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Micropost Force Sensor Array (MFSA) for Measuring Cell Traction Forces

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1 Introduction

Cell traction forces (CTFs) enable cell motility and maintenance of cell shape and therefore play an essential role in many biological processes such as angiogenesis, embryogenesis, inflammation, and wound healing. To determine CTFs at the sub-cellular level with high sensitivity, we developed a micropost force sensor array (MFSA) technology for measuring traction forces of single cells.

2 Materials and Methods

Fabrication of MFSA. The MFSA was fabricated by replica molding technique using a silicon wafer mold to cast PDMS. A 10:1 (w/w) mixture of Sylgard 184 (Dow Corning) PDMS prepolymer was poured onto the wafer, cured at 65 °C for 24 hr, and peeled off.

Finite element method (FEM) analysis. FEM was used to determine the deflection–force relationship of the microposts. The micropost was meshed with an element size of 0.5 μm. The bottom of the micropost was fixed, and a point force was applied to the top (**Fig. 1A**). Finally, the deflections of the micropost's top corresponding to different point force magnitudes were computed. The calculated deflections vs. forces were fit with a polynomial function (**Fig. 1B**).

Image acquisition and analysis. First, a set of images of microposts were captured using phase contrast microscopy (**Fig. 2**). By focusing at either the top or the bottom of microposts, two images were obtained, representing the locations of the top and the bottom of microposts, respectively. These two images are termed the *"force-loaded"* image and the *"force-independent"* image, respectively. A fluorescence image of a cell was recorded to define the cell region. A novel image acquisition and processing approach was developed by MATLAB coding to determine the displacements/deflections of individual microposts.

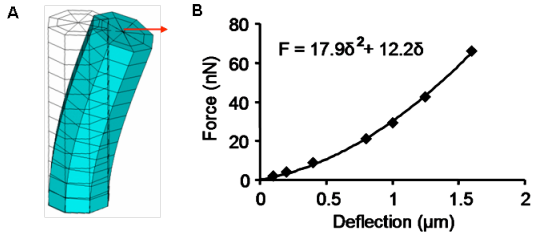

Figure 1 : A. Schematic of FEM model for a micropost with a lateral force exerted on its top; and **B.** Relationship between micropost deflection and lateral force, as determined by FEM.

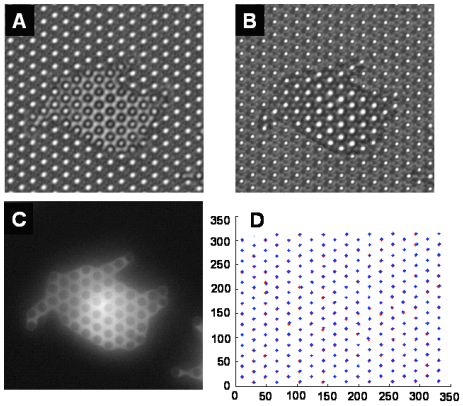

Figure 2 : "*Top-Bottom*" method for CTF measurement. **A. "Force-loaded" image; B. Force-independent image; C.** Fluorescence image of cell; and **D.** Overlaid centroids.

Culture experiments with MFSA. Smooth muscle cells (SMCs) from rat aorta were used in this study. The MFSAs were first treated with air plasma for 4 min and subsequently coated with 50 μg/ml human plasma fibronectin for 1 hr before cells were seeded. Cells were maintained in DMEM containing 10%

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FBS and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 atmosphere.

3 Results

Morphology and geometry of MFSAs. High quality MFSAs were obtained in this study (**Fig. 3A)**. The MFSAs consisted of an array of vertically standing PDMS microposts, 2 μm in diameter and 6 μm in height, with a center-to-center distance of 4 μm. Because of the physical characteristics, the microposts behave as individual waveguides and thus result in high contrast images with phase contrast microscopy (**Fig. 3B)**.

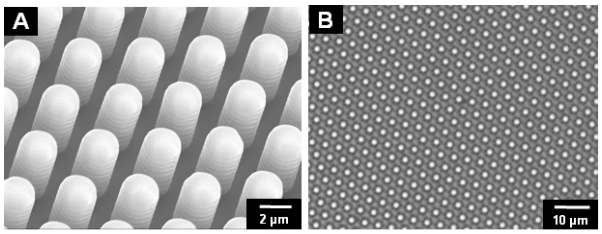

Figure 3 : A. SEM image; and **B.** Phase contrast microscopy image of a MFSA.

Resolution of deflection and force sensitivity. ***"Top-Bottom"*** method was developed to determine the displacements of microposts (**Fig. 3**). A spatial resolution of 70 nm has been achieved for the determination of micropost displacement. According to the FEM modeling results, this corresponds to force sensitivities of 0.9 nN.

Cell adhering on MFSA. Cells attached to and spread on the MFSA's top surface. The cells on the MFSA developed numerous stress fibers and focal adhesions that were formed exclusively at the contact sites between the cell and microposts (**Fig. 4A**). Detailed morphology of a spread cell on top of MFSAs was observed with SEM (**Fig. 4B**). Apparently, the cell established close contact with the microposts underneath and applied contractile force on the microposts. As a result, these microposts were bent, and the extent of bending (lateral deflection) at each micropost reflected the magnitude of CTF applied by the cell.

Measuring CTFs using MFSA. In a typical CTF map (**Fig. 5**), the displacement vectors as well as the cell boundary are superimposed on the top image of the MFSA to give a clear view of the direction and magnitude of CTFs. Apparently, the majority of CTFs applied on the microposts range from 1 to 4 nN, with some as high as 8 nN at certain positions close to the edge. All CTFs are centripetal.

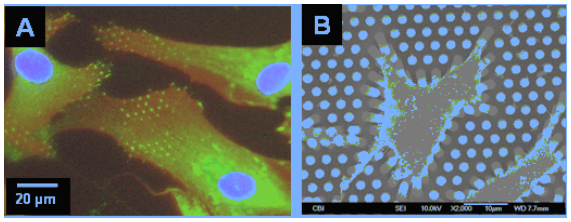

Figure 4 : A. Fluorescence microscopy image of HSFs cultured on a MFSA. Red, green, and blue colors represent actin filaments, focal contacts, and nuclei, respectively; and **B.** SEM image of rat aorta SMCs adhering to top of a MFSA.

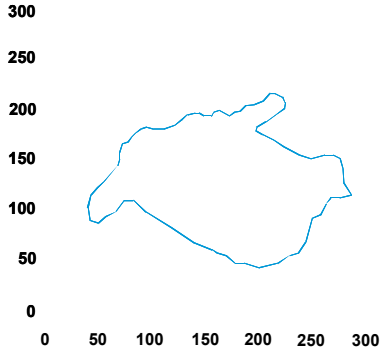

Figure 5 : A map of CTFs determined using "***Top-Bottom"*** method for a rat aorta SMC.

4 Conclusion

We have developed a MFSA technology, including high density micropost array, novel image acquisition and analysis method for determination of displacement of microposts, and non-linear micropost displacement/deflection-force relationship for determination of CTFs. The MFSA can achieve a spatial resolution of 70 nm and a force sensitivity of 0.9 nN. As such, this technology will be a useful tool for a variety of biological applications, including studying the effect of cell shape and cytokines on CTFs and using CTF as a "biophysical marker" to detect cancerous cells.

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