be locally compressed by a membrane-bound organelle located between the nucleus and the luminal membrane. Here, we defined the middle plane as the plane containing the nucleolus in the nucleus, luminal membrane, and abluminal membrane.

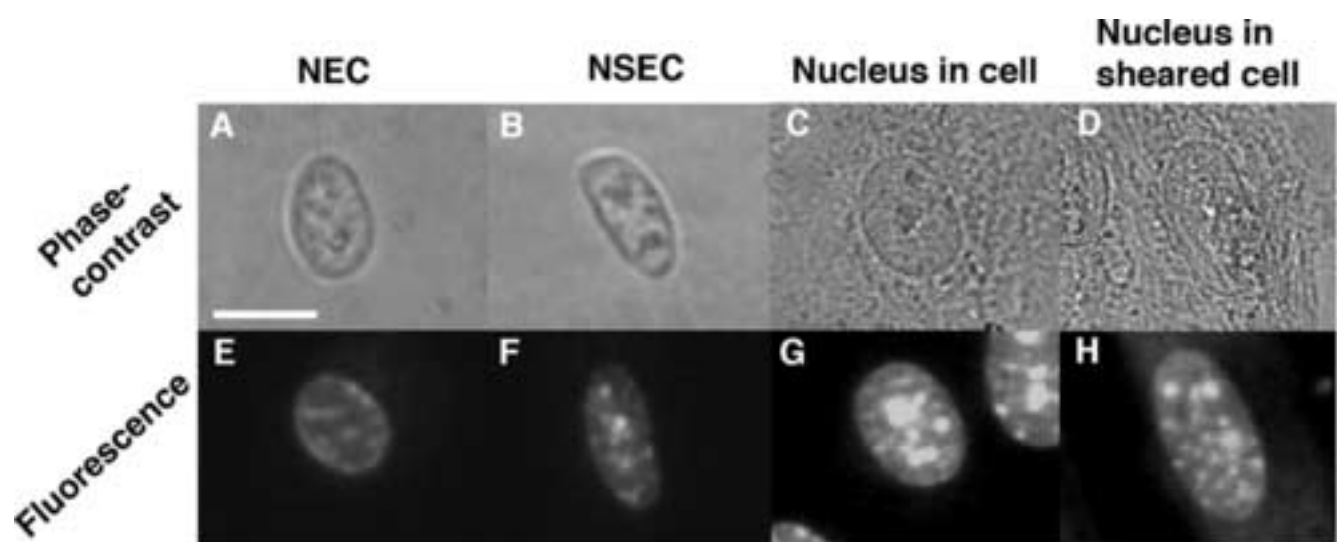
Fig. 9. Parameters determining the size of a nucleus approximated as an ellipsoid.

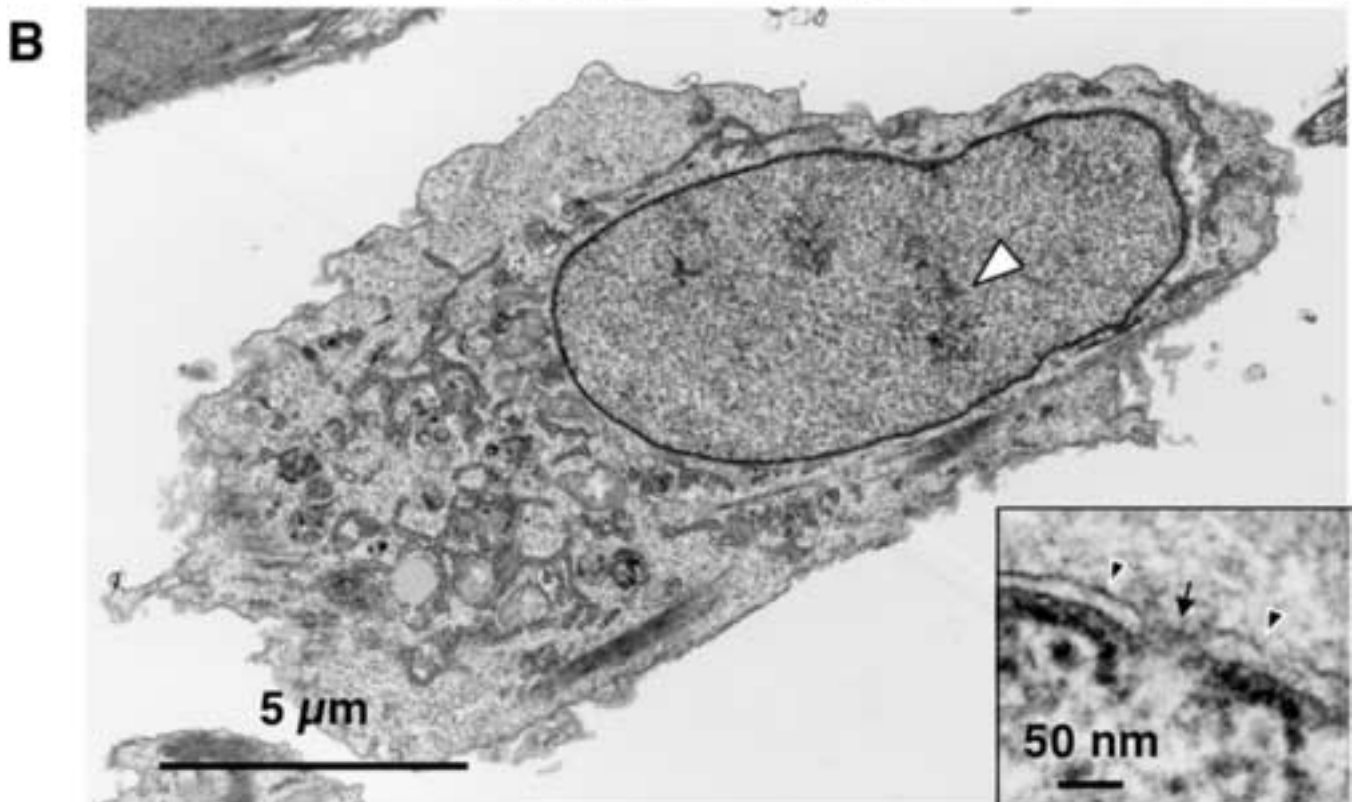
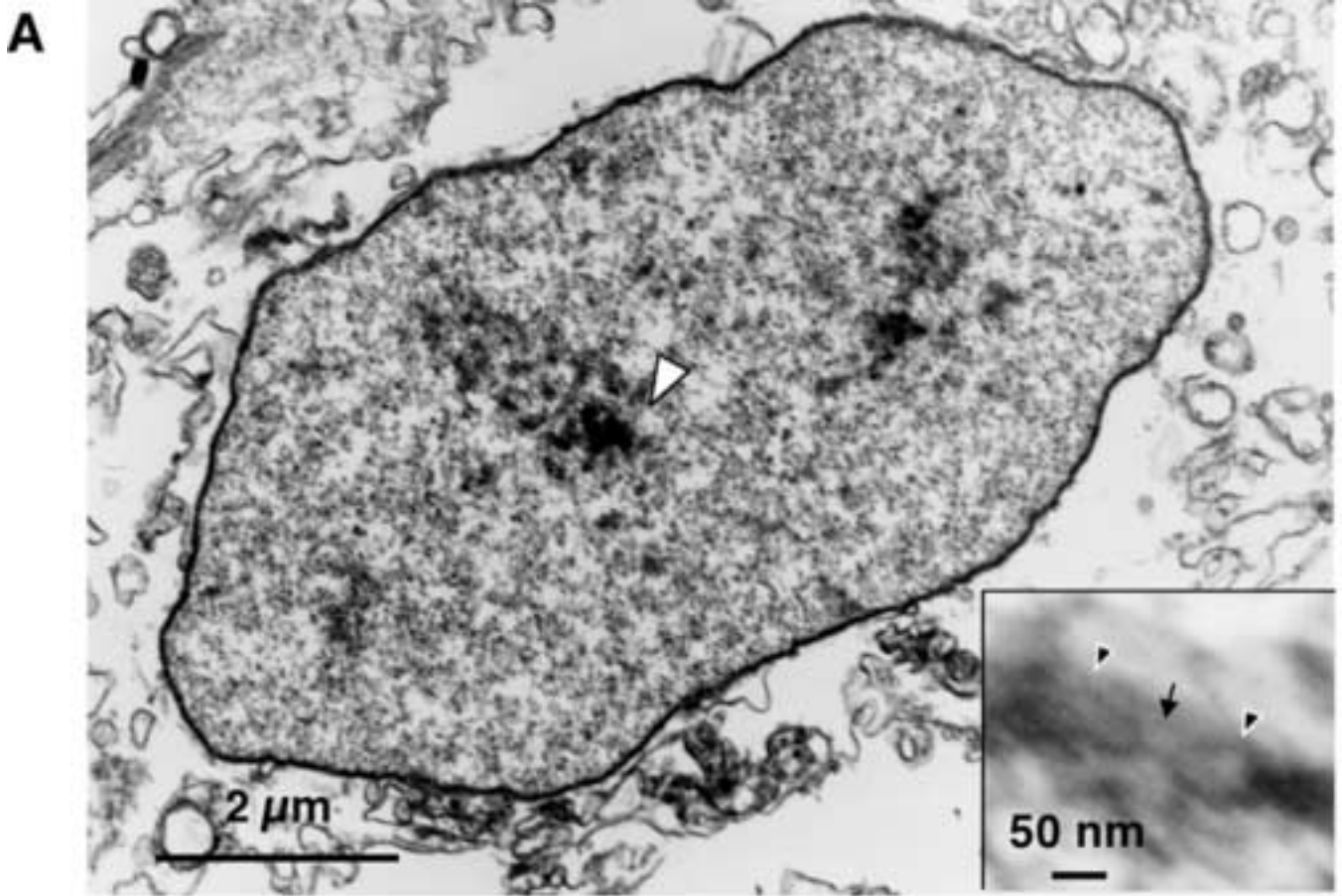
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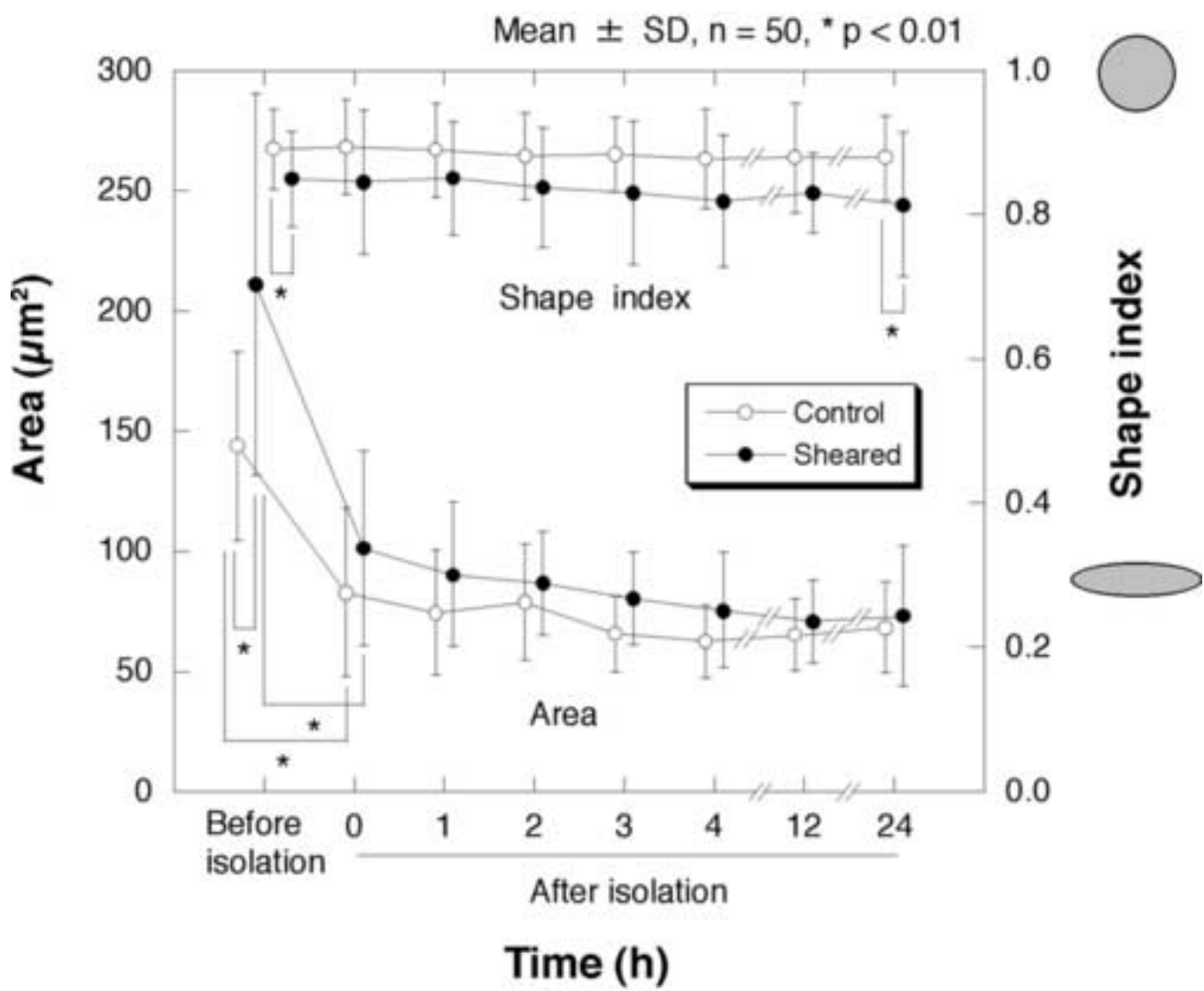


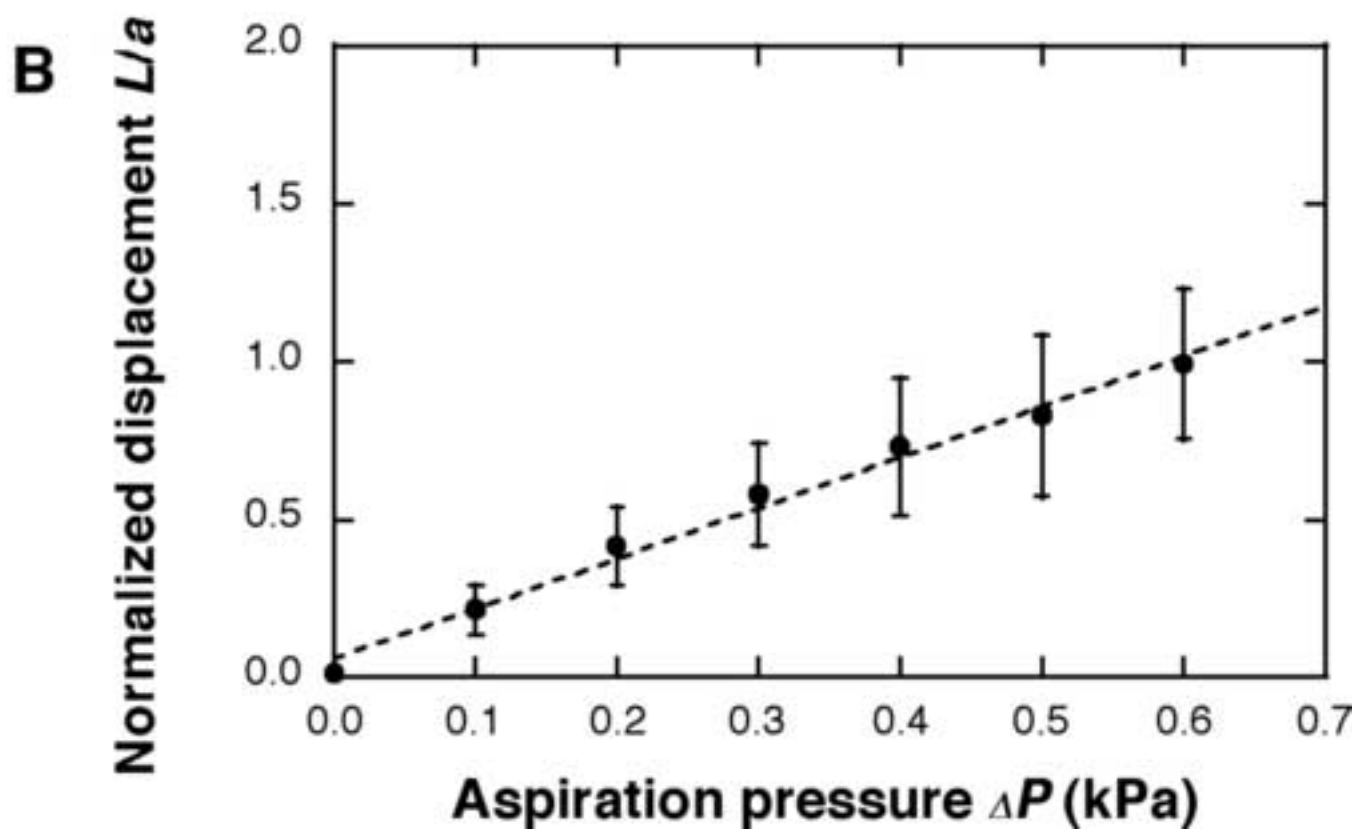
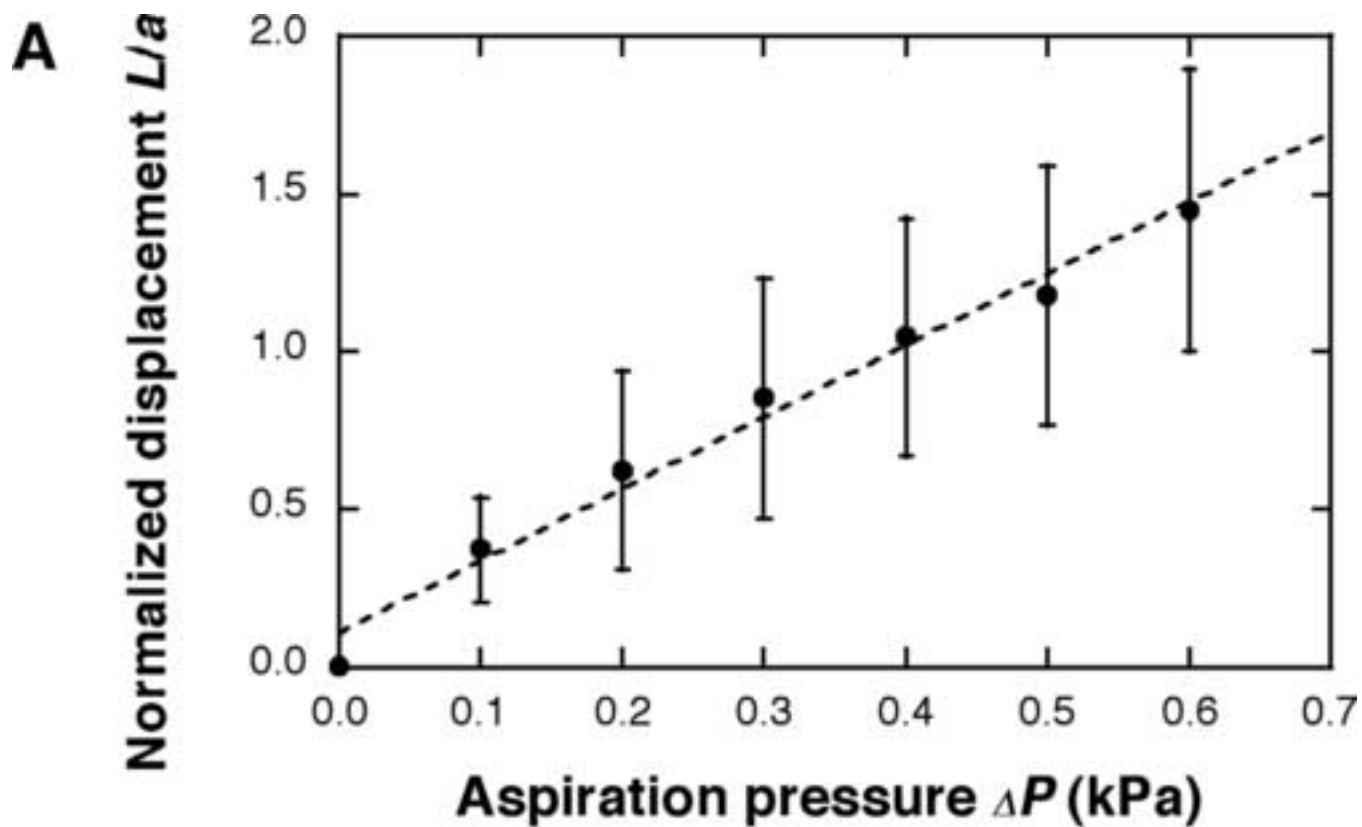
Figure(s)

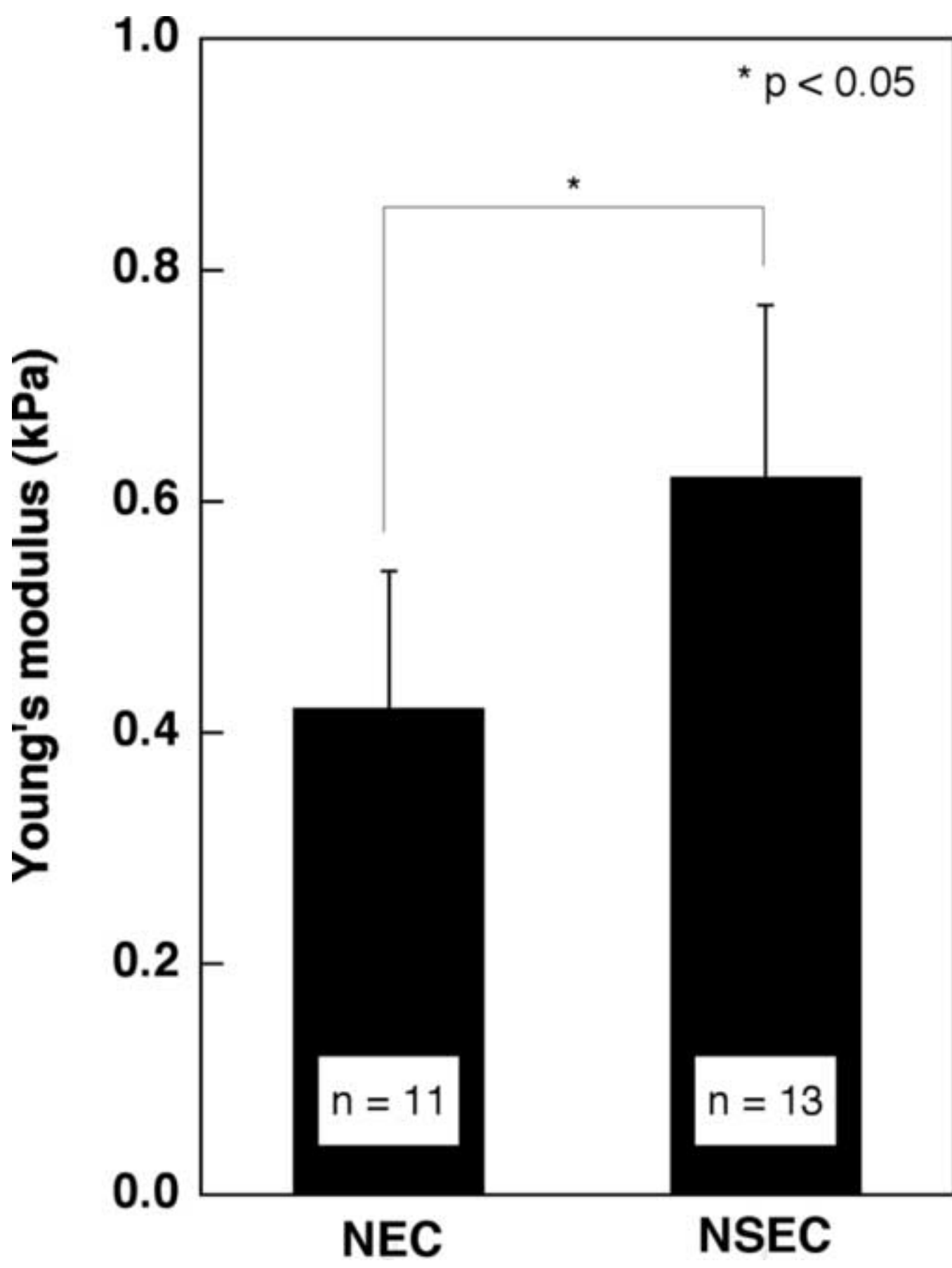
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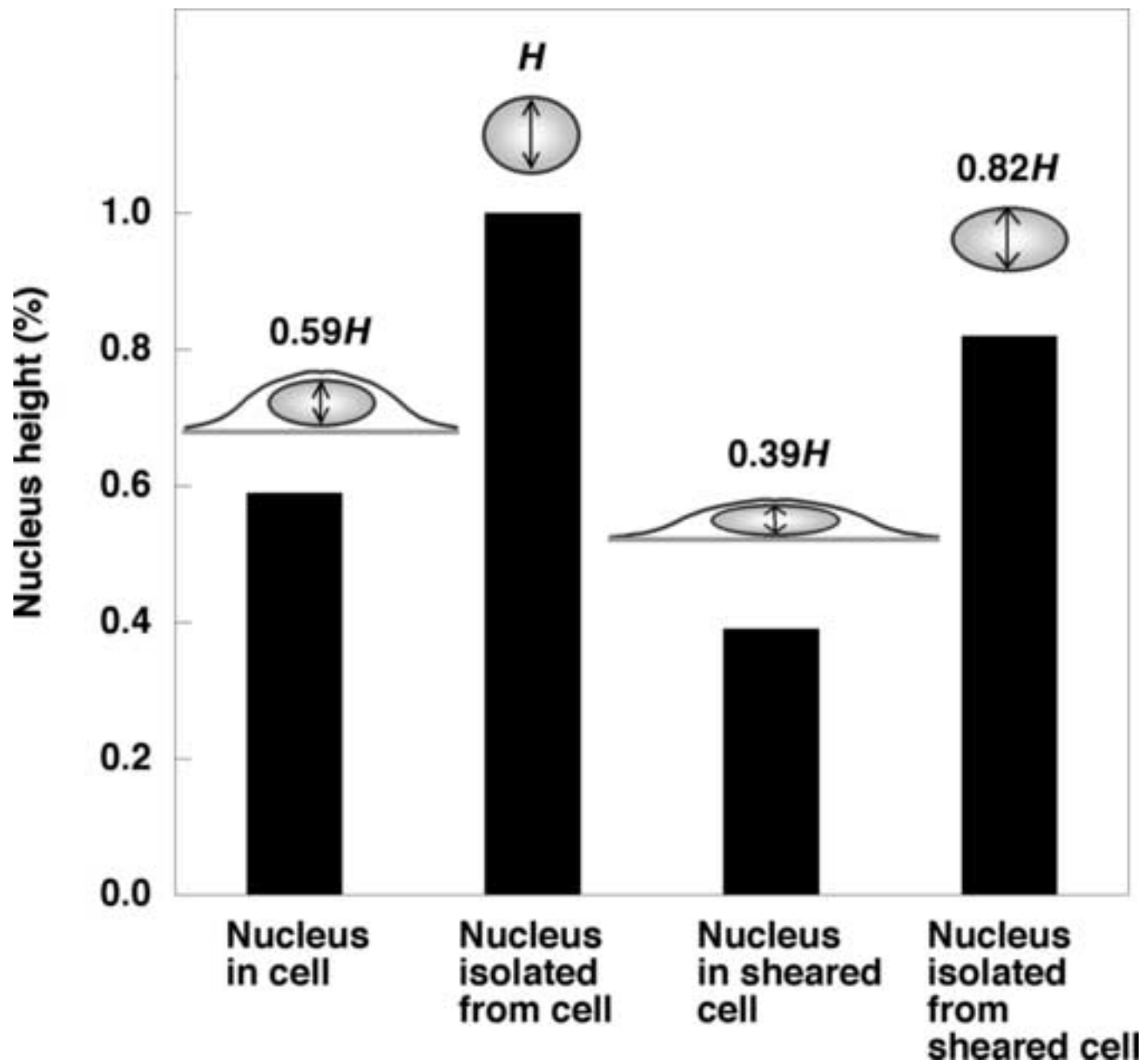


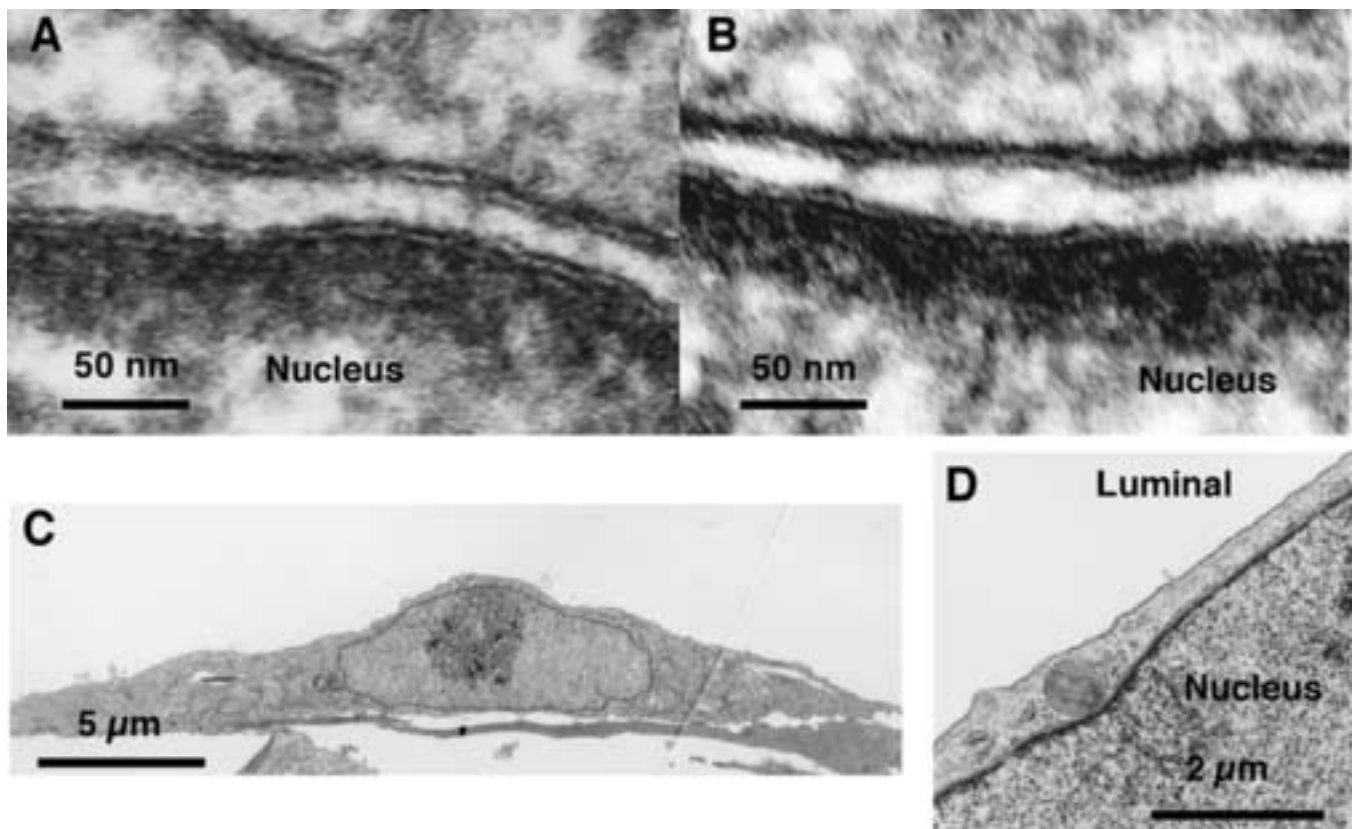












Figure(s)
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