CHAPTER IV

RESULTS AND DISCUSSION

4.1 Properties of the prepared PEMs

PDADMAC/PSS, PDADMAC/gelatin and chitosan/gelatin were successfully prepared on glass slides and PCL films. PDADMAC/gelatin and chitosan/gelatin were also formed on silicon wafers. Their properties, including film thickness, hydrophilic/hydrophobic property and stability of the films in culture medium solution were investigated. These surface characteristics play an important role in cell adhesion on biomaterials.

4.1.1 Film thickness

The films thickness of the 20-layer PEMs was determined by measuring the difference in the height between the top surface of PEM thin film and the bare glass slide surface as demonstrated in Figure 4.1. As revealed by the AFM analysis in tapping mode, Figure 4.2 shows the two-dimensional AFM surface topographic images of the prepared PEMs at the scan size of 10 μm.

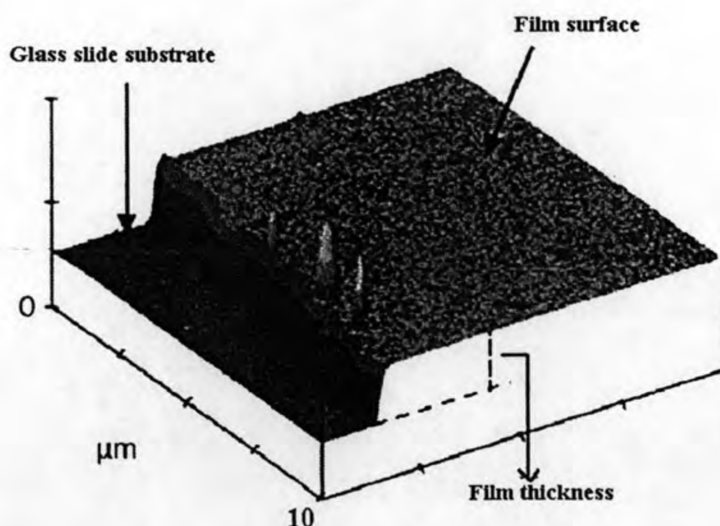


Figure 4.1 A three-dimensional AFM surface topographic image showing how the film thickness is measured

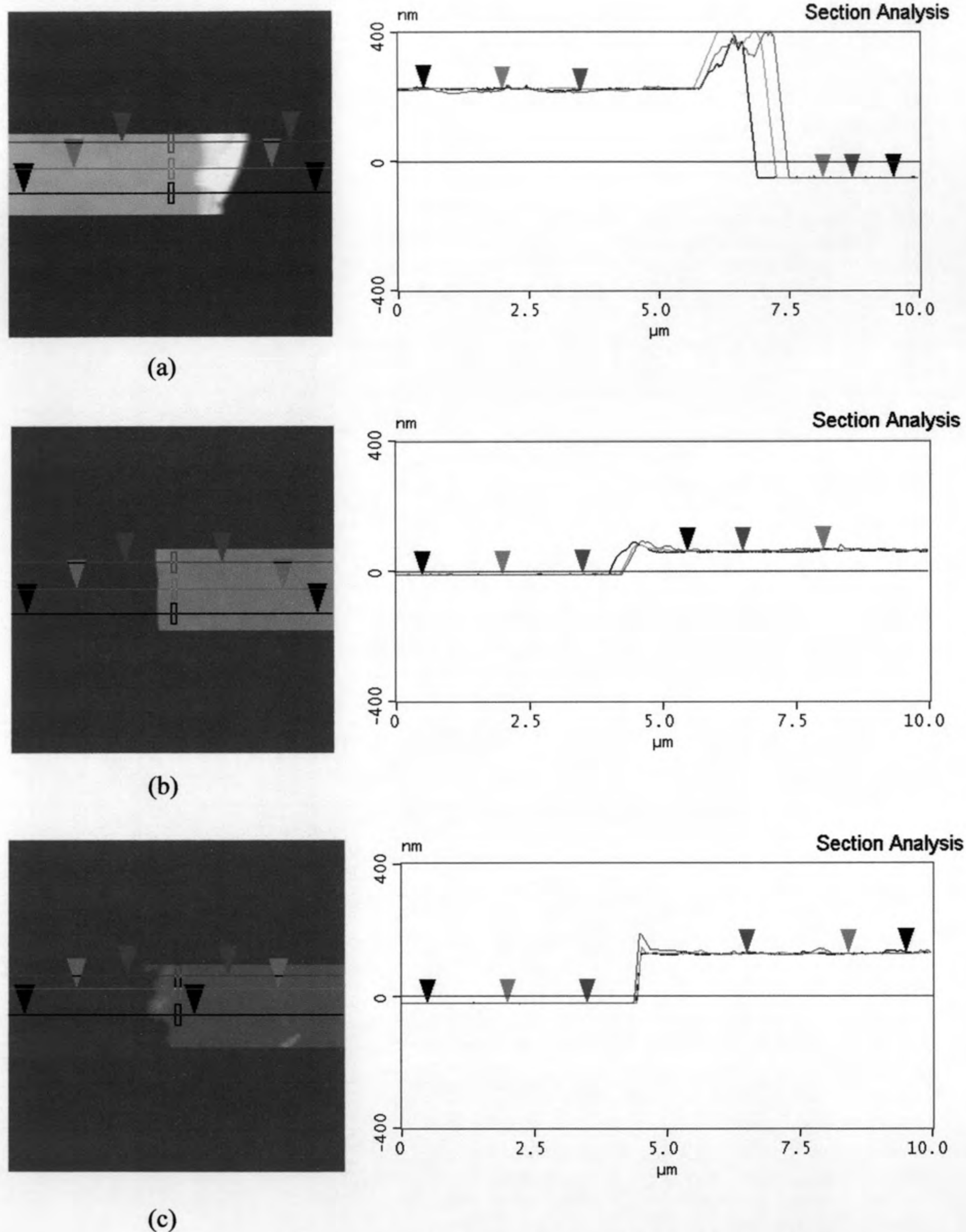


Figure 4.2 Two-dimensional AFM surface topographic images of (a) $(PDADMAC/PSS)_{10}$ (b) $(PDADMAC/gelatin)_{10}$ and (c) $(chitosan/gelatin)_{10}$

$(PDADMAC/gelatin)_{10}$, respectively. This can be explained according to the pervious work about the pH independent thickness when using strong polyelectrolytes, which are PDADMAC and PSS [39]. The influence of strong polyelectrolytes on multilayer built-up depends on the relative strength of the polymer/polymer ion pair. The strong interacting segments cannot be easily swollen. At the pH used in this experiment for PDADMAC/PSS (pH 7), PDADMAC/gelatin (pH 9), and chitosan/gelatin (pH 6) was one of the effects for different thickness and coverage onto the film surface since the same dipping time were used for each of the polyelectrolytes.

Table 4.1 Average thickness of different PEMs coated on glass slides

PEMs	**Film thickness (nm)**
$(PDADMAC/PSS)_{10}$	271.5 ± 6
$(PDADMAC/gelatin)_{10}$	73.1 ± 2
$(chitosan/gelatin)_{10}$	150.2 ± 2

Figure 4.3 shows the film thickness of PDADMAC/gelatin at 4, 8, 12, 16, 20 layers. The results demonstrated a near linear growth of surface thickness as the number of layers increased.

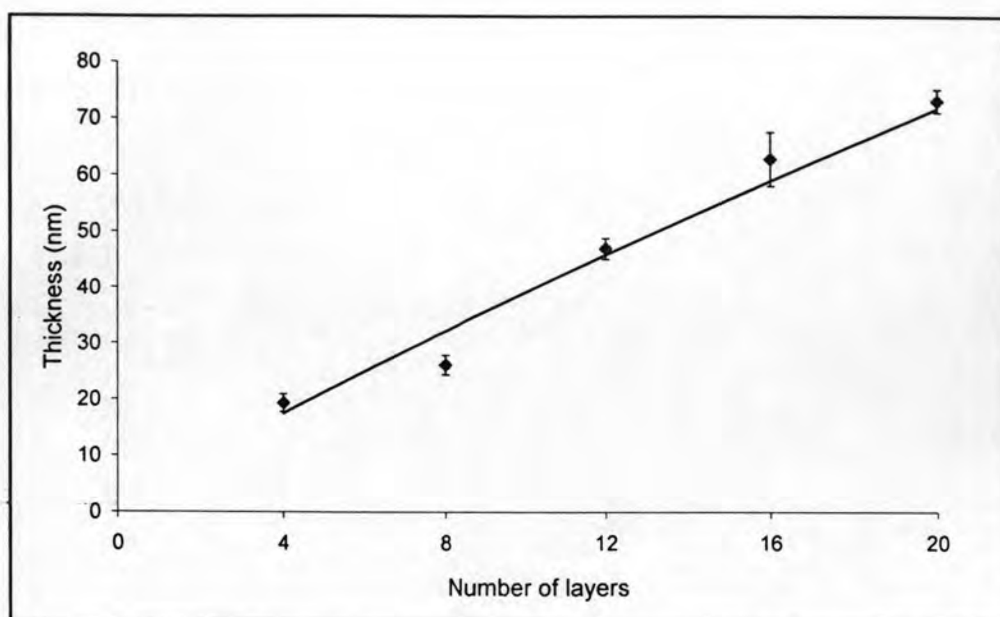


Figure 4.3 Thickness of $(PDADMAC/gelatin)_n$ on glass slide

4.1.2 Hydrophilic/hydrophobic property

Water contact angle measurement is a surface selective technique and can indicate the outermost layer properties. As first described by Thomas Young in 1805 [40], it is the interaction between the forces of cohesion and the forces of adhesion which determines whether or not wetting, the spreading of a liquid over a surface, occurs. On extremely hydrophilic surfaces, a water droplet will completely spread (an effective contact angle of 0°). This occurs for surfaces that have a large affinity for water (including materials that absorb water). On many hydrophilic surfaces, water droplets will exhibit contact angles of 10° to 30°. On highly hydrophobic surfaces, which are incompatible with water, one observes a large contact angle (70° to 90°). Some surfaces have water contact angles as high as 150° or even nearly 180°. On these surfaces, water droplets simply rest on the surface, without actually wetting to any significant extent. Young also developed the well-regarded Young's Modulus which is used to measure the stiffness of a material as well as Young's Equation which defines the balances of forces caused by a wet drop on a dry surface. If the surface is hydrophobic then the contact angle of a drop of water will be larger. Hydrophilicity is indicated by smaller contact angles and higher surface energy. Water has high surface energy by nature; it has polar and forms hydrogen bonds.

The hydrophobic/hydrophilic property of glass slides coated with $(PDADMAC/gelatin)_{10}$ were compared with those coated with $(chitosan/gelatin)_{10}$.

Figure 4.4 show the water contact angle at the alternate deposition sequence of PDADMAC/gelatin and chitosan/gelatin. At the odd layers, PDADMAC or chitosan is the outermost layer while at the even layers, gelatin is the outermost layer. The results indicated that the increase in hydrophobic was more significant when gelatin was the outermost layer than when PDADMAC was the outermost layer thus the contact angle jumped alternatively between 47^0 and 80^0 depending on the outermost layer component. With further increasing in the deposition cycle, the alternately changes still occurred. Similar behavior was observed in the case of chitosan/gelatin. For the initial seven coating layers, The changes of water contact angles were not clear. The contact angles when the outermost layer was gelatin were higher than when it was chitosan. However, the contact angles when the outermost layer was chitosan were higher than when PDADMAC was the outermost layer. The results indicated the significant difference in hydrophobicity of the prepared PEMs. Apparently, gelatin was more hydrophobic than PDADMAC and chitosan since gelatin is not a single chemical substance. The main constituents of gelatin are large and complex polypeptide molecules [34]. The monomeric representation of this polypeptide is (Gly–X–Pro)$_n$, where X is an amino acid. The chemical composition of this biopolymer has about ~53% (glycine, proline and hydroxyproline) its chain length is non-ionic leaving about ~16% of the chain length to be hydrophobic.

The water contact angles of PEMs coated PCL films are shown in Figure 4.5. In this case, layers of even number have gelatin and PSS as the outermost layer (layer zero is the virgin PCL films). The virgin PCL sheet, exhibiting a water contact angle of 50^0-55^0, showed a relatively hydrophobic surface. Similar trend is observed for the PDADMAC/gelatin and chitosan/gelatin, i.e., the contact angles was higher for the gelatin as the outer layer. The increasing water contact angles were probably due to the inherent (CH_3 group) hydrophobic nature of the PCL chemical structure [22]. However, for the PCL films coated with PDADMAC/PSS, the contact angles decreased dramatically compare to other cases. The decrease in contact angles resulted from the adsorption of PSS with polar sulfonate group interaction with the charged surface. When positively charged PDADMAC was absorbed onto PSS surface the contact angle decreased. The lower contact angles also reflected the hydrophilic nature of positively charged PDADMAC which has quaternarium ammonium group. It was suggested that different contact angle behavior was caused

ammonium group. It was suggested that different contact angle behavior was caused by electrostatic interaction, or more probably by differences in the change of gelatin hydration state or its molecular conformations upon adsorption.

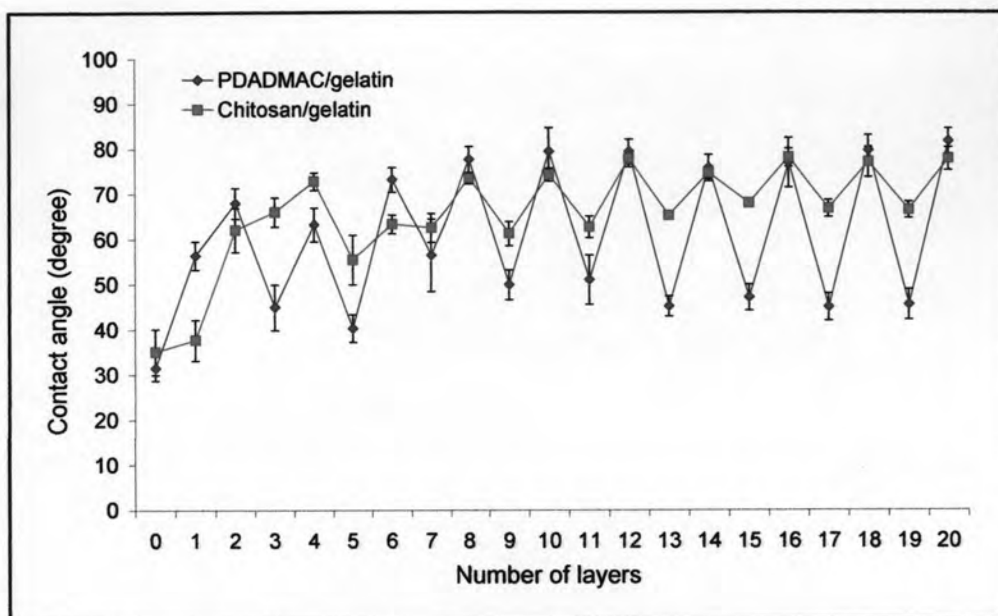


Figure 4.4 Water contact angles (sessile drop) of PEMs coated glass slides

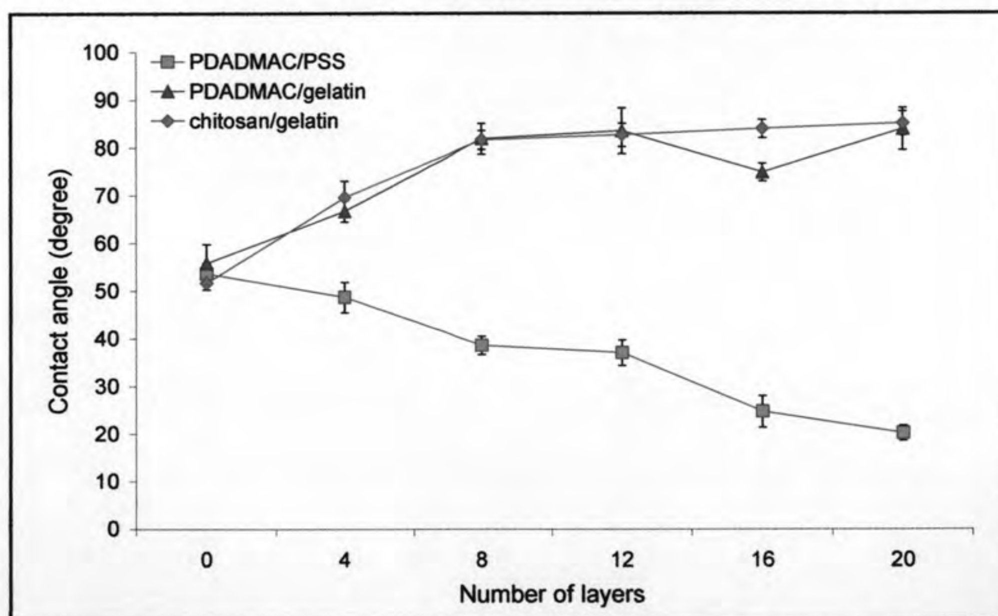


Figure 4.5 Water contact angles (sessile drop) of PEMs coated PCL films

The long-term stability of PDADMAC/gelatin in medium solution of pH 7.4 was due to the fact that the PDADMAC was pH independent [41]. By contrast, chitosan is a polycationic polymer, well known for its chelating properties. Chitosan molecules are rigid and high intrinsic stiffness due to abundant intra-molecular hydrogen bonds [36]. Therefore, reactions with negatively charge components, either ions or molecules can lead to the formation of network between polymeric chains.

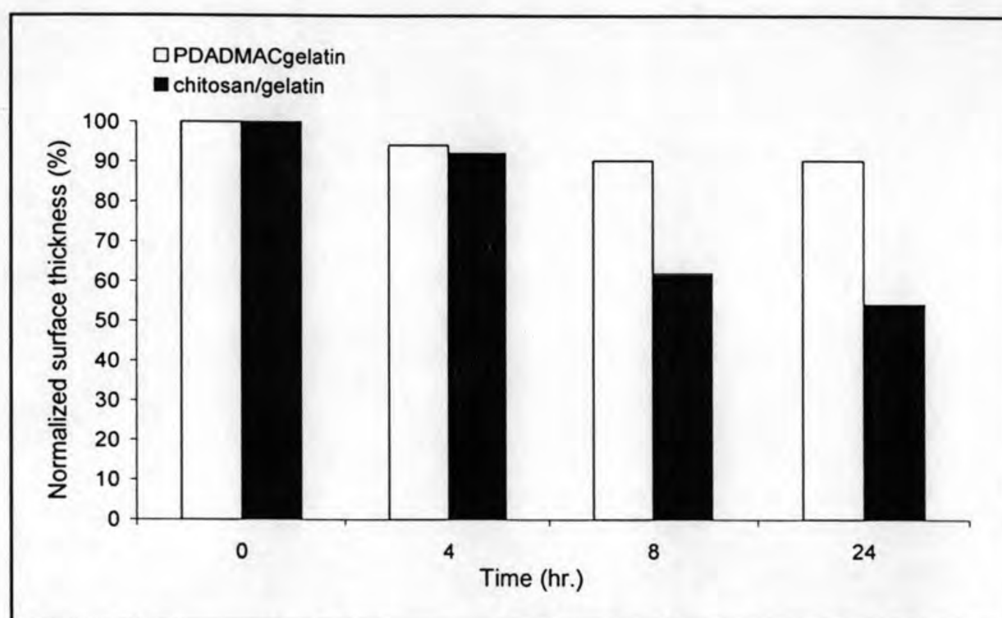


Figure 4.6 Normalized thickness as a function of dipping time in culture medium solution

Swelling is mainly influenced by ionic interactions between chitosan chains. In addition to its pH sensitivity, swelling ionic interactions through a shielding effect, which increases swelling and instable [42, 43]. Moreover, swelling is favored by the protonation and repulsion of chitosan free ammonium groups. If the pH is too high, the positive charge of chitosan is neutralized and the system capacity is not stable and decrease as shown in Figure 4.7. At pH 6 chitosan contains 50% $\sim NH_3^+$ and 50% $\sim NH_2$. When immersed PEMs in pH 7.4 solution, chitosan contains 30% $\sim NH_3^+$ and 80% $\sim NH_2$ and can neutralized or precipitation. To avoid chitosan precipitation, the pH of solution should not be higher than ≈ 6 [38, 44]. Therefore, the main disadvantages of chitosan were the lack of stability and the risk of dissolution of the system, due to pH sensitivity.

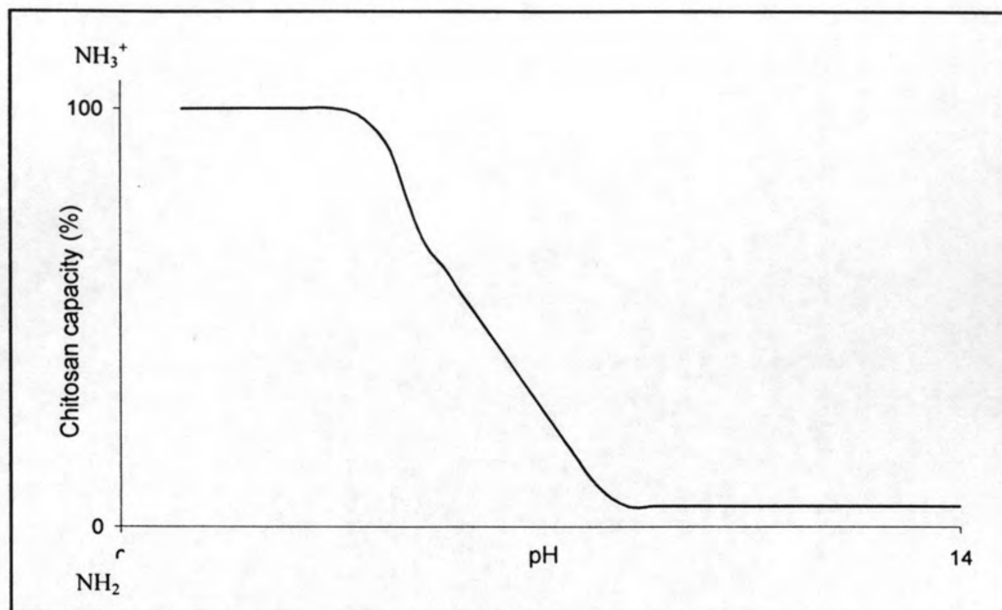


Figure 4.7 A titration curve showing pH-sensitive of chitosan.

The change of hydrophilic/hydrophobic property of PEMs films were also confirmed by the contact angles. The results shown in Figure 4.8 confirmed that, after immersing in medium solution at each interval, the PDADMAC/gelatin was more stable than chitosan/gelatin.

From the above results, it can be concluded that the use of chitosan/gelatin was limited by poor stability characteristics.

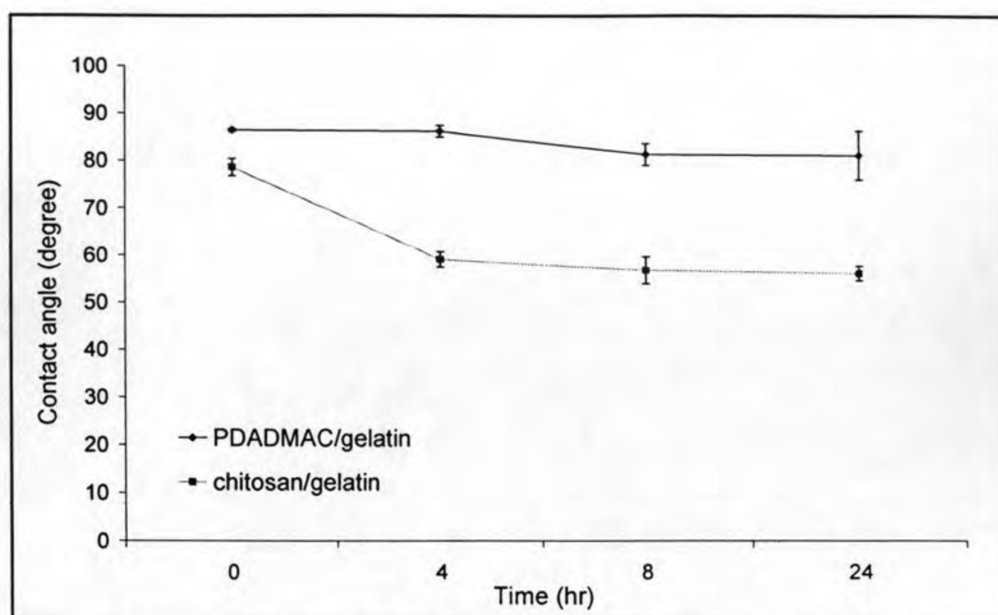


Figure 4.8 Change of contact angles as a function of dipping time in culture medium solution pH 7.4

4.2 Cell behaviors on PEMs coated glass cover slips

For biological evaluation of modification material by *in vitro* methods, an essential step is represented by the assessment of test with material-cell interaction. Surfaces improvement has been reported to enhance cell adhesion and other differention [32, 35]. PDADMAC/PSS was known to promote cell adhesion, proliferation and differention [31, 45]. In this research, $(PDADMAC/PSS)_4$ was used as the primer layer before coating the outermost layer. The outermost layer was negatively charged gelatin, positively charged gelatin and chitosan. Additionally, the different of the number of layers of $(PDADMAC/gelatin)_n$ and $(chitosan/gelatin)_n$ were also evaluated.

4.2.1 Viability of L929 cell on different PEMs

Cell attachment and cell viability are interesting cell behaviors. These behaviors allowed survival of cells to adhere on non-toxicity surface. To confirm that all of the PEMs coated were non-toxic to viable cells, MTT test was carried out. To investigate the viability of L929 cell, reduction of MTT reagent was assesses as an assay of mitochondrial redox activity. MTT reagent is a pale yellow substance that is reduced to a dark blue fomazan product. When incubating with viable cell by mitochondrial succinate dehydrogenate in complex II, which plays a critical role in

both oxidative phosphorylation and the tricarboxylic acid cycle [36]. Therefore, the production of fomazan can reflect the level of cell viability.

Figure 4.9 Molecular structure change of MTT reagent

Figure 4.10 shows the percentages of the number of viable cells attached on different film substrates over a period of 24 hr. The percentages of the number of living cells on PEMs coated on glass cover slips obviously increased compared with that on the tissue culture polystyrene plate (TCPS) used as a control plate. This demonstrated that these PEMs are useful for improving cell adhesion on scaffolds for tissue engineering.

The percentage of the number of living cells on $(PDADMAC/PSS)_4$chitosan was higher than those on $(PDADMAC/PSS)_4$, $(PDADMAC/PSS)_4$gelatin(+), $(PDADMACM/PSS)_4$gelatin(-) and $(PDADMAC/PSS)_4$chitosan/gelatin. There is no significant difference between the percentages of the number of living cells on $(PDADMAC/PSS)_4$gelatin(+) and on $(PDADMACM/PSS)_4$gelatin(-). The results suggested that the gelatin charges on the outermost layer did not affect the viable of cells. Chitosan promoted cell proliferation thus $(PDADMAC/PSS)_4$chitosan showed the highest percentage of all.

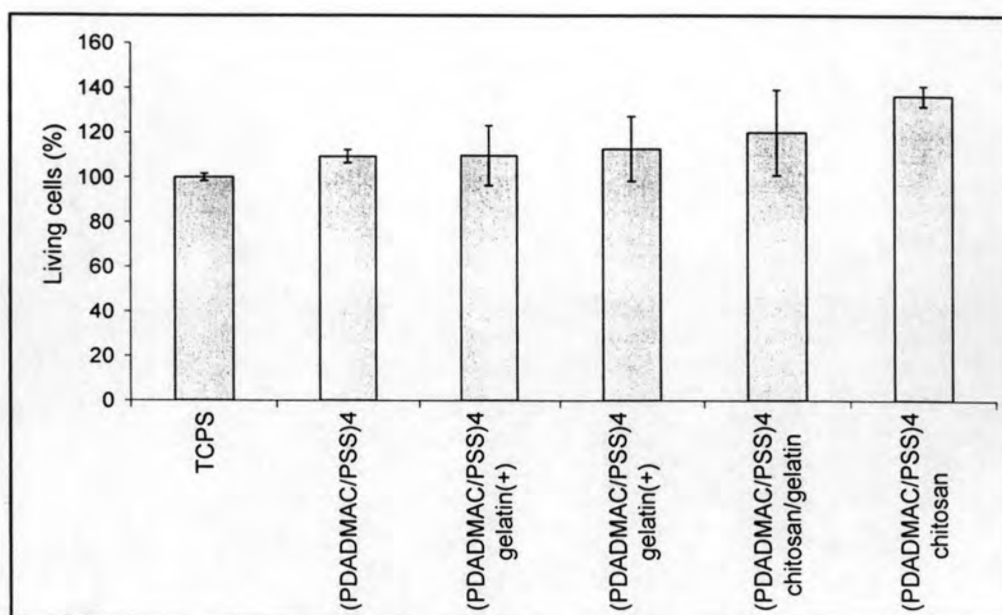


Figure 4.10 Viability of L929 cell cultures on the different outermost layers at 24 hr.

In order to assess the attachment of L929 cells on the different numbers of layers, $(PDADMAC/gelatin)_n$ and $(chitosan/gelatin)_n$ were coated on $(PDADMAC/PSS)_4$ primer coated glass cover slips and L929 cells were seeded at an equal density. Figure 4.11 demonstrates the percentages of the number of living cells on PEMs films at 24 hr. It was observed that there is no significant difference between $(PDADMAC/gelatin)_4$ and $(PDADMAC/gelatin)_8$. The percentage of living cells on $(chitosan/gelatin)_4$ seemed to be the lowest among all. It was reported that the combination between chitosan and gelatin caused the number of living cells to decrease than chitosan, especially at this time point [46]. It was assumed that gelatin reduced charge density of chitosan which was responsible for supporting cell attachment. Although use of $(chitosan/gelatin)_n$ coating resulted in the lowest number of living cells, the non-toxic chitosan/gelatin was still a candidate for support cell proliferation.

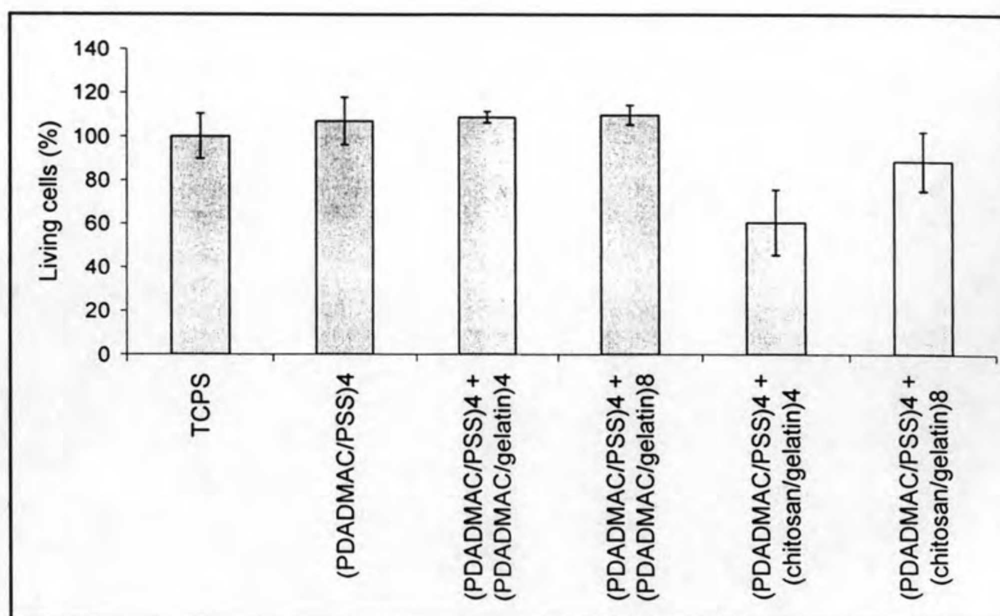


Figure 4.11 Viability of L929 cell culture on the different number of layers at 24 hr

4.2.2 Proliferation of L929cell on different PEMs coated

Cell proliferation assay was used to indicate the number of cell at the certain time interval. Cell proliferation is controlled by many factors such as, growth factors, receptors on the cell surface that connect to, signaling molecules that convey message from receptor to the nucleus and transcription factors bind to DNA, turning on or off the production of proteins [47]. The process of cell proliferation is shown in Figure 4.12. The first process of cell reproduction involves the replication of the parental cell's DNA. The second process issue is the separation of the duplicated DNA into two equally sized groups of chromosomes. The third process aspect of cell reproduction is the physical division of entire cells, usually called cytokinesis [48].

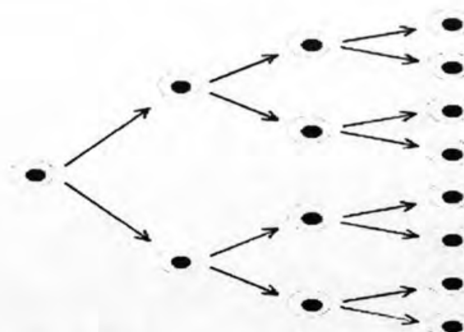


Figure 4.12 Process of cell proliferation.

The increasing number of living cells on the prepared PEMs after the cultured periods of 4hr, 8 hr, 24 hr and 48 hr is shown in Figure 4.13, suggested the successful proliferation of L929 that is cells can grow and reproduce on these PEMs. Like the cell viability, there was no significant difference between the percentages of the number of living cells on $(PDADMAC/PSS)_4$gelatin(+) and $(PDADMACM/PSS)_4$gelatin(-). According to Figure 4.10, the highest percentage of living cells was found when chitosan was at the outermost layer. This result is consistent with the previous study [46] which indicated that cell proliferation on chitosan membrane was the highest at any definite time point.

The effects of the number of layers on cell growth are shown in Figure 4.14 which also demonstrated the proliferation on all PEMs. The result indicated that the number of proliferate cells on each substrate increased with the increasing time. The percentages of the number of cells growth on $(PDADMAC/gelatin)_n$ were more than those on $(chitosan/gelatin)_n$. This was probably due to the fact that cells on $(chitosan/gelatin)_n$ did not stick very well in the beginning thus it was difficult to proliferate. Moreover, as suggested earlier, combination of chitosan and gelatin worsen cell growth as gelatin can reduce charge density of chitosan responsible for support cell adhesion [43, 44]. However at 48 hr, the cell growth on $(chitosan/gelatin)_n$ began to increase. From the result can be noted that, the number of top coating layers did not affect the cell proliferation.

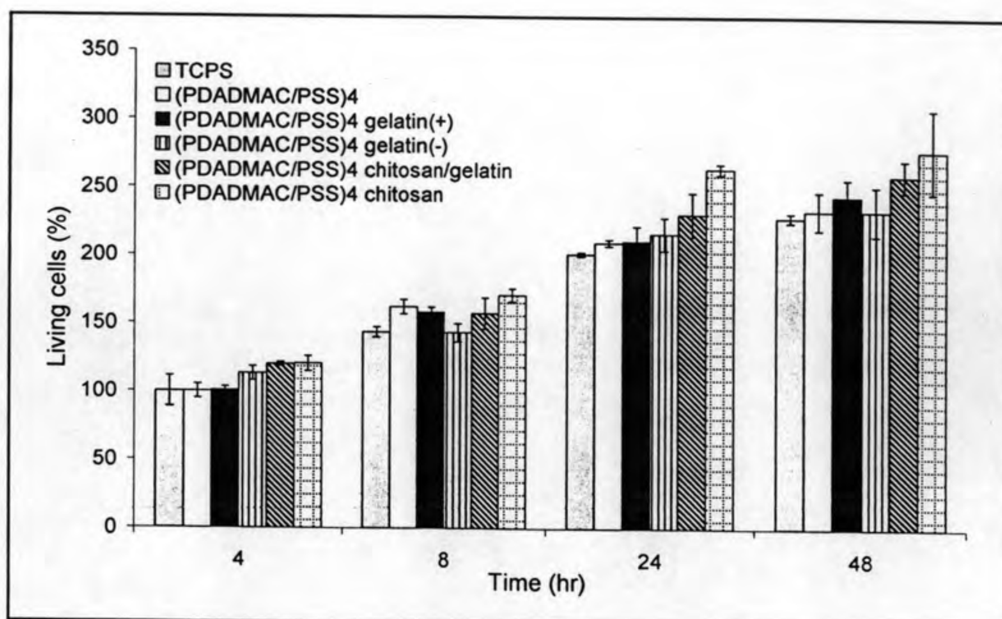


Figure 4.13 Proliferation of L929 cell cultured on the different outermost layers at 4, 8, 24, 48 hr, as determined by the activity of viable cells

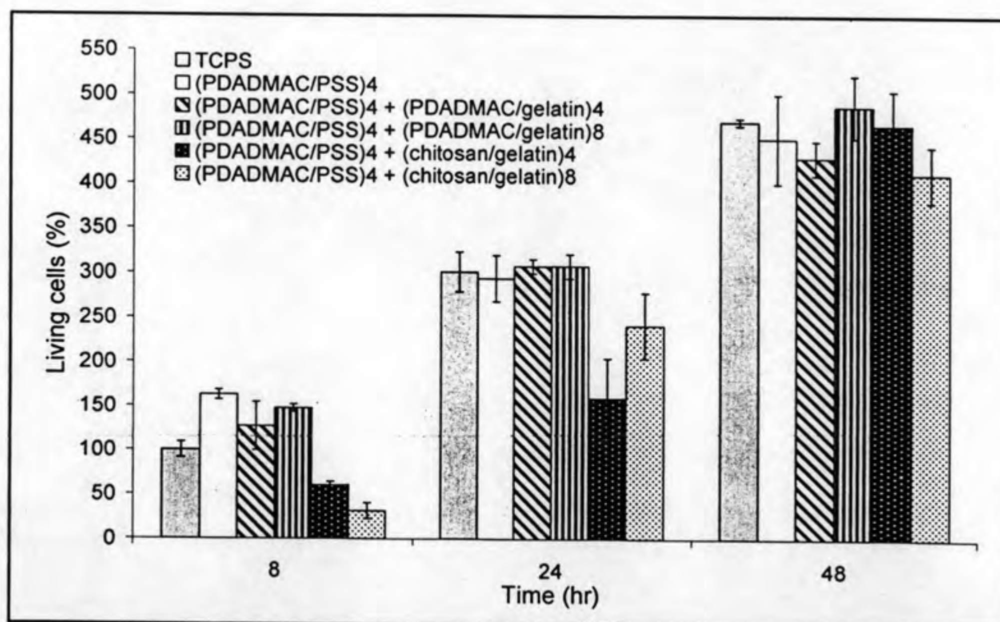


Figure 4.14 Proliferation of L929 cell cultured on the different number of layers at 8, 24, 48 hr, as determined by the activity of viable cells.

4.2.3 Cell morphology and spreading

Morphology of cell is primarily a matter of response to stimuli, which may be any one of a variety of influences that derive either from inside or outside the organism. In culture, the morphology indicates the status of the cells, both in terms of the health of the cells and in the case of primary isolates the differentiation state may be critical. Cell spreading and migration on substrates are the first sequential reaction when coming into contact with materials surface, which is crucial for cell survival. Figure 4.15 show the morphologies of L929 culture on PDADMAC/PSS primer layer, positively charged gelatin, negatively charged gelatin, chitosan/gelatin and chitosan on top layer after 4, 8, 24 and 48 hr. Indeed, cells covered to TCPS much greater degree in all cases. Negatively charged gelatin on the outermost layer was the most spread after 24 hr cell culture, followed by film coated by chitosan/gelatin. Compared between positively charged gelatins and negatively charged gelatin at 48 hr, the negatively charged gelatin were fully spread while gelatin positively charged still around shape. At longer culture time (i.e., 24 to 48), showed the cells adhered on positively charged gelatin and chitosan, some of both cells have spread and flattened but many are still rounded. This is probably due to an absence of strong cell-surface interaction via focal adhesion of this time point [20]. It has been reported that the outermost layer play a determinant role in cellular adhesion [50] and the physicochemical parameters may affect protein adsorption and thereby subsequent cellular behavior [51, 52]. A significant number of spreading between cells was observed which would eventually lead to a complete differentiation. Cell spreading structure was noticed in this study, which indicated the preferable, strong interaction between cell and PEMs coated. In addition at 48 hr, the gelatin negatively charge is more favorable for the cells spreading over than all film coated.

Figure 4.16 showed morphology and spreading of L929 cell that were cultured on primer layer, $(PDADMAC/gelatin)_4$, $(PDADMAC/gelatin)_8$, $(chitosan/gelatin)_4$, $(chitosan/gelatin)_8$ at different time in culture. The result shows that, as there was no PEMs coated on the control TCPS, cells spread evenly and showed normal morphology and confluence during interval time, whereas the first 8-24 hr in culture, the morphology of the cells on PEMs possesses a round shape. At longer period (48 hr), the cells on PDADMAC/gelatin expanded significantly. According to the result from Figure 4.14, PDADMAC/gelatin appeared to promote

both the viability and the proliferation of L929 cells. The results indicated the potential use of PDADMAC/gelatin to improve cell adhesion.

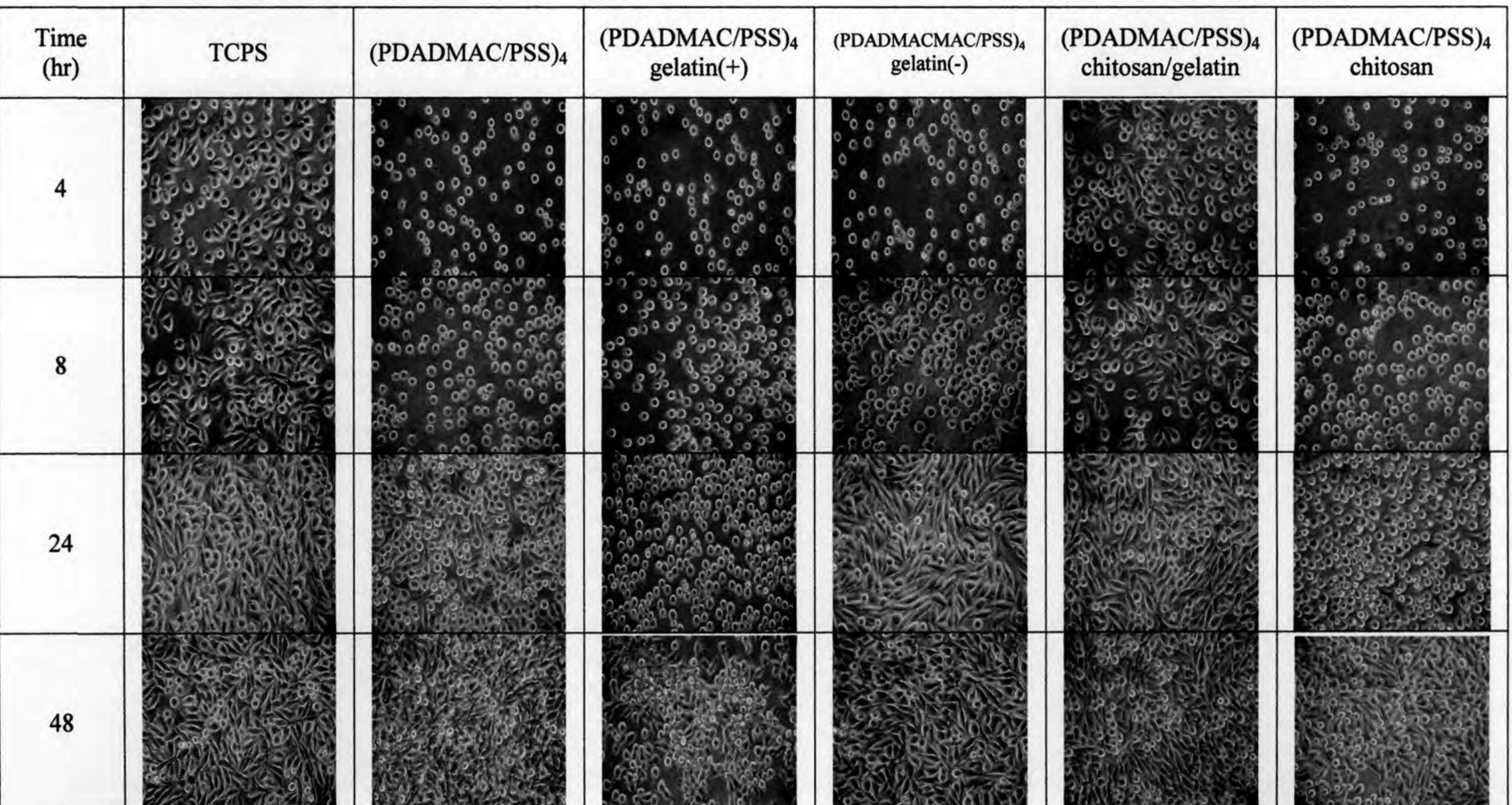


Figure 4.15 The morphology of L929 cells on the different outermost layers after 4, 8, 24 and 48 hr by light microscope (the magnification is 200x)

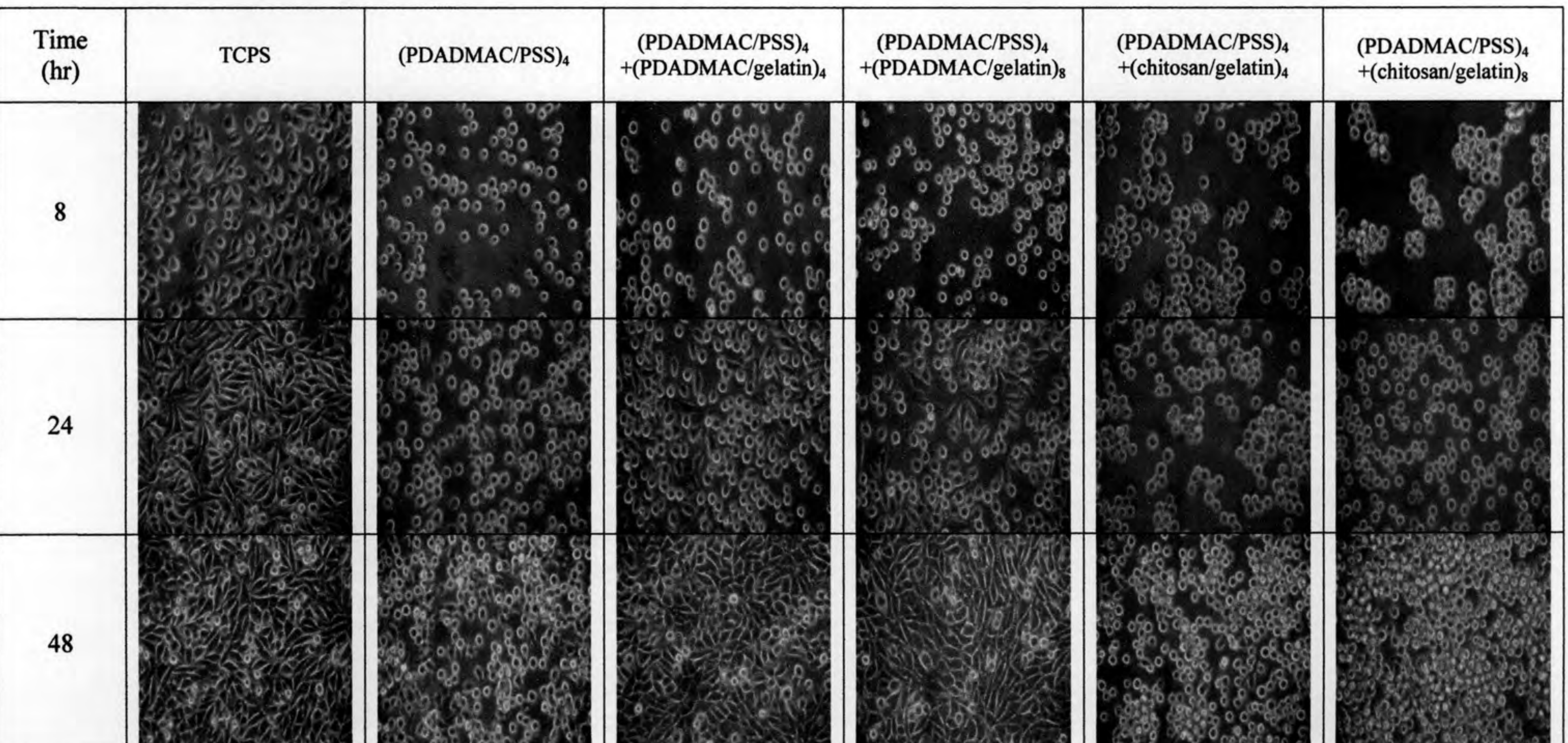


Figure 4.16 The morphology of L929 cells on the different number of layers after 8, 24 and 48 hr by light microscope (the magnification is 200x)

4.3 Cell behavior on PEMs coated PCL nanofibrous scaffolds

Tissue engineering is the used of a scaffold material to either induce formation of the new cells from the surrounding tissue or to act as a carrier or template for implanting the cells or other agents. From this viewpoint, electrospinning has recently been drawn strong attention in biomedical engineering, providing a basis for the fabrication of unique nanofibrous matrices and scaffolds. Among various types of nanofibrous scaffolds, PCL is one of the most interested materials, because of its biocompatibility, low cost, easy processability and slow hydrolytic degradation rate. It was reported that PCL is a good substrate for promoting cell adhesion and proliferation [22, 23]. Moreover, this novel structure provides a high level of surface area for cells to attach, due to its three-dimensional feature and its high surface area to volume ratio. In this part, PCL nanofibrous scaffolds were prepared and then coated with $(PDADMAC/PSS)_4$ primer. Then, PDADMAC/gelatin was selected as the top coat due to its good stability in culture medium solution and also its ability to promote cell adhesion.

4.3.1 Preparation of PCL solution

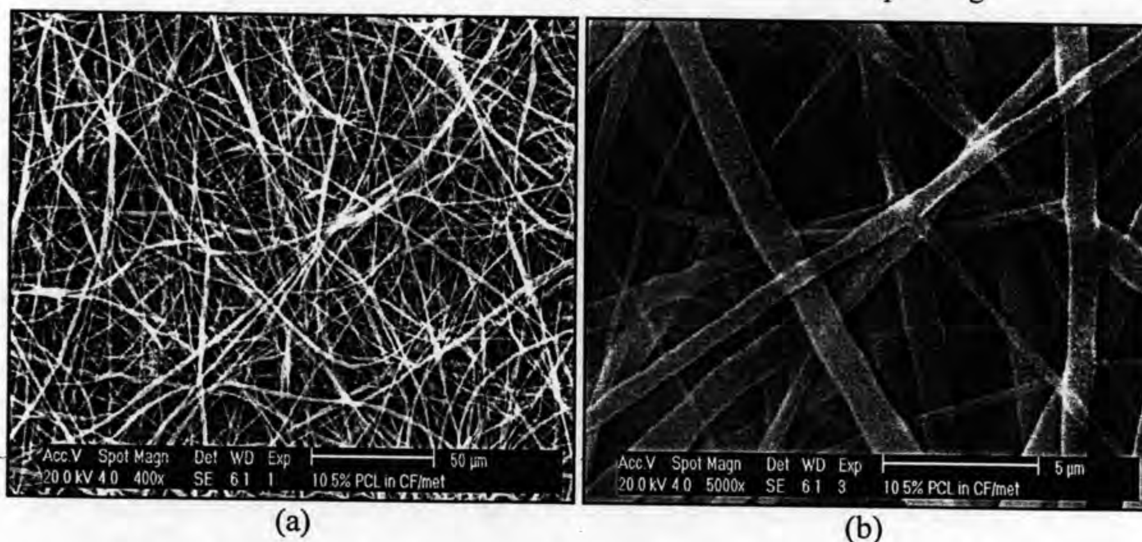
It is commonly known that the concentration of the polymer solution or the corresponding viscosity is one of the most important variables to control the fiber morphology [53] as well as other electrospinning parameters including applied voltage, distant between the needle tip and collector, solution flow rate, syringe and needle configuration, rotational speed were constant . In this study, the process parameters were selected from the preliminary work done in the laboratory and a previous study [23] that identified 10.5% w/v of the PCL solution, distance of 20 cm from the needle tip, applied voltage of 13 kV as the acceptable spinning parameters to obtain the continuous and bead free PCL fibers.

Solution of 10.5% w/v PCL was dissolve in methanol:chloroform (v/v 1:3). Prior to electrospinning, some properties such as viscosity and surface tension of the PCL solution were measured. The viscosity of the gelatin solution was 219.6 ± 0.424 cP whereas the surface tension was 26.191 ± 0.029 mN/m.

SEM images of PCL nanofibers are shown in Figure 4.17. The three dimensional fibrous mesh consists of fibers with the diameter ranging from 400 nm – 2 μm. Most of the fiber diameters are less than 1 μm, and the average diameter is 507 ± 251 nm. In addition to the broad fiber diameter distribution, the fiber often had non-

uniform diameter, i.e., the diameter varied along an individual fiber. The fibers were randomly oriented and interconnected void were presented.

It is anticipated that randomly-aligned nanofibrous scaffolds can be fabricated to any thickness by adjusting the collecting time. This is in consistent with the previous study report that the fiber orientation became disordered at the top layer of the spun mesh when the collecting time was longer than 30 min, which may due to the residual charges on the collecting fibers [12]. It is known that an electrically charged polymer jet can be created when the applied electrical force overcomes the surface tension of the polymer solution. After the polymer jet dried, it forms charged nano- or micro- size fibers. These residual charges seemed to be sufficient to exert a repel force on the next depositing fiber by changing its orientation or pushing it aside, leading to pores or voids between the random fibers. At a high magnification (Figure 4.17b), occasionally, two fibers were fused or bonded at their contact sides. The fibers exhibited these oriented, fibrillar morphology, indicative of a high degree of structure re-organization and randomness induced by the process of electrospinning.



(a) (b)

Figure 4.17 SEM micrographs of electrospun nonwoven obtained from 10.5% w/v PCL solution in methanol:chloroform (v/v 1:3). The collection time was 1 hr.

(a) Low magnification view at 400× (scale bar = 50 μm)

(b) High magnification view at 5000× (scale bar = 5 μm)

4.3.2 Viability and proliferation of L929 cell on fibrous scaffold

The nature and control of cell-biomaterial interactions influencing cell viability, proliferation and differentiation have been studied intensively since they provide the key to biocompatibility and the clinical success of an implanted device. Figure 4.18 showed the viability of cells on different PEMs at 24 hr as analyzed by MTT assay. When the percentage of the number of living cells on TCPS was normalized as 100%, the percentage of the number of living cells on the uncoated scaffold, scaffolds coated with $(PDADMAC/PSS)_4$ and with $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ were 103.52, 110.15 and 108.80, respectively. This result shows that L929 cells attached and adhered to the uncoated fibrous scaffold slower than to the scaffolds coated with $(PDADMAC/PSS)_4$ and $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$. There were no significant differences between $(PDADMAC/PSS)_4$ and $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ at this time point.

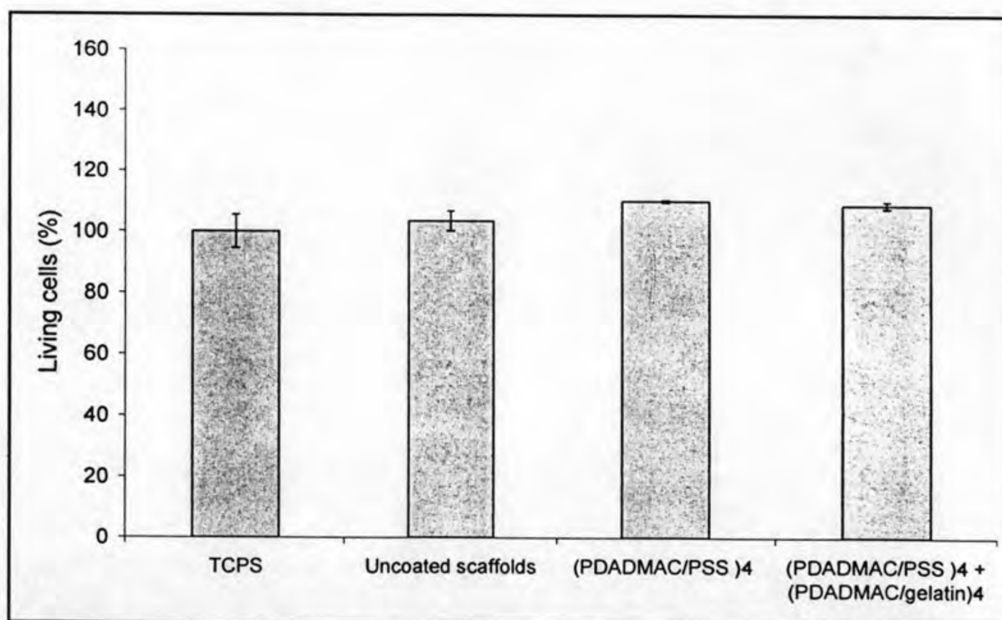


Figure 4.18 Viability of L929 cell culture on fibrous scaffold at 24 hr.

The proliferation of L929 cells on TCPS, uncoated scaffold, scaffold coated with $(PDADMAC/PSS)_4$ and scaffold coated with $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ are shown in Figure 4.19. Cells proliferated slower on all types of film coated during the first 24 hr. However, the longer culture time (48 hr) showed the number of cell proliferated on all types of film coated increased twice

faster. Cell growth on scaffolds coated with $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ was significantly higher than TCPS, uncoated scaffold and the scaffold coated with $(PDADMAC/PSS)_4$, although at first 4-24 hr, the trend was similar on the scaffold coated with $(PDADMAC/PSS)_4$. This was probably due to surface coating of PCL fibrous scaffolds with gelatin increased the proliferation rate of L929 cell after 24 hr relative to the uncoated scaffold. Gelatin has been widely used for production of cell support structures due to its cell adhesion properties and low antigenicity [55]. Improved cell adhesion and proliferation on the gelatin coated fibers may be conveniently explained by the presence of basic, negatively charged lysine and arginine residues in the denatured collagen molecule (which promoted electrostatic interaction with cell surface) and the presence of specific cell adhesion sites such as RGD sequences. Moreover fibroblast binding to gelatin-modified PCL fibers is anticipated directly via the prominent $\alpha_1\beta_1$ and $\alpha_2\beta_2$ intergrin receptors [20].

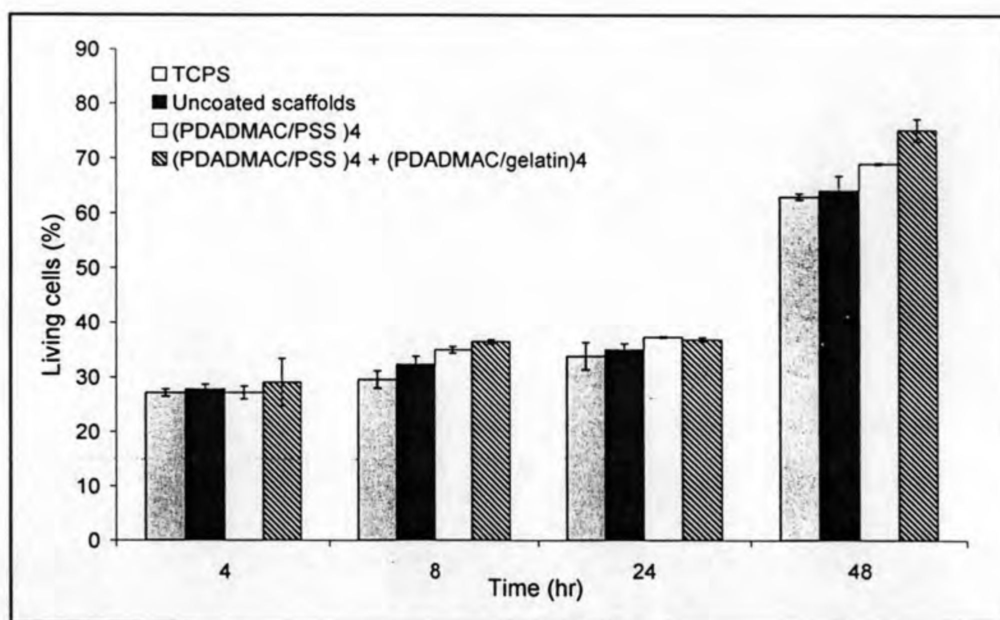


Figure 4.19 Proliferation of L929 cell cultured on fibrous scaffold at 4, 8, 24, 48 hour, as determined by the activity of viable cells.

4.3.3 Cell-scaffold morphology

The interaction between L929 cells and PCL nanofibers was investigated using a SEM. Although it can be observed that the area on the scaffolds that the cells occupied increased with the increasing culturing time, the number of cells could not be estimated from the obtained SEM images due to the fact that the cells were able to penetrate into the inner side of fibrous scaffolds. Figure 4.20 shows SEM images at a low magnification of 500× (scale bar = 50 μm) of cells that were cultured on the uncoated fibrous scaffold, the scaffold coated with $(PDADMAC/PSS)_4$ and the scaffold coated with $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ at different culture duration. At the first 8 hr, some of the cells were spread and flattened but many were still in round shape, indicating an absence of strong cell-fiber interaction at this time point. After 48 hr, the adherence of cells onto the scaffold coated with $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ was more densely distributed than that of the uncoated scaffold. SEM images at a higher magnification of 2000× (scale bar = 10μm) are shown in Figure 4.21. Clearly, during the first 4 hr of cell culture, the morphology of cells was mostly in round shape with slight trace of filopodia. At the longer time, the cells on modified fibrous scaffold, especially $(PDADMAC/PSS)_4$+$(PDADMAC/gelatin)_4$ expanded more, with an evidence of the anchoring ligrands reaching out to help supporting them on the fiber surface.

High porosity of nanofibrous scaffolds provides more structural space for cell accommodation and makes the exchange of nutrient and metabolic waste between the scaffold and environmental more efficient. The reason for cells entering into the matrix through small pore may be the pores in an electrospun structure, are formed by differently oriented fibers lying loosely upon each other [53]. When cell perform amoeboid movement to migrate through the pored, they can push the surrounding fibers aside to expand the hole as the small fibers offer little resistance to cell movement. These characteristics are fundamental criteria for successful tissue engineered scaffold. According to the results, cell seeded on the nanofibrous scaffolds were found to have appropriate interaction with their environment based on the following observation. First, the cells maintain a normal phenotypic shape, suggesting that the cells function biologically within this structure. Second, the cells favor this structure, so they attach onto the fibers and proliferated on the nanofiber network. Third, these cells crosslink the nanofibers and integrate with the surrounding fiber to form a three dimension cellular network. From this evidence, it can be concluded that

cell adhesion can be improved by coating PCL nanofibrous scaffolds with PEMs. The most suitable PEMs is $(PDADMAC/PSS)_4+(PDADMAC/gelatin)_4$.

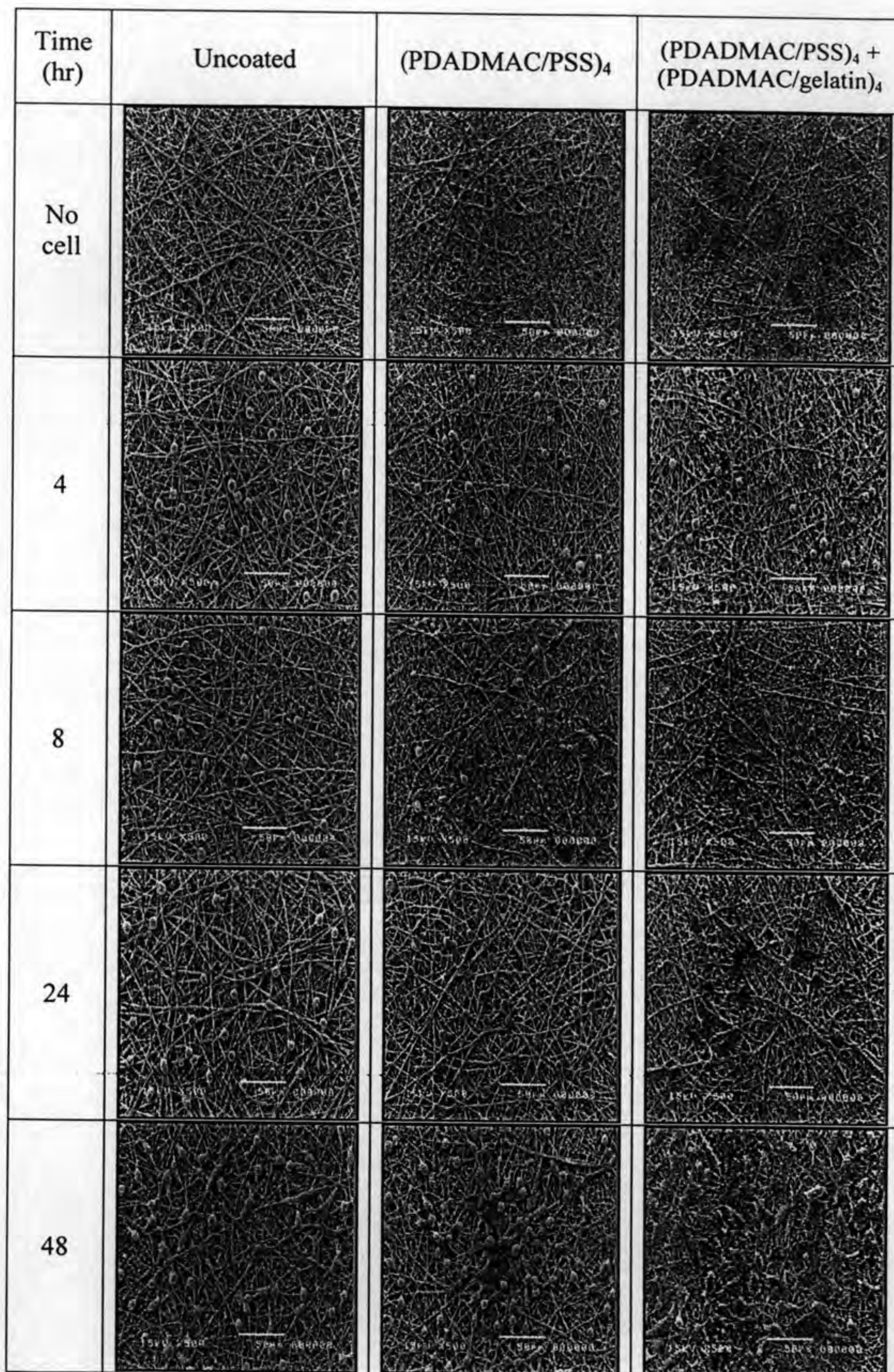


Figure 4.20 SEM micrographs of the L929 cells on nanofibrous scaffolds after 4, 8, 24 and 48 hr at 500× (scale bar = 50 μm)

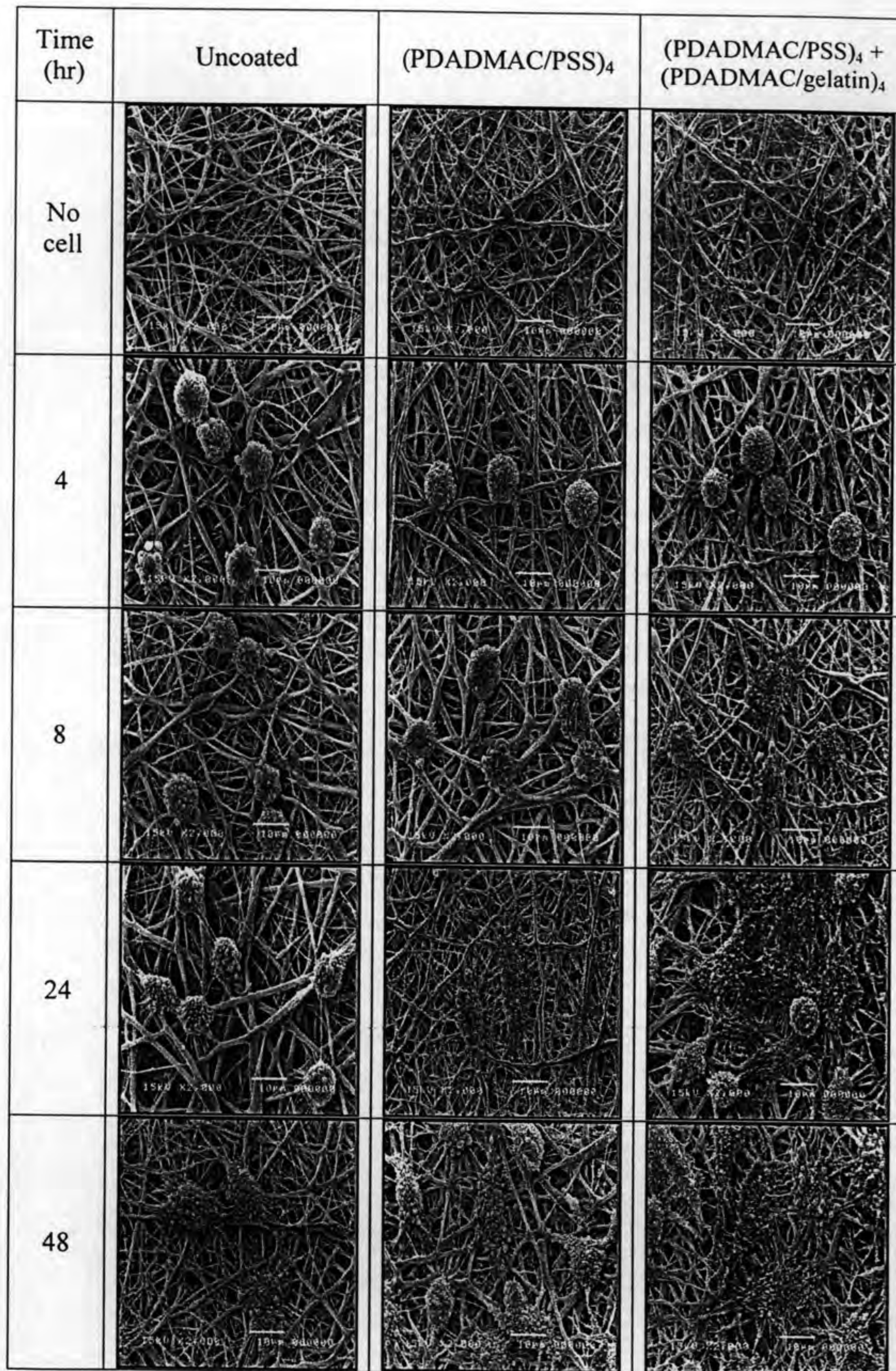


Figure 4.21 SEM micrographs of L929 cells on nanofibrous scaffolds after 4, 8, 24 and 48hr at 2000× (scale bar = 10 μm.)