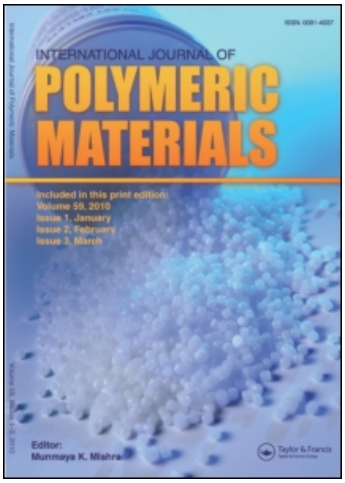

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Surface Modification of Nanoporous Poly(ε-caprolactone) Membrane with Poly(ethylene glycol) to Prevent Biofouling: Part I. Effects of Plasma Power and Treatment Time

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Surface Modification of Nanoporous Poly(ε-caprolactone) Membrane with Poly(ethylene glycol) to Prevent Biofouling: Part I. Effects of Plasma Power and Treatment Time

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Biofouling, a result of protein adsorption and cell adhesion on a surface, is detrimental to membrane performance. The objective of this study is to modify the polycaprolactone (PCL) membrane surface with poly(ethylene glycol) (PEG) to prevent fibroblast adhesion. To achieve this goal, oxygen plasma and PEG(400)-monoacrylate were used

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to graft the PEG onto the membrane surface through covalent bonding. Various plasma treatment conditions were investigated to optimize the PEG-grafting quality and to achieve minimum fibroblast adhesion. After the treatment, the water contact angle decreased significantly. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra indicated that PEG was successfully grafted onto the PCL membrane with the appearance of the PEG characteristic peaks. X-ray photoelectron spectroscopy (XPS) revealed that different plasma powers and treatment times changed the surface composition of membranes. To evaluate the applicability of this new strategy for the prevention of biofouling, NIH 3T3 fibroblast was used as a model biofoulant. Cell adhesion and morphology studies indicate that either lower plasma power or shorter treatment time is able to improve resistance to the cell adhesion. This simple and efficient method can be applied to inhibit biofouling on the membrane surface.

Keywords biofouling, oxygen plasma, poly(ethylene glycol) (PEG), polycaprolactone (PCL) membrane, surface modification

INTRODUCTION

In recent years, porous membranes have found a broad spectrum of applications, including water purification and biomedical devices [1,2]. Porous poly(ε-caprolactone) membranes have been widely applied to separation processes and controlled drug release due to their biocompatible and biodegradable properties [3,4]. However, biofouling can cause serious problems in several industrial and medical applications. Biofilm formation would reduce membrane performance, and increase operation and maintenance costs in water treatment processes [5–7]. In our previous study, the nanoporous PCL membranes can be utilized to achieve a zero-order drug release rate for implantable drug delivery devices [8]. However, implantation of biomedical devices can induce host reactions, including injury, blood-material interactions, provisional matrix formation, inflammation, granulation tissue, foreign-body reaction, and fibrous capsule development. During the development of granulation tissue, fibroblasts can proliferate and synthesize collagen around the implant site. Collagen can form a dense fibrous capsule surrounding the implanted devices in the late stage [9]. Extensive granulation tissue will lead to more fibrous capsulation and scar formation. Consequently, fibrous encapsulation around the implants can cause complications and poor healing [10].

It has been reported that the fibrosis around porous membrane-based biomedical devices can limit the long-term performance of the devices [11–14]. Moreover, fibrosis around a porous membrane can result in pore clogging, and therefore be detrimental to drug release [15]. Therefore, it is a crucial issue to prevent fibroblast adhesion to the surface of the nanoporous PCL membrane, for our membrane-base drug delivery device [7]. This study presents a novel, simple and efficient approach to modifying the surface of the nanoporous PCL membrane, in order to prevent fibroblasts from attaching

to the surface. Therefore, along with the surface modification, a long-term stable drug release can be expected.

Coating a device surface with biocompatible material may minimize the protein adsorption and cell adhesion, and therefore maintain the release performance of the implanted devices [16]. Preferred coating materials include poly(ethylene glycol) (PEG), albumin, collagen, etc. Among these materials, PEG has been the center of interest in the surface modification of implantable biomedical applications because it is a nontoxic, non-antigenic, non-immunogenic and FDA-approved material for internal consumption [17]. Moreover, PEG molecules are effective in reducing protein adsorption and cell adhesion because of the rapid mobility, the steric stabilization effect, and the minimal interfacial energy with water [18–21].

Surface modification with PEG molecules can be accomplished through physical adsorption [21–23] or covalent bonding [24–32]. The physical adsorption of PEG on a surface provides the simplest approach to obtaining the anti-biofouling property on the substrate. This interactive force between PEG and the surface could result from hydrogen bonding, hydrophilic/hydrophobic interaction, van der Waals force, etc. However, this kind of interaction is quite weak compared to a covalent bond. Therefore, the physically adsorbed PEG will not permanently remain on the substrate, and the physical adsorption is not an ideal method to create cell and protein-resistant surfaces for long-term biomedical applications [21]. On the other hand, covalent bonding of PEG onto the substrate is the most effective way to prepare a stable biocompatible surface. The strategies for covalent bonding of PEG molecules include silane coupling [24–26], wet chemical reaction [27,28], UV irradiation [29–31], and plasma discharge [32,33]. Silane coupling is usually used to graft PEG onto inorganic materials, such as glass, silicon, and silicon dioxide. Accordingly, silane coupling cannot be utilized to attach PEG on the polymeric PCL membrane surface. To successfully immobilize PEG on the surface, a wet chemical method usually requires high temperature and prolonged reaction time. This approach is applicable only if there are some reactive functional groups on the surface. Because PCL is an inert polymer and has a low melting point ($\sim$60°C), this type of approach is not suitable for our study. UV irradiation method usually involves complex and tedious procedures, which are needed to permanently attach PEG on the surface, so it is not a convenient and preferable method. Therefore, for the prevention of fibroblast adhesion, a simple and efficient approach to grafting PEG on the surface of the nanoporous PCL membrane is urgently sought.

In this study, a simple-step and time-efficient approach has been developed to immobilize PEG on the nanoporous PCL membrane surface by using PEG(400) monoacrylate and oxygen plasma. The schematic illustration of this method is shown in Figure 1. Initially, PEG(400) monoacrylate was physically adsorbed onto the surface. Subsequently, the application of oxygen plasma

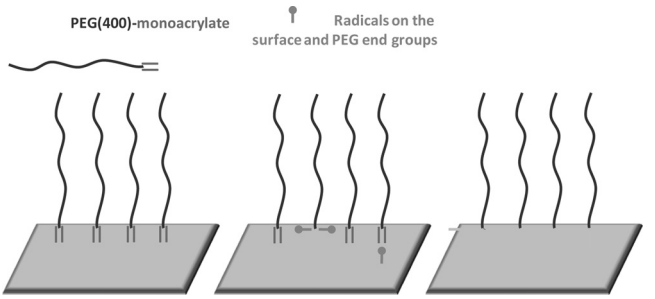

Figure 1: The scheme for the surface immobilization of PEG onto the nanoporous PCL membrane.

treatment might initiate polymerization of acrylate groups and produce radicals on the surface. PEG chains were then grafted onto the nanoporous PCL membrane surface due to the reaction between the acrylate groups and the radicals on the surface. In this protocol, plasma power and treatment time are critical. Therefore, operating parameters were explored to achieve a high quality of PEG grafting and obtain an anti-biofouling surface. The PEG-grafted nanoporous PCL membranes were characterized by using ATR-FTIR and the contact angle measurement. Furthermore, X-ray photoelectron spectroscopy was used to analyze the chemical composition of the surfaces. Short-term (6 h) fibroblast adhesion on unmodified and PEG-grafted surfaces was also evaluated in this work. The cell morphology on the PEG-modified surface was observed by using a fluorescence microscope. It should be noted that this proposed approach has several advantages. This surface modification process can be performed at room temperature so thermal-sensitive materials would not be subject to damage due to elevated temperature. With the application of plasma treatment, it would become simple to modify a variety of substrates, composed of inert polymers, by using this proposed method. Moreover, this proposed method is an efficient process because of the highly reactive double bonds provided by the acrylate groups.

MATERIALS AND METHODS

Chemicals and Reagents

Polycaprolactone ($M_n \sim 80{,}000$) was purchased from Aldrich Chemicals (Milwaukee, WI). 1,4-dioxane was obtained from Mallinckrodt Chemicals (Philipsburg, NJ). 2-methoxyethanol (ACS reagent, $\geqq 93\%$) was acquired from Sigma-Aldrich (St. Louis, MO). Polyethyleneglycol-400 monoacrylate (PEG(400)MA) was purchased from Monomer-Polymer and Dajac Labs (Feasterville-Trevose, PA). NIH 3T3 (mouse embryonic fibroblast cell line, CRL-1658) was purchased from American Type Culture Collection (Manassas,

VA). Dulbecco's modified Eagle medium (DMEM/F-12), Dulbecco's phosphate buffered saline (D-PBS), L-glutamine, sodium pyruvate, newborn calf serum (heat-inactivated), trypsin-EDTA (0.25% trypsin and 1 mM EDTA-4 Na), and calcein-AM were obtained from Invitrogen Inc. (Carlsbad, CA). Isopropanol was purchased from Fisher Scientific Inc. (Fair Lawn, NJ). All chemicals were used as received without further purification.

Preparation of the Nanoporous PCL Membrane

The nanoporous polycaprolactone membrane was prepared using a combination of thermally- and nonsolvent-induced phase separations [8]. To prepare a 20 wt% PCL casting solution, the PCL pellets were dissolved in a diluent which consisted of 15 wt% 1,4-dioxane as solvent and 65 wt% 2-methoxyethanol as nonsolvent. The solution was well-stirred and heated at 50°C for approximately 2 h. A general procedure for membrane preparation is described below. The PCL solution was cast on a Teflon plate. Subsequently, the cast film was immediately immersed in a 5°C coagulation bath, i.e., water. After 4–6 h, the membrane on the Teflon plate was removed from the bath, and isopropanol was poured onto the top of the solidified membrane. After 20–30 min, the membrane was separated from the Teflon plate. Then, the membrane was dried in air and further in vacuum.

PEG Grafting via Oxygen Plasma Technique

Although PCL is an inert polymer without any reactive functional groups, radicals can be easily generated on the membrane surface with the aid of oxygen plasma to produce free radicals. Because of the high reactivity of PEG(400) monoacrylate, a covalent bond can be formed from the reaction between the acrylate and PCL surface through the radicals. The following procedure was used to treat PCL membranes for PEG grafting. The nanoporous PCL membrane was firstly dipped into isopropanol and then deionized (DI) water to clean the surface. After the membrane was dried completely in air for around 2 h, it was soaked for 2 h in a 0.1 M PEG(400) monoacrylate solution using a solvent mixture, which consisted of 80% ethanol and 20% water. After the PEG(400)MA-adsorbed membrane was dried in air overnight, the membrane was exposed to oxygen plasma. The plasma-induced grafting process was conducted using the Techinics/Micro-RIE 800-II reactive ion etcher at a radio frequency of 30 kHz. During the plasma treatment, the oxygen flow rate was 20 standard cubic centimeters per minute (sccm) and the pressure inside the chamber was around 150 mTorr. To study the effect of the plasma treatment conditions on the biofouling resistance, the plasma power was adjusted from 25 to 100 W and the treatment time was varied from 5 to 20 sec. Following

the completion of the reaction, the treated membrane was immersed into a copious amount of isopropanol and then DI water to remove any organic residuals for further analysis.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

A Nexus 470® FTIR spectrometer with a zinc selenide crystal prism (Thermo Nicolet, Madison, WI) in the attenuated total reflection (ATR) mode was used for evaluation of chemical compounds on the modified and unmodified nanoporous PCL membranes. A small piece of the sample after surface treatment was mounted in the sample holder. Thirty-two scans were collected for each spectrum. Each scan was conducted from 400 to 4000 cm^{-1}. The background spectrum and the sample spectrum were obtained for analysis.

Contact Angle Measurement

To investigate the surface hydrophilicity, droplets of distilled water were placed on the surface of a membrane sample at room temperature. The water drop profile on the surface was captured with a high-performance charge-coupled device camera acquired from COHU (San Diego, CA). The contact angle was obtained by measuring the sessile drop profile analyzed with a MATLAB code [34].

X-Ray Photoelectron Spectroscopy

Surface compositions of the membranes were analyzed using the Kratos Axis Ultra X-ray photoelectron spectroscopy instrument with a monochromated Al K_α X-ray at a power of 130 W and a photon energy of 1486.7 eV. Each of the membrane samples was attached on a carbon tape, mounted on a sample holder and evacuated overnight in a chamber holder. Then, the sample was moved into the ultra-high vacuum ($\sim 10^{-9}$ Torr) analysis chamber. During the measurement, the charge neutralization was used with 2.1 A filament current, 2.3 V filament charge, and 1.3 V filament bias. A binding energy of 285.0 eV for C–H was used to compensate for the charging effects. High-resolution C1s core level (the 1 s orbital of carbon) spectra were taken with a power of 80 W passing through the detector and further resolved into individual peaks, i.e., C–H, C–O, C=O, and O–C=O, by using the software supplied by the manufacturer [35].

Fibroblast Adhesion Study

NIH 3T3 fibroblast cells were cultured in DMEM/F12, which was supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% v/v newborn

calf serum. Prior to the cell adhesion study, the membranes were fixed at the bottom of 48-well cell culture plates and sterilized with exposure to an ultraviolet light at a power of 30 W (Sylvania, Danvers, MA) overnight. The UV sterilization was verified to have no influence on surface properties and fibroblast adhesion. The cells were seeded onto the sterilized membrane surfaces at a density of approximately 1×10^5 cells/cm^2 and incubated at 37°C with 5% CO_2. After 6 h incubation, the cell-seeded membranes were removed from the cell culture plates and gently rinsed in fresh D-PBS to remove non-adherent fibroblasts. Subsequently, the membranes with attached cells were trypsinized with trypsin-EDTA for 10 min at 37°C, and the number of fibroblasts was counted using a hemacytometer. Cell counts were performed at least three times. Untreated PCL membranes were used for control.

Fibroblast Morphology Study

NIH 3T3 fibroblast morphologies on the PEG-modified PCL membranes were observed using fluorescent calcein-AM stain and a fluorescent microscope. The PCL membranes were treated with two distinct plasma conditions, i.e., at a power of 25 W for 5 sec and a power of 100 W for 20 sec. Initially, the cells at a density of 100,000 cells/cm^2 were seeded on the membranes which were already sterilized and fixed at the bottom of 48-well cell culture plates. After 6 h incubation at 37°C with 5% CO_2, the membranes were rinsed with D-PBS to remove the unattached cells. Then, a 200 μL of 10 μM calcein-AM in D-PBS was prepared to label the adherent fibroblasts on each of the modified membranes in the 48-well cell culture plate at 37°C. After 20 min, the membranes were rinsed with D-PBS and placed on the glass slide with a cover slip on the top. The cell morphology was visualized with a Nikon Eclipse TS100 fluorescent microscope.

RESULTS AND DISCUSSION

Attenuated Total Reflection-Fourier Transform Infrared Analysis

The plasma-based surface modification is generally used in order to improve surface properties like surface free energy for better adhesion properties and printability of frequently chemically quite inert and hardly wettable polymers. ATR-FTIR analysis was applied to the treated and untreated nanoporous PCL membranes to identify the chemical functional groups on the surface. Figure 2 shows the existence of the peak at 810 cm^{-1}, which can be attributed to the C=C stretching on the PEG(400)MA-adsorbed membrane surface without the plasma treatment. For the membrane which

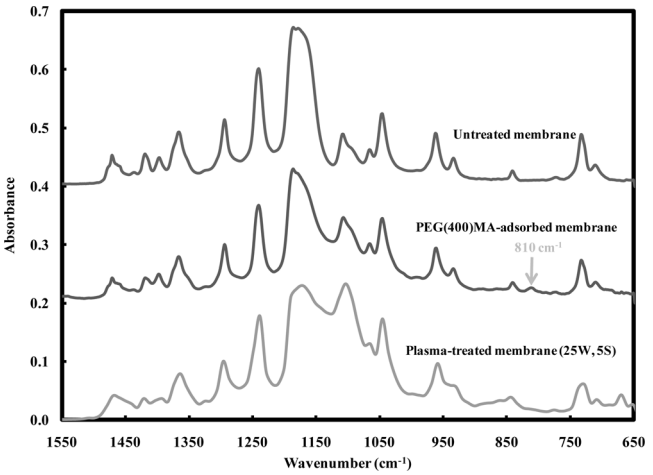

Figure 2: ATR-FTIR spectra of the untreated, PEG(400)MA-adsorbed and plasma-treated nanoporous PCL membranes.

was treated with the plasma at a power of 25 W for 5 sec, the spectrum displays the disappearance of the acrylate's double bond peak at 810 cm^{-1}, implying the complete conversion of the acrylate double bonds [36]. Therefore, a covalent bond was formed due to two possible reactions. One is the free-radical initiated polymerization of acrylate groups. The other is the reaction between the acrylate groups and the radicals on the membrane surface.

Figures 3 (a)–(c) compare the functional groups between the untreated and the PEG-modified membranes under different plasma treatment conditions. For the PEG-modified membranes, the spectra show the presence of the strong PEG characteristic peak at 1106 cm^{-1}, corresponding to C–O–C stretching vibration of the PEG. In the spectra of the PEG-modified membrane, the intensities of the peaks at 2866 cm^{-1} and 3440 cm^{-1}, ascribed to CH_2-stretching and OH-stretching of the PEGs, are relatively high compared to the unmodified membrane [37]. Therefore, ATR-FTIR analysis provides solid evidences that PEG was successfully grafted onto the nanoporous PCL membranes. Moreover, there exists no significant difference among the spectra of the PEG-modified membranes, which were treated with a variety of plasma conditions (25–100 W and 5–20 sec).

Contact Angle Measurement

Figure 4 shows the static water contact angles of the modified and unmodified PCL nanoporous membranes. For the pristine membrane, the contact angle was around 107°, suggesting that the surface was quite hydrophobic. Upon the plasma treatment, the water contact angle dropped to the average of 43°. This significant decrease in the contact angle indicates the presence

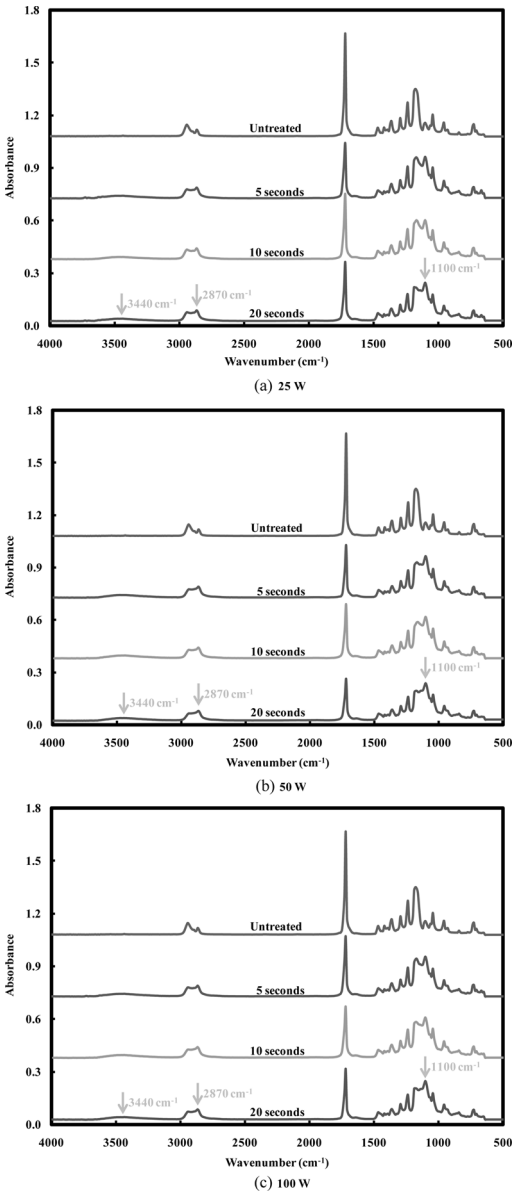

Figure 3: ATR-FTIR spectra of the pristine and PEG-modified nanoporous PCL membranes which were treated with oxygen plasma at power of (a) 25 W, (b) 50 W and (c) 100 W for 5, 10 and 20 sec.

of hydrophilic PEG on the surface. However, the contact angle still remained around 43°, when the plasma power and the treatment time were varied. Thus, further increasing the plasma treatment intensity had little influence on the wettability of the modified surfaces.

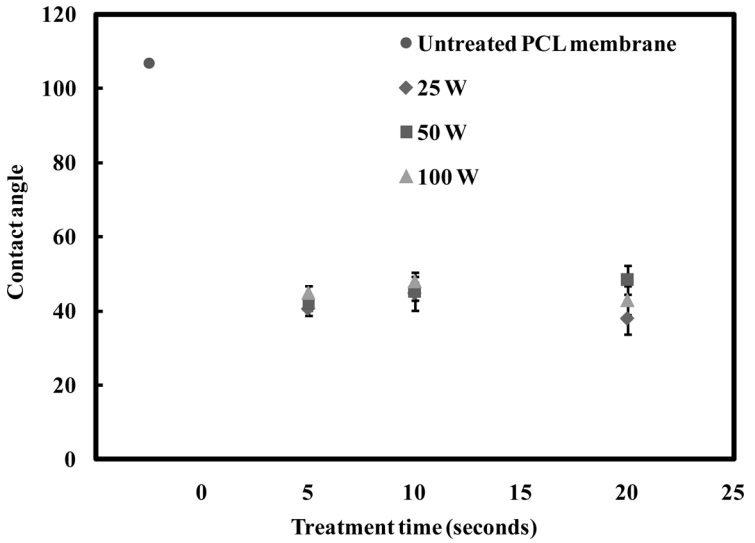

Figure 4: Static water contact angles of unmodified and PEG-modified nanoporous PCL membranes which were treated with oxygen plasma at the power of 25–100 W for 5–20 sec. The number of samples tested was 5 for the error bars shown.

Surface Elemental Composition Analysis

Surface roughness and chemistry are two major factors influencing contact angle measurement and cellular response to a specific surface. In our study, surface roughness, measured by atomic force microscopy, is not related to contact angle and various plasma treatment conditions (data not shown). Therefore, surface chemistry could be a major factor in determining the extent of fibroblast adhesion to a surface. XPS was used to investigate change in surface chemistry according different plasma powers and treatment times. Figure 5 illustrates the chemical compositions of the plasma-treated membranes as a function of plasma power (25, 50 and 100 W) and treatment time (5, 10 and 20 sec). High-resolution C1 s core-level spectra for all treated membranes can be resolved into three to four component peaks. The peaks were located at the binding energies of 285.0 eV for C–H, 286.4 eV for C–O, 288.0 eV for C=O, and 289.1 eV for O-C=O. For the unmodified PCL membrane, Figure 5(j) displays that the signal of C–H is much higher than the signals of C–O and O-C=O. This result is in agreement with PCL molecular structure. For the membranes which were treated with the oxygen plasma at a power of 25 W for 5, 10 and 20 seconds, there were only three component peaks involved in the spectra, which were C–H, C–O, and O-C=O. The signals of C–H and O-C=O could come from the backbone of PEG(400)MA and/or the nanoporous PCL membrane. The strong intensity of the C–O peak can provide the evidence that PEG was successfully grafted onto the nanoporous PCL membrane surface.

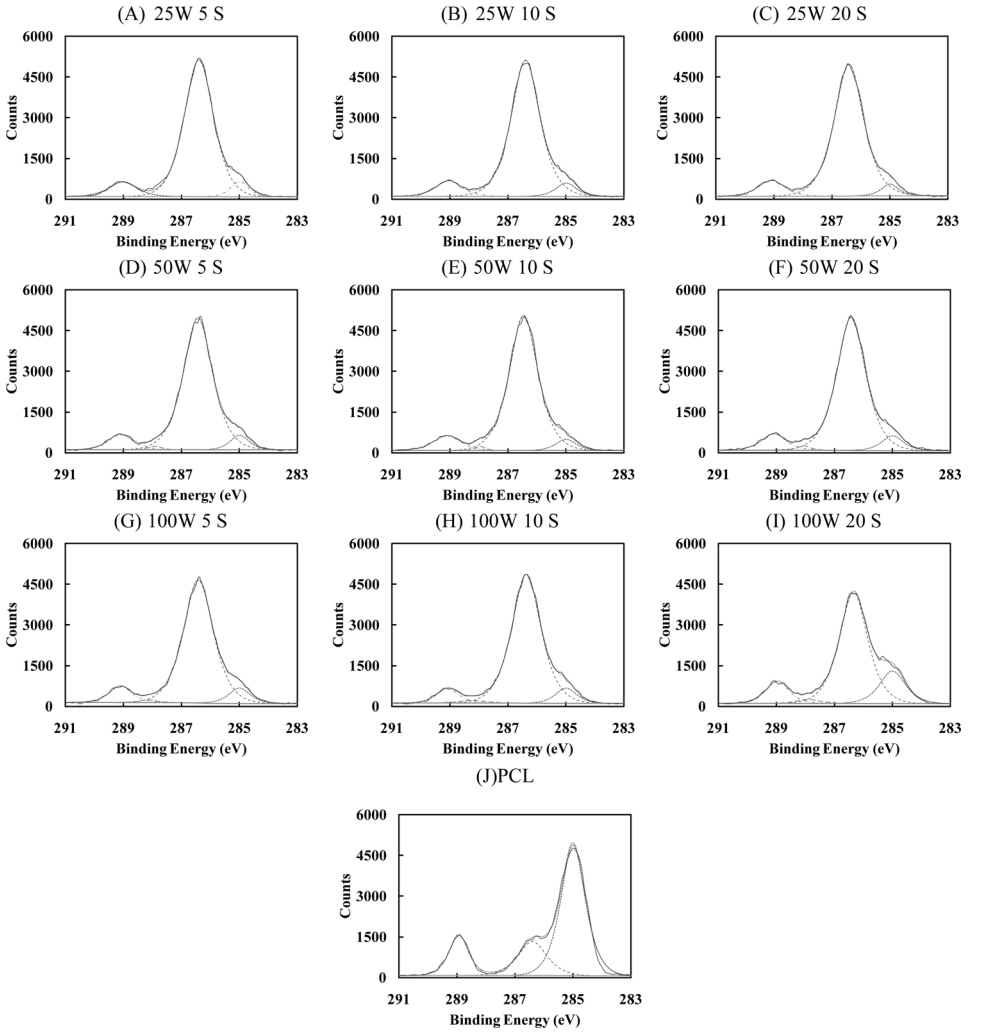

Figure 5: High-resolution C1s XPS spectra for the membranes which were treated with a variety of plasma conditions. (a) 25 W for 5 sec, (b) 25 W for 10 sec, (c) 25 W for 20 sec, (d) 50 W for 5 sec, (e) 50 W for 10 sec, (f) 50 W for 20 sec, (g) 100 W for 5 sec, (h) 100 W for 10 sec, (i) 100 W for 20 sec, and (j) PCL membrane without any treatment.

When the oxygen plasma power was increased from 25 to 50 and 100 W, a new species, C=O, started to appear on the surface due to the application of overdosed oxygen plasma. Additional radicals generated by the overdosed oxygen plasma could lead to the formation of C=O after exposure to air [38,39]. When the plasma power was fixed at 100 W and the treatment time was increased to 20 sec, significant increase in the intensity of the C–H peak and decrease in the intensity of the C–O peak were observed in the spectrum. These results can be attributed to the overexposure of the oxygen plasma.

The prolonged plasma treatment can result in a substantial etching process [40]. Due to the strong plasma power, some ether and/or hydroxyl (C–O) groups might be etched away from the PEG chains. As a result, a relatively low intensity of the C–O peak and a high intensity of the C–H peak can be seen in Figure 5 (i).

Based on the XPS results, Figure 6 shows the calculated ratio of carbonyl groups (C=O and O–C=O) to the ether and/or hydroxyl groups (C–O) as a function of plasma power and treatment time. For the unmodified PCL membrane, the ratio would be equal to 0.81. However, the ratios would be in the range of 0.1 to 0.2 for the PEG-modified membranes; these results indicate that the ether and/or hydroxyl groups (C–O) can be a major component on the membrane surface. Therefore, it may be concluded that PEG was successfully grafted on the membrane surface.

Carbonyl groups, for example C=O and O–C=O, might be a result of the over-dosage and overexposure to the oxygen plasma. The ether and/or hydroxyl group, C–O, can directly come from the PEG chains immobilized on the nanoporous PCL membrane surface. An increase in the ratio can suggest that a larger number of oxygen-containing functionalities is generated by the oxygen plasma and less intact PEG chains are grafted onto the surface. Therefore, this ratio can be indicative of the quality of PEG grafting under the oxygen plasma treatment.

As shown in Figure 5, the ratios became higher when both the plasma power and treatment time were increased. This indicates that higher plasma

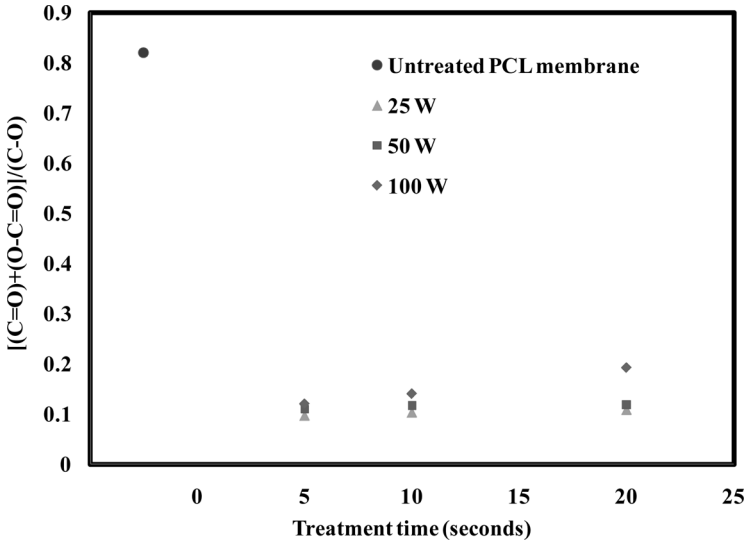

Figure 6: Effects of plasma power and treatment time on the ratio of C=O plus O–C=O to C–O.

power and longer treatment time might result in the breakage of the PEG chains and consequently a smaller number of ethylene glycol groups that could be attached onto the membrane surface. On the other hand, higher plasma power and longer treatment time might produce a lot of radicals on the membrane surface. When the membrane surface was exposed to air, the reaction between the radicals and air will lead to the formation of carbonyl groups, i.e., C=O and O–C=O. It is also likely that the breakage of PEG chains can react with air to form carbonyl groups under severe oxygen plasma treatment.

In this study, however, the lowest ratio was obtained with application of the lowest plasma power and the shortest treatment time. This implies that intact PEG can be grafted onto the membrane surface and few carbonyl groups would appear on the surface with the mildest plasma treatment condition. With the high quality of PEG grafting, the PEG-grafted nanoporous PCL membrane surface is supposed to better prevent fibroblast adhesion.

Fibroblast Adhesion Study

The effects of the oxygen plasma treatment conditions on biofouling prevention were examined by studying fibroblast adhesion to the PEG-modified PCL membrane surfaces. Figure 7 shows the ratio of fibroblast adhesion between a PEG-modified PCL membrane surface and the untreated PCL membrane surface for various oxygen plasma treatment conditions. As shown in this figure, either lower plasma power or shorter treatment time led to a

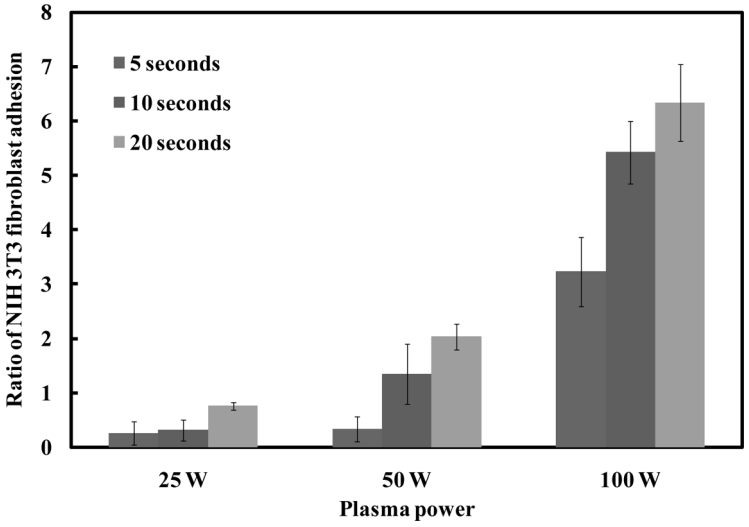

Figure 7: Ratio of NIH 3T3 fibroblast adhesion between a treated PCL membrane surface and the untreated PCL membrane surface for various oxygen plasma treatment conditions. The number of samples tested was 5 for the error bars shown.

reduction in fibroblast adhesion. For the PEG-modified membrane which was treated with oxygen plasma at the power of 25 W for 5 sec, there was a significant decrease in cell adhesion by around 72%, as compared to the untreated membrane. However, a more than 5-fold increase (greater than 6 times of the cell number) in fibroblast adhesion was observed when the PEG-modified membrane was exposed to the oxygen plasma at a power of 100 W for 20 sec. Therefore, the results indicate that the milder plasma treatment conditions, i.e., lower power and shorter treatment time, can result in less fibroblast adhesion.

The observed fibroblast adhesion is in agreement with the XPS results as shown in Figures 5 and 6. During the plasma process, higher power and longer treatment time might not only break PEG chains but also generate additional oxygen-containing functionalities. Figures 5(i) and 6 indicate that severe oxygen plasma treatment could etch away ethylene glycol groups and therefore make PEG chains shorter. It is reported that short PEG chains cannot prevent cell adhesion well enough in comparison with long PEG chains [41–43]. Because shorter PEG chains may have lower mobility, they cannot move so fast to decrease the contact time between proteins/cells and the surfaces. Thus, it is more likely that fibroblasts can adhere onto the membrane surfaces as in the case that the surfaces were treated with severe plasma conditions [21]. Also, low-molecular-weight PEGs can provide fewer polymer segments. When proteins and/or cells approach the surfaces, there is a minimal change in a conformation number and a configurational entropy. Therefore, the developed repulsive force would not be strong enough to prevent fibroblast adhesion.

Furthermore, based on the XPS results, a large number of carbonyl groups, such as aldehyde, ketone and carboxylic acid, were produced due to the severe plasma treatment. The carbonyl groups can promote significant protein adsorption with a specific confirmation, in turn, leading to a substantial increase in cell adhesion [44–46]. Thus, a drastic increase in fibroblast adhesion to the PEG-grafted surfaces was observed due to the severe oxygen plasma treatment. According to the result displayed in Figure 7, the 5-second oxygen plasma treatment at a power of 25 W could be sufficient enough to graft PEG onto the surfaces without too much formation of carbonyl compounds to prevent fibroblast adhesion.

Fibroblast Morphology Investigation

To further investigate the effects of plasma conditions on the interaction between fibroblasts and the treated membrane surfaces, the cell morphology on the membrane surfaces was observed by using fluorescent microscopy and calcein-AM stain. The PEG-modified nanoporous PCL membranes were treated with two distinct plasma conditions, namely 25 W for 5 sec and 100 W for 20 sec, and NIH 3T3 fibroblast morphologies on both cases were studied.

As shown in Figure 8(a), few cells adhered to the PEG-modified membrane surface, which was treated with oxygen plasma at a power of 25 W for 5 sec. Most of the adhered cells were still round without stretch as normal 3T3 cell growth pattern. This observation indicates that low affinity existed between fibroblasts and the membrane surface. However, Figure 8(b) displays that a large number of fibroblasts adhered to the PEG-modified nanoporous PCL membrane which was treated with oxygen plasma at a power of 100 W for 20 sec. Also, the extensive cell spreading suggests high affinity between the fibroblasts and the membrane surface. The observation of fibroblast morphology is in agreement with the results shown in Figures 5, 6 and 7.

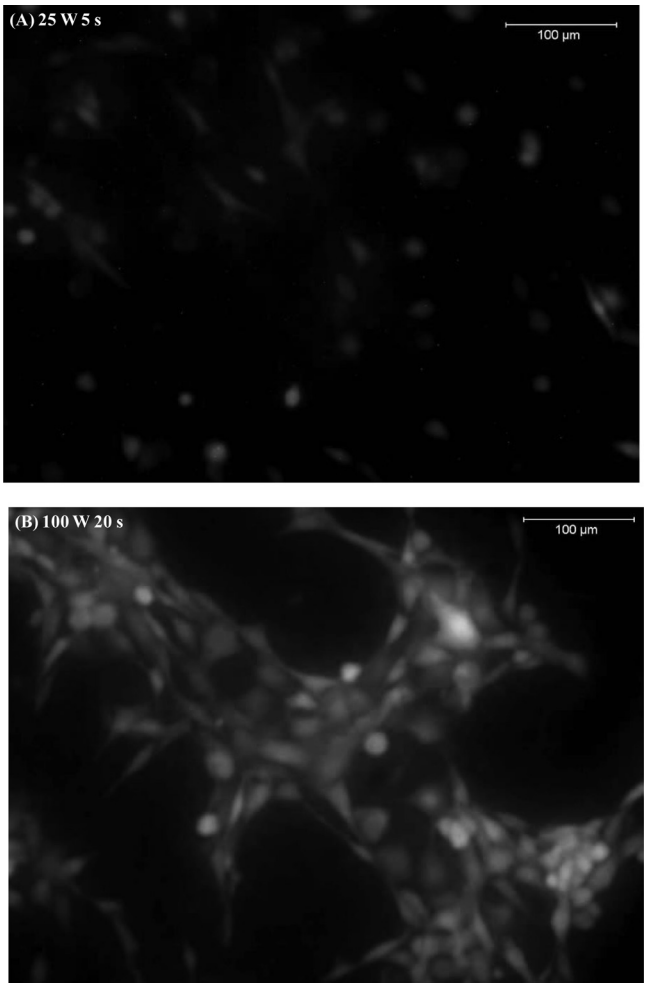

Figure 8: Fluorescence microscopy images of NIH 3T3 fibroblast adhered onto PEG-grafted nanoporous PCL membranes treated with two extremely distinct plasma conditions: (a) 25 W for 5 sec and (b) 100 W for 20 sec.

Cell attachment and morphology on a specific surface is considerably affected by adsorption of cell-adhesive proteins, such as fibronectin and vitronectin. The functional activity of adsorbed proteins can mediate cell function on a specific surface. García reported that contact angle is a poor indicator of the functional activity of adsorbed proteins. In his studies, it has been showed that surface chemistry plays a considerably important role in the adsorption and conformation of the proteins and, in turn, modulates cell adhesion and morphology [47–50]. Moreover, Groth et al. showed that the carboxyl group can improve the fibroblast adhesion and lead to the spread-out morphology on the surface [18]. Figure 6 indicates that a considerable formation of carbonyl groups, i.e., C=O and O−C=O, can be induced by using the severe oxygen plasma treatment. These two functional groups may promote the adsorption of cell-binding proteins with the proper conformation. Fibroblasts can consequently adhere to the membrane surfaces with the spread-out morphology. The change in surface chemistry should be the reason why the various extent of fibroblast adhesion was observed (Figure 7) with the similar measured contact angles (Figure 5) in this present study. As a result, the PEG-grafted membrane surface which was treated with the appropriate plasma condition, i.e., lower power and shorter treatment time, with less amounts of these two groups, had a positive effect on suppressing cell adhesion and spreading.

CONCLUSIONS

In this study, a new and simple approach to grafting PEG from PEG monoacrylate onto the nanoporous PCL membrane to prevent fibroblast adhesion has been developed. To accomplish the optimized PEG-grafting on the membrane surface, a variety of oxygen plasma treatment conditions were investigated. As a result, the water contact angle dropped from 107° for the unmodified membrane to an average of 43° for all PEG-modified membranes, revealing the appearance of the hydrophilic PEG on the surface. ATR-FTIR spectra provided evidence that the PEG was successfully grafted onto the membrane surface from the existence of PEG characteristic peaks at 1106, 2866, and 3440 cm^{-1}. Disappearance of the peak at 810 cm^{-1} suggested that complete conversion of acrylate group was achieved. XPS results showed that the surface composition was changed according to the different plasma treatment conditions. More gentle plasma treatment led to the lower ratio of C=O and O-C=O to C−O, suggesting that a higher quality of the PEG-grafting was obtained. The severe plasma treatment not only generated carbonyl groups on the surface but also broke the PEG chains. It is concluded that the plasma treatment condition can affect the surface chemistry and play an important role in the fibroblast adhesion to the surface. Therefore, with the optimization of plasma powers and treatment times, the PEG chains can be successfully

immobilized onto the nanoporous PCL membrane surface to inhibit biofouling problems.

It is found that either lower plasma power or shorter treatment time can cause a decrease in fibroblast adhesion. When the membrane was treated with the oxygen plasma at a power of 25 W for 5 sec, fibroblast adhesion was reduced by 72%, as compared to the untreated membrane. From the cell morphology study, few round fibroblasts were observed on the membrane which was treated with the mildest conditions (25 W for 5 sec), indicating a low affinity between the cells and the substrate. The approach of this investigation holds great potential to improve the performance of various membrane applications, such as water purification and biomedical devices, by reducing biofouling problems.

REFERENCES

[1] Ho, W. S. W., and Sirkar, K. K. (Eds.). (1992). *Membrane Handbook*, Chapman & Hall, New York, Kluwer Academic Publishers, Boston, reprint edition (2001).

[2] Desai, T. A., Hansford, D., and Ferrari, M. Characterization of micromachnined silicon membranes for immunoisolation, and bioseparation applications, *J. Membr. Sci.* **159**, 221–231 (1999).

[3] Tanaka, T., Tsuchiya, T., Takahashi, H., Taniguchi, M., and Lloyd, D. R. Microfiltration membrane of polymer blend of poly(L-lactic acid), and poly(ε-caprolactone), *Desalination* **1993**, 367–374 (2006).

[4] Lin, W. J., and Lee, H. G. Design of a microporous controlled delivery system for theophylline tablets, *J. Control. Release* **89**, 179–187 (2003).

[5] Baker, J. S., and Dudley, L. Y. Biofouling in membrane systems – A review, *Desalination* **118**, 81–90 (1998).

[6] Vrouwenvelder, J. S., and van der Kooij, D. Diagnosis, prediction and prevention of biofouling of NF and RO membranes, **139**, 65–71 (2001).

[7] Grelot, A., Grelier, P., Vincelet, C., Brüss, U., and Grasmick, A. Fouling characterization of a PVDF membrane, *Desalination* **250**, 707–711 (2010).

[8] Yen, C., He, H., Lee, L. J., and Ho, W. S. W. Synthesis and characterization of polycaprolactone nanoporous membranes via thermally- and nonsolvent-induced phase separations for biomedical device application, *J. Membr. Sci.* **343**, 180–188 (2009).

[9] Anderson, J. M. Biological responses to materials, *Ann. Rev. Mater. Res.* **31**, 8–110 (2001).

[10] Ratner, B. D., and Bryant, S. J. Biomaterials: Where we have been, and where we are going, *Ann. Rev. Biomed. Eng.* **6**, 41–75 (2004).

[11] Wisniewski, N., and Reichert, M. Methods for reducing biosensor membrane biofouling, *Colloids Surf. B* **18**, 197–219 (2000).

[12] Popat, K. C., Mor, G., Grimes, C., and Desai, T. A. Surface modification of nanoporous alumina surface with poly(ethylene glycol), *Langmuir* **20**, 8035–8041 (2004).

[13] Wang, Y., Ameer, G. A., Sheppard, B. J., and Langer, R. A tough biodegradable elastomer, *Nat. Biotechnol.* **20**, 602–606 (2002).

[14] Grayson, A. C. R., Shawgo, R. S., Johnson, A. M., Flynn, N. T., Li, Y., Cima, M. J., and Langer, R. A BioMEMS review: MEMS technology for physiologically integrated devices, *IEEE* **92**, 6–21 (2004).

[15] Walczak, R. J., Boiarski, A., Cohen, M., Teri, W., Melnik, K., Shapiro, J., Sharma, S., and Ferrari, M. Long-term biocompatibility of NanoGATE drug delivery implant, *Nanobiotechnol.* **1**, 35–42 (2005).

[16] Vachon, D. J., and Hellan, J. R. Coated implantable stimulation electrode and lead, US Patent No. (1994) 5,324,324.

[17] Harris, J. M. (1992). *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*, Plenum Press, New York.

[18] Faucheux, N., Schweiss, R., Lutzow, K., Werner, C., and Groth, T. Self-assembled monolayers with different terminating groups as model substrates for cell adhesion studies, *Biomaterials* **25**, 2721–2730 (2004).

[19] Nie, F. Q., Xu, Z. K., Huang, X. J., Ye, P., and Wu, J. Acrylonitirle-based copolymer membranes containing reactive group: Surface modification by the immobilization of poly(ethylene glycol) for improving antifouling property, and biocompatibility, *Langmuir* **19**, 9889–9895 (2003).

[20] Goddard, J. M., and Hotchkiss, J. H. Polymer surface modification for the attachment of bioactive compounds, *Prog. Polym. Sci.* **32**, 698–725 (2007).

[21] Lee, J. H., Lee, H. B., and Andrade, J. D. Blood compatibility of polyethylene oxide surfaces, *Prog. Polym. Sci.* **20**, 1043–1079 (1995).

[22] Bridgett, M. J., Davies, M. C., and Denyer, S. P. Control of staphylococcal adhesion to polystyrene surfaces by polymer surface modification with surfactants, *Biomaterials* **13**, 411–416 (1992).

[23] Amiji, M., and Park, K. Prevention of protein adsorption, and platelet-adhesion on surfaces by PEO/PPO/PEO triblock copolymers, *Biomaterials* **13**, 682–692 (1992).

[24] Popat, K. C., Mor, G., Grimes, C., and Desai, T. A. Poly(ethylene glycol) grafted nanoporous alumina membranes, *J. Membr. Sci.* **243**, 97–106 (2004).

[25] Flamme, K. E. L., Popat, K. C., Leoni, L., Markiewicz, E., Tempa, T. J. L., Roman, B. B., Grimes, C. A., and Desai, T. A. Biocompatibility of nanoporous alumina membranes for immunoisolation, *Biomaterials* **28**, 2638–2645 (2007).

[26] Tao, S. L., Popat, K. C., Norman, J. L., and Desai, T. A. Surface modification of SU-8 for enhanced biofunctionality, and nonfouling properties, *Langmuir* **24**, 2631–2636 (2008).

[27] Kiss, É., Samu, J., TÓth, A., and BertÓti, I. Novel ways of covalent attachment of poly(ethylene oxide) onto polyethylene: surface modification, and characterization by XPS, and contact angle measurements, *Langmuir* **12**, 1651–1657 (1996).

[28] Zhang, F., Kang, E. T., Neoh, K. G., Wang, P., and Tan, K. L. Reactive coupling of poly(ethylene glycol) on electroactive polyaniline films for reduction in protein adsorption, and platelet adhesion, *Biomaterials* **23**, 787–795 (2002).

[29] Wang, B., He, T., Liu, L., and Gao, C. Poly(ethylene glycol) micro-patterns as environmentally sensitive template for selective or non-selective adsorption, *Colloid Surf. B* **46**, 169–174 (2005).

[30] Cheo, S. H. Y., Wang, P., Tan, K. L., Ho, C. C., and Kang, E. T. Surface modification of natural rubber latex films via grafting of poly(ethylene glycol) for reduction in protein adsorption, and platelet adhesion, *J. Mater. Sci.-Mater. M.* **12**, 377–384 (2001).

[31] Altankov, G., Thom, V., Groth, T., Jankova, K., Jonsson, G., and Ulbricht, M. Modulating the biocompatibility of polymer surfaces with poly(ethylene glycol): Effect of fibronectin, *J. Biomed. Mater. Res.* **52**, 219–230 (2000).

[32] Song, Y. Q., Sheng, J., Wei, M., and Yuan, X. B. Surface modification of polysulfone membranes by low-temperature plasma-graft poly(ethylene glycol) onto polysulfone membranes, *J. Appl. Polym. Sci.* **78**, 979–985 (2000).

[33] Wang, P., Tan, K. L., Kang, E. T., and Neoh, K. G. Plasma-induced immobilization of poly(ethylene glycol) onto poly(vinylidene fluoride) microporous membrane, *J. Membr. Sci.* **195**, 103–114 (2002).

[34] Lu, C. M., Xie, Y. B., Yang, Y., Cheng, M. M. C., Koh, C. G., Bai, Y. L., and Lee, L. J. New valve, and bonding designs for microfluidic biochips containing proteins, *Anal. Chem.* **79**, 994–1001 (2007).

[35] Bai, Y. L., Koh, C. G., Boreman, M., Juang, Y. J., Tang, I. C., Lee, L. J., and Yang, S. T. Surface modification for enhancing antibody binding on polymer-based microfludic device for enzyme-linked immunosorbent assay, *Langmuir* **22**, 9458–9467 (2006).

[36] Ulbricht, M., Matuschewski, H., Oechel, A., and Hicke, H. G. Photo-induced graft polymerization surface modifications for the preparation of hydrophilic, and low protein-adsorbing ultrafiltration membranes, *J. Membr. Sci.* **115**, 31–47 (1996).

[37] Harder, P., Grunze, M., Dahint, R., Whitesides, G. M., and Laibinis, P. E. Molecular conformation in oligi(ethylene glycol)-terminated self-assembled monolayers on gold, and silver surfaces determines their ability to resist protein adsorption, *J. Phys. Chem. B* **102**, 426–436 (1998).

[38] Ha, S. W., Hauert, R., Ernst, K. H., and Wintermantel, E. Surface analysis of chemically-etched, and plasma-treated polyetheretherketone (PEEK) for biomedical applications, *Surf. Coat. Tech.* **96**, 293–299 (1997).

[39] Grace, J. M., and Gerenser, L. J. Plasma treatment of polymers, *J. Disper. Sci. Technol.* **24**, 305–341 (2003).

[40] Morra, M., Occhiello, E., and Garbassi, F. Contact angle hysteresis in oxygen plasma treated poly(tetrafluoroethylene), *Langmuir* **5**, 872–876 (1989).

[41] Zhu, B., Eurell, T., Gunawan, R., and Leckband, D. Chain-length dependence of the protein, and cell resistance of oligo(ethylene glycol)-terminated self-assembled monolayers on gold, *J. Biomed. Mater. Res.* **56**, 406–416 (2001).

[42] Iguerb, O., Poleunis, C., Mazéas, F., Compère, C., and Bertrand, P. Antifouling properties of poly(methyl methacrylate) films grafted with poly(ethylene glycol) monoacrylate immersed in seawater, *Langmuir* **24**, 12272–12281 (2008).

[43] Fan, X. W., Lin, L. J., and Messersmith, P. B. Cell fouling resistance of polymer brushes grafted from Ti substrate by surface-initiated polymerization: effect of ethylene glycol side chain length, *Biomacromolecules* **7**, 2443–2448 (2006).

[44] Siow, K. S., Britcher, L., Kumar, S., and Griesser, H. J. Plasma methods for the generation of chemically reactive surfaces for biomolecule immobilization, and cell colonization – a review, *Plasma Process Polym.* **3**, 392–418 (2006).

[45] Davidson, M. R., Mitchell, S. A., and Bradley, R. H. UV-ozone modification of plasma-polymerized acetonitrile films for enhanced cell attachment, *Colloid Surf. B* **34**, 213–219 (2004).

[46] García, A. J., Vega, M. D., and Boettiger, D. Modulation of cell proliferation, and differentiation through substrate-dependent changes in fibronectin conformation, *Mol. Biol. Cell.* **10**, 785–798 (1999).

[47] Michael, K. E., Vernekar, V. N., Keselowsky, B. G., Meredith, J. C., Latour, R. A., and García, A. J. Adsorption-induced conformational changes in fibronectin due to interactions with well-defined surface chemistries, *Langmuir* **19**, 8033–8040 (2003).

[48] Capadona, J. R., Collard, D. M., and García, A. J. Fibronectin adsorption, and cell adhesion to mixed monolayers of tri(ethylene glycol)- and methyl-terminated alkanethiols, *Langmuir* **19**, 1847–1852 (2003).

[49] Keselowsky, B. G., Collard, D. M., and García, A. J. Surface chemistry modulates focal adhesion composition, and signaling through changes in integrin binding, *Biomaterials* **25**, 5947–5954 (2004).

[50] Stephansson, S. N., Byers, B. A., and García, A. J. Enhanced expression of the osteoblastic phenotype on substrates that modulate fibronectin conformation, and integrin receptor binding, *Biomaterials* **23**, 2527–2534 (2002).