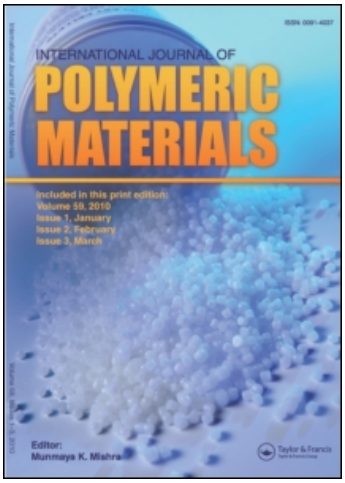

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Surface Modification of Nanoporous Poly(ε-caprolactone) Membrane with Poly(ethylene glycol) to Prevent Biofouling: Part II. Effects of Graft Density and Chain Length

Chi Yen[ab]; Hongyan He[b]; Zhengzheng Fei[ab]; Xulang Zhang[b]; L. James Lee[ab]; W. S. Winston Ho[abc]
[a] William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, Ohio, United States [b] NSF Nanoscale Science and Engineering Center for Affordable Nanoengineering of Polymeric Biomedical Devices, The Ohio State University, Columbus, Ohio, United States [c] Department of Materials Science and Engineering, The Ohio State University, Columbus, Ohio, United States

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Surface Modification of Nanoporous Poly(ε-caprolactone) Membrane with Poly(ethylene glycol) to Prevent Biofouling: Part II. Effects of Graft Density and Chain Length

Chi Yen,[1,2] Hongyan He,[2] Zhengzheng Fei,[1,2] Xulang Zhang,[2] L. James Lee,[1,2] and W. S. Winston Ho[1,2,3]

[1]William G. Lowrie Department of Chemical and Biomolecular Engineering, The Ohio State University, Columbus, Ohio, United States
[2]NSF Nanoscale Science and Engineering Center for Affordable Nanoengineering of Polymeric Biomedical Devices, The Ohio State University, Columbus, Ohio, United States
[3]Department of Materials Science and Engineering, The Ohio State University, Columbus, Ohio, United States

Biofouling is a common problem in wastewater treatments and medical devices. It is important to find a strategy to prevent biofouling and surface modification. This study presents a novel approach to modifying the surface of nanoporous poly(ε-caprolactone) membrane with poly(ethylene glycol) (PEG) to prevent biofouling problems. Oxygen

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Address correspondence to W. S. Winston Ho, Department of Materials Science and Engineering, The Ohio State University, 2041 College Road, Columbus, OH 43210-1178, United States. E-mail: ho@chbmeng.ohio-state.edu

plasma and poly(ethylene glycol)-monoacrylates (PEGMAs) were utilized in the surface modification process. Mouse embryonic fibroblast was used as a model biofoulant. The effects of the density and length of PEG chains on surface properties and cell adhesion were investigated. Contact angle measurements and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra illustrated that PEG can be successfully immobilized on the membrane surface. Membranes which were pre-treated with higher PEG concentrations can lead to higher grafting density and greater resistance against cell adhesion. The resistance against cell adhesion cannot be enhanced while the PEG concentration is higher than a certain point, i.e., 0.1 M. For different chain lengths, PEG(400)MA can provide higher resistance to cell adhesion than PEG(200)MA and PEG(1000)MA.

Keywords chain length, graft density, oxygen plasma, poly(ethylene glycol), polycaprolactone (PCL) membrane

INTRODUCTION

Nowadays, membranes have been extensively used in the fields of water treatment and medical devices [1,2]. However, biofouling caused by organism adhesion is a serious problem in several industrial and medical applications. Biofilm formation would worsen membrane performance and increase operation and maintenance cost in water treatment processes [3,4]. Therefore, anti-biofouling properties are highly required to improve the long-term stability of implantable medical devices, such as biosensors and drug delivery devices [5,6].

Porous poly(ε-caprolactone) membranes recently have been widely applied to separation processes and controlled drug release due to its biocompatible and biodegradable properties [7,8]. Our previous study has demonstrated that nanoporous PCL membranes can be successfully prepared for controlled drug release [9]. For the nanoporous PCL membranes, surface modification is a promising strategy to create anti-biofouling surface and to ensure the long-term stable drug release. A variety of polymers can be immobilized onto surfaces to provide anti-biofouling properties, such as polyacrylamide, poly(N,IV-dimethylacrylamide), poly(ethylene glycol), ethylene-vinyl alcohol copolymer, and poly(2-hydroxyethyl methacrylate). Among these polymers, PEG has drawn a lot of attention because it is a nontoxic, non-antigenic, non-immunogenic and FDA-approved material, especially for biomedical applications [10]. PEG chains can effectively prevent biofoulants, such as proteins and cells, from adhering to a specific surface. This is due to PEG's rapid mobility, the steric stabilization effect, and the minimal interfacial energy with water [11].

Currently, many technologies can be utilized to modify surfaces with PEG molecules, for example physical adsorption [12,13], silane coupling [14,15], wet chemical reaction [16,17], ultraviolet irradiation [18,19], and plasma discharge [20,21]. However, the bonding force formed through physical adsorption is usually quite weak between PEG molecules and surfaces.

Therefore, PEG molecules would easily detach from surfaces. For silane coupling methods, PEG molecules can be immobilized onto inorganic substrates. To modify the nanoporous PCL membrane surface, silane coupling methods would not be appropriate. Wet chemical reaction methods also cannot be utilized to modify the nanoporous PCL membrane surface, because high temperature is usually required to accomplish the successful surface modification process. Nevertheless, PCL has a quite low melting point (~60°C), and membrane structures could be altered during the reaction process due to elevated temperature. Furthermore, complex and tedious procedures are involved in the UV irradiation processes, such as degasification. Hence, the UV irradiation method also is not preferred.

This study presents a novel, simple and efficient approach to modifying the nanoporous PCL membrane surfaces with PEG molecules. In this study, oxygen plasma is employed to produce reactive groups on the membrane surface at room temperature. In addition, the reactivity between surfaces and PEG molecules can be enhanced with the use of double bonds provided by PEG-monoacrylates.

Part I of this study has shown that PEG chains can be successfully immobilized onto the PCL membranes with the use of oxygen plasma and PEG-monoacrylate [22]. In Part I, the effects of plasma treatment conditions (plasma powers and treatment times) on the surface properties and anti-biofouling performance is illustrated. As a result, an appropriate plasma treatment condition was obtained for further investigation. Moreover, it has been reported that the density and length of PEG chains can affect surface properties and anti-biofouling performance [23–27]. In Part II, the effects of the graft density and PEG chain length on surface properties and fibroblast adhesion were evaluated, with the use of the appropriate plasma treatment condition obtained in Part I. The grafting density is related to the concentration of PEG solution, and the grafting density could be well-controlled by varying the PEG-monoacrylate concentration [28,29]. In this investigation, 0.05–0.2 M PEG(400)MA solutions were used to explore the effect of grafting density on water contact angle, surface functional groups and the cell adhesion. Moreover, PEG(200)MA, PEG(400)MA and PEG(1000)MA were used to examine the relationship among PEG chain lengths, various surface properties and anti-biofouling performance. A model biofoulant, NIH 3T3 fibroblast was used to test the anti-biofouling performance of the PEG-modified nanoporous PCL membranes.

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, ≧93%) was acquired from Sigma-Aldrich (St. Louis, MO). Polyethyleneglycol-200 monoacrylate (PEG(200)MA), polyethyleneglycol-400 monoacrylate (PEG(400)MA) and polyethyleneglycol-1000 monoacrylate (PEG(1000)MA) were obtained from Monomer-Polymer & 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 membranes were produced using a combination of thermally- and nonsolvent-induced phase separations [1]. The PCL pellets were dissolved in a diluent which composed of 15 wt% 1,4-dioxane as solvent and 65 wt% 2-methoxyethanol as nonsolvent, to obtain a 20 wt% PCL casting solution. To obtain a homogeneous solution, the solution was well-mixed and heated at 50°C for approximately 2 h. An overall procedure for membrane preparation is described below. The homogeneous PCL solution was cast on a Teflon plate. Accordingly, 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 taken out from the bath, and isopropanol was poured onto the top of the solidified membrane. After 20–30 min, the membrane was removed from the Teflon plate. Then, the membrane was dried in air and further in vacuum.

PEG Grafting via Oxygen Plasma Technique

The nanoporous PCL membrane was firstly dipped into isopropanol and then deionized (DI) water to clean the surface. Then, the membrane was dried in air and further in vacuum overnight. To evaluate how grafting density affects surface properties and fibroblast adhesion, a series of concentrations of PEG(400)MA solutions were prepared. The nanoporous PCL membrane was soaked for 2 h in 0.05 M PEG(400)MA, 0.1 M PEG(400)MA and 0.2 M PEG(400)MA solutions using a solvent mixture, which consisted of 80% v/v ethanol and 20% v/v water. To study the effect of PEG chain length on surface properties and anti-biofouling performance, the nanoporous PCL membrane was soaked for 2 h in 0.1 M PEG(200)MA, 0.1 M PEG(400)MA and 0.1 M

PEG(1000)MA solutions. After the PEGMAs-adsorbed membranes were dried in air overnight, the membranes were 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, an oxygen flow rate of 20 standard cubic centimeters per minute (sccm) was used, and the pressure inside the chamber was around 150 mTorr. The plasma power of 25 W and the treatment time of 5 sec were used in the plasma treatment process. Following the completion of the oxygen plasma treatment, the treated membrane was soaked into a copious amount of isopropanol and then DI water to remove any organic residuals for later characterization.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Functional groups on the modified and unmodified nanoporous PCL membranes were evaluated using Nexus 470® FTIR spectrometer with a zinc selenide crystal prism (Thermo Nicolet, Madison, WI) in the attenuated total reflection (ATR) mode. 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 performed in a range of 400 to 4000 cm^{-1}. The background spectrum and the sample spectrum were obtained for analysis.

Contact Angle Measurement

To examine the surface hydrophilicity, droplets of 10 μL DI water were deposited on the surface of a membrane sample at room temperature. The water drop contour on the surface was captured with a high-performance charge-coupled device camera acquired from COHU (San Diego, CA). The contact angle was analyzed by measuring the sessile drop contour processed with a MATLAB code.

X-ray Photoelectron Spectroscopy

Surface compositions of the membranes were investigated 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 transferred 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 1s 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.

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 placed 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 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 attached cells were detached from the membranes by trypsinizing them for 10 min with trypsin-EDTA 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.

RESULTS AND DISCUSSION

It is reported that the grafting density of PEG chains is related to the concentration of PEG solution in which a solid substrate was soaked [17,28]. Accordingly, the density of PEG chains on the nanoporous PCL membrane surface was manipulated by varying the concentration of PEG(400)-monoacrylate solution. Prior to the oxygen plasma treatment, the nanoporous PCL membranes were soaked for 2 h in 0.05, 0.1 and 0.2 M PEG(400)-monoacrylate solutions. Afterward, the membranes were treated with the oxygen plasma with the same power and time, i.e., a power of 25 W for 5 sec. Figure 1 shows the variation of static water contact angle as a function of the concentration of PEG(400)-monoacrylate solution. The contact angle was 107° for the unmodified membrane; however, the contact angles were around 43° for the all modified membranes. A decrease in water contact angle indicates that the hydrophilic PEG was successfully immobilized onto the membrane surface. As shown in Figure 1, the density of PEG chains had no significant effect on static water contact angle.

Surface functional groups were analyzed using ATR-FTIR spectroscopy. Figure 2 shows the ATR-FTIR spectra of the unmodified membrane and modified membranes which were treated with different PEG(400)-monoacrylate concentrations. For the three modified membranes, there was an emergence of peaks at 1106, 2866 and 3440 cm^{-1}, which are attributed to the C-O-C

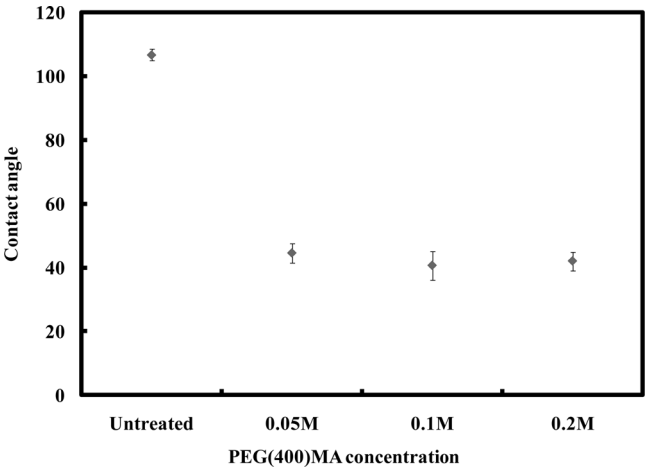

Figure 1: Variation of water contact angle as a function of grafting density/PEG(400)MA concentration.

stretching vibration, CH_2-stretching and OH-stretching of PEG, respectively. This confirms the presence of PEG on the membrane surfaces. As illustrated in Figure 2, the intensities of the three peaks increased while the concentration of PEG(400)-monoacrylate was increased from 0.05 to 0.1 M. However, there was no significant difference between the spectra of the membranes which were treated with 0.1 and 0.2 M PEG(400)-monoacrylate solutions.

Surface elemental composition of the oxygen plasma-treated membranes is displayed in Figure 3. When the membrane was pre-treated with the

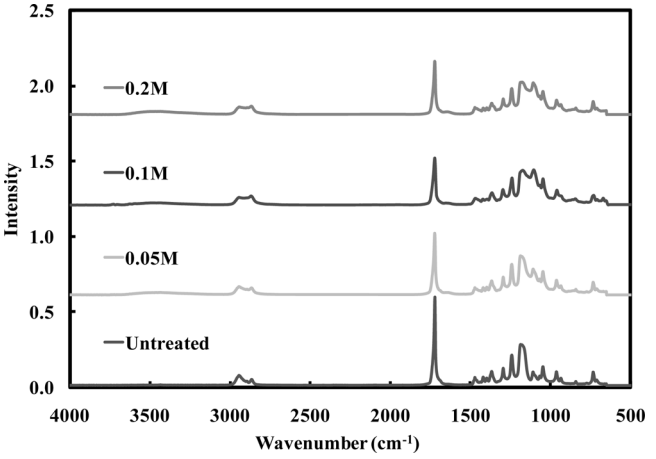

Figure 2: ATR-FTIR spectra of the untreated and PEG-modified nanoporous PCL membranes which were modified with various grafting densities/PEG(400)MA concentration (0.05–0.2 M).

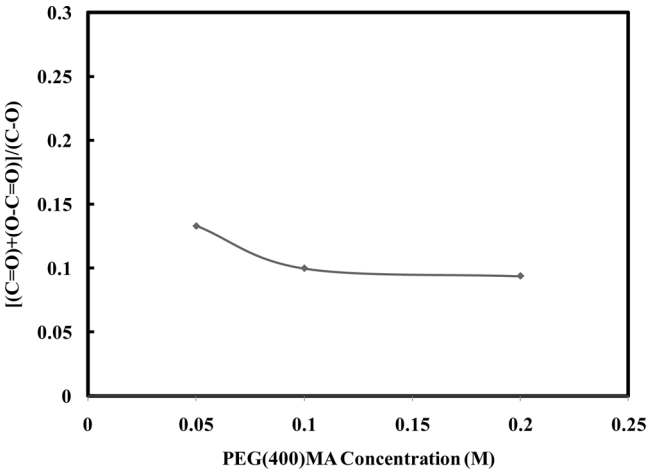

Figure 3: Surface elemental composition as a function of PEG(400)MA concentration.

0.05 M PEG(400)-monoacrylate solution, the membrane surface could be insufficiently covered by PEG(400)-monoacrylate molecules. Therefore, there would be a large PCL membrane area available for XPS detection. Moreover, there might be plenty of surface area exposed to oxygen plasma; and carbonyl groups (C=O and O-C=O) can be generated. The calculated ratio of carbonyl groups (C=O and O-C=O) to the ether and/or hydroxyl groups (C-O) can be indicative of the grafting density of PEG chains on the membrane surface. The ether and/or hydroxyl groups (C-O) would primarily come from PEG. The carbonyl groups (C=O and O-C=O) might be from the base material, poly(ε-caprolactone) and the oxygen-rich species produced by oxygen plasma. The calculated ratio would be reduced while the concentration of PEG(400)-monoacrylate solution were increased from 0.05 to 0.1 M. A decrease in this ratio can be ascribed to the higher PEG grafting density on the membrane surface. Nevertheless, there is no significant difference between these two ratios for the membranes pre-treated with 0.1 and 0.2 M PEG(400)-monoacrylate solutions. With the use of the 0.1 M PEG(400)-monoacrylate solution, the membrane surface could be already covered by PEG(400)-monoacrylate molecules quite well. The surface coverage of PEG(400)-monoacrylate molecules would not be enhanced by increasing the concentration to 0.2 M.

Figure 4 illustrates the relationship between fibroblast adhesion and the density of PEG chains. Fibroblast adhesion was reduced substantially while the concentration of PEG(400)-monoacrylate concentration was increased from 0.05 M to 0.1 M. However, there is little difference between the anti-biofouling ability of the membranes which were treated with 0.1 and 0.2 M PEG(400)-monoacrylate solutions. It is reported that an increase in the grafting density would lead to a decrease in cell adhesion [17,29]. As the

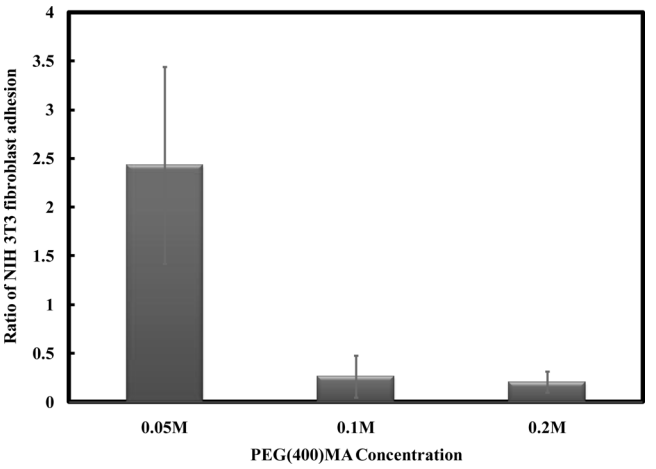

Figure 4: Ratio of NIH 3T3 fibroblast adhesion between the treated PCL membrane surface and the untreated PCL membrane surface for three PEG(400)MA concentrations, 0.05, 0.1 and 0.2 M.

grafting density was increased, the mutual inter-chain interaction would become more and more important. The mutual interaction could force each PEG chain to stretch out perpendicularly to the surface and provide sufficiently repulsive force to repel approaching fibroblasts [28]. However, it is observed that there is no considerable improvement in the prevention of fibroblast adhesion when the concentration of PEG(400)-monoacryate concentration was increased from 0.1 to 0.2 M. That is because 0.1 M PEG(400)-monoacrylate solution might provide adequate amount of PEG(400)-monoacrylate molecules to cover the membrane surface. Any increase in the concentration higher than 0.1 M would not improve the anti-biofouling ability.

To investigate how the length of PEG chains affect the surface properties and the prevention of fibroblast adhesion, 0.1 M PEG(200)MA, 0.1 M PEG(400)MA and 0.1 M PEG(1000)MA solutions were prepared. The nanoporous PCL membranes were soaked for 2 h in these three solutions. After drying, the membranes were exposed to oxygen plasma at a power of 25 W for 5 sec. ATR-FTIR analysis was used to characterize the surface functional groups of the untreated and PEG-modified membranes. Compared to the spectrum of the untreated membrane, the increased intensity of the peaks at 1106, 2866 and 3440 cm^{-1} can imply that PEGs were successfully immobilized on the membrane surface, for the three modified membranes. As shown in Figure 5, the intensity of these three peaks (1106, 2866 and 3440 cm^{-1}) became higher when the molecular weight of PEG chains was increased from 200 to 400 daltons. The increased intensity of the peaks can be attributed to more ethylene glycol groups being attached to the membrane surface after plasma treatment. However, the intensity decreased considerably when the

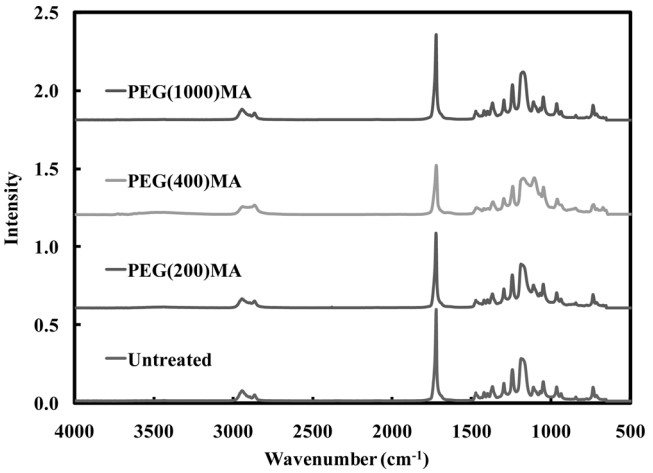

Figure 5: ATR-FTIR spectra of the untreated and PEG-modified nanoporous PCL membranes which were modified with various PEG-monoacrylate molecular weights.

molecular weight of PEG chains was increased from 400 to 1000 daltons. Among the spectra of these three modified membranes, the lowest intensity of PEG characteristic peaks (1106, 2866 and 3440 cm^{-1}) was obtained for the membrane pre-treated with a 0.1 M PEG(1000)MA solution. Based on the observation from Figure 5, it can be concluded that the fewest ethylene glycol groups can be attached to the membrane surface via the method presented in this study, when the membrane was pre-treated with a 0.1 M PEG(1000)MA solution.

The relationship between the contact angle and the length of the PEG chains is depicted in Figure 6. After plasma treatment, a significant decrease in water contact angle can be observed for the three membrane samples. When the molecular weight of PEG was increased from 200 to 400 daltons, the average contact angle would decrease from 53° to 41°. The reduction in water contact angle is due to more ethylene glycol groups on the membrane surface, which renders the surface more hydrophilic. However, the water contact angle would increase from 41° to 64° while the molecular weight of PEG was increased from 400 to 1000 daltons. Supposedly, the membrane surface should be more hydrophilic as the membrane was pre-treated with a 0.1 M PEG(1000)MA solution. As shown in Figure 5, the fewest ethylene glycol groups were attached to the surface of the membrane which was pre-treated with a 0.1 M PEG(1000)MA solution, compared to the other two solutions. That could be the reason why the highest contact angle would be obtained on the membrane pre-treated with a 0.1 M PEG(1000)MA solution.

The relationship between the fibroblast adhesion and the membranes which were pre-treated with different PEG-monoacrylates is shown in Figure 7. It was found that the amount of cell adhered on the modified surface

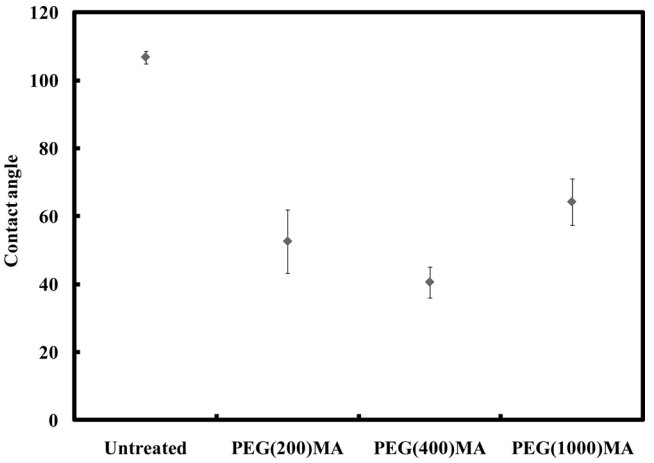

Figure 6: Variation of water contact angle as a function of the molecular weight of PEG-monoacryate.

would decrease with an increase in PEG molecular weight from 200 to 400 daltons. It was reported that short chains could form a mushroom-like structure and therefore could not provide sufficient repulsive force against biomolecule adhesion [15,27]. Compared to PEG(200), PEG(400) might be long enough to form a brush-like structure, and PEG chains can stretch out to prevent fibroblast from approaching to the membrane surface. When the membrane was pre-treated with a PEG(1000)MA solution, however, the opposite was observed. The membrane pre-treated with a PEG(1000)MA solution improved fibroblast adhesion instead of reducing the cell adhesion. As shown

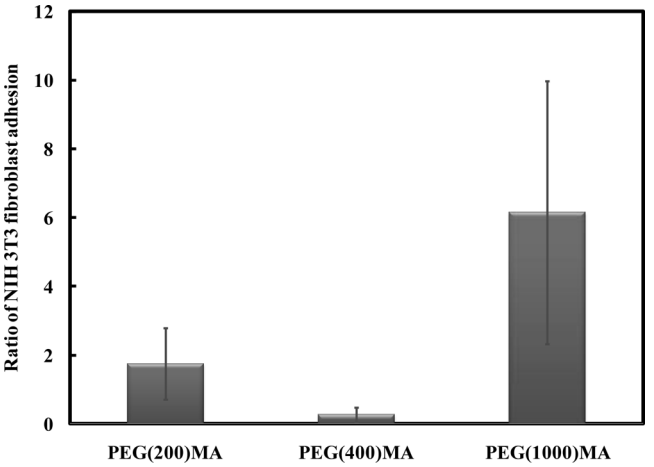

Figure 7: Ratio of NIH 3T3 fibroblast adhesion between the treated PCL membrane surface and the untreated PCL membrane surface for PEG(200)MA, PEG(400)MA and PEG(1000)MA.

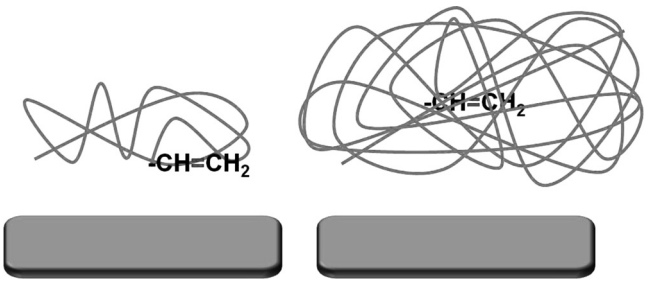

Figure 8: Schematic illustration of the relation between PEG molecular weight and surface reactivity.

in Figures 5, few ethylene glycol groups can be attached to the membrane surface. Hence, there might be not enough ethylene glycol groups to inhibit fibroblast adhesion. Furthermore, with pre-treatment of a PEG(1000)MA solution, the improved hydrophilicity (Figure 6) on the membrane surface can be more attractive to the fibroblast than the untreated/hydrophobic PCL membrane surface [30].

Figure 8 explains why it is more difficult to modify the membrane surface with PEG(1000)-monoacrylate using oxygen plasma in a solid-state reaction. In our modification process, the membranes were initially soaked in a PEG-monoacrylate solution, and then the pre-treated membranes were dried prior to oxygen plasma treatment. During the plasma treatment, the reactive functional group (monoacrylate; -CH=CH_2) plays an important role in the success of the PEG modification. With an increase in the molecular weight of PEG chains, it is more likely that the monoacrylate group would be embedded within the PEG chains. Therefore, the reactivity between the surface and PEG-monoacrylates would be lowered while the molecular weight of PEG chains was increased. As shown in Figures 5 and 6, the fewest ethylene glycol groups can be detected by ATR-FTIR and the highest contact angle can be obtained when the membrane pre-treated with a PEG(1000)MA solution, compared to PEG(200)MA and PEG(400) solutions.

CONCLUSION

The density and length of PEG chains are significant in determining the cellular response to a specific surface. This investigation presents the effects of graft density and PEG chain length on surface and nonfouling properties. Different concentrations (0.05, 0.1 and 0.2 M) of PEG(400)-monoacrylate solutions were used to pre-treat the nanoporous PCL membranes and to enable different amounts of PEG chains to adsorb on the surface. Q variety of surface

properties were characterized, after a solid-state reaction induced by oxygen plasma treatment. The grafting density was the lowest for the membrane pre-treated with a 0.05 M PEG(400)-monoacrylate solution. The surface properties were nearly identical for the membranes pre-treated with 0.1 and 0.2 M PEG(400)-monoacrylate solutions. Due to lower grafting density, the membrane pre-treated with a 0.05 M PEG(400)-monoacrylate solution would promote fibroblast adhesion instead of inhibiting it. However, when the concentration of PEG(400)-monoacrylate solution was greater than or equal to 0.1 M, the membrane can provide sufficient resistance against fibroblast adhesion. Because of similar surface coverage of PEG chains, the membranes, which were pre-treated with 0.1 and 0.2 M PEG(400)-monoacrylate solutions, could allow nearly the same extent of reducing fibroblast adhesion.

Along with oxygen plasma treatment, PEG(200)-monoacrylate, PEG(400)-monoacrylate and PEG(1000)-monoacrylate were used to investigate the effect of PEG chain length on the surface properties and the resistance against fibroblast adhesion. When the molecular weight of PEG chains was increased from 200 to 400 daltons, the anti-biofouling properties was improved significantly. However, the reactivity became low when the molecular weight of PEG chains was increased to 1000 daltons. Therefore, it is unlikely that PEG(1000)-monoacrylate can be grafted to the membrane surface. With insufficient PEG chains on the surface, the membrane pre-treated with PEG(1000)-monoacrylate was able to increase fibroblast adhesion rather than reduce it, due to enhanced hydrophilicity. Therefore, the medium chain length, i.e., PEG(400)-monoacrylate, would be preferable to achieve anti-biofouling surface for our newly developed surface modification method.

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