

Micropipette Aspiration for Studying Cellular Mechanosensory Responses and Mechanics

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Abstract

Micropipette aspiration (MPA) is a widely applied method for studying cortical tension and deformability. Based on simple hydrostatic principles, this assay allows the application of a specific magnitude of mechanical stress on cells. This powerful method has revealed insights about cell mechanics and mechanosensing, not only in *Dictyostelium discoideum* but also in other cell types. In this chapter, we present how to set up a micropipette aspiration system and the experimental procedures for determining cortical tension and mechanosensory responses.

Key words Micropipette aspiration, Cell mechanics, Cortical tension, Mechanosensing, Mechanosensory response

1 Introduction

It is well recognized that mechanical forces impact the behavior of biological systems, ranging from molecular (e.g., force-induced focal adhesion complex formation) to tissue levels (e.g., blood pressure in the vasculature) (1, 2). Methods such as atomic force microscopy (AFM) and optical traps have been utilized to study cell behavior in response to forces (3, 4). These methods, although powerful, primarily exert forces in the sub-nN range over sub- μm^2 surface areas of the cell. Compression is another form of cell deformation, and compressive stress may be applied using agarose overlay with a thin agarose sheet (5). Although a straightforward method to use, the specific amount of mechanical stress applied by agarose overlay can be tricky to estimate. In contrast, micropipette aspiration (MPA) can apply a specific amount of pressure to cells (ranging from pN/ μm^2 to nN/ μm^2) over several tens of μm^2 , allowing total forces of up to a few tens of nN to be applied to cells.

Given that many large-scale cell shape changes involve several nN of total force, MPA is an attractive method for studying these processes (6–8). In the past few decades, MPA has been widely used to introduce mechanical disturbance to cells and to probe their mechanics (9–12). MPA has a long history in studies that deciphered the mechanical properties of mitotic cells (13–17). Based on simple mechanics, the cortical tension and elastic Young's modulus of the aspirated cell can be determined (10, 18). Coupled with genetics and fluorescent protein-labeling techniques, this method also allows us to impose mechanical stress on cells and examine how cells respond to this mechanical perturbation (mechanosensing) by changing protein localization. Using MPA, previous studies indicate that dividing *Dictyostelium* cells are exquisitely sensitive to mechanical stress (19, 20). Proteins such as myosin II (a force-generating mechanoenzyme) and cortexillin I (an actin cross-linking protein) accumulate to regions of high mechanical stress imposed by MPA (19, 20). This method has been applied recently to the developing *Drosophila* embryo to show that myosin II accumulation is mechanical stress dependent (21). Therefore, MPA is a powerful technique that can be used to examine cell mechanics and mechanosensitive behavior, and to reveal the mechanoresponsive signaling pathways (22).

In this chapter, we will first describe the motorized MPA systems that we designed and constructed in our laboratory. We will detail the procedures for calibrating the MPA system and manipulating cells. Finally, we will explain the image analysis for extracting the necessary information for quantifying cortical tension and the mechanosensitive localization of fluorescently labeled proteins.

2 Materials

2.1 The Components of the MPA System

The MPA system can be constructed in different ways and with different levels of motorization. Our system is designed to offer a high level of control and precision and to allow mechanical stress to be imposed within seconds. Our design (not including the microscope, micromanipulators, micropipette puller, or microforge) can be constructed for less than US\$10,000.

2.1.1 Instruments

1. Microscope: Olympus IX81 or IX71 (with DIC and fluorescence imaging capability, 10 \times , 40 \times (NA1.3), and 60 \times (NA1.45) objectives, and a 1.6 \times optovar). The microscope is controlled with MetaMorph software for image acquisition. Image analysis is performed using ImageJ (<http://rsbweb.nih.gov/ij/>).
2. Micromanipulator: Sutter Instrument MP225.
3. Micropipette puller: MicroData Instrument PMP-102.
4. Microforge: MicroData Instrument MFG-5 Microforge-Grinding Center.

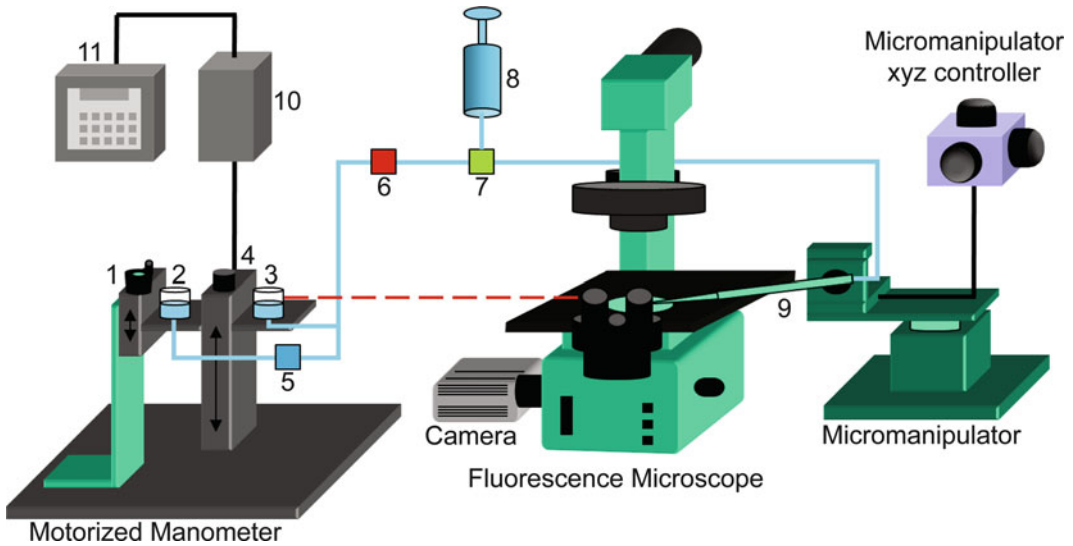


Fig. 1 The components of the MPA system. Individual components are labeled with a number and are described in Subheading 2.1. The *blue lines* connecting different components depict the polyethylene tubing that conducts water flow from the manometer to the micropipette. The *black lines* depict the electronic wiring that connects the components. Instruments that are crucial but not shown in this figure are the computer that communicates with the microscope imaging system, the power supplies for the motorized manometer and micromanipulator, and the vibration isolation table

2.1.2 MPA System Setup

The cartoon depiction of the MPA system setup is shown in Fig. 1. The numbers in Fig. 1 represent the part numbers described below.

1. Manual hand crank table (Part #1): Parker Bayside Manual Table SC100A-200. This part is mounted onto the Z-bracket (the L-shape structure; from Parker Daedal). The Z-bracket is the metal stand that supports the hand crank table, the linear slide (Part #4), and both of the water tanks (Parts #2, 3). The manual hand crank table allows vertical adjustment on the position of the platform that connects the water tanks and linear slide. This part is crucial for calibrating the manometer system.
2. Reference water tank (Part #2): Cylindrical water tanks (clear plastic, 8.5 cm height, 7.5 cm diameter) were custom-built in-house. The position of this water tank is fixed onto the manual hand crank table and is enclosed by a water tank holder, which also provides the support for the linear slide. The water level of #2 will remain constant and matches the level of the imaging platform during the experiment.
3. Movable water tank (Part #3): The position of this water tank is controlled by the keypad system (Parts #10, 11). When the position of #3 is lower than #2, an aspiration (negative, suction) pressure is applied by the micropipette (Part #9). When #3 is higher than #2, a positive pressure is applied by the

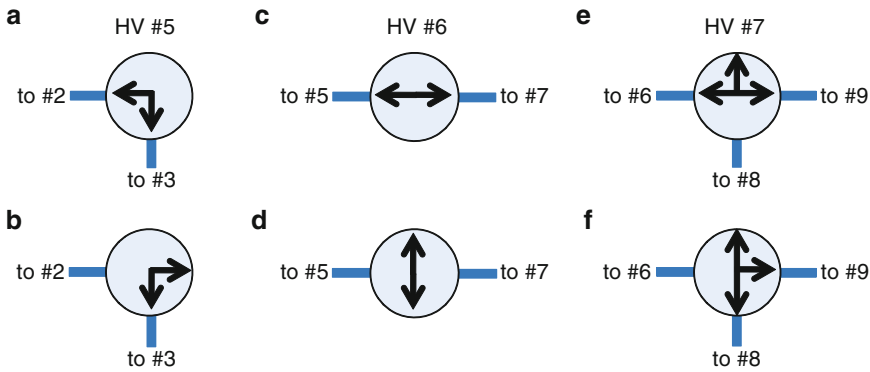


Fig. 2 Configurations of hand valves. The *circles* represent the hand valves (HVs, Part #5, 6, 7 in Subheading 2.1). *Arrows* represent the flow paths of the valves. *Blue lines* represent the water tubing which connects to the labeled components of Fig. 1. Panels (a) and (b) show the two configurations for HV #5. (a) The reference water tank (Part #2) and movable water tank (Part #3) are connected if HV #5 is in this configuration. HV #5 is set in this configuration during MPA system calibration (Subheading 3.4). (b) HV #5 is in this setting during experiment, which allows water flow only from #3 to the micropipette. Panels (c) and (d) depict the two configurations of HV #6. (c) This setting connects the water manometer to the rest of the system (“on”). (d) This setting isolates the manometer (“off”). Panels (e) and (f) show HV #7 which connects HV #6, the syringe (Part #8), and the micropipette (Part #9). (e) During experiment, HV #7 should be in this setting. (f) HV#7 should be in this setting when the syringe is in use

micropipette. The height difference between #2 and #3 defines the magnitude of pressure applied by the micropipette (see Note 1). When #3 is at the home position (zero pressure), the water surface of tank #2 and #3 has to be at the same level as well as the imaging stage (red dashed line in Fig. 1).

- Linear slide (Part #4): Parker Daedal Ballscrew Drive Linear Table (404T06XEMS series, NLD2H3L3C2M3E1B1R11P1). This part is supported by the water tank holder for #2. The motorized moving part of the slide is attached to the holder for water tank #3. The motor controls the movement of water tank #3 on the linear slide. There are three sensors attached to the linear slide (top, home, and bottom). These sensors are connected to the keypad controller system (Part #10). The top and bottom sensors mark the upper and lower limits that #3 can travel. The home sensor defines the position of #3 for the zero pressure. The position of the home sensor in Part #4 may have to be adjusted during the initial setup to align the water level in #2 and #3 with the imaging platform. It should not require any further adjustment once the system has been set up, provided there is no change of the position of the microscope imaging platform.
- 90° flow path 2-port hand valve (Part #5): Hamilton (86726) HV2-2 valve. When calibrating the system, water tanks #2 and #3 should be connected as shown in Fig. 2a. This allows the water from both tanks to flow from one to the other in order

to reach equilibrium. When the pressure of the system is equilibrated, valve #5 has to be in the configuration shown in Fig. 2b to only allow water flow from #3 to the micropipette during the experiment (see Note 2).

6. 180° flow path 2-port hand valve (Part #6): Hamilton (86725) HV1-1 valve. This part allows the isolation of the flow of water from the manometer to the rest of the tubing. The valve should be in the “on” or “connected” position (Fig. 2c) during system calibration and experiment. The valve should be turned off (Fig. 2d) when the system is not in use or when the tubing is flushed out by using the syringe (Part #8).
7. “T” flow path 3-port hand valve (Part #7): Hamilton (86727) HV3-3 valve. This valve dictates the fluid flow from either the syringe (Part #8) or the water manometer (provided that #6 is in the “connected” configuration). Valve #7 should be set as shown in Fig. 2e during system calibration and experiment. When using the syringe to flush out the tubing or when loading the micropipette, the valve should be set as indicated in Fig. 2f (see Note 3).
8. 30-mL syringe (Part #8): This syringe is used to push out the water to flush the tubing, especially when air bubbles are trapped in the valves, the tubing, or at the joint where the micropipette holder and the micropipette connect (see Notes 4 and 5).
9. Micropipette holder (Part #9): Sutter Instrument. This part is connected to the tubing that links to valve #7. A glass pipette with a μm -size tip is inserted into the holder (see Notes 6 and 7). The holder is held by a customized clamp shown in Fig. 3. This allows adjustment in the angle (θ in Fig. 3) of the micropipette relative to the microscope stage. The clamp holder is attached to the platform, which is manipulated by the micromanipulator to control the x - y - z position of the micropipette.
10. The keypad controller system (Part #10): IDC SmartStep23-FP. This part inputs the signal from the keypad (Part #11) to adjust the movement of water tank #3 attached on the linear slide.
11. Keypad (Part #11): IDC SmartStep I/O keypad. This part allows the user to run programs that control the movement of #3. It displays the change in position of #3 relative to home position in units of mm (see Note 8).

2.2 Imaging Chamber and Buffers

1. Reusable hollow rectangular imaging chambers made of anodized aluminum were custom-built in-house. The thickness of the chamber is about 1.5–2 mm (see Note 9). The chamber outer dimension is 32×70 mm and the inner dimension is 12×40 mm. The imaging chamber holds approximately 1 mL of sample.

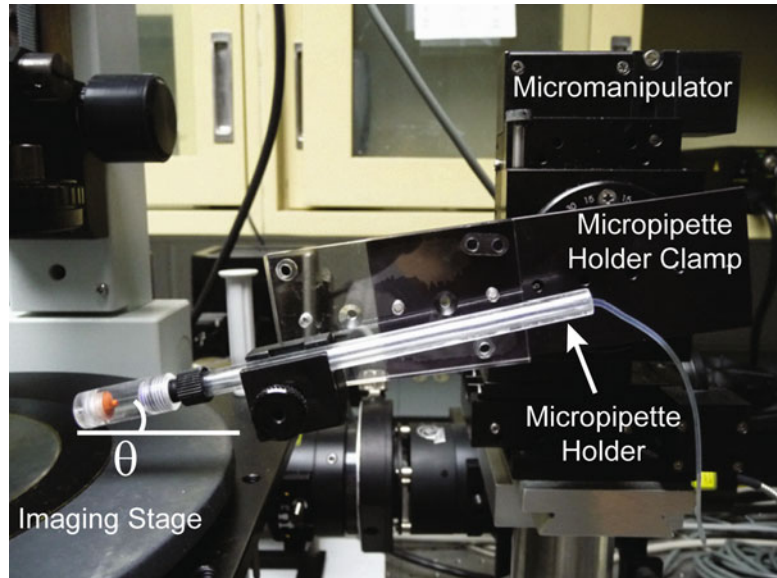


Fig. 3 Micropipette holder and customized micropipette holder clamp. The clamp is mounted onto the micromanipulator for controlling the x - y - z movement. The micropipette holder clamp can be adjusted to change the angle (θ) between the imaging stage and the micropipette. All the screws and knobs of the holder clamp must be securely tightened to reduce fluctuations of the micropipette during the experiment

2. Vacuum grease (Dow Corning high-vacuum grease) is required for attaching the cover glass to the imaging chamber and preventing leak.
3. Cover glass (No. 1.5 thickness, 22 × 60 mm).
4. To study the mechanosensitive accumulation of fluorescently labeled proteins, a nonfluorescent MES starvation buffer (50 mM MES, pH 6.8, 2 mM MgCl₂, 0.2 mM CaCl₂ in deionized water) is used for imaging. MES starvation buffer must be filtered and sterilized with a 0.22- μ m filter before use.

2.3 Cell Strains and Culture

1. Cells are cultured in 10-cm Petri dishes with Hans' enriched HL-5 medium (1.4× HL-5 enriched with 8% FM) with penicillin and streptomycin at 22°C.
2. Mutant or wild-type cells. For direct phenotype comparison, the corresponding parental strains in which the mutants are derived should be used as the control cell lines.
3. For protein mechanosensitive response studies, cells are transformed with an expression plasmid encoding a protein of interest fused to a fluorescent protein (e.g., GFP, citrine, or mCherry).
4. Depending on the transfected plasmid, 10–15 μ g/mL of G418 or 15–50 μ g/mL of hygromycin is used for selecting the transformed cells.

2.4 Micropipettes and MPA Calibration

1. Micropipette needles can be acquired commercially (World Precision Instrument. Fire-polished Pre-Pulled Glass Pipettes, TIP5TW1). However, we make our own micropipettes using a micropipette puller and microforge. The recommended inner diameter of the micropipette should be in the range of 4–6 μm , with 5 μm being ideal. For cortical tension measurements, the micropipette tip should be straight with a polished edge. For protein localization studies, the tip can be slightly slanted but still should be polished. A microforge is very useful for polishing the micropipette tips.
2. Thin-wall borosilicate glass tubing (1 mm outer diameter, 0.75 mm inner diameter, 10 cm length) from Sutter Instrument. This is the starting material to make micropipettes with the micropipette puller.
3. 1- μm blue polystyrene microbeads (#15712) from Polysciences, Inc. This material is important for calibrating the MPA system.

3 Methods

All experimental procedures described below are performed in a temperature-controlled room in which temperature is kept nearly constant at 22°C.

3.1 Imaging Sample Preparation

1. Spread vacuum grease along the inner edge of the imaging chamber to adhere a 22 \times 60 mm cover glass (No. 1.5). The cover glass is gently pressed against the imaging slide to allow homogeneous spread of the vacuum grease along the inner edge of the imaging chamber until no apparent gap exists. The sample will leak if the imaging chamber is not sealed properly.
2. 1 mL of log-phase ($2\text{--}4 \times 10^6$ cells/mL) *Dictyostelium* cells is added to the sealed imaging chamber. Cells are allowed to settle for 10–15 min.
3. Once cells have settled, the growth medium is gently removed using a p1000 pipette. Tilt the imaging slide to restrict the contact between the pipette tip and the imaging slide to one corner.
4. Gently wash the cells by adding 1 mL of MES starvation buffer to the corner of the imaging slide and allow to sit for a few seconds before removing. Then, gently add another 1 mL of fresh MES starvation buffer to the imaging chamber. Adding the buffer abruptly will cause the cells to dislodge from the surface.

For cortical tension studies, steps 3 and 4 can be ignored since the measurements are made in growth medium. For *Dictyostelium* cells, we have tested growth medium and several other buffers that span a ~4-fold range in osmolarity and have not detected any quantitative differences in cortical tension values for wild-type cells.

3.2 Loading the Micropipette

1. Make sure there is no air bubble in the tubing, micropipette holder, and valve joints (see Note 5). If there is a visible air bubble, loosen the micropipette holder tip and flush the system with the 30-mL syringe. Gently tap the tubing with your fingertip while flushing the syringe to dislodge any air bubble. Follow the air bubble and make sure it flows completely out of the micropipette holder tip.
2. To load the micropipette, first loosen the micropipette holder tip and squeeze out a drop of water, leaving it to hang at the opening of the micropipette tip. If this is neglected, an air bubble will be introduced into the micropipette holder when the micropipette is inserted.
3. Use a p20 pipette with a fine gel loading tip to fill the micropipette with MES starvation buffer (or growth medium for cortical tension measurements). Leave an excess hemispherical droplet at the opening of the micropipette. While filling with buffer, the loading tip should always be kept below the buffer level to prevent any air gap in the micropipette, and the micropipette should be handled carefully (see Note 10).
4. Insert the micropipette to the micropipette holder droplet to droplet. This is the easiest way to insert the micropipette while minimizing the chances of introducing an air bubble (see Note 6). The micropipette is inserted through the O-ring of the micropipette holder. The opening of the micropipette should be at the midway of the chamber in the micropipette holder. Tighten the micropipette holder tip. Wipe off any excess fluid.
5. Secure any screws or knobs of the micromanipulator clamp that holds the micropipette holder to prevent any unwanted motion (see Note 7).

3.3 Locating the Micropipette in the Imaging Field

1. Put the imaging chamber with cells on the microscope stage. Select the 40× magnification objective. Turn on the DIC channel to allow light to pass through the imaging stage.
2. Move the micropipette tip as close as possible to the light path either by adjusting the micropipette holder clamp or by using the micromanipulator.
3. Connect the micropipette with the 30-mL syringe (see Fig. 2f). Push a droplet of water out from the micropipette tip. This droplet will only be visible when the tip is directly at the light path. The droplet indicates the position of the micropipette tip. Make sure the droplet sits at the center of the light path.
4. Select 10× magnification. Lower the position of the micropipette using the micromanipulator so that the tip is immersed (see Note 11). Once the tip is immersed, adjust valve #7 so that the water manometer is connected to the micropipette (see Fig. 2e and Note 3).

5. Adjust the micromanipulator for fine movement when the micropipette gets close to the surface where the cells are. Coarse movements will disturb the cells. Position the micropipette tip at the center of the imaging field.
6. Change the magnification to 40 \times . With slight adjustment, the micropipette position should be at the center of the imaging field.

3.4 Calibrating the MPA System

1. The system should be calibrated every time before each session.
2. Locate the micropipette and make sure the valves are adjusted so that the micropipette is connected to the manometer.
3. Make sure the positions of the two water tanks are at the same level (home position). Valve #5 should be turned as shown in Fig. 2a to connect the two water tanks to allow the water level in both tanks to equilibrate (see Note 12).
4. Turn on the DIC channel and live image.
5. 1- μ m polystyrene beads are diluted 1:500 with filtered sterilized water. Add ~20–50 μ L of the diluted bead solution to the imaging chamber directly in the light path.
6. The live DIC channel will show microbeads gradually sinking to the surface of the cover glass.
7. Carefully move the micropipette to approach a free-floating microbead without introducing turbulence. Position the pipette opening right next to a free-floating microbead. If the bead does not move towards or away from the pipette opening, the system is nicely calibrated (the zero pressure is a true zero). It is normal to observe thermal fluctuation of the microbeads.
8. If the bead moves towards the pipette opening, this means the water level of the manometer is lower than the stage. Adjust the position of the manual hand crank table (Part #1) by rotating the lever so that the manometer can be raised. Slowly increase the height of the manometer. Check the movement of the microbead through the live DIC image. Continue to raise the manometer until the microbead stops moving towards the pipette. If the bead moves away from the pipette, lower the manometer.
9. Once the true zero pressure is achieved, disconnect the two water tanks by adjusting valve #5 as shown in Fig. 2b.
10. Use the keypad (Part #11) to control the position of the movable water tank (Part #3). If #3 is 0.5 mm lower than the reference water tank (Part #2), the bead will move into the pipette. If #3 is 0.5 mm higher than #2, the bead will move away from the pipette. The calibration is completed (see Note 13).

3.5 Cortical Tension Measurements

1. Prepare the imaging sample (see Subheading 3.1). The cells should be in growth medium on the imaging slide.
2. Put the sample on the microscope imaging stage.
3. Load the micropipette and locate it in the imaging field (see Subheadings 3.2 and 3.3).
4. Calibrate the MPA system (see Subheading 3.4).
5. Set up time-lapse acquisition using the DIC channel with 5-s intervals.
6. Record the room temperature (see Note 14).
7. Locate a healthy cell that has relatively few pseudopods or protrusions (see Note 15).
8. Gently dislodge the cell with the micropipette (see Fig. 4a).
9. Apply pressure to the cell and start imaging. Usually, 10–15 mm of water pressure is a good start.
10. Image the cells for five frames and then pause the acquisition. Increase the pressure by 5 mm (by lowering #3) and resume imaging. Record the pressure magnitude and the corresponding frame number. The cell cortex will slowly extend into the pipette due to the gradual pressure increase. Repeat this step until the equilibrium pressure (ΔP_c) is reached. At ΔP_c , the aspirated cell forms a hemisphere deformation inside the pipette. The radius of this hemisphere (L_p) equals to the pipette radius (R_p). The effective cortical tension (T_{eff}) of the cell can be quantified by the equation:

$$\Delta P_c = 2T_{\text{eff}}(1/R_p - 1/R_c)$$

where R_c is the radius of the cell at ΔP_c (see Note 16 and Fig. 4b). Other mechanical models may also be used to analyze MPA data (e.g., (6, 8, 18) and references therein).

11. For each cell, at least three measurements should be collected to average out any differential effects on the cell cortex. After reaching ΔP_c , wait for about 5 min to allow the cell to regain its stability before aspirating again. Alternatively, use the micropipette (at zero pressure) to maneuver the same cell so that the cell may be aspirated on a new region of the cortex. An example dataset is shown in Fig. 4c.

3.6 Quantification for Protein Mechanosensitive Accumulation

1. Prepare the imaging sample (see Subheading 3.1). The cells should be in MES starvation buffer on the imaging slide.
2. Put the sample on the microscope imaging stage.
3. Load the micropipette and locate it in the imaging field (see Subheadings 3.2 and 3.3).
4. Calibrate the MPA system (see Subheading 3.4).

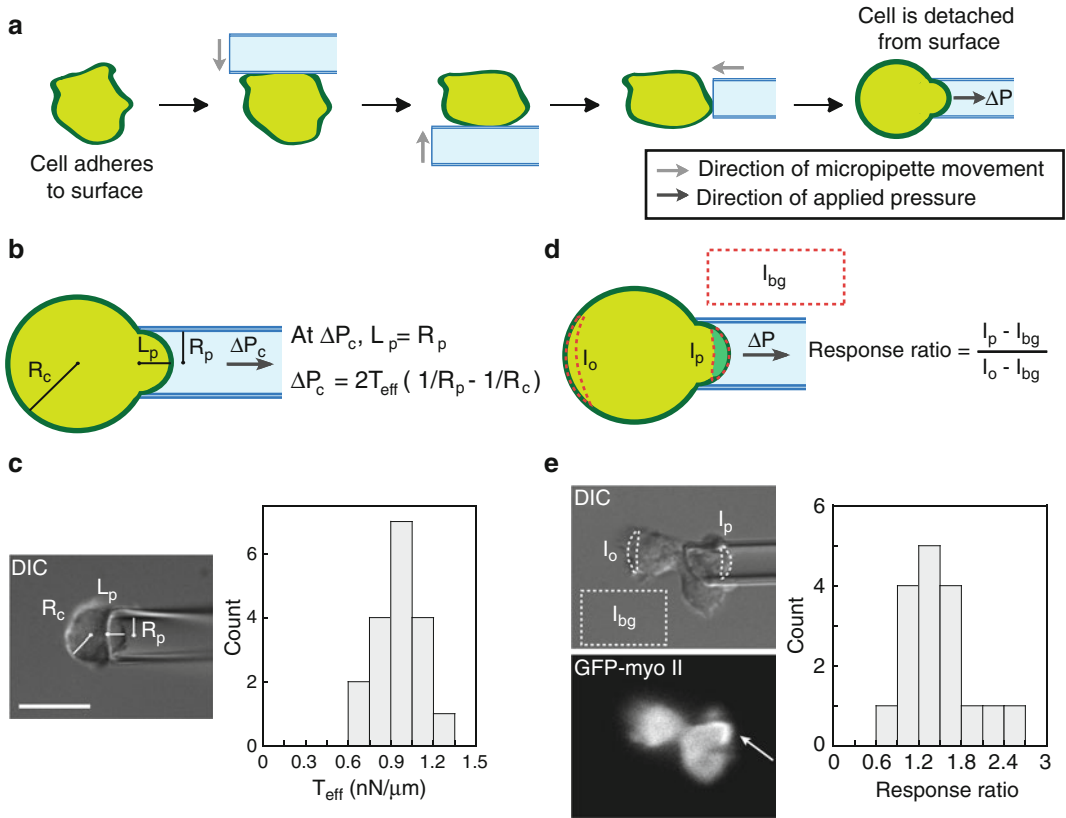


Fig. 4 Manipulation of cells by MPA for cortical tension and mechanosensitivity analysis. **(a)** This panel shows how to dislodge a cell from the cover glass surface. First, use the micropipette (zero pressure) to gently perturb one side of the cell (abrupt pipette movement will cause the cell to bleb or rupture). Move the pipette to the opposite side of the cell and gently perturb. Both sides of the cell should be less adherent due to the perturbation. Move the pipette to the side and gently dislodge the cell. Pressure (ΔP) can then be applied to aspirate the cell. **(b)** For cortical tension measurements, the pressure is increased until the aspirated cell shows a hemisphere inside the pipette. At this equilibrium pressure (ΔP_c), the length (L_p) of the region of the cell pulled into the micropipette equals the radius of the pipette (R_p). The radius of the cell at ΔP_c can be quantified by analyzing the acquired DIC image. The effective cortical tension T_{eff} is calculated from the equation shown in the panel. **(c)** The DIC image shows a wild-type cell during cortical tension measurement. The parameters labeled in the image are described in **(b)**. The histogram shows the distribution of measurements from 18 wild-type cells. The mean T_{eff} value is 0.93 ± 0.03 nN/ μ m. Scale bar represents 10 μ m applied to panels **(c)** and **(e)**. **(d)** To quantify the mechanosensory response ratio of a fluorescently labeled protein of interest, the mean pixel intensities of three different regions (I_p , I_o , I_{bg}) in the fluorescence image are measured. I_p is the mean pixel intensity of the cell cortex inside the pipette, I_o is the mean intensity of the opposite cortex, and I_{bg} is the background mean intensity of a large region devoid of cells. The mechanosensory response ratio is the background corrected I_p divided by background corrected I_o as shown by the equation in the panel. **(e)** The images from DIC and GFP channels show a dividing wild-type cell expressing GFP-myosin II during an MPA experiment. The parameters labeled in the DIC image are described in **(d)**. The histogram shows measurements from 17 wild-type dividing cells. The mean response ratio is 1.45 ± 0.10

5. Record the room temperature.
6. Set up multiple wavelength image acquisition with DIC and the corresponding fluorophore channels. Record the exposure time for each channel. Set the time interval to be 15–30 s (see Note 17).
7. Start imaging to acquire an image before the cell is aspirated.
8. Pause imaging then dislodge cell gently with the micropipette (Fig. 4a).
9. Apply pressure to the cell (see Note 18).
10. Lower the micropipette along with the aspirated cell so that the micropipette is gently pressed against the cover glass surface. This allows the cell and pipette to be right above the glass surface (see Note 19).
11. Resume imaging until the acquisition is complete.
12. Use ImageJ or another reliable image analysis software package to analyze the fluorescence images.
13. Measure the pixel intensity of the cell region inside the pipette (I_p), opposite from the pipette (I_o), and background (I_{bg} , an area devoid of cell). The mechanosensitive protein response ratio can be quantified by the following equation:

$$\text{Response ratio} = (I_p - I_{bg}) / (I_o - I_{bg}).$$

The cartoon depiction of this measurement is shown in Fig. 4d and an example dataset is shown in Fig. 4e (see Note 20).

4 Notes

1. Water tanks #2 and #3, syringe, and tubing should only be filled with sterile filtered, distilled water to avoid contamination. After an experiment, water tank #3 should be returned to its home position before shutting down the system.
2. The handle of the hand valve over time may get loose by itself. It can be tightened using a hex wrench. Valve #5 must be turned off (as shown in Fig. 2b) to allow water to only flow from the movable water tank #3 to the micropipette during the experiment. If one forgets to do so and leaves the two water tanks connected (as shown in Fig. 2a), the water level in the tanks will be altered and may destabilize the MPA system due to any shift in the position of #3. Full system calibration is required if one makes such a mistake.
3. During the experiment, one should make sure valve #7 is set as shown in Fig. 2c so that the micropipette is isolated from the syringe and connected to the manometer. If one forgets to do

so and leaves the valve as shown in Fig. 2f, there will be a constant inward pressure from the micropipette (valve #7 is most likely positioned below the imaging stage).

4. When the water in the syringe (Part #8) is depleted, carefully unscrew the syringe tip that connects the tubing. Completely remove the piston from the syringe and fill it with water. One can use a fingertip to block the syringe opening to prevent water flow. Once the water is filled, put the piston into the syringe. Invert the syringe so that the syringe tip faces up. Push the piston (tap the syringe if necessary) to free up any bubbles or air gap. Before connecting the tubing to the syringe tip, let the syringe tip face upward and gently squeeze out some water so that a hemispherical water droplet is formed at the tip. When linking the tubing connector, the syringe should remain vertical. While tightening the tubing connector, the piston of the syringe should be pressed simultaneously to ensure water flow. This will prevent air bubbles from being trapped at the tubing joint.
5. Because air is compressible, air bubbles can strongly affect the accuracy of the applied pressure. Therefore, great care must be taken to ensure that there are no air bubbles in the system. Occasionally, the user may have to disconnect the valve joint (while connected to the syringe to allow flushing water) and dislodge any air bubbles in the hand valve joints using a p200 pipette. Clear out any visible bubbles with the pipette tip while flushing. After removing all bubbles, use the syringe to push out a hemispherical water droplet on top of the valve joint opening. Screw in the tubing connector at the opening while pushing out water from the syringe to prevent any air gaps. Use an absorbent pad or some paper towels to absorb the excess water while performing this.
6. Air bubbles may be easily trapped at the joint where the micropipette and the pipette holder meet due to a poor technique of inserting the needle into the holder.
7. The knobs of the clamp that secure the pipette holder (Part #9) have to be tightened. The micropipette will be unstable during experiments due to loosen knobs. This will cause micropipette vibration and significantly impact image quality.
8. For Part #11, a backlit keypad is preferable for experiments that will be performed in the dark, such as during fluorescence imaging acquisition.
9. The thickness of the imaging chamber should not be too high. Thick imaging chambers prevent the ability to position the micropipette as close to parallel to the surface as possible (smallest possible θ in Fig. 3).
10. When handling micropipettes, do not touch the tip. This will result in breaking the fragile tip and may pierce the skin.

11. Sometimes surface tension will prevent the micropipette tip from being immersed. In this case, gently add a drop of MES starvation buffer (or enriched HL5 for cortical tension study) above the micropipette to break the surface tension.
12. Eyeball the top water level to see if it is the same for both of the water tanks. Also, use a ruler to check if the top water level in the water tanks matches the level of the imaging stage where the cell sample sits. As evaporation occurs, the water level drops over time. More filtered sterilized water may need to be added to the water tanks in order to recalibrate the system. If more water is added to the water tanks, make sure valve #5 is positioned to connect both tanks (Fig. 2a).
13. Any leak from the tubing or the valve joints will disturb the calibration. Take any necessary action to prevent the leak. Tubing and valve may wear out over time and have to be replaced.
14. The temperature should be recorded for each experiment session. *Dictyostelium* cells are temperature sensitive and are healthiest at 22°C.
15. Cells with fewer pseudopods or protrusions are more ideal for cortical tension measurements. We find that these cells are less likely to bleb or extend protrusions at the region where aspiration is performed. If the aspirated cell starts blebbing or extending abruptly into the pipette (often a behavior more common for some mutant strains), release the cell and locate another more stable cell.
16. For quantifying the effective cortical tension (T_{eff}), make sure that all the parameters (ΔP , R_p , and R_c) are in SI units.
17. The acquisition time interval (the time between each acquisition) may vary depending on the cell line. Certain cell lines are extremely photosensitive. A longer acquisition time interval may reduce the phototoxicity for these cell lines. Cells exhibit photodamaged behavior by rounding up. Ideally, the exposure time (the amount of time the shutter stays open to expose cells with excitation light) should be kept constant for all the experiments performed across all cell lines for direct comparison.
18. The required pressure for inducing protein accumulation does vary throughout the cell cycle and may vary from protein to protein in wild-type and different genetic strains. In dividing cells, 30–50 mm (29–49 nN/ μm^2) water pressure promotes wild-type myosin II mechanosensitive accumulation whereas 60–100 mm (0.59–0.98 nN/ μm^2) of water pressure is required for wild-type myosin II accumulation during interphase. Most proteins we have studied do not display mechanosensitive accumulation.
19. Fluorescence imaging is sensitive to any shift in the position of the pipette and out-of-focus effect. When the micropipette is

gently pressed against the cover glass surface, it stabilizes the position of the cell and the micropipette. The cell, however, should not adhere to the glass surface so that the mechanosensitive localization of the protein is independent of any effects from cell-substrate adhesion and cell motility (in case the cell tries to crawl out of the pipette). Therefore, the position of the micropipette should be gently shifted between frames by using the micromanipulator to ensure the cell remains non-adherent from the substrate.

20. Different proteins may have different levels of responsiveness to mechanical stress. For example, IQGAP2 responds to mechanical stress with a slightly lower magnitude (with a response ratio of 1.2–1.5) as compared to myosin II and cortexillin I (response ratio of 1.4–2). Nonresponsive proteins usually display ratios that are lower than 1. For example, soluble GFP or mCherry shows a ratio of 0.82. This ratio <1 is due to the fact that the thickness of the cell inside the micropipette is ~80% of the thickness of the opposing cortex. The image quality and the result of image analysis may be affected if the aspirated cell adheres to the outer surface of the micropipette (by wrapping around the micropipette). Rare cells that exhibit this behavior should not be analyzed.

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