CHAPTER IV
RESULTS AND DISCUSSION

4.1 Preparation of Poly(lactic acid) Electrospun Fiber Mats

Poly(lactic acid) (PLA; M_n = 80,000 g/mol) electrospun fiber mats were prepared via electrospinning technique under fixed conditions as mentioned in the previous chapter. Translucent electrospun PLA fibrous scaffolds with a thickness of 136 ± 5 μm were obtained. Morphological appearance and size of the individual fibers of the scaffolds were examined by JEOL JSM 5410LV scanning electron microscopy (SEM) (Figure 4.1). At least 100 readings of the fiber diameters from various SEM images were statistically analyzed using SemAphore 4.0 software, from which the arithmetic mean value of the individual fibers within the PLA fiber mats was determined to be 0.63 ± 0.1 μm.

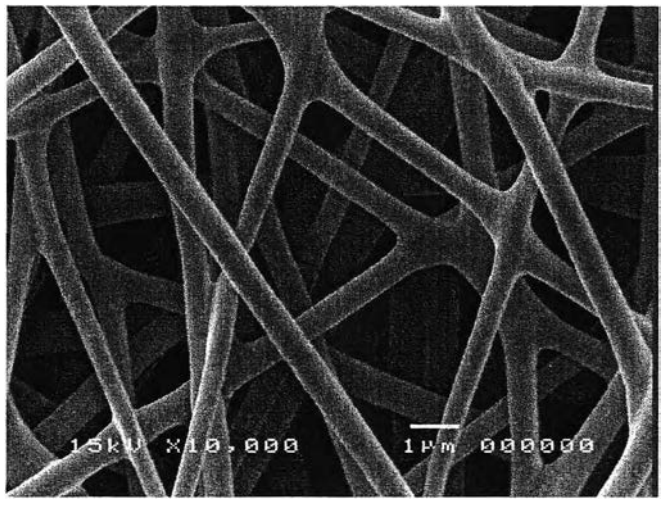


Figure 4.1 Selected SEM image of electrospun PLA fibrous scaffolds (magnification = 10000x; scale bar = 1 μm).

4.2 Surface Modification of Electrospun PLA Fibrous Scaffolds

By reaction with 1,6-hexanediamine (HMD), amino groups can be covalently introduced onto the surface of the electrospun PLA fibrous scaffolds. One

amino group (NH_2) of HMD reacts with an ester group (—COO—) of PLA to form the amide linkage (—CONH—), while another amino group is unreacted and free for the further reaction. These free NH_2 groups can be used as active sites through which proteins like collagen can be bonded to the surface using *N, N'*-disuccinimidyl carbonate (DSC) as a coupling agent. However, to avoid aggregation, two-step procedure was employed. The attached amino groups had been first activated with DSC with *N*-hydroxysuccinimide being lost from the reaction and the as-formed succinimidyl esters were later reacted with respective biomolecule, collagen, with *N*-hydroxysuccinimide again being cleaved from the reaction. The chemical pathway for the immobilization of biomolecule on the surface of e-spun PLA fibrous scaffolds is summerized in the Figure 4.2.

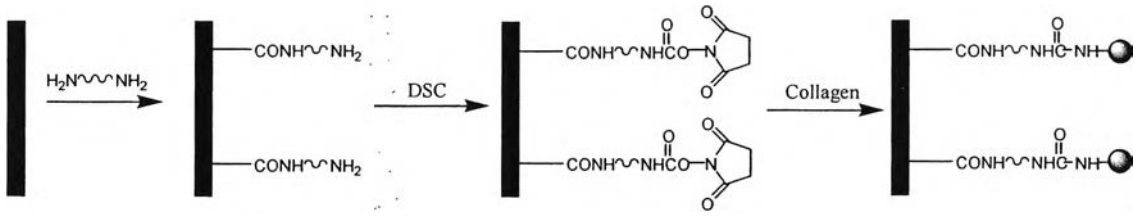


Figure 4.2 The chemical pathway for the immobilization of collagen.

4.3 Surface Characterization

4.3.1 Quantification of Amino Groups

The existence of free amino groups (NH_2) on PLA surface is a prerequisite for protein bonding in this modification method. It is important to confirm the existence of amino groups before protein is further introduced. Herein, ninhydrin is used as an indicator to confirm and quantify the $-NH_2$ moiety on the aminolyzed PLA surface. The NH_2 density on e-spun PLA fibrous scaffold surfaces was influenced by the concentration of 1,6-hexanediamine, aminolyzing time, temperature, and so on. Because the glass transition temperature (T_g) of the present PLA is ~62°C, the aminolyzing temperature should be lower than this value to maintain the shape of the material. Hence, 50°C was chosen to perform the following reactions. Figure 4.3 shows that NH_2 density increased with increasing 1,6-

hexanediamine concentration. However, when the concentration of 1,6-hexanediamine is equal to or greater than 0.1 g/ml, the mechanical properties of the e-spun PLA fiber mats became worse. Figure 4.4 shows that the surface-bound NH_2 increased with increasing aminolyzing time to reach a maximum value at about 15 min, and then decreased slightly. The decrease at the longer reaction time may be caused by a further reaction of the free amino groups on the terminal chain with other ester groups, or by the degradation of the superficial layer (Zhu *et al.*, 2006). Both of these situations will reduce the density of the surface amino groups and thus intensity of purple colored complex. To maintain enough mechanical properties for practical applications, PLA e-spun fiber mats were aminolyzed at 50°c for 15 min in a 0.04 g/ml 1,6-hexanediamine/isopropanol solution. According to the calibration curve obtained with 1,4-dioxane-isopropanol (1:1, v/v) solution containing 1,6-hexanediamine of known concentration, the NH_2 density on PLA e-spun fiber mats aminolyzed under these conditions was (3.7 ± 0.02) x 10^{-7} mol/cm^2. After immobilization with 0.5 mg/ml and 3.0 mg/ml type-I collagen, the average areal density of the amino groups on the surface of the PLA fibrous scaffolds were (4.52 ± 0.08) x 10^{-9} collagen and (3.21 ± 0.2) x 10^{-8}, respectively.(Table 4.1)

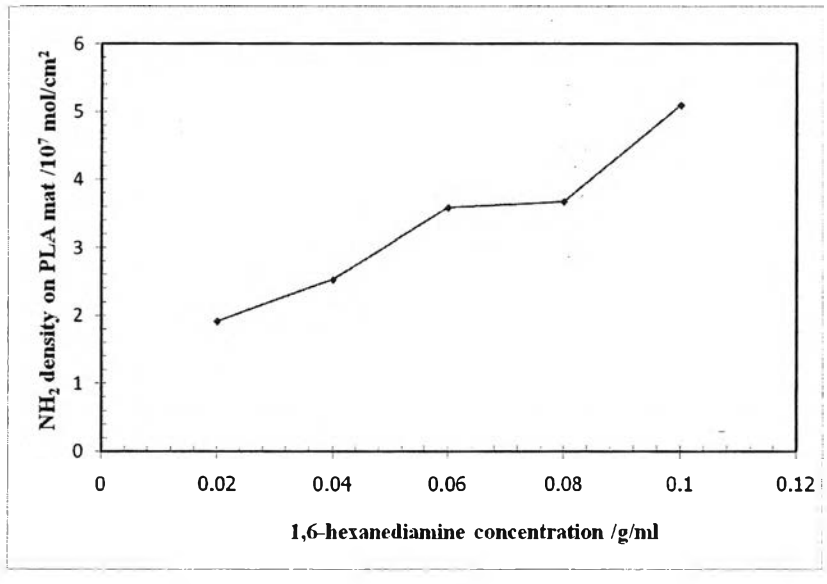


Figure 4.3 NH_2 density on PLA electrospun fiber mats as a function of concentration of 1,6-hexanediamine/isopropanol solution. The PLA mat was aminolyzed at 50°C for 8 min.

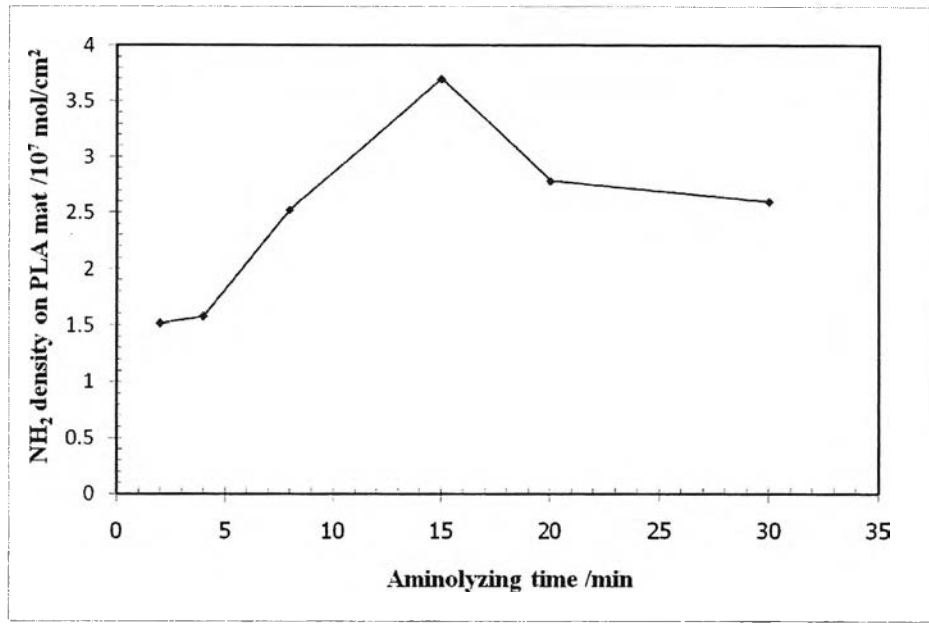


Figure 4.4 NH_2 density on PLA electrospun fiber mats as a function aminolyzing time. The aminolysis reaction took place at 50°C in 1,6-hexanediamine /isopropanol solution (0.04 g/ml).

Table 4.1 NH_2 density on the surface of the aminolyzed and collagen-immobilized PLA fibrous scaffolds

Sample	NH_2 concentration (mol·cm^2)
control PLA	-
Aminolyzed PLA[a]	$3.70 \pm 0.02 \times 10^{-7}$
Activated PLA[b]	-
Collagen Immobilized PLA[c1]	$4.52 \pm 0.08 \times 10^{-9}$
Collagen Immobilized PLA[c1]	$3.21 \pm 0.20 \times 10^{-8}$

[a]The PLA e-spun fiber mats was immersed in 0.04 g/ml 1,6-hexanediamine solution at 50 °C for 15 min.

[b]The aminolyzed PLA fibers were immersed in 0.1 M DSC solution for 3 h.

[c1,c2]The activated PLA fibers were immersed in collagen solutions 0.5 mg/ml and 3.0 mg/ml, respectively, for 24 h followed by the rinsing process.

4.3.2 Surface Wettability

To further evaluate the effect of aminolysis, surface wettability of the aminolyzed PLA fibrous scaffolds with respect to that of the neat PLA fibrous scaffolds was measured. Besides, water contact angle measurement was also used to evaluated the surface wettability of the collagen-bounded PLA fibrous scaffolds. Table 4.2 shows the surface wettability of unmodified PLA, aminolyzed PLA, activated PLA, collagen-immobilized PLA (without coupling agent; collagen = 0.5 mg/ml), collagen-immobilized PLA (collagen = 0.5 mg/ml), and collagen-immobilized PLA (collagen = 3 mg/ml) fibrous scaffolds. The water contact angle measured by the sessile drop method decreased slightly from 106.8° to 99.5° after the scaffolds were aminolyzed with 0.04 g/ml of HMD/IPA solution for 15 min. That is, introduction of the amino groups on the surface of the PLA fibrous scaffolds improved the hydrophilicity of the surface. After the aminolyzed PLA fibrous scaffolds have been activated with DSC, their surface became more hydrophobic as evidenced by the water contact angle of 112.5°. Water contact angle decreased substantially after the collagen was bonded. Figure 4.5 shows that the surface became much more hydrophilic after collagen immobilization. Water contact angle was 79.4° for collagen 0.5 mg/ml immersion and was 73.0° for collagen 3 mg/ml immersion. These result showed that the more collagen concentration used, the more collagen can be bonded to the PLA surface. It has been known that physisorption processes are virtually always involved in a coating procedure. In our case, we had tried physical coating of collagen on unactivated PLA fibrous scaffolds. Water contact angle measurements showed physisorbed collagen-coated PLA surfaces have contact angle almost the same as the aminolyzed PLA surfaces (99.5° and 92.1°) but much higher values than covalently bonded with protein surfaces. That means activation step is an important step in protein grafting process.

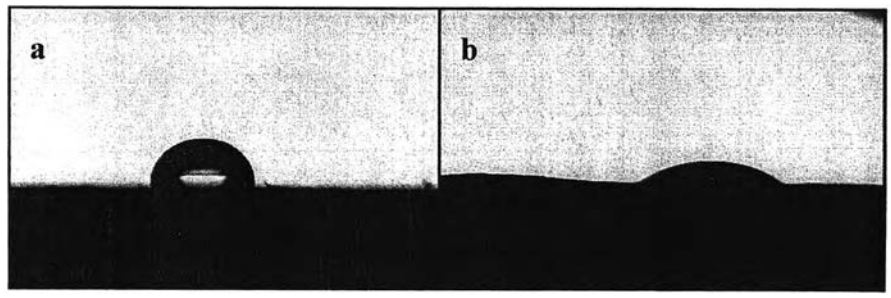


Figure 4.5 Water dropped on the surface of neat PLA fibrous scaffold (a), and PLA fibrous scaffold immobilized with 3.0 mg/ml collagen (b).

Table 4.2 Water contact angles of the control and modified PLA fibrous scaffolds

Samples	Water contact angle/degree (Sessile drop)
control PLA	106.8 ± 0.20
Aminolyzed PLA[a]	99.5 ± 0.17
Activated PLA[b]	112.5 ± 0.38
Collagen Immobilized PLA (w/o coupling agent)[c]	92.1 ± 0.19
Collagen Immobilized PLA[d1]	79.4 ± 0.30
Collagen Immobilized PLA[d2]	73.0 ± 1.18

[a]The PLA e-spun fiber mats was immersed in 0.04 g/ml 1,6-hexanediamine solution at 50 °C for 15 min.
[b]The aminolyzed PLA fibers were immersed in 0.1 M DSC solution for 3 h.
[c]The aminolyzed PLA fibers were immersed in 0.5 mg/ml collagen solutions for 24 h followed by the rinsing process.
[d1,d2]The activated PLA fibers were immersed in collagen solutions 0.5 mg/ml and 3.0 mg/ml, respectively, for 24 h followed by the rinsing process.

4.3.3 Chemical Analysis of Surface

ATR-FTIR spectra of PLA and modified PLA fibrous scaffolds are shown in Figure 4.6. There was a major absorption peak assigned to the ester carbonyl of virgin PLA appeared at 1755 cm^{-1}. However, the spectra of all of the modified materials are almost the same as the unmodified PLA. This may be regarded as a result of the extremely low concentration of introduced chemicals which presented within the sampling depth of ATR-FTIR (1-2 μm).

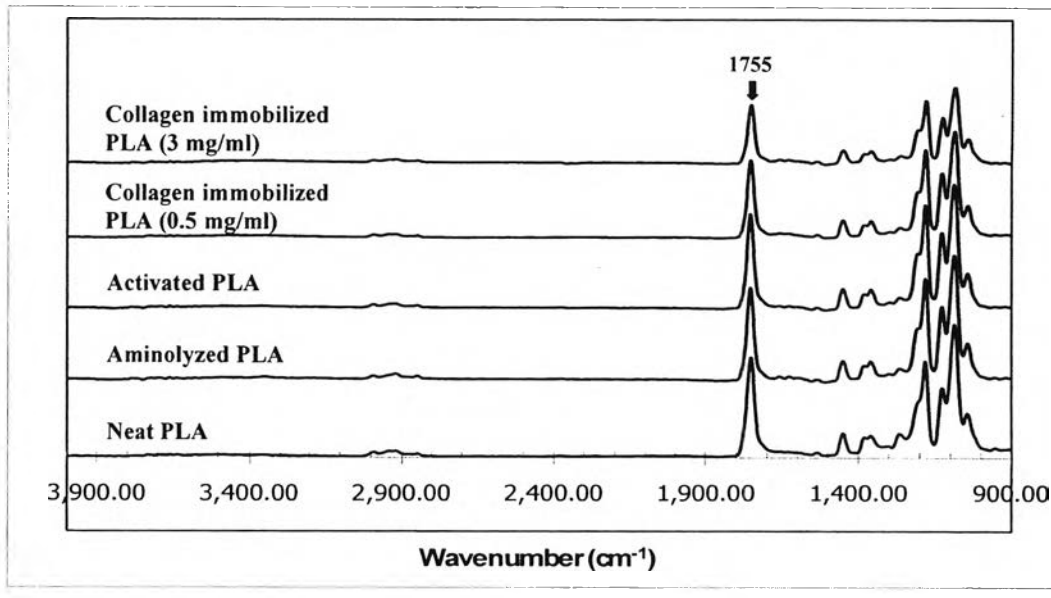


Figure 4.6 ATR-FTIR spectra of neat and modified PLA fibrous scaffolds.

Because of this problem, to confirm the success of aminolysis and immobilization of type-I collagen, X-ray photoelectron spectrometer (XPS) was used to evaluate the N_{1s}/C_{1s} ratio of the unmodified and modified PLA fibrous scaffolds. We expected that after aminolysis and collagen immobilization, the N_{1s}/C_{1s} ratio should increase.

4.3.4 Elemental Composition of the Surface

The surface alteration after the PLA fibrous scaffolds were modified was studied by XPS. To study the effect of the aminolyzing condition on the surface alteration, N_{1s}/C_{1s} ratios as a function of 1,6-hexanediamine concentration and aminolyzing time were evaluated. Table 4.3 shows that the more diamine concentration used, the more N_{1s}/C_{1s} ratio observed which could be due to increasing in NH_2 group concentration. Table 4.4 shows that the N_{1s}/C_{1s} ratio increased with increasing aminolyzing time to reach a maximum value at about 15 min, and then decreased slightly, which, in addition to the results on ninhydrin analysis method. Table 4.5 shows the alteration of N_{1s}/C_{1s} ratios after PLA scaffolds were modified. After aminolysis of PLA fibrous scaffolds, the N_{1s}/C_{1s} ratio was increased from 0 to 0.0290 because of NH_2 groups were introduced on the surface. The N_{1s}/C_{1s} ratio was increased to 0.0381 by reaction with DSC. It shows that the nitrogen concentration increased when succinimidyl esters was formed. Finally, it was obviously increasing

in N_{1s}/C_{1s} ratio after collagen immobilization due to the large amount of nitrogen atom in biomolecule structure was additionally introduced.

Table 4.3 N_{1s}/C_{1s} ratios as a function of 1,6-hexanediamine concentration

Diamine concentration (g/ml)	N_{1s}/C_{1s} ratio
0.02	0.0220
0.04	0.0237
0.06	0.0248
0.08	0.0283
0.10	0.0833

The aminolysis reaction took place at 50°c for 8 min.

Table 4.4 N_{1s}/C_{1s} ratios as a function of aminolyzing time

Aminolyzing time (min)	N_{1s}/C_{1s} ratio
2	0.0133
4	0.0200
8	0.0237
15	0.0290
20	0.0240
30	0.0238

The aminolysis reaction took place at 50°c in 0.04 g/ml HMD/IPA solution.

Table 4.5 N_{1s}/C_{1s} ratios of the control and modified PLA fibrous scaffolds

Sample	N_{1s}/C_{1s} ratio
control PLA	0.0068
Aminolyzed PLA[a]	0.0290
Activated PLA[b]	0.0381
Collagen Immobilized PLA[c1]	0.2349
Collagen Immobilized PLA[c1]	0.2401

[a]The PLA e-spun fiber mats was immersed in 0.04 g/ml 1,6-hexanediamine solution at 50 °C for 15 min.
[b]The aminolyzed PLA fibers were immersed in 0.1 M DSC solution for 3 h.
[c1,c2]The activated PLA fibers were immersed in collagen solutions 0.5 mg/ml and 3.0 mg/ml, respectively, for 24 h followed by the rinsing process.

4.4 Biological Characterizations

4.4.1 Cytotoxicity

The potential for use of these fiber mats as bone scaffolds was first assessed by an indirect cytotoxicity evaluation with mouse fibroblastic cells (L929) and mouse calvaria-derived pre-osteoblastic cells (MC3T3-E1), based on the initial 40,000 cells·cm^{-2}of cells seeded. Indirect cytotoxicity test was conducted on neat, aminolyzed, activated, collagen-immobilized PLA fibrous scaffolds. In this experiment, the PCL/HA that were prepared from solvent casting and particulate leaching technique, was chosen to be the positive control. Even though we were interested in using the obtained modified fiber mats as potential bone scaffolds, it was mandatory to test the materials with L929 just to comply with the ISO10993-5 standard test method. Figure 4.7 shows %viability obtained from MTT assay of L929 which were cultured with the 1, 3, 7 day- extraction media in comparison with those cultured with SFM (i.e. control). The viability of the cells that had been cultured with SFM at ay given time point was taken as the basis to arrive at the relative viability shown in the figure. Evidently, the viability of L929 for all types of modified PLA fibrous scaffolds exhibited slightly lower, in comparison with that of the control (100%). While the neat PLA scaffold and PCL/HA scaffold which used as positive

control exhibited comparable viability. In a similar manner was found for MC3T3-E1 (Figure 4.8). However, the viability of cells that were cultured with 7-day extraction media prepared from modified scaffolds were significantly lower, in comparison with that were cultured with 7-day extraction media prepared from the neat PLA scaffold and PCL/HA. Thus, we have to set other experiment to confirm the compatibility of the modified PLA fibrous scaffolds.

MC3T3-E1 were cultured in wells of a 24-well culture plate in 2% serum-containing MEM diluted with 7-day extraction medium for 1, 2, and 3 d to allow cell growth on the plate. Figure 4.9 shows that the viability of MC3T3-E1, cultured with 2% serum-containing MEM diluted with 7-day extraction media prepared by all types of modified scaffolds, was increased with increasing the culturing time in the respective media. All of the obtained results clearly suggested that all types of the PLA fibrous scaffolds, released no substances at levels that were harmful to both types of cells.

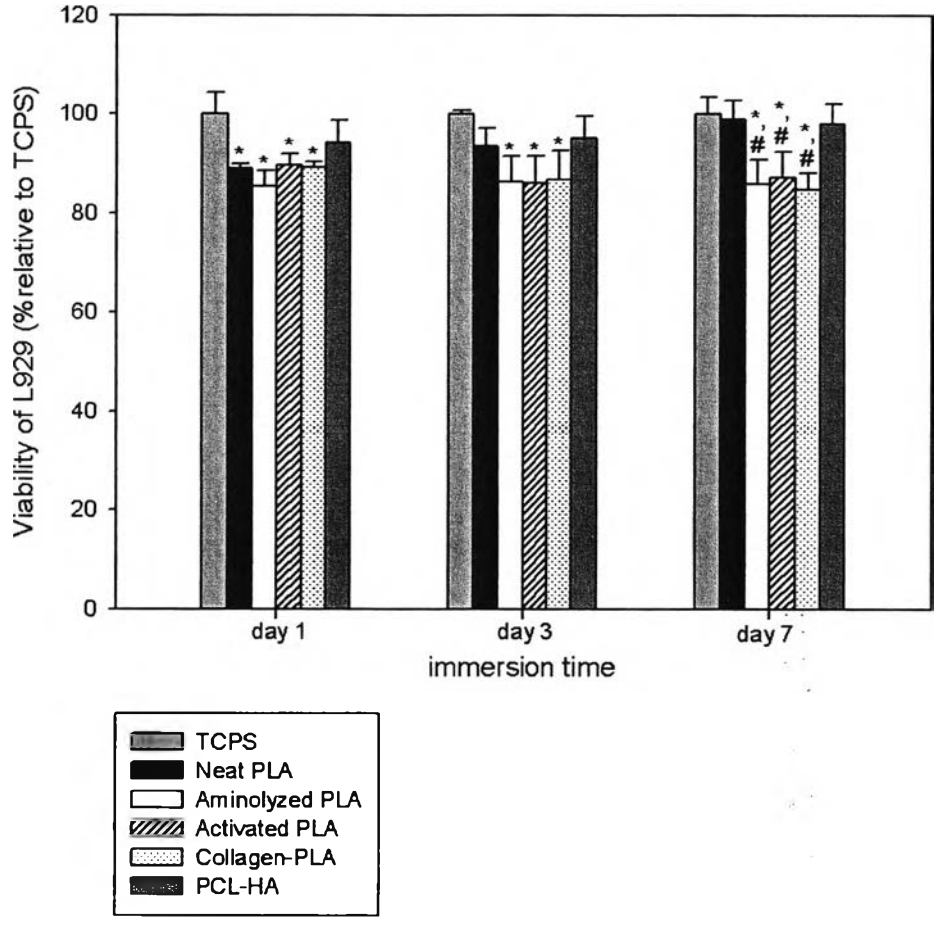


Figure 4.7 Indirect cytotoxic evaluation of neat PLA fibers, modified PLA fibers, and PCL/HA based on viability of mouse fibroblasts (L929) that had been cultured with the extraction media from each of these materials against the viability of the cells that had been cultured with the respective culture media for 1 day as a function of the incubation time of the extraction and the culture media of 1, 3, or 7 d. Statistical significance: $^{*}p < 0.05$ compared with control and $^{\#}p < 0.05$ compared to the neat PLA fibrous scaffolds at any given time point.

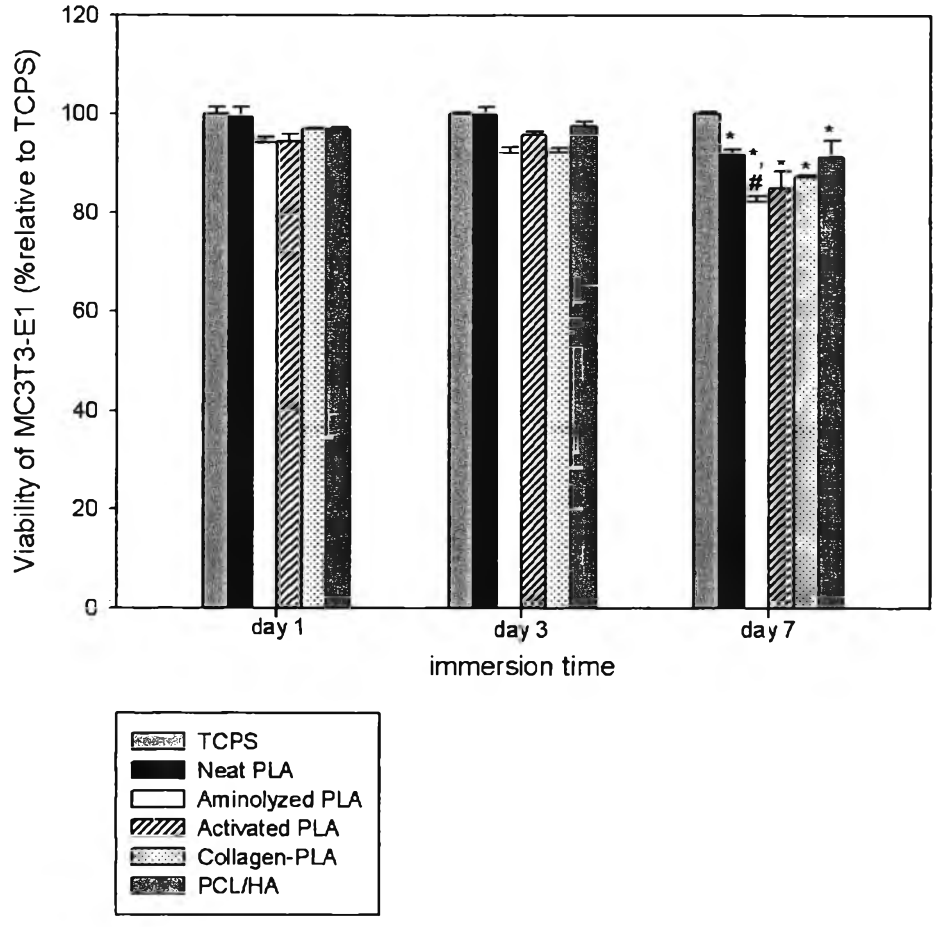


Figure 4.8 Indirect cytotoxic evaluation of neat PLA fibers, modified PLA fibers, and PCL/HA based on viability of pre-osteoblast (MC3T3-E1) that had been cultured with the extraction media from each of these materials against the viability of the cells that had been cultured with the respective culture media for 1 day as a function of the incubation time of the extraction and the culture media of 1, 3, or 7 d. Statistical significance: $^{*}p < 0.05$ compared with control and $^{\#}p < 0.05$ compared to the neat PLA fibrous scaffolds at any given time point.

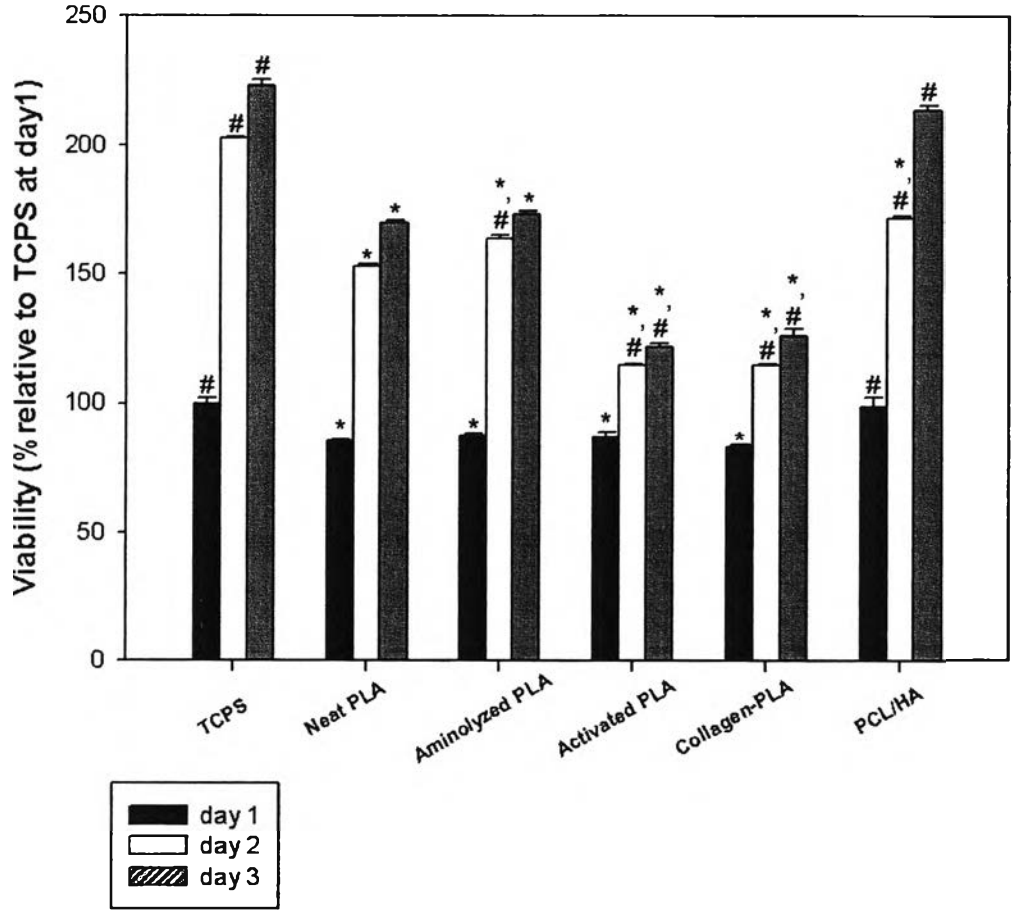


Figure 4.9 Indirect cytotoxic evaluation of neat PLA fibers, modified PLA fibers, and PCL/HA based on viability of pre-osteoblast (MC3T3-E1) that had been cultured with the 7-day extraction media from each of these materials with 2% serum-containing MEM against the viability of the cells that had been cultured with the respective culture media for 1, 2, and 3 day. Statistical significance: $^{*}p < 0.05$ compared with control and $^{\#}p < 0.05$ compared to the neat PLA fibrous scaffolds at any given time point.

4.4.2 Cell Attachment and Proliferation

The potential for use of the neat and the modified PLA fibrous scaffolds was further evaluated by observing their ability to support both the adhesion and the proliferation of MC3T3-E1. The viability of the cells that had been

cultured on the surface of TCPS for 2 h was taken as the basis to arrive at the relative viability shown in a figure. Figure 4.10 shows the attachment of MC3T3-E1 on the surfaces of TCPS, neat PLA, aminolyzed PLA, activated PLA, and collagen-immobilized PLA at 2, 4, and 16 h after cell seeding in terms of viability. On TCPS, the number of the attached cells increased from ~100% at 2 h after cell seeding to ~111% at 16 h after cell seeding, based on the initial number of cells seeded (60,000 cells·cm^{-2}). With regards to MC3T3-E1, the surfaces of all of the PLA fibrous scaffolds were inferior in supporting the attachment of the cells to that of TCPS. Specifically, the number of cells attached on these fibrous scaffolds was only ~50% in comparison with that on TCPS at any given time point. There were not significantly different in the viability among various types of the fibrous scaffolds.

Figure 4.11 shows the proliferation of MC3T3-E1 on the surfaces of TCPS, neat PLA, aminolyzed PLA, activated PLA and collagen-immobilized PLA on day 1, 2, and 3 after cell culture in terms of viability (%relative to TCPS at day1). On TCPS, the number of cells increased from ~100% on day1 after cell culture to ~187% (i.e., an increase of about 2 fold from the initial number of cells seeded) on day 3 after cell culture, based on the initial 30,000 cells·cm^{-2}of cells seeded. In comparison with that on TCPS, the viability of the cells cultured on various types of PLA fibrous scaffolds were significantly higher at any given time point. The viability of cells proliferated on these fibrous scaffolds, at day 3, was higher than TCPS of about 2 fold for neat PLA, aminolyzed PLA, and activated PLA and about 3.5 fold for collagen-immobilized PLA. Marked improvement was achieved with the collagen-immobilized PLA fibrous scaffolds on day 3 after cell culture.

The lesser viability of cells in the attachment period on various types of the fibrous scaffolds in comparison with that on TCPS could be due to the lesser number of cells that were able to attach on the rough surface of the fibrous scaffolds in comparison with the smoother and hydrophilic surface of TCPS. According to Figure 4.10, the introduction of the NH_2 groups on scaffold surface by aminolysis improved the adhesion of the cells could be due to the more hydrophilicity of the surface. The attachment of the cells was further improved with the reaction of aminolyzed PLA with DSC and the immobilization with collagen, respectively. On

the contrary, the greater number of cells in the proliferation period on all types of fibrous scaffolds could be because of high surface area to volume and high porousity of the e-spun fiber mats through which the cells were able to penetrate into the scaffolds. Among the various modified PLA fibrous scaffolds, the collagen-immobilized PLA fibrous scaffolds provided the most significant improvement in the ability to support the proliferation of the cells (Figure 4.11 shows the greatest proliferation rate on day 3) which could be due to the protein-containing and hydrophilic surface of the substrate.

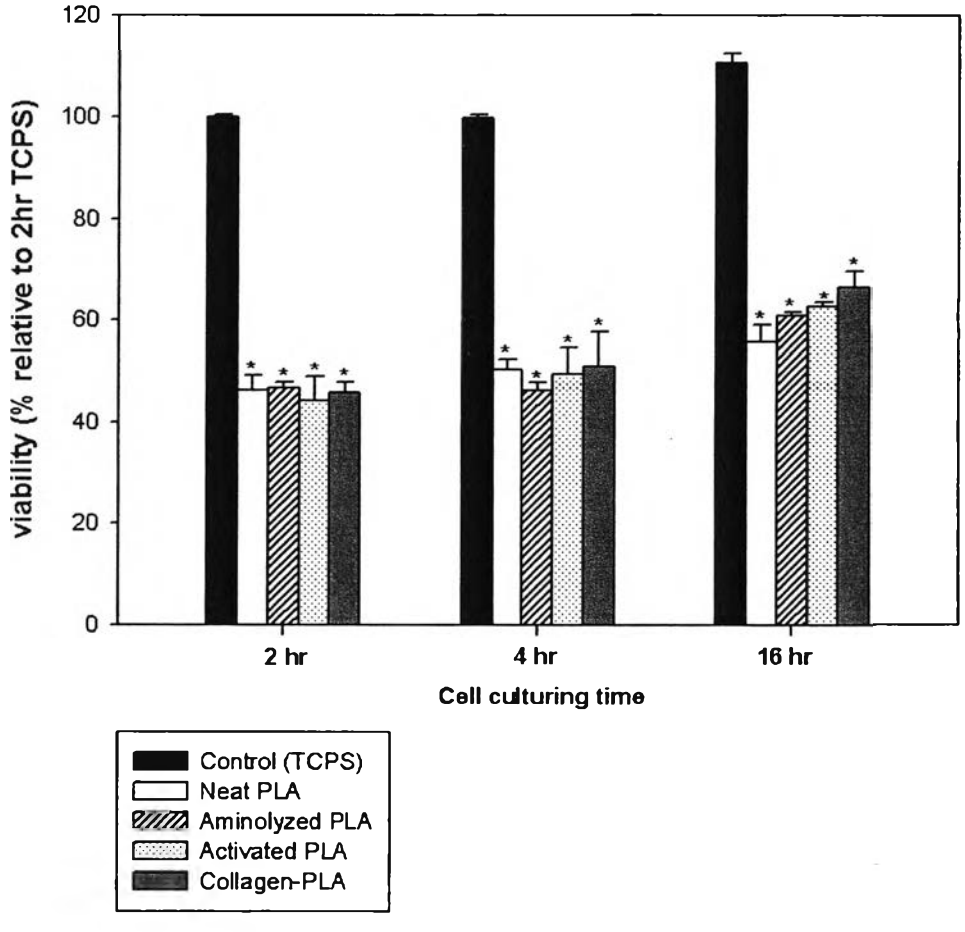


Figure 4.10 Attachment of MC3T3-E1 that had been seeded or cultured on the surfaces of TCPS and the neat and the modified PLA fibrous scaffolds for 2, 4, or 16 h. Statistical significance: $^{*}p < 0.05$ compared with control and $^{\#}p < 0.05$ compared to the neat PLA fibrous scaffolds at any given time point.

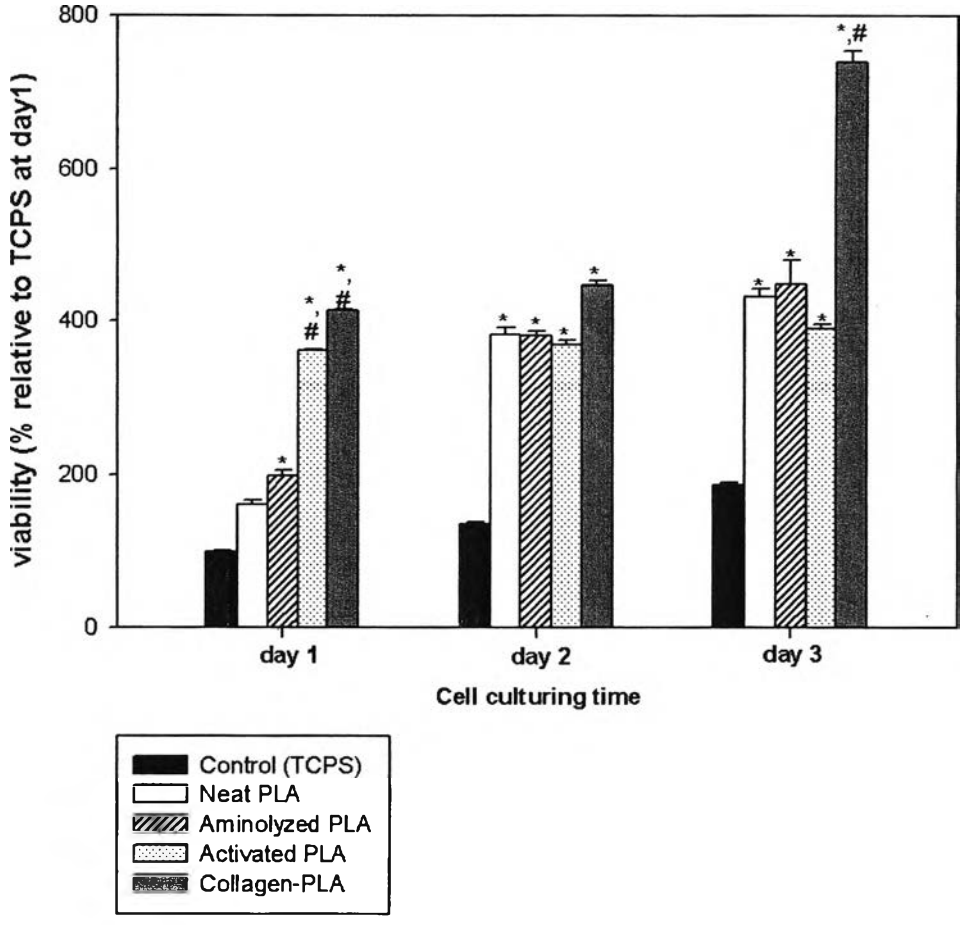


Figure 4.11 Proliferation of MC3T3-E1 that had been seeded or cultured on the surfaces of TCPS and the neat and the modified PLA fibrous scaffolds for 1, 2, or 3d. Statistical significance: $^{*}p < 0.05$ compared with control and $^{\#}p < 0.05$ compared to the neat PLA fibrous scaffolds at any given time point.

4.4.3 Cell Morphology

Table 4.6 shows selected SEM image (magnification = 500X; scale bar = 50 μm) and Table 4.7 shows selected SEM image (magnification = 2000X; scale bar = 10 μm) of MC3T3-E1 that were cultured on the surfaces of glass, neat PLA, activated PLA and collagen-immobilized PLA at different time points. The use of glass as the control instead of TCPS was due to the ease of taking the samples to SEM observation. These images provided snap shots in time that revealed the morphology of the cells and interaction between the cells and the tested surfaces. At 4 h after cell seeding, based on the initial 60,000 cells·cm^{-2} of cells seeded, the majority of the cells on the glass surface started to extend their cytoplasm, an evidence of the ability of the cells to attach on the surface. At 16 h after cell seeding,

expansion of the cytoplasm of the majority of the cells was evident. The majority of MC3T3-E1 that had been seeded on the surfaces of unmodified PLA fibrous scaffolds for 4 h was remained round, but a closer examination around the edge of the cells revealed an evidence of filopodia (i.e., slender cytoplasmic projections extending from the leading edge of the migrating cells that help the cells during their migration over the surface of a scaffolds). The majority of the cells were evidently expanded after 16 h of cell seeding. On the other hand, the majority of the cells seeded on the surfaces of various types of modified PLA fibrous scaffolds showed an evidence of the extension of their cytoplasm on the fibrous surface even at 4 h after cell seeding, with collagen-immobilized PLA surface did so to a greater extent. These results suggested that the cells prefer the fibrous surfaces of modified PLA over that of the unmodified. Specifically, the cells were seemed to prefer the collagen-containing than others.

Table 4.6 Selected SEM images of cultured specimens, i.e., glass (i.e., control), neat PLA, aminolyzed PLA, activated PLA, and collagen-immobilized PLA fibrous scaffolds at various time points after MC3T3-E1 were seeded or cultured on their surfaces (magnification = 500X; scale bar = 50 μm).

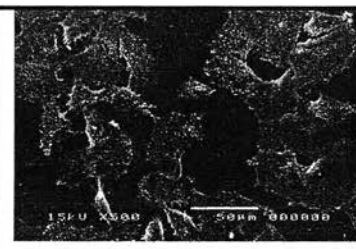
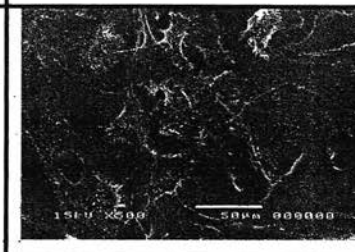
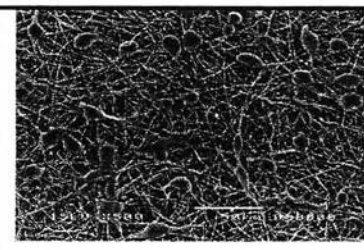
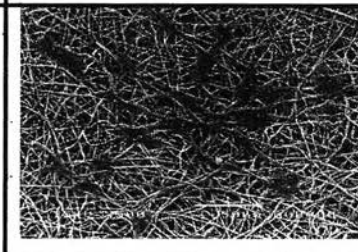
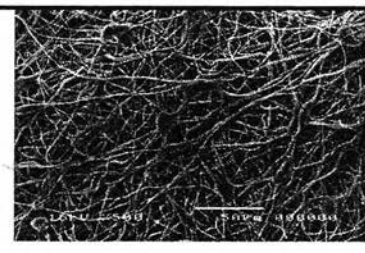
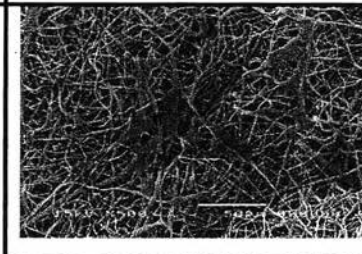
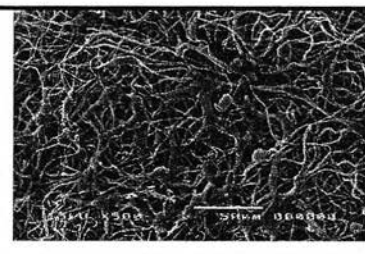
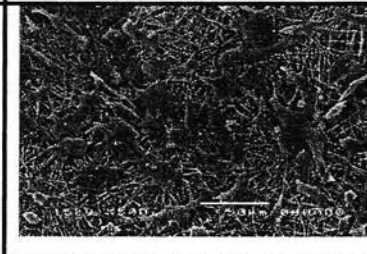
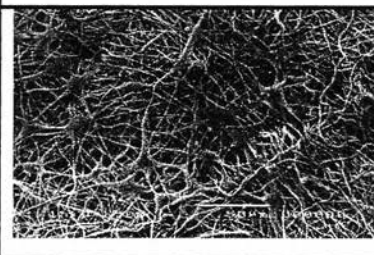
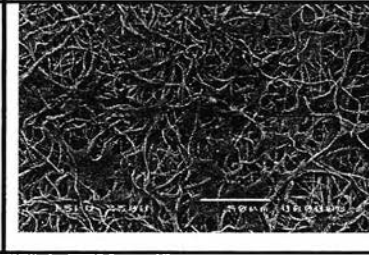
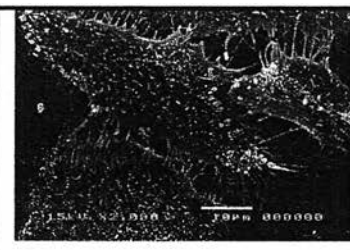
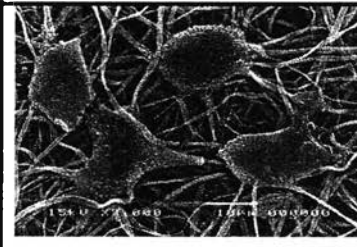
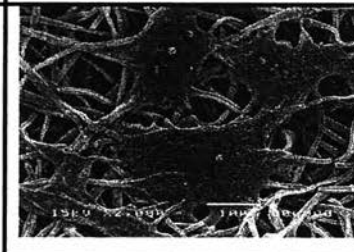
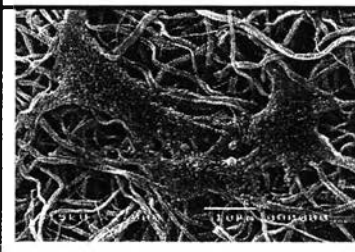
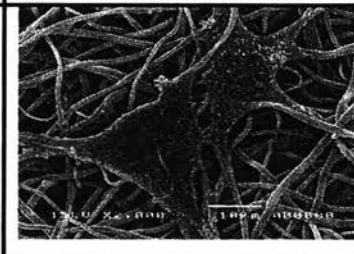
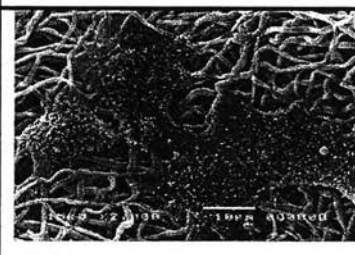
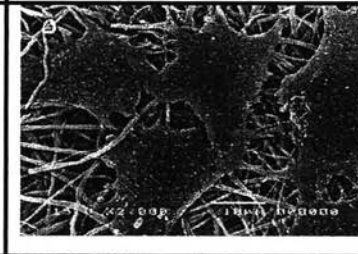
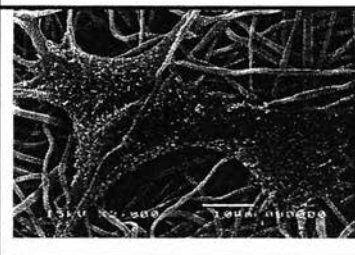
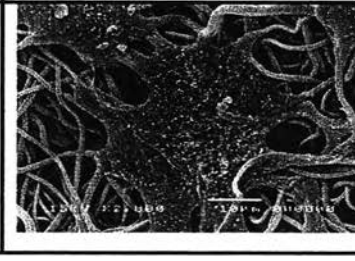
Materials	**Magnification = 500X** **Scale bar = 50 μm**	
	Cultured 4 hr	**Cultured 16 hr**
Glass (control)		
Neat PLA		
Aminolyzed PLA		
Activated PLA		
Collgen-PLA		

Table 4.7 Selected SEM images of cultured specimens, i.e., glass (i.e., control), neat PLA, aminolyzed PLA, activated PLA, and collagen-immobilized PLA fibrous scaffolds at various time points after MC3T3-E1 were seeded or cultured on their surfaces (magnification = 2000X; scale bar = 10 μm).

Materials	**Magnification = 2000X** **Scale bar = 10 μm**	
	Cultured 4 hr	**Cultured 16 hr**
Glass (control)		
Neat PLA		
Aminolyzed PLA		
Activated PLA		
Collgen-PLA		

4.4.4 Alkaline Phosphatase (ALP) Activity

The ability for these PLA fibrous scaffolds to support differentiation, in addition to attachment and proliferation, of cultured cells is another important aspect suggesting actual applicability of the scaffold. Alkaline phosphatase is a membrane bound enzyme and its activity is used as an osteoblastic differentiation marker, as it is produced only by cells showing mineralized ECM. The ALP activity of MC3T3-E1 on TCPS (i.e. controls), neat PLA, aminolyzed PLA, activated PLA, and collagen-immobilized PLA were monitored at 3, 5 and 7 days in culture (Figure 4.12). Apparently, the amount of ALP synthesized by the cells that were cultured on TCPS and all of the fibrous scaffolds increased with the initial increase in time in culture between day 3 and 5, reached a maximum level on day 5, and decreased with a further increase in culture after day 3. In comparison with other substrates, TCPS exhibited the highest ALP activity of MC3T3-E1. According to Figure 4.12, activated PLA and collagen-immobilized PLA fibrous scaffolds showed the highest ALP activity among the various fibrous scaffolds investigated at day 3 of cell culturing time. And at day 5 and day 7 collagen-immobilized PLA fibrous scaffolds exhibited the highest ALP activity.

The decrease in the ALP activity, after day 5 for the cells grown on both TCPS and PLA fibrous scaffolds, with a further increase in the culturing time can be due to cellular process switching onto further step (i.e. mineralization) (Choi *et al.*, 1996) Since ALP is not an exclusive protein synthesized by osteoblasts as it is also found in tissues of such organs as kidneys, small intestines, and placenta, the presence of ALP of MC3T3-E1 that were cultured on these substrates could not be used as the sole marker to confirm the osteoblastic phenotype of the cells (Tsukamoto *et al.*, 1992). From the obtained results, it was suggested that collagen-immobilized PLA fibrous scaffold was the best among the fibrous scaffolds that promoted both proliferation and differentiation of MC3T3-E1. However, additional long term experiments in order to clarify the effect of scaffolds on the differentiation of osteoblasts are currently underway.

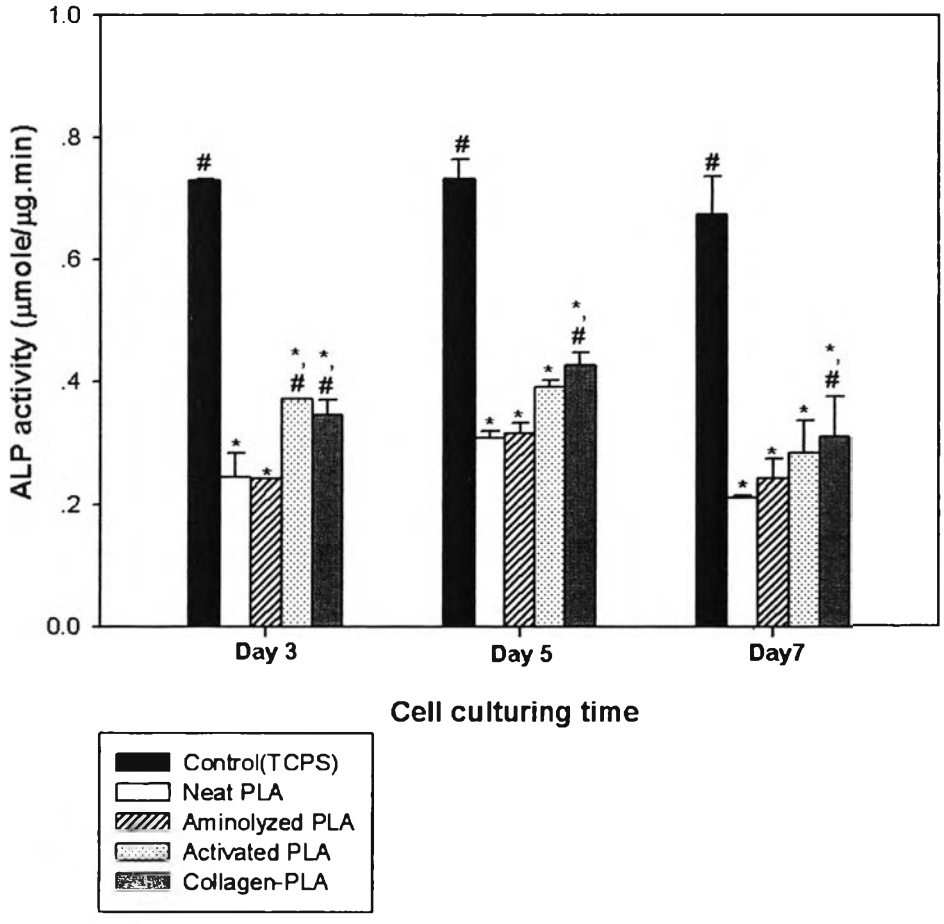


Figure 4.12 Alkaline phosphatase (ALP) activity of MC3T3-E1 that were cultured on the surfaces of TCPS and the neat and the modified PLA fibrous scaffolds for 3, 5, or 7 d. Statistical significance: $^{*}p < 0.05$ compared with control and $^{\#}p < 0.05$ compared to the neat PLA fibrous scaffolds at any given time point.