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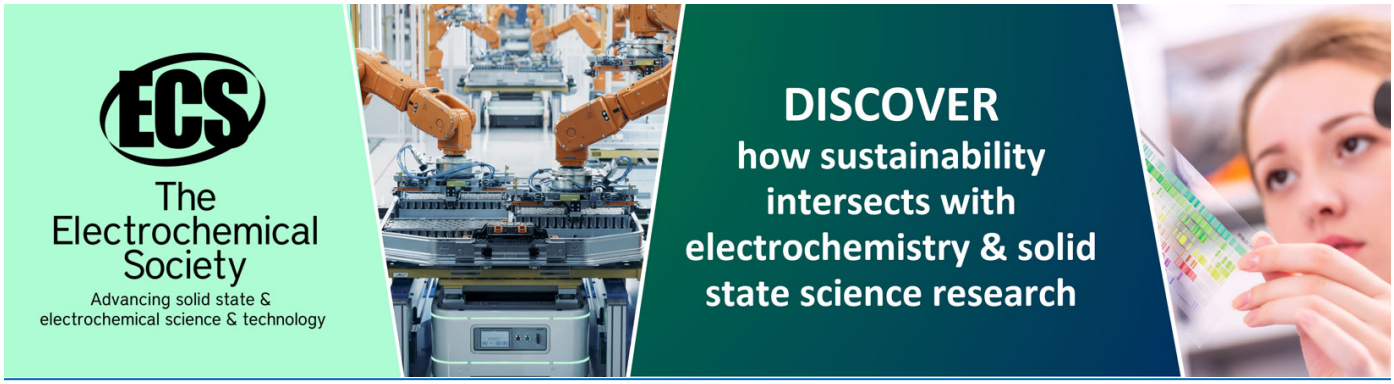


Wettability Effect of PECVD-SiO_x Films on Poly(lactic acid) Induced by Oxygen Plasma on Protein Adsorption and Cell Attachment

S Sarapirom[1], J S Lee[2], S B Jin[2], D H Song[3], L D Yu[1,4], J G Han[2] and C Chaiwong[1,4]

[1] Plasma and Beam Physics Research Facility, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[2] Institute for Plasma-Nano Materials, Center of Advanced Plasma Surface Technology, Sungkyunkwan University, 300 ChunChun-dong, Jangan-gu, Suwon 440-746, Korea
[3] Department and Research Institute of Dental Biomaterials and Bioengineering, College of Dentistry, Yonsei University, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, Korea
[4] Thailand Center of Excellence in Physics, Commission on Higher Education, 328 Si Ayutthaya Rd., Bangkok 10400, Thailand

E-mail: cchwng@gmail.com

Abstract. Surface wettability is an important property of biomaterials. Silicon oxide films have a wide range of applications due to a range of the properties such as the mechanical strength and surface wettability. This paper reports effect of the surface wettability of silicon oxide (SiO_x) films on protein adsorption and cell attachment and proliferation. SiO_x films were deposited onto poly(lactic acid) (PLA) substrate using plasma enhanced chemical vapor deposition (PECVD). Octamethylcyclotetrasiloxane (OMCTS:$Si_4O_4C_8H_{24}$) was used as a precursor with O_2 as a carrier gas. After deposition, the films were treated with O_2-plasma to adapt wettability. It was found that O_2-plasma enhanced the wettability of the films without changing the film thickness, while made the surface morphology slightly smoother. The polar component increased after O_2-plasma treatment as observed in the contact angle measurements. The surface energy of the films was calculated by means of the Owens-Wendt method to resolve the contributions of polar and dispersive components. The chemical structure was characterized using attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. The films were dense with a high Si-network structure. The reduced carbon content (-CH_n, Si-CH_3) and increased hydrogen content (-OH) of the O_2-plasma treated SiO_x films led to the polar components enhancing the SiO_x wettability. Adsorption of bovine serum albumin (BSA) on the films was investigated by using x-ray photoelectron spectroscopy (XPS). More BSA was adsorbed onto the O_2-plasma treated SiO_x films. Attachment and proliferation of MC3T3-E1 mouse pre-osteoblasts and L929 mouse fibroblasts cells on the SiO_x films were evaluated via MTT assay. The cells were attached more to the untreated SiO_x films but proliferated more on the surface of the O_2-plasma treated SiO_x films depending on the cell types.

1. Introduction

Biomaterials have been important in transplantation medicine, tissue engineering, and bioartificial tissues. 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 surfaces properties of biomaterials [1, 2], and each way depends on the objectives. For example, adhesive proteins (fibronectin, vitronectin or collagen) are immobilized onto different wettability surface for improving cells attachment or promoting specific interaction for biosensor/microarray [3-5], and bovine serum albumin is immobilized on hydrophobic and hydrophilic surface for blocking of non-pacific proteins [6]. Moreover, thin film coating on the materials can lead to excellent mechanical properties and biocompatibility of biomaterials. For instance, titanium dioxide improved cells-surface interaction [7], and silicon oxide films promoted selective adhesion of protein or cells to materials [8, 9].

Thin films of silicon-organic compounds are used in a range of applications. The characteristics of the films can be tailored by changing deposition process parameters. Polymer-like films are applied in optoelectronic devices, and protective coatings for different substrates. Quartz-like films can be used as diffusion barriers [10, 11]. In recent years, there is a growing interest in developing silicon oxide films for use in biomedical applications such as bioelectronics, and biofunctional materials. Plasma enhanced chemical vapor deposition (PECVD) is widely used to deposit silicon oxide films on polymeric biomaterials [12] because it can adjust the mechanical properties with the deposition parameters. Oxygen (O_2) plasma was used to adapt wettability of SiO_x films because O_2 plasma enhanced higher O_2-containing functionalities and more drastic morphology change leading to proteins and cells attachment [13, 14].

The adsorption of proteins in the blood plasma on the biomaterial surface is, in general, a dynamic phenomenon since there is a competition in the adsorption process of such proteins. Albumin (Alb) 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. In an aqueous environment, proteins will 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 undergoes conformation changes. According to the Vroman effect, low-molecular-weight protein such as Alb is first adsorbed on the surface then higher molecular weight proteins, such as fibronectin [15]. Thus, if Alb is adsorbed with irreversible bonds, the cell adhesion protein can follow and enhance cell attachment and proliferation.

In this work, we were interested in and thus studied effect 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 using PECVD. 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.

2. Experiment

2.1. Materials preparation and modification

Poly(lactic acid) (PLA) membranes of (thickness 50 μm), obtained from the School of Mechanical Engineering, Institute of Engineering, Suranaree University of Technology, Thailand were used as the substrate. The membranes were cut into 1 cm × 4 cm strips.

SiO_x films were deposited on the PLA membranes by PECVD in Sungkyunkwan University, Korea using octamethylcyclotetrasiloxane (OMCTS: $Si_4O_4C_8H_{24}$) with oxygen as a carrier gas. The details of the deposition has been described elsewhere [12]. In brief, the membrane substrate samples were put on a glass slide sample holder in the PECVD chamber and the chamber was evacuated to a base

pressure of 0.01 Pa. To remove the residues and increase films adhesion, the membranes were first sputtered using O_2-plasma at 3.3 Pa by a bottom electrode with a radio frequency (RF) power of 60 W for 20 seconds. After that, SiO_x films were deposited at an OMCTS vapor pressure of 3.3 Pa by a top electrode with an RF power of 120 W and a bottom electrode with an RF power of 60 W for 40 seconds. Then, for increasing the hydrophilicity, a part of the SiO_x films were further treated using O_2-plasma at 3.3 Pa and a bottom electrode with an RF power of 60 W for 60 seconds.

2.2. Surface characterization

The thickness of the deposited film was measured using an Alpha-Step IQ Surface Profiler (KLA-Tencor, USA). The film surface morphology was observed using atomic force microscopy (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 root mean square (Rms) and the surface-peak-to-valley roughness (Rp-v).

The static contact angles were measured with 2 µl of deionized water and diiodomethane (CH_2I_2), respectively, at room temperature. The image of the fluid drops was captured and analyzed by using the image analysis software. The surface energy was calculated using the Owens-Wendt equations [16, 17], which are expressed as follows:

$$\gamma_W\left(1+\cos\theta_W\right)=2\sqrt{\gamma_S^d\gamma_W^d}+2\sqrt{\gamma_S^p\gamma_W^p} \quad (1)$$

$$\gamma_D\left(1+\cos\theta_D\right)=2\sqrt{\gamma_S^d\gamma_D^d}+2\sqrt{\gamma_S^p\gamma_D^p} \quad (2)$$

where γ is the surface energy, θ is the contact angle, and the superscripts, *d* and *p*, represent the dispersive component and the polar component of the surface energy, respectively. The Subscript *S*, *D*, and *W* indicate solid, diiodomethane and water, erspectively. For water, γ_W = 72.8 mJ/m^2, γ_W^d = 22.1 mJ/m^2, and γ_W^p = 50.7 mJ/m^2. For diiodomethane, γ_D = 50.8 mJ/m^2, γ_D^p = 44.1 mJ/m^2, and γ_D^d = 6.7 mJ/m^2 [18].

The chemical structure of the film was characterized using attenuated total reflectance-Fourier transform infrared (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}.

2.3. Protein adsorption analysis

The bovine serum albumin (BSA)(A2153) 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 protein was immobilized onto the films by adding 1 ml of BSA solution (0.5% W/V in distilled water) into each well and then incubated for 20 min on a rocking shaker at room temperature. After incubation, the films were washed in distilled water for 20 min, first soaking and then rinsing 3 times. The films were dried in a new plate and left at room temperature for 12 h. Some films were analyzed for the chemical structure using x-ray photoelectron spectroscopy (XPS) (Shimadzu, Japan) [19, 20]. 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 the insight into the bonding.

2.4. Cell culture

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. In this investigation, 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

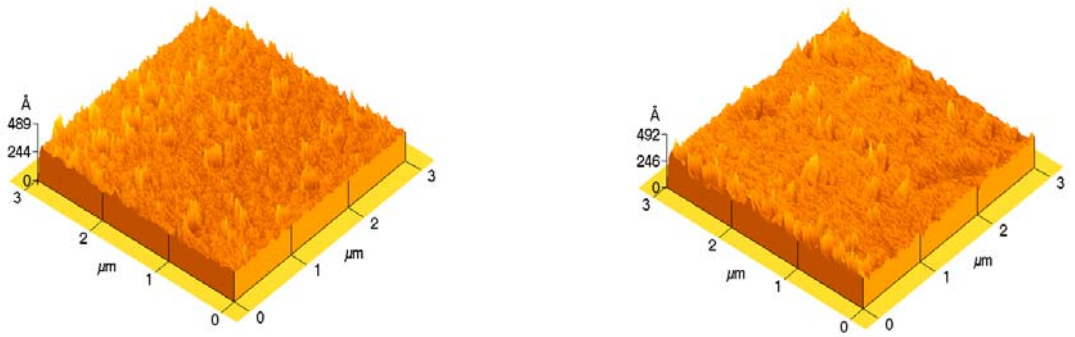
of the fibroblast cells was aimed at artificial tissue test and use of the osteoblast cells was aimed at scaffold test. The cell attachment and proliferation were evaluated using MTT assay. The films were first sterilized under ultraviolet (UV) light for 1 h (both faces). The cells were diluted to 5×10^3 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 at wavelengths of 544 and 590 nm. In these experiments, 3 replicates 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.

3. Results and discussion

3.1. Surface characterization

The thickness of the as-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. Surface morphology of the films was observed by AFM with a scanning area of 3μm × 3μm. Roughness data were obtained from different regions on each sample. Figure1 shows the AFM images of the as-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.

The material surface energy 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 [21]. Figure 2a shows the results of the contact angle measurements. The water contact angle of the as-deposited SiO_x films was 57.0 degree and after O_2-plasma treatment the water contact angle obtained was significantly decreased down to 2.7 degrees, showing a significant increase in the hydrophilicity of the film. Figure 2b 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 higher 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.



(a) (b)

Figure 1. AFM images of the surface morphology of (a) as-deposited SiO_x and (b) O_2-plasma treated SiO_x films.

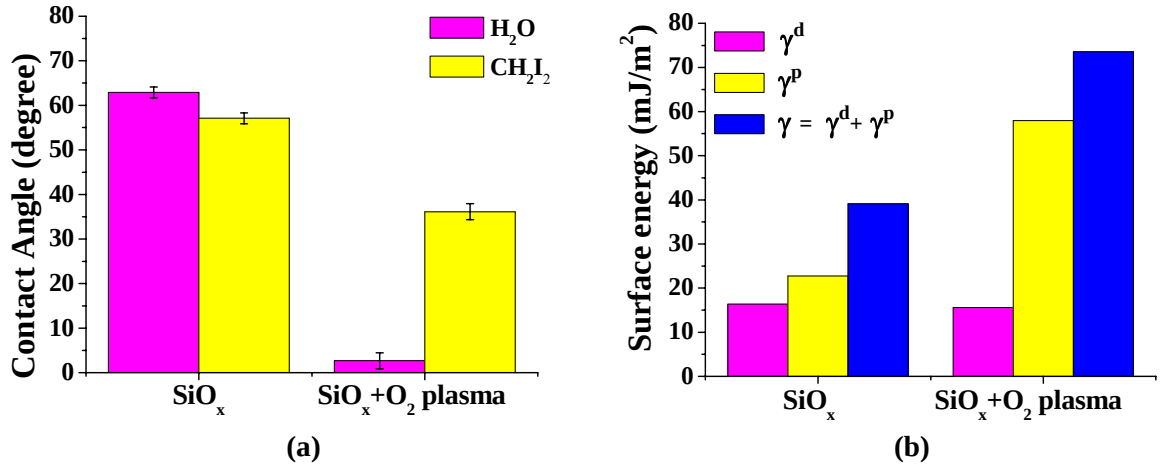


Figure 2. Surface properties of the SiO_x films. (a) Contact angle of water (H_2O) and diiodomethane (CH_2I_2) on the as-deposited SiO_x film and O_2-plasma treated SiO_x film. (b) Surface energy of the as-deposited SiO_x film and O_2-plasma treated SiO_x film, including dispersive components : γ_S^d and polar components : γ_S^p .

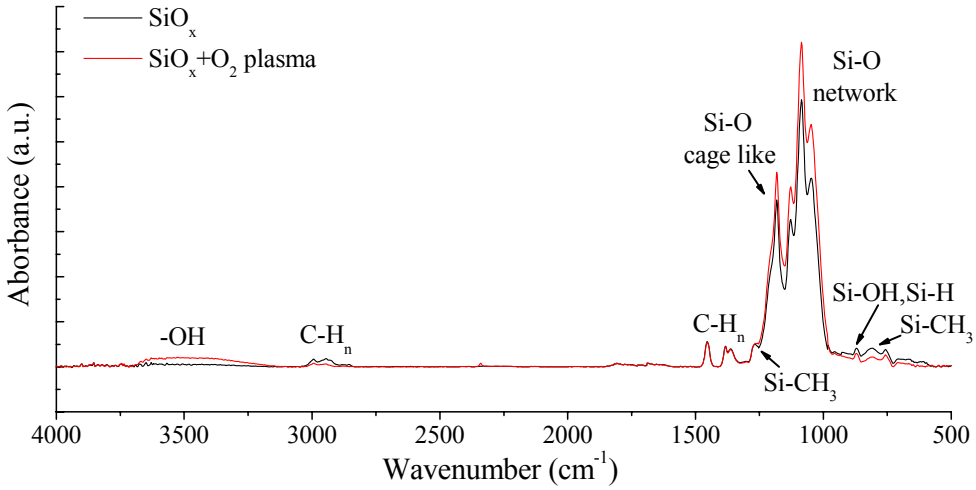


Figure 3. ATR- FTIR spectra of the as-deposited SiO_x and O_2-plasma treated SiO_x films.

The ATR-FTIR spectra of the as-deposited SiO_x film and O_2-plasma treated SiO_x film are show in Figure 3. The absorbance spectra of OMCTS 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 [10]. 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 $Si-(CH_3)_2$ [22]. 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 [23] 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 [22]. 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.

3.2. Cell culture

Cell attachment and proliferation of mouse fibroblast cells (L929) and mouse pre-osteoblast cells (MC3T3-E1) were observed in 1 day and 3-7 days, respectively, and the results are summarized in Figure 4. 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.

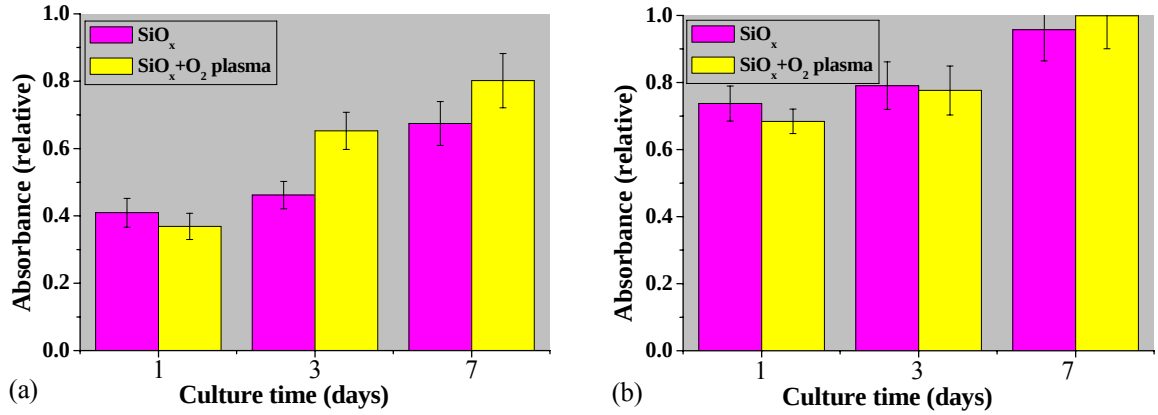


Figure 4. Relative absorbance of (a) L929 mouse fibroblast cells and (b) MC3T3-E1 mouse pre-osteoblat cells on the SiO_x and O_2-plasma treated SiO_x films measured by MTT assay after 1, 3, and 7 days, respectively.

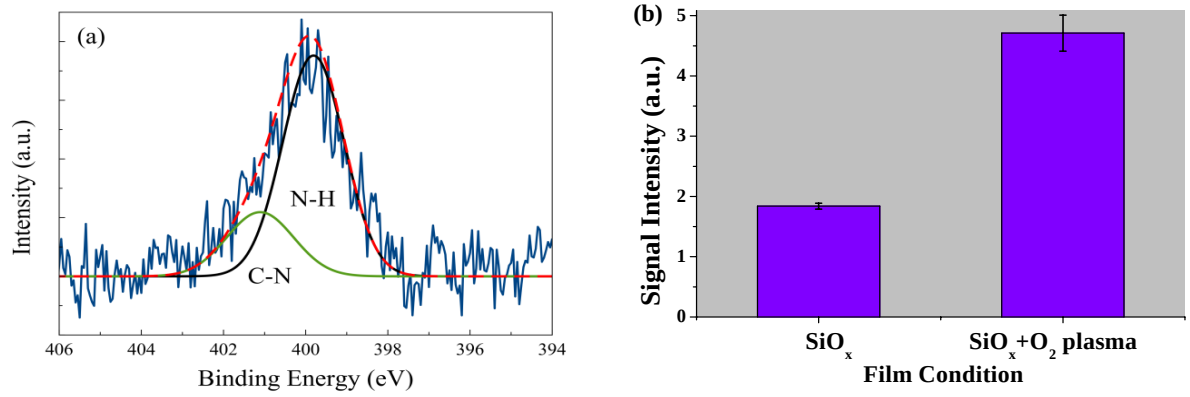


Figure 5. The concentration of the nitrogen bonds on BSA adsorbed on films SiO_x only and SiO_x treated with O_2-plasma measured by XPS. (a) An example of the original XPS spectrum with deconvoluted components. (b) The calculated N-signal intensity indicating the N concentration in the films.

3.3. Protein adsorption

Protein adsorption is a very complex process, depending on environment. There are three major factors: protein, surface and solution, and each factor depends on its properties. As several factors are present, it is quite difficult to focus on only a single factor. However, it may say correctly that the key factor of protein adsorption is the conformation change of proteins or peptides on substrate [24]. This is influenced by kinetic and thermodynamic considerations.

Applications of protein adsorption are almost related to blood plasma proteins. Alb 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 [25-28]. 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, Alb 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 Alb adsorbs on a water-free contact layer due to the hydrophobic effect with irreversible bond [29].

There have been many studies on controlling adsorption/desorption proteins to surface with the surface wettability. This study was focused on bovine serum albumin (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. Nitrogen peaks are caused by the presence of the amino acid sequence of BSA molecules. In the XPS spectrum the N1s peak was deconvoluted into two peaks, assigned to the N-H and C-N groups [30]. Figure 5 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 [31] studied the fibrinogen adsorption on $SiO_xC_yH_z$, TiOx 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.

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.

Acknowledgements

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