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Cell traction forces measurement based on BioMEMS microposts matrix

ZHOU Zhi-guo, LIU Zhi-wen, FAN Zhe-yi.*(School of Information and Electronics, Beijing Institute of Technology, Beijing 100081, China)*

Abstract: A review of cell traction forces (CTFs) measurement based on Biological Micro Electromechanical Systems (BioMEMS) microposts matrix is presented. CTFs are exerted by cells and transmitted to the underlying substrate through focal adhesions and close contacts, which is essential for cells movement. Cells probe the mechanical compliance of the extracellular matrix (ECM) in part by locally deforming it with nanonewton-scale traction forces. Precision measurement of CTFs is significant for many researches such as cell biology and tissue engineering and so on. Enabled by the advancement in BioMEMS technology, surface treated high aspect ratio Polydimethylsiloxane (PDMS) microposts matrix devices, which serve as BioMEMS sensors for detecting cellular nanoforces and studying in vitro cell mechanics, have been developed. Closely spaced vertical microposts matrixes were designed to encourage cells to attach and spread across multiple microposts, and to bend the microposts like vertical cantilevers as the cells locomote on the surface. Using this dense and discrete matrix of microposts rather than a conventional continuous substrate, CTFs can be directly measured and quantified by processing the microscopy images of the deformations of microposts. The resolution of the force was in tens of nN/μm scale. At first, the conventional CTFs measurement methods were concisely summarized. Then BioMEMS microposts matrix method was described in detail, including principle and fabrication process, surface treatment and cell experiment results. Furthermore, high aspect ratio structure collapse problem was investigated.

Key words: Cell Traction Forces; Biological micro electromechanical systems (BioMEMS) PDMS Microposts Matrix

基于 BioMEMS 微柱矩阵的细胞牵引力测量

周治国, 刘志文, 范哲意
(北京理工大学 信息与电子学院,北京 100081)

摘要: 本文综述了基于生物微机电系统(BioMEMS)微柱矩阵的细胞牵引力测量方法。细胞牵引力对于许多生物学过程非常关键,决定着许多细胞功能,包括细胞迁移、细胞外基质构建、信号传导等。细胞通过纳牛顿量级的牵引力使细胞外基质局部变形来探测其机械顺从性。精确测量细胞牵引力大小及分布对细胞生物学、组织工程等生物医学研究具有重要意义。BioMEMS 的发展使高深宽比聚二甲基硅氧烷(PDMS)微柱矩阵被开发出来作为传感器用来探测细胞纳牛力学及在体外研究细胞的机械性质。细胞贴附在微柱矩阵顶端,并且在多个柱顶端间延展迁移,这个过程会造成微柱发生如垂直悬臂梁般的弯曲形变。采用这种致密、垂直、离散微柱矩阵结构替代传统测量的连续介质,通过对微柱形变的显微图像处理,细胞牵引力可以被直接定性定量测量,精度可以达到数十 nN/μm 量级。首先简要介绍了传统细胞牵引力测量方法,接下来着重于论述基于 BioMEMS 微柱矩阵的测量方法,阐述了其原理、制作工艺、表面处理及细胞实验等。最后对微柱矩阵结构的坍塌问题进行了讨论。

关键词: 细胞牵引力; 生物微机电系统; PDMS 微柱矩阵
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作者简介:周治国(1977-),研究方向:生物力学。
通讯作者:刘志文,Tel:(010)68911432,E-mail:zwliu@bit.edu.cn。

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Traction forces, which are essential for cell movement, are exerted by cells and transmitted to the underlying substrate through focal adhesions[1,2] and close contacts[3,4] (Fig. 1). Cells probe the mechanical compliance of the underlying substrate in part by locally deforming it with nanonewton-scale traction forces[5]. CTFs are crucial to many biological processes. They play a fundamental role in many biological processes such as angiogenesis, embryogenesis, inflammation, and wound healing.

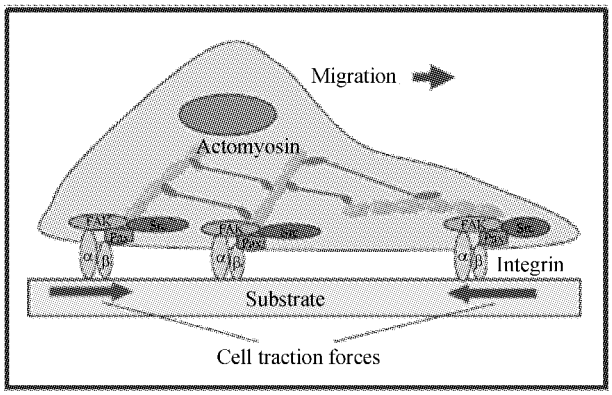

图 1 黏附细胞 CFTs 示意图[4]

Fig.1 An illustration of CTFs in an adherent cell[4].

Various methods have been developed to measure CTFs of both populations of cells and of single cells over last decades. According to techniques adopted the methods can be classified in two ways: active methods and passive methods. Active methods apply forces in order to make cells deformation which can be measured. Atomic Force Microscopy (AFM) method[6] uses a sharp tip attached to a flexible cantilever. The tip is used to probe the cell and the relative deformation of the cell and tip can then be used to estimate the force applied and the stiffness of the cell. However, scanning with too high a force on the tip might damage cell and many different tip shapes were used complicate replication of experiments in different laboratories. Micropipette method[7] uses gentle suction to a micropipette that is placed on the surface of the cell. The cell is deformed and the geometry of the resulting deformation together with the applied pressure is used to calculate the force applied. Mechanical properties of cells can then be inferred from this data. Passive methods use sensors to detect the forces generated by cells. Elastic substratum method was developed in ref.[8]. It's the first successful attempt to measure traction forces of individual cells using artificial flexible substrata. Different cells were cultured on thin films, polymerized with a flame, on the surface of silicone fluid. The films were coated with ECM proteins to promote cell adhesion and attachment. As the adherent cells exert forces on the sheets, they cause the surface to wrinkle, which can be easily visualized under a light microscope. Refs.[9] and[10] introduced flexible sheets with micropatterned dots or grids method. This method involves imprinting dots on the flexible sheet and observing the deformation of the grid from the ideal grid. Models of deformation can then be applied to the grid and the cellular forces inferred from the deformations produced.

Instead of using uniformly flexible substrata, an innovative approach has been developed. Enabled by the advancement in the BioMEMS technology, surface treated high aspect ratio PDMS microposts matrix devices, which serve as BioMEMS sensors for detecting cellular nanoforces and studying in vitro cell mechanics, have been developed. With micromachined mold on silicon wafers PDMS microposts matrix devices were fabricated by replica-molding. Cells were seeded on the closely spaced vertical microposts matrix, and encouraged to attach and spread across multiple microposts. Cells were observed crawling over the microposts, bending the microposts like vertical cantilevers, the deflections were detected with high precision under the light microscope. Using this dense and discrete matrix of microposts rather than a conventional continuous substrate, CTFs can be directly measured and quantified by processing the microscopy images of the deformations of microposts. The resolution of the forces was about in tens of mN/μm scale.

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1 Measurement principle and parameter optimization

Figure 2 shows the schematic drawing of the cell traction and deformation of a single micropost. In the linear regime of small deforMations, the micropost behaves like simple springs such that the deflection is directly proportional to the force exerted by the attached cell. The linear elastic theory of a cylinder of radius r, and length L, bent by the application of a lateral force F at its extremity then gives the following relation (Eq. 1, 2), where E, K, and Δx, are the Young's modulus, spring constant and the deflection of the micropost, respectively[11].

$$F = K\Delta x = \left(\frac{3\pi Er^4}{4L^3}\right)\Delta x \quad (1)$$

$$K = \frac{3\pi Er^4}{4L^3} \quad (2)$$

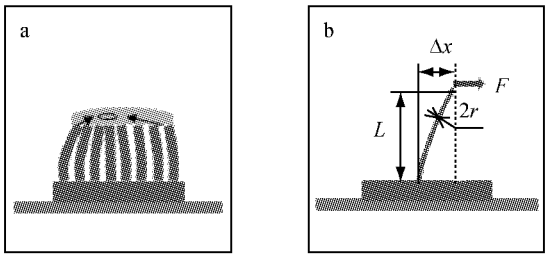

Fig.2 Schematic drawing of the cell traction on the top of microposts matrix and deformation of micropost
图 2 在微柱矩阵顶端细胞牵引和微柱型变示意图

Before fabrication, it is critical to optimize the parameters of microposts matrix to keep the structure stable and achieve high measurement resolution. To culture cells on microposts and prevent cells dropping down, the cell's diameter and the spacing of the microposts must be considered synthetically. Cells may hang up among microposts of low density and overhigh density may make microposts adhesion together when cells spread. Although higher aspect ratio can bring higher resolution, overhigh ratio may cause mechanical reliability problems. On the other hand low aspect ratio microposts are stiff. It is very difficult to detect the deformation displacement of the microposts. Usually the diameter of the microposts ranges from 1 to 3μm, the height ranges from 5 to 11 μm and spacing ranges from 3 to 9μm.

2 PDMS microposts matrix fabrication process

Figure 3 presents the key PDMS microposts matrix fabrication process steps adopting replica-molding method. The first step (Fig. 3a-c) is to design a conventional photolithography mask. The desired pattern is replicated in photoresist by photolithography. Tan et al.[12] used SU-8 (Microchem, Newton, MA) negative photoresist. After UV exposure and development, arrays of vertically-arranged SU-8 microposts were released and stand on the wafer as micromold. Using standard protocols for contact I-line UV soft lithography, it is very difficult to produce features smaller than this due to wavelength limitations, catastrophic adhesion failure and reflow of photoresist. Du Roure et al.[13] and Li et al.[14] adopted positive photoresist and followed by dry reactive ion etching (DRIE), also called the "Bosch" process. Silicon wafers were patterned with an array of cylindrical holes. Using this method, they were able to produce smaller dimension microposts with 1μm diameter, 5.2μm height and 2μm spacing. This is an impressive improvement in scale. However, there are two significant drawbacks to this approach. First, the equipment and infrastructure required for projection lithography and DRIE are too expensive to be readily available to most researchers and many institutions. Second, microposts produced in this manner are not entirely cylindrical and therefore cannot be considered to behave like the simple cantilever beams. Addae-Mensah et al.[15] improved to use standard contact I-line UV soft lithography with SU-8 negative photoresist and a contrast enhancement step to eliminate the deleterious effects of the air gap between the SU-8 and the photolithographic mask.

Before the second step PDMS (Sylgard 184, Dow- Corning) prepolymer needs to be prepared by

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mixing PDMS base and curing agent (volume ratio: 10:1) thoroughly and degassing with a vacuum pump for more than 20 min for casting.

The second step (Fig. 3d-g) is PDMS prepolymer casting. Method adopting negative photoresist needs cast twice. At first, prepolymer was poured over an array of SU-8 microposts made on silicon wafers, cured at 65℃ overnight, peeled off, oxidized in an air plasma for 1 min, and silanized to aid subsequent release of PDMS from the template. Then, PDMS prepolymer was poured over the template, degassed under vacuum, cured at 110℃ for 20 h, and peeled off the template. For silicon posts mold, the wafer was silanized to facilitate the release at first. Then PDMS prepolymer was poured over the silicon template, cured at 65℃ for 12 h, and peeled off from it.

The third step (Fig. 3h-i) is surface treatment. After demolding, PDMS microposts matrix was oxidized and sterilized in air plasma to make the PDMS surface hydrophilic. Using microcontact printing method[16,17], the desired area of microposts matrix was printed with ECM protein and fluorescently labeled for next cell experiment. Addae-Mensah et al. used quantum dot-labeling techniques allows precise localization and tracking of microposts using standard fluorescence microscopy, minimizing problems associated with discrimination of post-tops and cell edge artifacts generated by DIC microscopy and virtually eliminating time-dependent signal degradation.

3 Cell experiment

We merge cell experiment SEM images in one figure with the same scale bar for comparison (Fig. 4). Tan et al. constructed the mPADs (microfabricated post-array-detectors) with a radius of 3 μm, 11 μm height and 9 μm spacing, corresponding to a spring constant of 32 mN/m per micropost. Using DRIE method, the (FSA (microdimensional force sensor array) was developed by du Roure et al. The diameter of the micropost is 1 μm, the height of pores is 5.2 μm, and the central-to-central distance between two microposts is 3 μm. The high aspect ratio is near 6 and this ratio is the highest among the articles compared. The Young's modulus is 2 MPa and they reported a spring constant of 21.8 nN/μm. The MFSA (micropost force sensor array) was developed by Li et al. The diameter of the micropost is 2 μm, the height of pores is 6 μm, and the central-to-central distance between two microposts is 4 μm. The high aspect ratio is 3. In combination with image analysis algorithms, it's reported that the MFSA can achieve a spatial resolu-

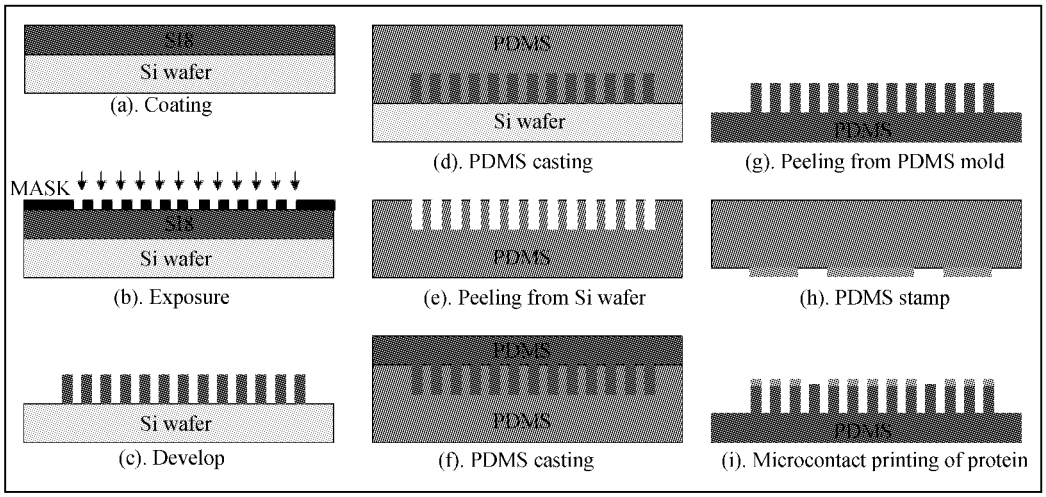

Fig.3 Cross-sectional view of the microposts matrix fabrication process steps using replica-molding method
图 3 采用复脱模方法制作微柱矩阵工艺步骤剖视图

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tion of 40 nm and a force sensitivity of 0.5 nN. The BoN (bed of nails) was developed by Addae-Mensah et al. The diameter of the micropost is 2 μm, the height of pores is 7 μm, and the central-to-central distance between two microposts is 5 μm. The high aspect ratio is 3.5 and the spring constant is 13.6 nN/μm.

4 Discussion

According to the Eq.1 and Eq.2, the higher aspect ratio can bring higher resolution. Actually researchers have been trying to improve fabrication technology to make higher aspect ratio microposts matrix. For example, the microposts matrix with 1 μm diameter and 20 μm the height corresponds to a spring constant of 0.04 mN/m per micropost. The force resolution is pretty good, but higher ratio may cause mechanical reliability problems. A few groups have explored fabrication of high aspect ratio microposts structures using PDMS[18-20]. Sharp[21] shows that the unique geometries of the high aspect ratio PDMS microposts structures may have lateral collapse and ground collapse problems. Lateral collapse refers to the collapse to each other and ground collapse refers to collapse to the ground substrate in one way or another. Based on Hui's model[22] the high aspect ratio PDMS microposts structures ground collapses under its own weight. According to the gravitational collapsing theory all fabricated PDMS microposts structures should be stable, which is in contrast to the experimental results. Actually the critical high aspect ratio is around 6 depending on the Young's modulus of PDMS in the air[23]. Even heating PDMS prepolymer for a long time or mixing PDMS base and curing agent with a different ratio, the Young's module doesn't change hugely[24].

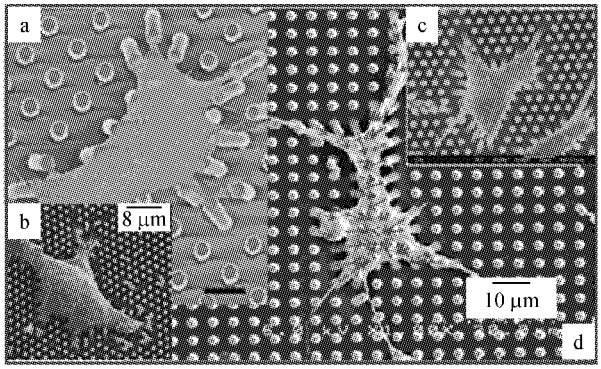

Fig.4 Cells on the top of microposts matrixes SEM images comparison with the same scale bar a. from Tan et al[12]; b. from du Roure et al.[13]; c. from Li et al.[14]; d. from Addae-Mensah et al.[15].

图 4 相同比例下细胞在微柱矩阵顶端 SEM 图比较 a. 来自 Tan et al[12]; b. 来自 du Roure et al.[13]; c. 来自 Li et al.[14]; d. 来自 Addae-Mensah et al.[15].

We adopted a handy procedure for fabricating high aspect ratio PDMS microposts matrix for cell traction forces measurement and collapse investigation. We found that the critical ratio can be higher in aqueous solution. Actually the cell experiments have been done in wetting environment and microposts matrixes with seeding cells were immersed in culture medium. We fabricated the microposts matrix in the aqueous solution and achieved higher ratio. We have designed the experiment to investigate the mechanical reliability problem and the critical ratio of PDMS microposts in the aqueous solution, such as ethanol and water. If we can avoid evaporation breaking the surface tension balance, the microposts can keep standing. In water the critical ratio can reach 10 in our experiment. Some unexpected forces such as shock, fluid flow and so on, will not lead to adhesion between microposts.

5 Conclusion

CTFs are crucial for an adherent cell to migrate, maintain its shape, organize ECM, probe physical environments, and generate mechanical signals. It is well recognized that a detailed knowledge of CTFs is important in understanding many fundamental biological processes. In this review, we provide a detailed presentation of the CTFs measurement using BioMEMS PDMS microposts matrix method. The measurement principle and model, fabrication technology, surface treatment,

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cell experiment and spring constant were described and compared in detail. The novelty in this field is remarkable and has provided many opportunities to investigate cell biomechanics although there are still some limitations need to be overcome. We hope the knowledge we gained from these studies will shed some insights into cell traction force measurement.

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References:

[1] Geiger B, Bershadsky A. Assembly and mechanosensory function of focal contacts [J]. Current Opinion in Cell Biology, 2001, 13: 584-592.

[2] Critchley DR. Focal adhesions - the cytoskeletal connection [J]. Current Opinion in Cell Biology, 2000, 12: 133-139.

[3] Adams JC. Cell-matrix contact structures [J]. Cellular and Molecular Life Sciences, 2001, 58: 371-392.

[4] Wang JHC, Lin JS. Cell traction force and measurement methods[J]. Biomechanics and Modeling in Mechanobiology,2007,6:361-371.

[5] Choquet D, Felsenfeld DP, Sheetz MP. Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages [J]. Cell, 1997, 88: 39-48.

[6] Weisenhorn AL, Khorsandi M, Kasas S, et al. Deformation and height anomaly of soft surfaces studied with an AFM [J]. Nanotechnology, 1993, 4: 106-113.

[7] Chien S, Sung KL, Skalak R, et al. Theoretical and experimental studies on viscoelastic properties of erythrocyte membrane [J]. Biophys J, 1978, 24: 463-487.

[8] Harris AK, Wild P, Stopak D. Silicone rubber substrata: a new wrinkle in the study of cell locomotion [J]. Science, 1980, 208: 177-179.

[9] Yang S, Saif T. Micromachined force sensors for the study of cell mechanics [J]. Rev Sci Instrum, 2005, 76: 044301-044308.

[10] Butler JP, Tolic-Norrelykke IM, Fabry B, et al. Traction fields, moments, and strain energy that cells exert on their surroundings [J]. Am J Physiol Cell Physiol, 2002, 282: C595-C605.

[11] Crandall SH. An Introduction to the Mechanics of Solids [M], ed. Lardner, T. J. (McGraw-Hill, New York), 1978, 511-576.

[12] Tan JL, Tien J, Pirone DM, et al. Cells lying on a bed of microneedles: An approach to isolate mechanical force [J]. PNAS, 2003, 100: 1484-1489.

[13] du Roure O, Saez A, Buguin A, et al. Force mapping in epithelial cell migration [J]. PNAS, 2005, 102: 2390-2395.

[14] Li B, Xie LK, et al. Development of micropost force sensor array with culture experiments for determination of cell traction forces [J]. Cell Motility and the Cytoskeleton, 2007, 64: 509-518.

[15] Addae-Mensah KA, Kassebaum NJ, Bowers, et al. A flexible, quantum dot-labeled cantilever post array for studying cellular microforces [J]. Sensors and Actuators a-Physical, 2007, 136: 385-397.

[16] Tan JL, Tien J, Chen CS. Microcontact printing of proteins on mixed self-assembled monolayers [J]. Langmuir, 2002, 18: 519-523.

[17] McGurk SL, Green RJ, Sanders GHW, et al. Molecular interactions of biomolecules with surface-engineered interfaces using atomic force microscopy and surface plasmon resonance [J]. Langmuir, 1999, 15: 5136-5140.

[18] Geim AK, Dubonos SV, Grigorieva IV, et al. Microfabricated adhesive mimicking gecko foot-hair [J]. Nature Materials, 2003, 2: 461-463.

[19] Delamarche E, Schmid H, Michel B, et al. Stability of molded polydimethylsiloxane microstructures [J]. Advanced Materials, 1997, 9: 741-746.

[20] Glassmaker NJ, Jagota A, Hui, et al. Design of biomimetic fibrillar interfaces: 1. Making contact [J]. Journal of the Royal Society Interface, 2004, 1: 23-33.

[21] Sharp KG, Blackman GS, Glassmaker NJ, et al. Effect of stamp deformation on the quality of microcontact printing: Theory and experiment [J]. Langmuir, 2004, 20: 6430-6438.

[22] Hui CY, Jagota A, Lin YY, et al. Constraints on microcontact printing imposed by stamp deformation [J]. Langmuir, 2002, 18: 1394-1407.

[23] Roca-Cusachs P, Rico F, Martinez E, et al. Stability of microfabricated high aspect ratio structures in poly(dimethylsiloxane)[J]. Langmuir, 2005, 21: 5542-5548.

[24] Gray DS, Tien J, Chen CS. Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's modulus [J]. Journal of Biomedical Materials Research Part A, 2003, 66A: 605-614.

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