Video Article

Biophysical Assays to Probe the Mechanical Properties of the Interphase Cell Nucleus: Substrate Strain Application and Microneedle Manipulation

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Abstract

In most eukaryotic cells, the nucleus is the largest organelle and is typically 2 to 10 times stiffer than the surrounding cytoskeleton; consequently, the physical properties of the nucleus contribute significantly to the overall biomechanical behavior of cells under physiological and pathological conditions. For example, in migrating neutrophils and invading cancer cells, nuclear stiffness can pose a major obstacle during extravasation or passage through narrow spaces within tissues.[1] On the other hand, the nucleus of cells in mechanically active tissue such as muscle requires sufficient structural support to withstand repetitive mechanical stress. Importantly, the nucleus is tightly integrated into the cellular architecture; it is physically connected to the surrounding cytoskeleton, which is a critical requirement for the intracellular movement and positioning of the nucleus, for example, in polarized cells, synaptic nuclei at neuromuscular junctions, or in migrating cells.[2] Not surprisingly, mutations in nuclear envelope proteins such as lamins and nesprins, which play a critical role in determining nuclear stiffness and nucleo-cytoskeletal coupling, have been shown recently to result in a number of human diseases, including Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, and dilated cardiomyopathy.[3] To investigate the biophysical function of diverse nuclear envelope proteins and the effect of specific mutations, we have developed experimental methods to study the physical properties of the nucleus in single, living cells subjected to global or localized mechanical perturbation. Measuring induced nuclear deformations in response to precisely applied substrate strain application yields important information on the deformability of the nucleus and allows quantitative comparison between different mutations or cell lines deficient for specific nuclear envelope proteins. Localized cytoskeletal strain application with a microneedle is used to complement this assay and can yield additional information on intracellular force transmission between the nucleus and the cytoskeleton. Studying nuclear mechanics in intact living cells preserves the normal intracellular architecture and avoids potential artifacts that can arise when working with isolated nuclei. Furthermore, substrate strain application presents a good model for the physiological stress experienced by cells in muscle or other tissues (e.g., vascular smooth muscle cells exposed to vessel strain). Lastly, while these tools have been developed primarily to study nuclear mechanics, they can also be applied to investigate the function of cytoskeletal proteins and mechanotransduction signaling.

Video Link

The video component of this article can be found at https://www.jove.com/video/3087/

Protocol

1. Substrate strain application

The measurement of normalized nuclear strain includes the preparation of strain dishes with transparent, elastic silicone membranes as cell culture surface, plating cells onto the dishes, and acquiring images of the cells before, during and after (uniaxial or biaxial) strain application.

Preparation of silicone membrane dishes and adherence of cells

1. Each strain dish consists of a custom-made bottomless plastic dish with a diameter of 3" and a plastic O-ring to hold a silicone membrane, which serves as the cell culture substrate. For preparation of the strain dishes, clamp a 4" x 4" piece of silicone membrane between the O-ring and the dish. Carefully cut away the excess membrane, rinse with deionized water, and autoclave the strain dishes.
2. Mark a reference point in the bottom center of the membrane (on the outside) before coating the silicone membranes with extracellular matrix molecules (e.g., fibronectin). This landmark will help identify the same cells during the strain experiments. (Optional: For uniaxial strain application, two parallel stripes of scotch tape are applied around the reference point to restrict deformation of the membrane in one dimension.)
3. To provide optimal cell attachment, coat the silicone membranes with 3 µg/ml fibronectin diluted in 10 ml PBS or any suitable extracellular matrix proteins. Cover the strain dish with an inverted 10cm polystyrene dish, and incubate the dishes over night at 4°C.

4. On the next day, rinse the membranes once with Phosphate Buffered Saline (PBS) to remove excess protein. Fill dish with 10ml of growth medium (Dulbecco's Modified Eagles Medium (high glucose) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) and set aside.
5. Mouse embryonic fibroblasts are trypsinized with 0.05% trypsin and seeded in growth medium at approximately 30% confluence onto the coated silicone membrane dish and incubate for 24 – 48 hours under normal culture conditions.

Substrate strain experiments

6. Set-up the microscope for experiments. The experiments are performed on an inverted microscope with a digital camera suited for fluorescence microscopy, phase contrast or DIC, using a 60x objective and appropriate image acquisition software (e.g., IPLab or Metamorph). An upright microscope is not suitable for this application. The strain device consists of a base plate that fits onto the microscope stage and holds a central cylindrical platen that serves to apply strain to the central section of the silicone membrane, a movable plate that holds the strain dish and that can slide up and down on four guidance pins, as well as a 5-lb weight plate to apply a load.
7. In order to visualize nuclei, incubate the cells in the strain dish with 1 µg/mL of Hoechst 33342 for 15 min at 37°C. Aspirate off the medium and replace with 15 ml phenol-red free growth medium (phenol-red free Dulbecco's Modified Eagles Medium (high glucose) with 25 mM Hepes, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin). Screw the strain dish into the dish holder plate. Carefully apply grease (Braycote 804 Vacuum Grease) to the perimeter of the bottom of the silicone membrane to ensure gliding of the membrane along the central platen. Make sure to keep the central section of the membrane clear.
8. Place the base plate onto the microscope stage. Mount dish holder plate carefully on the base plate. Assure that in the initial resting position, the silicone membrane of the strain dish loosely rests on the central platen.
9. First, focus on bottom of the silicone membrane and find the central black reference dot. The dot will serve as the starting point for all image acquisitions and aids in locating the same cells during and after stretch. We use a custom-written automated imaging program to store the positions of cells and to relocate these cells during the experiment, but this can also be achieved manually.
10. Starting from the dot, adjust the focus to visualize the cells and the top of the silicone membrane. Locate well-spread cells with centrally located nuclei and acquire a phase contrast and a fluorescence image of the nuclear Hoechst stain. The phase contrast image should focus on the cell outline and the silicone membrane, while the fluorescence images should focus on the central plane of the nucleus.
11. After acquiring images of 5 to 15 cells, move back to the central dot. Slowly apply the weight to the strain dish, resulting in uniform strain application in the center of the dish. The maximal applied substrate strain is limited by nylon spacers placed on the vertical alignment pins (guidance pins).
12. Focus on the bottom of the silicone membrane and locate the reference dot again. Starting from the dot, relocate the same cells and again acquire a phase contrast and a fluorescence image of the cells and nuclei under full strain, trying to closely match the focal planes of the initial images. This process should not exceed 10 min to avoid active remodeling and adaptation of the cell to the strained substrate.
13. After all the corresponding images have been acquired, move the microscope stage back to the starting point. Carefully remove the weight from the dish holder plate and allow the silicone membrane to relax. If necessary, gently push up the strain dish until it is in the initial position. Then acquire phase contrast and fluorescence images of the post-strain cells as described above for the strain images.

Analysis

14. Images of cells and fluorescently labeled nuclei before, during, and after strain application are analyzed to compute the normalized nuclear strain. In our laboratory, we use a custom-written MATLAB script for the analysis, but several alternative options are available. The analysis is performed in three steps.
15. First, to calculate the applied substrate strain, the positions of 3 to 6 control points located on the membrane are manually matched between the corresponding pre-, full-, and post-strain images. The MATLAB program then computes the applied membrane strain by comparing the positions of matching control points between the pre-strain and full-strain images and also the residual strain between the pre-strain and the post-strain images. At the same time, the control points are used to register the image pairs, which will help to detect damaged or detaching cell (see Figure 1).
16. In a second step, nuclei are manually selected using a separate MATLAB program that calculates nuclear strain for each individual nucleus by matching either nuclear size or intranuclear markers between corresponding pre-, full-, and post-strain fluorescent images. To account for small variations in applied membrane strain between different experiments, we express results as *Normalized Nuclear Strain*, defined as the ratio of induced nuclear strain to applied membrane strain that is computed for each nucleus. The MATLAB scripts are available from the Lammerding laboratory upon request.
17. Finally, each nucleus is validated, excluding measurements from cells that detach or become damaged during strain application (Figure 1).

2. Microneedle manipulation assay

Preparation of dishes, adherent cells, and microneedles

1. Incubate 35 mm glass bottom cell culture dishes with a low concentration of fibronectin (0.5ug/ml) in Hank's Buffered Salt Saline (HBSS) or any suitable extracellular matrix proteins for 2 hours at 37°C. Wash dishes with HBSS two times and add 2 ml of growth medium to the dish before proceeding to the next step.
2. Mouse embryonic fibroblasts are trypsinized with 0.05% trypsin and seeded in 2 ml growth medium seeded at 7.5×10^4 cells/ml onto the fibronectin-coated glass bottom dishes. Place cells back in incubator overnight. Place cells back in incubator overnight. One should optimize the number of cells to obtain single, adherent, non-confluent cells for other cell types.
3. Pull the microneedles, made of borosilicate capillaries, to tip diameters of approximately 1 to 3 µm with a commercial pipette puller (e.g., Sutter Instrument Company).

Microneedle manipulation experiment

4. The next day, incubate the cells with MitoTracker mitochondrial stain (600 µM; Invitrogen) and Hoechst 33342 nuclear stain (1 µg/mL) added to growth medium for 30 minutes in a 37°C incubator.

5. Wash the cells one time in HBSS for 5 minutes at room temperature and then add phenol-red free growth medium to the cells for imaging.
6. Acquire one image of the single cell without the microneedle inserted into the cytoskeleton in phase contrast, one fluorescent image of the Hoechst 33342 stain and one fluorescent image of the mitochondrial stain, with a 60x objective (0.70 N.A., Plan-Achromat) on an inverted microscope with a digital charge-coupled device camera.
7. Using a micromanipulator (e.g., InjectMan NI 2, Eppendorf), carefully insert the microneedle into the cytoplasm of a cell a fixed distance (typically 5 µm) away from the nuclear periphery and take one phase contrast image, one fluorescent image of the Hoechst 33342 stain and one fluorescent image of the mitochondrial stain. For this application, it helps to control the micromanipulator through a computer, e.g., Windows Hyperterminal, to achieve consistent micromanipulation procedures.
8. Move the microneedle, a specific distance (typically 10 or 20 µm) towards the cell periphery at 1 µm/sec while simultaneously collecting fluorescence and phase contrast images every 10 seconds. As the microneedle is moving to a specific distance toward the cell periphery. With the chosen parameters, as the microneedle is moving to a specific distance toward the cell periphery, this will correspond to 2-3 frames during the manipulation process.
9. Finally acquire additional images after the microneedle is removed from the cytoskeleton.

Analysis

10. Displacement maps are computed using a custom-written MATLAB script based on tracking fluorescently labeled features of the nucleus and cytoplasm. (The MATLAB script is available from the Lammerding laboratory upon request). The program uses a normalized cross-correlation algorithm between small image regions (approximately 10 µm x 10 µm in size and spaced 5 µm apart) in subsequent image frames. For each region center, the displacement in the x- and y-directions are computed as the shift between the original location and the newly identified position and displayed as a displacement vector and also stored as numerical values. From the displacement maps, average displacements within predefined regions can be computed. Note that cytoskeletal displacements are based on the fluorescence channel for cytoskeletal markers (e.g., MitoTracker mitochondrial stain), while nuclear displacements are computed from the fluorescence channel corresponding to the Hoechst 33342 signal. For our application, we routinely examine the following regions: *(i)* cytoskeletal strain at the strain application site, i.e., the microneedle insertion site; *(ii)* nuclear strain in a region inside the nucleus towards the strain application site; *(iii)* nuclear strain in a nuclear region away from the application site; and *(iv)* cytoskeletal strain in a cytoplasmic region across the nucleus. In addition, one can also directly measure nuclear elongation from phase contrast or Hoechst 33342 fluorescence image sequences. In this case, applied nuclear strain is calculated by dividing the nuclear elongation ($\Delta L = L - L_0$) by the initial length, L_0, where L is the final length of the nucleus at the end of strain application and L_0 is the initial length of the nucleus. For cells with an intact nucleo-cytoskeletal coupling, the nucleus will elongate towards the strain application site. In contrast, in cells in which nucleo-cytoskeletal coupling is disrupted, i.e., forces are transmitted less efficiently between the cytoskeleton and the nucleus, the nucleus is expected to elongate significantly less in the direction of the strain application site. Thus, decreased nuclear deformations in response to cytoskeletal strain application imply a (partial) uncoupling between the nucleus and cytoskeleton.

3. Representative results:

Substrate strain application

We acquired images before, during, and after strain application to mouse embryonic fibroblasts from heterozygous and homozygous lamin A/C-deficient (*Lmna*$^{+/-}$ and *Lmna*$^{-/-}$), and wild-type (*Lmna*$^{+/+}$) mice and subsequently computed the normalized nuclear strain for each cell. After analysis, the nuclei are validated and cells that become damaged or retract during strain application are excluded from the analysis. Figure 1A depicts nuclei of three cells that are valid, whereas Figure 1B depicts cells that should be excluded from analysis. Normalized nuclear strain data are pooled from at least three independent experiments (each containing measurements from ~5–10 nuclei) and compared with other cell or treatment groups by statistical analysis. Increased normalized nuclear strain indicates reduced nuclear stiffness, as seen in cells with reduced expression of the nuclear envelope proteins lamin A/C (Figure 2).

Microneedle manipulation assay

For the microneedle manipulation assay, we imaged nuclear and cytoskeletal displacements during localized cytoskeletal strain application. Cells that become damaged or detached are excluded from the analysis. For the analysis, we measure the magnitude of the nuclear and cytoskeletal movements towards the force application site in single, adherent cells. For example, in Figure 3, we track mitochondrial (marker for the cytoskeleton) displacements before and after cytoskeletal strain and then plot the displacements as vectors. Each vector represents the displacement computed as the shift between the original location and the newly identified position. Regions with low image intensity or insufficient texture (e.g., regions outside the cell) are excluded from the analysis. The cytoskeletal and nuclear displacements are then quantified in select areas at increasing distances from the strain application site (Figure 4, areas corresponding to the colored boxes in inset). In mouse embryonic fibroblasts with intact nucleo-cytoskeletal coupling, forces are transmitted through the entire cells, resulting in induced nuclear and cytoskeletal deformations that slowly dissipate away from the strain application site (Figure 4). In contrast, fibroblasts with disturbed nucleo-cytoskeletal coupling (or altered cytoskeletal organization) display localized displacements near the application site, as shown in Figure 4 and only little induced deformations further away. Comparable cytoskeletal strain application at the microneedle insertion site (orange box) is observed for both control fibroblasts (mCherry alone) and fibroblasts with a disrupted nucleo-cytoskeletal coupling (DN KASH). However, induced nuclear and cytoskeletal displacements (blue, yellow, and red boxes) at other regions were significantly smaller in the fibroblasts with disrupted nucleo-cytoskeleton coupling (DN KASH) than in control cells (mCherry alone) (Figure 4). Thus, decrease in cytoskeletal and nuclear displacements away from the strain application site, indicates that force transmission between the cytoskeleton and nucleus was disturbed.

Importantly, we have also validated that mitochondria are suitable cytoskeletal marker, by conducting microneedle manipulation on mouse embryonic fibroblasts transfected with GFP- or mCherry actin and GFP-vimentin and fluorescently labeled with Mitotracker Green or Red. Cytoskeletal displacement maps were calculated independently from the fluorescent signal of the mitochondria and the actin or vimentin cytoskeleton. The average absolute displacement was computed for four distinct cytoskeletal regions at increasing distances away from the strain application site. The slope and R-squared values were computed from the linear regression between the measurements obtained from

mitochondria and from actin or vimentin, respectively. For actin, the slope was 0.99 and the R^2 value was 0.986; for vimentin, the slope was 1.04 and the R2 value was 0.971, confirming that mitochondrial displacements serve as reliable indicators for cytoskeletal deformations.

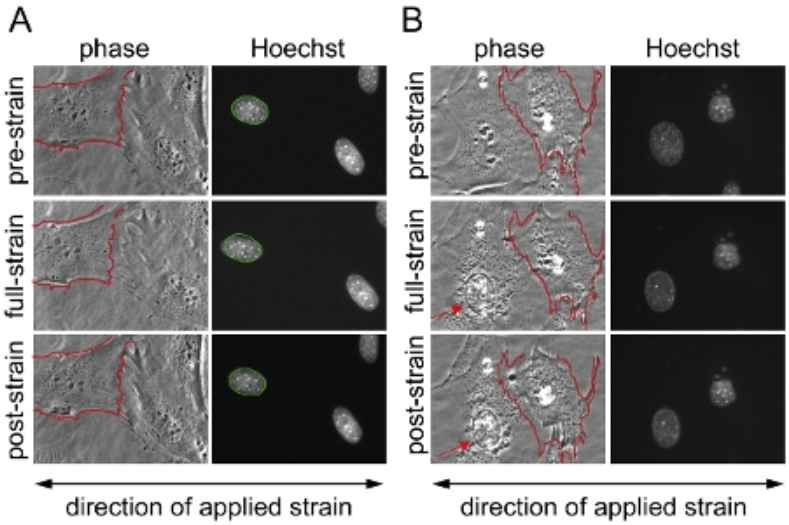

Figure 1. Substrate strain application on mouse embryonic fibroblasts (MEFs). Mouse embryonic fibroblasts spread over two distinct areas on the silicon membrane were imaged with phase contrast and fluorescence microscopy before, during and after application of 20% uniaxial strain. (A) Example of a successful experiment with valid nuclei from cells that survived the strain application without any damage or detachment and (B) example of cells that retract/partially detached during strain application; results from the cells depicted in (B) are excluded from the analysis. In (B), the cell on the left side shows signs of cytoskeletal damage and nuclear collapse (arrow), while the cell on the right side detaches partly and retracts during strain application. This can be an indication of excessive strain application. For better comparison, in (A) and (B) the border of one of the unstretched cell membranes is outlined in red and superimposed on the same cell during and after strain application. In (A) the border of the unstretched nucleus is outlined in green and superimposed on the same nucleus during and after strain application.

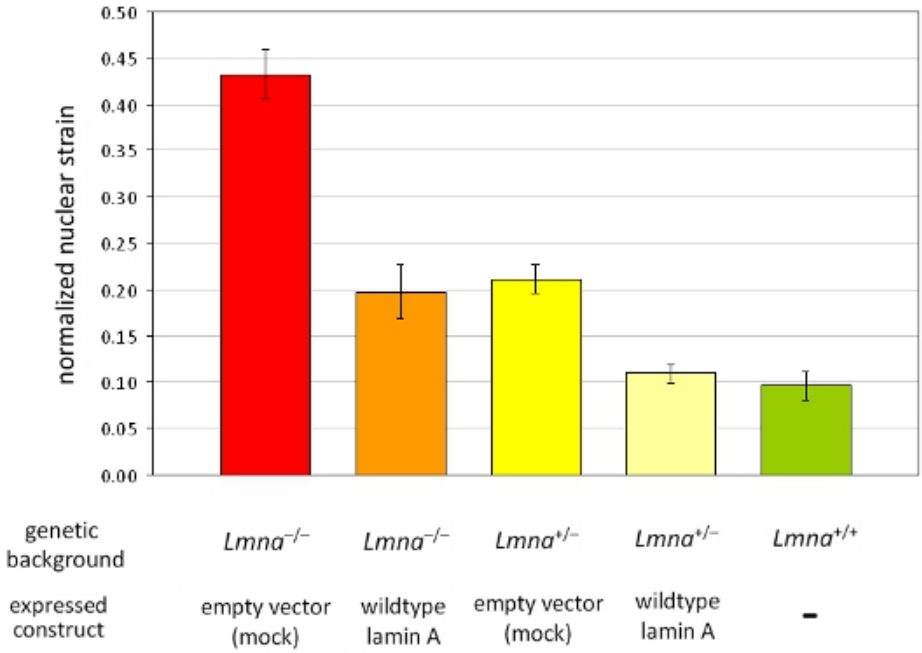

Figure 2. Analysis of normalized nuclear strain in a panel of different MEF cell lines. MEFs of the *Lmna*$^{-/-}$ and *Lmna*$^{+/-}$ genetic background ectopically expressing either an empty vector or wild-type lamin A were analyzed. In comparison to MEFs from wild-type littermates (*Lmna*$^{+/+}$), loss of lamin A/C expression results in decreased nuclear stiffness that can be fully restored by reintroduction of wild-type lamin A. Notably, reduced nuclear stiffness is reflected by increased values of normalized nuclear strain. The error bars represent standard errors.

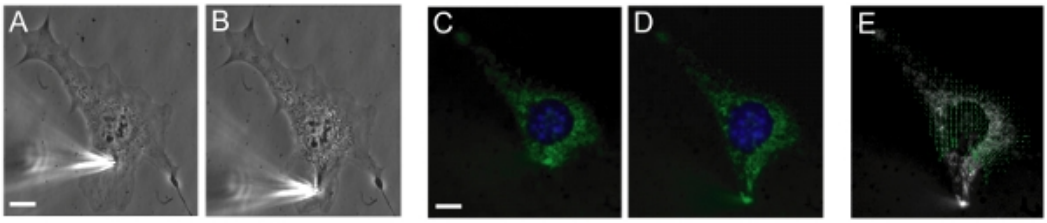

Figure 3. Microneedle manipulation assay to measure intracellular force transmission. Phase contrast (A, B) and fluorescence (C, D) images of a fibroblast labeled with nuclear stain (blue) and MitoTracker mitochondrial stain (green). A microneedle was inserted into the cytoskeleton at a defined distance from the nucleus (A and C) and subsequently moved towards the cell periphery (B, D). Cytoskeletal and nuclear displacements were quantified by tracking fluorescently labeled nucleus and mitochondria using a custom-written cross-correlation algorithm. (E) Displacement map of the final cytoskeletal (green) deformations computed from fluorescence image series; arrow length is magnified by 2x for better visibility. Scale bars, 10 μm.

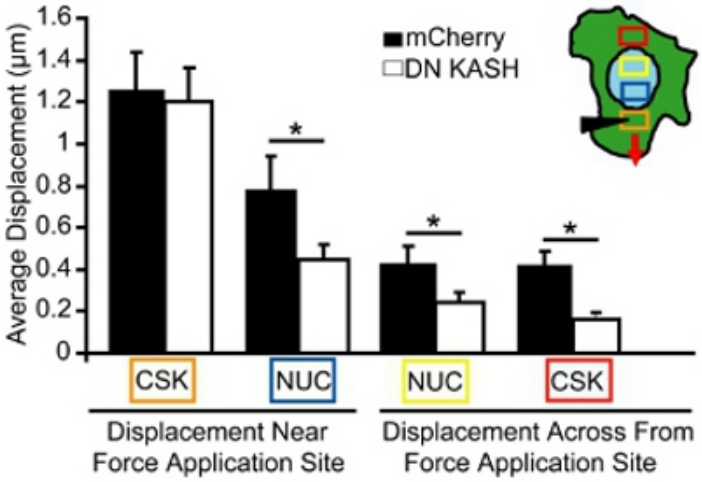

Figure 4. Analysis of intracellular force transmission during microneedle manipulation. Induced cytoskeletal and nuclear displacements during microneedle manipulation, measured in the areas corresponding to the colored boxes (inset in A). The orange box is the strain application site. Despite similar strain application in the cytoskeleton (orange box), induced nuclear and cytoskeletal displacements (blue, yellow, and red boxes) were significantly smaller in the mouse embryonic fibroblasts that with a disrupted nucleo-cytoskeletal coupling (DN KASH) compared to control (mCherry alone) cells.

Discussion

Substrate strain assay

Strain application has been successfully used by us and other groups to study induced nuclear deformations in cells subjected to mechanical stress and to investigate the contribution of specific nuclear envelope proteins to nuclear stiffness.[4-8] The advantage of this technique is that it probes mechanical properties of living nuclei in their normal cellular and cytoskeletal environment and that the substrate strain application resembles physiological load application as found in many tissues such as contracting muscle or blood vessel walls.[9] Furthermore, it enables strain application to many cells in parallel, increasing the number of cells that can be analyzed in a single experiment. One limitation of the substrate strain assay is that it does not allow direct measurements of nuclear stiffness. Instead, this method determines the relative stiffness of the nucleus compared to the surrounding cytoskeleton. Detailed analysis of induced nuclear and cytoskeletal strain in cells subjected to uniaxial substrate strain show that in wild-type cells, the cytoskeletal strain is comparable to the applied substrate strain, while the stiffer nucleus deforms significantly less.[4] Nonetheless, additional assays, such as the microneedle manipulation assay, may be necessary to assure that observed differences in nuclear deformation between different cell lines are not the results of altered nucleo-cytoskeletal coupling or cytoskeletal structure. Despite these limitations, measuring nuclear mechanics in intact cells rather than in isolated nuclei minimizes the risk of artifacts induced by osmotic effects, damage during the isolation procedure, or other changes associated with nuclear isolation. However, one stringent requirement for the substrate strain assay is that cells firmly adhere to the substrate. To improve cellular adhesion, dishes can be coated with different extracellular matrix proteins such as fibronectin, collagen or laminin at variable concentrations; we recommend determining the optimal coating conditions for each new cell type used in the experiments.

Furthermore, for optimal results, the applied substrate strain must be sufficiently large to detect nuclear deformations while minimizing damage to the cells. For mouse and human fibroblasts, we typically apply 5 % biaxial strain or 20% uniaxial strain without overt cell damage. In general, we find that cells tolerate uniaxial strain application better than biaxial strain application, as smaller changes in membrane area are required. As for the extracellular matrix coating, the optimal conditions of strain application should be determined for each new cell line to be tested.

In addition, we found that the experiments are sensitive to the cell density. Experiments should ideally be conducted on sub-confluent cells to minimize cell-cell interactions; however, cell densities that are too low often result in poor survival of cells in response to strain and in a low yield

of cells per experiments. On the other hand, a cell density that is too high makes it difficult to identify the same cells before, during, and after strain application. We recommend to test optimal cell density for individual cell types.

Another important advice for the experiments is that strain application should be limited to 10 minutes or less to avoid cellular adaptation, such as remodeling of cytoskeletal elements. To this end, the use of automated imaging software, e.g. IPlab, in combination with a motorized stage enables automatic re-localization of the cells on the strain dish and faster image acquisition. Furthermore, we use custom-written image analysis software (e.g. MATLAB) for data analysis. The software requires certain marker points on the membrane to calculate the applied substrate strain, such as the applied dot, small fluorescent beads or distinct irregularities on the silicon membrane. Finally, validation of each nucleus is required to exclude those of damaged or retracting cells, as the nuclear deformations measured in these cells are not representative. A possible adaptation of the substrate strain technique is to apply substrate strain to cells cultured in three-dimensional collagen gels, which allows analysis of nuclear mechanical properties under more physiological conditions, as well as to apply the method to more weakly adherent cells. Another variation is to use micropatterned substrates, e.g., round or rectangular patches of fibronectin on the silicon membrane, to achieve consistent cell spreading and alignment during the strain application. Finally, to better understand nuclear mechanics on a more physiological level, cellular strain can be applied on entire tissues instead of isolated cells. We are currently successfully using this technique to measure nuclear stiffness in model organisms such as *Drosophila melanogaster* or *Caenorhabditis elegans*.

Microneedle manipulation assay

The microneedle manipulation assay is a single cell-based method to probe intracellular force transmission by quantifying induced nuclear and cytoskeletal displacements after localized cytoskeletal strain application, thereby advancing an earlier approach pioneered by Maniotis and colleagues.[10] This technique offers several advantages over other localized force application methods such as magnetic tweezers or optical traps. For instance, strain can be applied directly to the cytoskeleton and can be modified varying the distance of the microneedle. The microneedle can also be positioned in the cell with high accuracy, making the experiment very reproducible, and the force application rate, which is directly related to the microneedle speed, can be precisely controlled by the programmed micromanipulator. In contrast, the magnetic beads used in magnetic tweezer and optical traps are often randomly localized on the apical cellular surface, and both optical and magnetic tweezers generate insufficient forces to result in large scale cytoskeletal deformations in many cell types. Although, the microneedle manipulation assay is an invasive technique and there is limited control over which cytoskeletal structures the microneedle adheres to, we can confirm the results with less invasive approaches (e.g. substrate strain). Thus, microneedle manipulation and substrate strain are two complementary assays that yield information about nuclear mechanics in intact living cells while maintaining normal nuclear and cytoskeletal architecture and preserving the correct chemical composition of the nucleoplasm and cytoplasm.

In the following, we suggest possible modifications or improvements. If cells are too well spread, the microneedle tip may break against the glass bottom dish when trying to position it within the thin (~1-2 µm) cytoskeleton. To avoid these problems, we recommend testing a range of concentrations of extracellular matrix (ECM) molecules, such as fibronectin, collagen, or laminin, to determine conditions that achieve sufficient cell adhesion without allowing the cells to spread too thin. Another modification could be to plate the cells on ECM-coated micropatterned surfaces to assure uniform cell spreading and orientation. An additional or complementary approach could be to plate cells on ECM-coated polyacrylamide gels and insert the microneedle through the cytoskeleton into the gel to provide uniform strain application across the cytoskeleton. The use of an intact and fine-tipped microneedle is absolutely essential for the success of the experiments. We routinely pull our own microneedles, and one can experiment with different shapes and sizes. Alternatively, one can also purchase individually packed microneedles with consistent geometry (e.g., Eppendorf Femtotips). In any case, it is important to assure that the microneedle remains undamaged during the experiments, as a broken microneedle tip, for example after making contact with the glass substrate, will damage cells. During the microneedle manipulation procedure itself, it is important to apply consistent speed and range of the microneedle movement, as the nucleus and cytoskeleton are viscoelastic materials with time-dependent behavior. We recommend using a computer-controlled micromanipulator, which will also assist in the coordination of microneedle movement and image acquisition. From our experience, Mitotracker can by cytotoxic, thus, limit the experiments to 30 minutes or use other fluorescent markers, such as GFP-actin or GFP-vimentin. One modification of the approach described here, particularly when studying nucleo-cytoskeletal coupling, is to insert the microneedle into the nucleus instead of the cytoskeleton and move it towards the cell periphery.

For all studies, due to the typically large cell-to-cell variability, experiments should be performed at least three independent times to obtain measurements from a minimum of 15–25 total valid cells.

Disclosures

No conflicts of interest declared.

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