CHAPTER IV
RESULTS AND DISCUSSION

4.1 The Effect of Degree of Crystallinity to Protein Adsorption

The 6 % (w/v) PCL films was prepared and annealed at 40 °C at different timing, i.e. 0 h, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h, and 24 h, to induce different degree of crystallinity. The X-ray diffractometer (XRD) was used to determine the degree of crystallinity of PCL films.

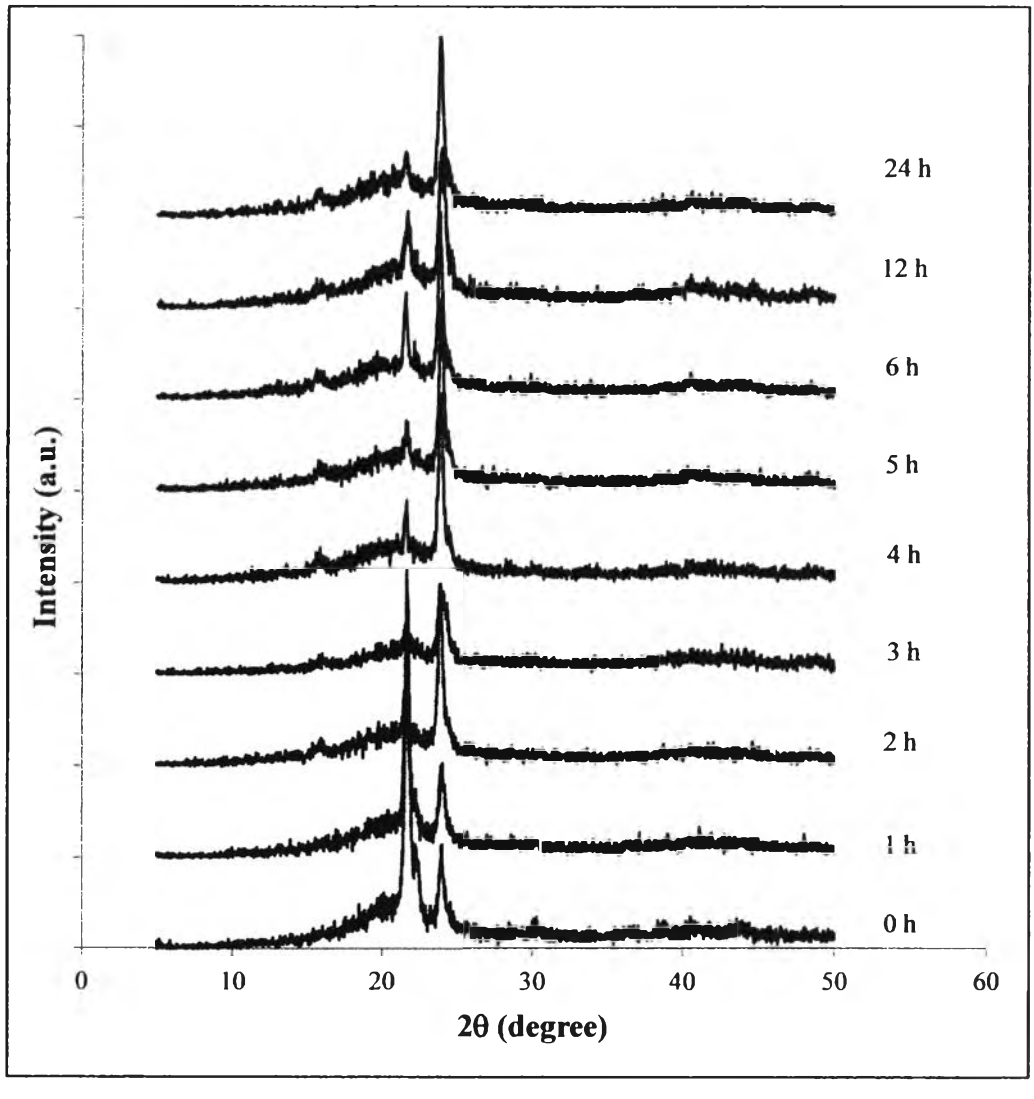


Figure 4.1 XRD result of PCL film annealed at 40 °C for different timing.

The percentage of crystallinity was calculated from the ratio of area under crystalline peak to the total area and was shown in Table 4.1 and Figure 4.2.

Table 4.1 The Percentage of Crystallinity

Annealing Time (h)	% Crystallinity (%)
0	49.02
1	54.56
2	54.92
3	57.39
4	66.64
5	66.35
6	65.00
12	64.19
24	66.70

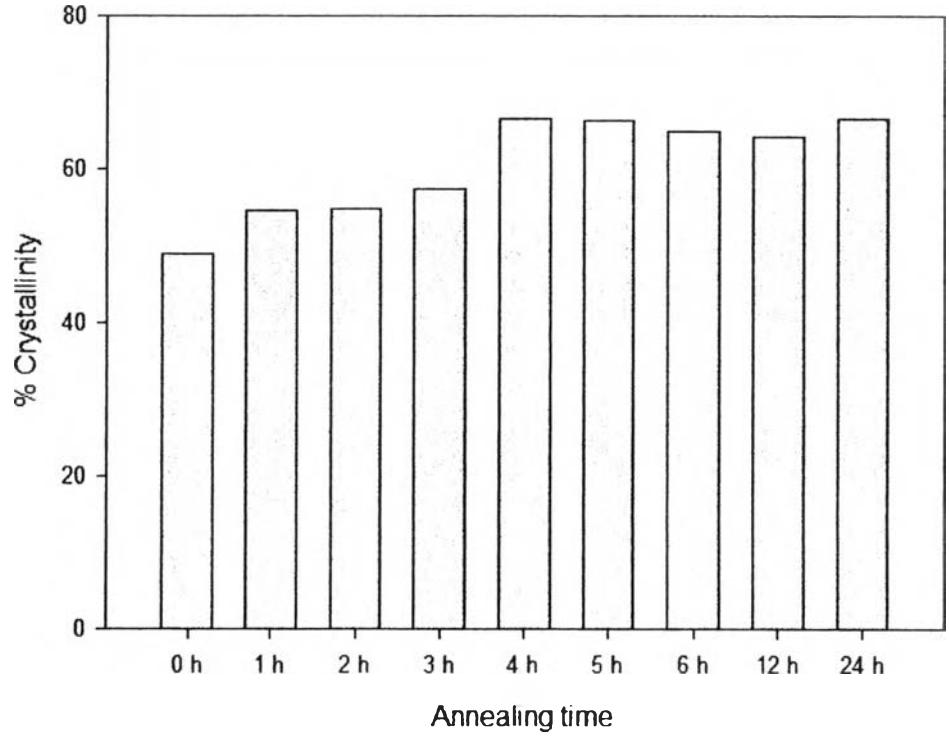


Figure 4.2 The percentage of crystallinity.

The result from XRD showed that the degree of crystallinity could be increased by increasing the annealing time. As the annealing temperature was higher than the glass transition temperature (T_g) but still lower than melting temperature (T_m) of PCL, the polymer chains were allowed to relax and move. This could result in better alignment with the neighboring molecules and created higher degree of crystallinity of the polymer. However, it was also observed that the degree of crystallinity did not increase further after annealing for 4 h where it started to reach its saturated point.

The PCL films with different degree of crystallization were immersed in 3 mg/ml of Bovine Serum Albumin (BSA) solution and the amount of protein adsorbed was shown in Figure 4.3.

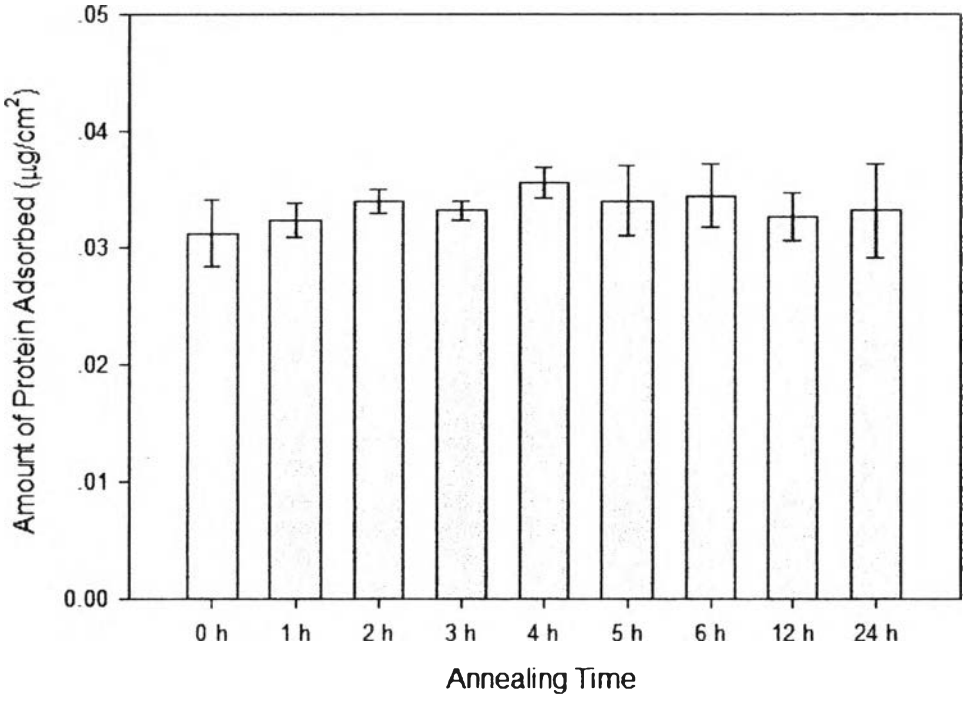


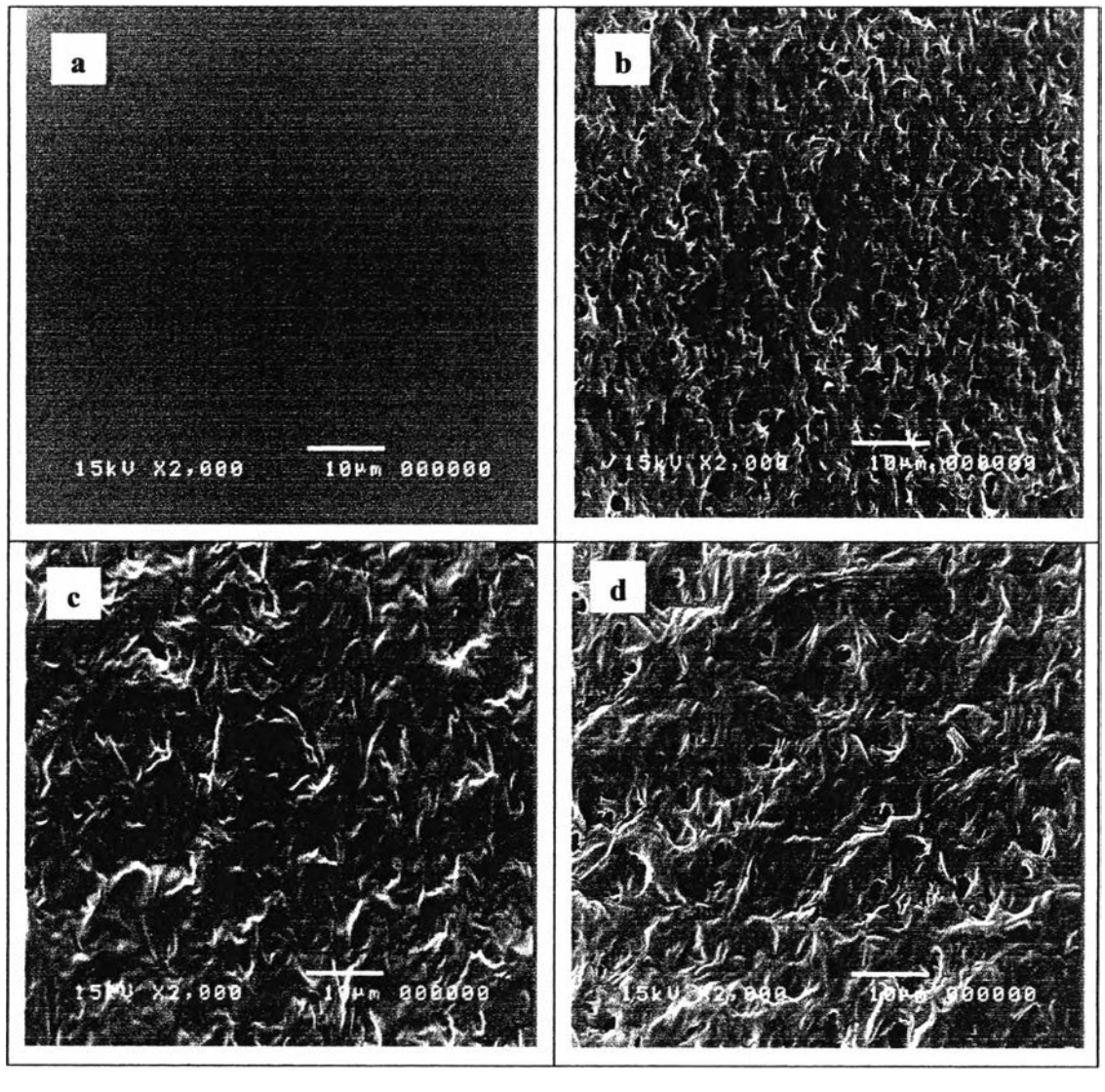
Figure 4.3 The amount of protein adsorbed on the surface of PCL film at various annealing time. *Significance at $p < 0.05$ with respect to the substrate at 0 h annealing time.

It was found that the amount of protein adsorbed on the surface of PCL film did not vary with the degree of crystallinity. The protein adsorption was the same in all types of film with different degree of crystallinity. Degree of crysatllinity does not have a significant role in the protein adsorption.

4.2 The Effect of PCL Surface Topography to Protein Adsorption

4.2.1 Surface Morphology of Neat Poly(ε-caprolactone) Film

Poly(ε-caprolactone) (PCL) films were prepared via solvent casting technique by dissolving PCL pellet (M_n = 80 000 g/mol) in different solvent system in order to induce different surface topology of the film.



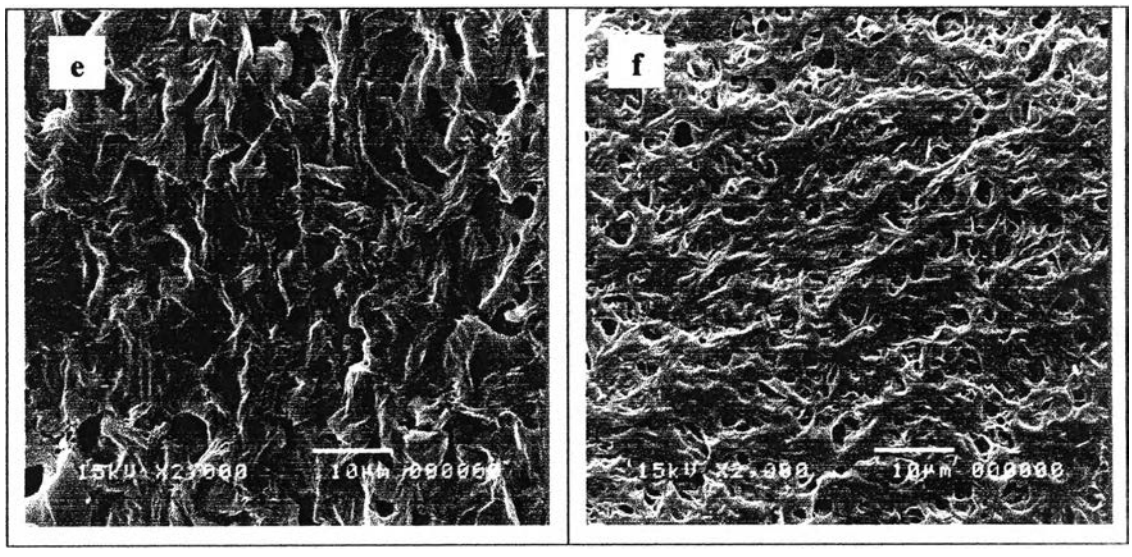


Figure 4.4 SEM images of the surface of PCL film casted from chloroform (a), acetone (b), tetrahydrofuran (THF) (c), 20:80 (v/v) EtOH:THF (d), 30:70 (v/v) EtOH:THF (e), and 40:60 (v/v) EtOH:THF (f).

The surfaces of PCL film were made to have different topologies by using different solvent system in the film casting technique. This could be explained by the difference in solubility parameter between the casting solvent and polymer. The solubility parameters of materials involved in this study were shown in Table 4.2.

Table 4.2 The solubility parameters

Type	**Solubility parameter** ($cal^{1/2}$ $cm^{3/2}$)
Polycaprolactone	9.2
Chloroform	9.21
THF	9.52
Acetone	9.77
Ethanol	12.92

Solubility parameter is a numerical value that indicates the degree of interaction between materials. Considering from the solubility parameters and the SEM result, the higher the difference between the solubility parameter of PCL and solvents, the rougher the surface of the film was observed. The solvents with similar value of solubility parameter as the polymer were regarded as good solvent. On the other hand, the solvents whose solubility parameters were different from the polymer were poor solvent and caused the phase separation. Phase separation formed pores in polymer membrane during solvent evaporation and caused the film surface to be rougher.

4.2.2 Surface Morphology After the Hydrolysis of PCL Film

The effect of surface modification to the surface morphology of the film was investigated by SEM as shown in Figure 4.5.

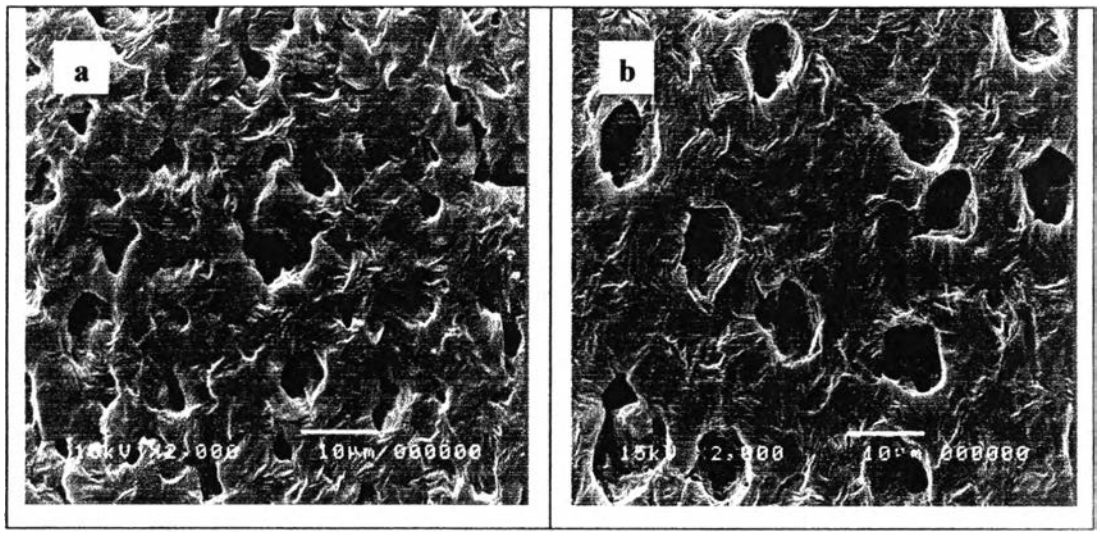


Figure 4.5 SEM images of the surface of PCL film casted from tetrahydrofuran and hydrolyzed by 1 M NaOH (a), and 5 M NaOH (b) (magnification = 2000X; scale bar = 10 μm).

The surface modification by hydrolysis caused the topology of the film to be rougher due to the pore formation. NaOH was able to hydrolyze the ester group on PCL. At higher the concentration of NaOH, the formed pores appeared to be larger.

4.2.3 Roughness

Atomic Force Microscopy was used to investigate and image the surface roughness. The roughness was described in usual roughness parameters, as the arithmetic average roughness (R_a), the root-mean-square roughness (R_q), and the difference in height between the average of the five highest peaks and the five lowest valleys along the profile (R_z).

According to the amplitude of the roughness parameters, as shown in Figure 4.6, the surface roughness of the PCL film was observed to increase with the increasing difference in solubility parameter between PCL and the solvent used. The coarsest surface belonged to the film casted from 40:60 (v/v) EtOH:THF. Also, the effect of hydrolysis was shown to increase the roughness of the surface with the increasing concentration of NaOH. From Figure 4.7, the selected images of the PCL film substrates taken by atomic force microscopy were shown. The different geometrical features of PCL film could be seen. The PCL film casted from chloroform, whose solubility parameter was the nearest to PCL's, appeared to be almost flat with some short peaks in a size of nanometer range. On the other extreme, the film casted from 40:60 (v/v) EtOH:THF appeared to be crinkled in a micrometer range.

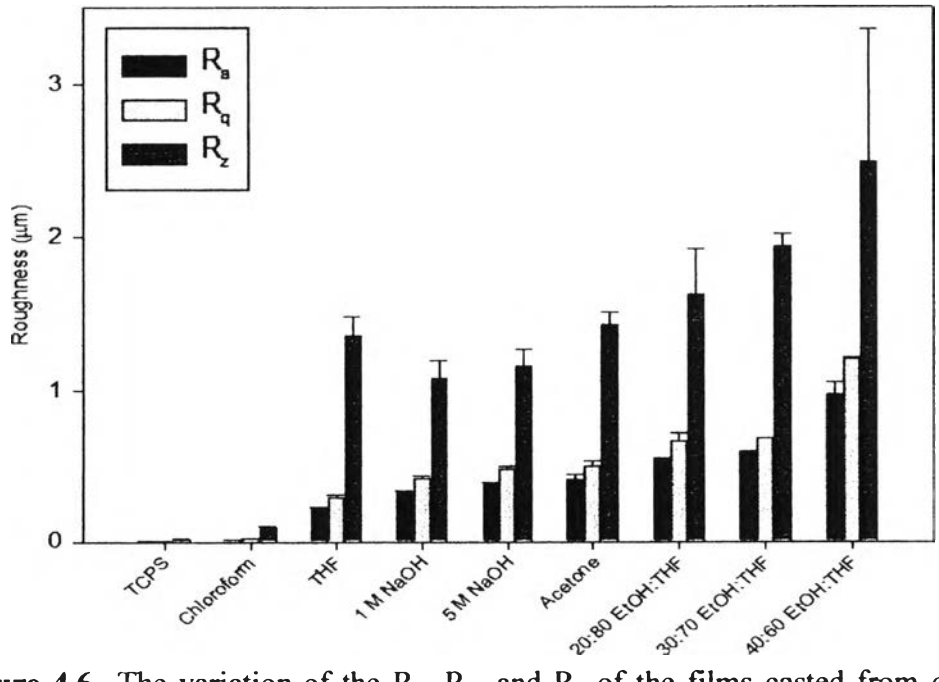
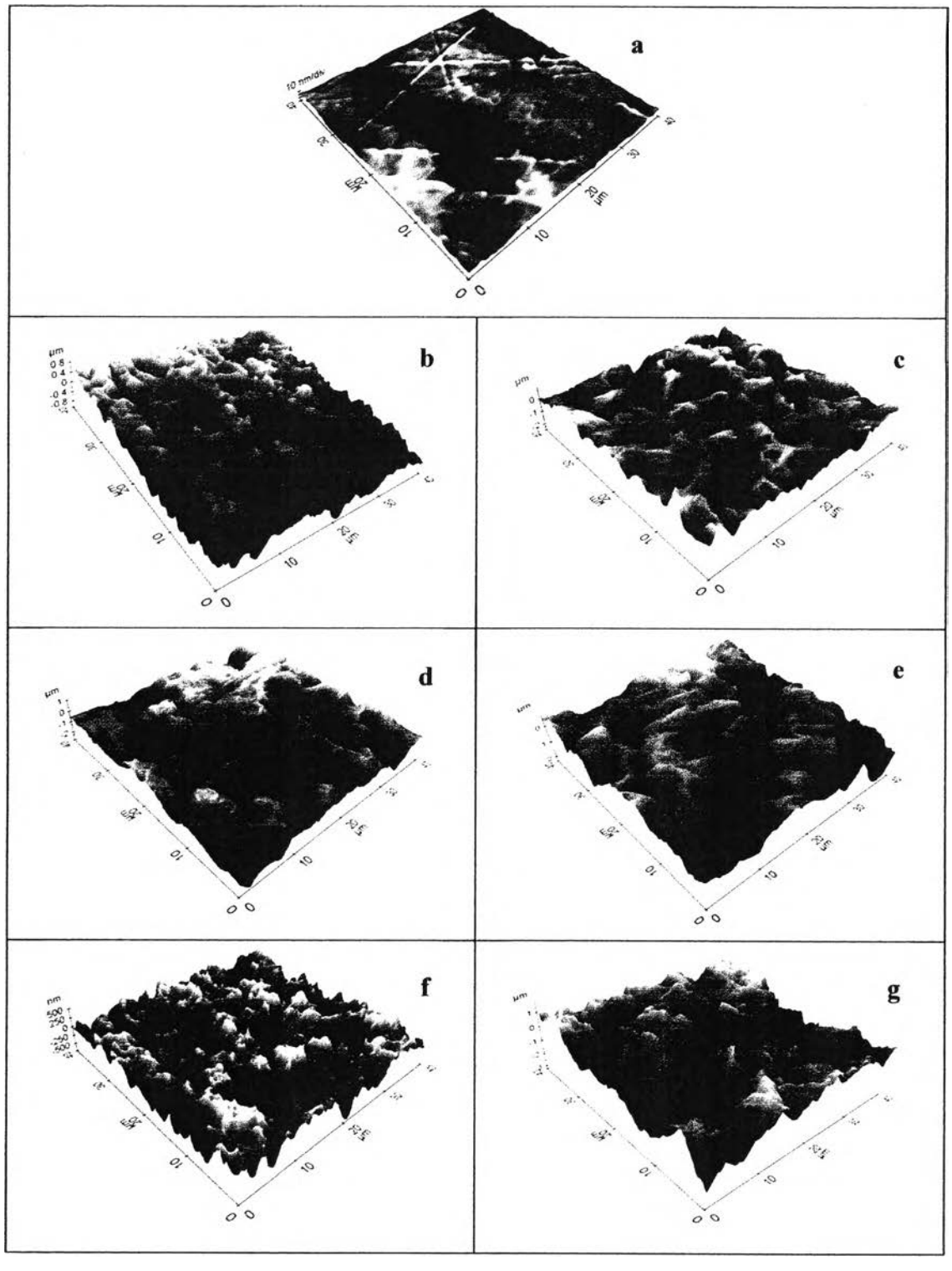


Figure 4.6 The variation of the R_a, R_q, and R_z of the films casted from different solvents.


a
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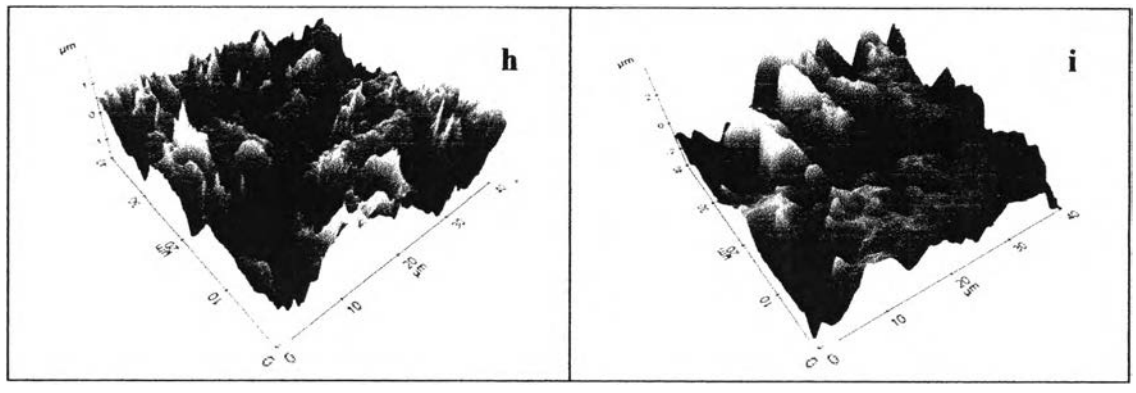


Figure 4.7 AFM pictures of the surfaces of PCL films casted from: TCPS (a), chloroform (b), THF (c), 1 M NaOH treated (d), 5 M NaOH treated (e), acetone (f), 20:80 (v/v) EtOH:THF (g), 30:70 (v/v) EtOH:THF (h), and 40:60 (v/v) EtOH:THF (i).

4.2.4 Thermal Analysis of PCL Film

The heat of reaction was investigated by DSC.

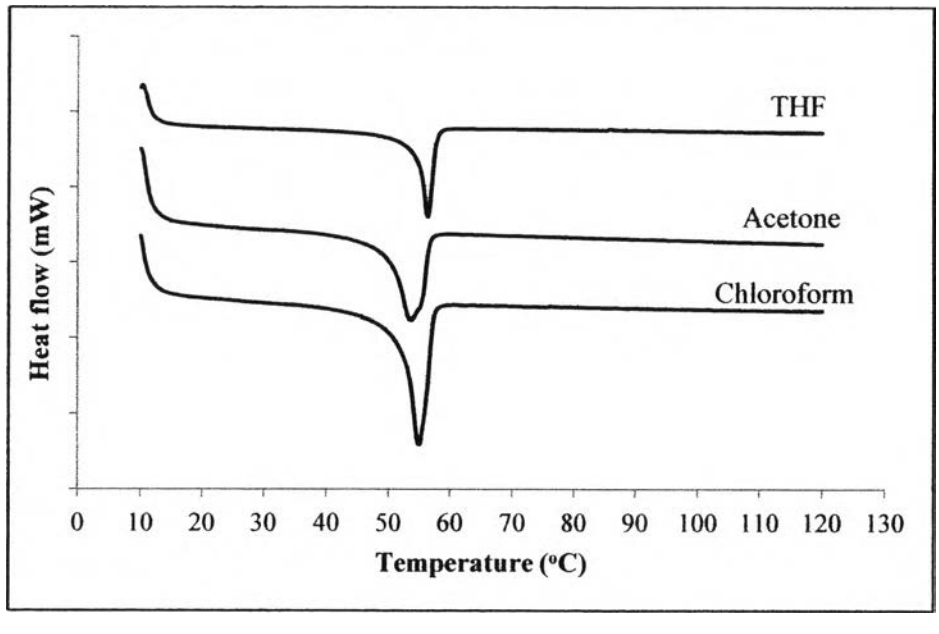


Figure 4.8 DSC result of films casted from different solvents.

The result from DSC showed that all films casted from different solvent had the same melting point near 60 °C.

4.2.5 Surface Wettability

Surface wettability of PCL film was evaluated by water contact angle measurement using sessile drop method. Table 4.3 showed that all the PCL films casted from different solvent had the same behavior of surface wettability in a way that the water contact angle decreased after the film had been aminolyzed by 1.5 M HMD/IPA solution for 8 h. This was an indication that the introduction of amino groups helped improve the hydrophilicity of the film. On the other hand, an activation of amino group by 0.1 M DSC/DMSO increased the water contact angle, indicating that the film was more hydrophobic. The water contact angle decreased again after an immobilization of protein, bovine serum albumin, showing that the biomolecules can improve the hydrophilicity of the film surface. The comparison of the effect of surface modification to surface wettability was shown in Figure 4.9. The appearance of the water droplet on the film casted from 40:60 EtOH:THF for neat PCL, aminolyzed PCL, activated PCL, and bovine serum albumin immobilized PCL was shown in Figure 4.10.

When the neat films casted from different solvents were compared, the same contact angle could be observed. The only exception was for the PCL film casted from 40:60 EtOH:THF which was more hydrophobic than the others as shown in Figure 4.11. This could be the result from its fine porous structure. Also, the hydrolyzed films certainly showed higher hydrophilicity due to the presence of hydroxyl group.

Table 4.3 The water contact angle of the neat PCL, aminolyzed PCL, activated PCL, and protein adsorbed PCL casted from different solvent

Solvent	Contact Angle (θ)			
	Neat PCL	**Aminolyzed PCL**	**Activated PCL**	**Protein Adsorbed PCL**
Chloroform	71.9 ± 0.7 ^{o}	67.2 ± 0.5 ^{o}	74.8 ± 0.4 ^{o}	60.8 ± 0.4 ^{o}
Acetone	72.7 ± 0.4 ^{o}	64.6 ± 0.8 ^{o}	77.5 ± 1.0 ^{o}	67.9 ± 0.6 ^{o}
THF	72.5 ± 0.7 ^{o}	64.9 ± 0.6 ^{o}	82.0 ± 0.6 ^{o}	73.7 ± 0.6 ^{o}
20:80 EtOH:THF	72.1 ± 0.4 ^{o}	61.6 ± 0.8 ^{o}	81.6 ± 0.9 ^{o}	69.1 ± 0.3 ^{o}
30:70 EtOH:THF	71.2 ± 0.7 ^{o}	55.0 ± 0.4 ^{o}	82.2 ± 2.1 ^{o}	63.6 ± 0.2 ^{o}
40:60 EtOH:THF	74.7 ± 0.1 ^{o}	46.9 ± 0.8 ^{o}	87.6 ± 1.3 ^{o}	62.6 ± 0.5 ^{o}
1 M NaOH	67.7 ± 0.4 ^{o}	58.2 ± 0.6 ^{o}	70.6 ± 0.8 ^{o}	55.1 ± 0.4 ^{o}
5 M NaOH	57.2 ± 0.9 ^{o}	44.9 ± 1.2 ^{o}	73.0 ± 1.0 ^{o}	67.5 ± 0.5 ^{o}

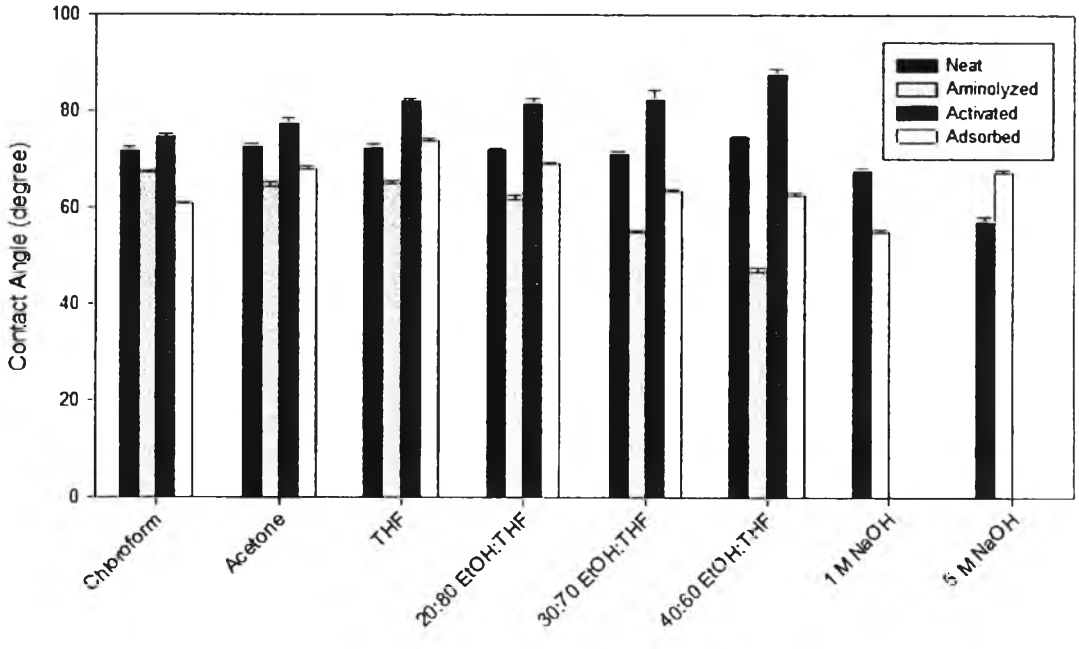


Figure 4.9 The comparison of the effect of surface modification to surface wettability.

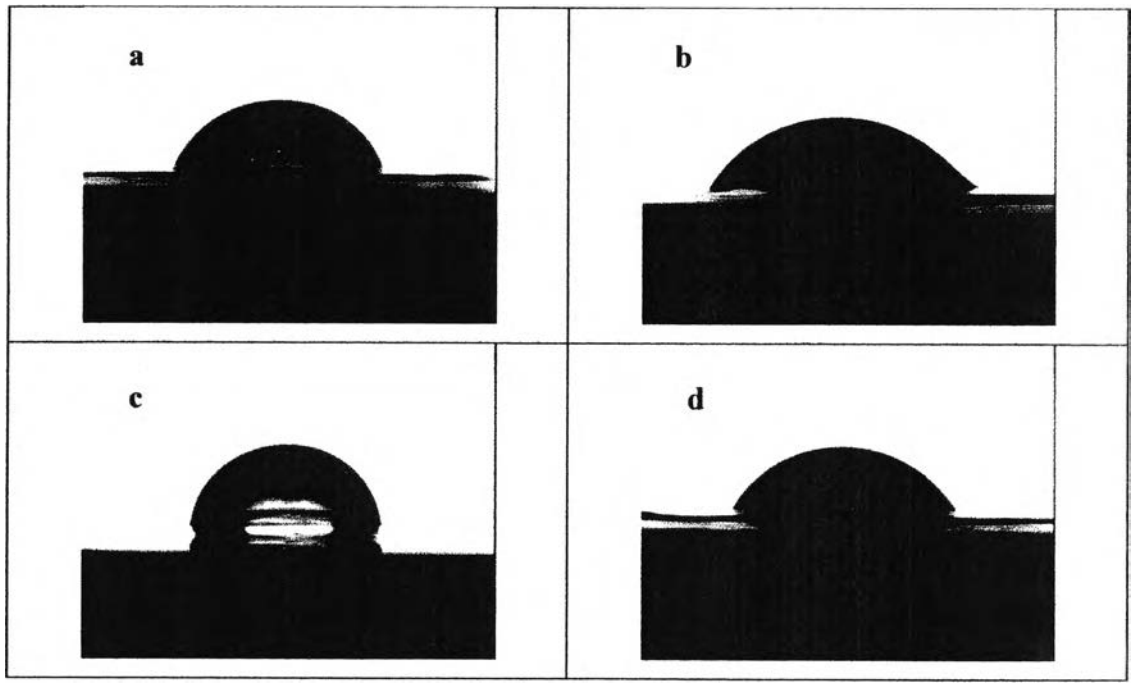


Figure 4.10 Water droplet on the surface of neat PCL (a), aminolyzed PCL (b), activated PCL (c), and protein adsorbed PCL (d) casted from 40:60 EtOH:THF.

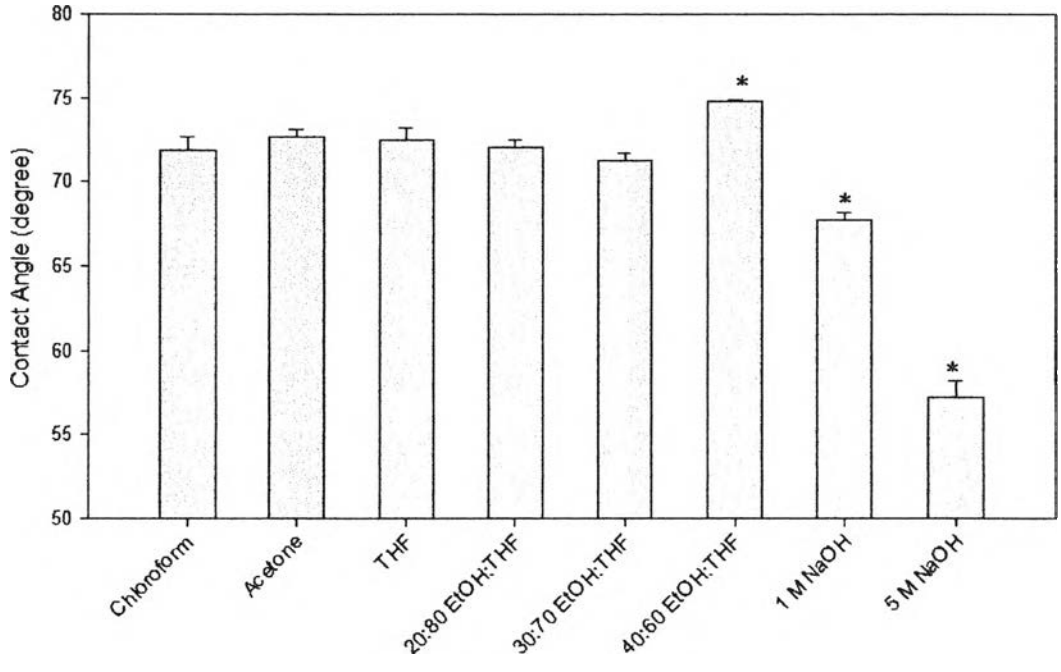


Figure 4.11 The water contact angle of neat films casted from different solvent system. *Significance at $p < 0.05$ with respect to the substrate casted from chloroform.

4.2.6 Surface Chemical Analysis

ATR-FTIR spectra of neat PCL casted from different solvent system were shown in Figure 4.12. The major absorption peak could be observed at 1755 cm^{-1} from the ester carbonyl of PCL. It was shown that, though casted from different solvent, they possessed the same spectra. The same absorption pattern can be used to confirm that PCL film casted from different solvent had the same functional groups due to the complete evaporation of the solvent.

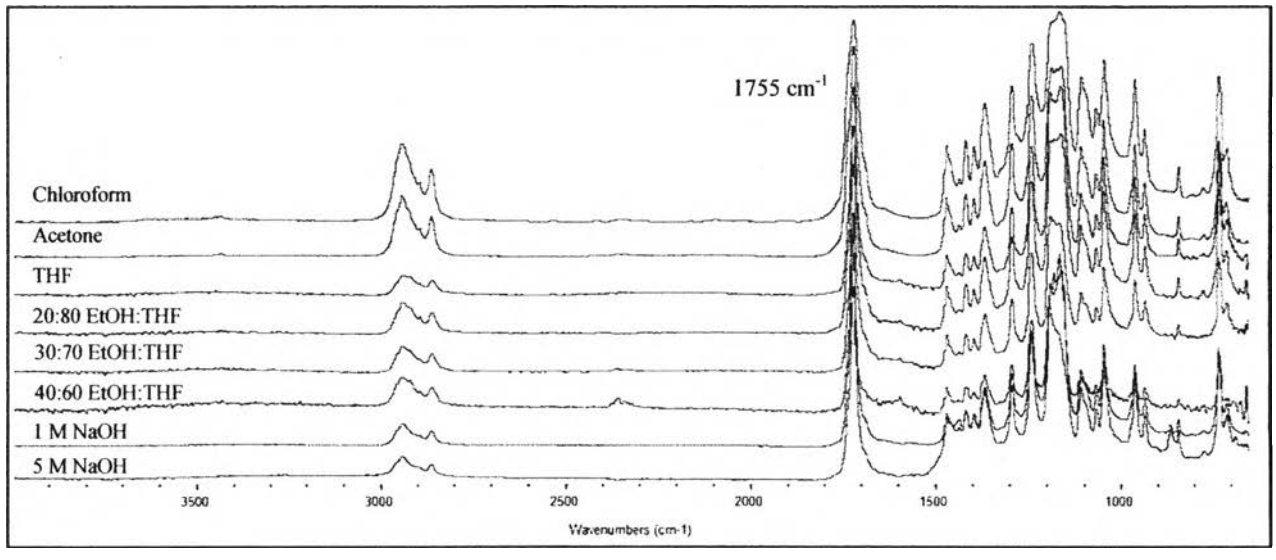


Figure 4.12 ATR-FTIR spectra of neat PCL films casted from different solvents.

After the protein immobilization, C=O stretching at 1650 cm^{-1} (amide I) and N-H stretching peak at 3300 to 3500 cm^{-1} was observed as shown in Figure 4.13 and 4.14.

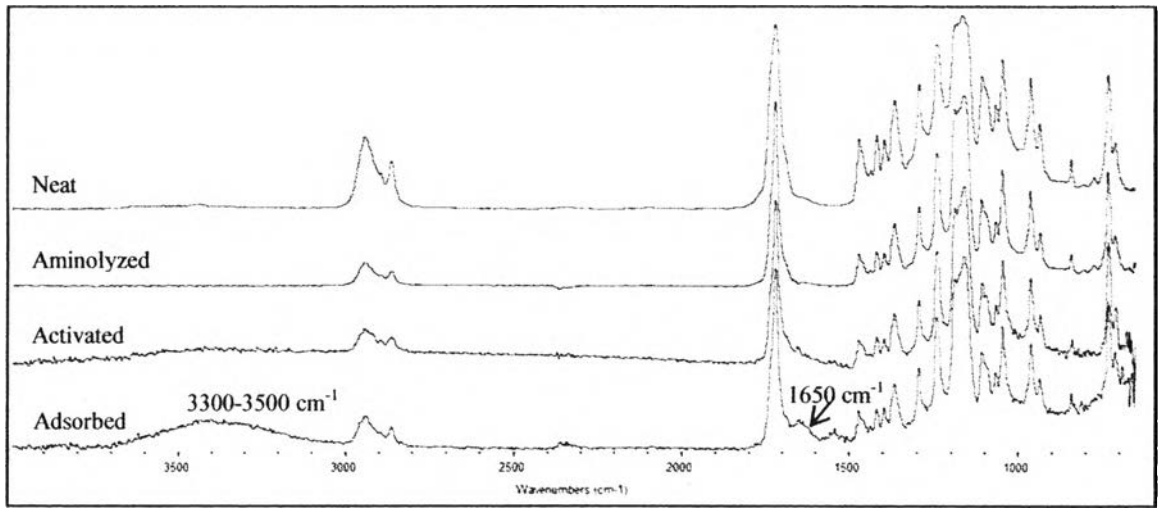


Figure 4.13 ATR-FTIR spectra of neat and modified PCL films casted from chloroform.

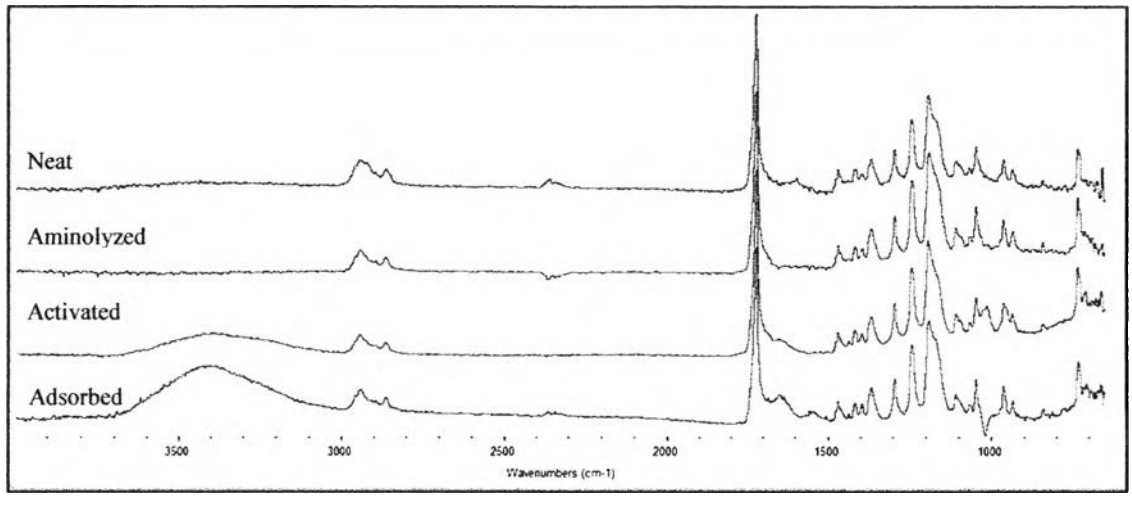


Figure 4.14 ATR-FTIR spectra of neat and modified PCL films casted from 40:60 EtOH:THF.

4.2.7 Protein Adsorption Test

The amount of protein adsorbed on the surface of PCL film was determined based on bicinchoninic acid method by using BCA protein assay kit and calculated against the standard curve. The adsorption isotherm of the adsorbed bovine serum albumin was shown in Figure 4.15.

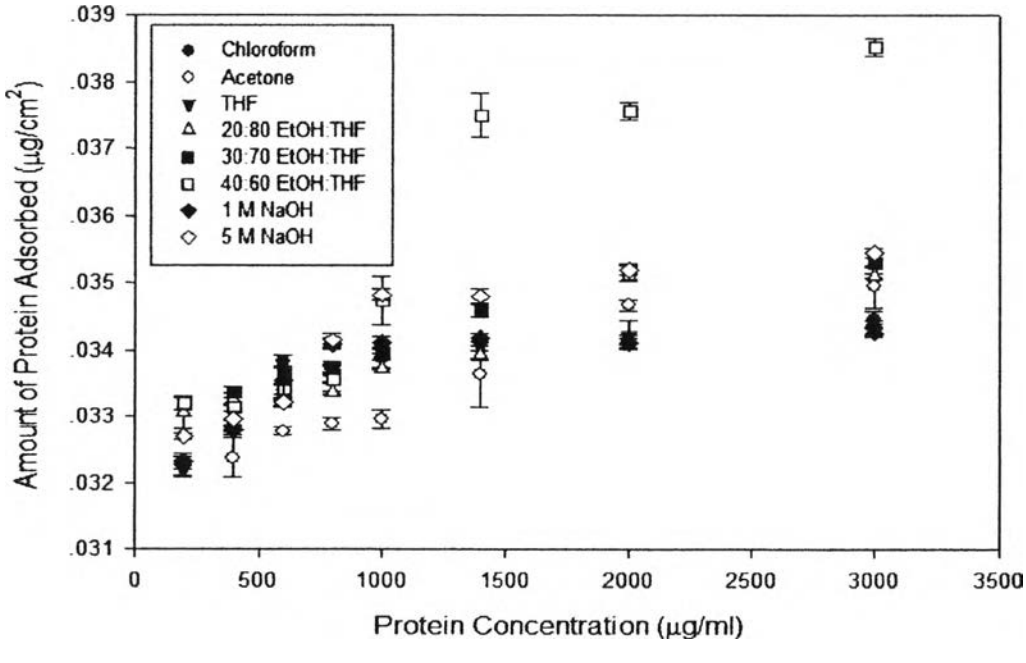


Figure 4.15 The adsorption isotherm of the adsorbed bovine serum albumin on the neat PCL films (diameter = 1.5 cm) casted from different solvent systems.

The adsorption isotherm of bovine serum albumin on the neat films showed that the amount of protein adsorbed increased with the increasing protein concentration until it began to level off at the protein concentration around 1400 μg/ml. The film with the roughest surface casted from 40:60 EtOH:THF had significantly higher amount of protein adsorbed. Protein preferred to adsorb more on the rough surface.

The effect of surface modification to protein adsorption was studied. The result of protein adsorbed on the roughest surface, i.e. casted from 40:60 EtOH:THF, was shown in Figure 4.16.

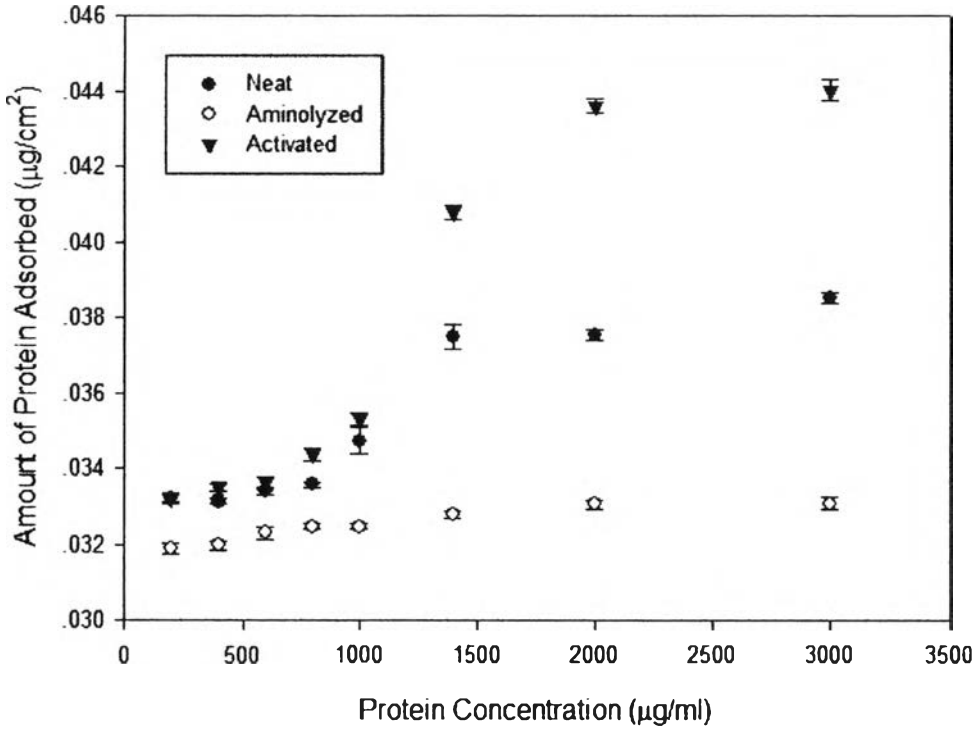


Figure 4.16 The adsorption isotherm of the adsorbed bovine serum albumin on the neat and modified PCL films (diameter = 1.5 cm) casted from 40:60 EtOH:THF.

The surface modification had the effect on the protein adsorption on PCL film. The aminolyzed film had the lowest amount of protein adsorbed. On the other hand, as can be expected, the activated film had the highest amount of protein. The amount of protein adsorbed can be enhanced by the activation of amino group on the aminolyzed film. The different amount of protein adsorbed on the surface

could be explained by the hydrophobic interaction. When flexible proteins as bovine serum albumin arrived at the hydrophobic surface, they undergo a conformational rearrangement to expose the inner hydrophobic groups which interact with the surface. Also, the local changes of the side chains can improve the ligand binding by unburied the active sites from the core. The hydrophobic interaction between BSA and hydrophobic surface is stronger than on hydrophilic surface.

4.3 Biological Characterization

4.3.1 Indirect Cytotoxicity Evaluation

The cytotoxocity test is important to evaluate the biomaterials because it determines the viability of living cells by detecting the toxic product released by the materials. The extraction media was prepared by immersing sample in Serum Free Media (SFM) for 1, 3, and 7 d in order to obtain the released product from the sample. The extraction media was then used to culture MC3T3-E1 for 1 d. After which, the MTT assay was performed to find the relative viability of cells. The MTT reagent composes of yellow tetrazolium salt that will turn to purple formazan crystal as it is incubated with the living cells.

4.3.1.1 Indirect Cytotoxicity Evaluation of Protein Adsorbed Films

Figure 4.17 showed the percent viability of cells from the protein adsorbed films. The result indicated that the cells cultured in the extraction media had the percent viability higher than 80% compared to the control, meaning that the protein adsorbed films were non-toxic and did not release the toxic substances to MC3T3-E1 cells.

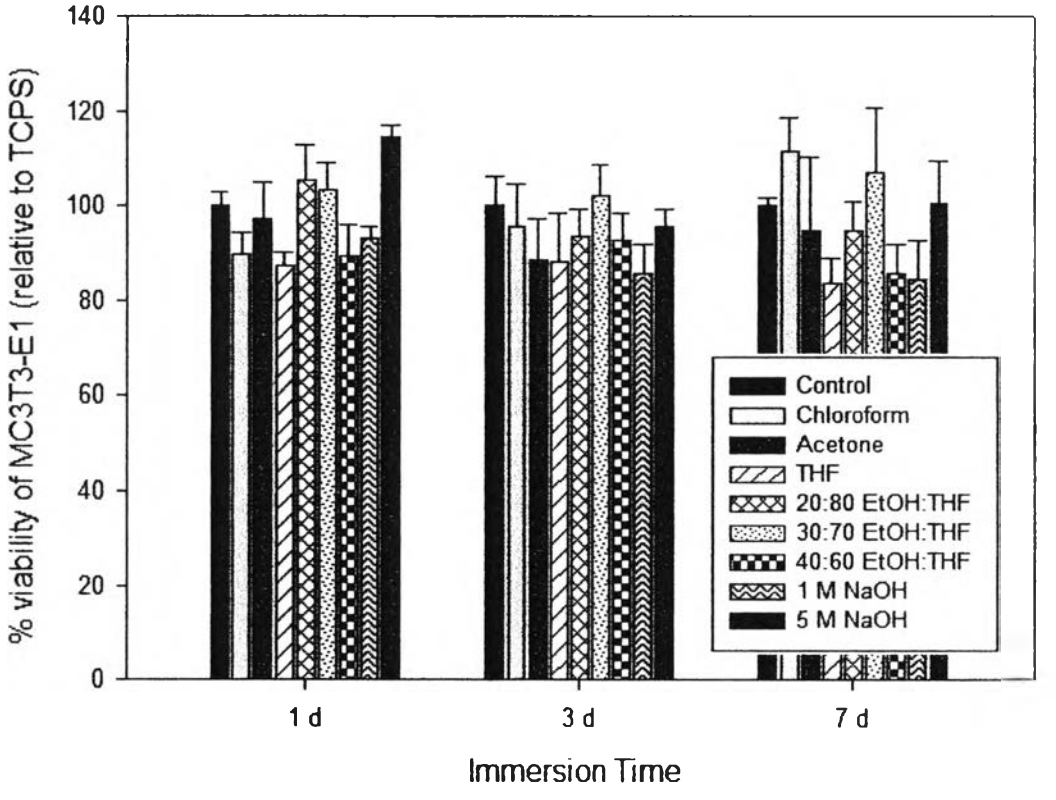


Figure 4.17 The percent viability of MC3T3-E1 of the control and the extract solution from protein adsorbed films. *Significance at $p < 0.05$ with respect to control.

4.3.1.2 Indirect Cytotoxicity Evaluation of Surface-Modified Films

The percent viability of cells cultured in the extraction media from the surface-modified (i.e. neat, aminolyzed, activated, and BSA-immobilized) films casted from chloroform, and 40:60 (v/v) EtOH:THF were represented in Figure 4.18. It was found that the percent viability of cells from all the materials were higher than 80% compared to the control which meant that the materials were non-toxic and did not release toxic substances. Moreover, the surface-modified films gave a slightly higher percentage of living cells than the control, indicating that the surface-modified films could also promote cell growth.

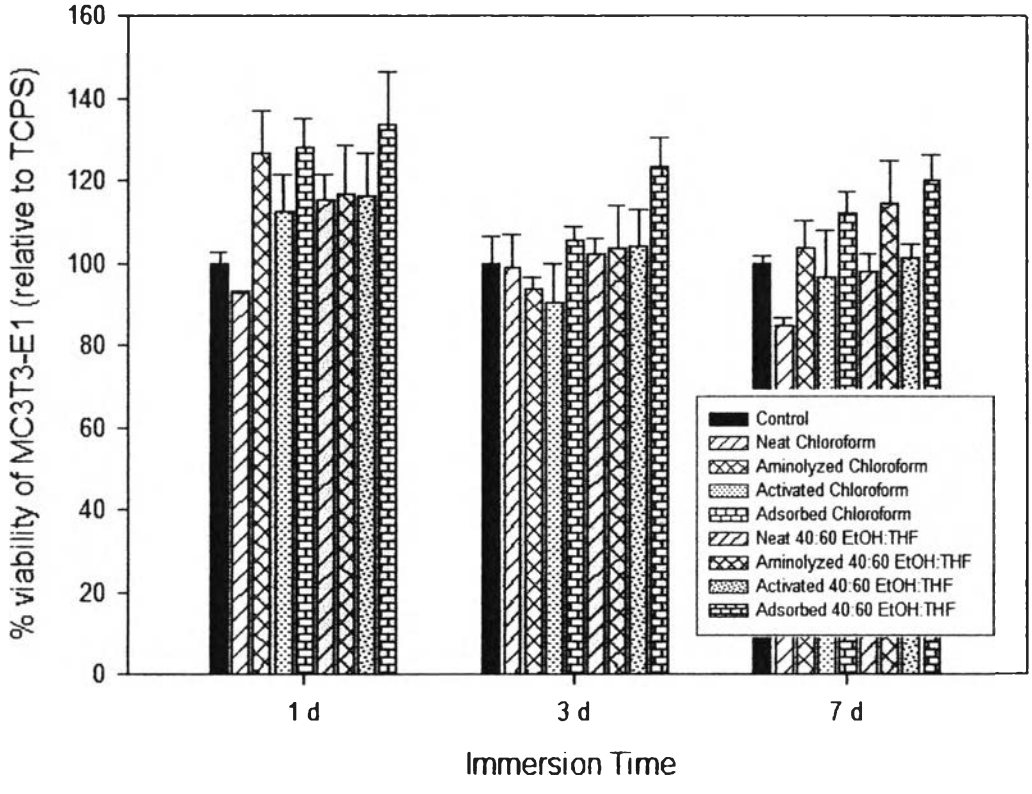


Figure 4.18 The percent viability of MC3T3-E1 of the control and the extract solution from surface-modified films. *Significance at $p < 0.05$ with respect to control.

4.3.2 Cell Attachment and Proliferation

Cell attachment was performed by culturing 40,000 cells of MC3T3-E1 on the materials and TCPS (as a control) for 4 h, 16 h, and 1 d.

In order to observe cell proliferation, MC3T3-E1 cells were seeded on the materials for 1, 2, and 3 d. The TCPS was used as a control. At each given time, the MTT assay was done to find the percentage of viable cells, in which the amount of living cells at 1 d was used as the basis to determine the relative viability.

4.3.2.1 Cell Attachment and Proliferation on Protein Adsorbed Films

It can be seen from Figure 4.19 that, for all types of materials, the percentage of living cells increased with the increasing time of culture. At 4 h and 16 h after cell seeding, most of the percent of viable cells was equivalent to that of TCPS, except for 30:70 (v/v) EtOH:THF, 40:60 (v/v) EtOH:THF, and the film treated with 5 M NaOH which showed higher percentage of viability. At all times, cells on the film casted from 40:60 (v/v) EtOH:THF seemed to have the highest percentage of viability and it should be able to support cell adhesion better than other materials.

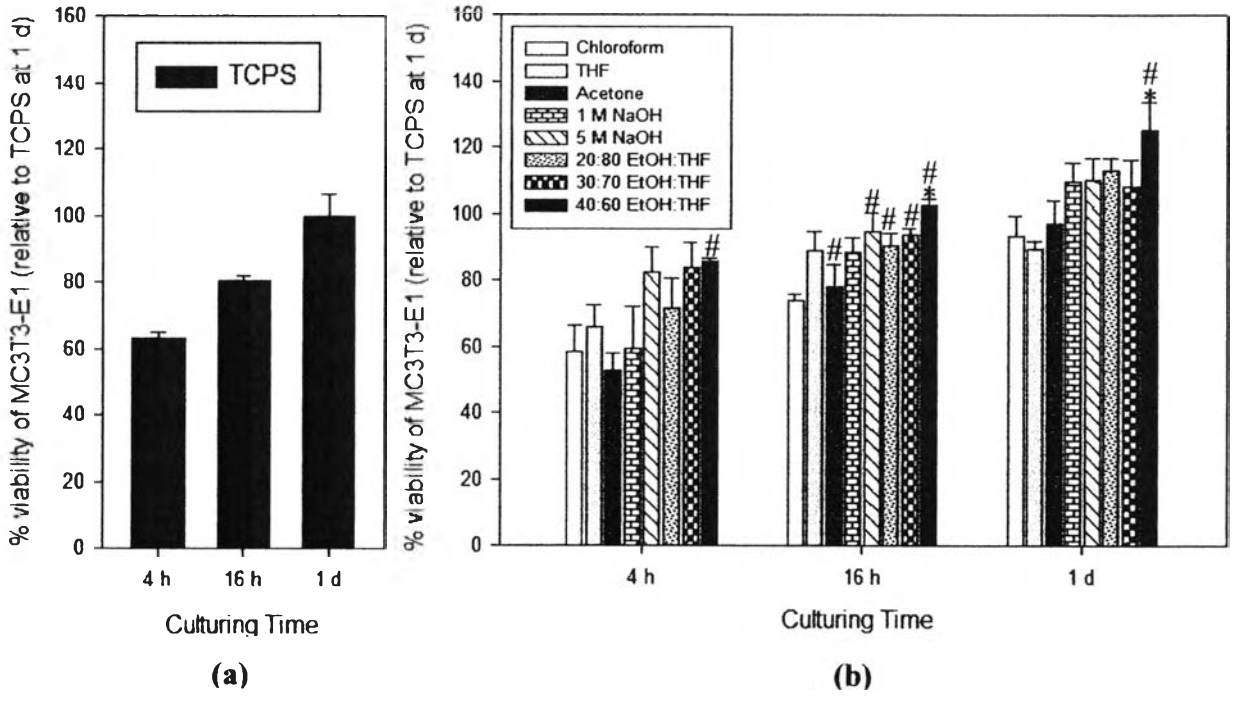


Figure 4.19 MC3T3-E1 attachment on the surface of TCPS (a), the protein-adsorbed films casted from different solvents (b) for 4 h, 16 h, and 1 d. *Significance at $p < 0.05$ with respect to control. #Significance at $p < 0.05$ with respect to the substrate casted from chloroform at any given time point.

For cell proliferation as can be seen from Figure 4.20, on day 1, the percentage of cell viability of the film casted from 30:70 (v/v) EtOH:THF, 40:60 (v/v) EtOH:THF, and the film treated with 5 M NaOH were slightly higher than TCPS. As cell seeding time increased, the percentage of viability from 30:70 (v/v) EtOH:THF, 40:60 (v/v) EtOH:THF, and the film treated with 5 M NaOH became significantly increased compared to other films. Especially on day 3, the viability of cells on the film casted from 40:60 (v/v) EtOH:THF was obviously higher than others and slightly higher than TCPS. This could be due to the significantly higher amount of protein adsorbed on the surface of the substrate.

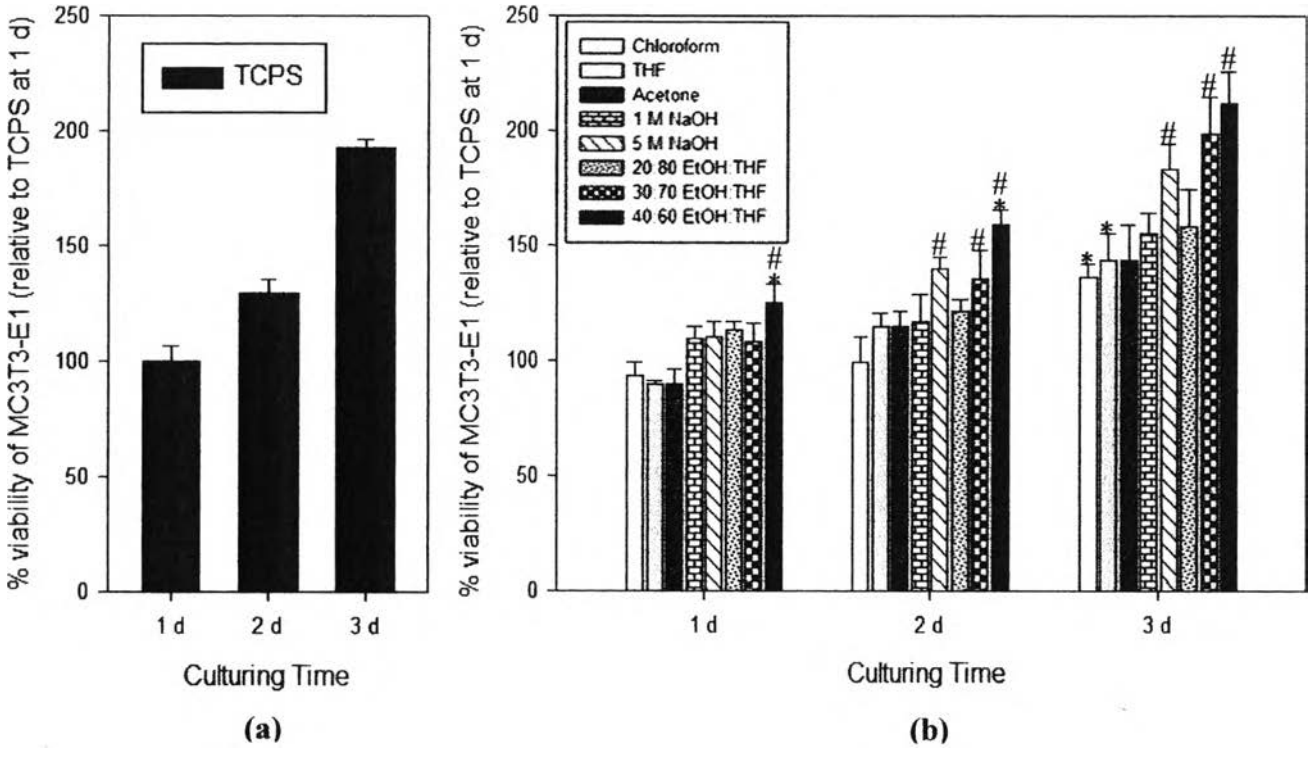


Figure 4.20 MC3T3-E1 proliferation on the surface of TCPS (a), the protein-adsorbed films casted from different solvents (b) for 1 d, 2 d, and 3 d. *Significance at $p < 0.05$ with respect to control. #Significance at $p < 0.05$ with respect to the substrate casted from chloroform at any given time point.

4.3.2.2 Cell Attachment and Proliferation on Surface-Modified Films

The result of cell attachment on the surface-modified films (ie. neat, aminolyzed, activated, and BSA-immobilized film) can be observed in Figure 4.21. All materials showed the increasing percentage of cell viability with increasing time. At 4 h and 16 h, the percentage of living cells on the BSA-immobilized film was greater than neat films. This indicated that BSA-immobilized film showed a better support for cell attachment more than other materials.

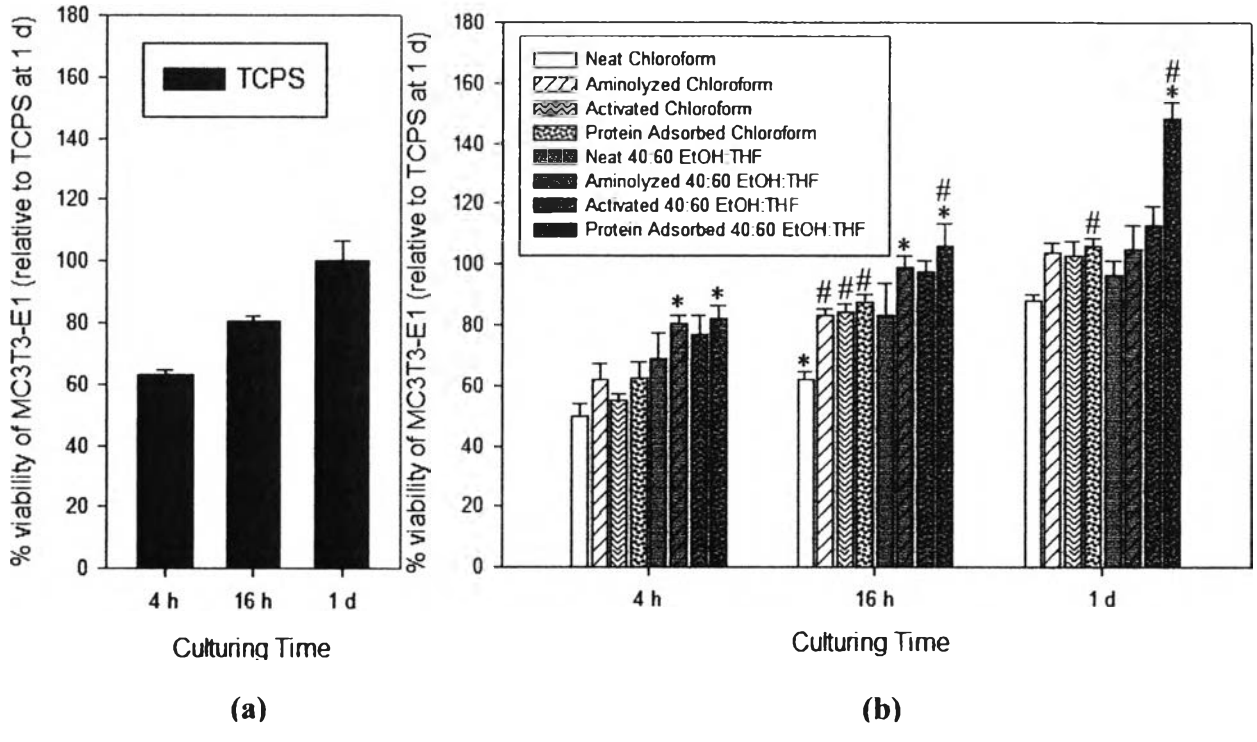


Figure 4.21 MC3T3-E1 attachment on the surface of TCPS (a), the surface-modified films (b) for 4 h, 16 h, and 1 d. *Significance at $p < 0.05$ with respect to control. #Significance at $p < 0.05$ with respect to the neat substrate of each solvent at any given time point.

The cell proliferation was shown in Figure 4.22. The amount of viable cells increased as the culturing time increased. MC3T3-E1 seeded on the film whose surface was BSA-immobilized showed better cell proliferation than others since day 1 onward. This could be due to better hydrophilicity of the film surface. There was a significant improvement of cell proliferation, especially on day

3. Moreover, when comparing 2 types of BSA-immobilized films (ie. the film casted from chloroform and from 40:60 (v/v) EtOH:THF), cells preferred to grow more on the film casted from 40:60 (v/v) EtOH:THF. This could be explained by higher amount of protein on the surface of the film casted from 40:60 (v/v) EtOH:THF.

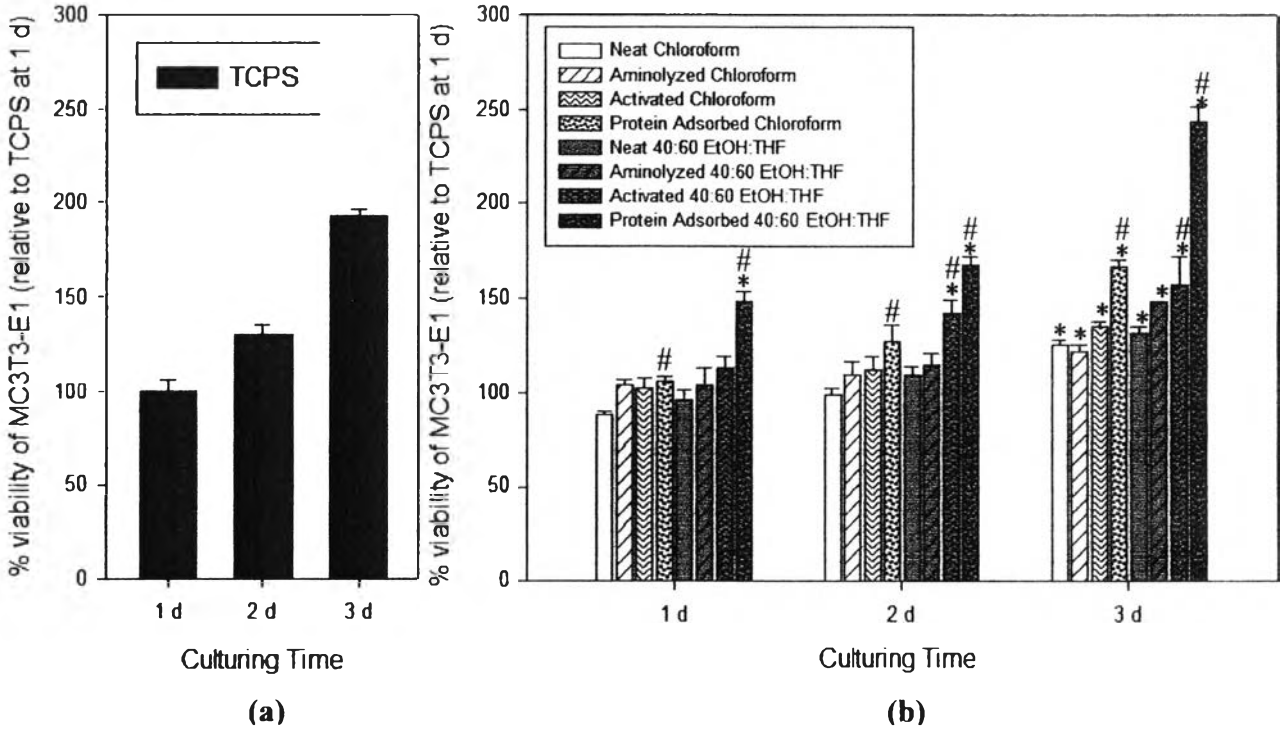


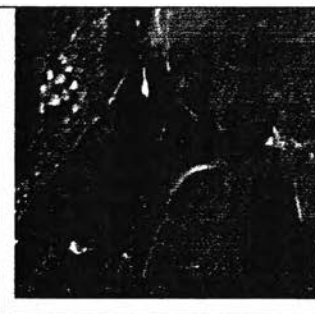
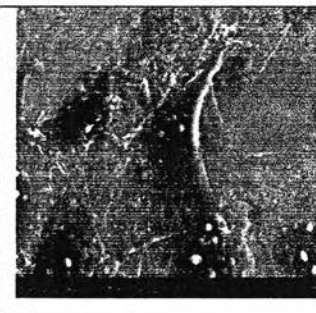
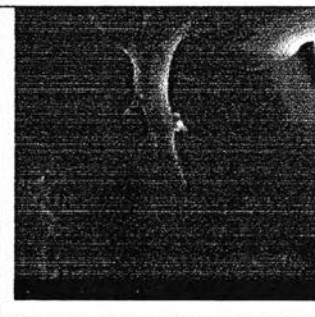
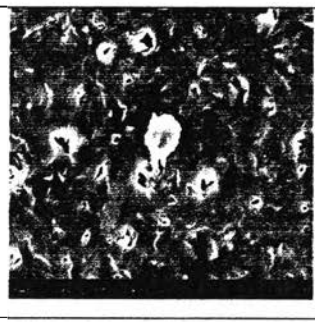
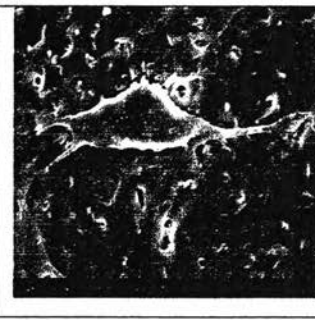
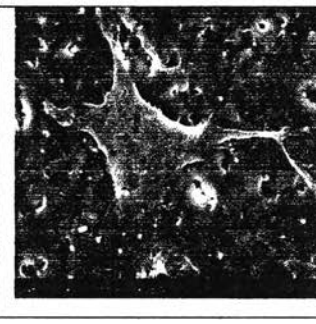
Figure 4.22 MC3T3-E1 proliferation on the surface of TCPS (a), the surface-modified films (b) for 1 d, 2 d, and 3 d. *Significance at $p < 0.05$ with respect to control. #Significance at $p < 0.05$ with respect to the neat substrate of each solvent at any given time point.

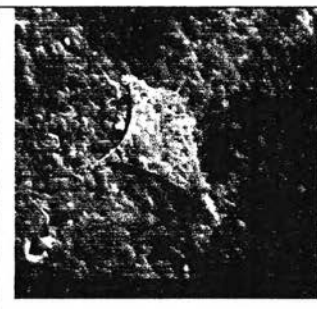
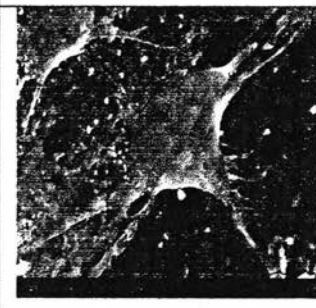
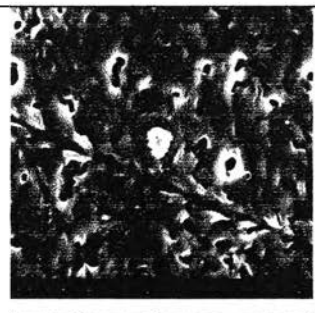
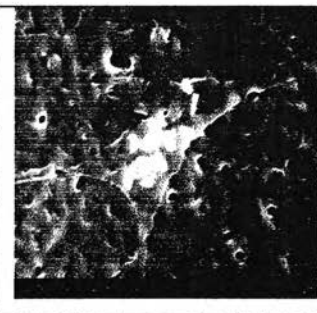
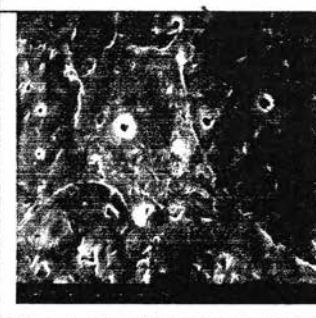
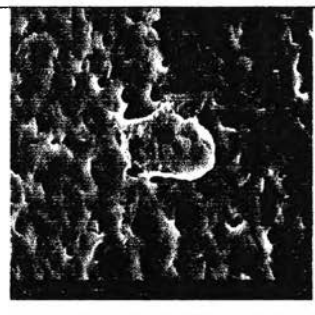
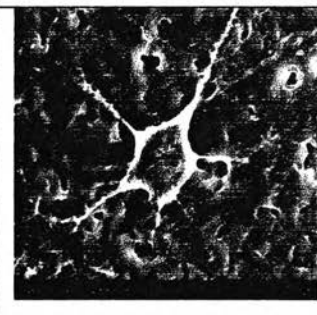
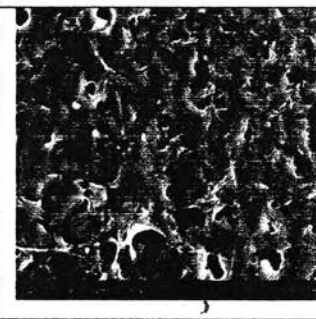
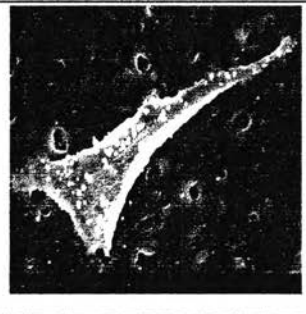
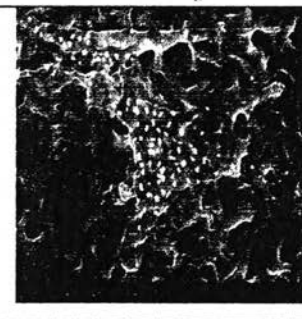
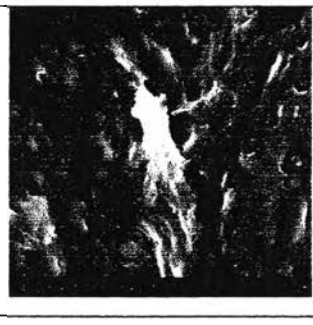
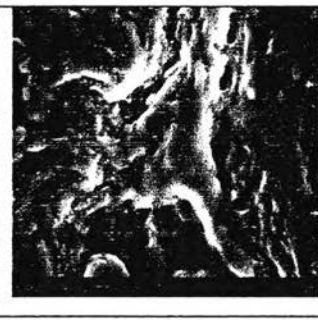
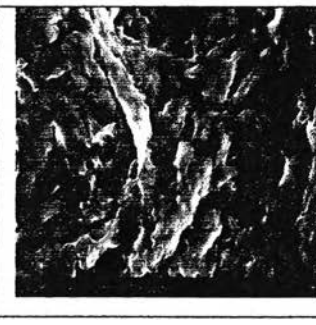
4.3.3 Cell Morphology Observation

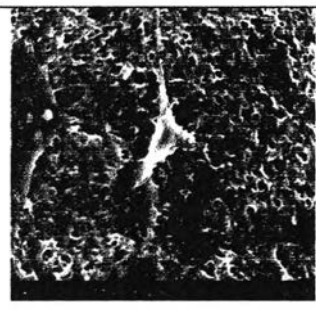
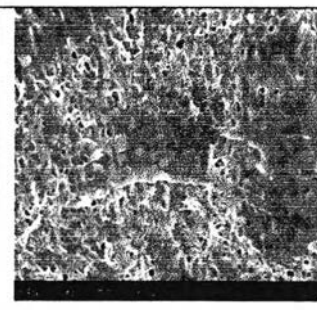
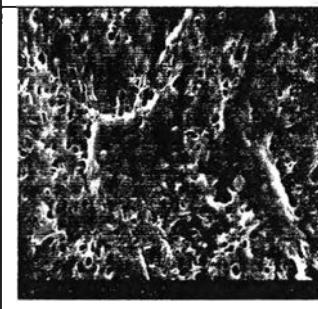
The effect of different surface topology to the cell morphology can be observed from the selected SEM images in Table 4.4. It can be seen that after 4 h of cell seeding, most of the cells were round, except for the PCL film casted from 40:60 (v/v) EtOH:THF which the cells started to spread. On day 1 and 3 after cell seeding, the cells cultured on all types of substrate showed the elongation and expansion of their cytoplasm which made cells be more flat. It can be clearly seen that cells could

proliferate more on the specimens with rough surface (i.e. the film casted from 40:60 (v/v) EtOH:THF) more than the smooth ones. This could be explained by higher amount of protein adsorbed on the rougher surface.

Table 4.4 SEM images of cultured specimens, i.e. TCPS (control), and the films casted from different solvents, at 4 h, 1 d and 3 d after the MC3T3-E1 cell seeding (magnification = 1500X)

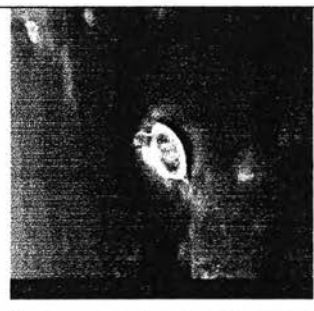
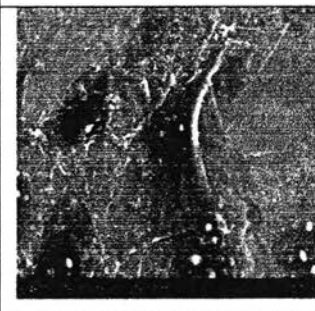
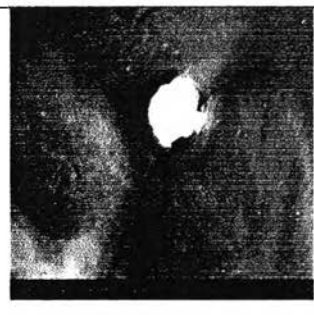
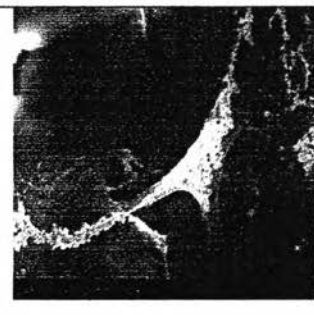
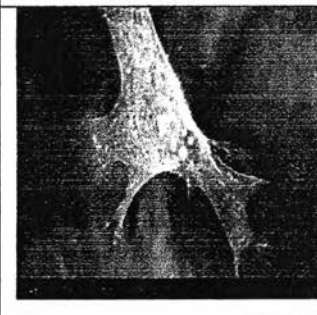
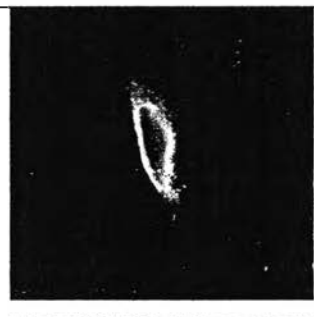
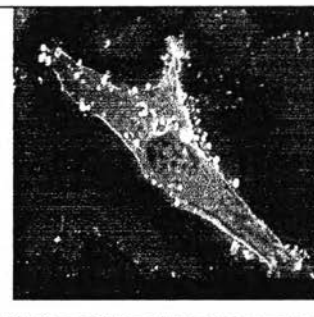
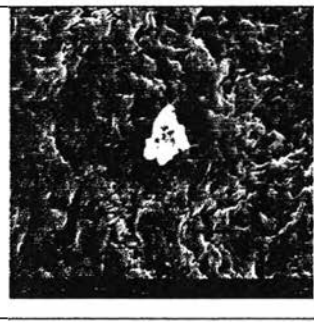
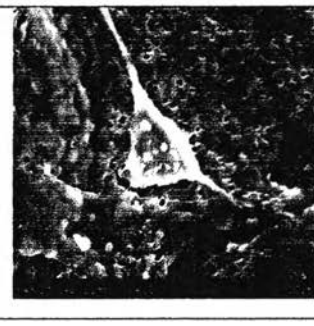
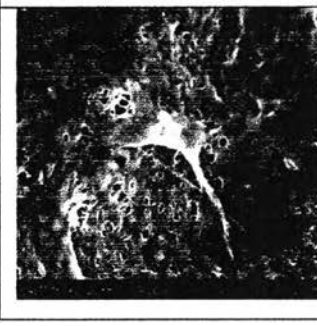
Solvents used in film casting	**Magnification = 1500X**		
	4 h	**1 d**	**3 d**
TCPS (control)			
Chloroform			
THF			

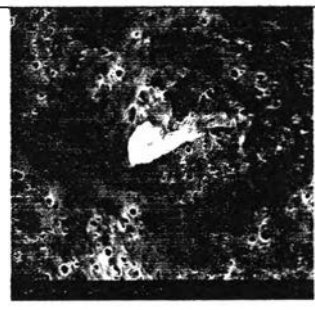
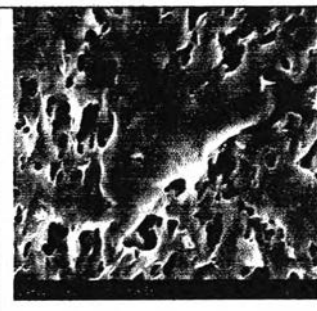
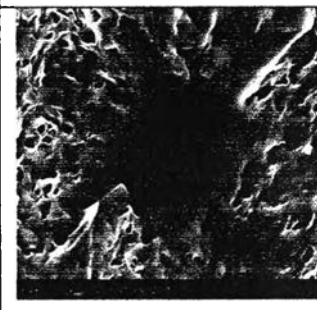
Solvents used in film casting	Magnification = 1500X		
	4 h	1 d	3 d
Acetone			
1 M NaOH			
5 M NaOH			
20:80 (v/v) EtOH:THF			
30:70 (v/v) EtOH:THF			

Solvents used in film casting	Magnification = 1500X		
	4 h	1 d	3 d
40:60 (v/v) EtOH:THF			

The effect of BSA-immobilized PCL film was compared with the neat PCL film to see the behavior of cells on the modified surface. Here, two types of PCL film were selected, which were the film casted from chloroform (smooth surface) and from 40:60 (v/v) EtOH:THF (rough surface). It can be seen from Table 4.5 that at 4 h after cell seeding, cell on neat PCL film casted from chloroform was still round but the cell on BSA-immobilized surface showed more expansion. This could be because after the immobilization of BSA, higher amount of protein was presented on the surface of the substrate, making it more suitable for cells to proliferate. The same trend also occurred with the film casted from 40:60 (v/v) EtOH:THF in a way that cells spread better on the BSA-immobilized surface than the neat surface. However, it can be clearly seen that rougher surface had better cell proliferation and cells preferred to expand more on rough surface than smooth surface.

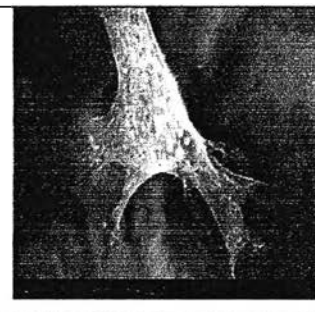
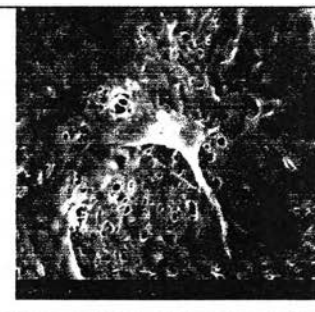
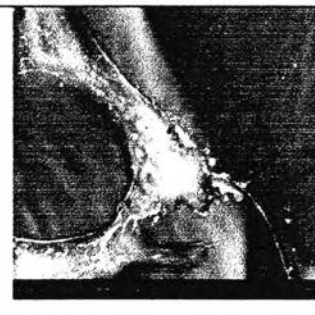
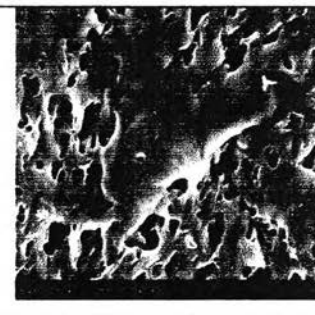
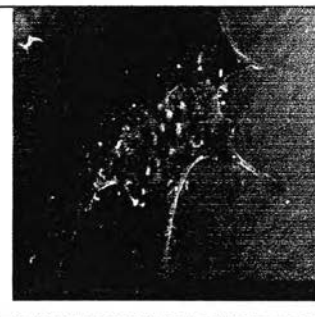
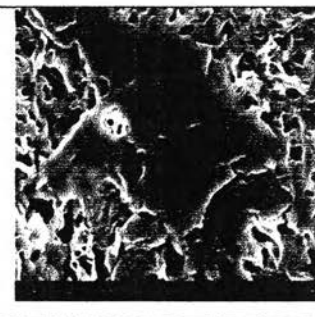
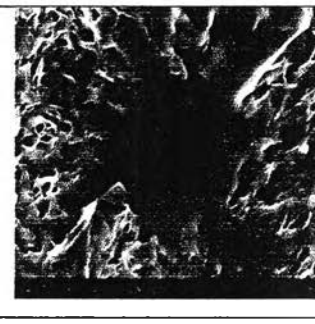
Table 4.5 SEM images of cultured specimens, i.e. TCPS (control), the neat and the BSA-immobilized films casted from chloroform and 40:60 (v/v) EtOH:THF, at 4 h, 1 d and 3 d after the MC3T3-E1 cell seeding (magnification = 1500X)

Materials	Magnification = 1500X		
	4 h	1 d	3 d
TCPS (control)			
Neat PCL casted from chloroform			
BSA-immobilized PCL casted from chloroform			
Neat PCL casted from 40:60 (v/v) EtOH:THF			

Materials	Magnification = 1500X		
	4 h	1 d	3 d
BSA-immobilized PCL casted from 40:60 (v/v) EtOH:THF			

A closer examination on the effect of each step of the surface modification (neat, aminolyzed, activated, and BSA-immobilized PCL films) to the cell behavior on day 3 after cell seeding was represented in Table 4.6. Once again, two types of PCL film were chosen, i.e. the film casted from chloroform (smooth surface), and from 40:60 (v/v) EtOH:THF (rough surface). It was found that modified PCL had better cell proliferation than neat PCL, especially on the BSA-immobilized PCL. And the rough surface showed better support to the cell. The presence of protein was more preferable for cell to attach and proliferate.

Table 4.6 SEM images of cultured specimens, i.e. the surface-modified films casted from chloroform and 40:60 (v/v) EtOH:THF, for 3 d after the MC3T3-E1 cell seeding (magnification = 1500X)

Materials at 3 d	Magnification = 1500X	
	Casted from chloroform	Casted from 40:60 (v/v) EtOH:THF
Neat PCL		
Aminolyzed PCL		
Activated PCL		
BSA-immobilized PCL		

4.3.4 Mineralization

In order to investigate the ability to promote the bone formation of the materials, the analysis on mineralization was done. The ability of calcium deposition on the TCPS (as control) and the scaffolds was evaluated by Alizarin Red-S staining after cell culture for 21 days. The stain was eluted with 10% cetylpyridinium chloride in 10 mM sodium phosphate. The eluting solution was read for its absorbance at 570 nm by spectrophotometer. The absorbance which is related to the redness can determine the amount of calcium deposition as shown in Figure 4.23. Qualitatively, the images of Alizarin Red-S staining on protein-adsorbed PCL films casted from different solvents were captured comparing to the stain on the neat PCL films (with no protein adsorbed), as can be observed in Figure 4.24. From the obtained results, all types of PCL films showed the red stain, indicating the calcium deposition. Especially, the PCL film casted from 40:60 (v/v) EtOH:THF which provided the highest red intensity, followed by the film casted from 30:70 (v/v) EtOH:THF. Although MC3T3-E1 cells cultured on TCPS gave a high attachment and proliferation, it had the lowest amount of mineral deposition. Moreover, the protein-adsorbed films exhibited higher intensities of stained minerals than the neat PCL films, showing that protein helped promoting differentiation of the cells.

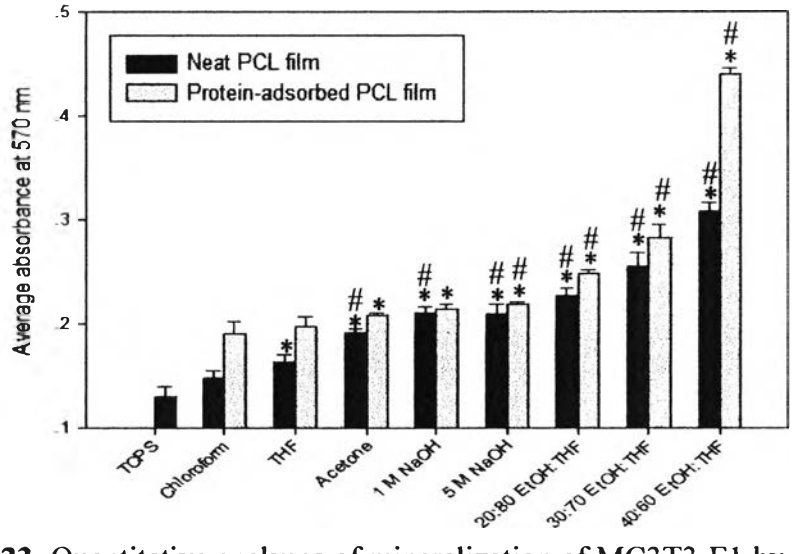


Figure 4.23 Quantitative analyses of mineralization of MC3T3-E1 by Alizarin Red-S staining on day 21 after being cultured on TCPS, protein-adsorbed PCL films, and non-protein-adsorbed PCL films. *Significance at $p < 0.05$ with respect to control. #Significance at $p < 0.05$ with respect to the substrate casted from chloroform.

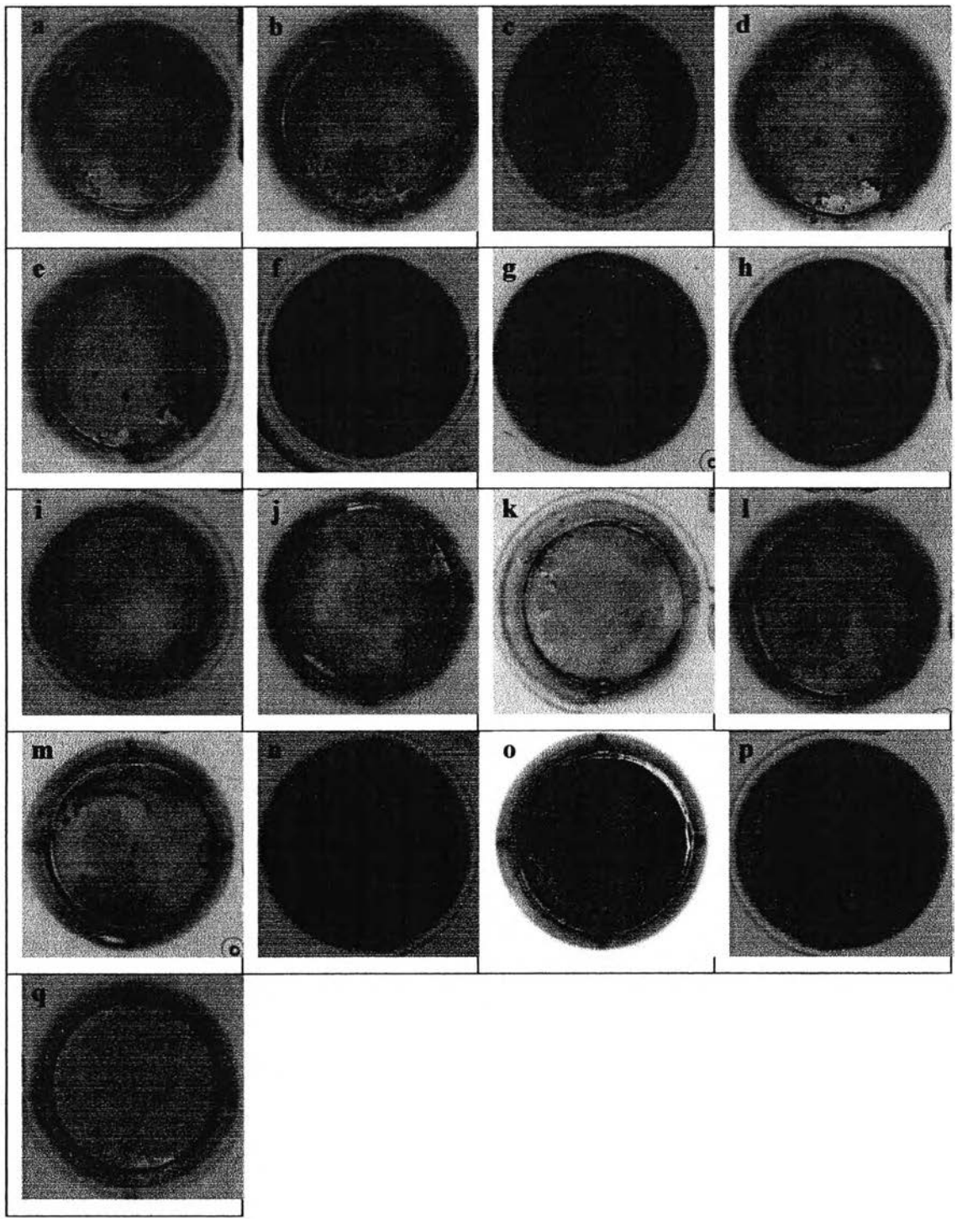


Figure 4.24 Images of Alizarin Red-S staining for mineralization of MC3T3-E1 on day 21: Bovine serum albumin-adsorbed films casted from chloroform (a), THF (b), treated with 1 M NaOH (c), treated with 5 M NaOH (d), acetone (e), 20:80 (v/v) EtOH:THF (f), 30:70 (v/v) EtOH:THF (g), 40:60 (v/v) EtOH:THF (h), and the neat

films casted from chloroform (i), THF (j), treated with 1 M NaOH (k), treated with 5 M NaOH (l), acetone (m), 20:80 (v/v) EtOH:THF (n), 30:70 (v/v) EtOH:THF (o), 40:60 (v/v) EtOH:THF (p), and TCPS (q) as a control.

From the results, it can be seen that introducing the protein as bovine serum albumin exhibited no cytotoxicity and also positively promoted cell attachment, proliferation and differentiation. Moreover, it had been shown that a higher amount of protein could be immobilized on the surface of the PCL film though a chemical modification process. The increased in cell promoting ability of the protein-adsorbed film could be attributed to the increased hydrophilicity, higher amount of protein adsorption on a non-uniform surface over a planar surface, and the rough topography of the surface itself. The obtained result demonstrated that the protein-adsorbed PCL film, especially on a rough film surface, can be potentially used as a scaffold for bone tissue engineering.