6. Discussions

6.1 Control of Aspect ratio

6.1.1 Silicon structure

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 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. If we change the power, pressure and flow rate of gases, the Photoresist/Silicon etching selectivity will be increasing. But there is a tradeoff if the selectivity is much higher, the profile of patterns will be worse.

Thus, we selected recipe as 10mT/350TCP/100BP/70Cl2/ 100HBr because it could keep sharp profiles if the aspect ratio was less than 4.5. The easy way to control the aspect ratio is by etching time. In figure 6.1 shows the diagram between etching time and aspect ratio. When the etching time was from 60 sec to 120 sec, there is a linear relation between both and aspect ratio increased from 1.58 to 4.55.But when the etching time was from 120 sec to 180 sec, we will get a flat curve and the aspect ratio only increase from 4.55 to 5.52 .By the way, the profile of patterns were getting worse by loading effects..

In figure 6.2 (a), the depth of silicon structure is 500 nm and the CD size is 110 nm. The aspect ratio was about 4.5. In figure 6.2 (b), the depth of silicon structure is 500

nm and the CD size is 375 nm. The aspect ratio was about 1.33. According to the experimental results, the aspect ratio could be controlled from 1.33 to 4.5 and keep good profiles.

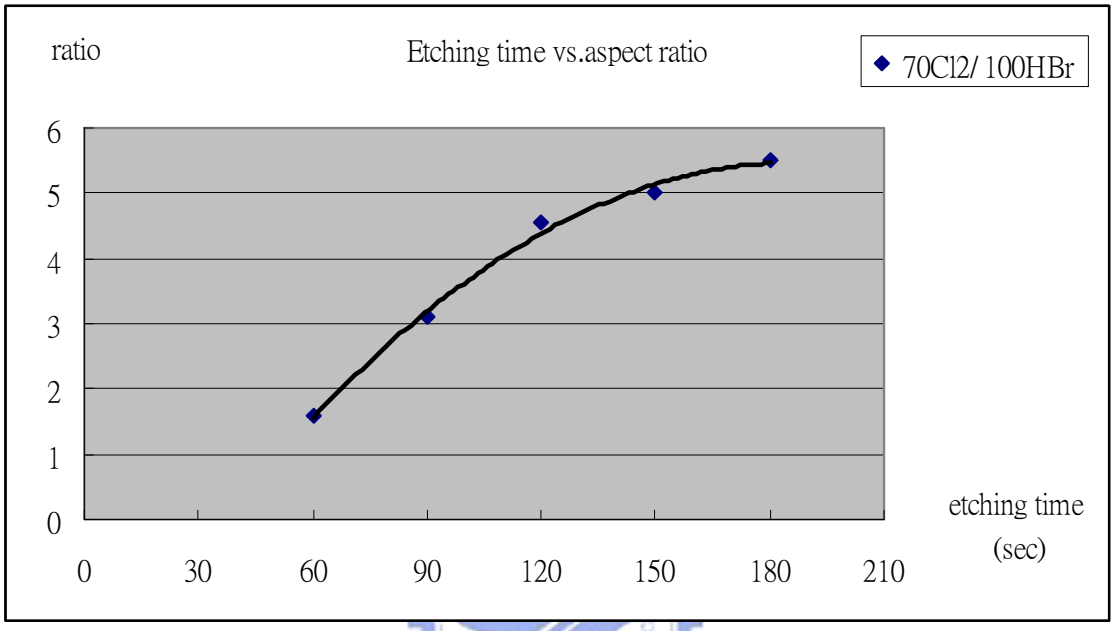


Figure 6.1 the diagram between etching time and aspect ratio.

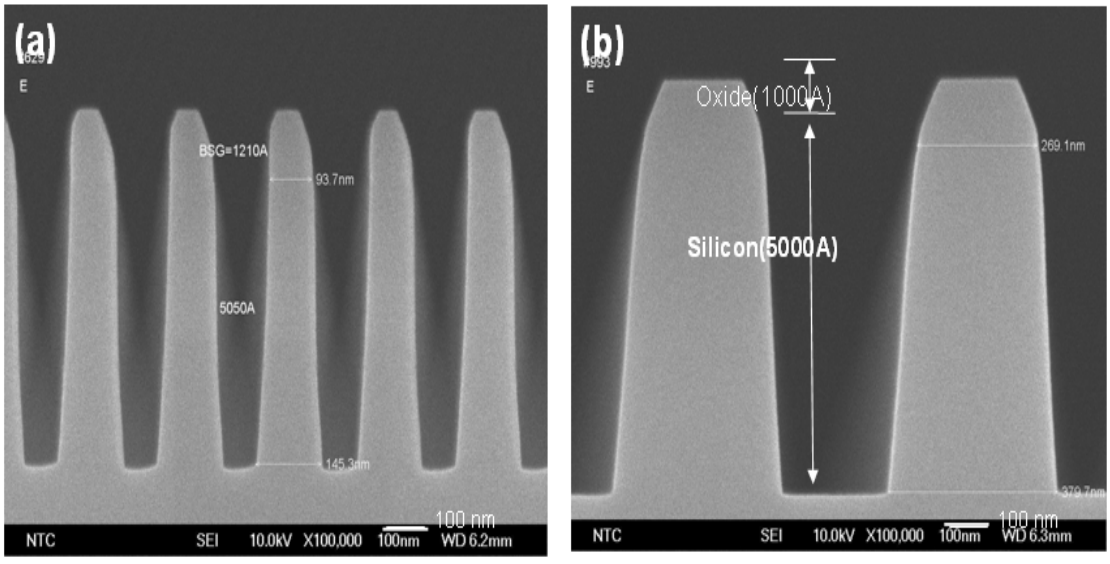


Figure 6.2 (a) the depth of silicon structure is 500 nm and the CD size is 110 nm. The aspect ratio was close to 4.5. (b) the depth of silicon structure is 500 nm and the CD size is 375 nm. The aspect ratio was close to 1.33.

6.1.2 PDMS structure

It is also very important for us to investigate the mechanism of different aspect ratio patterns transferring from silicon to PDMS structures. In this section, we select 0.3 um solid lines and two different dimensions (0.6:0.6 um and 0.6:1.2 um) of dash line patterns with two different depths of silicon structures (4000A and 12000A) to do the study. In fact, dash lines are very sensitive patterns than solid lines to detect the pattern top lose and sticking issues.

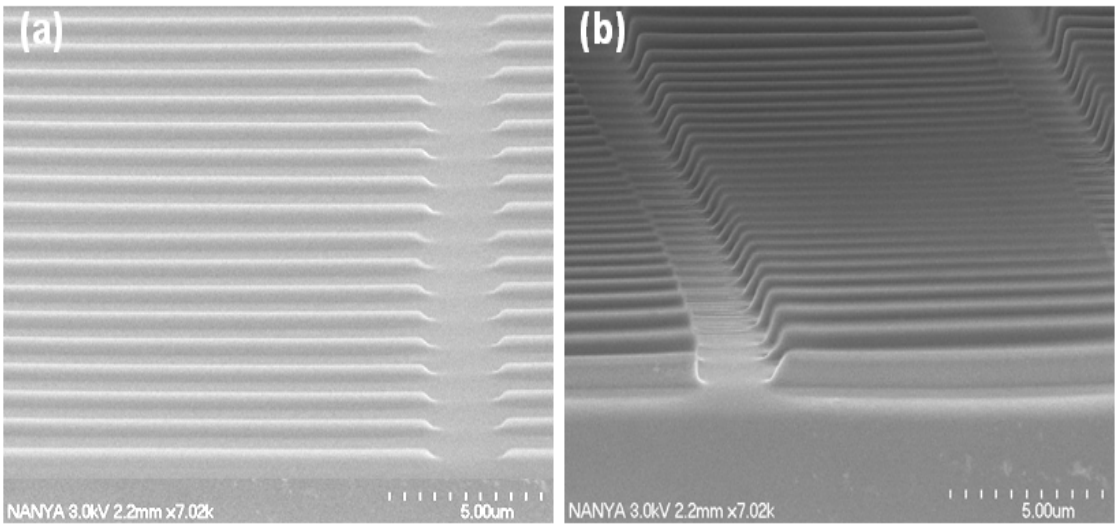


Figure 6.3 Stamping results (solid lines) in 70000X from (a) 4000A and (b) 120000A depth of silicon structures

In figure 6.3, 0.3 um solid line PDMS patterns were transferred from (a) 4000A and (b) 12000A depths of silicon structures in 70000X.The patterns seem good in both depths. In fact, the aspect ratio could reach to 4 when the depth of silicon structure is 12000A.

In figure 6.4, 0.6 um (1:2) dash line PDMS patterns were transferred from 4000A depth of silicon structures in (a) 14000X and (b) 30000X. Figure 6.3 (c) and (d) showed 10000X and 40000X PDMS patterns were transferred from 12000A depth of silicon

structures. With increasing 3 times aspect ratio of silicon structures, PDMS patterns could still keep the same aspect ratio and no extra top lose from stamping. The pattern sticking versus the increasing of aspect ratio seems not be a problem in these patterns.

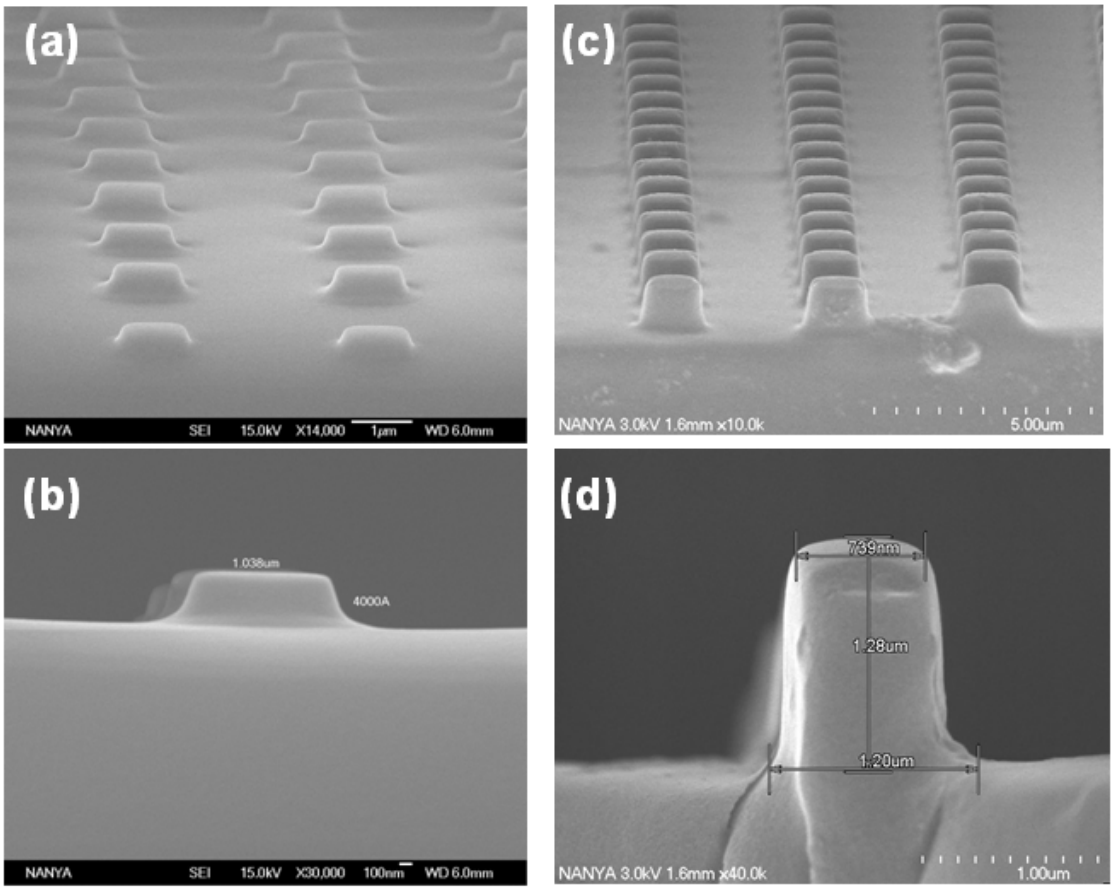


Figure 6.4 Stamping results (1:2 dash lines) in (a) 14000X and (b) 30000X from 4000A depth, (c) 10000X and (d) 40000X from 12000A depth of silicon structures.

In figure 6.5, 0.6 um (1:1) dash line PDMS patterns were also transferred from 4000A depth (right) and 12000A depth (left) of silicon structures. There are also similar results with 1:2 dimension patterns. Top lose and sticking of patterns were well controlled by buffer layers. But pattern collapsing was getting worse when the pattern dimension become smaller.

These facts also proved that the buffer layers could play good roles to prevent the pattern sticking and top lose issues when the depths were increased from 4000A to

12000A. If the pattern sizes were too small, the patterns are easily collapsing or distorting after the procedure of stamping. That means the micro contact printing has its limitation of high aspect ratio even we use AR3 to be buffer layers.

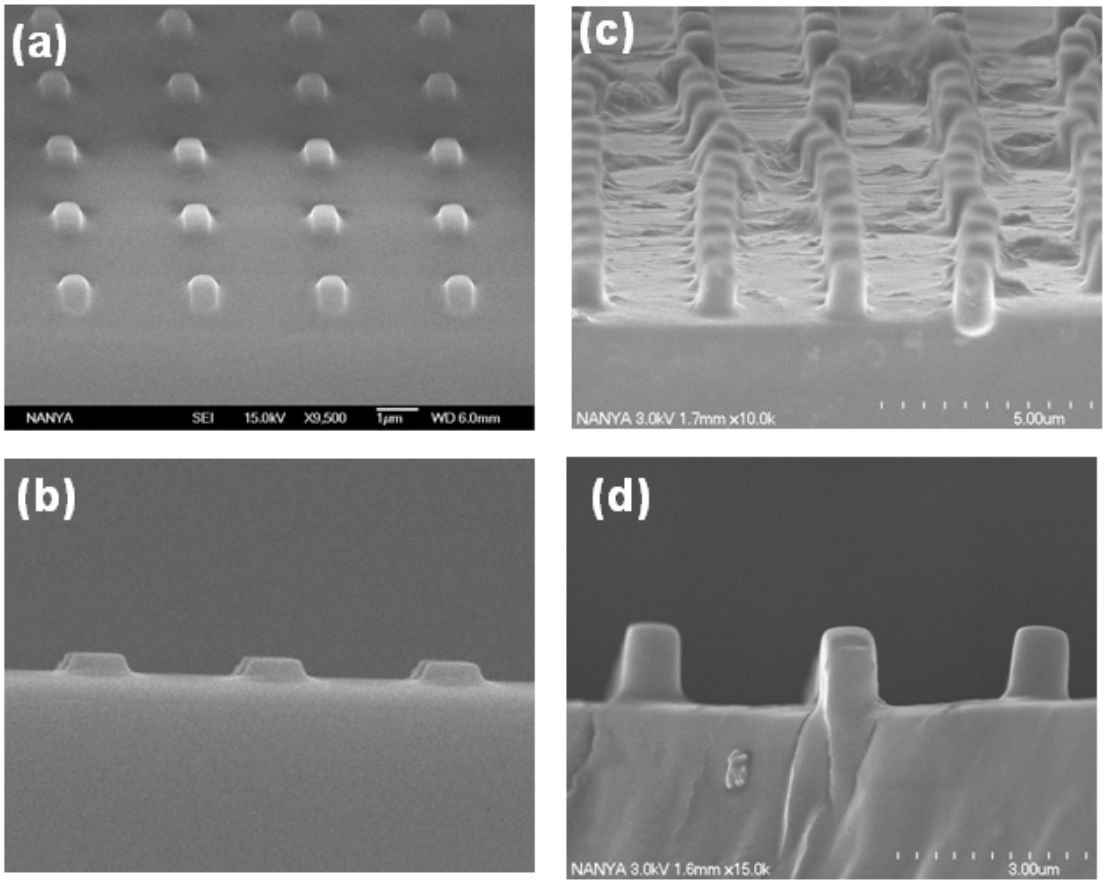


Figure 6.5 Stamping results (1:1 dash lines) in (a) 14000X and (b) 30000X from 4000A depth, (c) 10000X and (d) 40000X from 12000A depth of silicon structures.

6.2 Chemical mechanism of buffer layers

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. The chemical formula of PDMS is in Figure 6.6(b)

In figure 6.7(a), the surfaces of silicon will crosslink with PDMS. That is why 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 6.7(b). The forces of hydrogen bonding are lighter than crosslink but still cause the pattern sticking in sub-micro level. Thus, a buffer layer must be pre-formed before the PDMS coating to prevent the sticking issue. Several materials have been used such as Teflon, SDS solution (surfactants), HMDS supposing to avoid the sticking issues. But it is a pity that these materials could work in micro size but fail in sub-micro size level.

The AR3 layer was employed to be a buffer layer and successfully reduce the issue of pattern sticking in sub-micro level size. The chemical formula of AR3 is in Figure 6.6(a). In figure 6.7(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.

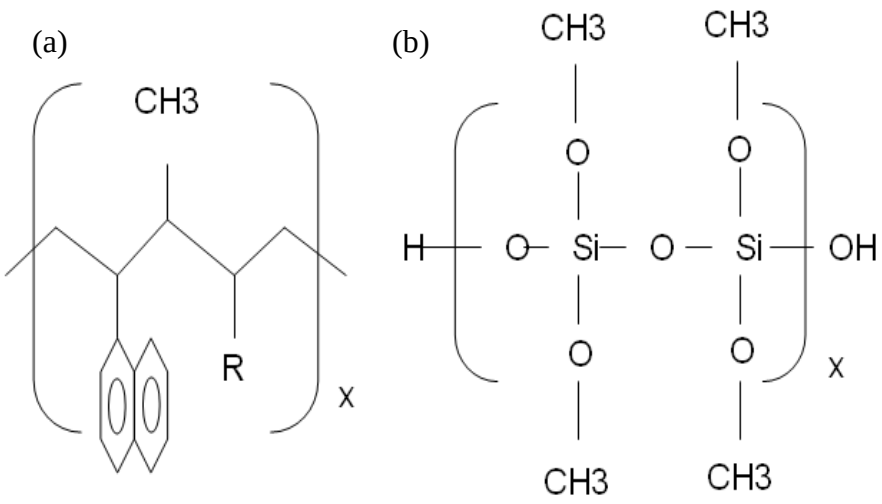


Figure 6.6 .The chemical formula of AR3 (a) and PDMS (b)..

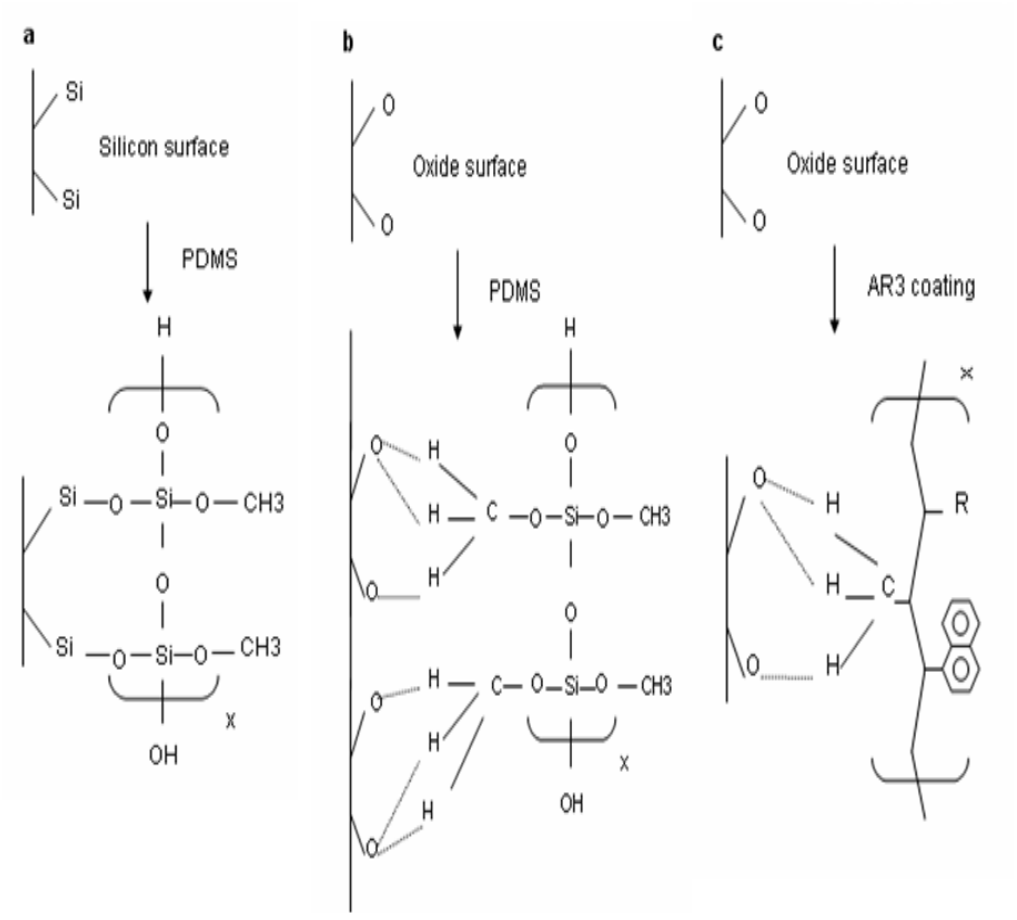


Figure 6.7.The mechanisms of PDMS sticking issue with (a) Silicon surface (b) oxide surface (c) oxide and AR3 pre-formed

Besides, AR3 is a conformal type of adhesion layers and covered along the surface topography of the substrates. Figure 6.8 shows the profile of silicon masters with AR3 coating in 0.6 um and 0.3 um. The profile of patterns will taper because different adhesion value between sidewall and planar areas. 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.

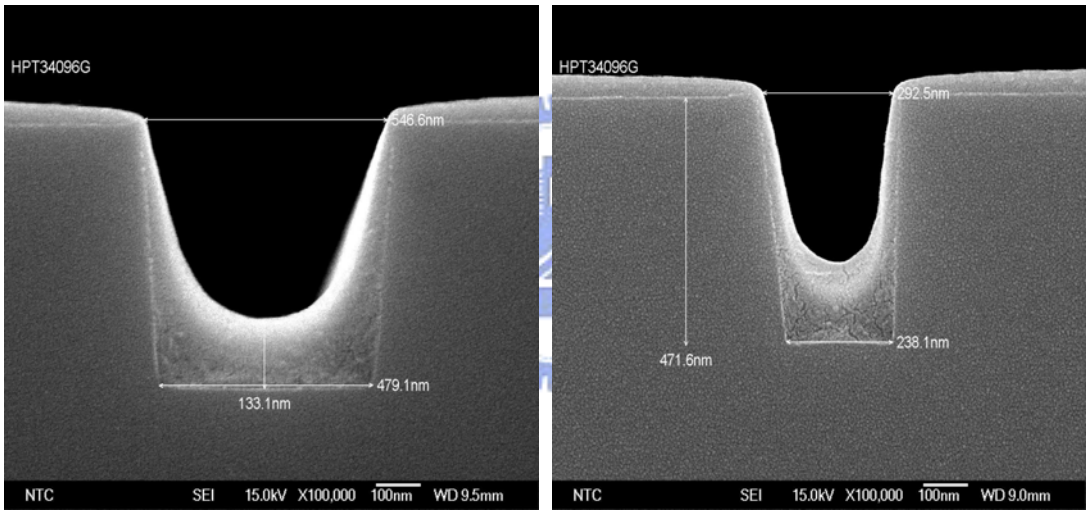


Fig.6.8. The profile of silicon master with AR3 coating. Pattern size is 0.6 um (Left) and 0.3 um (Right)

6.3 Accuracy comparisons

We have compared two different linewidth (0.3 um, 0.6 um) and four variable pitches in each process step (Table 6.1). 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 6.9).Finally, we got some summary in accuracy comparisons as below:

1. **Size of 0.6 um protein micropatterns are successfully printed in all pitches.**
2. **Size of 0.3 um protein micropatterns are printed but the equal line and space was getting worse in procedure of PDMS**
3. **If the pitch and size of patterns became smaller; the accuracy of PDMS and protein printing will be in decay.**

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. (Figure 6.9)

Table 6.1 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

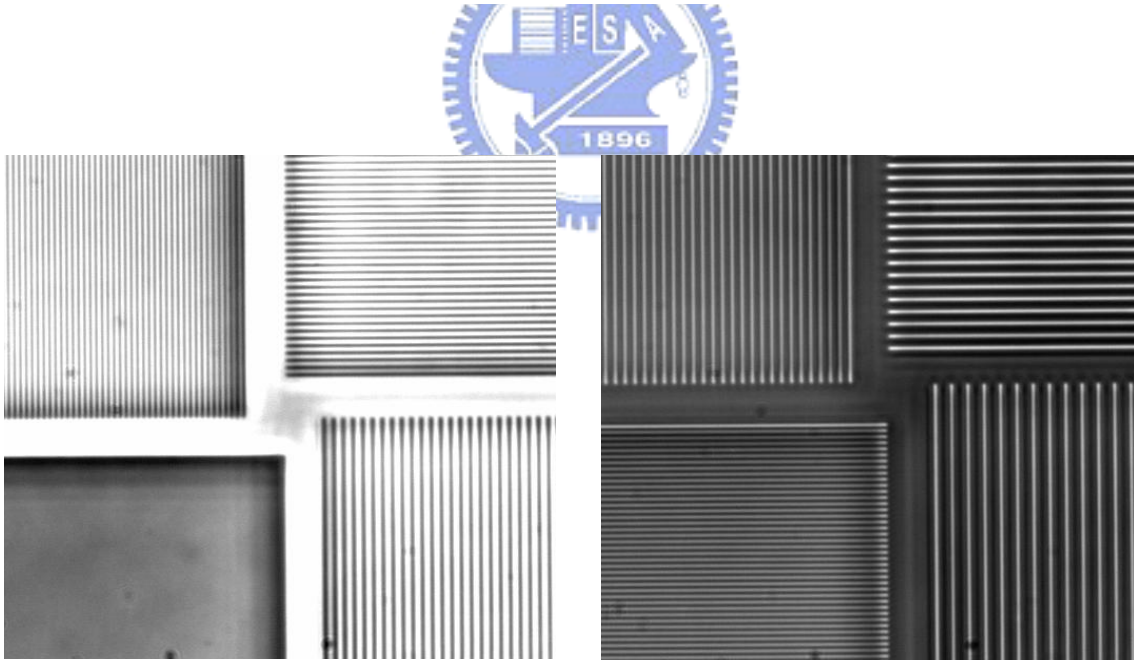
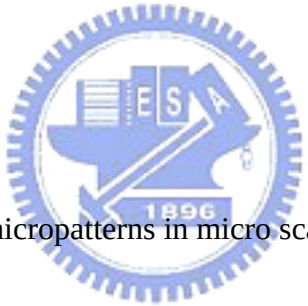


Figure 6.9.The top view pictures of 0.3 um through pitch (Left) and 0.6 um through pitch (Right) PDMS patterns

6.4 Cell biology applications

In this work, three kinds of cells (HEP-G2 cell, CHO- Tubulin and Macrophages) 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. Some properties of cell culture such as morphology, motility, dynamics and outgrowth will be observed and compared to each other.

6.4.1 HEP-G2 cells



When the size of protein micropatterns in micro scale, the HEP-G2 cells will be put on the patterns those size are 10 um, 5 um and 3um. After 1 hour and 24 hours, the cell will outgrowth along these three different sizes of patterns as figure 6.10. In sub-micro scale, (figure 6.11) they will be put on the surface of protein micropatterns. The sizes of protein micropatterns are 0.3 um and 0.6 um lines. They will be like globs and stay on the surface of patterns .After 24 hours; they still kept the same morphologies and no outgrowth on the patterns.

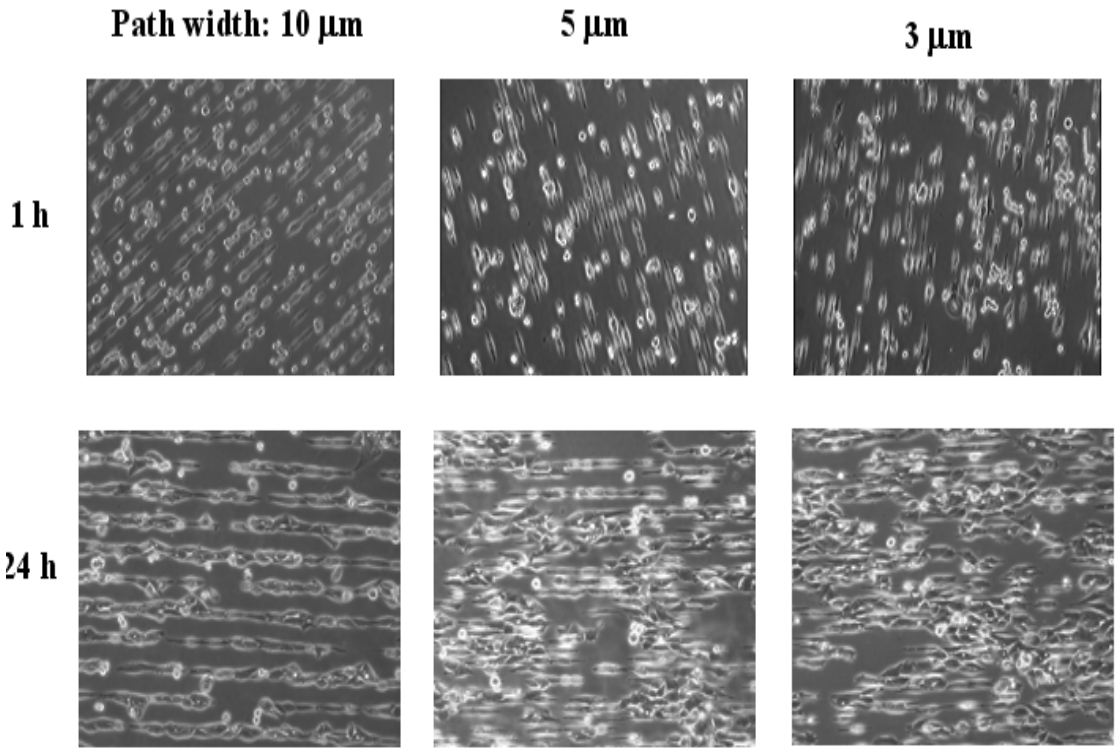


Figure 6.10 the HEP-G2 cells will be put on the patterns those size are 10 um, 5 um and 3um. After 1 hour (upper) and 24 hours (down), the cell will outgrowth along the patterns

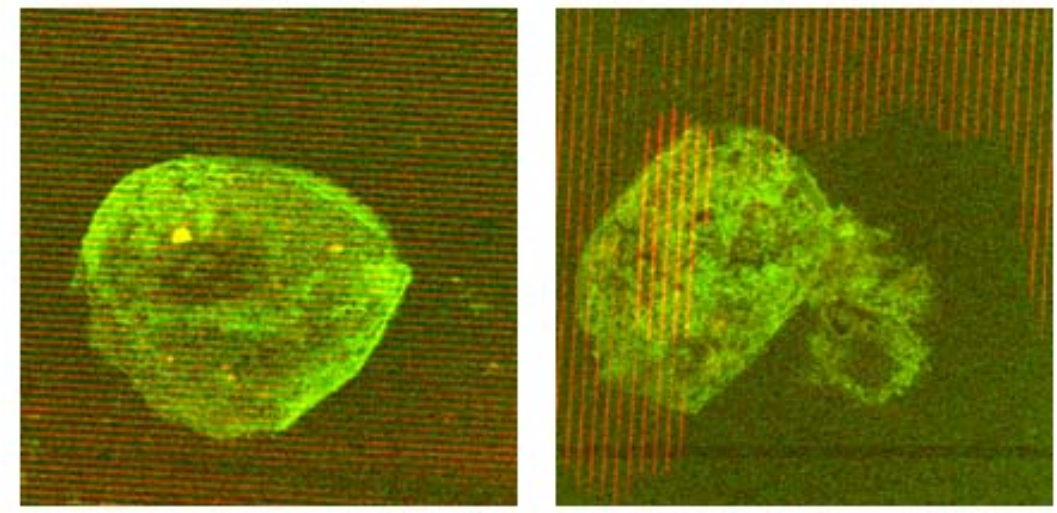
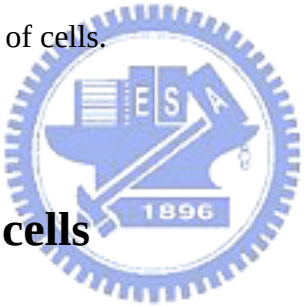


Figure 6.11 the HEP-G2 cells will be put on the patterns those size are 0.3 um (Left) and 0.6 um (Right). They will be like globs and stay on the surface of patterns. .After 24 hours; they still kept the same morphologies and no outgrowth on the patterns.

6.4.2 CHO-Tubulin cells

In this chapter, the dynamics of cells is also an important topic in sub-micro scale. The CHO-Tubulin cells will be observed in six difference types of patterns. From figure 6.12, CHO-Tubulin cells (Green) were attached on the protein sub-micro patterns (red).Figure (a) shows 0.6 um solid lines of protein micropatterns and figure (b) shows dash-lines of protein micropatterns. The figure (c) and (d) shows the cells attached on vertical solid lines and T-shape patterns. In these types of micropatterns, we did not see any difference for the dynamics of cells.



6.4.3 3T3 fibroblast cells

From figure 6.13, other cells (3T3 fibroblast) were attached on the 0.6 um small dash lines with solid line (3A) of protein micropatterns (a) and T-shape (4C) of protein micropatterns (b). The figure (c) and (d) shows the cells attached on horizontal and vertical solid lines (2C and 2D).

In these types of micropatterns, we did not see any difference for the dynamics of cells, too. But morphology of both cells were quite different, CHO-tubulin cells were rounding and 3T3 fibroblast cells were spraying on the sub-micro scale patterns.