CHAPTER 5

Plasma Polymerization of PLA Wettability Effect of PECVD-SiO_x Films on PLA for Protein Adsorption and Cell Attachment

5.1. General Introduction

Plasma polymerization has been used to deposit thin films with functional properties for various applications such as protective coating, electronic and optic device, lubricant surface or biomaterials [156-159]. Polymer-like films are applied in optoelectronic devices and protective coatings for different substrates. Quartz-like films can be used as diffusion barriers [160, 161]. One of the effective plasma polymerized film is silicon oxide films. Plasma enhanced chemical vapor deposition (PECVD) is used extensively to deposit silicon oxide films on various materials [162] because it can adjust the mechanical properties with the deposition parameters. Moreover, the PECVD is the choice of the coating industrial due to it can produce a high quality, good uniform and adhesion films to the substrates.

In recent years, there is a growing interest in developing silicon oxide films for use in biomedical applications such as bioelectronics and biofunctional materials. Biomaterials have been used in transplantation medicine, tissue engineering and bioartificial tissues. However, for advanced applications, they should not only be biocompatible but also specifically designed for peculiar cellular response, such as cell attachment, proliferation and differentiation. Common interaction between material and biological systems occurs in a wide range of applications. While biomaterial is in contact with a biological environment, protein adsorption first takes place before other processes such as platelet or blood cell adhesion and then cells contact with a protein layer on the material surface. Consequently, protein-surface interaction is critical to understanding for control and design of biomaterials.

There are several methods to modify the biomaterials surfaces property [73, 132] which depends on their objectives. The immobilized a number of proteins such as fibronectin, vitronectin or collagen was immobilized onto different wettability surface for improving cells attachment or promoting specific interaction for biosensor/microarray [11, 17, 92] and bovine serum albumin is immobilized on hydrophobic and hydrophilic surface for blocking of non-pacific proteins [163]. Commonly the immobilized process is more effective when combining with the plasma surface modification technique. The coated thin film is one of the modifications to the material surface, as thin film coating can lead to excellent mechanical properties and biocompatibility of biomaterials. Evermore, titanium dioxide improved cells-surface interaction [15] and silicon oxide films promoted selective adhesion of protein or cells to materials [164, 165].

Albumin is the most abundant protein in blood plasma and able to bind with other molecules. It is usually employed as a model protein to be studied in protein-surface interactions. Bovine serum albumin (BSA) is popular used for studying. In this an aqueous environment, proteins tend to adopt both a hydrophobic and a hydrophilic surface. Proteins can be irreversibly bound to a hydrophobic surface through the dehydration of the interface and undergo conformation changes. According to the Vroman effect, low-molecular-weight protein such as Albumin is first adsorbed on the surface. Following this are higher molecular weight proteins, such as fibronectin [74]. Thus, if Albumin is adsorbed with irreversible bonds, the cell adhesion protein can follow and enhance cell attachment and proliferation.

In previous study, we used the NH_3 plasma treated PLA to study the plasma surface functions. One of the unfair of control and the treated surface is the base type of surface. So the SiO_x films in the different functional surfaces that have the same physiology were choice to this study. The SiO_x films and wettability of SiO_x films were deposited on polymer by using PECVD. Oxygen (O_2) plasma was used to adapt wettability of SiO_x films. The O_2 plasma enhanced higher O_2-containing functionalities and more drastic morphology changes to the surface [32, 166]. Therefore in this work, we were interested in and thus studied effects of wettability of silicon oxide films on

protein adsorption, cell attachment and proliferation. Post-deposition O_2-plasma treatment was applied to modify the surface wettability of SiO_x films which were deposited on polymer. The research results would be beneficial to certain biomedicine application potentials such as polymeric cell-culture dishes and biosensors whose cell attachment and proliferation properties could be modified by depositing SiO_x thin films.

5.2. Experimental Procedure

5.2.1 Samples Preparation

The PLA films (NatureWork® with L/D ratios from 24:1 to 30:1) prepared from PLA resin by the standard calendering process. The film is clear with 50μm thickness. The PLA films were used as the substrate. They are cut into 1 cm × 4 cm strips for surface analysis and protein adsorption test. For cell attachment test, the films are cut into a circular shape in 1cm diameter which this size is fit to the cell culture plate. Then wiped the films with 95% ethanol and stored in a desiccator at 25±2ºC for 24 h prior to plasma treatment. The samples were place on the glass slide as in the Fig. 5.1 before being loaded in the chamber. The 2 mm x 10 mm tape was placed on the top left of PLA films for measurement of the thickness of the SiO_x films, then peeled off before measuring. The glass slides were cleaned with 95% ethanol and dried with nitrogen gas blow before being used. After treatment, the samples were put into the packaging quickly inside the chamber to prevent the environment contaminated.

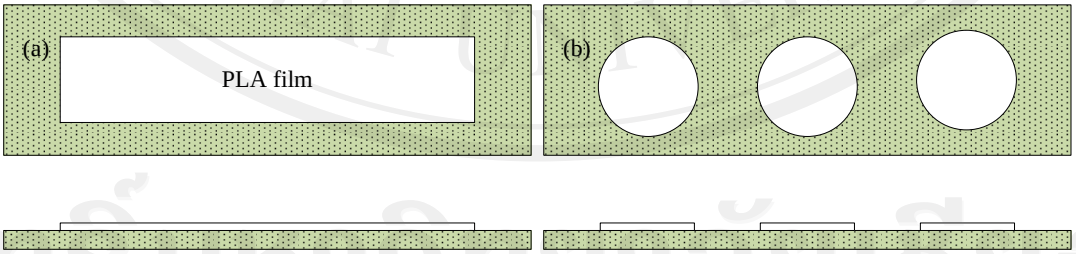


Figure 5.1 The sample preparation; PLA film put on the glass slide (a) 1 cm × 4 cm strips for surface analysis and protein adsorption test (b) a circular shape in 1cm diameter for cell culture.

5.2.2 Plasma Polymerization and Modification

SiO_x films were deposited on the PLA strips by PECVD at Sungkyunkwan University, Korea. The details of the deposition have been described in a previous chapter. The PLA substrate samples were put on a glass slide sample holder in the PECVD chamber. Then pumping the chamber evacuated to a base pressure of $1x10^{-2}$ torr. To remove the residues and increase films adhesion, the films were first sputtered using O_2-plasma at $9.5x10^{-2}$ torr by a bottom electrode with a RF power of 60 W for 20 seconds. Then SiO_x films were deposited by using octamethylcyclotetrasiloxane (OMCTS: $Si_4O_4C_8H_{24}$) as a precursor with oxygen as a carrier gas at pressure of $9.5x10^{-2}$. Applied a top electrode with an RF power of 120 W and a bottom electrode with an RF power of 60 W for 40 seconds. For the wettability SiO_x films, we deposit the SiO_x films like a base on PLA substrate. Then for increasing the wettability, O_2-plasma treatment was used. SiO_x films were continuing treated using O_2-plasma at $9.5x10^{-2}$ and applied a bottom electrode with an RF power of 60 W for 60 seconds.

5.2.3 Film Characterizations

The thickness of the deposited films was measured using an Alpha-Step IQ Surface Profiler (KLA-Tencor, USA), three replicas of each condition were used and each sample was scanned in 4 different regions. The film surface morphology was observed using AFM (Thermo Microscope Autoprobe CP-Research, USA). The AFM images were acquired in the contact mode using silicon tips with a scan rate of 1 Hz in air. The images were analyzed to measure the rms by three replicas of each condition and each sample was scanned in 3 different regions.

Static contact angles were measured using 2-µl droplets of deionised water and diiodomethane (CH_2I_2), respectively, at room temperature. Each sample was dropped in the 5 different regions and three replicas were measurement of each condition. Images of the fluid drops were captured and analyzed by using an image analysis software. The data was collected from both left and right side. The surface energy was calculated using the Owens-Wendt equations (Eq. 2.10-2.11).

The chemical structure of the film was characterized using ATR-FTIR spectroscopy (Bruker-Optics) with germanium crystal. Each spectrum was obtained using an average of 64 scans in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. Three replicas of each condition were scanned and each sample was scanned in 3 different regions.

The films were analyzed for the chemical structure using XPS (Shimadzu, Japan) [99, 167]. Survey spectra of XPS were acquired from 0-1200 eV, with a pass energy of 80 eV and step size of 1 eV. The core level spectra (high-resolution spectra) were obtained with a pass energy of 20 eV and step size of 0.1 eV. Elemental compositions were calculated from peak areas obtained from the survey spectra. All XPS peaks were referenced to a C_{1s} signal at a binding energy of 284.6 eV, corresponding to the C-C and C-H bonds in hydrocarbons. The peaks were deconvoluted into Gaussian components to gain insight into the bonding

5.2.4 Protein Adsorption

The BSA (A2153) powder was purchased from Sigma-Aldrich Corporation, Germany. Strips of the films were cut into 1 cm × 1 cm and placed into wells of a 4-well plate. The BSA solution (0.5% W/V) was prepared by adding the distilled water to BSA powder and mixed gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic protein. Then the protein was immobilized onto the films by adding 1 ml of BSA solution into each well and incubated for 20 min on a rocking shaker at room temperature. After incubation, the films were washed in distilled water, first soaking for 20 min on a rocking shaker at room temperature and then rinsing three times. The films were dried in a new plate and left at room temperature for 12 h.

5.2.5 Cell Culture

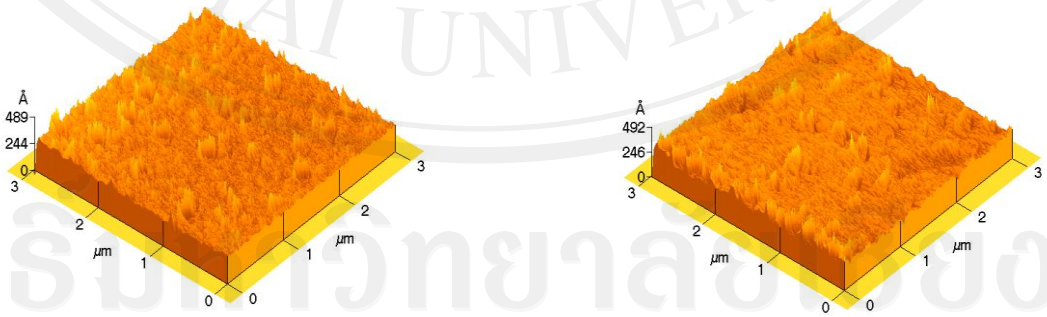
Mouse fibroblast cells (L929) and mouse pre-osteoblast cells (MC3T3-E1) were used to study cell cultivation on the surfaces of the as-deposited SiO_x and O_2-plasma treated SiO_x films. Use of the fibroblast cells was aimed at artificial tissue testing and use of the osteoblast cells was aimed at scaffold testing. Fibroblast cells are

normally used as a model of cell attachment and proliferation since they are the most common cells of connective tissues in animals and able to grow in a high rate. After treatment, the films were keeping to the packaging rapidly, however, the films still exposed to the environment then the films must be sterilized under UV light for 1 h (both faces) before cell culture. The cell attachment and proliferation were evaluated using MTT assay. Both of cell lines were diluted to 5×10^3 cells/ml. The culture medium was modified with 10% fetal bovine serum and 1% antibiotic antimycotic solution (WelGENE, Korea). The cells were cultured at 37°C in a 5%-humidified CO_2-atmosphere incubator. Then cell seeding films were measured with a fluorescence spectrometer. In these experiments, three replicas were used for calculating the percentage of each experiment. The cell attachment was observed in 1 day and cell proliferations were observed in 3 and 7 days.

5.3. Results and Discussion

5.3.1 Surface Characterizations

The thickness of the deposited SiO_x films was 48.2±0.5 nm and that of the O_2-plasma treated SiO_x films was 47.8±0.8 nm as measured by the surface profiler. The film thickness was decreased by O_2-plasma treatment insignificantly, indicating that the O_2-plasma treatment did not have noticeable effect on changing the thickness of SiO_x films.



(a) (b)

Figure 5.2 AFM images of the surface morphology of (a) as-deposited SiO_x and (b) O_2-plasma treated SiO_x films.

Surface morphology of the films was observed with a scanning area of 3μm × 3μm by AFM. Roughness data was scanned from different regions on each sample. Figure 5.2 shows the AFM images of the deposited SiO_x film and the O_2-plasma treated SiO_x film. The O_2-plasma treatment decreased the surface roughness (root-mean-square, R_{rms}) of the films from 26 nm to 19 nm due to plasma etching effect as the result related with the decreasing of the films thickness.

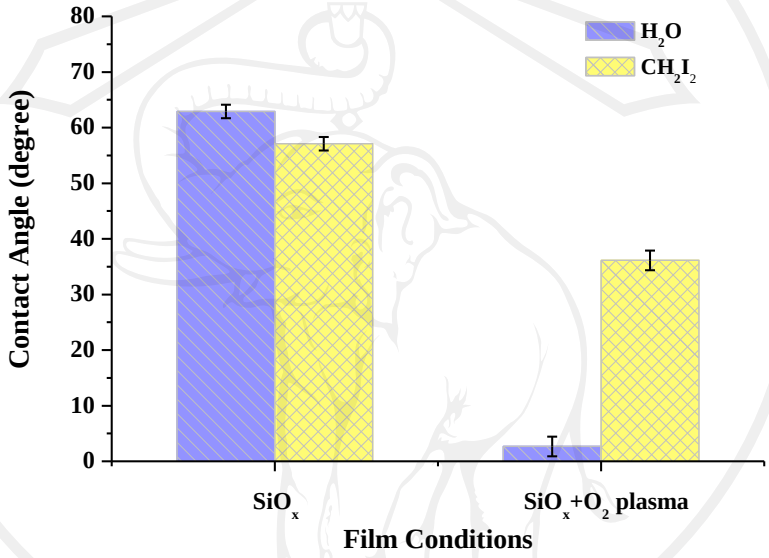


Figure 5.3 Contact angle of water (H_2O) and diiodomethane (CH_2I_2) on the as-deposited SiO_x film and O_2-plasma treated SiO_x film.

The surface energy of the films consists of polar and non-polar components. The polar component of surface energy comprises all other interactions due to the non-London forces. Polar molecules interact with dipole forces and hydrogen bonds. The dispersive or non-polar components of surface energy result from molecular interactions due to the London forces [148]. Figure 5.3 shows the results of the contact angle measurements. The water contact angle of the as-deposited SiO_x films was 57.0 degrees and after O_2-plasma treatment the water contact angle obtained was significantly decreased down to 2.7 degrees. These show a significant increase in the hydrophilicity of the film.

Figure 5.4 shows the components of the surface energy of the films. The polar components of the surface energy increased after O_2-plasma treatment. The total surface energy was increased after the O_2-plasma treatments, demonstrating that the surface of SiO_x became more hydrophilic. The enhanced hydrophilicity of the O_2-plasma treated SiO_x films was owing to increased polarity, as there were more polar components in the total surface energy. This could result from the incorporation of polar groups such as hydroxyl group on the surface.

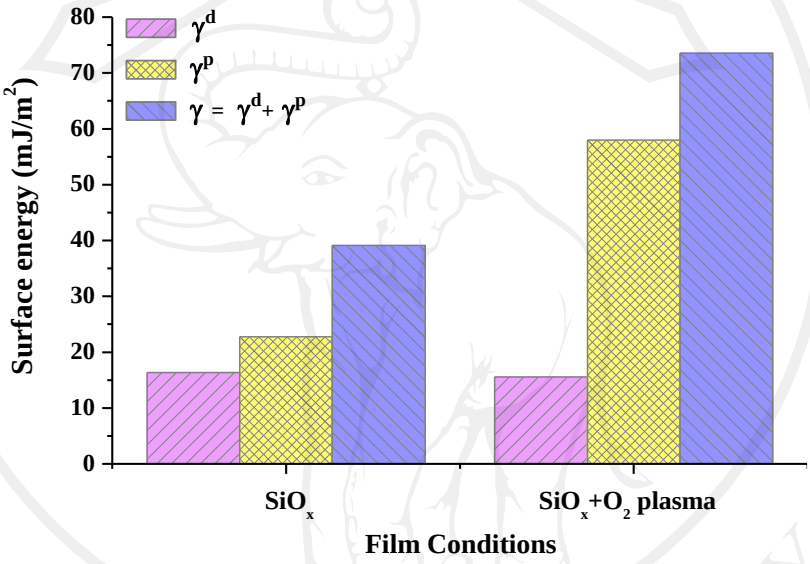


Figure 5.4 Surface energy of the as-deposited SiO_x film and O_2-plasma treated SiO_x film, including dispersive components : γ_S^d and polar components : γ_S^p .

The chemical structure in the deposited films was examinated by ATR-FTIR spectra. Figure 5.5 shows the spectra of the as-deposited SiO_x film and O_2-plasma treated SiO_x film. The absorbance spectra were collected from 500-1350 cm^{-1} and compared with the spectral data of the known structures. In the PECVD of the films, a high ion current density enhanced the film density and hardness [160]. In this case, the Si-O network structure and Si-O cage-like structure showed very strong absorption bands in the range of 960-1250 cm^{-1}. The Si-O structure comprised Si-O-Si (1050 cm^{-1}), ring link Si-O-C (1085 cm^{-1}), open link Si-O-C (1128 cm^{-1}) and cage link Si-O-C (1180 cm^{-1}). The alkyl groups as $Si-CH_3$ and $-CH_n$ were found in the small region. The peaks

at 754, 810 and 1268 cm^{-1} could be assigned to the Si-C stretching and the $-CH_3$ rocking modes as Si-(CH_3) and Si-(CH_3)$_2$ [168]. The small peak at 874 cm^{-1} could be assigned to Si-OH and Si-H which indicated the incorporation of some moisture into the oxide films [169] which were related to the broad peak of -OH bond between 3150 and 3600 cm^{-1}. The peak between 2830 and 3025 cm^{-1} of the $-CH_n$ stretching bond consisted of $-CH_2$ and $-CH_3$. The $-CH_n$ peaks at 1360, 1379 and 1449 cm^{-1} could be assigned to $-CH_2$, $-CH_3$ and $-CH_4$, respectively [168]. It was found that after O_2-plasma treatment, the $-CH_n$ stretching bond and the Si-CH_3 bond decreased, whereas the -OH stretching bond increased. This implied that the O_2-plasma treatment reduced the carbon content of Si-O films and hydrogen from moisture was incorporated into the oxide films and contributed to the polar surface to enhance the SiO_x wettability.

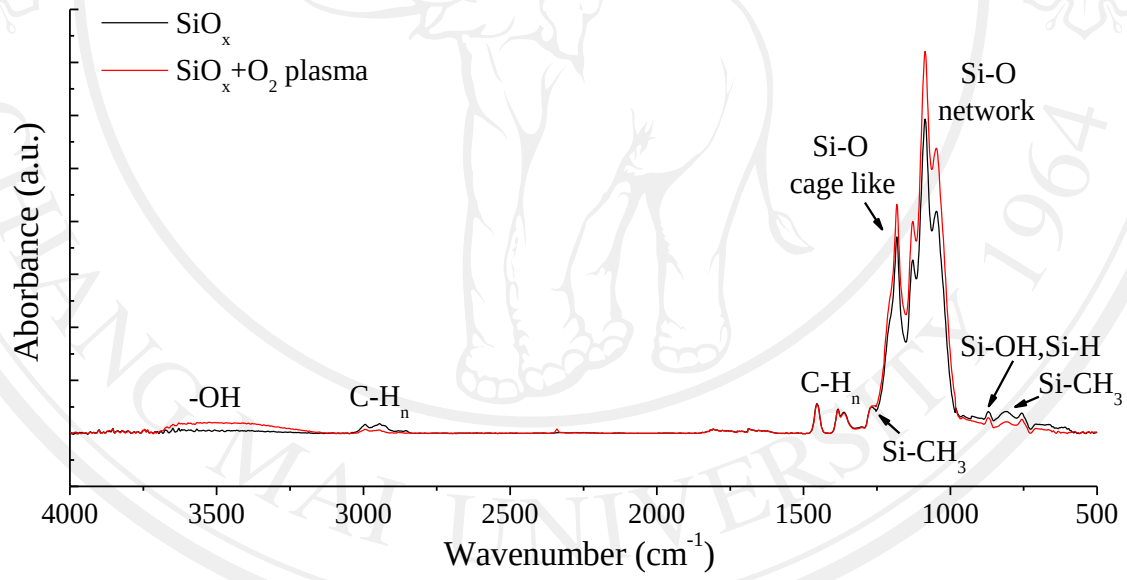


Figure 5.5 FTIR spectra of the as-deposited SiO_x and O_2-plasma treated SiO_x films.

5.3.2 Protein Adsorption

The protein adsorption plays a role in variety of biologically related process. The biomaterials are required to reduce the non specific adsorption of blood protein, as protein adsorption is a very complex process, depending on the environment. There are three major factors: protein, surface and solution, and each factor depend on its

properties. As several factors are present, it is quite difficult to focus on only a single factor. However, one may say correctly that the key factor of protein adsorption is the conformation change of proteins or peptides on substrate [170]. This is influenced by kinetic and thermodynamic considerations.

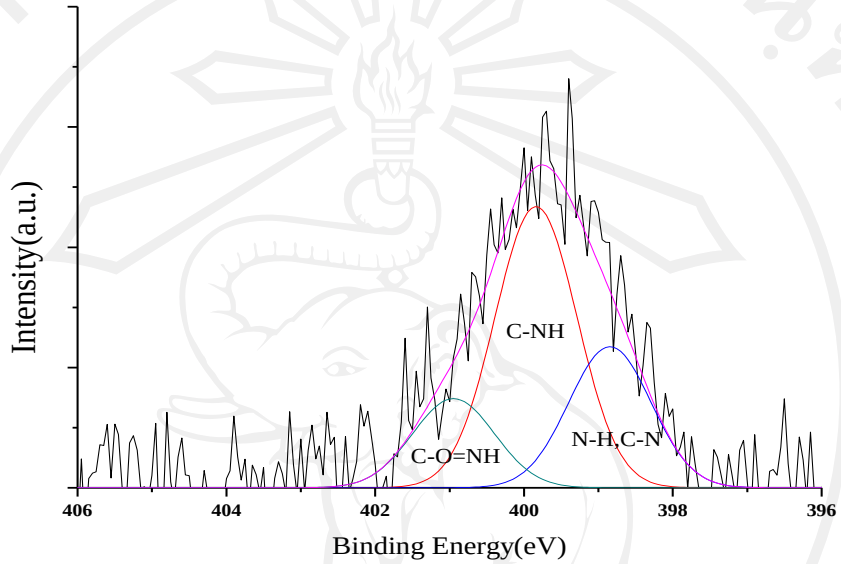


Figure 5.6 The concentration of the nitrogen bonds on BSA adsorbed on films SiO_x only and SiO_x treated with O_2-plasma measured by XPS. This is an example of the original XPS spectrum with deconvoluted components.

Albumin is the most abundant protein in blood with a high concentration of 45 mg/ml, a molecular weight of 66 kDa, and an isoelectric point of 5.5 which has a negative charge in blood of pH 7.4. It is a globular protein in a soft protein type, which has a low internal stability in aqueous solution. Generally, soft proteins adsorb on a hydrophobic surface rather than a hydrophilic surface, because the proteins tend to conserve their native structure on hydrophilic surface [155, 171-173]. When biomaterials come into an aqueous solution, a water shell will form on the surface in a microsecond. Dehydration of the surface occurs in the next step, and the water structure forms hydrogen bonds with hydrophilic surface. On the hydrophilic surface, albumin will adsorb on the water layer with less tightly bond namely reversible bond. On the hydrophobic surface, water shell cannot form on the surface and Albumin adsorbs on a water-free contact layer due to the hydrophobic effect with irreversible bond [31].

Many researchers study controlling adsorption/desorption proteins to surface with the surface wettability. This study was focused on BSA) adsorption onto SiO_x films with certain wettability. BSA was used to represent mammalian albumin. Some characteristics of BSA adsorption were investigated by XPS.

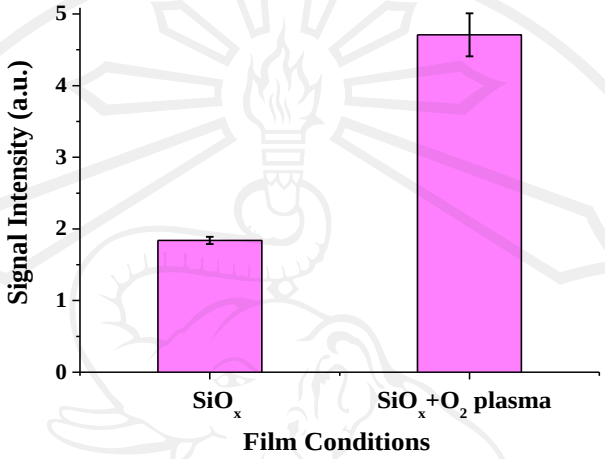


Figure 5.7 The calculated N-signal intensity indicating the N concentration in the films.

Nitrogen peaks are caused by the presence of the amino acid sequence of BSA molecules. In the XPS spectrum the N_{1s} peak was deconvoluted into three peaks, as in Figure 5.6, assigned to amino (C-NH) and peptide bound (C=O-NH) [149-151]. Figure 5.7 shows the concentration of the nitrogen bonds on BSA adsorbed on the SiO_x films and O-plasma treated SiO_x films measured by XPS. It is seen that the O-plasma-treated SiO_x films had more nitrogen than the untreated films, indicating the former absorbing more protein than the latter due to a lower energy band gap. The energy band gap has been rarely taken into account for a material surface factor of protein adsorption. Gandhiraman [81] studied the fibrinogen adsorption on $SiO_xC_yH_z$, TiO_x and SiO-TiO films with the surface factors of wettability, roughness and energy band gap. They found that fibrinogen adsorption was the highest on the low energy band gap.

5.3.3 Cell Culture

Cell attachment and proliferation of L929 mouse fibroblast cells and MC3T3-E1 mouse pre-osteoblast cells were observed in 1 day and 3-7 days, respectively, and the results are summarized in Fig. 5.8-5.9.

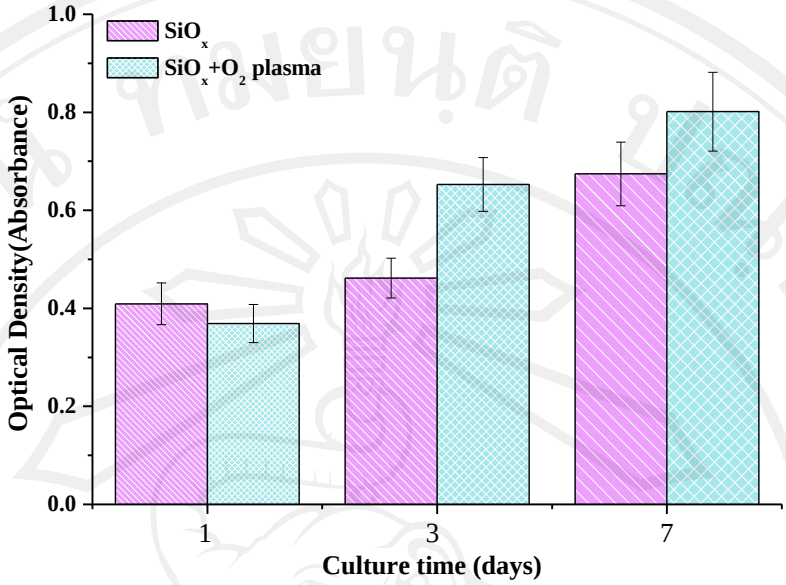


Figure 5.8 Relative optical density of L929 mouse fibroblast cells on the SiO_x and O_2-plasma treated SiO_x films measured by MTT assay after 1, 3 and 7 days, respectively.

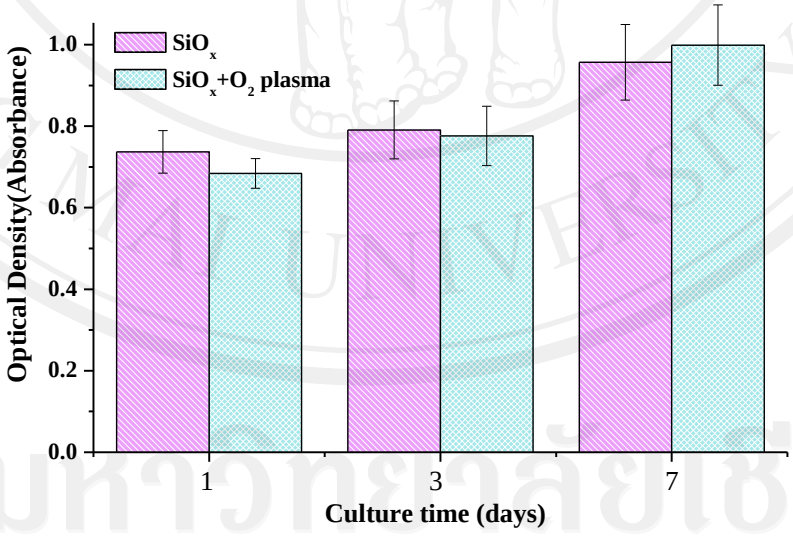


Figure 5.9 Relative optical density of MC3T3-E1 mouse pre-osteoblat cells on the SiO_x and O_2-plasma treated SiO_x films measured by MTT assay after 1, 3 and 7 days, respectively.

It showed both types of the cells attached on the as-deposited SiO_x films slightly more than on the O_2-plasma treated SiO_x films due to the lower BSA adsorption on the untreated films. The low BSA adsorptions resulted in the cell adhesion proteins more adsorbed on to the film surface and thus the cells could have more attachment. But, both types of the cells proliferated on the O_2-plasma treated SiO_x films more than on the untreated as-deposited SiO_x films. Mouse pre-osteoblast cells proliferated slower than mouse fibroblast cells. The results indicated that the cell attachment depended on the protein adsorption on the surfaces, while the cell proliferation depended on the surface wettability.

5.4. Conclusion

SiO_x films were deposited on PLA substrate using the PECVD technique and further treated with O_2-plasma to investigate the film surface wettability effect on the protein adsorption and cell attachment and proliferation. The O_2-plasma significantly enhanced the surface wettability of the SiO_x films without changing the thickness of the films but smoothing the surface morphology. Increase in the OH bond was responsible for the enhancement of the wettability due to hydrogen replacement and incorporation into the oxide films to contribute to the polar surface. BSA protein was more adsorbed on the hydrophilic SiO_x films treated by O_2-plasma with the low energy band gap, resulting in less cell attachment but cell-type-dependent cell proliferation, increased for the L929 mouse fibroblast cells but almost no changes for the MC3T3-E1 mouse pre-osteoblat cells. The results provide some hints for designing certain applications of SiO_x films on polymers in biomedicine.