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緒　　言

打ち抜き試験は小さな試験片で簡便に機械特性を評価できるため応用範囲が広い．例えば，最終製品や不具合による抜去品から試験片を作製できるだけでなく，コンポーネント内部での部位を指定して調べる[3]ことも可能である．具体的な試験法がASTMで規格化されている[1]が，試験治具や試験片の寸法許容幅が非常に厳しく設定されており（図１），規格を作成した研究グループの報告以外に規格に則った試験の報告はないとも言われている[6]．特に試験片厚さの寸法許容幅の設定が厳しいが，これは直接試験結果に影響を与えることに加え，試験片の保持条件にも影響を与えるためと考えられる．そこで，試験片厚さの寸法許容幅を緩和した場合の影響について検討を行い，より実用的で簡便な試験法の確立を目指した．

材料および方法

UHMWPE GUR1020のパウダーから40×40×0.5mmのシートを直接圧縮成型した．これをカッターで切断し，約６×６mmの試験片とした．各試験片の厚さをマイクロメータ（ミツトヨ，MDC-25MJ）により測定したところ，0.488mmから0.561mmであった．試験片は図２に示す治具に固定し，万能試験機（島津製作所，オートグラフAG-20ｋNG，ロードセル容量１kN）と半球状のポンチを用いてその中央を打ち抜いた．ポンチの直径（2.5mm）と試験速度（0.5mm/min）はASTM法と同様とした．また，図３に示すように，試験片にあわせた厚さのシク

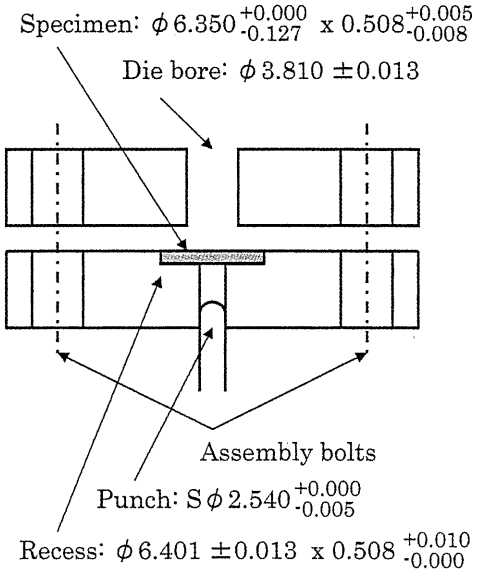

図１．Apparatus and specimen for tensile punch test prescribed by ASTM F2183-02.

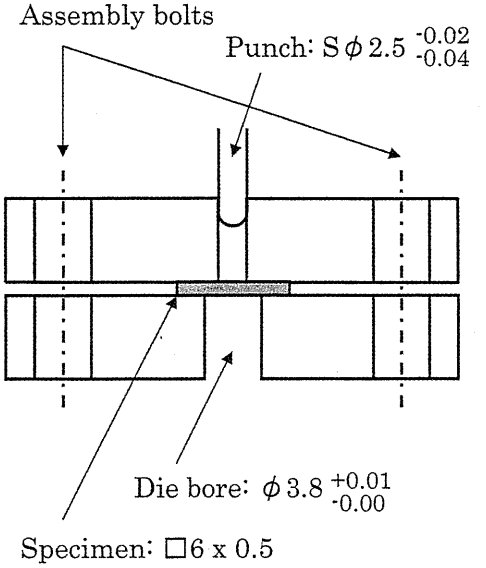

図２．Apparatus and specimen for tensile punch test used in this study.

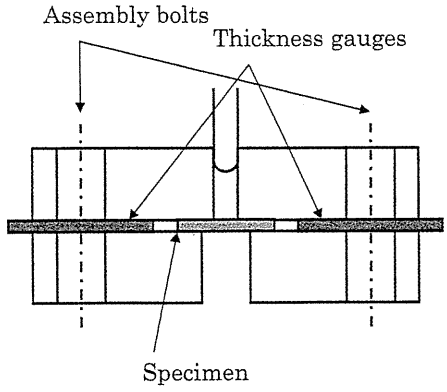

図3．Apparatus and specimen for tensile punch test with thickness gauge (thickness gauge method).

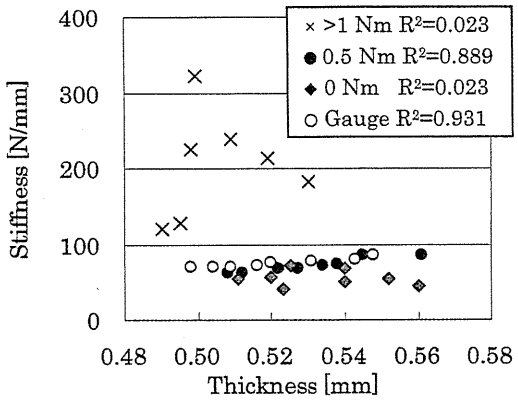

図4．Stiffness of virgin UHMWPE with various fixation methods. The results of tightening torque of 0.5 Nm (0.5 Nm) and thickness gauge method (Gauge) were highly related to specimen thickness. However, excessive tightening torque (>1 Nm) or insufficient tightening torque (0 Nm) lead to scattered results.

ネスゲージ（アズワン，100MY）をスペーサとして使用する方法（シクネスゲージ法）でも試験を行った．得られた荷重変位曲線から，剛性，初期最大荷重，破断のび，破断エネルギーを計算した．

試験片の厚さは得られる各種パラメータに直接影響を与えるほか，試験片を保持する際に試験片に生じる圧縮応力にも影響を与えると考えられたため，治具の締め付けはトルクレンチで行い，締め付けトルクによる影響，試験片厚さによる影響を調べた．また，本試験法の有用性について検討するため，生体脂質であるスクアレンの浸入による機械特性への影響を調べた．具体的には，一部の試験片をスクアレンに100℃で7日間浸漬し，同様に試験した．

結　　果

図4に試験片厚さと得られた剛性を示す．締め付けトルクが0.5Nmの場合とシクネスゲージ法では，試験片厚さと剛性の間にそれぞれR^2＝0.889，0.931と高い相関が見られるが，締め付けトルクが高い場合（1Nm以上）と低い場合（0Nm）は，得られた剛性がばらつき，試験片厚さとの相関もそれぞれR^2＝0.023，0.023と低いことがわかった．

ここで，UHMWPEの弾性率を900MPa，圧縮力が試験片全体に均等に加わると仮定し，試料を治具に固定した際に試料に加わる圧縮応力について検討した．本試験法の場合，締め付けトルクと軸力の関係式

$$T = F \times (d_1/2 \times (\mu/\cos\alpha + \tan\beta) + \mu \times d_2/2)$$

より，締め付けトルク1Nmの場合で74MPa，0.5Nmの場合で37MPaと計算された．ただし，Tは締め付けトルク，Fはネジ1本あたりの軸力，μはネジと治具との間の摩擦係数，d_1とd_2はそれぞれネジ部と座部の有効径，αとβはそれぞれねじ山の半角とリード角である．また，ASTM法とシクネスゲージ法の場合，治具やシクネスゲージは弾性率が高いため圧縮力による変形は無視でき，試験片は治具のくぼみ深さ，あるいはシクネスゲージの厚さと一致するまで圧縮変形すると考えられる．ASTM法では，試験片最大厚さ0.513mmと治具のくぼみの最小深さ0.508mmより，0～8.7MPaの圧縮応力が加わると計算される．シクネスゲージ法では0.01mm単位で用意されたゲージのうち，試験片厚さを超えない最大のゲージを用いたため，厚さの差の最大値は0.01mmであり，0～17MPaの圧縮応力が試料に加わると考えられる．

以上のことから，1Nm以上の締め付けトル

クでは試験片に過大な圧縮応力が加わり，塑性変形を生じたため，試験結果がばらついたものと考えられた．一方，ねじによる締結を行わずに試験をした場合（0 Nm），試験片の変形により治具が浮くことが目視で確認され，これが試験結果のばらつきにつながったものと考えられた．圧縮応力の値は，少なくともUHMWPEの降伏応力である20MPa未満であることが望ましいと考えられ，シクネスゲージ法が最も望ましいと考えられた．

図5～8にvirgin（締め付けトルク0.5Nm），virgin（シクネスゲージ法）およびスクアレン浸漬UHMWPE（締め付けトルク0.5Nm）の試験片厚さと各パラメータの関係を示す．剛性（図5），初期最大荷重（図6），破断エネルギー（図8）では，各パラメータは試験片厚さに依存するが，いずれも高い相関が見られることがわかった．一方，破断伸びは試験片厚さに殆ど依存せず，変動係数は最大でも3.7%と小さいことがわかった（図7）．スクアレンの浸入に

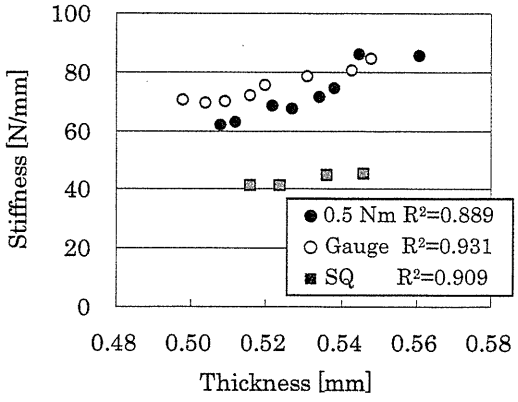

図5．Stiffness of virgin UHMWPE (0.5 Nm and Gauge) and squalene soaked UHMWPE (SQ). The results were highly related to specimen thickness. SQ showed lower stiffness than virgin UHMWPE.

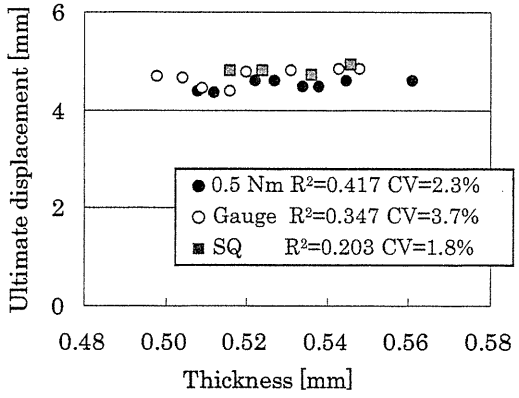

図7．Ultimate displacement of virgin UHMWPE (0.5 Nm and Gauge) and squalene-soaked UHMWPE (SQ). The results were highly related to specimen thickness. SQ showed a value similar to that of virgin UHMWPE.

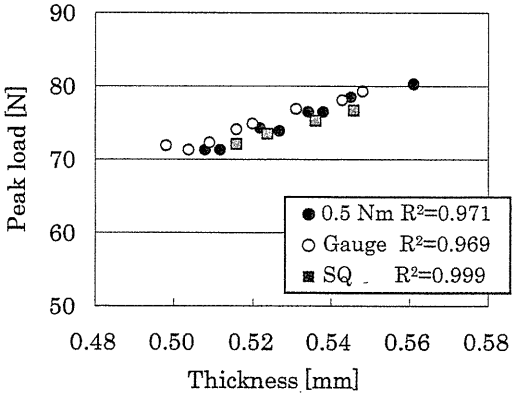

図6．Initial peak load of virgin UHMWPE (0.5 Nm and Gauge) and squalene-soaked UHMWPE (SQ). The results were independent of specimen thickness. SQ showed a value similar to that of virgin UHMWPE.

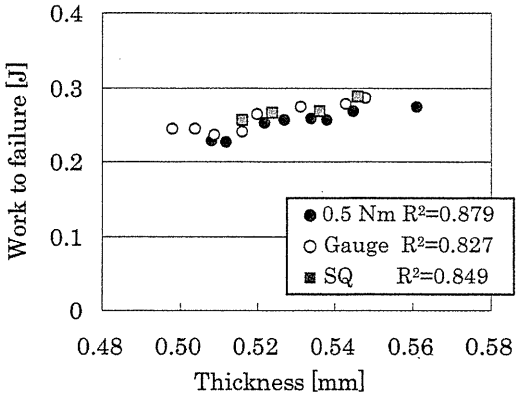

図8．Work to failure of virgin UHMWPE (0.5 Nm and Gauge) and squalene-soaked UHMWPE (SQ). The results were highly related to specimen thickness. SQ showed a value similar to that of virgin UHMWPE.

より初期最大荷重，破断伸び，破断エネルギーに大きな変化は見られなかったが，剛性の明らかな低下が見られた．

考　　察

試験片に加わる圧縮応力が過大になると，試験結果に影響を与えることがわかった．また，固定が十分でない場合も問題が生じることがわかった．シクネスゲージ法は，試験片に加わる圧縮応力を適切に制御することができ，有用であると考えられた．

本研究で算出したパラメータは厚さにより影響を受けるものが多かったが，その場合は試験片厚さと高い相関を示しており，統計的手法により解決が可能と思われた．例えば，各測定点から近似曲線を求め，厚さが0.5mmの点と交わる値をその試料の値とする方法が考えられる．また，2群の比較を行う方法としては，座標変換をする方法や多変量解析の適用などが考えられた．ただし，いずれの場合でも，同質の試験片を複数枚準備できることが必要である．

本試験法の応用例として，スクアレン浸漬UHMWPEの試験を行った．スクアレンは生体内でUHMWPEに浸入することが知られている[2]．スクアレンがUHMWPEに浸入すると，弾性率が大幅に低下することが報告されている[4]が，本研究の結果はこれと一致した．加えて本研究では，最大荷重などその他の引張特性にはあまり影響を与えないことが示され，スクアレンの浸入が直ちに不具合につながるという知見は得られなかった．一方，生体内ではスクアレン以外にも多様な脂質がUHMWPEに浸入することが報告[2]されているほか，スクアレンによるUHMWPEの劣化を示唆する報告[5]もあり，このような研究目的には試験片が小さく脂質を人工的に浸入させやすい本試験法が極めて有効であると考えられた．近年開発されているビタミンEの浸入による影響を評価するなどといった目的への応用も期待できる．

結　　論

本研究では，打ち抜き試験における試験片厚さの影響について検討を行った．試験片厚さのばらつきが原因で試験片を保持する際に加わる圧縮応力がばらつくと，試験結果に大きな影響を与えるが，トルクレンチを用いたねじの締結や，シクネスゲージ法によりばらつきを抑えることが可能であった．また，試験片の厚さは試験から算出される各種パラメータに直接的に影響を与えるが，試験片厚さと得られた各種パラメータの間には高い相関がみられ，同質な試験片が複数用意できるならば試験片厚さにばらつきがあっても十分に評価が可能であると考えられた．これにより，研究目的によっては試験片の製作などに困難があった規格化された従来の方法より簡便に試験ができ，適用範囲を広げることが可能であると考えられた．

＜謝　　辞＞

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Effects of 3,4-dihydrophenyl groups in water-soluble phospholipid polymer on stable surface modification of titanium alloy

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ABSTRACT

The surface of a titanium (Ti) alloy substrate was modified by a simple and quick process using a water-soluble polymer, and the effects of 3,4-dihydroxyphenyl (DHP) groups in the polymer side chain on the modification process were examined. The polymers (PMDP) composed of both 2-methacryloyloxyethyl phosphorylcholine (MPC) unit and 3,4-dihydroxyphenyl methacrylate unit were synthesized for surface anchoring. The Ti alloy substrate was coated with PMDP using an aqueous solution of the polymer. A PMDP layer with a thickness of 20 nm was formed on the Ti alloy substrate simply by dip coating for 10 s without drying. Even when the Ti alloy substrate with PMDP coating was immersed in the aqueous medium for 1 week, no change in the thickness was observed, i.e., the PMDP layer was bound to the surface very stably. Oxidation of the DHP groups reduced the stability of the polymer layer significantly. Thus, the DHP groups play a significant role in achieving stable binding. Protein was adsorbed on the Ti alloy substrate; however, this was not observed for the PMDP-coated Ti alloy substrate. In conclusion, we confirmed the effects of DHP groups in PMDP on the stability of the coating on the Ti alloy substrate. Moreover, we found that surface treatment using PMDP was simple, quick, and reliable, and thus, it has great potential for improving biofouling of Ti alloy substrates used in medical devices.

1. Introduction

Titanium (Ti) alloys have many desirable properties such as a relatively low Young's modulus, good fatigue strength, formability, machinability, and corrosion resistance. Accordingly, they have been widely used in biomedical devices and components since the late 1970s, especially in cardiac and cardiovascular applications (e.g., prosthetic heart valves, protective cases in pacemakers, implantable blood pumps, cardiovascular stents, and circulatory devices) [1]. However, Ti alloy substrates induce severe biological responses such as thrombus formation and tissue reaction [2]. As a result, anticoagulant therapy is necessary to minimize the risk of thromboembolic complications. Therefore, surface modification of Ti alloy substrates is indispensable for improving its thrombogenicity and tissue compatibility.

Protein adsorption is the first essential event followed by biological responses such as acute thrombus formation and inflammation and then fibrous encapsulation, bacterial adhesion, and infection [3]. It is generally believed that reducing protein adsorption on the substrates can significantly attenuate these adverse biological responses. One well-known polymeric material used to prevent protein adsorption is hydrophilic poly(ethylene glycol) (PEG) [4]. Indeed, PEG functions well under both in vitro and in vivo conditions for a relatively short period. However, because PEG-based materials are susceptible to degradation by spontaneous oxidation under physiological conditions, these systems lack long-term stability, which reduces their effectiveness as a surface modifier [5]. In other words, PEG-based materials are not suitable for use in implantable cardiovascular devices.

Another promising and effective way of preventing protein adsorption to attain biocompatibility is to prepare an artificial cell membrane surface on the substrates using phospholipid polymers. Such polymers have been synthesized using 2-methacryloyloxyethyl phosphorylcholine (MPC), which is a methacrylate monomer bearing the same polar group as that in the natural phospholipid molecules in the side chain [6,7]. Ishihara et al. developed a synthetic route for MPC in 1989 that has been successfully applied worldwide. MPC polymers show adequate stability both chemically and physically even when under in vivo conditions. Moreover, they have excellent thrombogenicity and tissue

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compatibility [8–12]. At present, MPC polymers are widely used for the surface modification of implantable medical devices and artificial organs [13–17].

There are many reports of surface immobilization of MPC polymers on Ti alloy substrates. However, these methods have many limitations for widespread practical use. Layer-by-layer assembly (LBL) involves complex multistep procedures [18], the self-assembled monolayer (SAMs) technique requires surface-specific interaction [19], and surface-initiated atom transfer radical polymerization (ATRP) needs unstable polymerization conditions [20]. Poly(MPC-co-*n*-butyl methacrylate (BMA)) (PMB) is a typical MPC polymer. The polymer suppresses non-specific protein adsorption, platelet adhesion, activation, and aggregation in whole blood, even in the absence of anticoagulants [8–11,21]. The coating procedure of PMB from its solution is relatively simple [8–10,16]. PMB can be tightly bound to the substrate by the drying process. More than 5 h of prehydration time is needed to enable the surface functionalities of PMB, although the time depends on the thickness of the PMB layer [22]. However, this prehydration process cannot be applied to medical devices such as cardiovascular stents and blood separation devices. Thus, for practical applications, it is desirable to use a more simple, convenient, and versatile method to immobilize MPC on Ti alloy substrate surface without prehydration.

Recently, to facilitate convenient adhesion of organic compounds to metal substrates, mussel-inspired chemistry has been widely investigated [23–25]. Mussels can rapidly and permanently adhere to all types of inorganic and organic wet surfaces in aqueous environments. Such adhesive properties rely on the repeated 3,4-dihydroxy-L-phenylalanine (DOPA) motif found in the foot protein of mussels [26]. Although the exact mechanism of adhesion is not fully understood, it has been widely speculated that the 3,4-dihydroxyphenyl (DHP) group of DOPA is responsible for the adhesion [27,28]. Lee [29] reported that the oxidation of the DOPA motif in the foot proteins dramatically reduces the strength of adhesion to metals. This mussel-inspired chemistry can be used for surface modification using polymers. When a polymer with DHP groups is in contact with a metal substrate, the thin polymer film is spontaneously deposited on the surface. The functionalization of the polymers imparts new characteristics to the metal substrate. In fact, it has been reported that PEG with DHP groups was used to modify a TiO_2 surface in the pH range 6.0–7.4 to reduce protein adsorption on the surface [30].

In this study, we synthesized water-soluble MPC polymers that have DHP groups in the side chain (PMDP). Surface modification of the Ti alloy substrate was carried out using an aqueous solution of the polymer. The surface characteristics and stability of the coated polymer layer were examined, and the effects of the DHP groups on the adhesion of PMDP have been discussed. Finally, we examined the reduction of protein adsorption on the surface of the Ti alloy substrate after modification using PMDP.

2. Materials and methods

2.1. Materials

Two types of water-soluble MPC polymer, poly(MPC-co-methacrylic acid (MAA)) (PMA), were obtained from NOF Co. (Tokyo, Japan) which were synthesized by conventional radical polymerization of MPC and MAA [31]. The compositions of the MPC units in PMA were 30 unit mol% (denoted PMA3) and 50 unit mol% (denoted PMA5). The number average molecular weight (Mn) of PMA3 and PMA5 was 2.7×10^5 and 3.2×10^5, respectively. Dopamine hydrochloride was purchased from Sigma–Aldrich (St. Louis, MO, USA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride (WSC) was purchased from Dojindo (Kumamoto, Japan). A Ti alloy substrate with thickness of 1.0 mm was purchased from Sumitomo Metals, Ltd. (Tokyo, Japan). The Ti alloy substrate was cut into 10 mm × 10 mm pieces and polished with #2000 and #3000 polish papers. The pieces were then rinsed in acetone and ethanol by sonication for 15 min. After drying in air, the substrates were cleaned using oxygen plasma apparatus (PR500 plasma reactor, Yamato Science, Tokyo, Japan) for 10 min before use. To test the resistance of the substrate surface to protein adsorption, bovine serum albumin (BSA, Sigma–Aldrich) was used without further purification.

2.2. Synthesis of phospholipid polymer

The water-soluble MPC polymer with DHP groups was synthesized by condensation reaction between PMA and dopamine hydrochloride. The reaction scheme is shown in Fig. 1. Dopamine hydrochloride and WSC were dissolved in 4 mL of PMA aqueous solution (5.0 wt%), and 96 mL of pH 6.0 buffered solution (potassium dihydrogen phosphate and sodium hydroxide) was added. The reaction was carried out at room temperature for 24 h under Ar gas atmosphere to prevent the oxidation of the DHP groups. The molar ratio [dopamine hydrochloride]/[COOH] was 2.0. After the reaction, the polymer solution was filtered using ultrafiltration membranes (Millipore Co., USA; molecular size cut off: 3.0×10^4) until there was no further release of unreacted dopamine through the membrane, which was confirmed by ultraviolet (UV, V-560, Jasco Co., Tokyo, Japan) adsorption. The polymer solution was freeze-dried. PMDP prepared from PMA3 are denoted as PMDP3, and that prepared from PMA5 are denoted as PMDP5. The chemical structure of these polymers was confirmed by both UV and Fourier transform infrared (FTIR) spectroscopy (FT/IR-615, Jasco) for 32 scans over the range 650–4000 cm^{-1} at a resolution of 4.0 cm^{-1}. The contents of the DHP groups in PMDP were calculated from the UV absorbance of the polymer aqueous solution at 280 nm by comparing with that of a given concentration of dopamine hydrochloride.

2.3. Surface modification on Ti alloy substrate with PMDP

The PMDP solution was prepared using the following aqueous media: pure water (pH about 5.5) and buffered solutions with pH 6.0 and 8.5. The Ti alloy substrate was coated with the PMDE solution by simply dipping it in the solution at room temperature for either 10 s or 24 h.

The surface of the substrate was analyzed by FTIR reflection adsorption spectroscopy to confirm that the substrate coated with the solution. The surface morphology was then observed using an atomic force microscope (AFM, Nihon Veeco, Tokyo, Japan) operated in the tapping mode. The measurements were performed under ambient conditions using a standard cantilever at a scan rate of 1.0 Hz. The root mean square (RMS) surface roughness was calculated from the roughness profiles.

Following the polymer adhesion process, a quartz crystal microbalance (QCM) sensor was used with dissipation monitoring (QCM-D, Q-Sense, Gothenburg, Sweden) and a fundamental resonant frequency of 5.0 MHz. The QCM is widely used to measure the change in mass (Δm) of materials/molecules attached to the surface of the QCM sensor via changes in the resonant frequency (Δf). The QCM-D can detect adsorbed mass up to a resolution of less than a few nanograms per square centimeter. The resonant frequency of the QCM sensor (f) depends on the total oscillating mass. When a thin film is attached to the QCM sensor, the frequency decreases; if the film is thin and rigid, the decrease in frequency is proportional to the mass of the film. Thus, the amount of the adsorbed material on a given surface can be measured by the decrease in the frequency of the oscillator. In this manner, the QCM operates as a very sensitive balance. The mass of the adhered layer can be calcu-

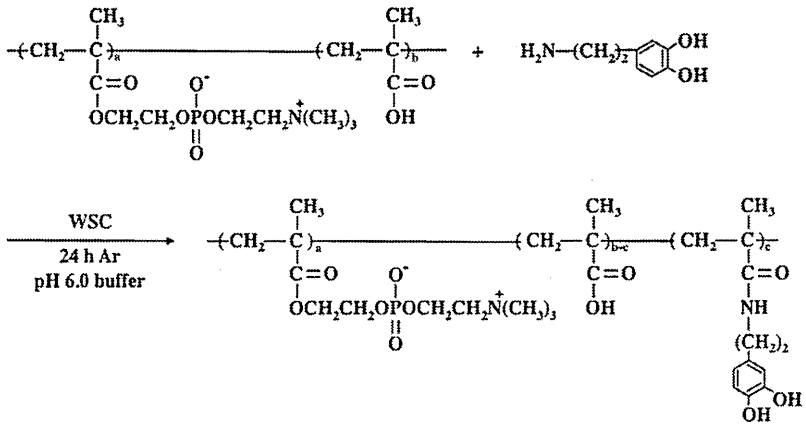

Fig. 1. Synthetic route of PMDP.

lated using the Sauerbrey equation [32], $\Delta m = -C \times \Delta fn/n$, where $C = 17.7\ \text{ng cm}^{-2}\ \text{Hz}^{-1}$, n is the overtone number ($n = 1, 3, 5, 7.$), and fn is the frequency of the overtone. Four resonant frequencies (overtones, $n = 1$, 3, 5, and 7) were used to detect the oscillation of the shearwave through the crystal at 5, 15, 25, and 35 MHz, respectively. The data from the seventh overtone is reported, because it contained minimum noise. The Ti-coated (Ti/Au) QCM sensor obtained from Q-Sense was cleaned using oxygen plasma for 10 min before use. The QCM sensor was exposed to the water solution until a stable baseline of the QCM signals was obtained. The QCM cell was then filled with 2.0 mg mL^{-1} of PMDP aqueous solution. After the PMDP solution was retained for 20 min in the QCM cell, phosphate buffered saline (PBS) solution was flowed to replace the PMDP solution and wash away the weakly adsorbed PMDP from the surface. The QCM signals were monitored throughout the procedure. All the measurements were performed at 37 °C and repeated at least three times.

2.4. Surface characterization and stability evaluation of the coating polymer layer

After coating, the Ti alloy substrates were immersed in water at room temperature for at least 2 days to evaluate the stability of the coating polymer layer. The hydrophilicity of the Ti alloy substrates before and after immersion in the PMDP solution was evaluated with a contact angle goniometer (CA-W, Kyowa Co. Ltd., Tokyo, Japan). The captive-bubble method was used to determine the static contact angle. Each Ti alloy substrate was immersed in water to equilibrate and then fixed horizontally on a metal plate. A small air bubble was attached to the surface of the Ti alloy substrates. The measurement was repeated five times for each substrate, and the average was calculated.

The thickness of the PMDP layer formed on the substrate was measured using an ellipsometer (J. A. Woollam Co., Inc., Tokyo, Japan) at an incident angle of 70° in the visible region. The thickness of the polymer coating layer was determined using a Cauchy layer model with an assumed refractive index of 1.49 at 632.8 nm.

A surface elemental analysis was carried out using an X-ray photoelectron spectroscope (XPS, AXIS-HSi165, Kratos/Shimadzu Co., Kyoto, Japan) with 15 kV Al Kα radiation source at the anode. The applied voltage was 15 kV, and the electric current was 10 mA. The take-off angle of the photoelectrons was maintained at 90°.

To examine the effects of oxidation of the DHP groups in PMDP, the PMDP aqueous solution was kept in air for spontaneous oxidization. After one month, the solution was freeze-dried, and the chemical structure of the remaining polymer was analyzed by both UV and FTIR spectroscopy. The polymer was dissolved in water again, and the solution was used for coating the Ti alloy substrate. The stability of the polymer layer was evaluated by ellipsometry.

2.5. Measurement of amount of protein adsorbed on Ti alloy substrate

The amount of BSA adsorbed on the PMDP3-coated surface was quantified using the QCM-D. First, a Ti-coated QCM sensor was used as a QCM-D. After flowing 2.0 mg mL^{-1} of PMDP3 aqueous solution through the QCM cell, the sensor was exposed to a PBS (pH 7.4) solution until a stable baseline of QCM signals was obtained. Then, 1.0 mg mL^{-1} of BSA in PBS was flowed to fill the QCM cell. After the BSA solution was retained for 20 min in the QCM cell, the PBS solution was flowed to replace the BSA solution and wash away the weakly adsorbed BSA from the surface. The QCM signals were monitored throughout the procedure. All the measurements were performed at 37 °C and repeated at least three times.

3. Results and discussion

3.1. Characterization of PMDP

We considered that the DHP groups were useful for binding the polymer after adsorption on the Ti alloy substrate from its aqueous solution. The IR spectra of the two PMDP polymers are shown in Fig. 2 with the starting materials, PMA and dopamine hydrochloride. The IR spectra of PMDP3 and PMDP5 were similar. The DHP group in PMDP was verified by the appearance of an absorbance

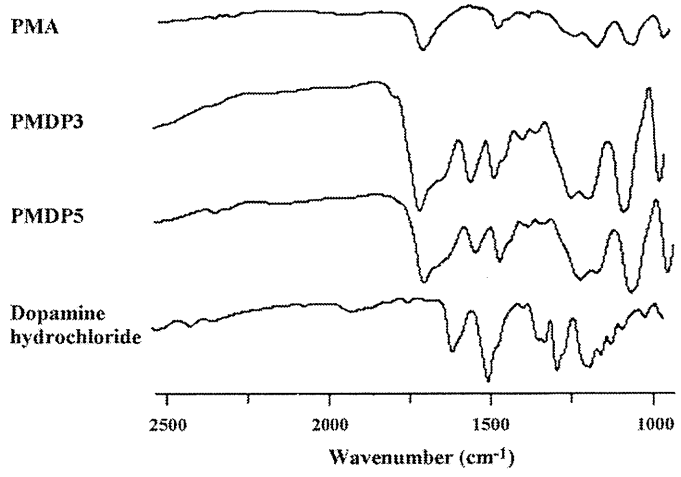

Fig. 2. IR spectra of PMA, PMDP, and dopamine hydrochloride.

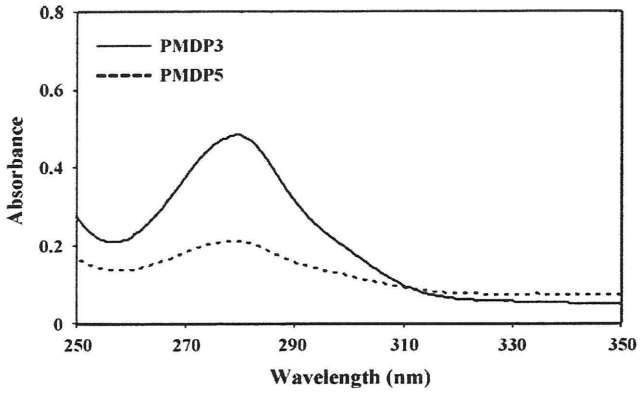

Fig. 3. UV absorption spectrum of PMDP aqueous solution (only the spectrum of PMDP3 is shown).

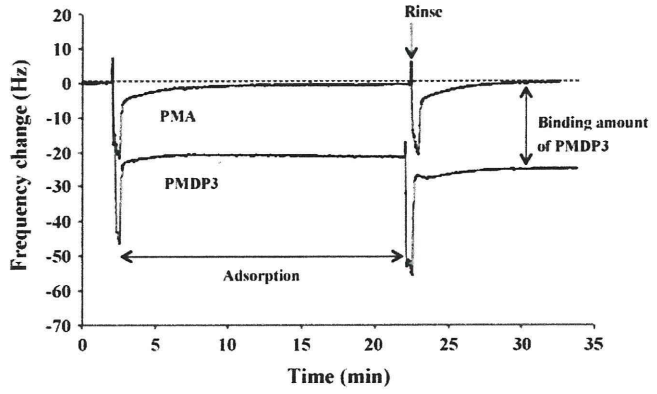

Fig. 5. Adsorption and binding process of PMDP3 and PMA3 on Ti-coated QCM sensor.

peak at 1553 cm^{-1}, which is attributed to the aromatic ring of dopamine hydrochloride. In addition, the presence of the ester carbonyl group of the methacrylate units in PMDP was verified by the appearance of an absorbance peak at 1715 cm^{-1}. The UV spectrum shown in Fig. 3 confirms the introduction of the DHP group in PMDP. An adsorption was observed at 280 nm, corresponding to the DHP groups. Absorbance calculations showed that the content of DHP groups was 4.0 unit mol% in PMDP3 and 2.0 unit mol% in PMDP5. The content of DHP groups in the polymer chain was less than expected. This was because of the solubility of dopamine hydrochloride and reactivity of carboxylate groups in PMA at this pH.

3.2. Surface modification on Ti alloy substrate with PMDP

The Ti alloy substrate was immersed in the aqueous solution of PMDP for different periods. After immersion in the PMDP solution for 10 s, the Ti alloy substrate was pulled out and dried under vacuum for observation with AFM. The AFM images are shown in Fig. 4. The RMS surface roughness of the original Ti alloy substrate was 1.0 nm, whereas that of the PMDP3-coated Ti alloy substrate was only 0.5 nm, indicating that the surface roughness can be reduced by this polymer coating process. The amount of polymer on the Ti alloy substrate was measured with QCM-D. As shown in Fig. 5, a small amount of PMA was deposited on the substrate (small change in frequency); on the other hand, 354 ng cm^{-2} of PMDP3 adhered to the substrate (20 Hz change in frequency). These results show that PMDP3 covered and adhered to the Ti alloy substrate immediately

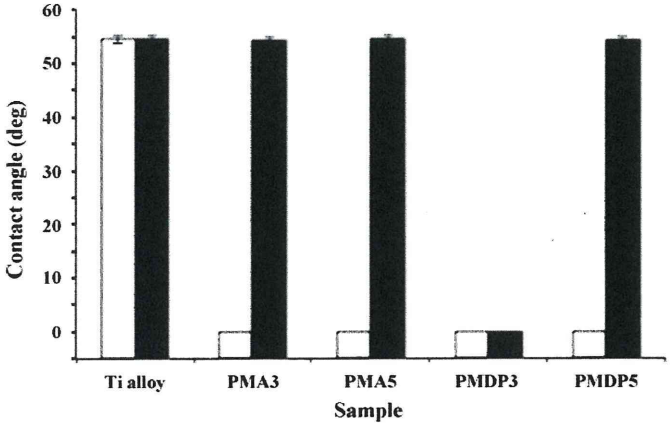

Fig. 6. Change in contact angle of Ti alloy substrate treated with PMA and PMDP by immersion in water for 2 days. Open column: Just after coating for 10 s. Closed column: After 2 days.

from its aqueous solution and formed a uniform coating layer via the dipping procedure. The peaks of DHP groups in the FTIR spectra also indicate the presence of PMDP3 on the Ti alloy substrate and ester carbonyl group after the coating procedure (data not shown).

The surface hydrophilicity was evaluated by performing contact angle measurements. PMDP is water-soluble, which means the polymer is quite hydrophilic. The contact angle was 54° on the original Ti alloy substrate, as shown in Fig. 6. After treatment

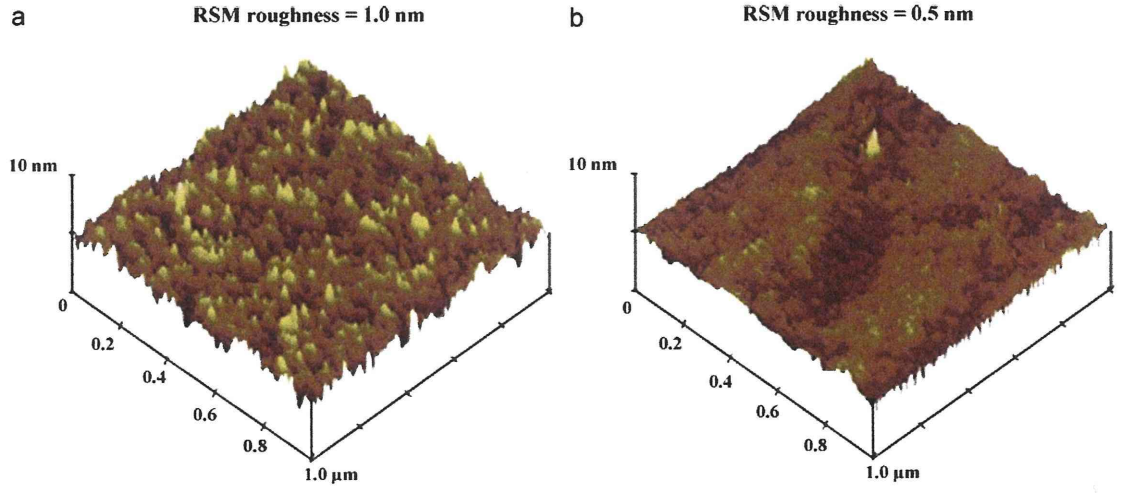

Fig. 4. AFM images of (a) original Ti alloy substrate and (b) Ti alloy substrate coated with PMDP3 by immersion for 10 s.

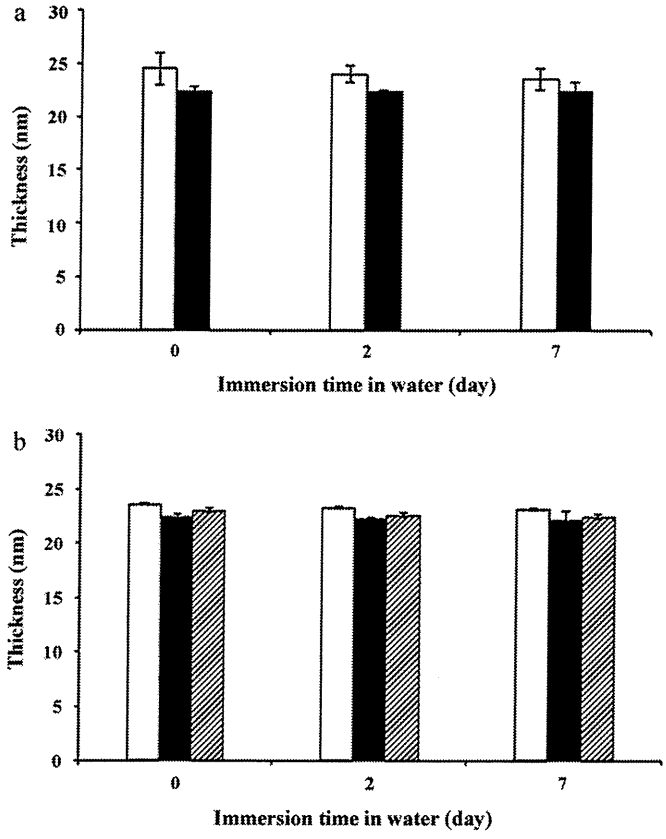

Fig. 7. Change in thickness of PMDP3 layer on Ti alloy substrate after immersion in water. (a) For different coating times (open column: 24 h; closed column: 10 s) and (b) with different pH (open column: pH 8.5 buffer; closed column: water; hatched column: pH 6.0 buffer).

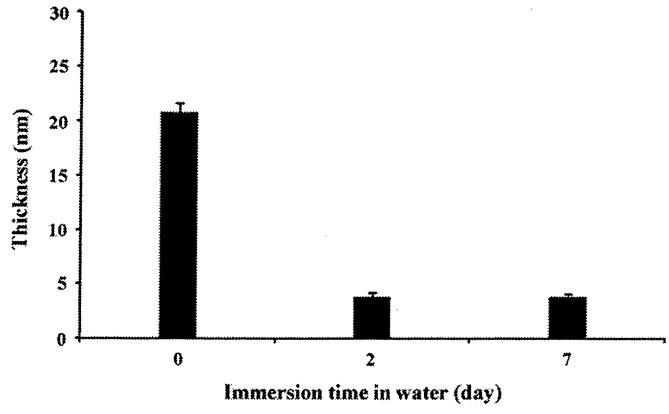

Fig. 8. Change in thickness of oxidized PMDP3 layer on Ti alloy substrate after immersion in water.

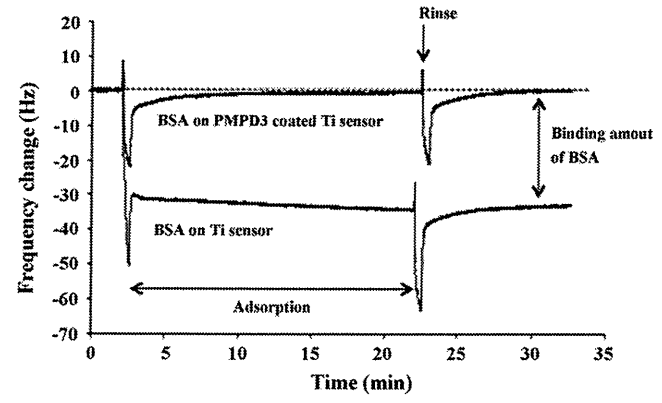

Fig. 9. Adsorption and detachment process of BSA on bare and PMDP3-modified Ti-coated QCM sensor.

with the PMDP3 solution, the contact angle decreased dramatically and reached 0°. This value was maintained even after the substrate was immersed in water for 2 days. This result suggests that PMDP3 remained on the substrate. On the other hand, in the case of PMA and PMDP5, the contact angle returned to 54° (the same as in the case of the original Ti alloy substrate) after immersion for 2 days. These polymers may be detached from the substrate. As shown in Fig. 7, the stability of the PMDP3 coating was confirmed by ellipsometry from the thickness change observed during the washing process. For both time periods (10 s and 24 h), in the case of the PMDP3 aqueous solution, the thickness of the coating layer did not change and minor differences because of the different coating periods and pH were observed. The signals of phosphorus atom at 133 eV and carbon atoms at 285–288 eV in the XPS spectra support the presence of PMDP on the Ti alloy substrate after 7 days immersion procedure (data not shown). Although the binding mechanism of the DHP group to the metal and metal oxide could not be clarified, the affinity of the DHP groups to the Ti alloy substrate was observed.

On the other hand, we considered that the reduced content of DHP groups because of oxidation may lead to instability of the polymer layer. The PMDP aqueous solution was spontaneously oxidized by air. Then, the chemical composition was studied by UV and FTIR spectroscopy. In the UV spectrum measured after oxidation, the intensity of the absorbance peak at 280 nm attributed to the aromatic ring decreased. Conversely, an IR absorbance peak appeared at 2852 cm^{-1}; this is attributed to ketone groups. The above results confirm that the DHP groups were converted to quinine groups. The oxidized PMDP solution was used as a coating solution. As shown in Fig. 8, the thickness of the coating polymer layer changed with the washing period, and the thickness decreased within 2 days. Thus, the oxidation reaction weakened the binding force of the polymer on the Ti alloy substrate and caused the coating polymer layer to become unstable. These results strongly support the notion that the DHP groups in PMDP play an important role in stabilizing the coating.

3.3. Protein adsorption resistance of Ti alloy substrate treated with PMDP

Resistance to protein adsorption is one of the most important properties of biomedical materials. The effects of coating with PMDP3 were evaluated using the BSA solution. BSA is the most highly concentrated protein in blood plasma. According to the QCM signals (Fig. 9), 530 ng cm^{-2} of BSA was adsorbed on the original Ti alloy substrate (30 Hz change in frequency), whereas after treatment with PMDP3, no QCM signal because of BSA adsorption could be detected. These results indicate that the resistance to protein adsorption can be improved by coating with PMDP3. The MPC polymer gave a phosphorylcholine-group-arranged surface [22,33]. The phosphorylcholine group is electrically neutral and hydrated with free-water-like water molecules [11,34,35]. Thus, both electrostatic interaction and hydrophobic interaction are extremely weak and resistance to protein adsorption on the surface is improved [36].

4. Conclusions

A uniform layer of PMDP3 can be deposited on a Ti alloy substrate simply by dipping for 10 s in a PMDP3 aqueous solution without further treatment. The DHP groups play an important role as molecular anchors for stabilizing the binding between the coat-

ing and the substrate. The reduction of protein adsorption on the surface treated with PMDP may induce significant suppression of biological responses, thus maintaining excellent biocompatibility of the MPC unit. In conclusion, simple and reliable surface treatment of a Ti alloy substrate was successfully carried out using bioinspired PMDP, and this method has the potential for application to high-performance cardiovascular implantable medical devices.

Acknowledgement

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PAPER

Quick and simple modification of a poly(dimethylsiloxane) surface by optimized molecular design of the anti-biofouling phospholipid copolymer

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The optimal molecular design of an amphiphilic copolymer composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) and dimethylsiloxane (DMS) units for modifying a poly(dimethylsiloxane) (PDMS) surface in a quick and simple manner was developed. Block- and random-type copolymers with three different compositions were each coated on a PDMS surface in a protic solution. The resulting surfaces were characterized by X-ray photoelectron spectroscopy, atomic force microscopy, contact angle measurement. From the results, the random-type copolymer containing 86% hydrophobic DMS unit was the most suitable molecular design to be stably coated on the PDMS surface. From view point of bioengineering application, it was confirmed that for optimal suppression of protein adsorption and cell adhesion on a PDMS surface, the surface should be coated by immersing it in the polymer solutions with a concentration of 30 mg mL^{-1} for more than 30 s.

1. Introduction

Poly(dimethylsiloxane) (PDMS) elastomers can be applied in various engineering fields such as bioengineering or microelectronics because of several attractive properties such as optical transparency, gas permeability, sufficient flexibility to form complicated shapes, and ease in designing at the microscale level by soft lithography.[1,2] Since their first medical application in bile duct repair, PDMS elastomers have been one of the most commonly used biomaterials for implants as well as base materials for diagnostic applications.[3,4] However, owing to the intrinsic hydrophobicity of PDMS elastomers, they cannot be safely used in blood contact devices or have long-term application in micro-fluidics as they undergo strong hydrophobic interactions with proteins.[5] Numerous research, involving chemical and physical methods, has been conducted with the aim of overcoming the abovementioned limitation. Chemical methods, particularly those involving grafting of hydrophilic polymer materials, have been reported as effective methodologies for preventing a large amount of protein adsorption.[6–8] However, chemical grafting generally requires multiple synthetic steps such as initiator formation, growth reaction, distillation process; this multiple-step procedure might prove to be a hindrance to the mass production of modified PDMS elastomers. Further, topological changes are often induced by the surface swelling of chemically modified PDMS surfaces.[9] Hence, several researches have been conducted for developing physical methods with a simplified and effective procedure.[10,11] However, they are device dependant and high vacuum states are normally required as a pre-treatment step, such as plasma treatment. This could also become a threshold point for massive modification or coating the inner space of a complicated shape such as a micro-fluidic system. Thus, a simple coating approach such as a coating and drying process can be employed as an alternative method for simplifying the procedure and for realizing shape-independent modification. These simple coating procedures using a polymer solution require several variable controls in the case of PDMS modification. For example, the PDMS elastomer is not wettable in polar solvents such as water or alcohol, which can dissolve various amphiphilic polymer modifiers, and undergoes significant dimensional changes in mixed non-polar solvents such as chloroform.[12] Moreover, the high molecular movement of PDMS molecules induced by a low glass temperature inhibits the stable immobilization of amphiphilic polymers on the PDMS surface. Thus, even a wettable amphiphilic polymer modifier is not easily immobilized on the PDMS surface in aqueous media but is stably immobilized on other hydrophobic substrates such as poly(methyl methacrylate), poly(ethylene terephthalate), or polystyrene.[13,14] Therefore, several characteristics such as molecular structure, compositions, wettability, stability, or surface roughness have to be comprehensively considered for satisfying the conditions for simple coating of the anti-biofouling PDMS surface. In this research, we synthesized block/random-type amphiphilic copolymers with different compositions of 2-methacryloyloxyethyl phosphorylcholine (MPC) and dimethylsiloxane (DMS) units for realizing the optimal molecular

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structure for a rapid and simple surface modification. Because the MPC is one of the most well-known hydrophilic and anti-biofouling materials,[15] a surface coated with an MPC polymer is expected to exhibit hydrophilic and anti-biofouling properties, as reported in several previous researches.[16–18] As the DMS unit has a high affinity for the PDMS elastomer, it was chosen as a stabilizing unit in order to maximize the hydrophobic interaction with the PDMS elastomer. For designing a molecular structure as a block/random-type with varying unit composition, we tested the wettability, stability, and the corresponding anti-biofouling properties of the modified PDMS surfaces. The final purpose of this research is to determine the optimized coating condition for a quick and simple modification of the PDMS elastomer by using a phospholipid copolymer with sufficiently high hydrophilicity and low surface roughness for realizing an anti-biofouling PDMS surface.

2. Materials and methods

2.1 Materials

MPC was synthesized as previously reported.[19] A Sylgard 184 silicone elastomer kit was purchased from Dow Corning (Midland, MI, USA). Dulbecco's phosphate buffered saline (PBS, without calcium chloride and magnesium chloride) was purchased from Invitrogen Corp. (Carlsbad, CA, USA). α,α′-Azobisisobutyronitrile (AIBN), bovine plasma fibrinogen, bovine serum albumin (BSA), and fluorescein isothiocyanate (FITC) labeled BSA were purchased from Sigma-Aldrich (St. Louis, MO, USA). A micro-BCA protein assay reagent kit was purchased from Pierce Chemical (Rockford, IL, USA), and 3-(methacryloyloxy)propyl-tris(trimethylsilyloxy) silane (MTS) was provided by Shin-Etsu Corp. (Tokyo, Japan). All the organic solvents (organic synthesis grade) were purchased from Wako Chemicals (Osaka, Japan) and used without further purification.

2.2 Preparation of PDMS elastomer

The PDMS elastomer was prepared as follows. A mixture of the PDMS precursor and cross-linker (10 : 1 by mass) was spread on a Petri dish and cured in a vacuum oven at 70 °C for 6 h after degassing. Next, the sample was cut into 10 × 10 × 2 mm quadrangles. The PDMS microchannel was prepared by pouring PDMS mixture onto a Si wafer mold (width: 100 μm and height: 50 μm) and heat treated by the same procedure as mentioned above. After the PDMS elastomers containing microline and washed glass substrates were peeled off, the elastomers were O_2 plasma treated and attached to each other, forming a micro-channel.

2.3 Synthesis of block-type copolymers

Block-type copolymers composed of PDMS and poly(MPC) (PMPC) was synthesized by atom transfer radical polymerization as previously reported.[20,21] A typical polymerization process (B2) could be described as follows: 0.232 mmol of the PDMS macroinitiator (Mn = 4.21 k) was placed into a 20 mL flask with 8.0 mmol MPC and 5 mL degassed methanol. The solution was bubbled with Ar for 10 min right after a mixture of 0.46 mmol of Cu(I)Cl and 0.928 mmol of 2,2′-bipyridyl was put into the solution. The flask was then purged with Ar gas and the homogeneous maroon solution was stirred at 25 °C until monomer conversion was over 90%. After the reaction, 15 mL of methanol was poured into the mixture and then filtered through 10 cm alumina column to remove the transition metal catalyst. A clear colorless solution was then slightly evaporated and reprecipitated in a large amount of diethylether and chloroform (7 : 3) mixed solvent followed by a dialysis process in water for a day. After freeze-drying, a white block copolymer was obtained. Three different compositions of block copolymers were synthesized by using different molecular weights of PDMS macroinitiator (1.19 k for B1 and 15.1 k for B3) with different in feed ratios of MPC monomer.

2.4 Synthesis of random-type poly(MPC-*co*-MTS) copolymers

A typical polymerization reaction was performed by adding 1 M of monomers in 30 mL of ethanol solution in a glass test tube. 5 mM of AIBN was added as an initiator, and the mixture was bubbled with Ar gas for 15 min. Next, the tube was sealed using an oxygen torch and placed in an oil bath at 60 °C for 18 h. The residual MTS was removed by dropping the reaction mixture in a large amount of acetone, and the residual MPC was removed by washing the collected white polymer with a large amount of water. Number averaged molecular weight (Mn) of synthesized copolymers were measured by size exclusion chromatography (SEC) using a JASCO (Tokyo, Japan) RI-1530 detector containing two connected gel columns (TSK-GEL Super HM-M) calibrated with PMMA standards in hexafluoroisopropanol (flow rate: 0.2 mL min^{-1}, 40 °C).

2.5 Wettability of copolymer solution on PDMS surface

Each copolymer was dissolved in ethanol at 30 mg mL^{-1}. 10 μL of each polymer solution was dropped onto the PDMS surface, and the contact angle of the polymer solution droplet was measured after intervals of 10 s and monitored for 1 min. The contact angle was calculated using a goniometer (Kyowa Interface Science Co., Tokyo, Japan). The load changes during the immersing process were monitored by dynamic contact angle measurement equipment[22] (DCA-100, Orientec Co., Ltd., Tokyo, Japan). A 5 × 2 mm cutting section of a PDMS substrate was immersed into the 30 mg mL^{-1} polymer solution at an immersion velocity of 10 mm min^{-1}. Each immersed substrate was then kept in the polymer solution for 5 min, extracted with the same velocity, and reimmersed for 1 more minute; the reimmersion process was repeated two more times.

2.6 Surface coating with copolymers

Each copolymer was dissolved in ethanol at 30 mg mL^{-1}. The PDMS elastomer was then put into each polymer solution for 3 min and naturally dried in a clean air box for 3 h. The dried samples were then thoroughly washed with fresh water and aged in water for 1 day to ensure their stability.

The micro-channel was coated as follows. Inlet and outlet holes were machined at each end of the microchannel using a drill; these holes were washed with ethanol, after which they underwent natural drying. Each polymer solution was then

injected through the inlet hole into the microchannel until it was filled. After 10 min, a syringe was used to inject fresh air into the microchannel to remove excess polymer solution; the microchannel was then naturally dried for 3 h. 1.5 mL of fresh water was then injected into the microchannel, and aged for 30 min. After then a protein adsorption test was conducted.

2.7 Characterization of coated PDMS elastomer

2.7.1 X-Ray photoelectron spectroscopy (XPS) measurement. The atomic ratio of the coated surfaces was investigated by XPS using magnesium Kα sources with a take-off angle of 90° (Kratos/Shimadzu, Kanagawa, Japan). The P/Si atomic ratio was calculated by integration of each peak area. More than 3 positions at each sample and more than 3 samples for each coating condition were measured.

2.7.2 Air bubble contact angle measurement. The hydrophilicity of each coated PDMS elastomer was investigated by measuring the air bubble contact angle in water. Each coated sample was fixed in water, and an air bubble was generated for interacting with the PDMS surface. After the air bubble was stabilized on the PDMS surface, the contact angle was calculated by a tangential method using a goniometer (Kyowa Interface Science Co., Tokyo, Japan).

2.7.3 Atomic force microscopy (AFM) imaging. The AFM images under wet conditions were analyzed using NanoScope IIIa (Nihon Veeco, Tokyo, Japan). The excitation frequency was in the range 7.8–9 kHz, and the scan rate and scan scales were 0.5 Hz and 100 nm, respectively. All the samples were aged in water for 1 day before observation, and the scanning size of each sample was 25 × 25 μm.

2.8 Protein adsorption test

2.8.1 Quantitative analysis of adsorbed protein. All the samples were aged in water for 1 day in order to ensure stable coating of the PDMS elastomer. The samples were immersed in a mixture of 0.3 mg mL^{-1} fibrinogen and 0.45 mg mL^{-1} BSA in PBS (pH 7.4) for 60 min at 37 °C and simply rinsed with fresh PBS. The adsorbed protein was detached in sodium dodecyl sulfate (SDS) (1 wt% in water) by sonication for 20 min; the protein concentration in the SDS solution was determined using the micro-BCA™ method.

2.8.2 Adsorption of protein in PDMS microfluidic device. The coated microchannel was washed with 1.5 mL of fresh water and aged for 30 min prior to the adsorption test. 4.5 mg of FITC-BSA was dissolved in 1 mL of PBS. 10 μL of protein solution was passed through the microchannel for 1 min. Next, 150 μL of fresh PBS was injected into the micro-channel to remove the excess protein solution. The microchannel was then naturally dried in a clean box, after which, it was observed using a fluorescence microscope (Axioskop2 plus, Carl Zeiss, Jena, Germany) at an exposure level of 1/3.5 s.

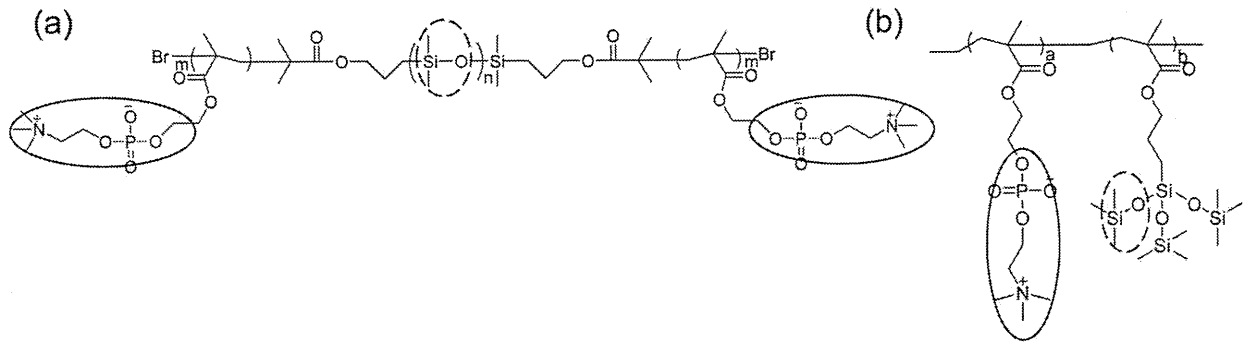
2.9 Cell adhesion test

The adhesion test of L929 fibroblasts (RCB 0081, Cell Bank, Japan) was conducted on the modified PDMS elastomer. Cells were grown for each PDMS substrate in 1 mL (3.5 × 104 cells mL^{-1}) of the minimum essential medium (Gibco BRL Life Technologies, Eragny, France) supplemented by 10% fetal bovine serum (FBS). All the samples were stored in a 100% humidified incubator at 37 °C with 5% CO_2 for 2 days. After then, all the PDMS elastomers were observed using an optical microscope (Olympus Optical Co. Ltd., Tokyo, Japan).

3. Results and discussion

In this research, two types of copolymers containing MPC and DMS units were designed to determine the optimal molecular structure of the surface modifier. Scheme 1 shows the molecular structure of block- and random-type copolymers composed of MPC and DMS groups. MTS in random-type copolymer contains three DMS groups with a methyl chain at the end. On the basis of their DMS unit compositions, the synthesized copolymers were classified into three categories, namely, less than 30%, from 30 to 70%, and over 70% of DMS units, respectively. Table 1 shows the resulting molecular profile of the synthesized copolymers. As shown, each of the three different compositions of the block- and random-type copolymer were synthesized by atom transfer radical polymerization and conventional radical polymerization. Copolymers containing less than 70% DMS were very well dissolved in ethanol. However, the solution containing B3 which contains more than 70% DMS, was a slightly opaque solution; this is possibly due to aggregation of polymer chains. In the case of R3, most of the polymer was clearly dissolved in ethanol and a small amount of undissolved polymer settled down in the container. This is possibly due to limitations intrinsic to conventional radical polymerization. Since the growth rate of AIBN-mediated polymerization is considerably rapid and non-controllable, some part of the polymer chain may contain excess amount of MTS as compared to that in the feed ratio; these types of extreme hydrophobic chains might settle down in ethanol. In any case, the R3 solution was filtered to remove precipitates prior to its application in the characterization or coating process.

The wettability of the PDMS elastomer in the polymer solution was estimated. In this study, the contact angle between each polymer solution and the PDMS surface was monitored to verify whether the polymer solution promotes wettability. Fig. 1 shows the monitored contact angle for each polymer solution. In the case of a pure ethanol drop, the initial value of the contact angle with the PDMS surface was maintained throughout the entire contact time; this indicates that wettability does not improve with contact time. On the other hand, for both block- and random-type copolymer solutions, the contact angle gradually decreased from its initial value, indicating that the wettability of PDMS surface gradually improves with contact time. Because all copolymers are amphiphilic owing to the presence of the hydrophobic DMS and hydrophilic MPC units, they probably act as a surfactant at the hydrophobic interface of the PDMS elastomer in a polar solvent in a time-dependent manner. In the case of a block-type copolymer solution, the initial contact angle



Scheme 1 Molecular structure of (a) ABA-type block copolymer (A: PMPC, B: PDMS) and (b) random type poly(MPC-*co*-MTS).

of B2 and B3 has an even higher value than that of pure ethanol, whereas B1 shows almost the same value as the solvent. It is considered that the large amount of DMS in B2 and B3 results in the macromolecular aggregation in the polar solution. Therefore, most of the polymer aggregation in B2 or B3 might possess the hydrophilic PMPC block segment as an outer shell; this aggregation is probably the reason for B2 and B3 to experience a more repulsive initial contact at the hydrophobic PDMS surface than the pure solvent. On the other hand, B1 shows the smallest contact angle as compared to B2 and B3 throughout the entire contact time. Since B1 containing 11% of DMS block segment in the middle of the polymer chain, this relatively small amount of hydrophobic portion might be contributed to the hydrophobic contact with a large area of PDMS surface rather than forming a macromolecular aggregation to each other. A reverse phenomenon was observed in the case of random-type copolymer solutions (Fig. 1b). At the initial contact time, only the R1 solution shows a higher contact angle than the pure ethanol solvent, which indicates a more repulsive initial contact with the PDMS surface. Because a random-type copolymer does not contain a large hydrophobic portion, only parts such as the DMS block segment in the block-type copolymer, it is thought that the hydrophobic interactions with the PDMS surface have to be considered in a point of overall polarity of random-coiled polymer chains in the solution. Since R1 contains the lowest composition of the DMS unit, its polarity may be the highest among the random-type copolymers; this high polarity is thought to induce the initial repulsive contact with the PDMS surface. In general, it was confirmed that all copolymers promote the wettability of the polymer solution at the PDMS interface in a time-dependent manner. Further, the minimal composition of DMS in the block-type copolymer promotes the initial wettability of the polymer solution whereas that in the random-type copolymer produces an opposite effect.

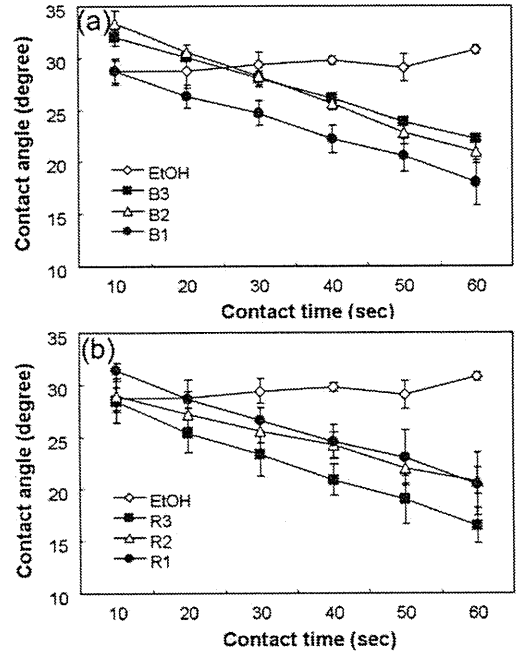


Fig. 1 Contact angle between the PDMS surface and the ethanol drop containing (a) 30 mg mL^{-1} of block copolymer and (b) 30 mg mL^{-1} of random copolymer.

Table 1 Molecular profile of synthesized copolymers

Symbol	Unit Composition (%, NMR) MPC	DMS	Solubility in Ethanol (30 mg ml^{-1})	Mn (× 10^3, SEC)	(× 10^3, NMR)	PDI (SEC)
B1	89	11	○	26.4	39.3	1.23
B2	53	47	○	23.0	23.1	1.35
B3	24	76	△[a]	[c]	33.9	[c]
R1	88	12	○	107		2.60
R2	41	59	○	185		2.57
R3	14	86	○[b]	209		1.96

[a] Opaque solution. [b] Partially opaque. [c] Calculated by NMR due to the solubility problem.

The PDMS elastomer was coated by the immersing method using the polymer solution. In order to disregard the effect of low concentration, all the polymer solutions were prepared with a sufficiently high concentration (30 mg mL^{-1}). The changes in the surface properties on immersing in the polymer solution were estimated by confirming the changes in a hysteresis loop during three successive immersing processes (Fig. 2). In the case of pure ethanol, the hysteresis loop shows no changes during the repeated immersion process, which indicates no significant conformation changes on the PDMS surface. In contrast, for copolymer solutions, the hysteresis loop showed a significant change at the first cycle of the immersion process, indicating that the surface property changed with the immersion into the polymer solution; this change is possibly induced by the adsorption of polymer molecules on the PDMS surface. Further, the hysteresis area was significantly decreased for each copolymer solutions. This indicates that the ethanol solvent feel equilibrated state between the treated PDMS surface and the polymer solution because of the enhanced wettability on the PDMS surface.

In order to estimate the coating efficiency and the stability in water, the surface element of treated PDMS was analyzed by XPS before and after washing with water. The resulting atomic ratio is shown in Fig. 3. For the PDMS surfaces coated with a block-type copolymer, only a little amount of MPC polymer was detected in the case of B1 immediately after coating, whereas a large amount of coated polymer was detected in the case of B2 and B3. Although B1 solution increased the wettability of the PDMS elastomer, it did not provide a stable coating from the ethanol solution. This indicates that in addition to the wettability of the substrate, its affinity with solvent must be considered when designing a coating agent. On the other hand, a large amount of polymers were coated from ethanol on PDMS elastomer in the case of B2 and B3 solutions. This indicates that the polarity of B2

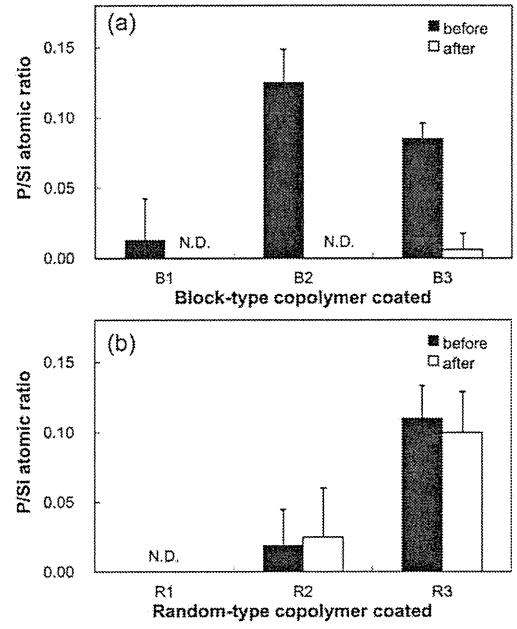


Fig. 3 P/Si atomic ratio of PDMS surface coated with (a) 30 mg mL^{-1} of block copolymer and (b) 30 mg mL^{-1} of random copolymer for 3 min. This ratio is determined before and after washing the surface with water.

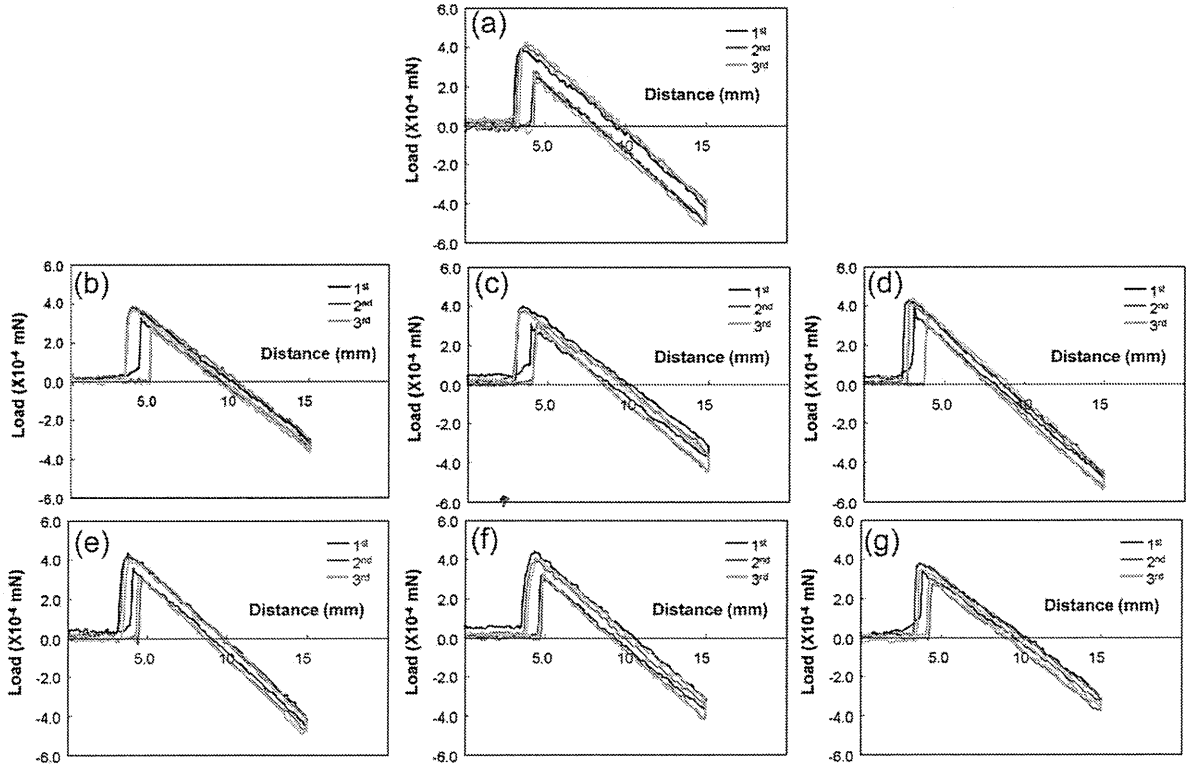
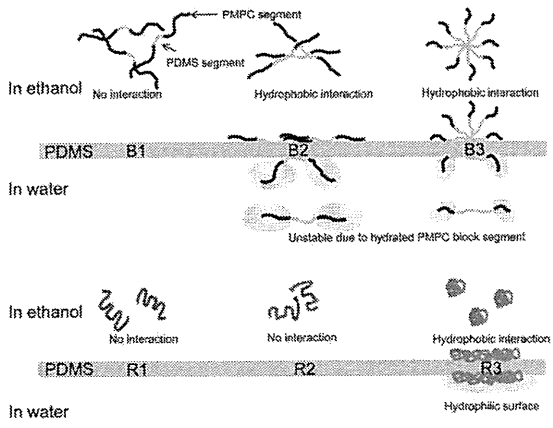


Fig. 2 Changes in hysteresis loop during three successive immersions in (a) pure ethanol and in 30 mg mL^{-1} of (b) B1, (c) B2, (d) B3, (e) R1, (f) R2, and (g) R3 solutions.

and B3 was hydrophobic enough to overcome the affinity with solvent for coating on the PDMS surface. Even though both B2 and B3 show a good coating efficiency from a polar solvent, the stability in the aqueous media was not satisfied. After the PDMS surfaces were thoroughly washed with water, most of the block-type copolymer was removed from them. Because both B2 and B3 did not show good solubility with water (turned opaque), the result has to be considered with respect to molecular structure. It is thought that the PMPC block segment on the PDMS surface is heavily hydrated when the substrate is immersed in water.[15] Therefore, a strongly hydrophobic PDMS surface might easily repel the PMPC block segment in water; this repulsion is probably the reason for the instability of block-type copolymers on the PDMS surface (Scheme 2). In all cases, a block-type copolymer synthesized in this research did not seem to be a suitable molecular design for developing a stabilized surface modifier of the PDMS elastomer. Fig. 3b shows the atomic ratio of the PDMS surface coated with random-type copolymers. Obviously, R1 and R2 were not effectively coated on the PDMS surface in ethanol, possibly for a similar reason as that in the case of B1. On the contrary, a large amount of R3 was coated on the surface right after the coating process and most of the R3 molecules were retained even after the surface is thoroughly washed with water. This indicates that a higher content of hydrophobic DMS in a random-type molecular structure is a key factor in maximizing the coating efficiency of the polar solvent.

In order to confirm the effect of the polymer concentration on the coating efficiency, R3 in various concentrations was tested as a coating solution. Fig. 4a shows the result of the P/Si atomic ratio measured after water washing. Clearly, it was confirmed that the amount of coated polymer was almost saturated when the polymer concentration was more than 5 mg mL^{-1}, and a slightly increased amount of P/Si was detected along with the concentration increase. This result indicates that the concentration of the polymer solution plays an important role in completely coating the PDMS surface with the R3 polymer solution. The effect of the immersing time in the 30 mg mL^{-1} R3 polymer solution was also investigated to obtain the optimized coating condition; the resultant P/Si atomic ratio is shown in Fig. 4 b. From the result, there was almost no coating effect when the immersion time was 10 s. However, the P/Si atomic ratio was



Scheme 2 Schematic explanation of interactions between solvent-copolymers and PDMS substrate.

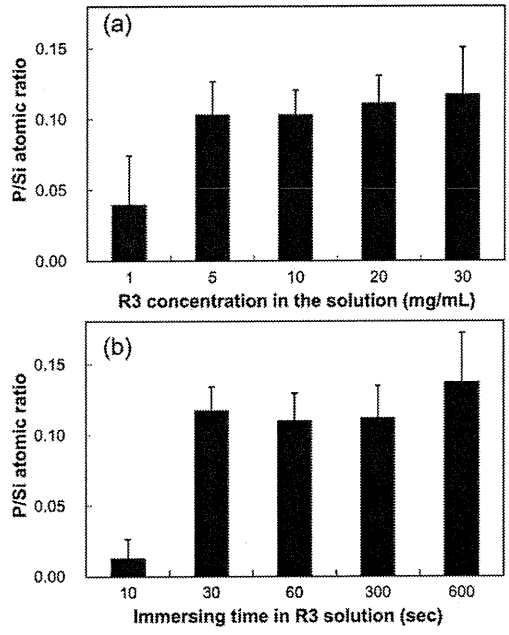


Fig. 4 P/Si atomic ratio of PDMS surface coated with different coating conditions. (a) Coated for different concentrations of R3 solution and (b) coated for different immersion time in a 30 mg mL^{-1} R3 solution after washing with water.

almost saturated when the coating time was over 30 s; this indicates that a large amount of polymer adsorption onto PDMS surface requires an immersion time of more than 10 s. In general, hydrophobic surfaces are completely covered with a large amount of protein because of hydrophobic interactions within a few seconds in PBS. The kinetics of this sort of interaction is generally controlled by physicochemical factors such as temperature, pH, surface charge, or ionic strength, *etc.*[23] Since random-type copolymer adsorption onto PDMS surface is considered due to hydrophobic interactions, quite similar to that of the protein adsorption process, this time-dependent coating efficiency could possibly be varied by controlling the above-mentioned physicochemical factors in ethanol.

A topological analysis of the coated PDMS surface was carried out by conducting AFM observations in water; the results are shown in Fig. 5. All the PDMS elastomers were immersed into the polymer solution for more than 10 min in order to prevent insufficient coating induced by the abovementioned time-dependent properties. The non-coated PDMS surface is flat, as expected. In the case of the PDMS surface coated with 1 mg mL^{-1} R3 solution, only a limited amount of polymer adsorption was observed on the surface. This clearly indicates that the immersion time is not a sufficient factor for completely coating the PDMS surface; this result is in good agreement with the XPS data. When the PDMS elastomer was coated with the 5 mg mL^{-1} R3 solution, the overall PDMS surface was covered with the polymer, as shown in Fig. 5 c. However, a hole-like surface indentation was commonly observed on the coated surface; this indentation is possibly due to the polymer concentration being insufficient to completely coat the surface. On the other hand, The PDMS surface coated with the 30 mg mL^{-1} polymer solution was observed to be almost completely covered; this result once again confirms the XPS result, *i.e.* 30 mg mL^{-1} polymer solution

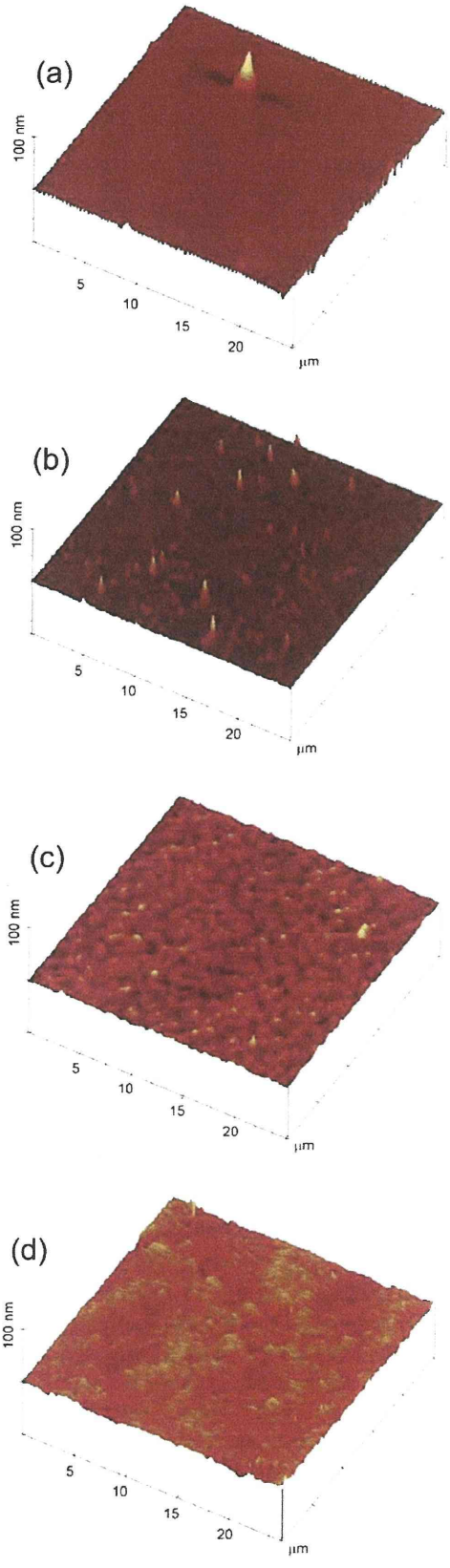


Fig. 5 AFM topological image of (a) bare PDMS surface and of a PDMS surface coated with (b) 1 mg mL^{-1}, (c) 5 mg mL^{-1}, (d) 30 mg mL^{-1} R3 solution.

was the optimized concentration. It is considered that the large amount of protein adsorption is mainly due to hydrophobic interactions between the amino acid residue and hydrophobic surfaces. Other physicochemical factors such as surface charge or ionic strength also have a considerable effect on the protein adsorption behavior.[24] However, the zwitterionic phosphorylcholine group and the DMS units are electrically neutral and the ionic strength of PBS is not changed in any of the experimental steps in this research. Thus, in this study, the degree of hydrophilicity was primarily considered as an important factor in suppressing the protein adsorption. The hydrophilicity of the coated surface was investigated by measuring the air bubble contact angle in water; the result is shown in Fig. 6. The bare PDMS surface shows an air bubble contact angle of approximately 80°, which indicates strong hydrophobicity in water. When the PDMS surface was coated with the 1 mg mL^{-1} R3 solution, the hydrophobicity of the PDMS surface did not improve significantly, even though the air bubble contact angle was increased slightly. As confirmed from the AFM topological images, a large area of the bare PDMS surface still remained when the surface was coated with the 1 mg mL^{-1} R3 solution. The coated surface remains a hydrophobic surface possibly because of the presence of this residual bare surface. In contrast, PDMS surfaces coated with R3 solution with a concentration of over 5 mg mL^{-1} show significantly improved hydrophilicity, and the degree of hydrophilicity was almost saturated when the polymer concentration was over 5 mg mL^{-1}. The results of the surface elemental analysis and AFM also indicated that the minimal concentration of R3 solution required for the complete coating of the PDMS surface was 5 mg mL^{-1}. This indicates that once the surface is covered with the polymer, its hydrophilicity is not significantly improved even when the polymer concentration is increased.

Fig. 6 also shows the relative amount of protein adsorbed on each coated surface, as calculated by the micro-BCA™ experimental method. When the surface was coated by the 1 mg mL^{-1} R3 solution, the amount of protein adsorption was decreased to 60% of the bare PDMS surface. The amount of adsorbed protein continuously decreased with the increasing polymer concentration. The surface coated with the 30 mg mL^{-1} polymer solution could suppress approximately 70% of the adsorption on the bare PDMS surface; this value was slightly above the detection limit of micro-BCA™. Because the surface elemental analysis data and the subsequent hydrophilicity of the coated surface was almost saturated for polymer concentrations of over 5 mg mL^{-1},

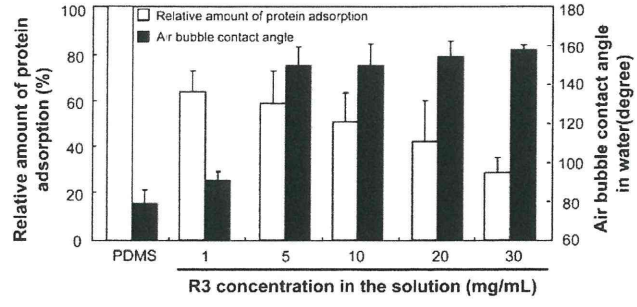


Fig. 6 Air contact angle in water and relative amount of protein adsorption on each PDMS surface. Coating time: 10 min.

it was expected that the amount of protein adsorption could also be minimized from the surface coated with the 5 mg mL^{-1} R3 solution. However, the amount of protein adsorption on the surface with 5 mg mL^{-1} solution is almost twice that on the surface coated with the 30 mg mL^{-1} solution. This indicates that in addition to the surface hydrophilicity, other factors such as surface morphology observed by AFM, must be considered for optimizing the physical coating process as previously discussed in other literature.[9] Even though the surface elemental analysis and the hydrophilicity of coated surface is almost saturated for polymer concentrations over 5 mg mL^{-1}, a hole-like surface indentation was commonly observed on the coated surface with the 5 mg mL^{-1} as described above in the AFM images. It is very well known that significant protein adsorption is detected even on hydrophilic polymer surfaces.[25] This type of adsorption is thought to be due to geometrical factors of polymer surfaces, such as chain mobility, intermolecular distance, or interstitial vacancies.[9] Primary protein adsorption is mainly dominated by the intermolecular distance between coated polymers. Based on the AFM observation, considerable primary adsorption might easily occur on the PDMS surface coated with the 5 mg mL^{-1} R3 solution rather than that coated with a 30 mg mL^{-1} R3 solution.

One of the major applications of PDMS in advanced bioengineering field is to fabricate the microfluidic chip. To enhance the performance of the microfluidics, non-specific adsorption of biological components should be avoided. In order to confirm the coating efficiency of the designed polymer, the PDMS microchannel was modified using the R3 solution based on the condition discussed in the above result. Fig. 7 shows the fluorescence microscopy images obtained after the protein adsorption test using the FITC-labeled BSA solution. Obviously, the bare PDMS channel shows a significant amount of protein adsorption on its surface. On the other hand, the amount of protein adsorption was significantly decreased almost to the background level when the channel was coated with 30 mg mL^{-1} R3 solution. This indicates that coating with R3 solution at a suitable concentration effectively suppresses the protein adsorption in PDMS-based materials such as a PDMS microchannel.

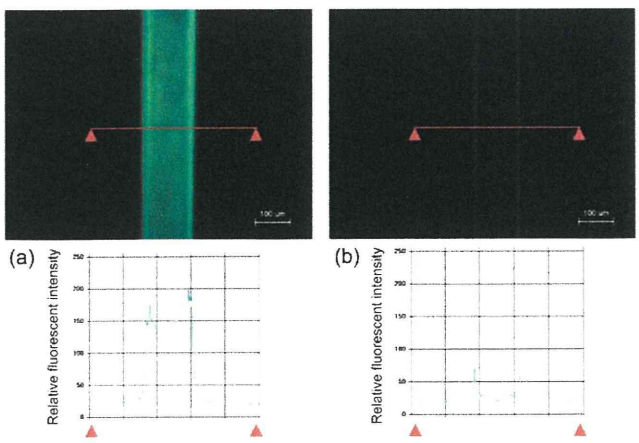


Fig. 7 Fluorescence microscopy image and relative fluorescence intensity of (a) bare PDMS micro-channel and of the PDMS microchannel coated with (b) 30 mg mL^{-1} R3 solution, obtained after the FITC-labeled BSA adsorption test.

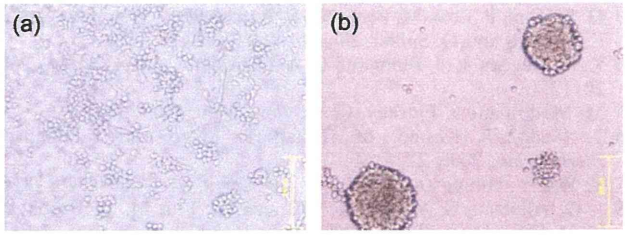


Fig. 8 Optical microscopy image of adhered cells on (a) bare PDMS surface and (b) PDMS coated with R3 (30 mg mL^{-1}, 3min) solution.

The cellular responses on the PDMS surface also play an important role in designing various types of biomedical devices such as cell-based drug screening systems or oxygen-permeable cell analysis systems.[26] Therefore, uncontrollable cellular attachment on the material surface must be prevented by proper surface modification. Fig. 8 shows the optical microscopy images of the PDMS substrates after performing the L929 cell adhesion test. Contrary to the significantly adhered cells on a bare PDMS surface, no adhered cells were observed on the PDMS surface treated with R3. Instead, we could observe cell aggregates flowing on the surface which is normally observed cell morphology on MPC polymer treated surface. The primary phenomenon required for cell adhesion on the material surface is a large amount of protein adsorption.[27] Since the R3-treated PDMS surface shows a significantly decreased amount of adsorbed proteins (Fig. 6), non-specific cellular adhesion could be effectively suppressed by MPC polymer coating.[28,29]

3. Conclusion

Modification of the surface of a PDMS elastomer in a simple and efficient manner was investigated by designing various compositions of block- and random-type MPC copolymers. The level of the hydrophobic DMS unit as a random-type component should be high (>70%) in order to stably immobilize the polymer on the surface in aqueous media. The immersion time and polymer concentration were also found to be important factors for realizing the optimal coating condition for suppression of large amount of protein adsorption and for inducing non-cell adhesive properties on the PDMS surface. Namely, molecular structure, unit composition, immersing time, and concentration of polymer solution must be comprehensively considered in optimizing the coating process on the PDMS surface.

4. Acknowledgments

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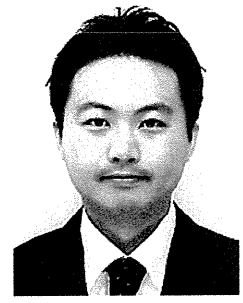
MPCポリマー処理架橋ポリエチレン人工関節の実用化



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1. はじめに

関節は運動機能を支える重要な器官であり，外傷や疾患による関節機能障害は日常の生活動作に大きな支障をきたす。人工関節置換術は，機能障害をきたした関節を人工関節に置き換える手術である。高齢化が進む現在，患者の痛みを取り除き運動機能を回復させる治療法として，手術件数は飛躍的に増加している[1]。しかし，医療技術の進歩と社会基盤の整備などで平均寿命の延伸が進んでいる一方で，人工関節の耐用年数（寿命）は一般に約15～20年といわれており，手術を受けた患者は，生涯のうちに人工関節の入れ換えの手術（人工関節再置換術）が必要となる可能性がある。特に中年期以前の患者の場合，複数回の再置換術が予測されることから，他の治療を受けながら，ある程度高齢になるまで疼痛や日常生活動作障害を我慢して過ごす場合も見られる。しかし，最近では患者の価値観や生活の質が尊重されるようになり，40歳代後半から50歳代でも，快適な生活を送るための一手段として人工関節置換術が選択されることも少なくない。したがって，活動性が高く充実した生活を実現するための人工関節の果たす役割は大きく，その長寿命化が期待されている。

人工関節の耐用年数は，患者の活動度や体重など，様々な要因で規定されるが，材料の側からは，関節摺動面から生じるサブミクロンサイズの摩耗粉が引き起こすインプラント周囲の骨溶解（osteolysis）と，これに続発する弛み（loosening）が主因の一つとされている[2]。骨溶解は摩耗粉発生数に依存する現象であることが明らかとなっており，人工関節の長寿命化を図るには，関節摺動面からの摩耗粉の産生を減らして弛みを阻止することが重要である。したがって，関節摺動面を構成する超高分子量ポリエチレン（PE）や金属・セラミックスの改良，摺動面材料の組み合わせの変更など，様々な研究が行われてきたが，根本的な解決策は得られておらず，可及的速やかに解決すべき課題である。

そこで，筆者らは，2-メタクリロイルオキシエチルホスホリルコリン（MPC）ポリマーを架橋PE（CLPE）臼蓋ライナー表面にナノメートルスケールでグラフトするバイオミメティクスの研究から生まれた革新的な技術“Aquala® technology”による人工股関節用臼蓋ライナー（MPC処理CLPE）を開発した[3)~5)]。MPCは，細胞膜を構成するリン脂質分子に着目し分子設計されたメタクリル酸エステルで，石原らが大量合成法を確立した。このMPCを重合して得られるMPCポリマーを用いて基材表面を処理すると，容易に人工細胞膜構造を構築できる[6)]。この表面は優れた抗血栓性および組織適合性を発揮するとともに，親水性であることから，水の薄膜層を形成する。これらの特性を利用した様々な医療デバイス（血管拡張ステント，ソフトコンタクトレンズ，人工肺，埋め込み型人工心臓）が開発されており，既に国内外で承認を受け臨床使用されるなど，MPCポリマーの生体内での機能性の確認，安全性の担保はなされている。

2. 生体関節軟骨の模倣

生体の関節軟骨の表面では，ヒアルロン酸，プロテオグリカンなど凝集体がブラシ構造を持つゲル層を形成し，この表面ゲル層が水和潤滑という機能により良好な摩擦・摩耗特性を示すと考えられている（図1）[7)]。また，関節軟骨の最表面にはナノメートルスケールのリン脂質分子層が存

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