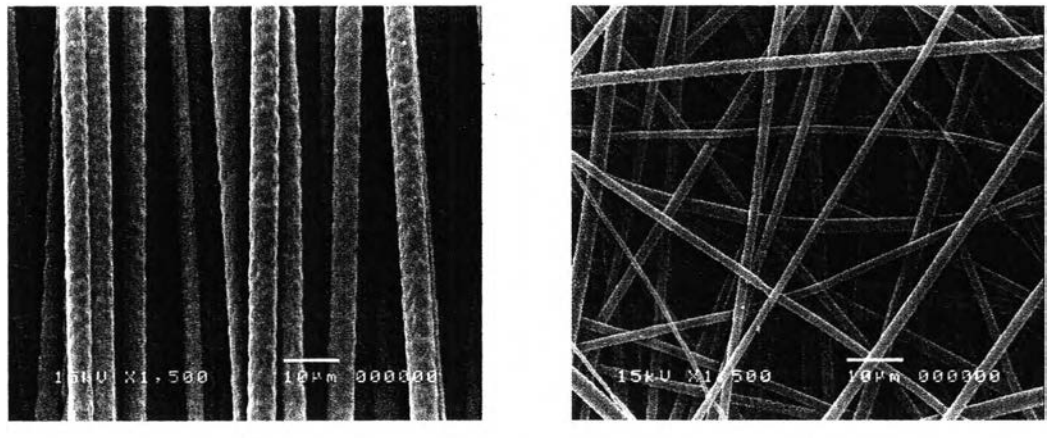
CHAPTER IV
RESULTS AND DISCUSSION

4.1 Electrospun Fabrications of Aligned and Random Scaffolds

Poly(lactic acid) (PLA; Mn~200,000 $gmol^{-1}$) electrospun fiber meshes were prepared via electrospinning technique under fixed conditions as mentioned in the previous chapter. Morphological appearance and size of the individual fibers of scaffolds were examined by JEOL JSM 5410LV scanning electron microscopy (SEM) (Figure 4.1). At least 100 readings of fiber diameters from various SEM images were statistically analyzed using SemAphore 5.0 software, from which the arithmetic mean value of the individual fibers within the PLA aligned and random fiber meshes were determined to be 1.744 ± 0.795 μm and 2.069 ± 0.577 μm respectively. Degree of fiber orientation, the PLA aligned fiber meshes had value of the degree of fiber alignment in the range of 86 – 90° (Figure 4.2).



(a) (b)

Figure 4.1 Scanning electron micrographs of electrospun PLA fibers, (a) aligned fibers (AF_1), and (b) random fibers (RF). (Magnification = 1500x; scale bar = 10 μm).

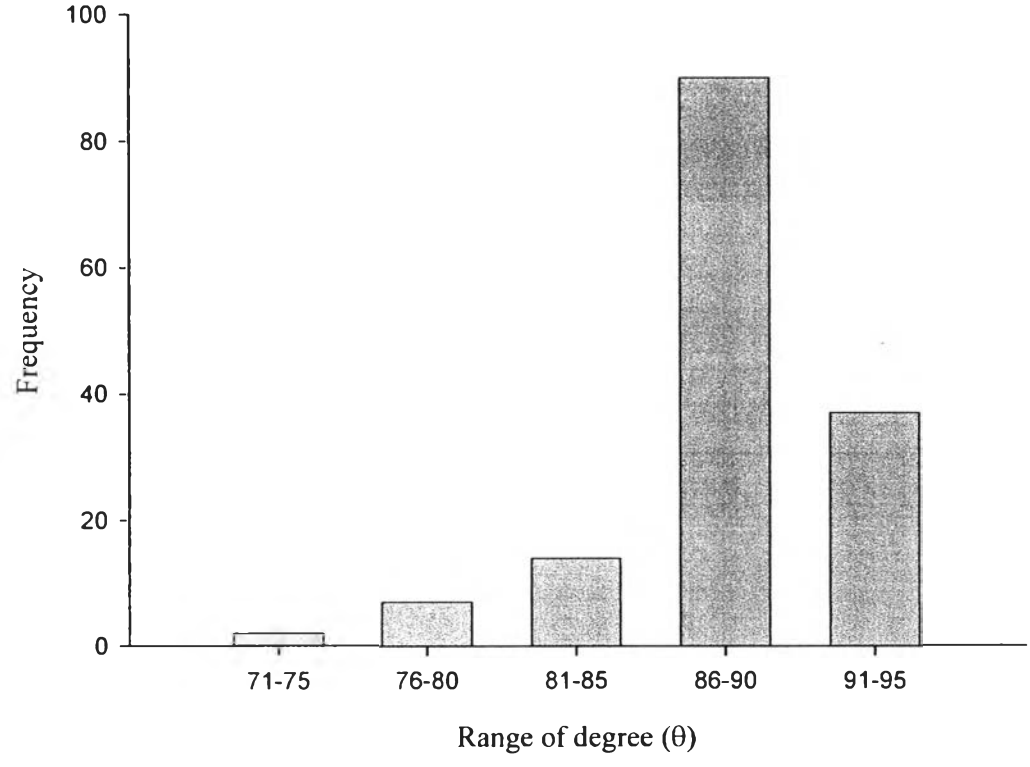


Figure 4.2 Degree of fiber orientation in aligned PLA fibers.

4.2 Determination of Surfactant Adsorption Isotherm

The adsorption isotherm of DBSA on alignment PLA fibers at pH 4 are presented in Figure 4.3. It can be observed that the adsorption of DBSA on alignment PLA fibers conforms to S-shaped adsorption isotherm for an ionic surfactant on neutral substrate. The amount of DBSA adsorbed on alignment PLA fibers were found to sharply increase initially with an increase in DBSA concentration. As the DBSA concentration increased further, the slope started to become more gradual leading eventually to a constant value. In admicellar polymerization process, the concentration of surfactant in the system should be just below or near its CMC to avoid emulsion polymerization. From the adsorption isotherm, a concentration of 1.0 mM. DBSA, which is close to the CMC of DBSA, was chosen below CMC 0.8 mM. for subsequent polymerization reactions.

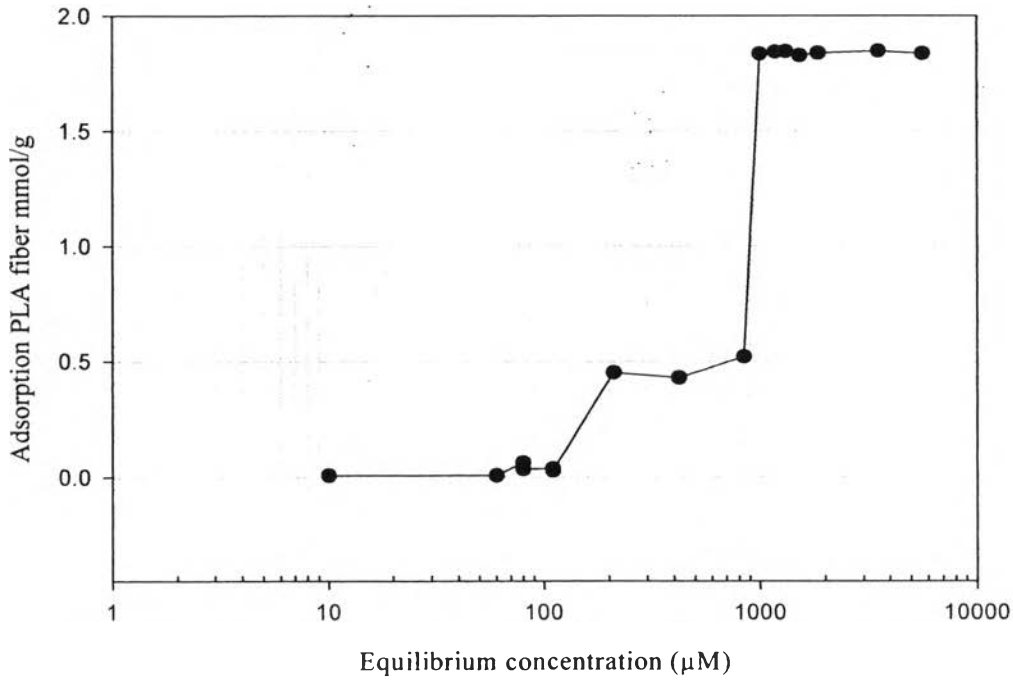


Figure 4.3 Adsorption isotherm of DBSA on PLA fibers (Temp. = 30° C, time = 15 h, pH 4).

4.3 Polypyrrole Coating Fabrication by Admicellar Polymerization

4.3.1 SEM Micrograph of the Treated PLA Fibers Surface

SEM images of PLA fibers and polypyrrole-coated PLA fibers are presented in Figure 4.4 (a), (b), (c), and (d) respectively. SEM micrographs of the aligned and random PLA fibers show a clear and relatively smooth fiber surface. Figure 4.4 (c) and (d) show the presence of polypyrrole (PPy) –coated on PLA fibers surface. The SEM images show that the PPy are coated on the surface of the PLA fibers, and excess PPy particles on the PLA fibers due to difficulty in removal of surfactant. The average diameter of PPy-coated aligned and random fibers were 4.229 ± 1.492 μm. and 6.389 ± 0.997 μm. respectively.

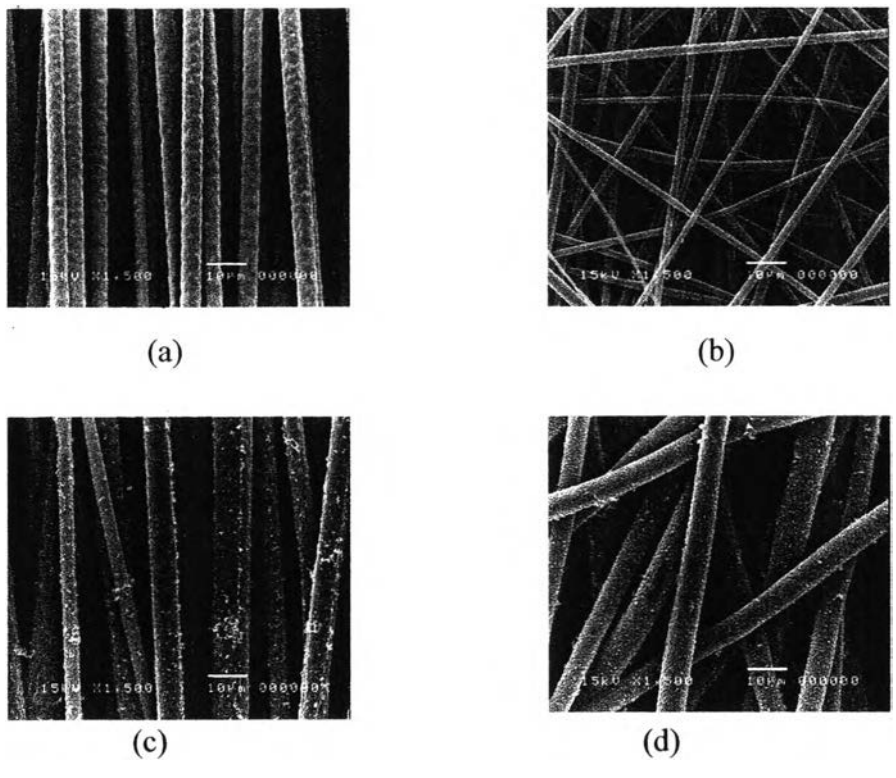


Figure 4.4 SEM micrographs of (a) untreated aligned PLA fibers, (b) untreated random PLA fibers, (c) PPy-coated aligned PLA fibers, and (d) PPy-coated random PLA fibers (Magnification = 1500x; scale bar = 10 μm).

4.3.2 Effect of Monomer Concentration

The coloring effect of varying the amount of pyrrole is shown in Figure 4.5 the DBSA concentration was fixed at 0.8 mM. For ratio of 1:1 and 1:2, the color of PLA aligned fibers were not changed relatively to black (uncompleted polymerization) when compared to the other ratios of 1:4, 1:6, 1:8, 1:10 and 1:12.

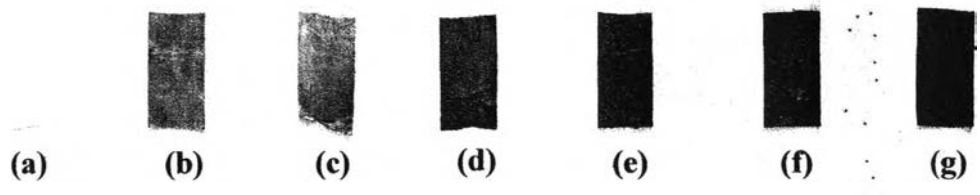
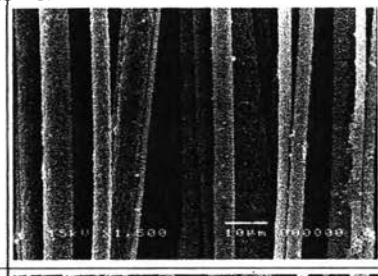
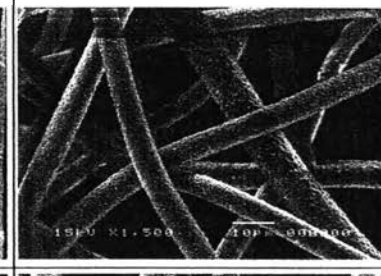
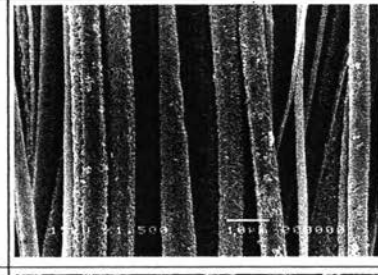
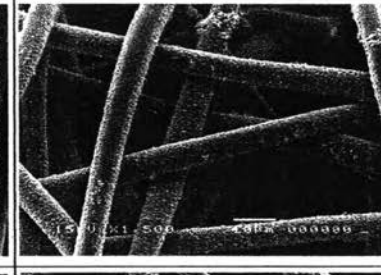
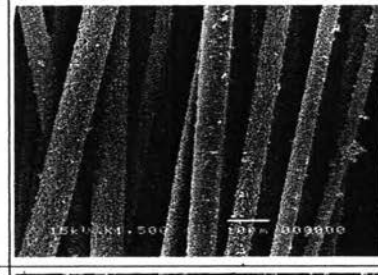
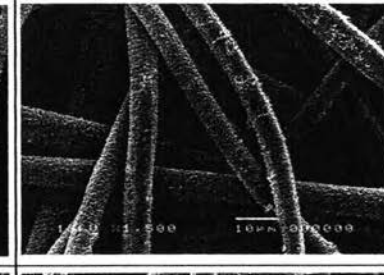
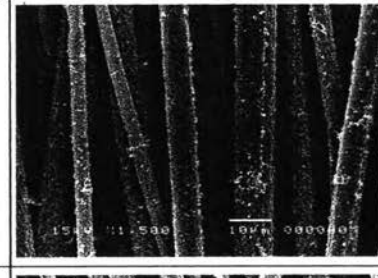
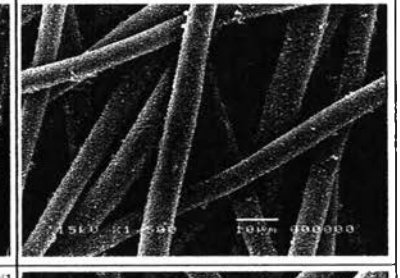
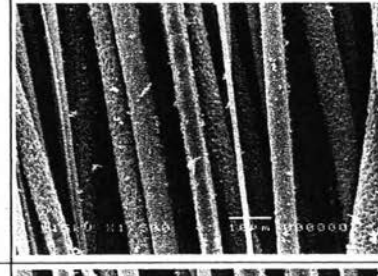
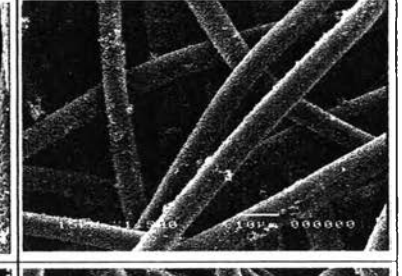
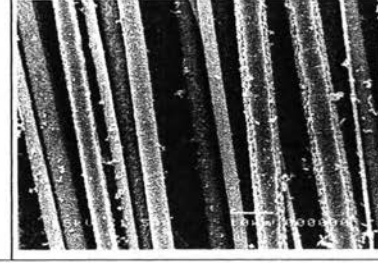
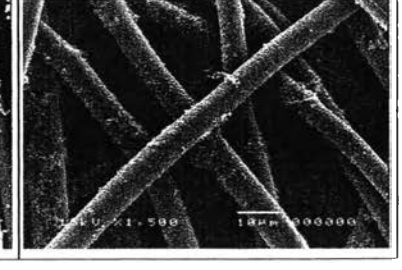


Figure 4.5 The pictures illustrated the coloring of PPy-coated aligned PLA fibers by varied the ratio of DBSA: Py monomer. (a) 1:1, (b) 1:2 , (c) 1:4, (d) 1:6, (e) 1:8, (f) 1:10 and (g) 1:12.

While The effect of varying the amount of pyrrole is shown in Table 4.1. In SEM micrographs not only found the excess PPy particles were occur on the PLA fiber due to difficulty in removal of surfactant and also Nisara *et al.* proved that excess monomers could have partitioned into the admicelle and reacted to form additional polymer which could have contributed to the increase in fiber thickness as shown in Figure. 4.6. Degree of fiber orientation, the PPy-coated PLA aligned fiber meshes had value of fiber alignment in range of 86 – 90°. Figure 4.7 indicated that aligned PLA fiber meshes are more stable in retaining its shape when polymerized with PPy.

Table 4.1 Scanning electron micrographs of electrospun PPy- coated PLA fibers

DBSA: pyrrole monomer	PPy-coated PLA AF	PPy-coated PLA RF
1:2		
1:4		
1:6		
1:8		
1:10		
1:12		

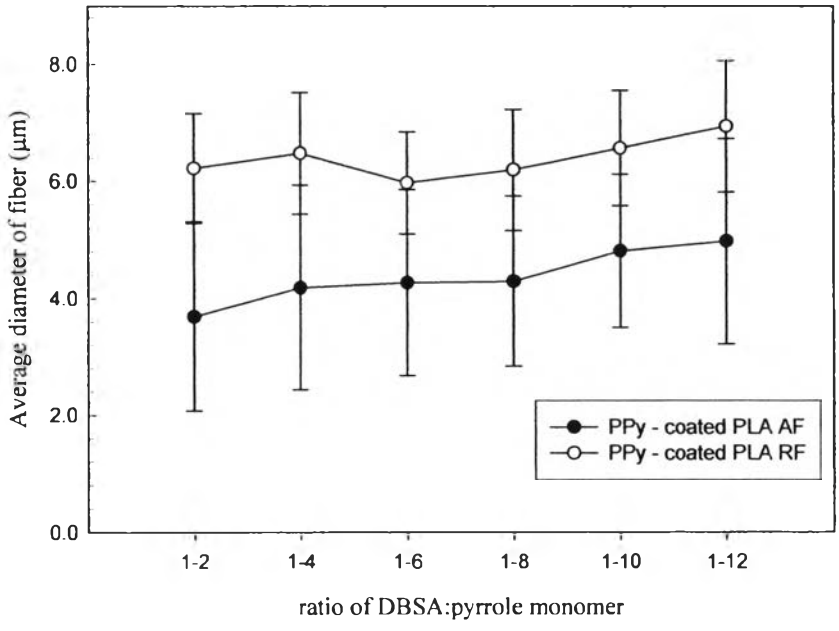


Figure 4.6 Relation between average diameter of fiber and pyrrole monomer.

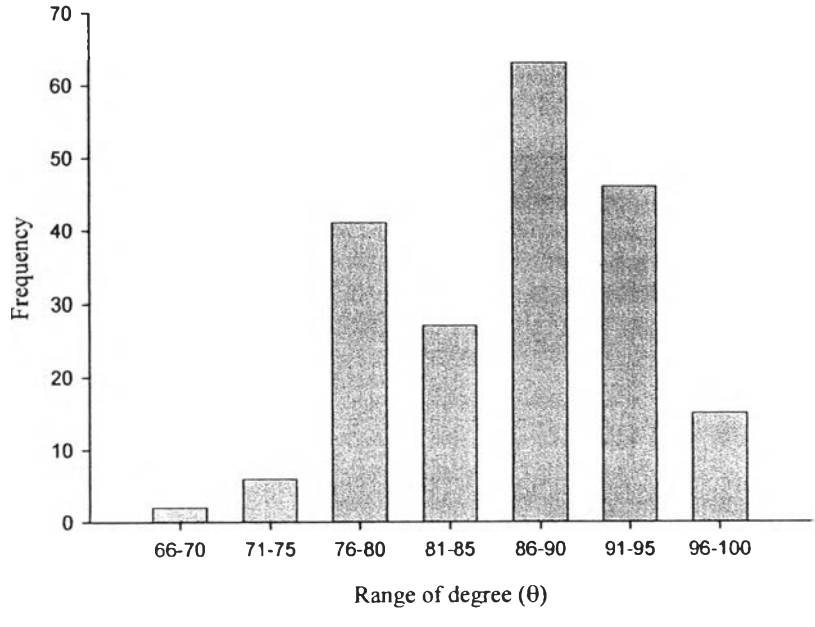


Figure 4.7 Degree of fiber orientation in PPy-coated on aligned PLA fibers.

4.4 Surface Conductivity

4.4.1 Effect of Varies Pyrrole Monomer for Coating by Admicellar Polymerization

The surface conductivity of PPy-coated on aligned and random PLA fibers obtained from the use of DBSA:Py ratios of 1:4, 1:6, 1:8, 1:10 and 1:12 respectively. Figure 4.8 shows that when the ratio DBSA:Py increases the conductivity could increased. On the contrary, conductivity would decrease after ratio of 1:8 due to these value were insensitive to the direction of measurement, which was reasonable because the PPy–coated PLA random fibers were randomly oriented and had multiple contacts among coated fibers. At the same time, conductivity values of PPy–coated aligned PLA fibers varied is always greater than PPy-coated random PLA fibers mainly due to the continuity of the electron flow path along the PPy. These results suggest that current is conducted primarily along the fiber axis.

Furthermore, laminin coated on PPy–coated aligned PLA fibers had value of conductivity 1.63×10^{-3} S/cm. The Result shown that conductivity was decrease after laminin coated on PPy–coated aligned PLA fibers due to laminin is protein that has value conductivity lower than PPy so effect to conductivity was decrease.

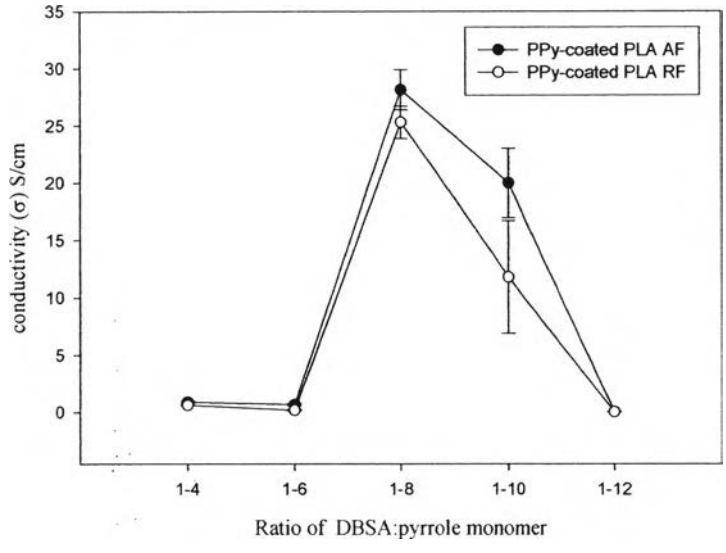


Figure 4.8 Conductivities of PPy-coated aligned PLA fibers (PPy-coated PLA AF) and PPy-coated random PLA fibers (PPy-coated PLA RF) using different monomer concentration with 0.8 mM. DBSA, and Ferric chloride:oxidant ratio of 1:1.

4.4.2 Effect of Storage Time Polypyrrole Coating by Admicellar Polymerization

Storage time was use to study de-doping of polypyrrole characterization. Figure 4.9 shows storage time obtained from PPy-coated aligned PLA fiber meshes at ratio 1:8. Result shown that increase storage time in month, the measured surface conductivity of fiber decreased. Because of the PPy-coated aligned PLA fiber meshes occurred reaction of H^+ (protons) transfer of PPy to ester carbonyl of aligned PLA fiber meshes. Therefore surface conductivity decreased when increasing the storage time.

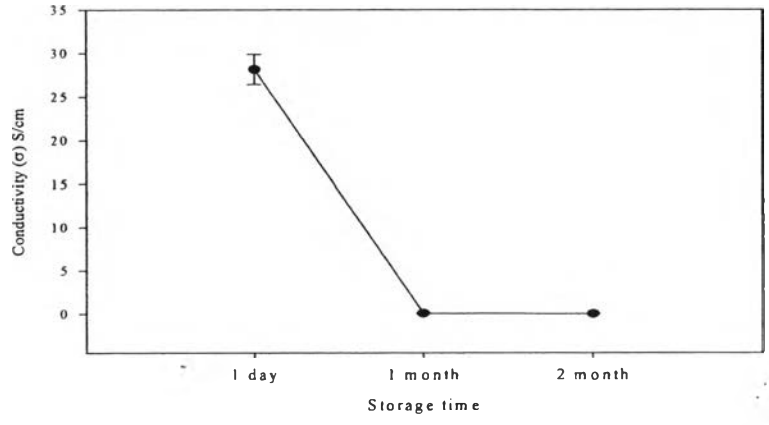


Figure 4.9 Conductivities of PPy-coated aligned PLA fibers at ratio 1:8 and Ferric chloride:oxidant ratio of 1:1.

4.5 Surface Wettability

Contact angle measurement was used to study the hydrophilic characteristic of the obtained PPy-coated PLA fiber meshes. Contact angle measurement were carried out on the samples subjected to compare between PLA fiber meshes and PPy-coated PLA fiber meshes condition. Table 4.2 shows average contact angles obtained from PLA fiber meshes and PPy-coated PLA fiber meshes samples. Results shown that after treated PLA fiber meshes, the measured contact angles of fibers increased. This result shown in Figure 4.10 corresponded to the improvement of hydrophobicity of the PPy-coated PLA fiber meshes. The aligned and random PLA fibers meshes have an average contact angle of 125.0° ± 0.68 and 146.4° ± 0.74 respectively. The PPy-coated aligned and random PLA fiber meshes exhibited more hydrophobic character, after admicellar polymerization, with contact angle 146.1° ± 1.08 and 151.1° ± 0.74 respectively.

On the contrary, the effect of immobilize surface wettability of laminin coated on (PPy-coated aligned PLA fibers) with respect to that of PPy-coated aligned PLA fibers were measured. Results shown that after immobilized by laminin that can introduction of amino groups on the surface of PPy-coated aligned PLA fibers improved the hydrophilicity of the surface, with contact angle 97.1° ± 0.20. This result shown in Figure 4.11 and Table 4.3.

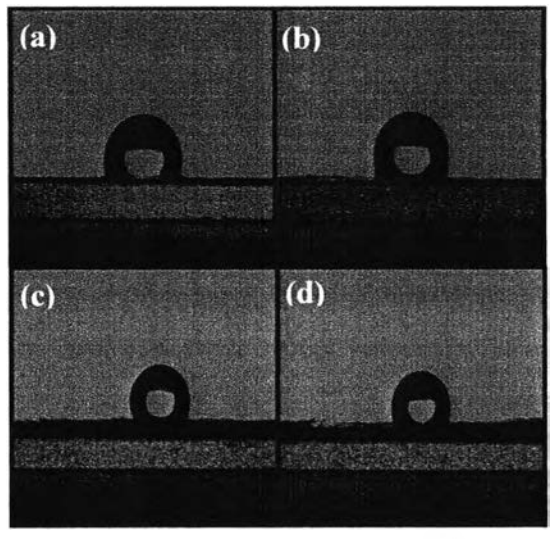


Figure 4.10 Water dropped on the surface of aligned PLA fiber meshes (a), random PLA fiber meshes (b), and PPy-coated aligned PLA fiber meshes (c), and PPy-coated random PLA fiber meshes (d).

Table 4.2 Average contact angle of PLA fiber meshes and PPy-coated PLA fiber meshes

Samples	Average contact angle (Θ)deg
Aligned PLA fiber meshes[(a)]	125.0° ± 0.68
PPy-coated aligned PLA fiber meshes [(b)]	146.1° ± 1.08
Random PLA fiber meshes [(c)]	146.4° ± 0.74
PPy-coated random PLA fiber meshes [(d)]	151.1° ± 0.74

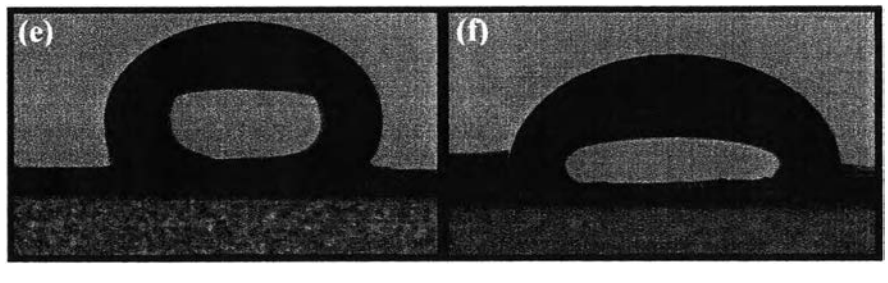


Figure 4.11 Water dropped on the surface of PPy-coated aligned PLA fiber meshes (e), and laminin coated on (PPy-coated random PLA fiber meshes)(f).

Table 4.3 Average contact angle of PPy-coated aligned PLA fiber meshes and Laminin coated on (PPy-coated aligned PLA fiber meshes)

Samples	Average contact angle (Θ)deg
PPy-coated aligned PLA fiber meshes[e]	146.1° ± 1.08
Laminin coated on (PPy-coated aligned PLA fiber meshes)[f]	97.1° ± 0.20

4.6 Chemical Analysis of Surface

ATR-FTIR spectra of aligned PLA fiber meshe, PPy-coated aligned PLA fiber meshes, and laminin coated on (PPy-coated aligned PLA fiber meshes) are shown in Figure 4.12. There was a major absorption peak assigned to the ester carbonyl of aligned PLA appeared at 1755 cm^{-1}. PPy-coated aligned PLA fiber meshes synthesized with DBSA as anionic dopant. The appearance of a strong band from 1520 to 1610 cm^{-1} is characteristic of skeletal C-C that stretches from the pyrrole ring. PPy-coated aligned PLA fiber meshes doped with sulfonic acid anions also showed absorption bands corresponding to the sulfonate groups at 1020-1050 cm^{-1} and 1140-1212 from S=O symmetric and asymmetric stretches respectively. However, the spectra of all are almost the same as aligned PLA fiber meshes. This may be regarded as a result of the extremely low concentration of introduced chemicals which presented within the sampling depth of ATR-FTIR.

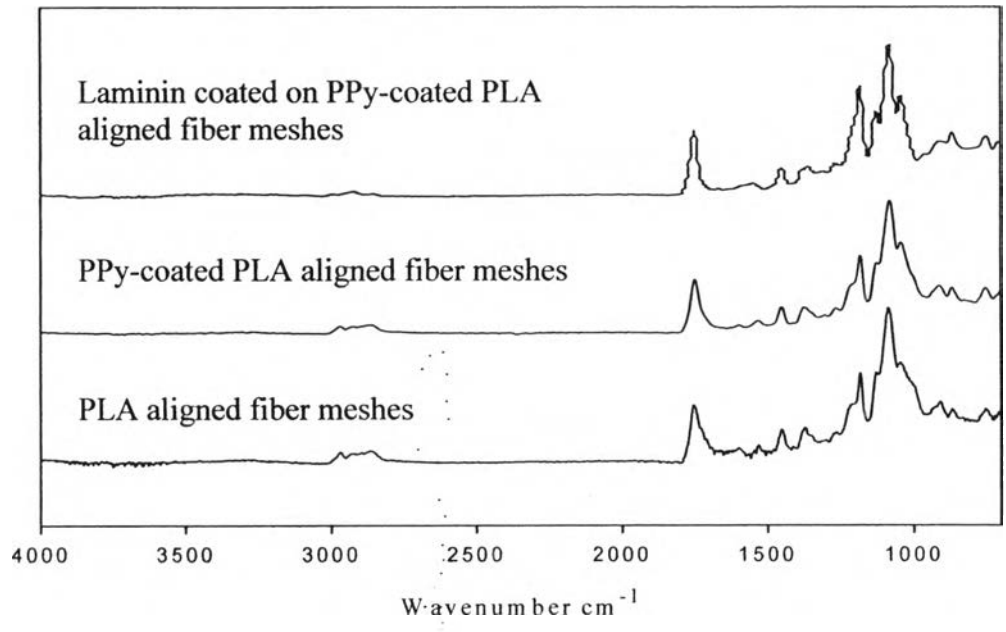


Figure 4.12 ATR-FTIR spectra of PLA aligned fiber meshes, PPy-coated PLA aligned fiber meshes and laminin coated on (PPy-coated aligned PLA fiber meshes).

Because of this problem, to confirm the success of PPy coated on PLA fibers and immobilization of laminin, X-ray photron spectrometer (XPS) was used to evaluated the N_{1s}/C_{1s} ratio and doping level of PPy-coated PLA aligned fiber meshes. These expected that after coated by PPy. the N_{1s}/C_{1s} ratio and doping level should increase.

4.7 Elemental Composition of the Surface

To characterize the surfaces of the fibers, XPS analysis was performed. Table 4.4 and Figure 4.15 summarizes the elemental compositions of the sample surfaces. For the aligned PPy–coated PLA fiber meshes found nitrogen, chlorine, and sulfur atoms were detected, whereas they were absent in the aligned (uncoated) PLA fiber meshes samples. Doping level was calculated for PPy-coated aligned PLA fiber meshes sample Doping levels of the PPy components were calculated from the atomic ratio of Cl and S to N from the high-resolution XPS spectra. The doping level

of PPy–coated PLA fiber meshes were 0.33 and close to that for oxidized PPy (Jae, Y. L. *et.al., 2009*).

High resolution C_{1s} of PLA fiber meshes were deconvoluted into C-C/CH(284.6eV), CO(286.6eV) and O-C=O (288.7eV). The C-O and O-C=O peaks diminished after polypyrrole coating. The C_{1s} high resolution scan for PPy-coated PLA fiber meshes were deconvoluted into C-C/CH(284.6eV), C-N(286eV), C=N(287eV) and C=N+(288.9eV) shown in Figure 4.13. Table 4.6 shows that the N_{1s}/C_{1s} ratio increased after PPy-coated on PLA aligned fiber meshes, the N_{1s}/C_{1s} ratio was increase from 0.0093 to 0.0585 because of amino groups from polypyrrole were introduced on the surface.

In addition, High resolution N_{1s} scan was performed as shown in Figure 4.14 for PPy-coated aligned PLA fiber sample, new peaks were detected and they are assigned as =N-(398.6eV), -NH-(399.8eV), C-N+(400.6eV) and C=N+(401.4eV). very low N intensity was observed for aligned (uncoated) PLA fiber meshes sample.

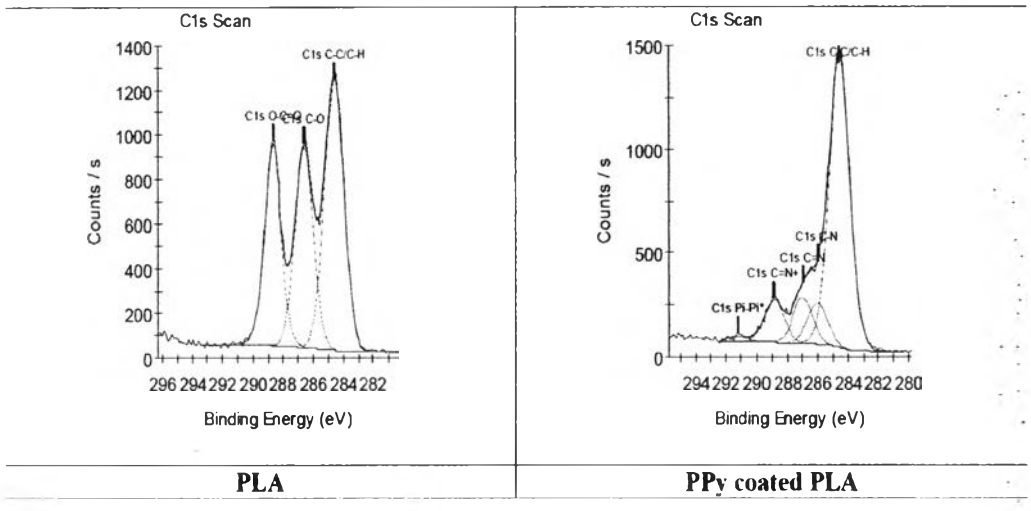
Table 4.4 Elemental compositions and doping levels of both aligned PLA fiber meshes and PPy-coated PLA fiber meshes using XPS analysis

Element	PLA (At%)	PPy-coated PLA (At%)
C	64.7	75.2
N	0.6	5.1
O	34.7	18
Cl	-	-
S	-	1.7
Doping level[a]	-	0.33

[a]Doping level = (Cl + S)/N

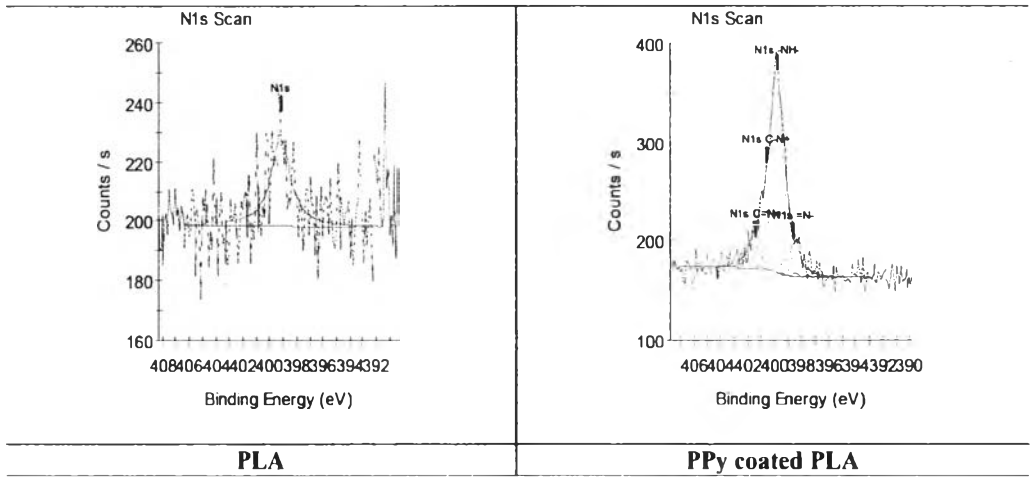
Table 4.5 N_{1s}/C_{1s} ratios of the PLA and PPy-coated PLA aligned fiber meshes

Sample	N_{1s}/C_{1s} ratio
PLA aligned fiber meshes	0.0093
PPy-coated PLA aligned fiber meshes	0.0585



PLA **PPy coated PLA**

Figure 4.13 XPS high resolution spectra of C_{1s}.



PLA **PPy coated PLA**

Figure 4.14 XPS high resolution spectra of N_{1s}.

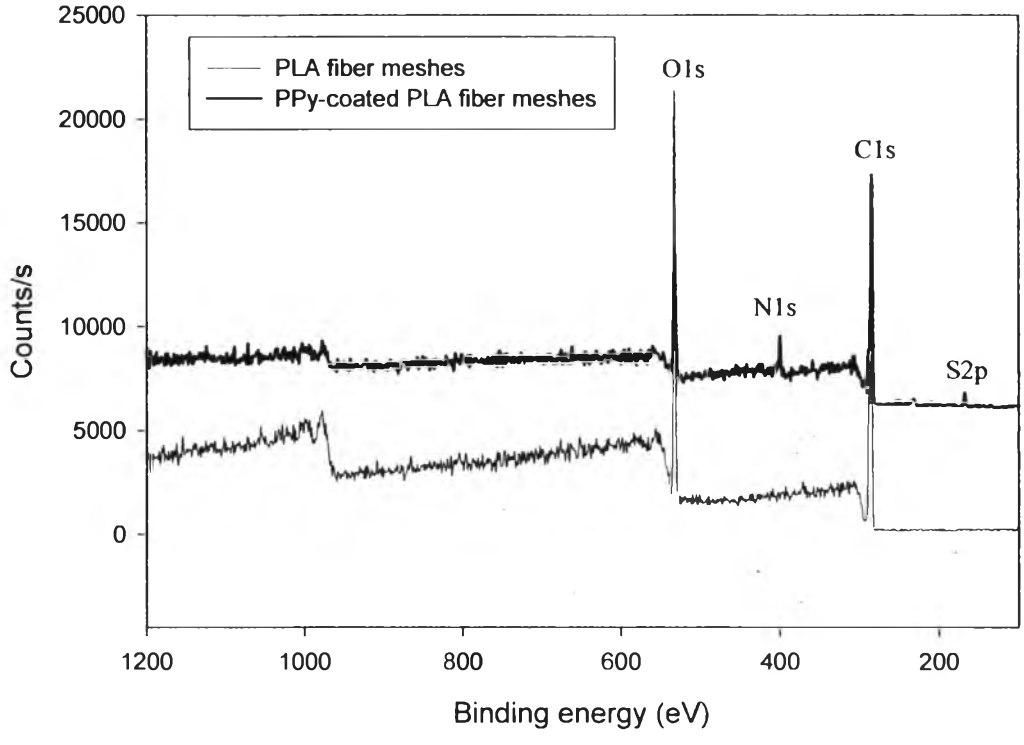


Figure 4.15 XPS survey spectrum obtained on as-received aligned PLA fiber meshes and PPy-coated aligned fiber meshes.

4.8 Biological Characterizations

4.8.1 Cytotoxicity Test

Cytotoxicity is a basic property of a biomaterial. Figure 4.16 shows the viability of the cells obtained from MTT assay after the cells had been cultured with extraction media from both the PLA fiber meshes and the PPy-coated PLA fiber meshes specimens as compared with that obtained after the cells had been cultured with the fresh SFM. The viability of the cells were reported as the percentage with respect to that of the control(100%). Evidently, the viability of Neuro 2a cultured with the extraction media from both PLA fiber meshes and PPy-coated fibers meshes specimens were equivalent to that of the cells cultured with fresh SFM, implying the biocompatibility of these materials toward Neuro 2a. In the case of both PLA fiber meshes and PPy-coated PLA fiber meshes, showed that these materials also implied that aligned and random PLA fiber meshes and aligned PPy-

coated PLA fiber meshes were cytocompatible and did not release cytotoxic substance in culture medium towards Neuro 2a.

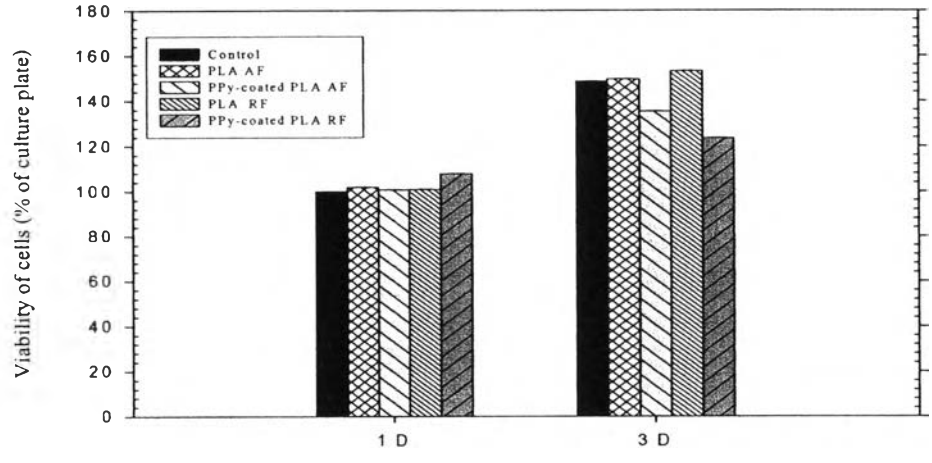
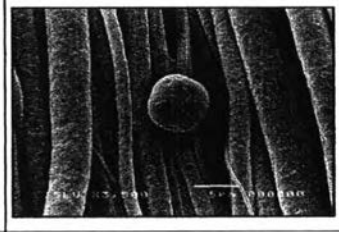
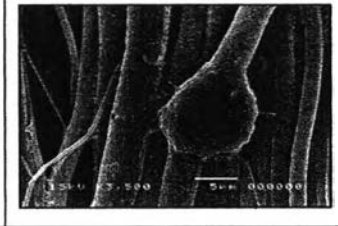
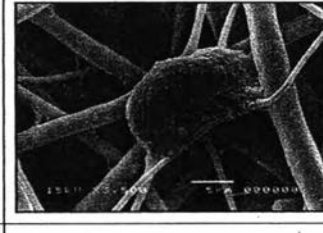
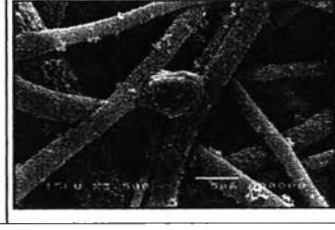
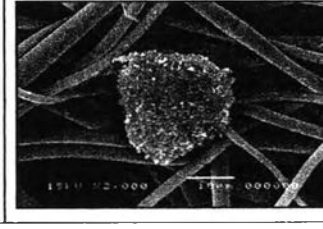


Figure 4.16 Indirect cytotoxicity evaluation of various electrospun fiber meshes based on the viability of Neuro 2a that were cultured with the extraction media from these materials for 1 and 3 Days. The viability of the cells that were cultured with fresh culture medium (SFM) (i.e., control) was used as the reference to arrive at the viability of the attached cells shown in the figure.

For confirming the cytoxicity test (MTT assay) results, with regard to cell interaction and spreading, Neural Stem Cells were cultured on various scaffolds and were observed by SEM images as shown in Table 4.6 at four hours, cell initially changed from their original round shape to elongated and spindle-like shape. After three days of culturing, most of cells started to expand and elongated along with alignment of fiber mat. Although PPy-coated random PLA fiber meshes show the lowest cell viability on the cytoxicity test with Neuro 2a after three days, it did not show cytotoxic towards Neuro 2a because the materials still shows more than 80% of viability to Neuro 2a. However, it did not show to be viable towards Neural Stem Cells.

Table 4.6 SEM images of Neural Stem Cell on various scaffold materials at different time in the culturing

	4 hours	3 day
Aligned PLA fibers		
PPy-coated aligned PLA fibers		
Random PLA fibers		
PPy-coated random PLA fibers		

4.8.2 RT-PCR Analysis

Gel electrophoresis following RT-PCR using the designed primers shown in Figure 4.17 revealed snapshot in applied stimulate electric current on scaffolds for the expression of c-Fos gene, a neural stem cell-specific gene, of rat hippocampal neural stem cell on 2 hours after the cells were cultured on laminin coated on (PPy-coated aligned PLA fiber meshes). The expression of GAPDH gene was used as an internal control. Normalization of the band intensities of the c-Fos gene to those of the GAPDH gene revealed the relative amount of the c-Fos gene expressed in the cells when they were cultured in different condition. Apparently, c-Fos gene expression in the cells that were grown under electrical stimulus of 100mV was significantly greater than those grown unstimulated shown in Figure 4.17.

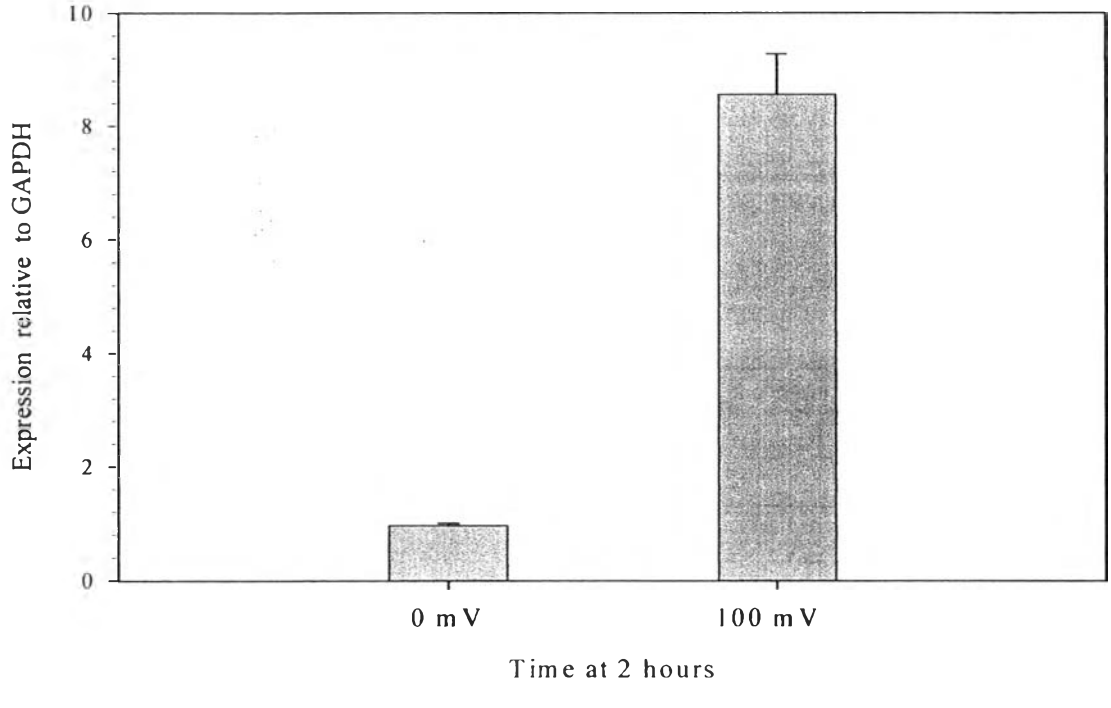


Figure 4.17 Expression of c-Fos gene in rat hippocampal neural stem cell on electrical stimulation 2 hours cultured on the surfaces of laminin coated on (PPy-coated aligned PLA fiber meshes)