

Investigation of the growth of focal adhesions using protein nanoarrays fabricated by nanocontact printing using size tunable polymeric nanopillars

This article has been downloaded from IOPscience. Please scroll down to see the full text article.

2011 Nanotechnology 22 265302

(<http://iopscience.iop.org/0957-4484/22/26/265302>)

View [the table of contents for this issue](#), or go to the [journal homepage](#) for more

Download details:

IP Address: 140.109.214.4

The article was downloaded on 20/07/2011 at 16:23

Please note that [terms and conditions apply](#).

Investigation of the growth of focal adhesions using protein nanoarrays fabricated by nanocontact printing using size tunable polymeric nanopillars

Chiung Wen Kuo, Fan-Ching Chien, Jau-Ye Shiu, Shih-Min Tsai, Di-Yan Chueh, Yu-Sheng Hsiao, Zong-Han Yang and Peilin Chen

Research Center for Applied Sciences, Academia Sinica, 128, Section 2, Academia Road, Taipei 115, Taiwan

E-mail: peilin@gate.sinica.edu.tw

Received 26 October 2010, in final form 28 March 2011

Published 17 May 2011

Online at stacks.iop.org/Nano/22/265302

Abstract

Here we describe a simple approach to create various sizes of protein nanoarrays for the investigation of cell adhesion. Using a combination of nanosphere lithography, oxygen plasma treatment, deep etching and nanomolding processes, well-ordered polymeric nanopillar arrays have been fabricated with diameters in the range of 50–600 nm. These nanopillar arrays were used as stamps for nanocontact printing to create fibronectin nanoarrays, which were used to study the size dependent formation of focal adhesion. It was found that cells can adhere and spread on fibronectin nanoarrays with a fibronectin pattern as small as 50 nm. It was also found that the average size of focal adhesion decreased as the size of the fibronectin pattern was reduced.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Cell adhesion plays an important role in many cellular processes including migration, proliferation, differentiation and apoptosis. It is known that cells adhere to other cells or surrounding extracellular matrices (ECM) where cell adhesion provides the physical support to cells allowing cells to sense their microenvironments and to regulate their behaviors [1–3]. *In vitro*, cells do not attach directly to the substrate surfaces. They bind to the ECM molecules through transmembrane protein integrins, which connect ECM molecules and cytoplasm proteins that attach the actin cytoskeleton to the membrane. Initially, the adhesion contacts are located around the cell periphery forming small focal complexes. Later, the focal complexes evolve into larger focal adhesions, which provide the anchor points for the cytoskeletons. To understand the cell adhesion, various techniques have been developed to precisely control the physical and chemical environments around cells on the micro- and nanoscale [4, 5]. It has been demonstrated that by

restricting the footprint of a cell on the patterned adhesive surfaces at micrometer scale, the growth and death of the cells can be regulated [6]. The polarization and motility of cells can also be manipulated by the patterned surfaces. The assembly of focal adhesion was found to depend on the cell shape, which could be engineered through the underneath patterns [7–9]. Recently, it was discovered that the fate of stem cells could also be regulated through the manipulation of the cell shape, and stiffness and topography of the ECM [10].

To investigate the cell adhesion on substrates, one of the most commonly used approaches is to immobilize the ECM or ligand molecules on a spatially well-defined area. Several techniques have been demonstrated capable of creating ECM patterns at micro- and nanoscale, including micro- and nanocontact printing [11–15], electron beam patterning [16], dip-pen lithography [17], electrowetting [18, 19], and the self-assembly of diblock copolymer [20–23]. For example, dip-pen lithography has been employed to create protein nanoarrays with 200 nm diameter. When these protein nanoarrays were used in the cell culture, it was found that

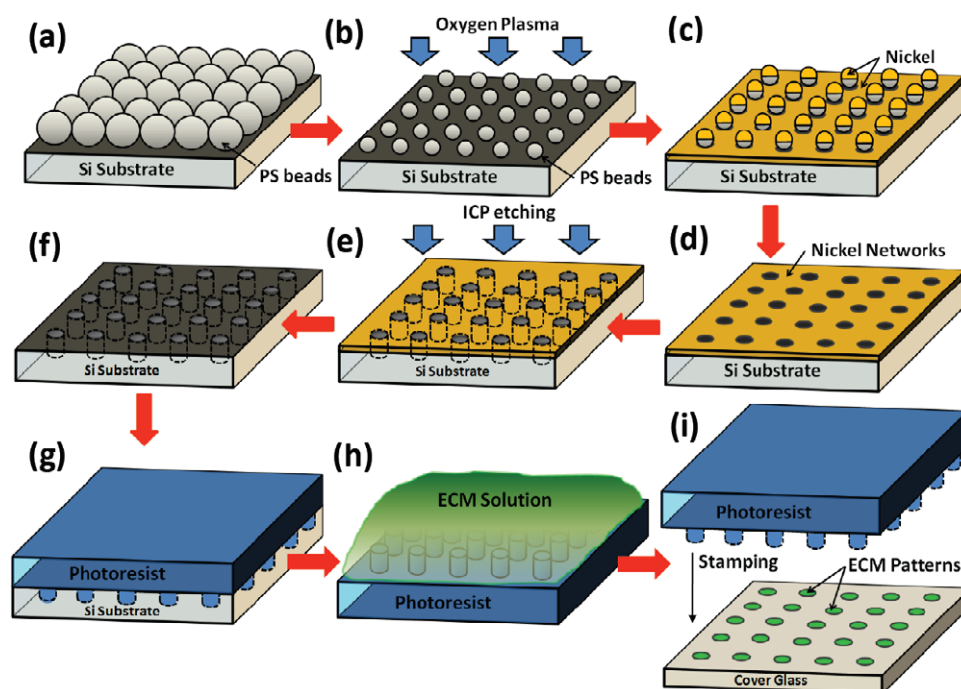


Figure 1. Schematic for producing protein nanoarrays using size tunable nanosphere lithography and nanocontact printing. (a) Nanosphere lithography was used to create well-ordered patterns. (b) The size of the polystyrene beads was reduced to the desired dimension by oxygen plasma. (c) A layer of nickel was deposited onto the size-reduced polystyrene beads. (d) The polystyrene beads were removed by sonication. (e) The patterns of nanoholes were transferred into silicon substrates in an induced coupled plasma (ICP) etcher. (f) After removing the nickel layer by nickel etchants, silicon nanoholes can be obtained. (g) SU-8 nanopillar arrays were obtained by peeling off the SU-8 photoresist from the silicon template. (h) To create protein nanoarrays, SU-8 nanopillars were placed in the PBS solution containing fibronectin. (i) The fibronectin nanoarray can be obtained by gently placing the SU-8 nanopillar on the glass substrate.

cells could attach and spread on the protein nanoarrays but not completely [17]. In another example, integrin binding ligands, arginine–glycine–aspartic acid (RGD) peptides, were patterned on the surfaces using gold nanoparticles and self-assembly of diblock copolymer micells where the separation distance between gold nanoparticles could be tuned by monomer repeating units. Because the size of the gold nanoparticles was small enough, it was possible to allow only one integrin molecule to bind to each gold nanoparticle. It was observed that the separation distance between RGD peptides larger than 73 nm would result in limited cell adhesion and spreading and the formation of focal adhesion and actin stress fibers was greatly reduced [20]. The proliferation and differentiation of cells as well as gene delivery and expression can also be regulated by tuning the ligand density in alginate gels [24, 25]. Despite these fruitful results, it remains difficult to rapidly create large-area protein nanoarrays with an arbitrary pattern size.

Here we describe a simple technique based on size tunable nanosphere lithography to create protein nanoarrays. Nanosphere lithography [26, 27], which utilizes the self-assembly of monodispersed colloidal nanoparticles, has been demonstrated to be capable of fabricating size and shape tunable silicon nanostructures with feature sizes of less than 10 nm [28, 29]. These nanostructures have been used as stamps to replicate nanostructures by nanoimprint lithography [30]. However, to print the protein nanoarrays on glasses, an alternative stamp is needed. To produce protein nanoarrays, we

have employed a nanomolding process [31, 32], which utilized the silicon templates fabricated by nanosphere lithography, to create size tunable polymeric nanopillars. These polymeric nanopillars were then used as the stamps to print the protein nanoarrays by nanocontact printing.

2. Experimental section

With a combination of oxygen plasma treatment, it has been shown that the periodicity and the size of nanostructures can be tuned independently using nanosphere lithography [32, 33] where the diameter of the nanostructures can be controlled by oxygen treatment time while the separation distance can be changed by using different sizes of nanospheres. In this experiment, the fabrication process was modified for producing polymeric nanopillar arrays, which were used as the stamps for nanocontact printing. The schematic for producing protein nanoarrays using size tunable nanosphere lithography is illustrated in figure 1. To create polymeric nanopillars, monodispersed polystyrene beads purchased from Bangs Laboratories, Inc. (Fishers, IN) were diluted in a solution of surfactant Triton X-100 (Aldrich) and methanol (1:400 by volume). This solution was then spin-coated on the silicon substrates to form hexagonally close packed two-dimensional colloidal crystals (figure 1(a)). To create monolayer close packed nanostructures, the speed of the spin-coater was about 2500 RPM. The size of the polystyrene beads was reduced to the desired dimension by oxygen plasma (Oxford, Plasmalab

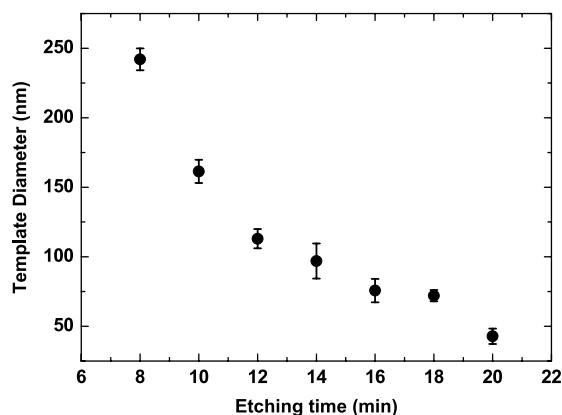


Figure 2. Diameters of silicon nanohole templates (figure 1(f)) as a function of oxygen plasma treatment time (figure 1(b)). The diameter of polystyrene beads used in this experiment was 400 nm.

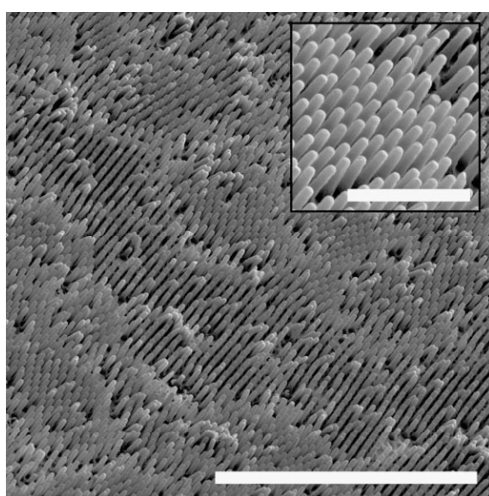


Figure 3. The SEM image of SU-8 nanopillars fabricated using 400 nm polystyrene beads. The diameter of the SU-8 nanopillars was measured to be 200 nm. Bar: 10 μm . Inset: bar: 2 μm .

80 Plus, United Kingdom) with 20 sccm of oxygen at a pressure of 50 mTorr as shown in figure 1(b). A layer of nickel (50 nm thick) was deposited onto the size-reduced polystyrene beads (figure 1(c)). Nickel was used here due to its availability. Other metals, such as gold and chromium, can also be used. The polystyrene beads were then removed by sonication in dichloromethane and acetone solution respectively for 10 min leaving nickel nanoholes on the substrates (figure 1(d)), which served as the etching mask in a deep etching process (figure 1(e)). The patterns of nanoholes were transferred into silicon substrates in an induced coupled plasma (ICP) etcher (Samco, RIE10ip). The gases used in the deep etching process were CF_4 and Ar and the ICP power was 150 W. The depth of the silicon nanohole templates could be controlled by the etching time. After removing the nickel layer by nickel etchants, silicon nanoholes can be obtained (figure 1(f)). To create polymeric nanopillars, a nanomolding process was used where the SU-8 2015 photoresist (MICROCHEM) was spun on silicon nanoholes and placed in a home-made vacuum system overnight to remove the air trapped inside the template.

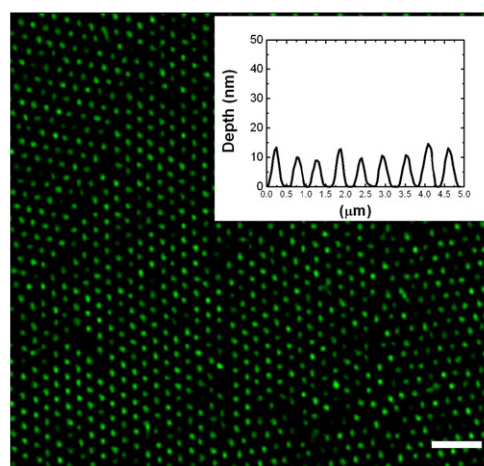


Figure 4. Confocal image of FITC-conjugated fibronectin nanoarrays prepared by nanocontact printing using nanopillars with 200 nm diameter. Bar: 2 μm . Inset: AFM line scan of fibronectin nanoarray of the same sample but in a different area.

The SU-8 photoresist was then exposed to UV radiation and baked at 200 °C for 30 min. SU-8 nanopillar arrays were obtained by peeling off the SU-8 photoresist from the silicon template (figure 1(g)). The SU-8 nanopillar arrays were then used as stamps in the nanocontact printing process to fabricate fibronectin nanoarrays. To create protein nanoarrays, SU-8 nanopillars were placed in the PBS solution containing fibronectin (50 $\mu\text{g ml}^{-1}$) for 1 h (figure 1(h)) and blown dry before stamping on the cover slips. To print the fibronectin nanoarray, the SU-8 nanopillars with fibronectins were placed on the cover slip and pressed gently by finger tips (figure 1(i)). For comparison, the fibronectin patterns in the micrometer scale were fabricated using standard photolithography and microcontact printing. The cover slips patterned with fibronectins were further placed in 1% BSA solution for blocking purposes. The scanning electron microscopic (SEM) images were recorded in a FEI Nova 200 NanoSEM. The silicon nanohole arrays can be used to create SU-8 nanopillars more than 20 times and each SU-8 nanopillar array can be used to print protein nanoarrays more than ten times.

Chinese hamster ovary (CHO-K1) cells were seeded on the cover slips with fibronectin patterns using Ham's F-12K medium supplemented with 10% (v/v) fetal bovine serum (FBS) and were incubated in incubators at 37 °C and 5% CO_2 . To visualize the focal adhesions, cells were stained with actin/cytoskeleton and a focal adhesion staining kit (CHEMICON). The cells were first fixed using 4% paraformaldehyde. Anti-vinculin was used to label the focal adhesion and TRITC-conjugated phalloidin was used to stain the cytoskeleton. The fluorescence images were taken on a confocal microscope (TCS SP5, Leica). The commercial software Image-Pro Plus was used to measure the size of focal adhesion. The atomic force microscopy (AFM) images of fibronectin arrays and cells on fibronectin nanoarrays were taken by JPK AFM (NanoWizard II) using cantilever (ULTRASHARP, MIKROMASCH) with a force constant of 0.1–0.4 N m^{-1} and resonance frequency of 17–24 kHz.

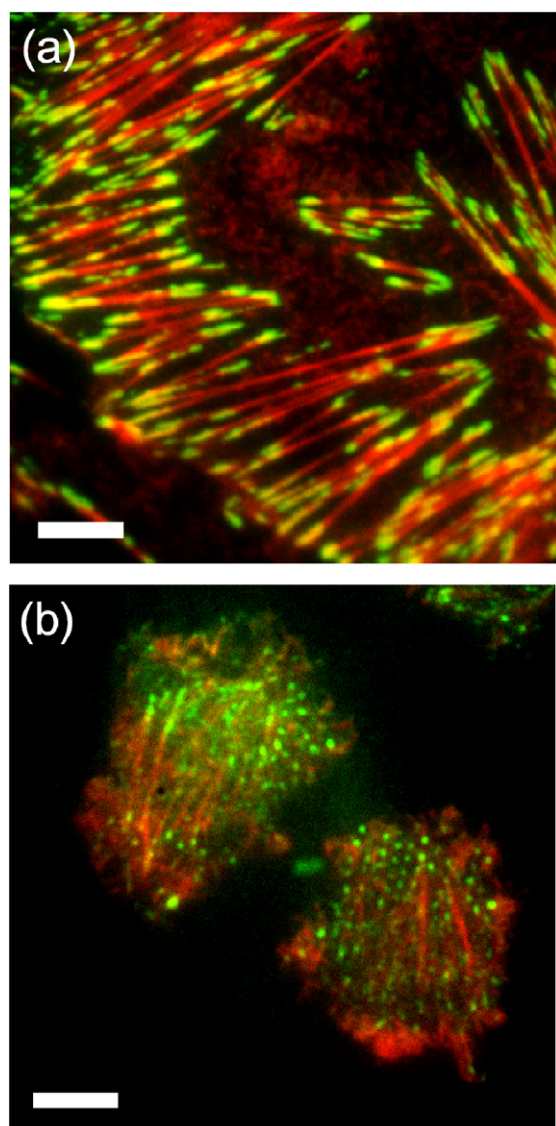


Figure 5. Fluorescence images of CHO cells stained with focal adhesion (vinculin) and actin on (a) flat glass and (b) 400 nm fibronectin nanoarrays with 890 nm separation distance. Actin was stained with TRITC and vinculin was labeled with FITC. Bar: 5 μm .

3. Results and discussions

Size tunable nanosphere lithography has been utilized to create size tunable silicon nanopillar arrays inside microfluidic channels [33] where size-reduced polystyrene beads have been used as the etching masks. In the size tunable nanosphere lithography, the separation distance between nanostructures is determined by the size of the nanospheres and the size of the nanostructures is governed by the oxygen plasma treatment. To create protein nanoarrays, nanopillars may be used to print protein solution on the substrate. However, the silicon nanopillars were not suitable for printing applications because of their hardness. Therefore, it is desirable to fabricate nanopillars using soft materials such as polymers. To fabricate polymeric nanopillars, we slightly modified the preparation process for the silicon nanomold where the nanohole arrays were first created in the silicon substrates by a deep etching

process using a metallic mask prepared by size tunable nanosphere lithography. Then these negative nanostructures were utilized to create polymeric nanopillars by nanomolding using curable polymers. To observe the protein nanoarrays on an optical microscope, polystyrene beads with diameters of 890, 500 and 400 nm were used to fabricate the templates for the nanopillars. Shown in figure 2 is the plot of the diameter of the silicon templates as a function of oxygen plasma etching time where 400 nm polystyrene beads were used. It can be seen that the diameters of the nanoholes in the silicon substrate could be systematically tuned from 250 to 50 nm using oxygen plasma treatment. When the diameter of the silicon nanohole was larger than 250 nm, part of the hole was connected to other holes forming continuous nanostructures in the nanomolding process. On the other hand, prolonged oxygen plasma treatment produced irregular shapes of nanoholes. Nanopillars with diameters larger than 250 nm or smaller than 50 nm could be produced by selecting the proper size of polystyrene beads. These silicon nanohole arrays were then used to create polymeric nanopillars by nanomolding.

In this experiment, we have fabricated polymeric nanopillars using SU-8 photoresist. A typical SEM image of the polymeric nanopillars is illustrated in figure 3 where SU-8 nanopillars with a diameter of 200 nm and a height of 1 μm were produced by curing the SU-8 photoresist in the silicon template formed by 400 nm polystyrene beads. Since the surfaces of SU-8 photoresist are hydrophobic, a very large water contact angle (up to 160°) [31] on the polymeric nanopillars was observed. These nanopillars were used to print protein nanoarrays via nanocontact printing. Shown in figure 4 is the confocal image of the FITC-conjugated fibronectin nanoarray printed by SU-8 nanopillars with a 200 nm diameter and 500 nm separation distance. As seen from the confocal image, well-ordered hexagonal close packed structures were reproduced by the nanocontact printing. To measure the size of the protein nanoarrays, AFM was used. The average height of the fibronectin nanoarrays was measured to be 11.5 nm with 20% variation in height and the periodicity was about 500 nm as indicated in the inset of figure 4, which closely resembled the dimension of the SU-8 nanopillar array. This result suggests that the polymeric nanopillar array produced by this approach can be used to create large-area high-resolution protein nanoarrays.

It is known that when cells are cultured on ECM patterns, the shape of the cells will be regulated by these patterns. However, if the pattern size was smaller than 20 μm , the cells would undergo apoptosis [6]. Massia *et al* [34] suggested that cells could proliferate on smaller ECM patterns as long as the separation distance between these patterns remained small. Pesen *et al* also reported similar results [16] where the fibroblast cells were found to attach and spread on 100 nm fibronectin patterns with 1 μm separation distance. However, no stress fiber was observed. In an effort to understand the spacing between the integrin ligands for cell spreading and the formation of focal adhesion on the nanometer scale, Spatz [20–23] *et al* have developed a unique patterning technique to control the spacing between RGD peptides, integrin binding ligands, using the self-assembly of diblock

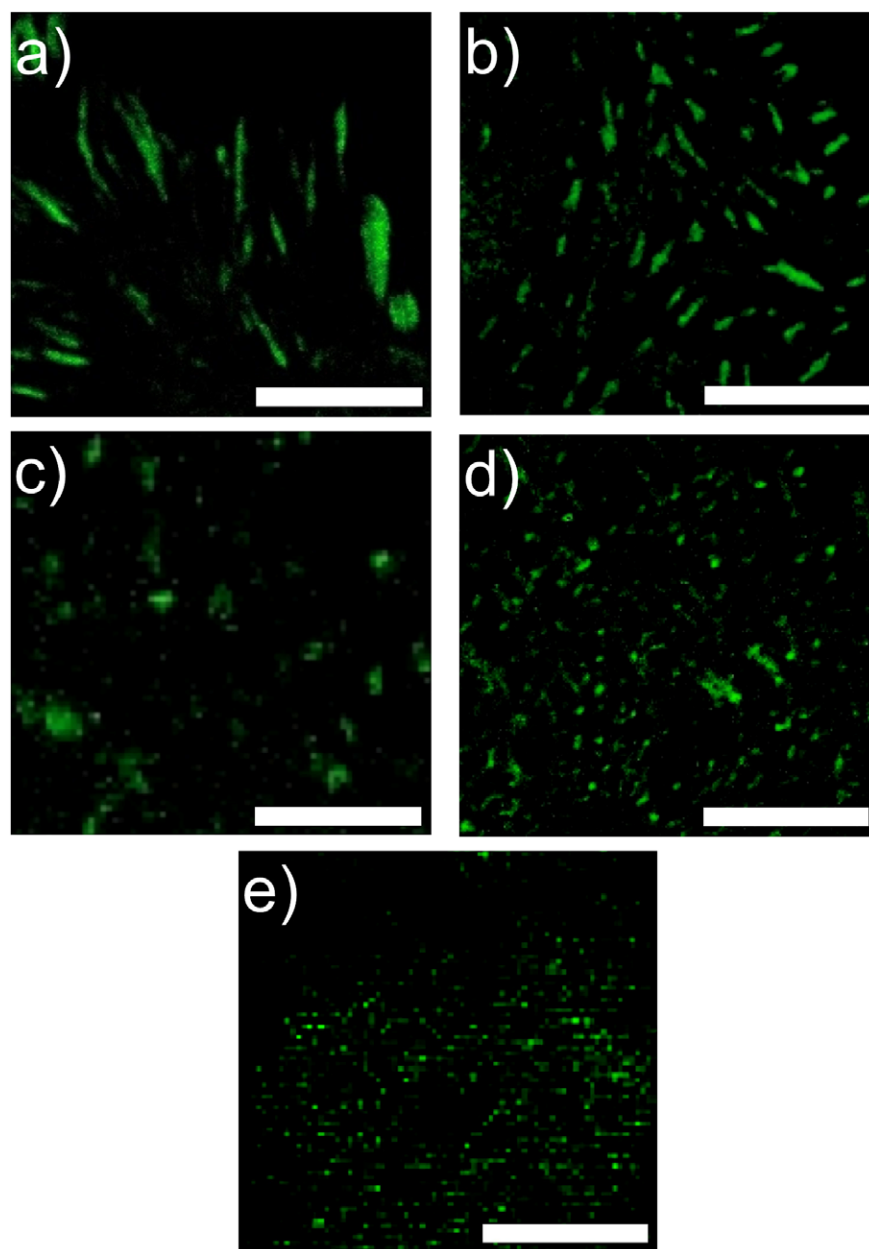


Figure 6. The confocal images of focal adhesions of CHO cells on (a) 100 μm , (b) 5 μm , (c) 2 μm , (d) 200 nm and (e) 100 nm fibronectin patterns. Vinculin molecules were labeled with FITC. The separation distances between fibronectin patterns were 100 μm , 5 μm , 2 μm , 400 nm and 400 nm, respectively. Bar: 10 μm .

copolymers. It was found that cell spreading and focal adhesion dynamics were regulated by the spacing between RGD peptides and the gradient of RGD peptide spacing could also induce the cell polarization and migration. When the separation distance between RGD peptides was larger than 73 nm, both the formation of focal adhesion and actin stress fibers were greatly reduced. However, it is not clear what is the minimum surface area required to form focal adhesion. To understand how cells behave on the ECM patterns in a size dependent manner, we have cultured CHO cells on different sizes of fibronectin nano- and micropatterns. The fluorescence images of CHO cells on a flat substrate and the substrate patterned with fibronectin nanoarrays are illustrated in figure 5

where the focal adhesions of cells were stained with green and actins were labeled with red. On the flat substrate, CHO cells formed large focal adhesions, which are located at both ends of the actin fibers. In contrast, the size of focal adhesions of CHO cells on the 400 nm fibronectin arrays with 890 nm separation distance was much smaller than those observed on the flat substrates. However, CHO cells could still adhere and spread on the fibronectin nanoarrays and stress fibers were also observed. Instead of forming focal adhesions at both ends of actin fibers, the focal adhesions on the nanopatterns were connected by the actin fibers. A similar result was reported previously [35] where the focal adhesions were found to be restricted to the micrometer size patterns (3 and 1 μm) whereas

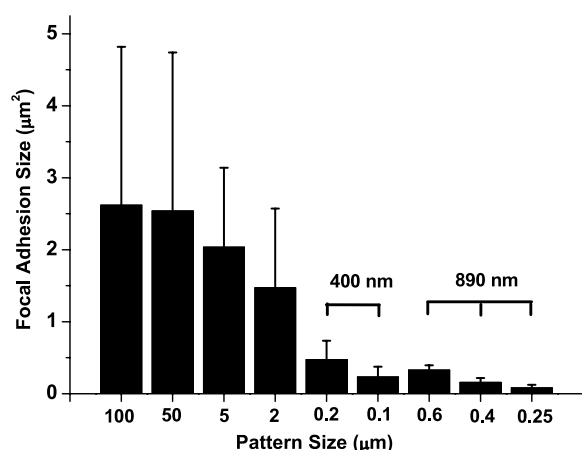


Figure 7. Average size of focal adhesion formed on various fibronectin patterns. The size of focal adhesion was roughly the same for pattern sizes larger than 50 μm . However, the size of focal adhesion on fibronectin patterns smaller than 5 μm was found to decrease with the size of the fibronectin pattern.

focal adhesions on the nanometer size patterns were bridged by actin fibers. It has been pointed out that the focal adhesions on micrometer size patterns could mature to withstand the pulling force during the cell spreading. However, it requires several actin bundles to stabilize the focal adhesions with nanometer size.

In this experiment, we have investigated the formation of focal adhesions of CHO cells on six different fibronectin micropatterns with pattern sizes of 100, 50, 20, 10, 5, 2 μm . The patterns were separated by the same dimension as their pattern size. As for nanopatterns, 890 nm polystyrene beads were used to create 600, 400 and 250 nm patterns whereas nanopatterns of 200 and 100 nm were prepared by 400 nm polystyrene beads. Shown in figure 6 are the confocal images of focal adhesions of CHO cells on various fibronectin patterns. When CHO cells were cultured on the fibronectin patterns larger than 50 μm , typical elongated focal adhesions with lengths of up to 5 μm were observed. The cell morphology was stretched and elongated. When the pattern sizes were reduced to 20 and 10 μm , the cells were found to confine to individual micropatterns. The cell morphology became round and the sizes of the cell were slightly larger than the patterns they were plated. No clear focal adhesion nor actin fiber was observed. The cells on these patterns underwent apoptosis and eventually detached from the surfaces. However, when the pattern size was further reduced to 5 μm where the separation between patterns was also 5 μm , CHO cells started to spread across several fibronectin patterns. The formation of focal adhesions was restricted on the area patterned with fibronectins while the actin fibers were found to cross several patterns connecting focal adhesions in different fibronectin patterns. The shapes of the focal adhesions resembled those observed on the flat surfaces. As the size of the fibronectin further reduced to a few hundred nanometers, the shape of the focal adhesion became symmetrical and the size of the focal adhesion also decreased. The spreading area of the cells was smaller than the cells on the micrometer fibronectin pattern. The average size of focal

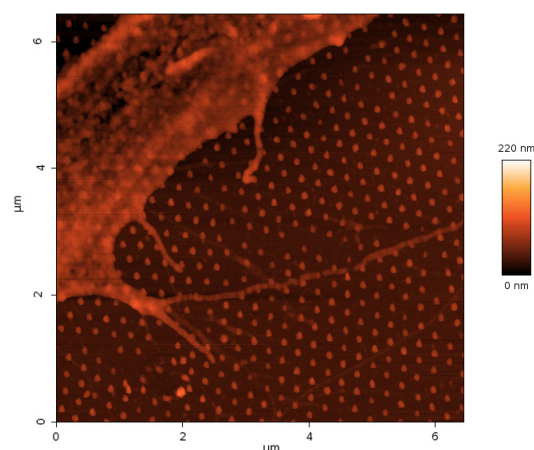


Figure 8. AFM image of a CHO cell on a 50 nm fibronectin nanoarray. It can be seen that the protrusions of cells followed the fibronectin patterns.

adhesion is summarized in figure 7. From figure 7, it is evident that the size of focal adhesion was roughly the same when the size of fibronectin patterns was larger than 50 μm . In other words, the CHO cells were not constrained by fibronectin patterns larger than 50 μm . They responded to these patterns as if they were on the flat substrates. When the size of the fibronectin patterns was smaller than 5 μm , the formation of focal adhesion was compromised as indicated by the reduced size of focal adhesion. As the size of the fibronectin patterns further decreased, the average size of the focal adhesion also decreased. It is known that transmembrane proteins, such as integrins, first bind to ECM molecules to form a focal complex. The focal complex matures to focal adhesion by both integrin clustering and recruiting more adhesion proteins to stabilize the focal adhesions. Since the footprint of the integrin binding sites was confined to the area patterned with fibronectin, their ability to mature to regular focal adhesion was greatly undermined. It may require the formation of actin networks to stabilize the focal adhesion on the nanopatterns [35]. The fact that the size of the focal adhesion did not scale linearly or quadratically with the pattern size indicates that more complicated focal adhesion dynamics are involved for cells adhering on fibronectin nanoarrays. Note that the size of focal adhesions for cells on the patterns fabricated using 890 nm polystyrene beads was smaller than those on patterns fabricated by 400 nm polystyrene beads because some of the focal adhesions formed in the fibronectin nanopatterns with a separation distance of 400 nm could not be distinguished due to the diffraction limit. One may need the help of newly developed nanoscopy [36] to investigate the detailed focal adhesion dynamics.

While the confocal microscope was used to provide the location of focal adhesion molecules in cells on the nanopatterns, its spatial resolution was not good enough to distinguish how the cells respond to even smaller nanopatterns. In this experiment, an AFM has also been employed to investigate the topographic information of cells on the fibronectin nanoarrays. Shown in figure 8 is a CHO cell cultured on a 50 nm fibronectin nanoarray, which was the

smallest fibronectin pattern fabricated in this experiment. It was found that the protruding edges of the cells always advanced to the area patterned with fibronectin and the edges of the cells always bound to the fibronectin nanoarrays. This result suggests that the shape and the polarization may be guided by ECM patterns even at such a small scale.

4. Conclusion

In summary, we have fabricated polymeric nanopillars with diameters ranging from 50 to 600 nm using a combination of nanosphere lithography, oxygen plasma treatment, deep etching and nanomolding processes. These polymeric nanopillar arrays have been used to create various sizes of fibronectin nanoarrays by nanocontact printing. When CHO cells were cultured on the fibronectin nanoarrays, it was found that cells can adhere and spread on all the fibronectin nanoarrays tested in this experiment and the protruding edges of the cells followed the fibronectin nanopatterns even with a pattern size as small as 50 nm. The average size of focal adhesion was observed to decrease as the diameter of fibronectin nanopatterns decreased. The size tunable nanosphere lithography offers several advantages in preparing well-ordered protein nanoarrays for many potential applications in cell biology and biosensors including (1) fast fabrication where stamps could be prepared within hours and protein arrays could be printed within seconds. (2) Diameter and separation distance of protein nanoarrays could be tuned independently, and (3) this technique is a high throughput and high-resolution printing process where large-area protein nanoarrays with a pattern size as small as 50 nm could be produced within seconds. The only drawback for using size tunable nanosphere lithography to create protein nanoarray is the defects (less than 10%) in the periodic patterns due to the self-assembly process.

Acknowledgments

This research was supported, in part, by National Science Council, Taiwan under contract 97-2628-M-001-010-MY3 and Academia Sinica Research Project on Nanoscience and Technology.

References

- [1] Morgan M R, Humphries M J and Bass M D 2007 *Nat. Rev. Mol. Cell. Biol.* **8** 957–69
- [2] Bershadsky A, Kozlov M and Geiger B 2006 *Curr. Opin. Cell Biol.* **18** 472–81
- [3] Zamir E et al 2000 *Nat. Cell. Biol.* **2** 191–6
- [4] Chen C S, Jiang X and Whitesides G M 2005 *MRS Bull.* **30** 194–201
- [5] Sniadecki N J, Desai R A, Ruiz S A and Chen C S 2005 *Ann. Biomed. Eng.* **34** 59–74
- [6] Chen C S, Mrksich M, Huang S, Whitesides G M and Ingber D E 1997 *Science* **276** 1425–8
- [7] Jiang X, Bruzewicz D A, Wong A P, Piel M and Whitesides G M 2005 *Proc. Natl Acad. Sci.* **102** 975–8
- [8] Brock A, Chang E, Ho C C, LeDuc P, Jiang X, Whitesides G M and Ingber D E 2003 *Langmuir* **19** 1611–7
- [9] Chen C S, Alonso J L, Ostuni E, Whitesides G M and Ingber D E 2003 *Biochem. Biophys. Res. Commun.* **307** 355–61
- [10] Guilak F, Cohen D M, Estes B T, Gimble J M, Liedtke W and Chen C S 2009 *Cell Stem Cell* **5** 17–26
- [11] Ruiz S A and Chen C S 2007 *Soft Matter* **3** 168–77
- [12] Khademhosseini A, Jon S, Suh K Y, Tran T-N T, Eng G, Yeh J, Seong J and Langer R 2003 *Adv. Mater.* **15** 1995–2000
- [13] Bernard A, Renault J P, Michel B, Bosshard H R and Delamarche E 2000 *Adv. Mater.* **12** 1067–70
- [14] Hyun J, Ma H, Zhang Z, Beebe T P Jr and Chilkoti A 2003 *Adv. Mater.* **15** 576–9
- [15] Li H W, Muir B V O, Fichet G and Huck W T S 2003 *Langmuir* **19** 1963–5
- [16] Pesen D, Heinz W F, Werbin J L, Hoh J H and Haviland D B 2007 *Soft Matter* **3** 1280–4
- [17] Lee K B, Park S-J, Mirkin C A, Smith J C and Mrksich M 2002 *Science* **295** 1702–5
- [18] Shiu J Y and Chen P 2005 *Adv. Mater.* **17** 1866–9
- [19] Shiu J Y and Chen P 2007 *Adv. Funct. Mater.* **17** 2680–6
- [20] Arnold M, Cavalcanti-Adam E A, Glass R, Blummel J, Eck W, Kantlehner M, Kessler H and Spatz J P 2004 *ChemPhysChem* **5** 383–8
- [21] Arnold M et al 2008 *Nano Lett.* **8** 2063–9
- [22] Cavalcanti-Adam E A, Volberg T, Micoulet A, Kessler H, Geiger B and Spatz J P 2007 *Biophys. J.* **92** 2964–74
- [23] Huand J, Grater S V, Corbellini F, Rinck S, Bock E, Kemkemer R, Kessler H, Ding J and Spatz J P 2009 *Nano Lett.* **9** 1111–6
- [24] Lee K Y, Alsberg E, Hsiong S, Comisar W, Linderman J, Ziff R and Mooney D 2004 *Nano Lett.* **4** 1501–6
- [25] Kong H J, Hsiong S and Mooney D J 2007 *Nano Lett.* **7** 161–6
- [26] Haynes C L and Van Duyne R P 2001 *J. Phys. Chem. B* **105** 5599–611
- [27] Shiu J Y, Kuo C W, Chen P and Mou C Y 2004 *Chem. Mater.* **16** 561–4
- [28] Kuo C W, Shiu J Y and Chen P 2003 *Chem. Mater.* **15** 2917–20
- [29] Kuo C W, Shiu J Y, Chen P and Somorjai G A 2003 *J. Phys. Chem. B* **107** 9950–3
- [30] Kuo C W, Shiu J Y, Cho Y-H and Chen P 2003 *Adv. Mater.* **15** 1065–8
- [31] Shiu J Y, Whang W T and Chen P 2008 *J. Adhes. Sci. Technol.* **22** 1883–91
- [32] Kuo C W, Shiu J Y, Chien F C, Tsai S M, Chueh D Y and Chen P 2010 *Electrophoresis* **31** 3152–8
- [33] Kuo C W, Wei K H, Lin C H, Shiu J Y and Chen P 2008 *Electrophoresis* **29** 2931–8
- [34] Massia S P and Hubbell J A 1991 *J. Cell Biol.* **114** 1089–100
- [35] Arnold M, Schwieder M, Blummel J, Cavalcanti-Adam E A, Lopez-Garcia M, Walther P, Kessler H, Geiger B and Spatz J P 2009 *Soft Mater* **5** 72–7
- [36] Hell S W 2007 *Science* **316** 1153–8