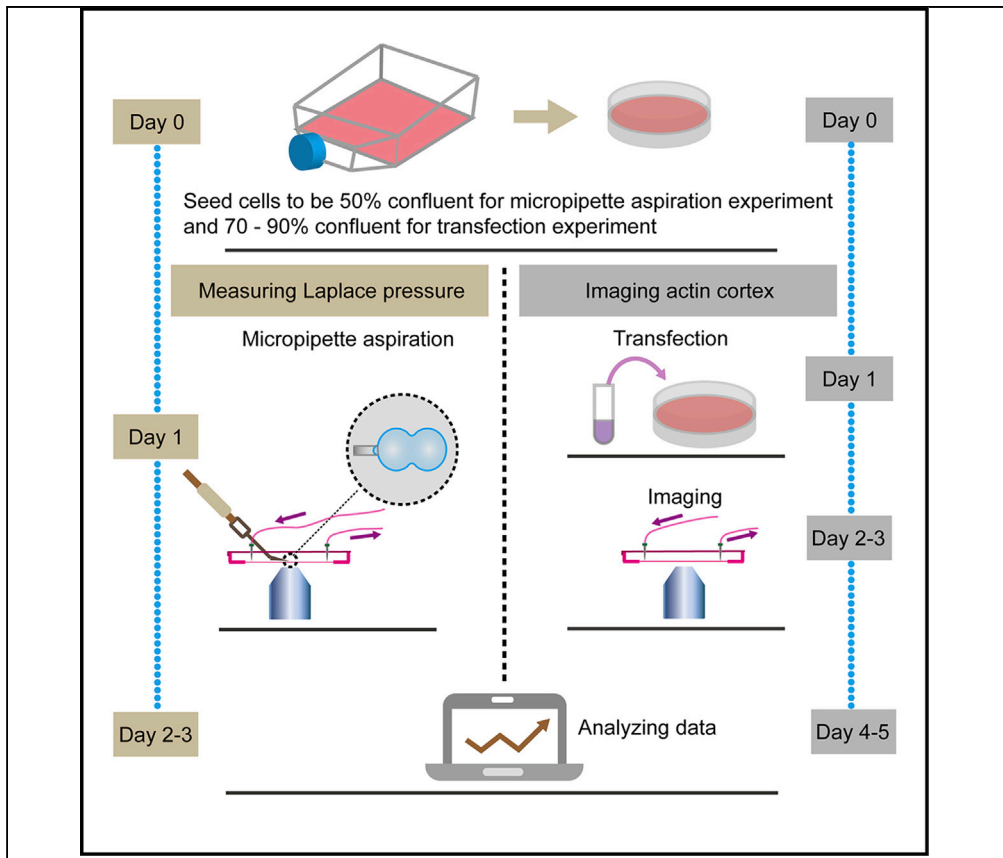


Protocol

Measuring Laplace pressure and imaging the actin cortex during cytokinesis in cultured cells



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Highlights

Using a micropipette to measure Laplace pressure in cultured cells

Imaging the dynamic response of actin cortex during cytokinesis

Correlation of actin cortex dynamics with changes in Laplace pressure during cytokinesis

Laplace pressure is an important regulator of cell dynamics and behavior during cytokinesis. Here, we provide a protocol to measure Laplace pressure in cultured cells using a micropipette and describe the steps for imaging the actin cortex during cytokinesis. The quantification steps allow tracing dynamic change in Laplace pressure and displaying dynamic response of the actin cortex during cytokinesis in HeLa cells. This protocol can be applied to any cultured cell type during various stages of cell division.

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Protocol

Measuring Laplace pressure and imaging the actin cortex during cytokinesis in cultured cells

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SUMMARY

Laplace pressure is an important regulator of cell dynamics and behavior during cytokinesis. Here, we provide a protocol to measure Laplace pressure in cultured cells using a micropipette and describe the steps for imaging the actin cortex during cytokinesis. The quantification steps allow tracing dynamic change in Laplace pressure and displaying dynamic response of the actin cortex during cytokinesis in HeLa cells. This protocol can be applied to any cultured cell type during various stages of cell division.

For complete details on the use and execution of this protocol, please refer to Wang et al. (2021).

BEFORE YOU BEGIN

Construction of the micropipette aspiration system and the medium replacement device

⌚ Timing: 1–2 days

Prepare components to set up the micropipette aspiration system and the medium replacement device. Then assemble them together with a living cell workstation to perform the experiments under culture conditions (Figure 1).

1. Prepare the following materials and equipment for the micropipette aspiration system and the medium replacement device.
 - a. EVOS® FL Auto Imaging System: 10× (NA1.2), 100× (NA1.4, oil-immersion type);
 - b. Living cell workstation (60 cm × 80 cm × 60 cm, Maworde);
 - c. Piezo-driven xyz micromanipulator: SN-PCZ-50R (World Precision Instruments, Inc.);
 - d. Micropipette puller: PC-100 (Narishige);
 - e. Microforge: MF-900 (Narishige);
 - f. Displacement measurement system: laser displacement sensor (LK-G500, Keyence), data acquisition card (USB3106A, ART), computer;
 - g. Two height gauges with hand wheel, measuring range 0–1,500 mm, measurement accuracy ± 0.04 mm and resolution 0.01 mm;
 - h. Two circular flat plates;
 - i. Two cylindrical water reservoirs (clear plastic, 12 cm height, 8 cm diameter);
 - j. Two 3-way T-port hand valves;



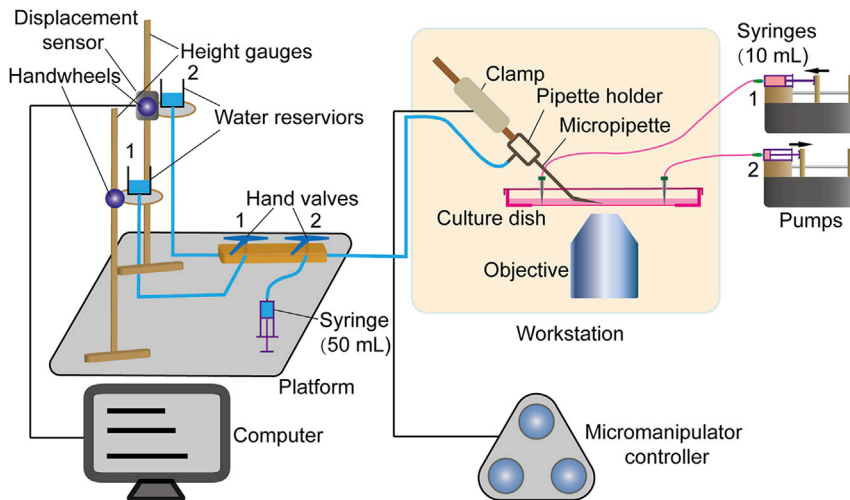


Figure 1. Components and set-up of the micropipette aspiration system and the medium replacement device

The micropipette is secured in a pipette holder and is navigated with a three-dimensional micromanipulator. The pressure applied to micropipette is controlled by vertically changing the height of water reservoirs connected to micropipette holder. Two syringes are loaded onto two pumps, respectively, one for aspirating old media and the other for providing new media.

- k. A 50 mL syringe;
 - l. Two 10 mL syringes;
 - m. Two syringe needles with blunt tips (21G);
 - n. A micropipette holder;
 - o. Five silicone tubes;
 - p. A glass-bottom Petri dish (35 mm);
 - q. Hot melt adhesives;
2. Set up the micropipette aspiration system.
 - a. Mount two circular flat plates onto each height gauge to support two water reservoirs, which are clamped by four fixtures on the plates.
 - b. Mount a laser displacement sensor to one of the two circular flat plates to measure real-time displacement of the water reservoir fixed on flat plate. Then connect the laser displacement sensor to data processing module, which consists of the data acquisition card and the host computer. The data acquisition card performs data synchronous acquisition work and sends the displacement data to the host computer for processing, graphing, and storing.
 - c. Fix the two water reservoirs onto the flat plates, and connect them through a 3-way T-port hand valve #1.
 - d. Connect the syringe (50 mL) to a 3-way T-port hand valve #2, which is then linked to valve #1 and micropipette holder, as shown in [Figure 1](#).

Note: Ensure the leakproofness of tube joints and valve joints to prevent water leakage, which significantly influences accuracy of the measurement results.

- e. Fill the two water reservoirs and the syringe (50 mL) with water.

Note: Fill the water reservoirs, syringe, and tubes with the sterile filtered, distilled water to avoid contamination. In addition, vacuum degassing helps to remove tiny entrained gas bubbles in water.

- f. Adjust the height of both water reservoirs and switch the valves #1 and #2 so that the tubes are filled up with water.

g. Pump the syringe (50 mL) to flush out the tubes and remove air bubbles if necessary.

△ **CRITICAL:** Make sure there are no air bubbles in the system since the compressible air bubbles strongly affect the measurement accuracy. To this end, use the syringe to dislodge any air bubbles.

△ **CRITICAL:** When the water in the syringe (50 mL) is used up, unscrew carefully the tube from syringe tip. Once the water is refilled, insert the piston into the syringe. Invert the syringe and push the piston up slowly, forcing the air out of the syringe. While connecting the tube to the syringe tip, the syringe is placed vertically and make sure a hemispherical water droplet has formed at its tip by gently pushing the piston. This will prevent air bubbles from being trapped at the tube joint.

h. Clamp the micropipette holder to the micromanipulator.

Note: Make sure the knob of the clamp that secures the micropipette holder is tightened, since the micropipette vibration due to loosen knob significantly affects image quality.

3. Set up the medium replacement device.

a. Heat up the tip of a knife over the alcohol lamp outer flame of around 650°C for 10 s. Then cut a U-shape notch in the lid of a plastic Petri dish (Figure 2A).

Note: To ensure high-resolution images when using a high power objective (e.g., 100×), the glass-bottom Petri dish is adopted.

Note: Keep the size of notch as large as possible to ensure adequate space for subsequent micropipette aspiration experiments.

b. Heat up the tip of an obsolete needle over the outer flame of an alcohol lamp for 5 s. Then drill a small-diameter vertical hole through each side of the remainders of the lid.

Note: Position the two holes on each side of the remainders of the lid, which helps to keep the cut lid balanced when covering the Petri dish.

c. Insert two syringe needles with blunt tip through each hole down vertically to the glass bottom of Petri dish.

△ **CRITICAL:** Make sure that the blunt tips of syringe needles reach to the glass bottom, rather than the plastic part around it. This ensures that all the media in the Petri dish can be sucked out.

d. Use hot melt adhesives to fix the needles in the corresponding holes.

e. Load two syringes (10 mL) onto two syringe pumps, one for aspirating old media and the other for providing new media.

f. Connect respectively the two syringe needles to two syringes mounted on syringe pumps.

4. Assemble the micropipette aspiration system, the medium replacement device, the living cell workstation, as well as the EVOS FL Auto Imaging System to construct an experimental platform.

a. Put the EVOS FL Auto Imaging System into the living cell workstation.

b. Put the micromanipulator of micropipette aspiration system on an adjustable holder in the living cell workstation.

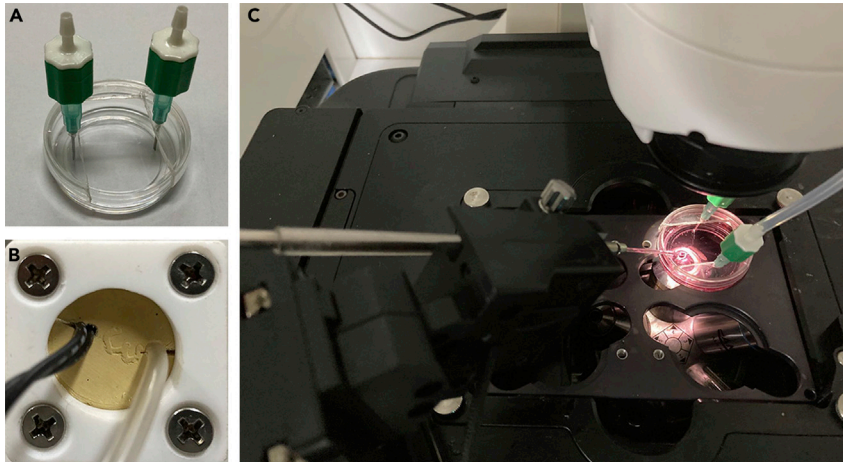


Figure 2. Assembled experimental platform consisting of the micropipette aspiration system, the medium replacement device, the living cell workstation, as well as the EVOS FL Auto Imaging System

- (A) Two syringe needles insert into small-diameter holes drilled on the cut lid.
 (B) Prefabricated hole of living cell workstation, which is used to allow connection wires and silicone tubes to pass it through, is sealed tightly with the rubber plug.
 (C) Equipment layout in the living cell workstation.

△ CRITICAL: Before placing the micropipette holder into the living cell workstation, regulate the 3-way T-port hand valves #1 and #2 to prevent water from flowing out to impair the EVOS FL Auto Imaging System and micromanipulator.

Note: The interior space of living cell workstation should be large enough to place other modules of this experimental platform, e.g., the EVOS FL Auto Imaging System, and the micromanipulator.

Note: Seal tightly the prefabricated hole of living cell workstation, which is used to allow connection wires and silicone tubes to pass it through (Figure 2B).

- c. Place appropriately the cut lid of medium replacement device in the living cell workstation (Figure 2C).

Note: Select softer silicone tubes that connect the syringe needles fixed in the holes of cut lid to syringes mounted on syringe pumps.

Cell culture

⌚ Timing: 1 week

5. Prepare the following materials and equipment for cell culture.
 - a. Fetal bovine serum (FBS) (100 mL).
 - b. Dulbecco's modified eagle medium (DMEM) containing L-glutamine (500 mL).
 - c. Phosphate buffered saline (PBS) (500 mL).
 - d. Trypsin-EDTA (0.25%, 10 mL).
 - e. Flasks (25 cm²).
 - f. Centrifuge tubes (15 mL).
 - g. Serological pipettes (2 mL and 10 mL).
 - h. Water bath kettle.
 - i. CO₂ incubator.

- j. Centrifuge.
 - k. Electronic pipette.
6. Prepare complete culture medium.
 - a. Thaw a bottle of FBS (100 mL) at 4°C.
 - b. Prepare the complete DMEM medium with 90% DMEM and 10% FBS.
 7. Culture HeLa cells.
 - a. Put cryogenic vials in a 37°C water bath kettle to thaw cryopreserved HeLa cells. Spray the thawed vial with 75% ethanol and wipe carefully. Then transfer thawed liquid contents into a 15 mL sterile centrifugal tube prefilled with 10 mL complete culture medium.
 - b. Centrifuge at 110 g for 3 min, discard supernatant, resuspend cells with 5 mL complete medium, which is then transferred into a cell culture flask.
 - c. Culture HeLa cells in the CO₂ incubator at 37°C until the cells reach 80–90% confluency.
 - d. Discard the culture medium, rinse the cell with 10 mL prewarmed PBS.
 - e. Remove PBS, then add 1 mL 0.25% Trypsin-EDTA solution to the cells and incubate at 37°C for 1–2 min until the cells become round-shaped.
 - f. Remove Trypsin-EDTA solution, add 5 mL fresh complete culture medium and disassociate cells from the flask by gently pipetting.
 - g. Transfer the cells to a 15 mL sterile centrifugal tube, and centrifuge cells at 110 g for 3 min at room temperature. Discard supernatant and resuspend cells in fresh complete culture medium. Then subculture them at a ratio of 1 : 4.

Note: Prewarm the culture medium to 37°C before use and maintain aseptic operation to avoid contamination.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
pCMV/pCAG-LifeAct Plasmids	lbidi	Cat#60101
Chemicals, peptides, and recombinant proteins		
Dulbecco's Modified Eagle's Medium (DMEM)	Solarbio	Cat#31600
Fetal Bovine Serum (FBS) (Australia origin)	Solarbio	Cat#11011-6123
Opti-MEM	Gibco	Cat#11058021
Penicillin-Streptomycin Liquid	Solarbio	Cat#P1400
Trypsin-EDTA(0.25%)	Gibco	Cat#25200072
Phosphate-Buffered Saline (PBS)	Solarbio	Cat#P1020
Sucrose	Solarbio	Cat# S8271
Lipo8000	Beyotime	Cat#C0533
Plasmid Maxi Preparation Kit	Beyotime	Cat#D0026
Experimental models: Cell lines		
HeLa Cells	ATCC	CCL-2
Software and algorithms		
ImageJ	National Institutes of Health	https://imagej.nih.gov/ij/
ORIGINPRO® 2021b	OriginLab	https://www.originlab.com/
EVOS FL Auto Cell Imaging Software	Invitrogen	N/A
Other		
NEST T25 Cell Culture Flasks, Sterile	NEST	Cat#707003
35 mm Glass-Bottom Culture Dish	Nunc	Cat#150682
15 mL Centrifuge Tubes	NEST	Cat#601002
Polystyrene Microbeads	NanoMicro Tech.	Cat#LBWNP-100

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Borosilicate Glass Capillaries	WPI	Cat#2301334
Microelectrode Filling Needle	WPI	Cat#MF28G67-5
Syringes, 10 mL, 50 mL	N/A	N/A
Syringe Pumps	Longer	Cat# 05.03.13A
EVOS FL Auto Cell Imaging System	Invitrogen	N/A
Living Cell Workstation	Maworde	N/A
Displacement Sensor	Keyence	N/A
Micropuller	Narishige	N/A
Microforge	Narishige	N/A
Water Bath Kettle	N/A	N/A
Magnetic Stirrer	N/A	N/A
Centrifuge	N/A	N/A
Benchtop	N/A	N/A
CO ₂ Incubator	Wiggins	N/A
−80°C Freezer	N/A	N/A
3-Axis Micromanipulator System	WPI	N/A

STEP-BY-STEP METHOD DETAILS

Cell preparation

⌚ Timing: 2 days

Prepare cells in glass-bottom plastic Petri dishes for subsequent micropipette aspiration experiments.

1. Repeat the steps 3d - 3g in the Cell culture section to disassociate the HeLa cells.
2. Plate cells in glass-bottom Petri dishes and incubate in a humidified 37°C, 5% CO₂ incubator for 24 h, at which point cells exhibit about 50–60% confluency as observed by light microscopy.
3. Rinse the HeLa cells 2–3 times with prewarmed PBS to wash away nonadherent cells and debris, and then add 2 mL fresh complete culture medium and incubate in a CO₂ incubator for 2–3 h.

⚠ **CRITICAL:** Cells undergoing mitosis, on which we focus here, could be rinsed away due to poor adhesion to the culture dishes. To make it easier to find HeLa cells undergoing mitosis and to ensure the experimental efficiency, incubate cells for 2–3 h after rinsing.

Note: Make sure to rinse the cells to remove any debris that could block the micropipette.

Prepare and mount micropipette

⌚ Timing: 15–30 min

Make micropipette from borosilicate glass capillary tubes (TW150-4, World Precision Instruments, Inc.) using the micropipette puller and microforge, as described in the following. Then mount the micropipette onto the pipette holder that allows fine control of the pipette movements in three dimensions.

4. Fabricating glass micropipettes ([Methods video S1](#)). [Troubleshooting 1](#) and [2](#)
 - a. Stretch the micropipette from a glass capillary (i.d./o.d. = 1.12 mm/1.5 mm) using a micropipette puller. To fabricate a pipette with an inner diameter of 1–2 μm and a taper length around 2 mm, we adopt the two-stage pull mode and use the following settings—Distance 1: 3 mm, Distance 2: 6 mm, Heat 1: 65 V, Heat 2: 60 V, Weights: 250 g.

Note: The shape of micropipette depends strongly on its properties and the parameter settings of micropipette puller.

- b. Cut the taper of the pulled micropipette at the desired micropipette radius (about 1.5–2 μm) by placing it against a hot metal wire in microforge. Then polish the end of micropipette by bringing it close to the heated bead to remove the sharp edges.

△ **CRITICAL:** For micropipette tip with a radius too large, the whole cell can be easily swallowed into the micropipette even for a small aspiration pressure. By contrast, membrane-cortex detachment occurs frequently due to the higher aspiration pressure needed to suck the cell into the micropipette tip with a radius too small. It thus goes against the experiment measuring for the tip radius which is set too large or too small. According to our experience, the appropriate radius of the micropipette tip, depending on the cell type, ranges from 1/4 to 1/3 of the radius of the HeLa cell.

- c. Place the micropipette in micropipette puller. Adjust the position of micropipette so that its tip protrudes about 5 mm beyond the spiral-shaped heating elements of micropipette puller, as shown in [Figure 3A](#).
- d. Heat up the tip of the micropipette and bend it using another obsolete micropipette to an angle (typically 35–40 degrees) at which the tip will be allowed to approach the cell at a very shallow angle, as close to horizontal as possible.

Optional: Micropipette can be passivated with, e.g., a β -casein solution to reduce adhesion of cells to the glass.

△ **CRITICAL:** The micropipette tip should be smooth to make a good seal to the plasma membrane.

△ **CRITICAL:** Keep the length of the bent part of micropipette tip as short as possible (5–8 mm) to increase the operating space as much as possible.

Note: When handling a micropipette, great care should be taken to avoid touching the tip. This will break the fragile tip and lead to the risk of inserting it into the body.

Note: The fabricated micropipette can be stored for months at room temperature in a dust-free container. If no dust-free storage container is available, micropipettes have to be prepared on the day of the experiment. We recommend having extra on hand in case one is broken.

Note: The micropipette tip can be also bent by using the microforge. In this approach, the filament of the microforge is heated up and progressively brought closer to the micropipette tip until it bends to the required angle ([Backholm and Bäumchen, 2019](#)).

5. Filling the micropipette.

- a. Prepare a solution composed of 70% PBS and 30% FBS (10 mL).

Note: The solution should be supplemented with 30% FBS so as to allow the cell membrane to move smoothly into the micropipette.

- b. Immerse the micropipette tip in the solution, which rises up to fill the tip by capillary action.
- c. Backfill the shank of the micropipette using a microelectrode filling needle and a syringe until a hemisphere droplet is formed at the back end of the micropipette ([Figure 3B](#)).

△ **CRITICAL:** Great care must be taken to ensure that there are no air bubbles in the micropipette. When backfilling the micropipette, make sure that a hemisphere droplet is finally formed at the back end of the micropipette. This will avoid trapping air bubbles at the joint where the micropipette and the pipette holder meet.

Note: The micropipette can also be filled from the back end by gently tapping on its shank to get rid of the air bubbles in the tip.

6. Mount the micropipette onto the pipette holder. [Troubleshooting 3](#)
 - a. Dismount the pipette holder from micromanipulator and take it out from the living cell workstation.
 - b. Position the pipette holder so that its outlet is flush with the liquid level in the water reservoirs.
 - c. Switch the hand valves #1 and #2 to connect the micropipette holder to one of the water reservoirs.
 - d. Loosen the micropipette holder tip and squeeze out a hemispherical water droplet at the holder outlet by slowly lowering the height of the micropipette holder ([Figure 3C](#)).
 - e. Insert the micropipette to the micropipette holder, and tighten the micropipette holder to ensure firm grip and good sealing. Wipe off any excess fluid ([Figure 3D](#)).

△ **CRITICAL:** Make sure that there are no air bubbles trapped during the micropipette installation.

Note: Do not tighten the knob of micropipette holder too much to avoid breaking the micropipette.

7. Remount the micropipette holder onto the micromanipulator.

△ **CRITICAL:** Make sure that the micropipette holder is tightly clamped to reduce fluctuations of the micropipette during the experiment.

△ **CRITICAL:** Control the hand valves #1 and #2 to prevent water from flowing out before remounting the micropipette holder.

Note: Carefully monitor the position of the micropipette in case it is broken during the entire experimental procedure.

Locate the micropipette and calibrate the micropipette aspiration system

⌚ **Timing:** 15 min

Move the micropipette tip into the center of the field of view, and focus on the tip. Then calibrate the micropipette aspiration system to ensure a null-pressure condition when the micropipette tip contacts with the cell surface.

8. Locate the micropipette in the microscope field. [Troubleshooting 4](#)
 - a. Turn on the anti-vibration table.
 - b. Put the culture dish with HeLa cells on the stage of the microscope, and observe using the 10× objective in the phase contrast mode.
 - c. Adjust the angle (40–45 degrees) of the pipette holder to ensure the micropipette tip is positioned approximately parallel to the bottom of the plate.
 - d. Bring the micropipette to a safe distance above the culture dish and into the field of view using the coarse adjustment on the micromanipulator. Then set this reference point as the home position and store this position on the micromanipulator keyboard.

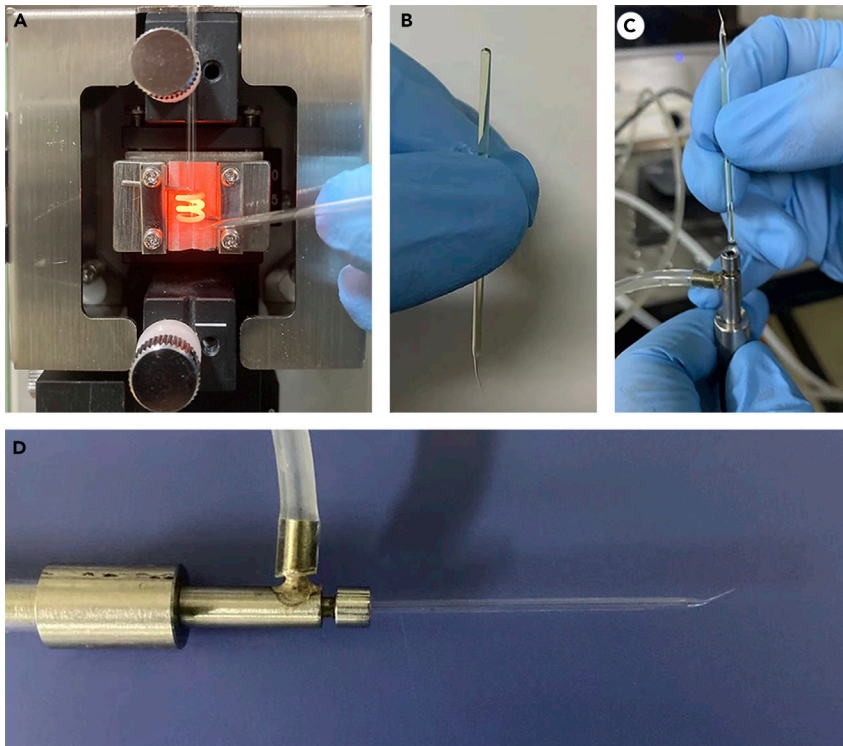


Figure 3. Micropipette fabrication and installation

(A) Heat up the tip of the micropipette with the spiral-shaped heating elements of micropipette puller and bend it to a required angle using another obsolete micropipette.
 (B) Fill the micropipette until a hemisphere droplet is formed at the back end of the micropipette.
 (C) Insert the micropipette to the micropipette holder.
 (D) Assembly of components including micropipette holder, micropipette, and connecting tubing.

- e. Slowly bring down the micropipette until it enters the culture medium.
- f. Use the micromanipulator in fine adjustment mode to lower the tip to a point just above the cell layer (50–100 μm). And then move the micropipette tip to the center of microscope field.

△ CRITICAL: Once the micropipette tip is immersed into the culture medium, use the fine adjustment on the micromanipulator to lower the tip to minimize the potential for breaking it on the bottom of the dish.

- g. Set this reference point as the target position, and store this position on the micromanipulator keyboard.

9. Check the micropipette.

- a. Focus on the micropipette tip by adjusting the 10 \times objective.
- b. Switch the hand valves #1 and #2 to connect a water reservoir #1 to the micropipette.

Note: Make sure that the two water reservoirs are isolated from each other in this inspection.

- c. Apply a pressure (e.g., 1,000 Pa) to the micropipette by raising the water reservoir to check whether the tip is blocked, damaged or poorly polished.

△ CRITICAL: Ensure the pressure is large enough to form a conical-shaped jet at the micropipette tip (Figure 4A) especially when checking whether the tip is damaged or poorly polished. Specifically, the shape of the jet changes if the tip is damaged or poorly polished (Figure 4B).

Note: Alternatively, one can also switch the objective to higher magnification (e.g., 100×) to check whether the micropipette is damaged (right panel in [Figure 4](#)).

d. Return the water reservoir #1 to its original position after the inspection.

10. Calibrating the micropipette aspiration system. [Troubleshooting 6](#)

- a. Add 25 μ L polystyrene microbeads (1 μ m, 5% w/v; NanoMicro Tech., China) diluted 1 : 400 in filtered sterilized water to the culture dish.
- b. Carefully move and position the micropipette tip next to a suspended microbead.
- c. Switch hand valve #1 to connect the water reservoir #2 to both water reservoir #1 and micropipette.

Note: Make sure that the two water reservoirs connect to each other during calibrating the micropipette aspiration system.

- d. Adjust the height of the water reservoir #1 so that this microbead does not move toward or away from the micropipette tip.
- e. Switch the hand valve #1 so that only the water reservoir #2 connects to the micropipette after calibrating the micropipette aspiration system.

△ CRITICAL: During this calibration process, care should be taken that no microbeads stick to the walls of the micropipette. If the microbeads stick to the walls of the micropipette, connect the 50 mL syringe to the micropipette by switching the hand valve #2, and then gently pump the syringe to flush out the microbeads.

△ CRITICAL: Remember to switch the hand valve #1 to disconnect the two water reservoirs and to allow water only flow from the water reservoir #2 to the micropipette once the micropipette aspiration system has been calibrated. Forgetting this operation will directly lead to errors in calculating aspiration pressure.

Note: Calibrate the micropipette aspiration system before each experiment.

Measure the Laplace pressure of HeLa cells undergoing cytokinesis in media with different osmolarities using micropipette aspiration experiment

⌚ **Timing:** 1 day

Determine the Laplace pressure of cytokinetic cells incubated in hypotonic, isotonic or hypertonic medium by virtue of the micropipette aspiration experiment, the image and data analysis, as well as the medium replacement device.

11. Prepare the experimental media with different osmolarities.

- a. Determine the mass of sucrose for the sucrose solutions with different osmolarities according to the equations: mass of solute = molar mass \times moles of solute, and osmolarity = molarity \times n , where molarity = moles of solute / liter of solution, the van't Hoff factor $n = 1$ for sucrose. For example, to prepare 1L of sucrose solution with osmolarity of 300 mOsm/L, we need dissolve 102.69 g (342.3×0.3) of sucrose in water.
- b. Weigh out sucrose of required mass into the beaker, then pour 500 mL of deionized water into the beaker and dissolve sucrose using magnetic stirrer.
- c. Transfer the sucrose solution into a 1L graduated cylinder, and then fill the graduated cylinder to the 1L mark with deionized water.
- d. Pour the solution back into the beaker followed by full agitation, and then store the sucrose solution for use.

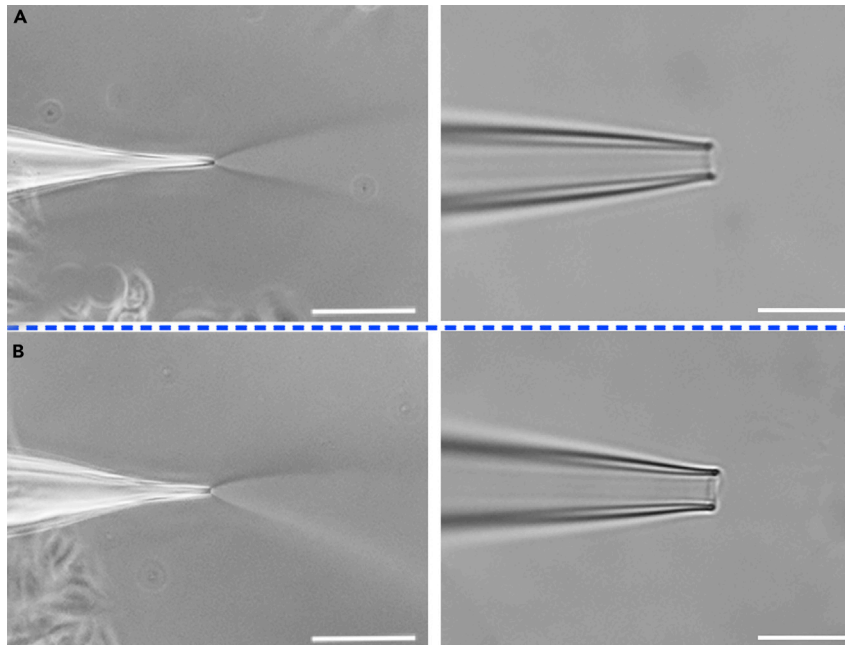


Figure 4. Check the micropipette

Check the micropipette by directly observing the shape of the jet at the micropipette tip (left) and by closely scrutinizing with higher-magnification objective (right). Here, it shows a finely and a poorly polished micropipette tip in (A) and (B), respectively.

Scale bars, 100 μm (left), 10 μm (right).

- e. Repeat the steps 11a - 11d to prepare sucrose solutions with osmolarities of 120, 210, 300, 390, and 480 mOsm/L.
 - f. Mix the sucrose solutions with the isotonic culture medium (300 mOsm/L) in a ratio of 2 : 1 to establish 180, 240, 300, 360, and 420 mOsm/L media, wherein the osmolarities of the mixture is calculated by $(\text{osmolarity of sucrose solution} \times 2 + \text{osmolarity of isotonic culture medium}) / 3$.
12. Perform the micropipette aspiration experiment. [Troubleshooting 4, 5, 6, 7, and 8](#)
- a. Start up the displacement measurement system equipped with a laser displacement sensor, a data acquisition card, and a computer.
 - b. Shift the micropipette to the home position using the micromanipulator.
 - c. Place a new culture dish with HeLa cells on the microscope stage.
 - d. Cover the culture dish with the cut lid of medium replacement device.

Note: Put the cut lid in the right position, as shown in [Figure 2C](#), to avoid the lid-micropipette contact and to ensure the adequate space for subsequent micropipette aspiration experiments.

- e. Carefully move the microscope stage to find a round-shaped (M phase) HeLa cell with visible chromosomes on the equatorial plate. Then place it in the center of the visual field.

△ CRITICAL: In the process of moving the microscope state to find the desired cells, care the culture dish not to contact the micropipette tip.

Note: Do not select the cells close to the blunt tips of syringe needles inserted in the cut lid.

- f. Position the micropipette to the target position using the micromanipulator.
- g. Switch to the 100× oil-immersion objective.

Note: Add a drop of immersion oil on the 100× objective before switching.

- h. Slowly move the micropipette until its tip gently touches the cell membrane.
- i. When this cell starts forming cleavage furrow and enters into cytokinesis, grab the cell by applying suction with the water reservoir #2.

△ CRITICAL: Gently adjust the position of the water reservoir during the micropipette aspiration experiment.

Note: The initially applied pressure for grabbing cell depends strongly on the cell type. For HeLa cells studied here, 10–15 mm of water pressure is a good choice.

- j. Increase the suction pressure stepwise by changing the position of the water reservoir #2 until it reaches a critical value P_c , at which the aspirated part of the cell forms a hemisphere with the radius of the micropipette tip (Methods video S2). Start imaging and record the displacement of the water reservoir #2.

Note: Start imaging and record the displacement at the same time.

- k. Fill the syringe #1 of the medium replacement device with prewarmed hypotonic, isotonic or hypertonic media (37°C) as required.

Note: Prewarm the hypotonic and hypertonic media to 37°C in a water bath kettle before using.

- l. Pump out the original media with syringe #2, then inject the hypotonic, isotonic or hypertonic media with syringe #1 as required. Keep adjusting the suction pressure to make the aspirated part of the cell in the micropipette to be roughly hemispherical in shape during exchanging media and throughout cytokinesis (Figures 5A–5C).

△ CRITICAL: Rapidly exchange media within, e.g., 20 s.

- m. Release the cell when the contractile-ring contraction completes, and shift the micropipette to the home position.
- n. Repeat the steps c–m as many times as required for other cells undergoing cytokinesis.

△ CRITICAL: The cell culture dish needs to be replaced every time the micropipette aspiration experiment is repeated. Remember to replace the original medium in the syringe #1 with fresh, prewarmed medium as well.

- o. After the experiment, switch the hand valve #1 to disconnect the two water reservoirs from the micropipette, then unmount and empty the micropipette.

13. Calculate the cortical tension and the Laplace pressure.
 - a. Open images using ImageJ, an open source image analysis software.
 - b. Set the scale to micrometers using the Set Scale command from the main menu.

Note: Do not skip step b.

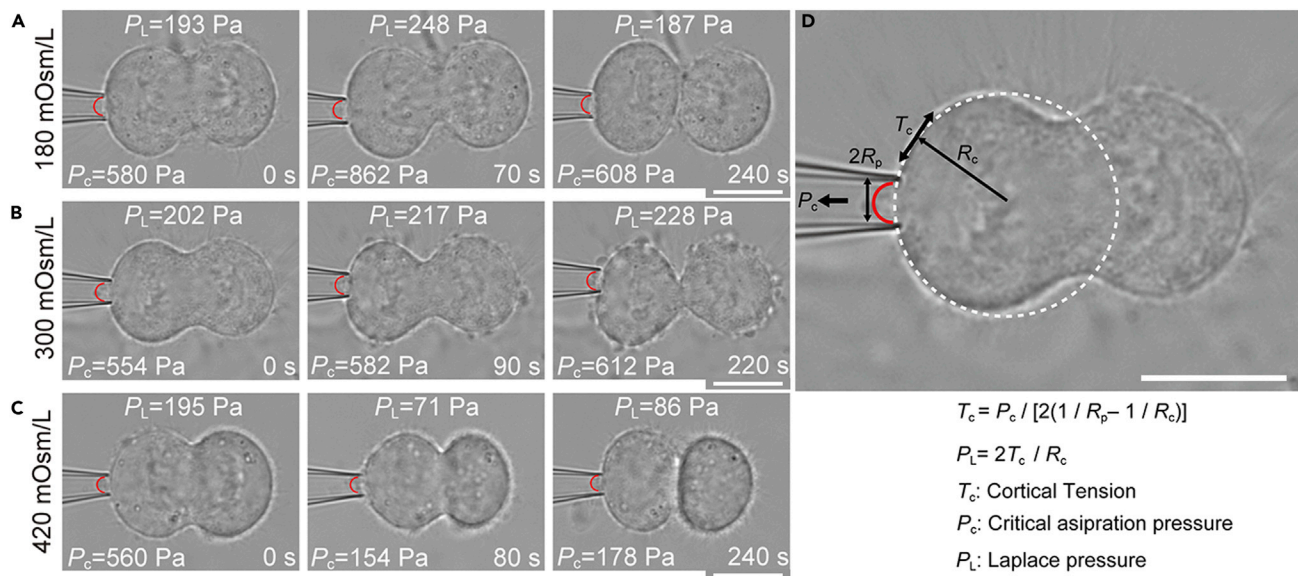


Figure 5. Measuring the Laplace pressure of HeLa cells during cytokinesis

(A–D) Measurement of the dynamic Laplace pressure with micropipette aspiration system for HeLa cells undergoing cytokinesis in hypotonic (A, 180 mOsm/L), isotonic (B, 300 mOsm/L) and hypertonic (C, 420 mOsm/L) media. The parameters used in the text and the formula used to calculate the cortical tension and the Laplace pressure are given in (D). Scale bars, 10 μ m.

- c. Measure the radius R_p of the micropipette and the radius R_c of curvature of the interface in contact with micropipette, as shown in Figure 5D.
- d. Retrieve the displacement data of water reservoir #2, and calculate the critical pressure P_c corresponding to each image processed above with ImageJ.

Note: Make sure the points in time for both the processed images and retrieved displacement data are the same.

- e. Calculate the cortical tension from the Laplace equation $T_c = P_c / [2(1 / R_p - 1 / R_c)]$ with the measured parameters (Maître et al., 2015; Tinevez et al., 2009).
- f. Calculate the Laplace pressure of the cell undergoing cytokinesis according to the equation $P_L = 2 T_c / R_c$.

Image actin cortex and analyze cortex density distribution for cells undergoing cytokinesis in media with different osmolarities

⌚ Timing: 2–3 days

Transfect HeLa cells with Lifeact-GFP plasmid for further imaging actin cortex and analyzing the density distribution of the cortex of cytokinetic cells incubated in hypotonic, isotonic or hypertonic medium.

14. Cell seeding and plasmid transfection. Troubleshooting 9, 10, 11, and 12
 - a. Seed 5×10^5 – 6×10^5 HeLa cells per glass-bottom Petri dish (35 mm) in 2 mL of complete growth medium. Incubate cells overnight in a humidified 37°C, 5% CO₂ incubator.

⚠ **CRITICAL:** The optimal cell density, an important factor influencing transfection efficiency, varies for different cell types. For HeLa cells, generally 70–90% confluency provides good results.

Note: It will be detrimental to the transfection efficiency if the HeLa cells seeded in dishes are cultured more than 24 h before transfection.

- b. Prepare mixture composed of the 125 μL Opti-MEM® medium, 2.5 μg Lifeact-GFP plasmid, 4 μL Lipo8000™ transfection reagent according to the manufacturer's instructions.

△ CRITICAL: In order to achieve the best transfection efficiency, the amount of reagents and plasmid used should be tested (Jiang et al., 2021).

- c. Transfect the cells with mixture prepared in step b.
 - d. Culture cells 24–36 h after transfection. Use the fluorescence microscope to directly verify whether the transfection efficiency of HeLa cell meets the experimental requirement.
15. Image the actin cortex for cells undergoing cytokinesis in media with different osmolarities (Figure 6A–6C). [Troubleshooting 13](#)
- a. Repeat the process 6 to rinse the transfected HeLa cells 2–3 times with prewarmed PBS to wash away nonadherent cells and debris, and then add fresh complete culture medium and incubate at 37°C (5% CO₂) for 2–3 h.
 - b. Repeat the steps 15c - 15e to find a round-shaped (M phase) transfected HeLa cell using the 10× objective and place it in the center of the visual field.
 - c. Switch to the 100× oil-immersion objective.
 - d. When this cell begins to form cleavage furrow and enters into cytokinesis, start imaging in bright field, green channel.

Note: Regulate the exposure time and the light intensity to reduce the decrease in the fluorescence intensity due to quenching.

- e. Repeat the steps 15k - 15l to exchange media.
 - f. Stop imaging when the contractile-ring contraction completes.
 - g. Repeat the steps a - f many times as required for other cytokinetic cells incubated in new dishes.
16. Image processing and analysis.
- a. Repeat steps 16a - b to open fluorescent images using ImageJ.
 - b. Select the Freehand Line (line width 10 pixels) and trace HeLa cell contour.
 - c. Go to Analyze → Plot Profile from the main menu to quantify the intensity distribution along the cell contour.
 - d. Go to Data → Save Data from the plot to save the experiment data for further analysis using ORIGINPRO® 2021b.

EXPECTED OUTCOMES

We provide here a detailed, step-by-step protocol, which has enabled us to describe the dynamic, quantitative variations in the Laplace pressure during cytokinesis and to relate this variability to physiological influences. By optimizing the micropipette aspiration system, we realize the dynamic, quantitative measurement of the Laplace pressure during cytokinesis. In order to regulate the Laplace pressure and study the cellular response to the change of the Laplace pressure, we design a medium replacement device by which the cytokinetic HeLa cells can endure the hypotonic and hypertonic osmotic shocks by controlling the extracellular osmolarities. Further, we take the actin cortex as an example to discuss the effect of the Laplace pressure on the cellular behavior, and combine the live-cell imaging and the designed medium replacement device to display the density distributions of the actin cortex of the plasmid transfected HeLa cells undergoing cytokinesis in hypotonic, isotonic or hypertonic media. Based on the quantitative measurement of the Laplace pressure of HeLa cells undergoing cytokinesis in the media with different

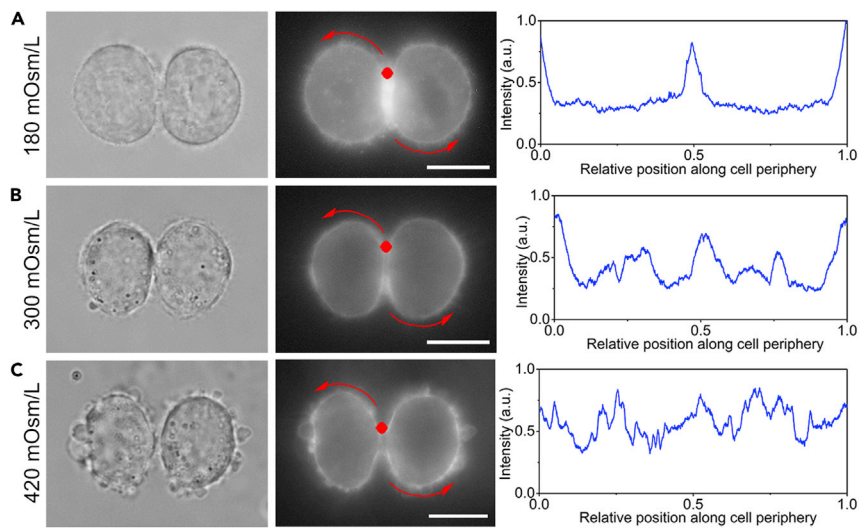


Figure 6. Changes in actin cortex distribution in response to the change in the Laplace pressure

(A–C) Experimental results showing the actin cortex for HeLa cells incubated in hypotonic (A, 180 mOsm/L), isotonic (B, 300 mOsm/L) and hypertonic (C, 420 mOsm/L) media when cytokinesis finishes. The Figures are arranged in three columns, from left to right: (i) bright field images; (ii) fluorescence images of actin in Lifeact-GFP transfected cells; (iii) actin cortex density distributions measured counterclockwise from the central furrow (red dot). a.u., arbitrary units. Scale bars, 10 μm .

osmolarities, the dynamic response of actin cortex to the change in the Laplace pressure is then established.

This protocol can be utilized to measure the dynamic Laplace pressure in other cell cycle phases during mitosis for different cell lines and extended to address further research questions regarding the intrinsic relation between the Laplace pressure and other cellular activities in combination with other technologies as required.

LIMITATIONS

In this protocol, we adopt a commonly used method to control suction pressure in micropipette aspiration systems through manually adjusting the height of a water reservoir. In order to measure the Laplace pressure for cells evolving from cleavage furrow emergence to contractile-ring contraction accomplishment, one has to keep adjusting the suction pressure to make the aspirated part of the HeLa cell in the micropipette to be roughly hemispherical in shape throughout cytokinesis. It is very difficult for an inexperienced user to accomplish such an operation. Even for a skilled operator, operation failure often occurs as well. In addition, it will easily result in the cell rupture and the failure of the experiment even only a few sharp edges are left on the micropipette tip, which contacts with cell membrane throughout the cytokinesis. Therefore, the micropipette tips must be finely polished with stringent quality.

TROUBLESHOOTING

Problem 1

The tip of micropipette is too long or too short (step 4).

Potential solution

If the micropipette tip needs to be shorter, make the weight heavier, or decrease the heater value. On the contrary, if the micropipette tip needs to be longer, make the weight lighter, or increase the heater value.

Problem 2

The tip of micropipette is closed (step 4).

Potential solution

Cut the end of the pulled micropipette by placing it against a hot metal wire in microforge.

Problem 3

The air bubbles are trapped in the micropipette after mounting it onto the pipette holder (step 6).

Potential solution

Gently pump the syringe (50 mL) to dislodge any visible air bubbles in the micropipette.

Problem 4

The tip of the micropipette vibrates during the micropipette aspiration experiment (steps 8 and 12).

Potential solution

Turn on the anti-vibration table.

Problem 5

The laser displacement sensor is unavailable (step 12).

Potential solution

The real-time displacement of the water reservoir can also be recorded using a digital height gauge that allows measurements to be sent to a data collector. If the function of sending data is unavailable, the digital height gauge can be equipped with a camera instead.

Problem 6

The micropipette tip is blocked by debris floating in the media even before the micropipette aspiration experiment (steps 10 and 12).

Potential solution

On the one hand, repeatedly insert the micropipette through the air-media interface to remove the debris stuck on the micropipette tip. On the other hand, temporarily apply a positive pressure inside the micropipette by syringe to push debris back out of the tip and into the media. Additionally, to prevent blockage with debris, carefully rinse each dish of cells with PBS to remove the debris during cell preparation, and apply a slight positive pressure by the water reservoir before immersing the micropipette in the process of calibrating the micropipette aspiration system.

Problem 7

The micropipette tip is always above the cell and cannot be placed in contact with the cell when positioning it to the cell using the micromanipulator (step 12).

Potential solution

Gently adjust the micropipette holder clamp to increase the angle of the micropipette relative to the microscope stage.

Problem 8

One is unable to grab the cell using micropipette (step 12).

Potential solution

This can attribute to different reasons. On the one hand, gently increase the suction pressure. On the other hand, check whether the micropipette tip is clogged by debris or closed. If so, refer to the Potential solution of Problems 2 and 5.

Problem 9

The Opti-MEM® medium is unavailable (step 14).

Potential solution

The serum-free and antibiotic-free DMEM medium can be used as an alternative.

Problem 10

There is no Lipo8000™ transfection reagent available (step 14).

Potential solution

The alternative transfection reagents such as Lipofectamine 3000 (Invitrogen™, Cat#L3000015) or Entranster™-H4000 (Engreen, Cat#4000-5) can be used as a candidate.

Problem 11

The transfection efficiency of HeLa cell is low (step 14).

Potential solution

First, make sure that the cells are cultured in dishes no more than 24 h before transfection. Second, optimize the cell density so that the HeLa cells are 70–90% confluent on the day of transfection. Finally, optimize the ratio and the amount of reagents and plasmid.

Problem 12

The cell viability decreases after transfection (step 14).

Potential solution

Adjust the amount of reagents used or reduce the incubation time of cells with transfection mixture.

Problem 13

The fluorescence intensity decreases during the imaging experiment due to fluorescence quenching (step 15).

Potential solution

Optimize the exposure time and the light intensity, or increase the time interval between image frames.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Fan Song (songf@lnm.imech.ac.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate unique code.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xpro.2022.101239>.

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AUTHOR CONTRIBUTIONS

Conceptualization, L.L. and F.S.; Investigation, X.H.W. and L.L.; Writing—Original Draft, X.H.W. and L.L.; Writing—Review & Editing, L.L. and F.S.; Supervision and Funding Acquisition, L.L. and F.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Backholm, M., and Bäumchen, O. (2019). Micropipette force sensors for in vivo force measurements on single cells and multicellular microorganisms. *Nat. Protoc.* 14, 594–615.

Jiang, P., Ren, L., Zhi, L., Hu, X., and Xiao, R.P. (2021). Protocol for cell preparation and gene delivery in HEK293T and C2C12 cells. *STAR Protoc.* 2, 100497.

Maire, J.L., Niwayama, R., Turlier, H., Nédélec, F., and Hiragi, T. (2015). Pulsatile cell-autonomous contractility drives compaction in the mouse embryo. *Nat. Cell Biol.* 17, 849–855.

Tinevez, J.Y., Schulze, U., Salbreux, G., Roensch, J., Joanny, J.F., and Paluch, E. (2009). Role of cortical

tension in bleb growth. *Proc. Natl. Acad. Sci. U S A* 106, 18581–18586.

Wang, X., Li, L., Shao, Y., Wei, J., Song, R., Zheng, S., Li, Y., and Song, F. (2021). Effects of the Laplace pressure on the cells during cytokinesis. *iScience* 24, 102945.