Video Article

Microfabricated Post-Array-Detectors (mPADs): an Approach to Isolate Mechanical Forces

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Abstract

In this video, we will present our approach to measure cellular traction forces using a microfabricated array of posts. Traction forces are generated through myosin-actin interactions and play an important role in our physiology. During development, they enable cells to move from one location to the next in order to form the early structures of tissue. Traction forces help in the healing processes. They are necessary for the proper closure of wounds or the migration and crawling of leukocytes through our body. These same forces can be detrimental to our health in the case of cancer metastasis or vascular growth towards a tumor. The most common method by which to study cells in vitro has been to use a glass or polystyrene dish. However, the rigidity of the substrates makes it impossible to physically measure cell traction forces, and there are relatively few methods to study traction forces. Our lab has developed a technique to overcome these limitations. The method is based on a vertical array of flexible cantilevers, the stiffness and size scale of which are such that individual cells spread across many cantilevers and deflect them in the process. The pillars we use are 3 μm in diameter, 10 μm tall, and are configured in a regular array with 9 μm center-to-center spacing. But these physical dimensions can be readily varied to accommodate a variety of studies. We start with a silicon master, but the final posts are made out of silicone rubber called poly (dimethyl siloxane), or PDMS. We can measure the deflections under a microscope and calculate the magnitude and direction of traction forces required to produce the observed deflections. We call these substrates microfabricated post-array-detectors, or mPADs. Here, we will show you how we fabricate and use the mPADs to assess modulations of cellular contractility.

Video Link

The video component of this article can be found at https://www.jove.com/video/311/

Protocol

Silicon Master Mold Fabrication
Lithography
Materials:

- 75 mm Silicon wafers
- SU-8 2 Negative Photoresist (Microchem, Boston, MA)
- N2

Method:

1. Dehydrate Si wafer at 120 °C for 2 hours.
2. UV ozone treat surface for 7 minutes to remove organics.
3. N2 blow surface to remove particulates.
4. Apply SU-8 2 to cover 70% of surface (~10 ml for 75 mm wafer).
5. Spin at 2000 rpm for 20 seconds with 300 rpm/s acceleration.
6. Place the wafer on a hotplate and ramp from room temperature to 95 °C.
7. Softbake the photoresist for 15 minutes at 95 °C.
8. Flood expose wafer for 20 - 25 mJ/cm2 dose at 365 nm (I-line) to form the bottom layer.
9. Apply SU-8 2 for second photoresist layer.
10. Spin at 550 rpm for 20 seconds with 300 rpm/s acceleration for an approximately 11 μm thickness film.
11. Place the wafer on a hotplate and ramp from room temperature to 65 °C.
12. Softbake the wafer at 65 °C for 5 minutes.

13. Ramp the hotplate from 65 °C to 95 °C.
14. Softbake the wafer for 55 minutes at 95 °C.
15. Cool wafer to room temperature for 20 minutes.
16. Align mPAD mask on wafer and expose for 95 - 100 mJ/cm2 dose at 365 nm (I-line).
17. Place the wafer on a hotplate and ramp from room temperature to 95 °C.
18. Post-exposure bake the photoresist for 25 minutes at 95 °C.

Develop
Materials:

- 100mm glass dishes (5)
- Propylene glycol methyl ether acetate (PGMEA)
- Isopropanol (IPA)
- N2

Method:

1. In a chemical hood, fill five dishes with PGMEA (2), IPA, and Hexane (2).
2. Hold wafer in 1st PGMEA for 10 seconds
3. Agitate wafer for 50 seconds
4. Transfer wafer to 2nd PGMEA with meniscus of PGMEA.
5. Agitate for 5 seconds.
6. Transfer wafer to IPA with meniscus of PGMEA.
7. Agitate for 20 seconds.
8. Transfer wafer to 1st IPA.
9. Agitate for 5 seconds.
10. Transfer wafer to 2nd IPA.
11. Agitate for 5 seconds.
12. Quickly remove wafer from 2nd IPA with meniscus of IPA and dry with N2
13. Inspect pattern.
14. Hardbake wafer at 120 °C for 14-20 hours to cross-link SU-8.
15. Dice the wafer with a diamond cutter
16. Mount the wafer on a glass slide using epoxy
17. Fluorosilanize the wafer (see instructions in replication molding)

Replication molding of mPADS
Negative Mold
Materials:

- Disposable aluminum weighing boat
- Polydimethylsiloxane (PDMS) base & curing agent (Dow, Midland, MI)
- Razorblade
- Silane: (tridecafluoro-1,1,2,2-tetrahyrooctyl)-1-trichlorosilane
- Glass slide
- Glass Pasteur Pipette

Method:

1. Mix PDMS 10:1 by weight and degas in under vacuum for 1 hour to remove bubbles.
2. Place silicon master mold in aluminum boat.
3. Pour 50 – 60 g of PDMS over master mold in aluminum boat.
4. Degas under vacuum for 30 minutes to remove bubbles.
5. Blow off surface-trapped air bubbles in PDMS with quick blast of N2.
6. Place boat in convection oven at 110 °C for 15 minutes until PDMS is firm.
7. Cut away aluminum boat.
8. With razorblade, trim away PDMS underneath silicon master.
9. Peel PDMS away from silicon master slowly and with constant motion in one direction to avoid destroying the master.
10. Inspect silicon master for damage.
11. Cut negative mold into 4 pieces.
12. Repeat steps 1-10 for a large batch of negative molds.
13. Oxygen plasma treat negative mold for 1.5 minutes to activate surface, or
14. Ozone treat negative mold for 10 minutes to activate the surface.
15. Place negative molds in dessicator with mPAD features exposed.
16. Spread 250-500 µL of silane on glass slide in center of dessicator
17. Place glass pasture pipette with 150-200 µL of silane in the tip inside dessicator.

18. Vacuum chamber to evaporate silane and coat negative molds for >14 hours.

mPADS
Materials:

- Polydimethylsiloxane (PDMS) base & curing agent (Dow, Midland, MI)
- Wide-mouth disposable transfer pipettes
- 2” x 3” Glass slides
- Glass Scribe
- N2

Method:

1. Mix PDMS 10:1 by weight and degas in under vacuum for 1 hour to remove bubbles.
2. lace silanized negative molds into ‘cross’
3. Pipette PDMS into negative molds so that the features are fully covered to a 1mm thickness.
4. Wait 30 minutes for any air bubbles in the PDMS to rise to the surface.
5. In 30’ downtime, dust 22x22mm (No. 2) glass coverslips with a stream of N2.
6. Oxygen plasma treat glass slides halves for 1.5 minutes to clean and activate glass
7. Blow off surface-trapped air bubbles in PDMS with quick blast of N2.
8. Gently lay edge of glass coverslip on PDMS with plasma cleaned side facing the PDMS. Slowly lower the glass slide so that the PDMS fully contacts the entire glass surface and avoid air bubbles getting trapped underneath the glass slide.
9. Place assembled molds in convection oven at 110 °C for 20 hours to fully cure.
10. Remove molds from oven and let them cool to RT.
11. Hold glass slide with one hand on top and slowly peel negative away the negative mold from the bottom to release the mPAD.
12. Inspect the mPAD under a microscope for collapsed posts.
13. Re-silanize mold via plasma and silane every four castings. The negative mold lifetime for good replication is approximately 6-20 casts.

Seeding Cells onto mPADS
Stamping
Materials:

- PDMS stamps for uCP (see Tien, J & Chen, C. S., in Methods of Tissue Engineering, 2001, pp. 113-120.)
- Ethanol (100% and 70%)
- Sterilized Deionized Water
- 50 ug/ml Human Fibronectin in sterile deionized water (BD Biosciences)
- 5 ug/ml DiI Solution filtered with 0.2 µm membrane (D-3886, Molecular Probes, OR)
- 2% Pluronics F127 (BASF, Mount Olive, NJ)
- 1X Phosphate Buffer Solution.
- 100 mm x 25 mm petri dish (4)
- 150 mm x 25 mm petri dish (2)
- Aluminum foil
- N2
- Tweezers
- Sterile Hood

Method:

1. Trim excess PDMS from mPADs
2. If the PDMS stamp has been used previously, sonicate stamps in 100% ethanol for 5 minutes to scrub away contaminating proteins.
3. In sterile hood, hold stamps on their sides with tweezers and dry with N2 after dipping stamps in fresh 100% ethanol and sterile water
4. Place in 150 mm petri dish with working side up.
5. Aliquot drops of fibronectin solution on each stamp. Start with the corners on each of the stamps, then the edges, and lastly the interior regions. Initially the PDMS surface of the stamp is hydrophobic, but becomes hydrophilic as the protein is adsorbed. Working “outside to inside” changes the contact angle of the PDMS so that a lower volume of fibronectin solution is needed to fully coat the stamp.
6. Let sit for 1 hour in the hood for protein adsorption.
7. Wash stamps with DI water by pouring in dish and letting the water level rise over the stamps.
8. Wash stamps in 100 mm petri dish filled with DI water.
9. Remove and dry with N2.
10. Ozone treat mPADS for 7 minutes to activate the surface for stamping.
11. Under hood, stamp mPADS by placing stamp edge on mPAD and gently lowering to make full contact. Lightly tap the top of the stamp with tweezers to ensure all areas have good contact but be careful not collapse the posts.
12. Carefully remove stamps with tweezers.
13. Submerge mPADS in 100 mm petri dish with 100% Ethanol for ~15 seconds. Keep microneedles from dewetting during transfer by transferring quickly between dishes.
14. Submerge mPADS in 100 mm petri dish with 70% Ethanol for ~15 seconds.

15. Submerge mPADS in 100 mm petri dish with DI water for ~15 seconds.
16. Submerge mPADS in second 100 mm petri dish with DI water for ~15 seconds.
17. Submerge mPADS in 150 mm petri dish with DI water.
18. Submerge mPADS in 150 mm petri dish with 100 mL of DiI solution.
19. Cover with aluminum foil and soak for 1 hour.
20. Submerge mPADS in 100 mm petri dish with DI water for ~15 seconds.
21. Submerge mPADS in 150 mm petri dish with 10 mL of 2% Pluronics and 90 mL of PBS for total concentration of 0.2% Pluronics.
22. Cover and soak for 1 hour.
23. Submerge mPADS in 100 mm petri dish with DI water for ~15 seconds.
24. Submerge mPADS in second 100 mm petri dish with DI water for ~15 seconds.
25. Submerge mPADS in third 100 mm petri dish with PBS for ~15 seconds.

Seeding
Materials:

- Chosen cell line
- Trypsin-EDTA (0.25% Trypsin with EDTA•4Na, Gibco Cat. No. 25200-056)
- Growth Medium
- 1X Phosphate Buffer Solution.

Method:

1. In sterile hood, place mPADS in 100 mm petri dish and fill with 17 mL medium.
2. Place petri dish in incubator to warm to 37°C
3. Suspend cells via trypsin
4. Aspirate repeatedly to break up cell clusters.
5. Pipet cell suspension into the petri dish with the mPADS. Add cells such they will remain single cells (without cell-cell contacts) at time of analysis. The exact amount of cells to add is cell-type specific and depends on cell spread area and growth rate.
6. Gently disperse cells in petri dish with slow pippetting.
7. Place in incubator for 2 hours to allow cells to settle and adhere onto microneedles.
8. Inspect mPADS to see if cells have spread. The cells will look flattened and span six or more posts.
9. If cells are spread, transfer mPADS to new 100 mm petri dish with 21 mL of fresh medium.
10. The mPADS are ready for your experiment.

Staining and Mounting mPADS
Fixing
Materials:

- Deionized water
- Paraformeldahyde (16%) vial
- 10X Phosphate Buffer Solution.
- 1X Phosphate Buffer Solution.

Method:

1. In a 50mL centrifuge tube, add 30 mL of DI, 10 mL of paraformeldahyde (entire vial), and 4.5 mL of 10X PBS for the fixing solution.
2. Add ~25 mL of fixing solution to a 100 mm petri dish.
3. Gently submerge mPAD with cells into fixing dish and let incubate for 20 minutes.
4. Submerge mPADS in 100 mm dish with 1X PBS three times.
5. Ready for immunostaining and mounting.

Analyzing Cellular Forces on mPADS
Confocal Imaging
Materials:

- Confocal Microscope
- 63X Objective
- Filter cubes for secondary antibodies
- Green filter cube (Rhodamine) for DiI imaging of microneedles.

Method:

1. Place mPADs on stage and find single cells with 300 ms exposure, no binning.
2. Image tops of microneedles for TOP IMAGE.

3. Using z-control readout, capture image of microneedles for BOTTOM IMAGE.
4. Image any other channels you have stains in (Hoechst, phalloidin etc.)

Image Analysis
Materials:

- MATLAB with Image Processing Toolbox

Method:

1. Rotate and register top and bottom images
2. Measure centroids of bottom (undeflected) and top (deflected) positions
3. Calculate the displacement
4. Using the spring constant for the posts (known, depends on post geometry), calculate force vector at each post
5. Error analysis is based on the ability to resolve forces, and depends on the image resolution and centroid detection resolution
6. Arrange the data as per your customized needs – e.g., force magnitudes and/or direction, as a time course, many measurements across different conditions etc.

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