A New Device for Two-Dimensional Cell Confinement

Ran Rao*, Haoxiang Yang

Department of Modern Mechanics, CAS Key Laboratory of Mechanical Behavior and Design of Materials, University of Science and Technology of China, Hefei, Anhui 230026, China

raoran@mail.ustc.edu.cn

*Corresponding author

Keywords: confinement ECM stiffness mechanobiology polyacrylamide

Abstract: Cells in living organisms are in a restricted environment. Many studies have revealed different phenotypes of cells in different restricted niches. In order to study the effect of this microenvironment on the physiological activity of cells, we have designed a restricted device that can independently control ECM stiffness and the degree of physical confinement. Such device can not only precisely adjust the substrate stiffness from 1kPa to 40kPa, but also can give a micro confinement to cell which can achieve to about 3 microns. Using this device to study cancer cell migration and stem cell differentiation may reveal some new characteristics of cells in confined spaces.

1. Introduction

All cells in the human body are in restricted niches, resulting from intercellular restrictions and cell-to-basement membrane limitation. Especially during the metastasis of cancer cells and the development of stem cells, this restricted niche can affect the degree of cancer migration and differentiation. And many studies have shown that ECM stiffness is another important factor affecting the osteogenic and adipogenic differentiation rate of stem cells and the migration of cancer cells.

In order to simulate the restricted microenvironment of cells in vivo for research, many experiments use three-dimensional cell culture, but the results are not ideal. This is because many experimental results change as the culture dimension reaches three dimensions-usually due to changing the modulus of the substrate in the three-dimensional substrate, and also changing the physical limitation of the gel network. These two factors are difficult to decouple, which makes studying cell behavior very difficult.

Here we have designed a device that independently adjusts the stiffness and restricted height of the bottom layer, which is different from the previous ones. We use photolithography technology to manufacture polydimethylsilane (PDMS) micro-pillars to make precise adjustments to the cells on the substrate. Further, we determine the hardness of the substrate by adjusting the acrylamide hydrogel ratio of the substrate. In view of the limited effect of this device, it provides a new method for studying the physiological activities of cells under limited conditions.

All cells in the human body are in restricted niches, resulting from intercellular restrictions and cell-to-basement membrane limitation. Especially during the metastasis of cancer cells and the development of stem cells, this restricted niche can affect the degree of cancer migration and differentiation.

And many studies have shown that ECM stiffness is another important factor affecting the osteogenic and adipogenic differentiation rate of stem cells and the migration of cancer cells.

In order to simulate the restricted microenvironment of cells in vivo for research, many experiments use three-dimensional cell culture, but the results are not ideal.

This is because changing the cell modulus often changes the pore size of the hydrogel network, which changes the degree of space limitation of the cell in the hydrogel. These two factors are difficult to decouple, which causes the results to be very different from those of the 2D cell culture.

Here we have designed a device that can independently regulate the stiffness and restricted height, which is different from the previous ones. We use photolithography technology to manufacture polydimethylsilane (PDMS) micro-pillars to make precise adjustments to the cells on the substrate. Further, we determine the hardness of the substrate by adjusting the acrylamide hydrogel ratio of the substrate. In view of the limited effect of this device, it provides a new method for studying the physiological activities of cells under limited conditions.

2. Fabrication of confinement Slide

For geometrical constraints, micro-shims must be molded under the limit slide in the PDMS layer to control the distance between the slide and the culture substrate.

In order to obtain reliable limits, the geometry of these shims needs to be controlled. We use photolithography to etch fixed height micropillars on silicon wafers. We use the previous designed mode [1]to calculate and simulate the arrangement and distance of these micro-pillars, and to ensure the stability of the capping equipment. The height of these micropillars is typically 5 microns., The minimum is 3 microns. 3 microns is the limiting limit, below which it may not be able to support the weight of the pillar and may be close to another plane.

2.1 Manufacturing

Prepare 10 g of PDMS mixture with a PDMS / crosslinker (A / B) ratio of 8/1 (w / w). The bubbles can be removed by centrifugation for 30min, or you can use a vacuum pump for one hour. Placing PDMS in a uniform thin layer on a silicon wafer can also use a homogenizer to prepare a thin layer. Bake the mold with PDMS on a hot plate for 15 minutes at 95 ° C or 12 hours at 65 ° C.

2.2 Slide treatment

Remove the PDMS film from the mold and cut it neatly according to the size in the experiment to ensure that it is smaller than the size of the rubber surface. The bottom surface of the glass cylinder used as a cover weight and the side of the PDMS film without the micro-pillar array were then placed in plasma cleaner for surface treatment. Within 5 minutes after the plasma treatment, the two surfaces were bonded together without gaps and compacted to make full contact. Subsequently, it was immersed in a pLL-g-PEG solution for at least 1 hour for a non-sticky treatment.

3. Fabrication of PA substrate

Polyacrylamide hydrogel is the most common biocompatible material. It has a tunable modulus that can simulate the modulus in vivo. By adjusting the ratio of Acr and Bis, the modulus can be controlled from 0.1kpa to 40kpa. Modulus [2]. Here we mainly introduce the production of hydrogel substrates that can be seeded with cells.

3.1 Prepare amino-silanated coverslip(s)

Add 200 μL of APTES to the aforementioned petri dishes in turn, and shake them by hand.

Let stand for 15min. This step is to use the special affinity of silicon for oxygen in the silane structure to promote the connection of silane to the surface of quartz glass, thereby modifying the surface of the quartz with amino groups.

Add 2mL of PBS and 40μL of 25% glutaraldehyde solution to the petri dish in turn, shake them in a circle, and let stand for 30min. The nature of the reaction is that the aldehyde group and amino group generate imine. PBS is used because this reaction of glutaraldehyde is most reactive in slightly alkaline environments.

Allow to dry for one to twenty minutes and put it in a dry dish for future use, theoretically it can be used for 2 days.

3.2 Regulate and measure the modulus of PA gel

PA gels are produced by mixing various acrylamide and bis-acrylamide concentrations. A table of expected modulus of elasticity values given the input concentrations of acrylamide and bis-acrylamide is provided (Table 1.)

PA gel modulus of elasticity was quantified using atomic force microscopy (AFM)(Figure1.), which is a nano-indentation method of calculating elasticity. This technique has been extensively detailed elsewhere [3,4].

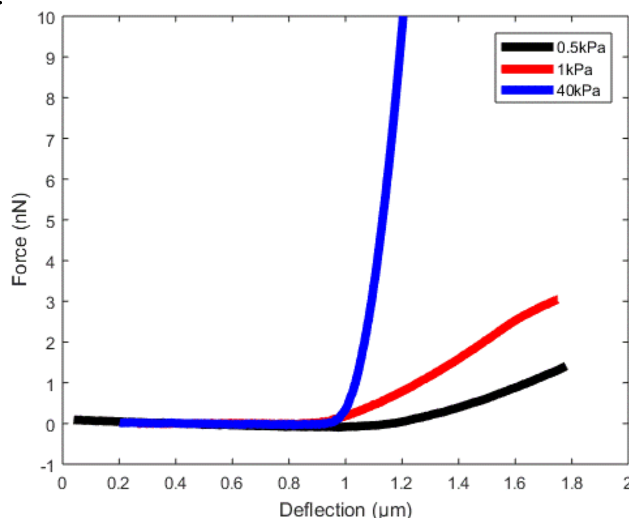

Figure 1. PA hydrogel measured by AFM Force-Deflection Curve

Table 1. PA Hydrogel Ratio and Young's Modulus

Desired Young's Module (kPa)	Acrylamide (wt %)	Bis-Acrylamide (wt %)	Measured Young's Module Average (kPa)	Relative Average Deviation
0.50	3	0.078	0.552	1.04%
1.00	3	0.150	0.963	1.87%
40.0	8	0.400	36.50	4.41%

Figure 2. Assembled restricted device

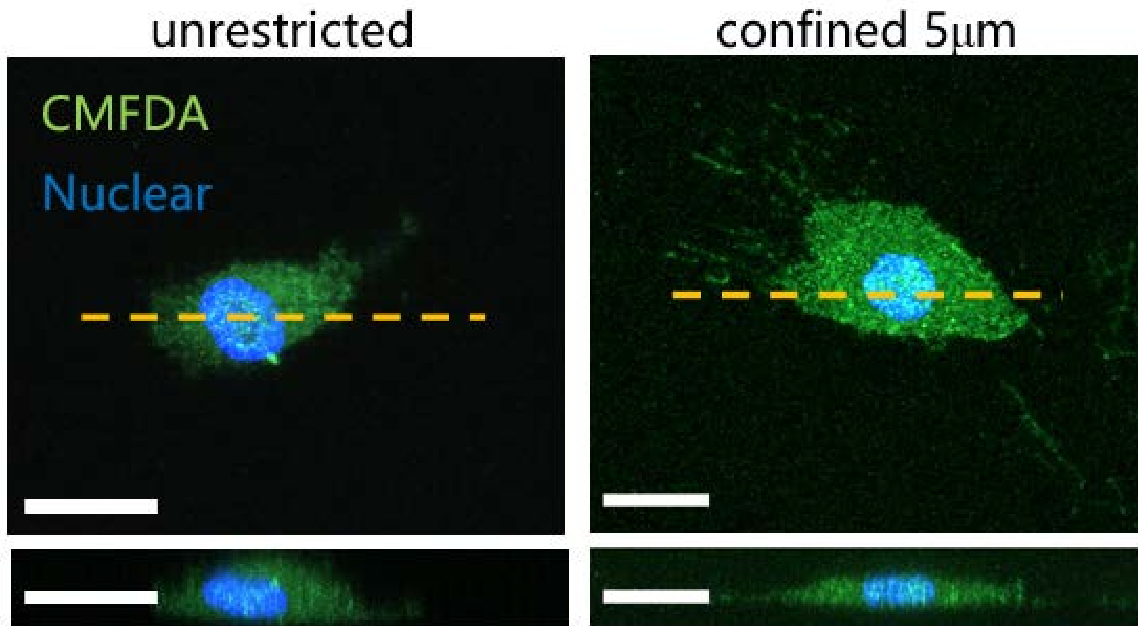

Figure 3. Cells cultured in a restricted device

4. Assembly of the Device and deformation of cell in restricted devices

To investigate the effects of substrate stiffness and confinement on cell, we subjected cells to gels with defined Young's modulus and introduced confinement with a non-adhesive PDMS surface.

The assembly diagram of the capping device is as follows. The confocal disk with a hollow glass bottom is fixed with a sealing film, and the glass column can be lowered by adjusting its size (Figure 2). Note that to cover the column very gentle to avoid damaging the cells, so the cells are confined in two parallel planes, and the height of the cells can be precisely controlled.In the interphase, the height of hMSC cells was 10.6 ± 0.36 mm. To characterize the device, we labeled the cytoplasm with the fluorescent indicator CellTracker Green CMFDA (a nuclear marker (Hochest)) and reconstructed the 3D cell morphology of the confocal microscope image (Figure 3) When in a restricted state, the cells on the soft surface are 5.2 1.1 mm high (n = 29, N = 2) and their nuclei are about 3.3 0.7 mm (n = 32, N = 2). In both cases, the cells are completely constrained and the nucleus is significantly deformed. The above results show that the restricted device effectively and accurately restricts cells and can guarantee cell viability.

5. Conclusion

Limiting parameters are becoming increasingly important in cell biology research, and tools that allow independent control of different limiting parameters while remaining simple enough for use in biological procedures are critical.

However, profiling the unique role of these two methods is critical. Because of the lack of an ECM scaffold, they cannot be independently changed, which severely limits these parameters. This has caused many contradictory phenomena and experimental results. To systematically address these unmet needs, we have developed a unique microculture platform that enables independent study of the effects of ECM stiffness and containment on cells. We have discussed how to confine cells in a reversible manner between two parallel surfaces separated by a defined gap. In this article, we describe a method for limiting cell populations in a controlled environment (stiffness) that can be easily combined with specific observation techniques and molecular biology protocols, and we hope that these protocols will help answer a certain These questions also lead to innovative ideas on how to study cells in complex environments.

References

[1] Le Berre, M., Aubertin, J., & Piel, M. Fine control of nuclear confinement identifies a threshold deformation leading to lamina rupture and induction of specific genes. Integra tive Biology. (2012) 1406–1414.

[2] Engler A J, Sen S, Sweeney H L, et al. Matrix elasticity directs stem cell lineage specification. Cell. (2006)126(4): 677-689.

[3] Rotsch, C. and Radmacher, M. Drug-induced changes of cytoskeletal structure and mechan ics in fifibroblasts: An atomic force microscopy study. Biophys. J. (2000) 78:520-535.

[4] Rotsch, C., Jacobson, K., and Radmacher, M. Dimensional and mechanical dynamics of active and stable edges in motile fifibroblasts investigated by using atomic force microscopy.PNAS. (1999) .