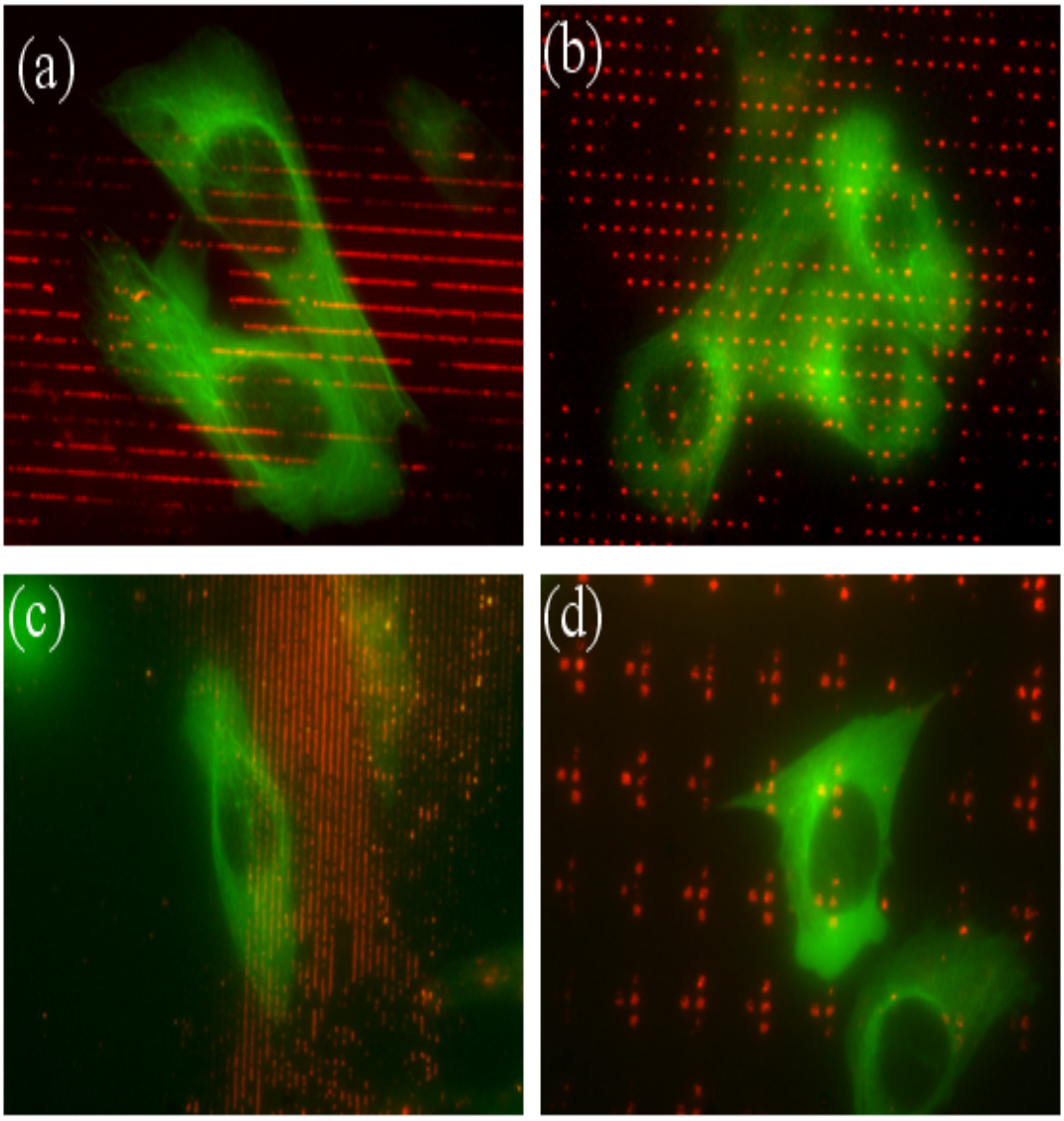

Figure 6.12 CHO-Tubulin cells (Green) were attached on the protein sub-micro patterns (red).Figure (a) shows 0.6 um solid lines, figure (b) shows dash-lines ,figure (c) and (d) shows the cells attached on vertical solid lines and T-shape patterns

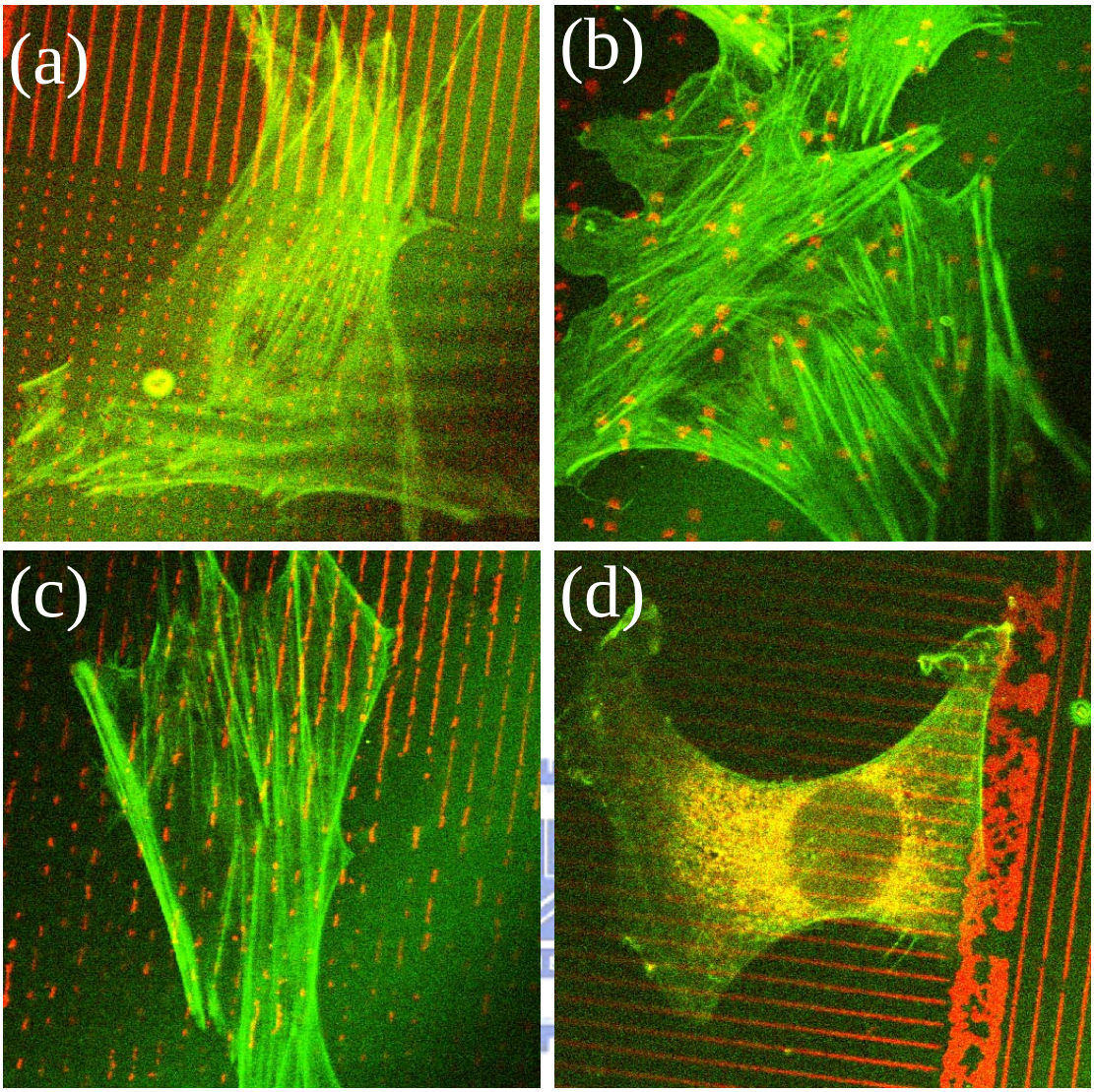

Figure 6.13 3T3 fibroblast cells (Green) were attached on the protein sub-micro patterns (red).Figure (a) shows 0.6 um small dash lines with solid line (3A) of protein micropatterns and figure (b) shows T-shape (4C) of protein micropatterns . The figure (c) and (d) shows the cells attached on horizontal and vertical solid lines (2C and 2D) (fluorescence image).

6.4.4 Macrophage cells

In this chapter, the motility dynamics of cells is also observed in sub-micro scale. The Macrophage cells will be put on 0.3 um line patterns (Left) and homogenous substrate (Right) and observed. From figure 6.14, we could observe that the Macrophages have migrated and rotated on these positions by fluorescence microscope in these two conditions. In 0.3 um sub-micro patterns, the cells are individually located on the surface with a low-speed rotate velocity. But in homogeneous substrate, the cells are closely located on the surface with a high-speed rotate velocity. We suppose the sub-micropatterns will reduce the adhesion between cells and surface and cause the migration and rotation speed down.

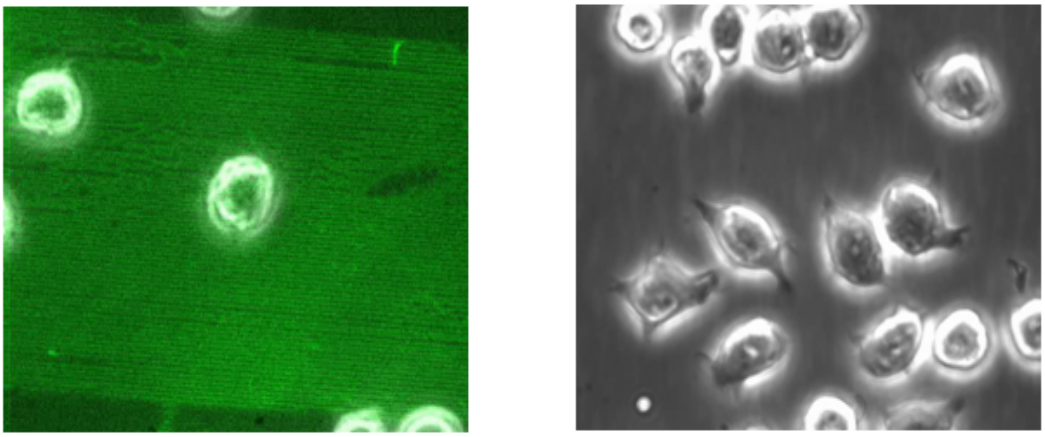

Figure 6.14 The Macrophage cells were put on 0.3 um line patterns (Left) and homogenous substrate (Right)

Chapter 7 Conclusions

In this thesis, we propose our idea —fabricating protein micropatterns with accuracy of sub-micro resolution by DUV photography and Micro Contact Printing (μ CP) method, comparing the pattern accuracy with variable size ,pitch and different shape in each steps of μCP method and observing the neuron and cell outgrowth in these successfully fabricated sub-micro protein patterns.

We successfully demonstrated the realization of fabricating protein sub-micro patterns for cell biology applications. To successfully generate the protein sub-micro patterns, some new designed procedures were involved into the DUV photolithography with micro contact printing process, such as TEOS film, RIE etching and AR3 coating. For the requirements of flexible aspect ratio, TEOS film was added as a hard mask between photoresist and substrate for increasing the etching selectivity. According to our experimental results, the aspect ratio could be offered from 1.5 to 4.5 by RIE recipe tuning. When the pattern size is into sub-micro level, the sticking issue between silicon and PDMS will be getting worse. Besides, sticking problem also has very strong dependence with pattern sizes, pitches and shapes. We analyze the chemical mechanisms and select suitable material to be used as the buffer layer to avoid the crosslinking from these two materials. The AR3 layer was employed to be a buffer layer and successfully reduce the issue of pattern sticking.

Several test patterns with different shapes; variable sizes, and pitches were designed and fabricated. In each steps, the patterns were measured and analyzed. By the accuracy comparison Table 6.1, almost all test patterns could be generated into protein

patterns except 1:1 0.3 um line and space. That might be due to the size and pitch dependence with resolution thus causing the patterns fail.

The results demonstrate that protein sub-micro patterns have been fabricated by DUV photolithography with micro contact printing. It provides a stable, accurate and workable platform to generate functional surfaces for cell biology investigations.

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Sub-microfabrication of Protein Micropatterns for cell biology applications

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Abstract

We successfully demonstrated the realization of fabricating protein sub-micro patterns for cell biology applications. To successfully generate the protein sub-micro patterns, some new designed procedures were involved into the DUV photolithography with micro contact printing process, such as TEOS film, RIE etching and AR3 coating. For the flexible aspect ratio requirements, TEOS film was added as a hard mask between photoresist and substrate for increasing the etching selectivity. According to our experimental results, the aspect ratio could be offered from 1.5 to 4.5 by RIE recipe tuning. When the pattern size is into sub-micro level, the sticking issue between silicon and PDMS will be getting worse. Besides, it also has very strong dependence with pattern sizes, pitches and shapes. We analyze the chemical mechanisms and select suitable material to be the buffer layer to avoid the crosslinking from these two materials. The AR3 layer was employed to be a buffer layer and successfully reduce the issue of pattern sticking. After that, we observed the neuron and cell outgrowth in these special designed protein sub-micro patterns. The investigated patterning process that combining with DUV photolithography and micro contact printing could be used to generate functional surfaces for cell biology applications

1. Introduction

During nerve regeneration, injured neurons often exhibit a significant capacity for re-growth, but the repair usually fails because the newly developed neurites can not be directed to appropriate target locations where they can form functional synapses with other cells. The long-distance targeting that is characteristic of nerve cells suggests that neurite growth and regeneration might be steered by adhesive pathways and topographic channeling. In developing embryo, neurons in vivo are presented with a complex array of guidance cues that direct neurons extend their processes in a highly oriented fashion. [1] These guidance cues that are functional bio-molecule of extracellular matrix (ECM) protein such as laminin one of the most effective to control neuronal cell growth in vitro. Thus, the accuracy of micropatterns would be very important to precisely control aspects of neurite growth in vitro.

In the last two decades, several techniques have been developed to generate patterns of functional bio-molecules on artificial surfaces to be used for biosensors, for cell biology studies and tissue engineering applications. The use methods include local deposition of molecules using ink-jet techniques and other microfluidic systems, micro contact printing (μCP, soft lithography) technique, photochemical patterning technique and photolithography technique (Ex. lift-off and plasma etching techniques). [2]

Currently, the most popular technique is micro contact printing (μCP, soft lithography). Here a polymer printing stamp, usually PDMS, PolyDiMethySiloxane is cast using a master that is produced with photolithography and silicon etching techniques. The stamp is then used to imprint bio-molecules from an aqueous solution onto a culture substrate. The printing process can be carried out rapidly without the need for expensive equipment and allows the transfer of molecules to surfaces in a wide concentration range with high efficiency, and also on curved surfaces. [2, 3]

But some further investigations, sub-micro patterns are indeed required such as artificial neuron networks combining with thin-film microstructure, where distances between outgrowing neurons and electrode surfaces may significantly influence the signal transfer. [2]

In some cell growth and migration studies, cells were shifted from growth to apoptosis by using substrates that contained ECM coating adhesive islands of decreasing size. The results illustrate that the growth index depends on the size of islands [4, 5]. Sub-micro patterns could not only offer new opportunities to push the growth rate but also other new applications of cell biology.

Recently, investigators have employed high resolution patterns techniques, such as photolithography techniques adapted from IC or MEMS industries [6]. The resolution has been pushed into sub-micro by KrF scanner, etc (DUV process) and globally be used in fabricating [6]. It makes possible to fabricate sub-micro protein patterns by integrating two regions, micro contact printing and DUV photolithography.

In this paper, we design several sizes, pitches, and shapes sub-micro patterns to verify the accuracy of process and compare in each step. Then, cell outgrowth will be observed in these functional protein patterns.

2. Experimental

2.1 Mask layout design

In our approach, the layout of fabricating sub-micro protein patterns that we design is illustrated in Table 1. 6 set of patterns to be plotted; including line patterns (0.3 and 0.6um), line combing dash line patterns, T shape (1.8um) and dash line patterns (0.6um). Each of them has 4 steps variation with different pitch.

2.2 New procedures of Micro contact printing method

In this work, we use the DUV lithography to fabricate sub-micro patterns. Through our related researches, photoresist base is used as the master [4]. But in DUV lithography, the thickness of photoresist is usually less than 1um.That means the aspect ratio will be limited.

Upon this, we re-design the procedure of Micro contact printing (μCP) method and add TEOS as a new film stacking above silicon structure of wafer. The TEOS is an oxide film and will be a hard mask to increase the etching selectivity between DUV photoresist and silicon.

According to our experimental results, the aspect ratio could be increasing from 1.5 to 4.5 (Figure 1)

Finally, we will use the new procedure of Micro contact printing (μCP) method to fabricate sub-micro protein patterns and compare the pattern accuracy with variable size (0.3, 0.6 um), pitch (1:1~1:4) and different shape in each step.

Table 1 The list of mask layout design.

No.	Shape (Unit : um)		Space (Unit : um)
1A	Solid Line (0.3 × 900)	Space	0.3
1B			0.6
1C			0.9
1D			1.2
2A	Solid Line (0.6 × 900)	Space	0.6
2B			1.2
2C			1.8
2D			2.4
3A	[Solid+Dash]Line : (0.6 × 375 : 0.6 × 1.2)	Space	0.6
3B			1.2
3C			1.8
3D			2.4
4A	T shape : (4 pieces 1.8 × 1.8 um square)	Space	0.6
4B			1.2
4C			1.8
4D			2.4
5A	Middle Dash Line : (0.6× 12)	Space	0.6
5B			1.2
5C			1.8
5D			2.4
6A	Long Dash Line : (0.6 × 60)	Space	0.6
6B			1.2
6C			1.8
6D			2.4

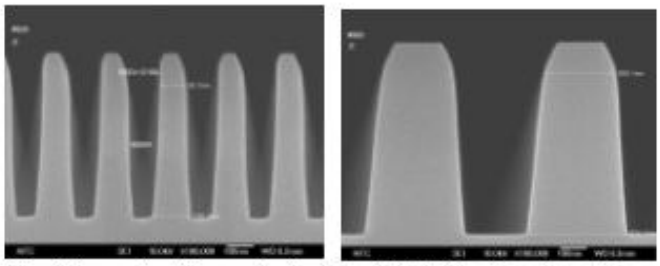


Fig.1 Aspect ratio controlled by TEOS film hard mask

As shown in figure 2, the rectangular frame marked by dashed outline represents the new procedures of Micro contact printing (μCP) method we re-designed. We deposited a TEOS film with 0.3 um thickness above the silicon structure of wafer. After

photolithography, we use RIE etching and transfer of the patterns into the TEOS film, then using RIE etching again and the patterns have been successfully transfer into substrate of silicon.

After all, a new step is added in photolithography process. In figure 3, the AR3 layer is used to be buffer layer to avoid sticking issue between silicon and PDMS and gets a silicon base of master.

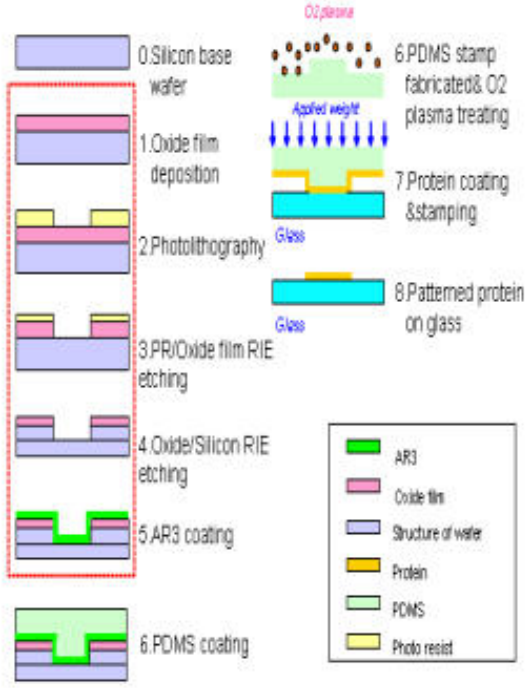


Figure 2 Schematic illustration of the procedure of silicon base of Micro Contact Printing

During the new procedures, we could control the aspect ratio by tuning etching recipe. In theory, we can get 5 times Photoresist/Silicon etching selectivity by TEOS hard mask.

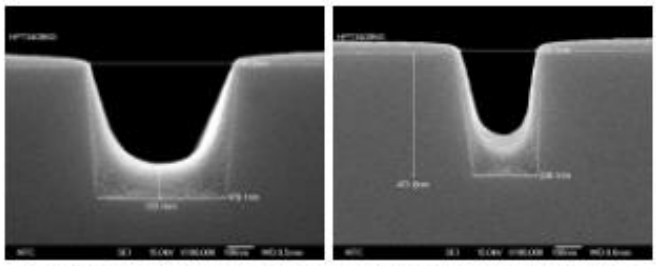


Fig.3 .The profile of silicon master with AR3 coating. Pattern size is 0.6 um (Left) and 0.3 um (Right)

2.3 DUV photolithography

In this work we use KrF (λ=248 nm) scanner for fabricating sub-micro patterns. Silicon with TEOS films of wafers was primed with HMDS. The UV82 (Shipley) photoresist were spun to the thickness (0.6 um) and baked at 130°C for 90 sec. The DUV scanner is S206B (Nikon)and the illumination we choice is NA=0.82,σ=0.85 for exposure. After exposure, the samples were post-exposure-baked at 130°C for 90 sec and developed by developer track ACT-8 (TEL) for 2 min. Then the photoresist masters were generated.

2.4 Hard mask and RIE etching

We deposited a TEOS film with 0.3 um thickness above the silicon structure of wafer. After photolithography, we use RIE etcher LAM 9400 (LAM) with recipe 10mT/350TCP/100BP/70Cl2/ 100HBr and transfer of the patterns into the TEOS film, then using RIE etcher again and the patterns have been successfully transfer into substrate of silicon.

2.5 Buffer layer coating

After all, the samples have to be re-coated an anti-reflection layer AR3 (Shipley) 700A and baking at 130°C for 90 sec as a buffer layer to avoid sticking issue between silicon and PDMS and get a silicon base of master.

2.6 PDMS stamp

Then we prepared by casting the liquid prepolymer of PDMS against a master that has a patterned relief structure. After that, The PDMS stamp is treated in oxygen plasma cleaner (Diener electronic) evacuated with a mechanical roughing pump for 20 s at 100W prior to protein immobilization.

2.7 Protein micropatterns

Laminin solution (50 μg /ml in PBS) is dropped on the surface of the patterned stamp for 4 hours at room temperature to allow for protein adsorption. The stamps were rinsed with diluted PBS and distilled water and then blown dry with a nitrogen blow-off gun. The stamp is placed in contact with 2.2 cm2 glass coverslip for 2 min. The coverslips are then immersed in sterile PBS before cell plating.

3. Results and Discussions

Protein sub-micro patterns have successfully been generated by DUV lithography with micro contact printing method. We will analyze and compare each processing steps in the following.

3.1 Photolithography

From figure 4, 6 sets of patterns were generated by lithography process. The exposure condition is NA=0.82,σ=0.85 (Nikon S206) with 6000A resist thickness (Shipley UV-82)

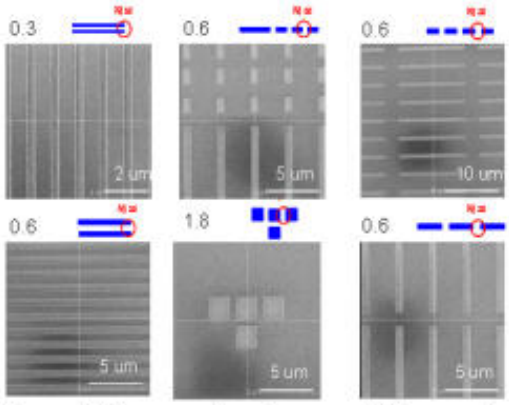
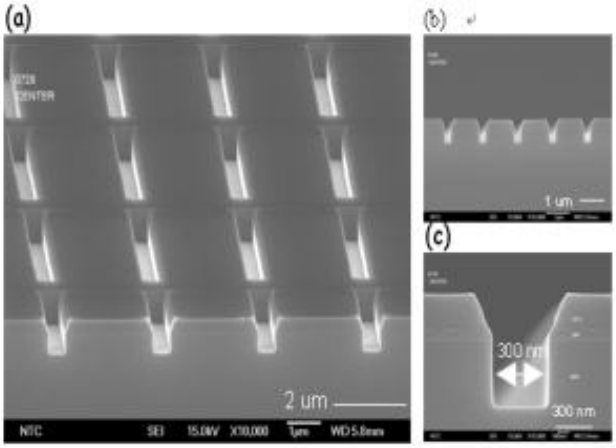


Figure 4 .The top view pictures of 6 sets of patterns by lithography process (Unit: um)

3.2 Silicon layers

In figure 5, 0.6 um dash line pattern (5B) could be generated in RIE etcher (LAM 9400). Figure (a) shows the cross section in 5 degree tilt. (b) and (c) show the cross section with 15kvX15000 and 15kvX75000.The profile of oxide film is taper with 30 degree and sharp in silicon structure. The thickness of oxide film and



silicon structure are 2400A and 4600 A

Figure 5 .The cross-section pictures of dash lines (5B) after RIE etching

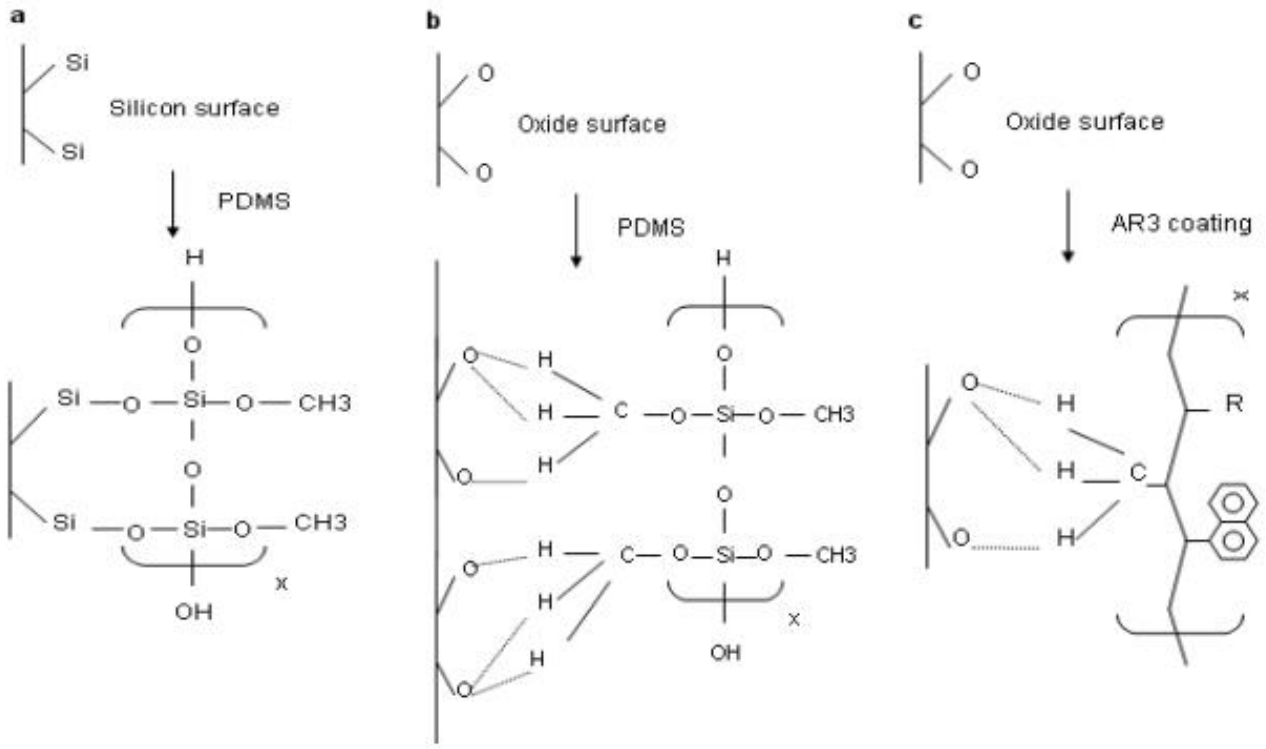


Figure 6 .The mechanisms of PDMS sticking issue with (a) Silicon surface (b) oxide surface (c) oxide and AR3 pre-formed

3.3 Chemical mechanism of buffer layer

A problem of the sub-micro-size micro contact printing process is that fine patterns such as narrow dense lines start pattern missing and sticking with PDMS layers in the procedure of PDMS stamp fabrication.
In figure 6(a), the surfaces of silicon will crosslink with PDMS. When the pattern size is into sub-micro level, the sticking issue will be getting worse. The oxide film could not avoid the sticking issue because the hydrogen bonding still happened between both as shown in Figure 6(b). Thus, a buffer layer must be pre-formed before the PDMS coating to prevent the sticking issue.
The AR3 layer was employed to be a buffer layer and successfully reduce the issue of pattern sticking. In figure 6(c), it could not only be immobilized on the oxide film because of hydrogen bonding and separated between PDMS and oxide film but also low binding between AR3 and PDMS. Besides, AR3 is a conformal type of adhesion layers and covered along the surface topography of the substrates (Figure 3). It is also a stable and elastomeric polymer. For these advantages, it is very useful to avoid the sticking issue and guarantee the designed pattern completely to be printing down to the PDMS layers.

3.4 PDMS layers

As figure 7, 6 sets of patterns could be generated by silicon masters stamping. Figure 8 shows the cross section of short dash lines (3C) PDMS patterns. The PDMS patterns have taper profiles. From the results, the reliability of the sub-micro patterns is good and no more sticking issues in this procedure. The limit of resolution of the combined process is estimated to be less than 0.3 um.

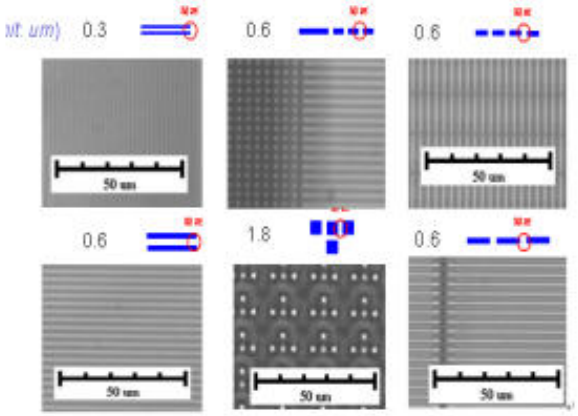


Figure 7 .The top view pictures of 6 sets of PDMS patterns by masters. (Unit: um)

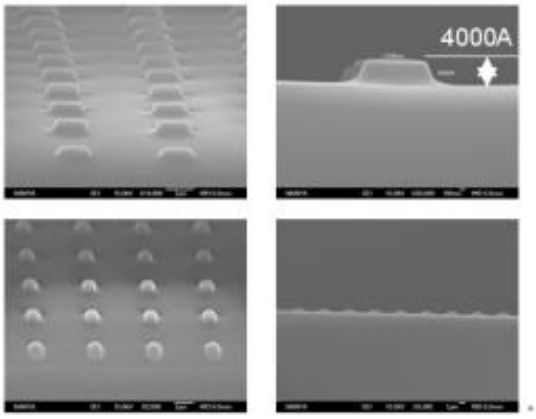


Figure 8 .The cross-section pictures of short dash lines (3C) PDMS patterns

3.5 Protein layers

Figure 9 shows that 6 sets of protein patterns were generated by PDMS stamping process. The 0.3 um patterns are not so good (Up-Left) and the other 0.6 um patterns are good.

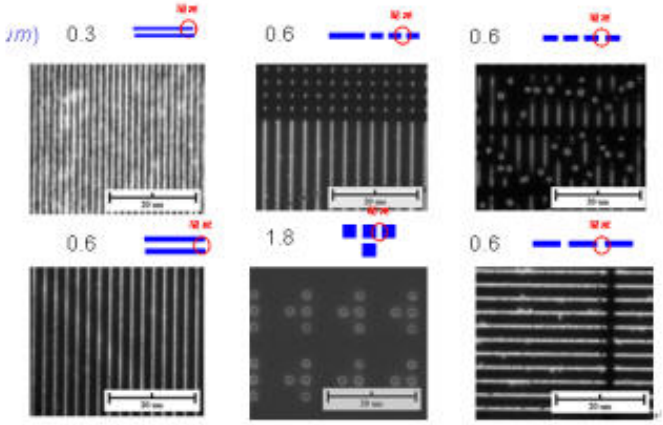


Figure 9.The top view pictures of 6 sets of protein patterns

3.6 Accuracy comparisons

We have compared two different linewidth (0.3 um, 0.6 um) and four variable pitches in each process step (Table 2). We got some results as below.
First, the silicon base could offer enough accuracy in every pitch in 0.6 um linewidth but in 0.3 um linewidth, the equal line and space was getting worse in procedure of PDMS. The same trend will be found in procedure of protein patterns. That means the pitch and size of patterns became smaller; the accuracy of PDMS and protein printing will be in decay. (Figure 10)
Moreover, the size of 0.3 um and 0.6 um linewidth protein micro patterns are both successfully printed in each pattern shapes as well as in all variable pitches.

Table2. Comparison tables in each step of process

O Good Δ Not so good X poor

Method\ Design	Size (um)	0.3				0.6			
	Space/Line	1	2	3	4	1	2	3	4
	No.	1A	1B	1C	1D	2A	2B	2C	2D
New Method Si base	Resist	O	O	O	O	O	O	O	O
	Silicon	-	-	-	-	-	-	-	-
	PDMS	Δ	O	O	O	O	O	O	O
	Protein	X	Δ	O	O	O	O	O	O

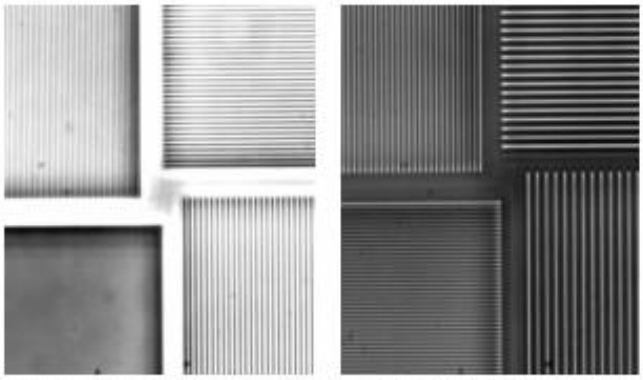


Figure 10.The top view pictures of 0.3 um through pitch (Left) and 0.6 um through pitch (Right) protein patterns

3.7 Cell biology applications

In this work, the outgrowth of cells gave direct evidence for the functionality of the sub-micro protein patterns. All the six sets of protein patterns were able to be utilized for outgrowth of cells. Two protein micropatterns with 0.3 um line and T shape structures were generated following the process we re-designed as illustrated in the figure 11(a) and (b) ,respectively. The cells (Macrophages) were directly attached on the positions that were marked by the arrows.
From the figure 11(c) and (d), we could observe that the Macrophages have migrated and rotated on these positions by fluorescence microscope. The findings prove that the DUV photolithography combining micro contact printing could generated sub-micro protein patterns and be used in cell investigations.

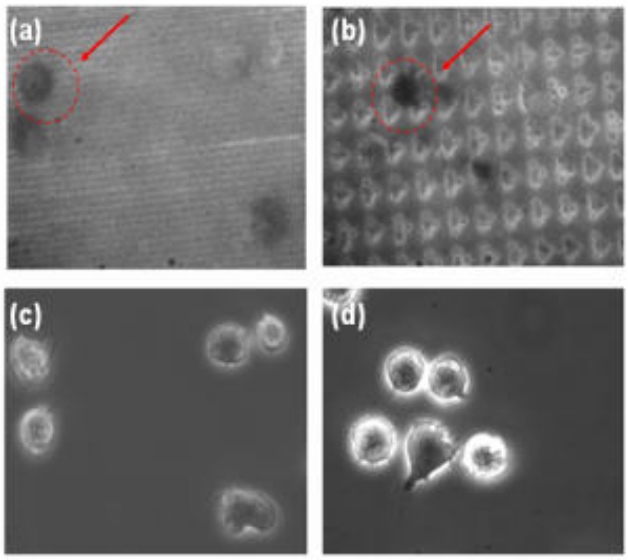


Figure 11 Macrophage cells were attached on the 0.3 um line of protein micropatterns (a) and T-shape of protein micropatterns (b) as marked by the arrows. The figure (c) and (d) shows the magnified pictures of Macrophage cells outgrowth on the positions marked by arrows in figure (a) and (b) (fluorescence image).

4. Conclusions

We successfully demonstrated the realization of fabricating protein sub-micro patterns for cell biology applications. To successfully generate the protein sub-micro patterns, some new designed procedures were involved into the DUV photolithography with micro contact printing process, such as TEOS film, RIE etching and AR3 coating. For the flexible aspect ratio requirements, TEOS film was added as a hard mask between photoresist and substrate for increasing the etching selectivity. According to our experimental results, the aspect ratio could be offered from 1.5 to 4.5 by RIE recipe tuning. When the pattern size is into sub-micro level, the sticking issue between silicon and PDMS will be getting worse. Besides, it also has very strong dependence with pattern sizes, pitches and shapes. We analyze the chemical mechanisms and select suitable material to be the buffer layer to avoid the crosslinking from these two materials. The AR3 layer was employed to be a buffer layer and successfully reduce the issue of pattern sticking.

Several test patterns with different shapes, variable sizes, and pitches were designed and fabricated. In each steps, the patterns were measured and analyzed. By the accuracy comparison Table 2, almost all test patterns could be generated into protein except 1:1 0.3 um line and space. That might be due to the size and pitch dependence with resolution thus causing the patterns fail.

The results demonstrate that protein sub-micro patterns have been fabricated by DUV photolithography with micro contact printing. It provides a stable, accurate and convenient platform to generate functional surfaces for cell biology investigations.

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　　88.3 ~ 89.6　世大積電 光罩技術作業處 製程工程師
　　89.6 ~ 89.10　台積電　光罩製作二部　製程工程師
現　職：89.10~93.　南亞科技 模組開發處 黃光模組開發課　專案經理

專　長：
1. 製作流程改善
2. 先進製作技術開發
3. 良率提昇與改善
4. 製程標準化建立

發表文獻：

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發明第 161143 號　檢測光罩機台修正精確度之方法
發明第 165606 號　檢測光罩機台修正精確度之方法
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