Video Article

Stretching Micropatterned Cells on a PDMS Membrane

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URL: http://www.jove.com/video/51193
DOI: doi:10.3791/51193

Keywords: Bioengineering, Issue 83, micropatterns, stretching, forces, PDMS, microscopy, polarity, mechanical forces

Date Published: 1/22/2014

Citation: Carpi, N., Piel, M. Stretching Micropatterned Cells on a PDMS Membrane. *J. Vis. Exp.* (83), e51193, doi:10.3791/51193 (2014).

Abstract

Mechanical forces exerted on cells and/or tissues play a major role in numerous processes. We have developed a device to stretch cells plated on a PolyDiMethylSiloxane (PDMS) membrane, compatible with imaging. This technique is reproducible and versatile. The PDMS membrane can be micropatterned in order to confine cells or tissues to a specific geometry. The first step is to print micropatterns onto the PDMS membrane with a deep UV technique. The PDMS membrane is then mounted on a mechanical stretcher. A chamber is bound on top of the membrane with biocompatible grease to allow gliding during the stretch. The cells are seeded and allowed to spread for several hours on the micropatterns. The sample can be stretched and unstretched multiple times with the use of a micrometric screw. It takes less than a minute to apply the stretch to its full extent (around 30%). The technique presented here does not include a motorized device, which is necessary for applying repeated stretch cycles quickly and/or computer controlled stretching, but this can be implemented. Stretching of cells or tissue can be of interest for questions related to cell forces, cell response to mechanical stress or tissue morphogenesis. This video presentation will show how to avoid typical problems that might arise when doing this type of seemingly simple experiment.

Video Link

The video component of this article can be found at http://www.jove.com/video/51193/

Introduction

The cells composing a tissue in higher organisms are subject to mechanical tensions and stretching forces coming either from the external environment or from surrounding cells[1,2]. Cells must adapt to and resist these forces in order to maintain tissue integrity. Such forces are also important for tissues morphogenesis during development[3,4]. Applying mechanical forces on cultured cells is a way to mimic what might happen in a tissue, but with a quantitative and independent control of cell shape and cell deformation[5,6]. For this, several techniques might be used. One can press on the cells (the whole cell or part of it), for example using AFM or derivatives[7,8] or stretch the substrate the cells are growing on.

The method described in this paper demonstrates how to stretch a plane substrate plated with cells. This technique was originally developed to assess the role of forces exerted on mitotic mammalian cells[9]. Mitotic cells stay connected to the substrate through retraction fibers and stretching the membrane exerted a force on those fibers, which in turn provoked the rotation of the mitotic spindle. The interest of combining adhesive micropatterns and stretching is to achieve independent control of forces and shape of individual cells. It is for example possible to stretch an ovoid cell into a perfectly isotropic round shape, while uniaxial stretch is applied. If the cells are not platted on micropatterns, uniaxial stretching results in cell elongation, with most cells having a long axis aligned with the stretch axis. It is then difficult to separate the effect of the long axis alignment and the effect of the stretch applied to the cells.

The device is suitable for any live cell imaging, including long time lapse fluorescent microscopy, and drugs can be added during the experiment. The deep UVs micropatterning method[10] was described in details in Azioune *et al.*[11] Patterning on PDMS was described in Azioune *et al.*[12] The present stretching protocol is a video version of Carpi *et al.*[13]

Protocol

1. Passivation of the PDMS

1. Cut a piece of PDMS approximately 35 mm x 20 mm from a pre made sheet (for instance, GelPak, as listed in the table of materials).
2. Remove the top and the bottom protective layers of plastic (if necessary) and use tweezers to place the PDMS in a plastic (not cell culture treated) Petri dish.
3. Wash the PDMS with 70% ethanol for 5 min on a rotator at 30 oscillations/min.
4. Dry the surface by flowing air on it.
5. Illuminate with deep UV (λ = 180 nm) for 5 min at a distance from the UV bulbs of about 5 cm (see Materials sheet for lamp reference; parameters will vary for different lamps).

6. Prepare 200 µl of EDC/NHS solution for each PDMS piece (this must be prepared just before use because the reactivity of the solution decays in a matter of hr). For 1 ml of 0.05 M MES + 0.5 M NaCl buffer at pH 6.0, add 11.5 mg of Sulfo-NHS and 19.2 mg of EDC.
7. Transfer the PDMS sheets from the Petri dish to the lid of the Petri dish, which has not been illuminated. This will ensure that the surroundings of the PDMS are very hydrophobic and facilitate the next step.
8. Add EDC/NHS solution and incubate for 15 min at room temperature.
9. Rinse EDC/NHS with water.
10. Add PLL-*g*-PEG solution (0.5 mg/ml in HEPES 10 mM pH 8.6) and incubate from 3 hr to overnight at room temperature.
11. Rinse the PLL-*g*-PEG with water. Passivated PDMS (functionalized with PLL-*g*-PEG) can be stored for several days at 4 °C.

2. Patterning of the PDMS

1. Take a PDMS sheet and place it on a synthetic quartz photomask bearing the microfeatures for patterning (see **Figure 1**). Place the PDMS side bearing the PLL-*g*-PEG facing the chrome side of the photomask.
2. Illuminate for 7 min through the photomask at a distance from the UV bulbs of about 5 cm.
3. Add water onto the mask + PDMS and peel the PDMS slowly off the mask.
4. Incubate with fibronectin solution at 50 mg/ml in HEPES buffer at pH 8.6 for 1 hr at room temperature. It is possible to use others ECM proteins, but these have not been tested with this protocol.
5. Rinse with PBS.

3. Mounting the Device

1. Mount the previously passivated PDMS onto the stretching device (see Discussion for troubleshooting).
    1. Attach one side of the PDMS to the fixed part of the stretcher.
    2. Fix the other side to the mobile part of the stretcher without clamping too much of the PDMS sheet.
2. Cut a rectangle of PDMS (22 mm x 19 mm) in a thick PDMS stab and cut another rectangle inside in order to have a pool (or frame) that will retain the cells and media (see **Figure 2**).
3. Add silicone grease under this PDMS pool and place it on top of the PDMS sheet to create a medium retaining pool. The grease will allow the gliding of the pool over the PDMS sheet during stretching.

4. Patterning the Cells

1. Detach the cells from a 50% confluency culture flask with Versene.
2. Count the cells and resuspend them at a concentration of 200,000 cells/ml.
3. Add 1 ml of cell suspension in the pool (see Discussion for additional details).
4. Let the cells bind to the patterns for 10-30 min (depending on the cell type, RPE-1 cells will take 10 min and HeLa cells 20 min).
5. Gently flush the floating cells with equilibrated medium (equilibrate medium in incubator).
6. Let the cells spread for a few hr on the patterns (RPE-1 will need at least 2 hr; HeLa cells will need 3 hr).
7. Add a coverslip on top of the pool to avoid evaporation and medium leakage in case the PDMS breaks.
8. Put the device on an inverted microscope and start imaging. Avoid the use of oil immersion objectives as it will not work due to focusing problems.

5. Stretching

1. Turn the micrometric screw while correcting the stage position in the x-, y-, and z-axis (mainly x). The stage position needs to be corrected to counteract the widening of the PDMS and the loss of focus. (See "During the stretch" paragraph in the DISCUSSION).

Representative Results

The technique presented in this video protocol allowed the application of forces on the retraction fibers of mitotic mammalian cells. Indeed, during cell division, at the mitotic stage, mammalian cells retract to take the shape of a sphere and leave behind thin actin cables surrounded by membrane which are attached to the substrate. These cables (retraction fibers), are the memory of the cell geometry before going into division. Making micropatterns with deep UV through a photomask on PDMS thin film (**Figure 1**) allowed the geometrical control of cell adhesion. By stretching the substrate at the onset of metaphase, some of these retraction fibers were pulled away from the cell body, resulting in a mechanical forces applied on the mitotic cell's cortex (**Figure 3**). These forces were shown to govern the orientation of the spindle axis[9].

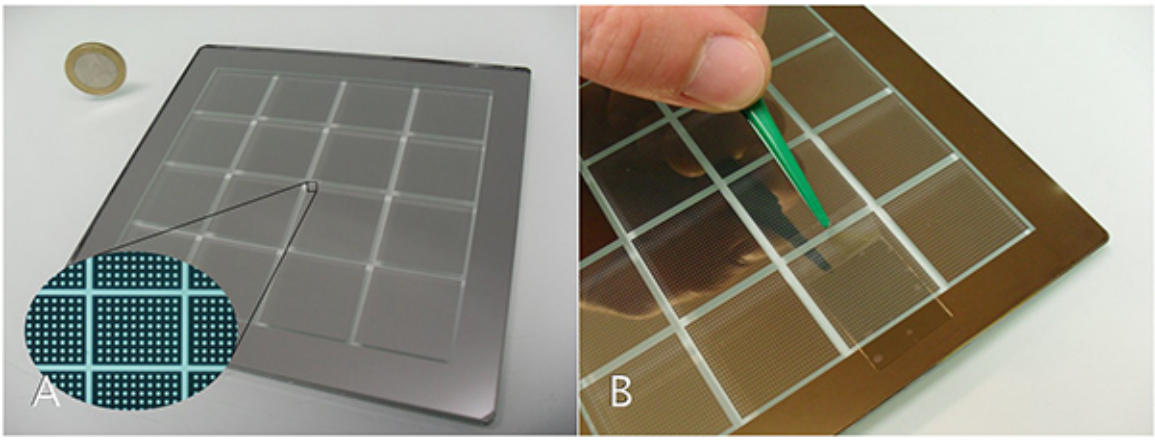

Figure 1. Synthetic Quartz Photomask bearing micropatterns features. A) Typical photomask used for deep UV patterning. It is a 10 cm x 10 cm binary mask and the features are typically of about 20 µm wide for single cell patterning, with a submicron resolution. Squares visible by naked eye are the size of a 10X objective microscope field of view. The blowup shows the features corresponding to single cell patterns (here discs). Features are transparent to let deep UVs pass through. **B)** The PDMS is applied on the photomask while carefully avoiding the formation of bubbles. The passivated PDMS side is in contact with the metallic side of the photomask. The micropatterns will be printed all over the PDMS. Click here to view larger image.

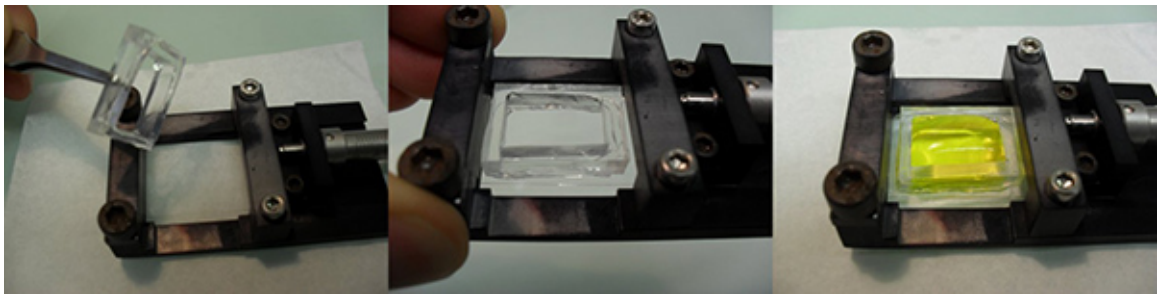

Figure 2. The pool. A PDMS pool is applied on the PDMS sheet. Grease is covering the bottom perimeter of the pool. The capacity of the pool is around 2 ml of media. The pool is filled with culture medium (here water with fluorescein appears in yellow). To avoid evaporation, a coverslip can be added on top. Click here to view larger image.

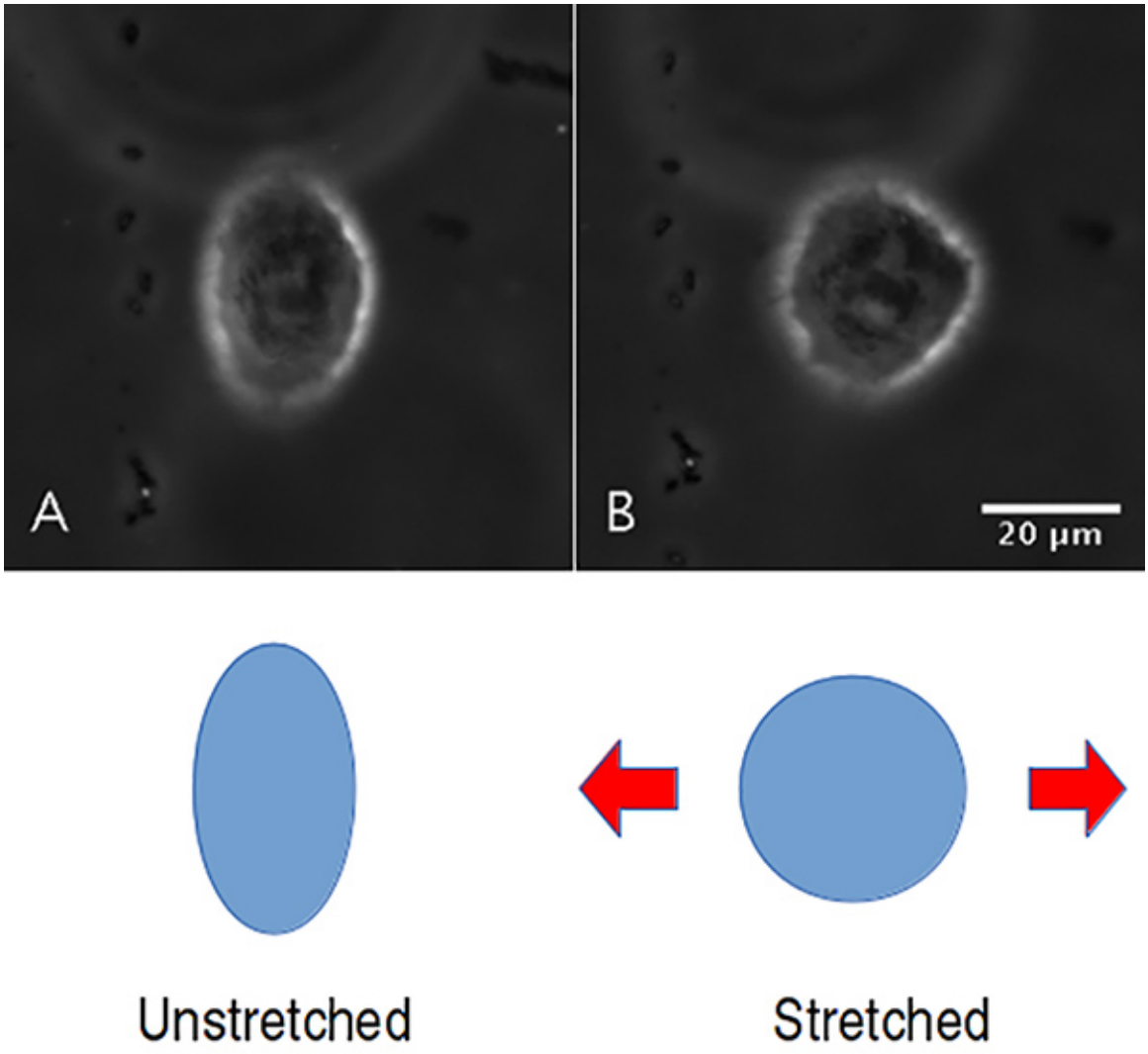

Figure 3. Deformation of a stretched cell. Phase contrast images of micropatterned RPE-1 cell on PDMS surface **A)** Before stretching. The cell is plated on an oval pattern. To achieve this, the patterning is done with the PDMS being stretched during the impression of round patterns through the photomask. A broken down photomask is used (the piece of mask is about 1.5 cm in diameter). To avoid breaking down a mask, use an oval pattern on the photomask and print it on an unstretched PDMS sheet. **B)** After stretching. The substrate is stretched such as the pattern becomes a circle. Click here to view larger image.

Discussion

Although this technique has been used numerous times and is thoroughly tested, there are several critical steps that can lead to a failed experiment.

About the PDMS:

For this work, GelPak, a commercially available thin PDMS sheet, was used. Alternatively PDMS sheets can be cast directly from PDMS mix. We recommend using GelPak because it is more reproducible, and is less likely to break compared to custom made PDMS.

About the device:

The stretching device used in this protocol was designed "in house", but one can also apply the micropatterning technique on commercially available stretchers like FlexCell. Custom made options are more versatile and a lot cheaper.

Mounting the device:

The mounting of the PDMS can lead to breaking. It is also easy to invert it and there is no way to tell which side is which. If the PDMS is clamped too much, it becomes shorter and this can lead to breaking (as the tension will be greater for the same distance of stretch). Make sure the screws are well tightened or the PDMS sheet will slip as soon as the stretch begins or later on during the experiment.

Adding the cells:

For detaching the cells from the culture substrate, it is better to use EDTA 0.02% in PBS rather than trypsin. Using EDTA will allow faster rebinding of the cells on the patterns. When cells are added to the PDMS, they must be well separated from one another in order to obtain individual cells binding to the pattern. Once they are in suspension, pipette them several times with a 200 µl tip; this will break up aggregates. About 200,000 cells must be added onto the 4 cm^2 surface in order to have binding to each pattern. Use a small volume so the cells fall quickly onto the surface. If there are not enough cells, many patterns will remain empty. The non attached cells must be flushed out by media removal as soon as enough cells are bound to the patterns. The time before the flush can vary between cell types but 15 min is generally enough for the cells to begin attaching to the patterns. If the cells are left to attach for too long, patterns will be occupied by 2 or more cells. This step is described in[13].

During the stretch:

Be extra careful, while stretching, to avoid losing the position of the region of interest. Indeed, during the stretch, the PDMS will become wider, and the stage position needs to be readjusted to compensate. A combination of a motorized stretching device and software control of the stage can be developed to automatically compensate for this effect[14]. The simple and versatile manual stretcher presented here can be upgraded to achieve such automated compensation.

The PDMS pool can leak if the silicon grease was not placed properly around the surface. Make sure the setup is correctly sealed by putting PBS inside and watching for any leak before plating cells on it.

Our stretcher allows a stretch of about 30%, but custom designed stretchers could allow for a higher degree of stretching. Be careful as the higher the strain, the higher is the risk of PDMS layer breaking.

Limitations:

One of the main limitations in the technique presented here is the lack of easy automatic stretching/destretching using a motor. Nevertheless, it is possible to adapt an actuator instead of a manual micrometric screw. This implementation, coupled with a specific programing of the microscope control can allow compensation for sample displacement during stretching.

Another drawback is the difficulty of having high resolution imaging, because using oil immersion objectives induces movements of the soft substrate when the objective moves too fast. This can be solved using water (but then evaporation is an issue for long term experiments) or very fluid oil.

Modifications and future applications:

In collaboration with Yanlan Mao and Nic Tapon from the Cancer Research Institute (London, UK), we developed another version of the stretcher which allows two layers of PDMS on top of each other, with the sample being in the middle, in order to pinch and stretch whole tissues.

Disclosures

The authors declare no competing financial interests.

Acknowledgements

This work was founded by Institut Curie, Paris, France. The mechanical stretcher was designed by Damien Cuvelier (Institut Curie) and is manufactured by GREM (mecanique-grem.com). The patterning on PDMS was developed by Ammar Azioune (Bordeaux II University).

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