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Assays to measure nuclear mechanics in interphase cells

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Abstract

The nucleus is the characteristic hallmark of all eukaryotic cells. The physical properties of the nucleus reflect important biological characteristics, such as chromatin organization or nuclear envelope composition; they can also directly affect cellular function, for example, when cells pass through narrow constrictions, where the stiff nucleus may present a limiting factor. We present two complementary techniques to probe the mechanical properties of the nucleus. In the first, nuclear stiffness relative to the surrounding cytoskeleton is inferred from induced nuclear deformations during strain application to cells on an elastic substrate. In the second approach, nuclear deformability is deduced from the transit time through a perfusion-based microfabricated device with constrictions smaller than the size of the nucleus. These complementary methods, which can be applied to measure nuclear stiffness in large numbers of living adherent or suspended cells, can help identify important changes in nuclear mechanics associated with disease or development.

Keywords

Nucleus; microfluidics; cell/nuclear mechanics; deformation

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KEY REFERENCE (optional)

Basic protocol 1:

(Caille et al. 1998),(Lammerding et al. 2004)

Basic protocol 2:

(Whitesides et al. 2001), (Huang et al. 2011), (Lammerding et al. 2004), (Rosenbluth et al. 2008)

INTERNET RESOURCES (optional)

<http://www.jove.com/details.php?id=3087>

This is a JoVE article: Biophysical Assays to Probe the Mechanical Properties of the Interphase Cell Nucleus: Substrate Strain Application and Microneedle Manipulation. (Lombardi, Zwerger, et al. 2011)

INTRODUCTION

The mechanical properties of the nucleus have recently gained increasing interest, as it is now emerging that changes in nuclear structure and mechanics are associated with a variety of human diseases and also occur during normal differentiation and development (Zwerger et al. 2011). The nucleus often occupies a large fraction of the cell volume and houses the genomic DNA. It is enclosed by the nuclear envelope, consisting of two lipid bilayers, the outer and inner nuclear membranes, and an underlying dense protein meshwork, the nuclear lamina. The nuclear lamina is composed of lamin proteins and connected to the inner nuclear membrane via nucleus-specific transmembrane proteins such as emerin, lamin B receptor (LBR), and SUN proteins (Holaska 2008). Importantly, the nucleus is physically connected to the surrounding cytoskeleton via nesprins and SUN proteins at the nuclear envelope, enabling continuous force transmission between the extracellular matrix and the nuclear interior (Lombardi & Lammerding 2011). The mechanical behavior of the nucleus is primarily determined by contributions from the nuclear lamina and the nuclear interior. The lamina deforms elastically and can stretch significantly under stress (Kris Noel Dahl et al. 2004). The nuclear interior, consisting of chromatin and chromatin-associated proteins, has characteristics of a viscoelastic medium (Rowat et al. 2008). In most normal cells, the nucleus is significantly stiffer than the surrounding cytoplasm. As such, the mechanical properties of the nucleus often govern the overall mechanical behavior of cells during large cell deformations. Nuclear deformability may even constitute a limiting factor in the cell's ability to migrate through narrow constrictions, for example, during cancer cell metastasis or white blood cell migration through tissues (Friedl et al. 2010). Furthermore, mutations in nuclear envelope proteins such as lamins cause a large number of human diseases (Simon & Wilson 2011), including muscular dystrophy and dilated cardiomyopathy, and it has been suggested that increased nuclear fragility could contribute to the often muscle-specific disease phenotypes. Modifications in nuclear envelope composition during development or differentiation are also associated with changes in nuclear mechanics (Pajerowski et al. 2007). Therefore, characterizing the mechanical properties of the cell nucleus is an important factor in understanding the function and behavior of normal and diseased cells.

Here, we present two independent and complementary approaches to measure nuclear mechanics. In the first, adherent cells are subjected to substrate strain and nuclear stiffness is inferred from the induced nuclear deformations. In the second approach, suspended cells are perfused through microfluidic channels with constrictions smaller than the size of the nucleus, so that the transit time through the constrictions is indicative of nuclear stiffness. The best choice of which technique to use (or to use both) will depend on the cells under investigation and the specific aims of the study.

BASIC PROTOCOL 1 NUCLEAR DEFORMATION DURING APPLIED SUBSTRATE STRAIN

Observing induced nuclear deformation resulting from stretching adherent cells can provide important information on the mechanical properties of the nucleus and nucleo-cytoskeletal force transmission. In the assay described in this protocol, the cell is stretch by applying uniform strain to the cell's substrate. The associated forces are transmitted across the focal adhesions through the cytoskeleton and the nuclear envelope to the nuclear interior. Previous studies indicate that the resulting nuclear strain is closely correlated to the applied substrate strain of the substrate (Caille et al. 1998),(Lammerding et al. 2004). Importantly, since the nucleus is normally significantly stiffer than the surrounding cytoskeleton, the induced nuclear strain is typically only a fraction of the applied membrane strain, and the cytoskeleton absorbs most of the applied strain. Hence, the ratio of the induced nuclear strain to the applied membrane strain can give a reliable measurement of the nuclear

stiffness relative to the surrounding cytoskeleton. Low nuclear strain values correspond to a stiff nucleus (or soft cytoskeleton), whereas larger nuclear strain values indicate softer, more deformable nuclei. In these experiments, the substrate strain can be applied bi-or uni-axially, mimicking physiological conditions. For example, cells in the lung and bladder are typically exposed to bi-axial strain, whereas muscle cells are predominantly stretched in only one direction, along the muscle fiber. In our experience, we found that uniaxial strain application is often preferable for the substrate strain experiments, since many cells can tolerate uniaxial strain application better than biaxial strain (presumably because it requires a smaller increase in total plasma membrane area), so that higher strains can be applied, which allows for better quantification of the induced nuclear deformations.

The microscope-mounted strain device described in this protocol is a custom-designed system (Fig. 1). Such a device can be built in a basic machine shop at a reasonable cost. Alternatively, one can use a commercially available system (see Materials for suggestions). However, many of the available devices have the limitation that they use thick silicone membranes, which can reduce image quality and resolution. The custom-built strain device described in the following protocol consists of a strain dish with an elastic silicone membrane at the bottom on which cells are cultured and a platform that is mounted on an inverted microscope. The bottom rim of the strain dish has an outer thread to fix the strain dish in the holder plate. The silicone membrane is secured to the strain dish with a plastic O-ring, which tightly fits into the groove of the strain dish from the bottom side.

The membrane is stretched by applying a weight to the top of the strain dish, pushing it down over a stationary platen in the base plate. This results in a uniform biaxial strain field in the center of the silicone membrane. Vertical metal pins in the base plate help to guide the strain dish in the holding plate and keep it aligned parallel to the base plate and objective. The maximum applied strain can be adjusted by limiting the vertical displacement of the strain dish and holding plate by placing nylon spacers between the base plate and the holding plate. The strain dish can be easily modified for uniaxial strain application by applying two parallel strips of Scotch tape to the membrane (See Fig. 2). Since the silicone membrane cannot deform where the Scotch tape is applied, the area between the two strips of tape is subjected to uniform uniaxial strain.

The cells are stained with a cell permeable blue fluorescent DNA dye (Hoechst 33342), which allows high resolution imaging of nuclear shape. Phase contrast and fluorescence images of several membrane sections are imaged before, during and after strain application. Marking the center of the strain dish with a fine-tip marker on the bottom of the silicone membrane can aid in locating (and re-locating) specific membrane sections for the repeated imaging, as the central dot serves as a landmark, and the positions relative to this landmark remain relatively constant during strain application.

Image analysis is typically performed off-line following the strain experiments. The applied membrane strain is calculated by comparing phase contrast images of the silicone membrane between pre- and full-strain images; nuclear strain is computed by comparing fluorescence images of the cell nucleus. Comparison between pre-strain and post-strain phase contrast images of the cells can serve to identify cells that became damaged or detached during the strain application that should be excluded from the analysis.

Materials

Reagents and Solutions

Phosphate buffered saline(PBS)or Hank's Balanced Salt Solution(HBSS), both Ca/Mg-ion-free

Fibronectin or other suitable extracellular matrix proteins to coat silicone membrane

Trypsin

Appropriate cell culture medium, e.g., Dulbecco's modified Eagle medium (DMEM) supplemented with fetal calf serum (FCS) and penicillin/streptomycin.

HEPES buffered, phenol red free imaging medium, e.g., Dulbecco's modified Eagle medium (DMEM) without phenol red and with HEPES

Hoechst 33342

Equipment

Custom-built strain device consisting of microscope-mounted base plate, dish holding plate, and components for strain dish (Fig. 1). Alternatively, one can use commercially available systems, e.g., from Flexcell International Corporation, the Cell Stretcher by Electron Microscopy Sciences, or the STREX instruments from B-Bridge International.

Silicone membrane: 0.005-inch-thick silicone sheeting in 12×12-inch sheets (Gloss/Gloss non-reinforced silicone sheeting; Specialty Manufacturing Inc., Saginaw, MI, USA)

Chemically inert, silicone-impermeable grease (Braycote804; Castrol, Irvine, CA, USA)

Weight plate (~5 lb) to place on strain dish for strain application (Fig. 1)

Scotch tape (19 mm width)

Fine-tip black permanent marker suitable to mark silicone membrane

Ethanol prep pad: 1.1×2.6-inch

Kimwipes or other tissues

70% ethanol

Deionized water to rinse assembled strain dishes

Autoclavable bags

Microscope

- Inverted epifluorescence microscope equipped for fluorescence and phase contrast image acquisition with light sensitive CCD camera
- Filter sets for DAPI/Hoechst 33342
- 60× non-immersion phase contrast objective with long working distance
- Linear encoded motorized stage (optional)
- Image acquisition software
- Image analysis software, e.g., ImageJ, Adobe Photoshop or MATLAB(Mathworks)

Note: DIC objectives can be used as an alternative for phase contrast; however, in our experience, DIC illumination is more sensitive to deviations from the ideal Koehler illumination that can occur with custom-build strain devices, resulting in reduced image quality that can make it difficult to analyze the transmitted light images.

We have also successfully used (water) immersion objectives for the strain experiments; however, care must be taken that the silicone membrane is not accidentally stretched over

the objective, particularly when applying strain, as this could cause additional local strain in the substrate. When using immersion objectives, it is best to lower the objective when applying the weight to the strain dish and the refocusing once full membrane strain is reached.

Protocol steps

Preparation of the strain dishes before plating cells

1. Prior to assembling the strain dish clean all components, including the O-ring, with water and soap, wipe with 70% ethanol, rinse thoroughly with tap water, then deionized water, and air dry components.
2. Mount silicone membrane on the clean strain dish using the plastic O-ring. We typically place a 3×3 inch piece of silicone membrane over the plastic O-ring lying on a clean surface and press the strain dish from the top over the plastic O-ring with the membrane. The O-ring is held in place in the groove of the strain dish together with the membrane. Cut excess membrane with scissors as close to the strain dish as possible so that the excess membrane cannot get into the outer thread of the strain dish when mounted into the holding plate.

After placing the membrane on top of the O-ring ensure that the membrane is wrinkle free, which would cause leakages during cell culture or strain-experiments.

3. Clean the top (inner) surface of the membrane by wiping a Kimwipe soaked in 70% ethanol in a circular motion from the center to the periphery of the dish, ending by wiping the dish walls in the inside of the dish. If necessary, clean the outside of the silicone membrane.
4. Repeat the previous cleaning step with a sterile ethanol prep pad.
5. Rinse the device under running deionized water and let it air dry.
6. Place dishes in autoclavable bags and autoclave using the dry cycle for glass and plastic.
7. Once dishes are sterilized and have cooled down to room temperature, open autoclave bag inside a sterile biosafety cabinet and place each strain dishes in an inverted 10-cm sterile disposable plastic dish.
8. Invert the dish, so that the bottom of the silicone membrane faces up. Use a fine-tip black marker to draw a small dot at the center of the silicone membrane, which will serve as the landmark to re-locate cells during and after strain application.
9. For uniaxial strain experiments, place two parallel pieces of Scotch tape (~20 mm ×19 mm) centered around the black dot, approximately 5 mm apart.
10. Turn over the strain device in upright position, so that the inside of the silicone membrane faces up and cover the strain dish with a sterile disposable petri dish.
11. Pipette 10 ml of 2 µg/mL fibronectin in sterile PBS into the strain dish; gently swirl to ensure that the entire membrane is covered.
12. Incubate overnight at 4°C. Alternatively, incubate for 2 hours at 37°C

Ensure that the surface is completely covered with the coating solution.

13. Aspirate the fibronectin solution and rinse membrane once with sterile PBS. Fill dishes with 10 mL of appropriate cell culture medium and place in incubator to pre-warm.

When handled outside the biosafety cabinet, strain dishes should always be kept inside an inverted sterile 10-cm disposable plastic petri-dish to minimize risk of contamination.

Plating cells for experiments—In our experiments, we have successfully used mouse embryo fibroblasts, human skin fibroblasts, breast epithelial cells, and mouse myoblasts and myotubes for the strain experiments. The conditions below were optimized for mouse embryo fibroblasts. Other cell types may require some adjustments. For fibroblasts, reseed cells every two to three days at 60–80% cell confluency in cell culture flask (e.g., T-75 flask). Avoid growing cells to total confluency.

1. Remove culture medium and rinse cells once with sterile PBS or HANKS without calcium and magnesium for one minute at room temperature.
2. Detach cells with trypsin/EDTA solution for 5 min. We typically use 1 mL for a T-75 flask.
3. Add 2 to 5 mL of culture media, containing 10% fetal bovine serum, to the trypsin/EDTA suspension to inactivate trypsin. Gently mix by pipetting up and down to breakup cell clusters and achieve single cell suspension. For a T-75 flask, we add 4 mL of media.
4. Spin-down cell suspension at $230\times g$ for 5 min at 4°C and remove supernatant.
5. Resuspend cell pellet in 5 mL cell culture media and count cell density with a hemocytometer.
6. Seed cells in the prepared strain dishes to achieve a desired cell confluency of approximately 20 to 30% within 1 to 2 days. The cell density should be high enough to contain several cells per field of view while at the same time low enough to minimize cell-cell contacts.
7. Maintain cells overnight or longer in cell culture incubator to achieve sufficient cell attachment before using the strain dish for experiments.

Preparing the cultured cells for stretch experiment

1. Add Hoechst 33342 directly to the strain dish with cell culture medium to a final concentration of $1.5\text{ }\mu\text{g/mL}$; gently swirl to mix and incubate for 15 minutes at 37°C in a cell culture incubator.

The final concentration of Hoechst 33342 maybe optimized for each cell type to achieve bright nuclear fluorescence labeling while avoiding cytotoxic effects.

2. Replace Hoechst-containing medium with 15 mL of phenol red-free imaging medium containing HEPES.

Setting up the microscope-mounted strain device

1. Remove strain dish from incubator. Gently apply a thin layer of Braycote grease along the periphery of the bottom of the silicone membrane with a gloved finger.

Make sure to keep the center of the dish free of any grease, as this could interfere with the imaging process.
2. Carefully screw strain dish into the holding plate, making sure not to spill any medium.

3. Position strain device base plate on the microscope stage. Insert appropriate spacers onto the guidance pins of the base plate. Switch microscope to high power long working distance objective. We typically use a 60× objective, but have also successfully conducted experiments with a 40× water immersion objective.

Depending on the design of the strain device, you may require an objective extension tube (e.g., from Thor Labs) to account for the altered imaging plane.

4. Carefully position the holding plate with strain dish onto the baseplate and slowly lower it until the silicone membrane rests on the central platen.
5. Adjust microscope stage position so that the landmark dot on the silicone membrane is centered over the objective. The system is now ready for image acquisition.

Imaging and strain application

1. In phase contrast mode, locate the landmark dot at the bottom of the silicone membrane and acquire an image of the reference point for later identification.
2. Focus on the cells plated on the silicone membrane and identify a field of view with 1 to 5 healthy looking, well-spread cells with the nucleus located in the cell center. Acquire a phase contrast and a fluorescence image. For the phase contrast image, focus on the cell boundary and the silicone membrane; for the fluorescence image, focus on the center cross-section of the fluorescently labeled nucleus.

Make sure to remember the location of the field of view relative to the reference landmark dot.

3. Move the field of view along an imaginary line perpendicular to the intended strain direction (in the case of uniaxial strain application), as the relative positions along this line will not change during strain application. Take note of the relative position of the field of view relative to the landmark dot so that the same cells can be found again.

Leave the phase contrast images open in the acquisition program to help identify the same fields of view later. Acquire 10 to 15 pictures of different areas. This pre-strain acquisition phase should take no more than 5 to 10 minutes.

4. Carefully place weight plate on top of strain dish to apply membrane strain. Wait approximately 1 min for viscoelastic effects to dissipate.
5. Focus on the bottom of the silicone membrane and search for the landmark spot. Comparison with the previously acquired images will aid in this task. Acquire full-strain image of reference dot.
6. Focus on cells on the silicone membrane. Re-locate cells acquired during pre-strain imaging, taking images in phase contrast and fluorescence for each field of view, making sure to match the focal positions of the pre-strain images. Use the images of the first set of image pairs to find the same field of view and to adjust the fine focus.

Do not exceed more than 15 minutes of full strain application to prevent cytoskeletal remodeling and other cellular adjustments to the strain application.

7. Carefully remove the weight plate. If necessary, gently lift up strain dish, until it loosely rests on the silicone membrane on the platen, matching the position during pre-strain imaging. Wait for 1–2 minutes for the cells and membrane to relax.
8. Acquire final set of image pairs (post-strain) following the procedure above. Use the previously acquired pre-strain images to help find the same cells again and to match the focal position.
9. Remove strain dish from the microscope and repeat process with the next strain dish.

The entire imaging procedure should take less than 30 minutes.

Protocol variation: Simple acquisition protocol with motorized stage

1. In phase contrast mode, find the landmark dot on the bottom of the silicone membrane.
2. Acquire an image of the landmark dot to make it easier to identify the same spot during and after strain application.
3. Set the stage position to zero at this location; all other recorded positions will be relative to this location.
4. Focus on the cells above (or slightly to the side) of the landmark dot. Acquire phase contrast and fluorescence images for the first field of view as described under the basic protocol.
5. Move the stage in the direction perpendicular to the intended uniaxial strain direction and find the next field of view with suitable cells. Acquire pictures in phase contrast and the DAPI/Hoechst channel as described above. Store the stage position of each field of view in the image acquisition program.
6. Repeat the above step for 6 to 10 additional fields of views. The entire process should take less than 10 minutes.
7. Apply the substrate strain as described in the basic protocol. Wait for 1 to 2 minutes for viscoelastic effects to dissipate. During that time, locate the landmark dot and focus on the same spot on the membrane. Use the reference image acquired in step 2 for orientation.
8. Reset the stage coordinates to zero in the acquisition program.
9. Use the motorized stage and the stored (relative) stage positions to revisit the previously acquired fields of view. If necessary, manually adjust the focus and stage position to closely match the original view. Again, take phase and fluorescence images as described under the basic protocol. Proceed with the other fields of view in the list of recorded stage positions.

Do not exceed 10 min of strain application.

10. Carefully remove the weight from the strain dish as described under the basic protocol and wait 1 to 2 minutes for viscoelastic effects to dissipate. During this time, bring the landmark dot into the field of view.
11. With the landmark dot closely matching the previously acquired field of view (see step 2), re-zero the stage coordinates. Acquire the same set of image pairs for each position as described above.

Image analysis with ImageJ or Photoshop—A typical set of images acquired in matching fields of view is shown in Figure 3. The cell was imaged in phase contrast and fluorescence (Hoechst 33342) mode in pre-, full-and post-stretched conditions. The uniaxial strain application is clearly visible by the displacement of distinct speckles on the silicone membrane in the phase contrast field and by elongation of the nucleus in the strain direction in the fluorescence images.

1. Calculate the applied membrane strain by measuring the distances between matching image features on the membrane in image pairs acquired before and during strain application. Compare image pairs acquired before and after strain application to confirm that the membrane relaxed back to the pre-stretched shape and cells did not become damaged or detached. Exclude cells with obvious differences in morphology from further analysis.

In our experience, the silicone membranes usually contain sufficient intrinsic speckles and other distinguishable features that can be used for the membrane strain computation. It is important to only use features firmly attached to the membrane (and not floating particles) and located outside the cells. It is best to use several pairs of features to make sure that the computed results are consistent. The entire process can be further optimized with image processing algorithms implemented in MATLAB or similar programs. For uniaxial strain application, the pairs of speckles should be located along the strain direction, as displacements in the perpendicular direction should be minimal.

2. Calculate the induced nuclear strain by comparing the length of the nucleus measured before, during, and after strain application in corresponding image-sets. Alternatively, pictures can be overlapped in Photoshop and the pre-stretched image adjusted by free transformation and stretching to the full-stretched image. This process is repeated for each cell in the field of view.

For mouse cells, the bright condensed heterochromatin spots (chromocenters) can be used to align the images of the nuclei, or to measure intranuclear distances.

Note that we here define strain as the relative increase in size, e.g., the change in length divided by the initial (unstrained) length.

3. Repeat the above steps for the image sets acquired at additional positions. The membrane strain should be fairly uniform between different fields of view and can be averaged to compute the mean membrane strain.
4. Calculate the normalized nuclear strain for each cell by dividing the induced nuclear strain by the applied membrane strain.

$$\text{normalized nuclear strain} = \frac{\text{nuclear strain}}{\text{applied membrane strain}}$$

Improvement—When performing a large number of experiments, it is helpful to automate parts of the image acquisition and the image analysis. A protocol for the use with a motorized stage that makes it easier to revisit previously recorded fields of view is included above. For the image analysis, we have developed several MATLAB scripts to compute nuclear and membrane strains. These scripts are available from the authors upon request.

To ensure that all cells have a similar size, shape, and orientation, contact printing can be used to deposit extracellular matrix molecules in only precisely defined areas.

The current protocol uses a one-step strain application, and cells cannot be imaged during the strain application process. Motorized strain devices can help alleviate this problem, although changes in focal position and membrane displacement can impose further challenges.

BASIC PROTOCOL 2 ASSESSING NUCLEAR DEFORMABILITY WITH A MICROFLUIDIC PERFUSION DEVICE

The mechanical properties of viscoelastic objects, such as nuclei and cells, can be determined by observing their dynamic deformation under stress. A commonly used method to probe nuclear mechanics is micropipette aspiration, in which the stiffness of the nucleus is determined by gently aspirating the nucleus into a micropipette with a tip diameter smaller than the size of the nucleus (typically a few microns) and quantifying the time-dependent deformation into the pipette opening (Kris Noel Dahl et al. 2004). The stiffer the nucleus is, the longer it will take to (partially) aspirate it into the narrow opening of the pipette. The approach presented here is based on a similar principle. Intact cells or isolated nuclei are perfused with a constant pressure gradient through microfluidic channels containing precisely defined constrictions narrower than the size of the nuclei. Since the nucleus is significantly stiffer than the surrounding cytoskeleton, nuclear deformability will largely determine the transit time through the narrow constrictions. While cells with highly deformable nuclei can rapidly pass through the constrictions, cells with stiffer nuclei will take longer or even be unable to transit through the constrictions altogether. A major advantage of this device over the aforementioned techniques is that hundreds of nuclei can be probed within a few minutes because of the rapid cell throughput and the many channels that can be imaged in parallel.

These perfusion devices are made of polydimethylsiloxane (PDMS) by soft lithography. In a first step carried out in a cleanroom facility, a silicon wafer spin-coated with photoresist is exposed to UV light through an optical mask containing the desired channel layout and subsequently developed. Subsequently, in a well-equipped laboratory, a PDMS replica is cast from the master and bonded to a glass coverslide to create a microfluidic device. Cells are then perfused through the device and their ability to pass through narrow channels is assessed as a measure of nuclear mechanics.

Materials

Reagents and Solutions

- SU-8 2010 photoresist (Microchem)
- SU-8 Developer
- Isopropanol
- Sylgard 184 with silicone elastomer base
- 0.2 M HCl
- 70% ethanol
- Deionized water
- Bovine Serum Albumin (BSA) (see recipe)
- Phosphate Buffered Saline (PBS) (see recipe)

Pluronic I-127 (optional)
Trypsin
Dulbecco's Modified Eagle Medium (DMEM)
Fetal Bovine Serum (FBS)
10% bleach in water

Equipment

Microfabrication facility with the following tools: mask generator, contact aligner, molecular vapor deposition
10 mm (diameter) silicon wafer
Oven
Hot plate
150 mm (diameter) plastic petri dish
Desiccator
Packing tape
Utility knife
Cutting mat
0.75 mm (diameter) Uni-Core hole-puncher (Harris)
Glass coverslips (22 × 50 mm, thickness 1.5)
Expanded plasma cleaner, 115 Volts (Harrick Plasma)
Premium vacuum pump, 115 Volts (Harrick Plasma)
5 and 15 ml syringes
0.2 µm(pore size) syringe filter
0.015" (inner diameter), 1/32" (outer diameter) pre-cut natural PEEK tubing for microfluidics (Ing)
Forceps (rounded tip)
Cells for analysis (1 ml cell suspension in PBS with BSA at a density of 3×10^6 cells/ml)
Cell culture incubator
Hemocytometer
Centrifuge
40 µm (pore size) cell strainer
15 ml Falcon tube
Compressed air tank with 5% CO₂
2 stage brass analytical pressure regulator, 0–25 psi (Airgas)
5/16" (inner diameter) PVC tubing, 40' length
0.05" (inner diameter) PVC tubing, 2' length

Pressurized cap for 15 ml Falcon tube for microfluidics (World Precision Instruments, FLUIWELL-1C-15ML)

Cytochalasin D (optional)

High speed microscope camera, 60 frames per second or higher; for example: PIKE F-032 1/3" CCD FireWire. B Monochrome Camera with up to 208 frames per second (Edmund Optics)

Inverted microscope with camera mount

Image acquisition software

Image Analysis software (ImageJ)

Protocol steps

Designing and microfabricating the device

- 1 Design a mask using CAD software for your device (See Fig. 4). The device consists of an inlet followed by a filter to prevent passage of aggregated cells and dust, followed by a bifurcation into several parallel constriction channels. The constriction channels consist of a sequence of constrictions narrower than the cell nucleus. In our devices, these constrictions are 3, 5 or 8 μm wide. Narrower constrictions are used for smaller or more deformable cells (e.g., neutrophils), while larger constrictions are suitable for fibroblasts or other larger cells. Two wider bypass channels are included in the device. These channels serve to maintain an approximately constant perfusion rate (and pressure drop across the constriction channels) in case some of the constriction channels become blocked with cells or debris. If the device is set up with a constant pressure perfusion, rather than constant flow rate, as in our set-up, these bypass channels are not necessary. The device also contains an extra inlet that can be used to flush the channels with PBS if the constriction channels get clogged. Features are also integrated into the design around the inlet to serve as guides while punching holes.

It is critical that an aspect ratio (channel width:channel height) of no more than 4:1 is maintained for any features in the channels to prevent the PDMS ceiling from collapsing into the channels. Thus, for a channel height of 8 μm , the channel width should not exceed 32 μm .

- 2 Create the appropriate mask design onto a 5 inch mask in the microfabrication facility using a mask writer.

Several companies also offer to custom-produce high resolution masks for a reasonable price.

- 3 Bake 4 inch silicon wafers at 200°C for at least 20 minutes and allow wafers to cool for a few minutes. Pour enough SU-8 2010 on the wafer centered on the spin-coater to cover half the diameter of the wafer, then spin the wafer at 500 rpm for 10 seconds (with 100 rpm/second acceleration), then 3,000 rpm for 30 seconds (with 300 rpm/second acceleration).

If another type of photoresist is used, follow the manufacturer's spin-coating recommendations to obtain a 10 μm thick layer of photoresist.

- 4 Allow the SU-8 photoresist to relax at room temperature for 10 minutes.

- 5 To remove any remaining solvent, bake the wafers on a hot plate at 60°C for 10 minutes, allowing the hot plate to ramp up from room temperature with the wafers placed on it.

Take care not to exceed the temperature.
- 6 Expose the photoresist-coated wafers in a contact aligner with a dose of 250 mJ/cm². The exact exposure time will depend on the specifications of the instrument. Allow the resist to relax for one hour post-exposure.

The exposure dose used here is double the manufacturer's recommended dose, as we have found that higher doses improve binding of the SU-8 features to the silicon wafer and reduce the appearance of cracks without any ill-effects on the structures. However, this may not be the case for all designs.
- 8 Post-bake the photoresist coated wafers for 3.5 minutes at 90°C. Allow the wafers to cool down on the hot plate which has been switched off to ensure a slow cool-down.

The structures in the resist should now be visible to the naked eye.
- 9 Develop the wafer in SU-8 developer for 5 to 10 minutes, then rinse the wafer thoroughly with isopropanol and dry with a stream of nitrogen.

If a white precipitate appears upon rinsing with isopropanol, the SU-8 has not been completely dissolved and the wafer should be rinsed with SU-8 developer and then placed in a dish with fresh SU-8 developer, since precipitate in the developer will result in white streaks on the wafer.
- 10 Hard bake the wafer at 200°C for 30 minutes.
- 11 Coat the wafer with an anti-stiction coating, such as (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane, a fluorinated silane (also known as FOTS). This coating helps prevent PDMS from becoming permanently stuck to the wafer.

Molding the PDMS and assembling the microfluidic device

1. Place the wafer in a 150 mm (diameter) plastic petri dish, with the photoresist features facing up. Combine approximately 6g of curing agent and 54 g of silicone elastomer base of the Sylgard 184 kit in a disposable container (10% solution by weight). Mix vigorously for a few minutes and then degas the solution under vacuum in a desiccator for 10 to 30 minutes to remove air bubbles. Pour the degassed solution on top of the wafer; the PDMS layer should be 3–5 mm thick to ensure that inlet and outlet tubing can be properly connected to the final device. Place the dish with the wafer and PDMS solution in the oven at 65°C for 2 hours to cure PDMS.

Optionally, once the solution has been poured on top of the wafer, it can be placed under vacuum as well, although this step is usually not necessary. Degassing can also be performed by pouring the solution into a Falcon tube and centrifuging it.
2. Slowly peel off the cured PDMS from the wafer. Tape a piece of packing tape to a lab bench (adhesive side up) and then place the side of the PDMS that was in contact with the wafer onto the packing tape to minimize dust accumulation on the PDMS.

3. Cut devices out of the PDMS on a cutting mat with a utility knife, while the packing tape is still adhered to the PDMS. Punch inlet and outlet holes in each device at the designated areas using a 0.75 mm diameter Uni-Core hole-puncher.
4. Clean glass coverslips or glass slides by placing them in a 0.1 M HCl solution for at least 10 minutes. Rinse the glass slides with deionized water, followed by ethanol, then water again. Dry under a stream of compressed air. Only dry glass slides close to the time they are going to be used to minimize the possibility of dust particles.

Glass slides in the HCl solution will quickly sink to the bottom of their container. Ensure that there is solution in between the pieces of glass and the container by swirling the solution occasionally. Prevent oil in the compressed air system from being sprayed on glass slides by connecting a filter in series with the tubing leading from the compressed air source.

5. Immediately prior to plasma treatment, remove the tape from the devices.
6. Place a piece of glass and PDMS, with microstructure side up, side by side in the plasma cleaner. Treat with plasma for 5 minutes at the highest setting (200 Watts). Release the vacuum, remove the glass slide and PDMS from the plasma chamber, and immediately bring the two into contact with each other, pressing both parts firmly together. Place the device on a 95°C hot plate for 10 minutes, then allow to cool at room temperature for 10 minutes.

Check if the device has adhered properly by gently trying to peel the PDMS off from the glass slide; if the PDMS becomes detached, then there is a bonding problem. To mediate bonding problems, eliminate sources of dust and oil. For example, wipe-down the inside of the plasma cleaner and keep the door closed when not in use. Additionally, gloves are often coated with a surfactant to prevent them from sticking to each other in their packaging; wash gloves thoroughly with 70% ethanol to remove this coating.

Filling and priming the microfluidic device

1. Prepare approximately 10 mL of 20 mg/ml bovine serum albumin (BSA) suspended in phosphate buffered saline (PBS). Filter the solution in a laminar flow hood (biosafety cabinet) using a 0.2 µm syringe filter to sterilize it.

The BSA does not dissolve quickly; stir in order to more efficiently suspend the solute. An alternative to the BSA solution is a solution of 0.2% I-127 Pluronic in PBS.

2. Passivate each device with 20 mg/ml BSA in PBS solution to prevent cells from adhering to the channel walls: using a clean sterile syringe, aspirate 1 ml of the sterile filtered BSA solution. Connect 0.015 inch (inner diameter) PEEK tubing to the syringe using a pair of forceps and then insert tubing into the device inlet. Firmly press on the syringe plunger until solution can be seen coming out of the other holes of the device. Continue injecting solution until air bubbles stop appearing in the liquid coming out of the device. Do not remove tubing from the inlet. The device should not contain any air bubbles.

The extent to which the device has been filled can be examined under a standard bright field microscope at low magnification. Bubbles generally form near constriction points and appear lighter in color than the channels that are filled with solution. Continue injecting BSA solution until all bubbles have disappeared.

3. Inject additional solution using the syringe every 5–10 minutes in order to refresh solution in the device channels and to prevent the inlets and outlet from drying out. Leave the solution in the device for approximately 30 minutes to ensure proper BSA coating.

BSA adsorbs onto the surfaces in the microfluidic device, making them less adherent to cells and preventing excessive clogging of the channels.

Preparing the cell lines

1. While priming the microfluidic devices, prepare cell lines to be examined. Each experiment requires at least 1 ml of cell suspension, with a cell density of approximately 3×10^6 cells/ml. Typically, a confluent T-75 cell culture flask is more than sufficient to provide the required number of cells.
2. Rinse plated cells with PBS and then detach cells using a trypsin solution for 5 minutes at 37°C. Once the cells are detached, deactivate the trypsin with medium containing serum.
3. Count cells using hemocytometer.
4. Centrifuge cells for 4 minutes at 1,500 rpm and resuspend them in 20 mg/ml BSA in PBS solution at a density of 3×10^6 cells/ml.

Make sure to repeatedly pipette up and down cell suspension to break up any cell clumps and to obtain single cell suspension, as cell clusters can clog the microfluidic channels.

5. Remove any remaining cell clusters by passing the cell suspension through a cell strainer with a 40- μ m pore size.
6. Assess cell size. Pipette approximately 30 μ L of cell suspension from each sample onto a glass slide and cover with a coverslip. Acquire 4 to 5 images with a 10 $\times$ objective in brightfield mode, so that 100–300 cells are imaged. Use an image analysis program, e.g., ImageJ, to determine the cell size (i.e., cross-sectional area) distribution for each population.

To avoid any confounding factors such as difference in cell size, it is important to only compare samples with similar cell size distributions. Image cells immediately after pipetting on the glass slide, before the cells have attached and spread.

7. (optional) Treat cells in suspension with cytochalasin D or similar drugs to completely disrupt the (actin) cytoskeleton, thereby further minimizing the effect of cytoskeletal stiffness on overall cell deformability.

Perfusing cells

1. Transfer 1–2 ml of cell suspension to a Falcon tube. Attach the tube to a pressure cap for 15 ml tubes. The pressure cap seals and pressurizes the Falcon tube with the cell suspension.

A constant flow rate device, e.g., a syringe pump, can also be used to perfuse cells through the microfluidic device. However, if there is significant clogging of the device due to dust particles or cell aggregates, this will result in an increased flow rate in channels that are not blocked. A constant pressure apparatus will maintain a constant flow rate in all channels, even when some of them become blocked.

2. Insert PEEK tubing into the pressure cap so that the tubing inlet is submerged in the cell suspension near the bottom of the Falcon tube.

Cells tend to accumulate in clumps at the very bottom of the Falcon tube. The cell suspension reservoir should be gently agitated every 5 minutes to keep cells in suspension.

3. Use the 2-stage regulator on the gas tank to set the pressure in the Falcon tube to approximately 5 psi. Once the cell suspension has filled the tubing and starts flowing out the end, connect the tubing to the inlet of the microfluidic device (See Figure 5). At the same time, connect a syringe with the 20 mg/ml BSA in PBS solution to a small length of tubing (approximately 1 ft) and connect the tubing to the inlet of the flushing channel. The syringe can be used to flush clogged channels if necessary.
4. Mount perfusion device on a microscope with video-capture capabilities. Set microscope to bright field mode and locate constrictions channels of the device. Orient device and camera so that parallel constriction channels can be simultaneously monitored.

Note microchannels that have become blocked by cells and other debris – it is normal for several channels to become blocked during an experiment. As long as a few constriction channels remain open during the observation period, devices can be used for experiments.

5. Preview device using the camera and image acquisition software (See Figure 6). Set the rate of frame capture between 50 fps and 100 fps, depending on the speed that the cells traverse the constrictions. As an approximate guideline, the minimum transit duration should be 2 to 5 frames.
6. Wait for the pressure in the device to equilibrate before acquiring the first video. This may take up to 10 minutes.

The length of tubing leading from the compressed air tank to the cell suspension reservoir is compliant and can store energy via elastic deformation. Therefore, the pressure gradient will be transient whenever there is a perturbation to the system, including when the pressure is initially turned on and the reading at the tank may not be representative of the pressure elsewhere in the system. This transient pressure can most accurately be monitored by taking pressure measurements near the cell suspension reservoir over time and waiting for the pressure to level off. A manometer is a simple and inexpensive way to accurately track the pressure just before the cell suspension reservoir. A manometer can be constructed by connecting a 500 ml bottle to the tubing system close to the device inlet. Connect tubing coming from the compressed air tank to one side of the bottle and connect another length of tubing to the other side, which will lead to the device inlet. Connect a final piece of tubing to the bottle that will be submerged in the manometer fluid. Use a saturated sugar aqueous solution to increase the density of the fluid in the manometer to approximately 1.3 g/ml and add food coloring to increase fluid visibility. The increased density will result in a lower fluid height in the manometer upon pressurization of the system. For a pressure of 5 psi, the sugar solution height will be approximately 2.7 meters, or 8.9 feet, as opposed to 3.5 meter, or 11.5 feet, for water alone. Wait for the fluid height to equilibrate to help ensure that there is little difference in pressure between experiments.

7. Acquire several videos of 60 to 90 second duration. Flush channels in between videos as necessary; when cells become lodged in the microchannels, firmly press the plunger of the syringe connected to the flushing channel to remove debris. Wait several minutes for the pressure to equilibrate again before acquiring another video sequence.
8. After finishing perfusion of one cell line, the next cell line should be perfused through a separate microfluidic device using the same protocol. The pressure regulator should be adjusted based on manometer readings to ensure uniform pressure for all experiment. Additionally, new or sterilized tubing should be used for each cell line. The new tubing should be the same length as in the previous experiment.

Tubing may be cleaned and sterilized by perfusing 10% bleach through the tubing. After 5 minutes, perfuse deionized water through each tubing length to remove bleach residue.

Analyzing Data

1. Measure the time that it takes for each cell to travel through the first constriction of the microfluidic device and the time needed to travel through the entire length of a constriction channel with an image analysis program such as ImageJ. Either of these two transit times may be used for analysis.

Cells usually take the longest amount of time to pass through the first constriction, as the initial deformation occurs here. Cells keep their general deformed shape as they pass through subsequent constrictions and, therefore, often travel through these constrictions much faster.

2. Sum the number of frames needed for each cell to pass through the constriction channels, or first constriction, and convert this to a total cell transit time based on the frame rate.

This step can be done manually in an image analysis software program like ImageJ. Cell tracking can also be automated to quickly analyze large data sets, as described previously (Rosenbluth et al. 2008).

3. Visually verify each transit event in the video sequence. Make sure to include only valid events in the final analysis. Cells should enter the constriction channels one at a time and traverse the entire channel before another cell enters the channel. Cells often pause and deform at each constriction, with the longest pause and largest change in conformation occurring at the first constriction. Examples of invalid events that should be excluded from analysis include: multiple cells traversing one constriction channel at the same time, cell conglomerates clogging a constriction channel, large dust particles, which may be mistaken as cells, or broken-up pieces of cells flowing through the channels.

Expect between 20 and 300 cells, in total, traversing the constriction channels per one minute of video.

4. Compare the distribution of transit times between different cell populations using appropriate statistical tests.

REAGENTS AND SOLUTIONS

Dulbecco's PBS

Dissolve the following in 1 L of deionized water:

0.2 g Potassium Chloride (KCl)

0.2 g Potassium Phosphate monobasic (KH₂PO₄)

8 g Sodium Chloride (NaCl)

2.16 g Sodium Phosphate dibasic (Na₂HPO₄·7H₂O)

Check pH and adjust to 7.4 if necessary. Autoclave solution to sterilize. Alternatively, commercial solutions without Calcium or Magnesium can be used.

BSA solution

Add 200 mg of BSA powder to a 50 ml Falcon tube and add PBS to obtain a final volume of 10 mL. Mix vigorously until BSA is fully dissolved. Sterilize by passing solution through a 0.2µm syringe filter. BSA powder should be stored at 4°C. The 20 mg/mL solution of BSA in PBS should be stored at 4°C and has a shelf-life of 1 month.

COMMENTARY

Background Information

Several techniques have been developed to probe the mechanical properties of nuclei [see (Rowat et al. 2008; Lammerding et al. 2007) for recent reviews]. These include single-cell based techniques such as atomic force microscopy on living cells, micropipette aspiration of suspended cells, and microneedle manipulation, in which the deformation of the nucleus is observed when strain is locally applied to the part of the cell, as well as global techniques such as cell compression, in which the deformation of a cell sandwiched between two plates is observed. Some of these techniques can be applied to isolated nuclei, which has the advantage of probing the nucleus directly without the confounding influence of the cytoskeleton. However, nuclear isolation can damage the nucleus and may also alter the mechanical properties of the nucleus, e.g., by osmotic swelling (Kris Noel Dahl et al. 2004).

In the techniques presented here, measurements can be carried out on many cells over a short period of time. In the case of the cell stretching experiment, the number of cells that can be probed is limited to the number of that cells can be observed in the field of view and imaged in the relative short time of stretching (less than 10 minutes). When acquiring cells from several nearby fields of view, this corresponds to circa 10 to 15 cells per experiment. In the perfusion technique, hundreds of cells can be observed in a matter of minutes. This latter technique can also be used with isolated nuclei or in the presence of cytoskeletal-disrupting drugs to reduce the effect of the cytoskeleton on the perfusion time.

Critical Parameters and Troubleshooting

Protocol 1: Stretching—It is essential to thoroughly clean the strain dishes before plating cells. Our experience shows that this is one of the most critical steps in the experimental procedure and the most common reason cells fail to grow on the silicone membrane. Another important parameter is cell density. We found that a critical cell density is necessary to achieve viable cells after plating. We recommend conducting pilot studies on the membranes with increasing cell densities to find an optimal setting that ensures high cell viability while minimizing cell-cell contacts.

The maximal membrane strain that can be applied without causing cell damage may vary between different cell types, as some cells can tolerate more membrane strain than others. This parameter should be optimized before performing experiments. We found that for fibroblasts, 5% biaxial or 20% uniaxial strains are generally well tolerated by most cells.

The choice of extracellular matrix type and concentration may be adjusted to achieve maximal cell adhesion for the experiments. Note that cells that are spread very thin may be more sensitive to strain application.

We have successfully acquired additional color channels during the experiments (typically only before strain application), for example, to identify cells expressing specific GFP- or mCherry-fusion proteins or that have been genetically modified.

Protocol 2: Perfusion—Many factors need to be taken into consideration in the device design, including the size of the constrictions, which will depend on the size of the cells or nuclei that will be perfused, and additional features such as filters for larger particles, flushing channels, and inlets for localized chemical perfusion. One additional aspect that needs to be considered in the design is appropriately sizing structures to avoid collapse of wide channels. This can be achieved by including support pillars in the design located within the channels without blocking the flow. We found that an aspect ratio, i.e., the ratio of channel width to its height, of 4:1 prevents PDMS collapse in most applications. In the case of a device with channels 10 μm tall, this means that the distance between the outer edges of two pillars in a single row should be no more than 40 μm apart.

Poor bonding of PDMS to glass slides will invariably lead to leaky devices. The most likely causes of poor bonding, in our experience, are dirtied bonding surfaces and inadvertently eliminating the plasma treatment by contacting plasma treated surfaces with gloves or tweezers. To troubleshoot this problem, ensure that all work surfaces are clean and dust-free before beginning work. Placing a piece of packing tape on the microstructured side of the PDMS can also help eliminate dust contamination. Tweezers gloves, hands, and any other material that may be in close contact with the PDMS or glass slide should be thoroughly washed with soapy water and ethanol.

It is essential for data analysis that the cell populations being analyzed are similar in size. Populations with larger cells will have longer average transit times. To ensure the validity of data, confirm that the populations are similar in size by measuring the area of non-adhered cells on a glass slide. Take 4 to 5 images of each glass slide using the 10 $\times$ objective of a bright field microscope, then compare the cell size distributions of the different cell populations.

Another key to obtaining consistent data is to ensure that the applied perfusion pressure in each device is uniform between experiments. The pressure reading at the inlet to the device might not always match the pressure reading at the air tank, due to the elastic tubing and compressible air. To monitor the pressure in the device more closely, pressure readings should be conducted near the inlet of the device. For example, the pressure can be monitored by connecting a pressure gauge to the tubing or by constructing a simple manometer (see Figure 5).

If alternative channel heights are desired, the microfabrication protocol will need to be modified. The manufacturer's recommendations for spin-coating speeds and accelerations appropriate for the height desired should be followed as a starting point. The wafer processing will also need to be modified; the baking times and exposure times will increase or decrease if the desired structures are taller or shorter. The protocol may need troubleshooting to obtain structures that are well-adhered and not cracked. Parameters to modify include the wafer cleaning procedure, the wafer dehydration procedure, the post-spinning, post-exposing and post-baking relaxation times of the resist, and the baking temperatures and times.

Anticipated Results

Basic protocol 1—The normalized nuclear strain reflects the nuclear stiffness relative to the cytoskeletal stiffness. Typical values for nuclear strain are 0.1 for mouse embryo fibroblasts for uniaxial strain application (20% strain) and 0.05 to 0.1 for human skin fibroblasts for similar levels of uniaxial strain. Normalized nuclear strain values of 0.3 and higher indicate extremely deformable nuclei, e.g., in lamin A/C-deficient cells. Occasionally, we observe single cells with nuclear strain values as high as 1, but this is quite rare. Nuclear strain values of 0 correspond to extremely stiff nuclei that do not show any nuclear deformation under strain. Negative nuclear strain values mean that the nucleus contracted during strain application and often indicate cells that became damaged or detached during strain application.

Since the normalized nuclear strain reports nuclear stiffness relative to the cytoskeletal stiffness, it is important to evaluate cells potential changes in cytoskeletal structure and mechanics when comparing results from different cell lines. This can be done by magnetic bead microrheology (Lammerding et al. 2004) and complemented by immunofluorescence analysis of cytoskeletal filaments.

Basic protocol 2—For cells passing through narrow constrictions with a diameter smaller than the size of the nucleus, the deformation of the large and relatively stiff nucleus presents the rate-limiting step in the transit process. Thus, cells with stiffer, less deformable nuclei will take longer to pass through the narrow constrictions than cells with more pliable nuclei. When perfusing mouse embryo fibroblasts through 5 μm wide constrictions, we typically observe transit times between 20 to 200 milliseconds, with some cells taking as long as a few seconds to pass the constrictions. In addition to comparing the mean transit times, it can also be helpful to plot a histogram of the transit times for each cell line, as such a histogram can reveal the fraction of cells that become (transiently) stuck in the narrow constrictions. Lastly, performing additional experiments with perfusion devices with constrictions larger than the nuclear size can serve as an important control, as differences in nuclear mechanics between populations should have no effect on transit times with these settings.

Time Considerations

Basic protocol 1

- assembling strain devices: 2 hours
- culturing cells: 3 days
- stretching cells: 1 hour per dish

Basic protocol 2

- microfabrication: 1 day
- microfluidic device fabrication: 3 hours
- perfusion experiment: 2 hours

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Figure 1.

Overview of the strain device: (1) strain dish with the mounted silicone membrane (2). The membrane is held in place by a plastic O-ring (3). The strain dish is inserted into the dish holder plate (4), which contains four nylon sliders (5) to slide up and down on vertical pins (6) fixed in the base plate (7). The vertical moving range, and thereby the maximum applied membrane strain, are limited by nylon spacers (8). The membrane is stretched over the stationary Teflon-impragnated delron platen (9). The platen contains a central opening wide enough to fit an objective (10) through it. The dish should contain enough imaging medium (11) that during stretch the membrane does not dry out. (A) Device in un-stretched condition as required for pre- and post-stretch images. (B) Device in full-stretch condition, by application of weight to the strain device, illustrated by the arrows pointing down. The membrane stretch is limited by the nylon spacers (8).

Figure 2.

Overview of the applied membrane strain. View from the top or bottom of the strain dish during strain application. **(A)** Membrane with central landmark. Note the uniform radial (bi-axial) strain field. **(B)** Membrane with central landmark and two parallel Scotch tape stripes (in gray) next to the landmark, one on each side of the landmark. The Scotch tape, which locally restricts the deformation of the silicone membrane, results in a uniaxial strain field between the stripes.

Figure 3.

Phase contrast and Hoechst fluorescence images of a cell before, during, and after uniaxial strain application. The top row shows the cell before strain application. The center row shows the cell during full strain application ($\approx 20\%$ strain in the x-direction). The arrow heads in the phase contrast images indicate how much the speckles on the membrane move during the strain application. The Hoechst image illustrate the induced nuclear deformation during stretch; the dashed line indicates the maximal extension during full strain application. The bottom row of images depict the cell after strain application. The membrane has returned to the pre-stretched condition and the cell did not retract, since it has the same shape as in the pre-stretched image. Scale bar: 25 μm .

Figure 4.

Perfusion device design. **(A)** Overview of the entire device, with the pillars needed to support the PDMS ceiling removed for viewing purposes. **(B)** Close-up of device constrictions and bypass channels with pillars to reveal additional details. **(C)** Further close-up view of the constrictions channels. **(1)** Flushing inlet, to clear blocked constriction channels. **(2)** Filter to eliminate large dust particulates and cell aggregates. **(3)** Flushing channels. **(4)** Inlet for cell suspension. **(5)** Bypass channels to maintain constant pressure in constriction channels. **(6)** Constriction channels or microchannels. **(7)** Device outlet.

Figure 5.

Experimental setup of perfusion device. (1) Pressurized air tank (5% CO₂) with (2) two-stage pressure regulator to control perfusion pressure. (3) PVC tubing with 5/16" inner diameter. The tubing may be 40' in length or longer, depending on the distance between the tank and microscope. (4) Manometer or other device for measuring pressure just before the tubing enters cell suspension reservoir. (5) PVC tubing with 0.05" inner diameter. This tubing only needs to be a few feet in length, as its primary purpose is to provide the correct sized connection to the pressurized cap on the cell suspension reservoir. (6) Cell suspension reservoir – composed of a 15 ml Falcon tube with a pressurized cap with sealed opening for tubing. (7) PEEK tubing with 0.015" inner diameter. (8) Perfusion-based microfluidic device, see Figure 4 for details. (9) Inverted bright field microscope with 10× objective and high-speed camera.

Figure 6.

(A) Image of 8 parallel constriction channels in bright field mode. Constrictions are 8 μm in width. Cells are perfused from the top and exit the channels at the bottom as indicated by the arrows. (B) Time-series of a mouse embryonic fibroblast cell passing through a channel with 5 μm -wide constriction at a pressure of 10 psi, acquired at 100 frames per second. The limiting step for the cell to travel through the channels is the deformation time to pass the first constriction. Subsequent constrictions are traversed substantially faster.