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Constructing Biomembrane-mimic Interface for Cell Separation in Microfluidic Chip

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Protein and cell adsorptions on the microfluidic surface are serious problems of the cell separation chip, especially in quartz/glass chip for separating different cells from a heterogeneous sample such as blood. The phospholipid polymers containing 2-methacryloyloxyethyl phosphorylcholine (MPC) units are a kind of biomembrane mimic polymers with regulation properties of biological components such as proteins and cells. In this research, we synthesized a negatively charged MPC polymer, PMBSSi, composed of MPC, n-butyl methacrylate(BMA), potassium 3-methacryloyloxypropyl sulfonate (PMPS) and 3-methacryloxypropyltrimethoxysilane (MPTMSi), to improve the function of PMSi composed of MPC, and MPTMSi, which was synthesized previously. The ξ- potential of PMBSSi coated surface showed -24.2±2.5mV (in pH7). It is a quite large value than that of the PMSi coated surface. The stability of PMBSSi coating was good for long time rinsing around 45hrs by the creation of chemical bonding between MPC polymer and SiO_2 substrate. The coated surface was effective for suppressing protein adsorption, which is due to the orientation of phosphorylcholine unit on the surface. The result suggests that PMBSSi has excellent property on anti bio-adsorption even though surface has negative charge.

Key words: negatively charged MPC polymer, quartz/glass, microfluidic, cell separation

1. INTRODUCTION

Quartz and glass are attractive and popular substrate materials for fabrication of microfluidic devices, because of their excellent optical and high insulating properties, and characteristics of inertness towards various solvents [1]. However, protein and cell adsorptions are serious problems when quartz and glass are used as substrates of cell separation chips, especially those for separating different cells from a raw and heterogeneous sample such as blood [2].

It is well believed that surface coating is the most feasible and hopeful strategy to create anti-adsorption surface in microfluidics. Current methods of quartz/glass coating include hydrophilic polymer coating [3, 4], gold nanoparticles coating [5], silanization[6,7] and polymer grafting[8]. These methods are usually transferred from the surface chemistry of classical capillary electrophoresis and are mainly applied in capillary electrophoresis on microchips for protein and DNA analysis. Regarding to the cell separation chip, there is not so much information. Moreover, these methods more or less have some drawbacks, for example, the modification process is complex and laboring, or the reduction of adsorption is not sufficient, or the modification is just dynamic not permanent and need recovering after operation.

The phospholipid polymers containing 2-methacryloyloxyethyl phosphorylcholine(MPC) units are a kind of biomembrane structure mimic phospholipid polymers with regulation properties of biological components such as proteins and cells in various surface modifications[9,10,11].

In our previous study, for constructing a permanent and anti bio-adsorption interface for a cell separation chip fabricated on the quartz/glass, a MPC polymer, PMSi shown in Fig.1a, composed of MPC, 3-methacryloxypropyltri-methoxysilane(MPTMSi), was molecular designed and synthesized to coat the quartz/glass surface. In the polymer, the MPC unit is for suppressing the protein and cell adsorption on the substrates, while the MPTMSi unit is a silane coupling agent, which can be chemically bonded with SiO_2 surface [2].

Another problem in microfluidic cell separation chip is electroosmotic-flow (EOF) control. EOF is the most successfully applied micropump in many applications to drive microflow [12, 13]. However, to achieve high pressure, the EOF pump requires high voltage, usually several kV, which might result negative effect on the living cells, when it is used in the cell separation chip [14]. The relationship of pressure parameters of EOF pump is usually approximately described as Eq.(1) [14], where ΔP is the pump pressure, ξ is the surface zeta potential, ε_r is the specific dielectric constant, ε_0 is the dielectric constant in vacuum, E is the electric field intensity, and L

is the capillary length, r is the capillary diameter. Clearly, the pressure is directly proportional to the surface zeta potential ξ.

$$\Delta P = 8\xi\varepsilon_r\varepsilon_0 EL / r^2 \quad (1)$$

Thus, theoretically, increasing surface ξ-potential is an effective way to achieve high EOF pressure pump with low-voltage. From this view, derived from PMSi, we newly designed a negatively charged MPC polymer PMBSSi, composed of MPC, n-butyl methacrylate (BMA), potassium 3-methacryloyloxypropyl sulfonate (PMPS) and MPTMSi. Since the PMPS unit of PMBSSi has anionic group, which can adjust the ξ-potential of the modified surface, the function of PMSi coating was improved to obtain minus ξ-potential.

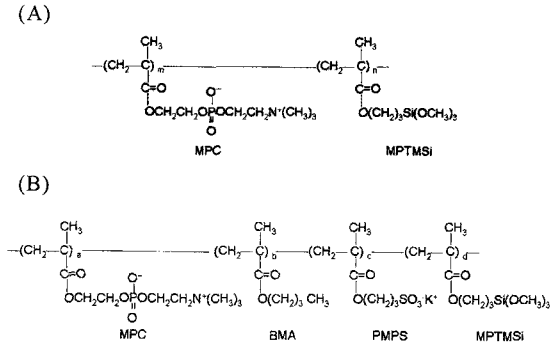

Fig.1 The chemical structures of (A) PMSi and (B) PMBSSi

2. EXPERIMENTAL

2.1 Materials

MPC was synthesized by the method reported before [15]. BMA (Kanto chemicals, Japan) was reagent grade and used after vacuum distillation (bp68.5℃/32mmHg). MPTMSi (Kanto Chemicals) and PMPS (Tokyo Kasei Kogyo Co., Japan) were used without further purification. α, α'-Azobis- isobutyronitrile (AIBN) was purchased from Kanto chemicals. Fluorescein isothiocyanate conjugated (FITC) - albumin was purchased from Sigma-Aldrich. Dulbecco's Phosphate- Buffered Saline (pH7.1, Invitrogen Co.) was used. 20×20×1.1 (mm) Pyrex glass plates (Toshin Riko, Japan) and 20×20×0.5(mm) quartz plates (Sendai Quartz &. Glass, Japan) were used.

2.2 Synthesis of MPC copolymer

Poly(MPC-co-BMA-co-PMPS-co-MPTMSi) (PMBSSi, Fig. 1b) was synthesized by a conventional radical polymerization technique. The desired amounts of MPC, BMA, PMPS, and MPTMSi were placed in a polymerization tube. AIBN and ethanol were added to the tube as the polymerization initiator and solvent, respectively. After the tube was sealed, the copolymerization was carried out at 60 ℃ for 15h. The polymer was recrystallized from ether/chloroform (7/3, v/v) solution and dried in vacuo. Poly (MPC-co-MPTMSi) (PMSi, Fig.1a) was synthesized as the same method with MPC and MPTMSi. The structures of two polymers were identified by ^{1}H NMR (table I).

Table I Synthesis and Characterizationof MPC polymers

Abb	Mole fraction [MPC/BMA/PMPS/MPTMSi]		Yield (%)	Solubility	
	in feed	in copolymer[a]		H_2O	EtOH
PMSi	90/-/-/10	80.4/-/-/19.6	72	+	+
PMBSSi	50/35/5/10	46/32/9/13	84	+	+

[Monomer]=0.5 mol/L, [AIBN]=5 mmol/L; Polymerization was carried out at 60 ℃ for 15h; +:soluble.
[a] determined by ^{1}H NMR

2.3 Surface coating process

After cleaning by ultrasonic treatment in ethanol solution and O_2 plasma treatment, the plate (quartz or glass) was dipped in the polymer (PMSi or PMBSSi) solution (ethanol as solvent) for overnight. Then taken out and dried under nitrogen condition.

2.4 Surface analysis

The plate (coated or not coated) surface was analyzed by an X-ray photoelectron spectroscopy (XPS, Axis-His, Shimadzu/KRATOS, Japan) to determine the phosphorylcholine and sulfonate unit composition on the surface. The take-off angle of the photo-electron was 90°. The surface morphology was characterized by an atomic force microscopy (AFM, SPI-3800, Seiko Instruments Inc., Japan) using the taping mode with a force constant of 15N/m. The surface ξ-potential was measured in 10mM NaCl solution condition using an electrophoretic light-scattering spectrophotometer (ELS 8000, Otsuka Electron Co., Japan) with a plate cell.

2.5 Coating stability investigation

Four coated plates were fixed by wires and then put in 1000ml beaker with 1000ml PBS (pH7.1) solution under the 300 rpm stirring and keep all the plates at the same rinse condition. The plates were taken out after 5h, 15h, 25h, and 45h rinse. The rinsed plates were analyzed by XPS.

2.6 Protein adsorption test

The plate (coated or not coated) was fully immersed in ion-free water overnight for equilibrium, and then incubated in 0.045g/100ml (PBS, pH7.1) FITC-albumin solution, at 4 ℃ for 1h. After incubation, the plate was rinsed in 1000ml PBS solution (pH 7.1) by stirring method (as described in 2.5)under 300rpm for 5min (repeat once). The plate was dried and the adsorbed protein was checked by fluorescence imaging using an Axioskop 2 optical microscope (Zeiss, Germany). The fluorescence imaging was observed under exposure time, 1/5.5s and 1s, respectively with ISO 200.

3. RESULTS AND DISCUSSION

3.1 Effective coating by simple coating process

Although the MPC copolymers have already successfully applied in various surface modification and achieved fine results in anti adsorption of biological components, the researches on MPC polymer modification in microfluidic systems are just a

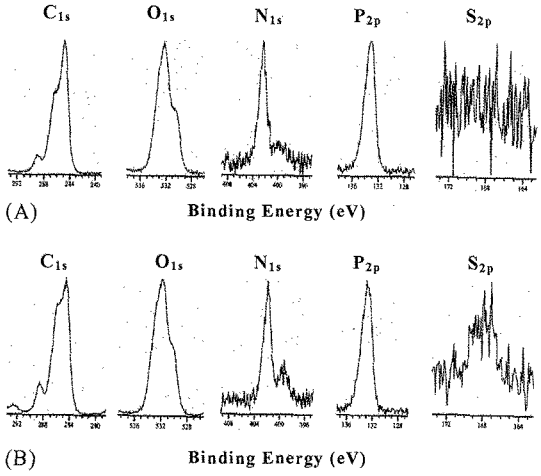

Fig.2 XPS spectra of quartz surface coated by (A). 0.3wt% PMSi and (B). 0.3wt% PMBSSi

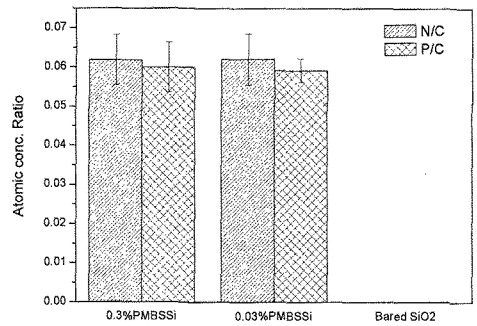

Fig.3 XPS result of atomic concentration ratios of nitrogen vs. carbon(N/C) and phosphor vs. carbon (P/C) on quartz surface coated by PMBSSi with different concentration.

beginning and not sufficient [2,16,17]. Silanized MPC polymers (PMSi, PMBSSi) were designed for quartz or glass substrate microfluidic chip, because they can chemically bond with the SiO_2 surface after hydrolysis [2]. PMSi and PMBSSi were dissolved in water, ethanol and methanol, which make them easily applied to coat the microchannel. The coating process was very simple: for plate coating, only saturating the plate in the coating solution at room temperature is enough for achieve an effective coating (Fig.2); similarly, for microchannel coating, directly irrigating the solution into channels by syringe can also obtain a wholly coated inner surface. Even using really low concentration of 0.03 wt% PMBSSi (its MPC unit composition 46% is far lower than that of PMSi 80%, shown in Table I) solution coating, the phosphorylcholine groups in the MPC units of the copolymer were also proved to be oriented on the quartz surface(Fig.3). The same result was got in glass substrate coating (data not shown). The surface morphology of coated plate and bared plate as observed in Fig.4 was nearly of no difference, so the coating is a homogenous coating and do not change the morphological

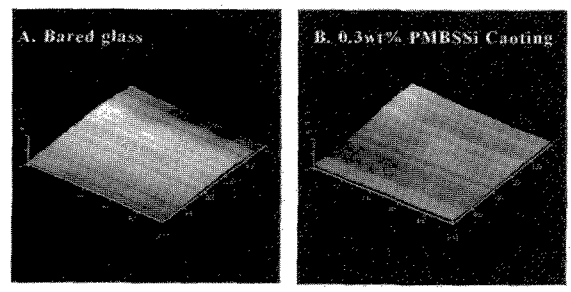

Fig.4. AFM images of bared glass and 0.3wt%.PMBSSi coated glass. The scale bars of z-axis are 40 nm.

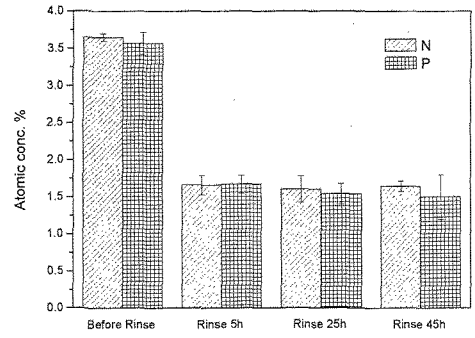

Fig.5 XPS result of atomic concentration of nitrogen (N) and phosphor (P) on glass coated by 0.3wt% PMBSSi in the condition of 300rpm stirring rinse

characteristics of the original substrates.

3.2 Permanent coating

The surface coating can be generally divided into two types, the dynamic coating and permanent coating [1]. In contrast to dynamic coatings, permanent coatings are often regard as the most effective way for reducing the analyte-wall interactions and modify the EOF [1]. We used a stirring rinse method to investigate the coating stability. The shear stress generated by stirring flow in the experiment condition is obviously much stronger than that of microflow in microchannel. The result (Fig. 5, PMSi coating data not shown) shows that the atomic concentrations of nitrogen and phosphor on the coated surface were reduced nearly half by 5 h rinse, but after 5h, the atomic concentrations nearly did not change even after 45h rinse. Thus MPC units in the copolymer still remained on the surface, although experienced a long time rinse with high shear stress flow. It is the silanization groups in both MPC copolymers that are the nature of this stable coating. From this aspect, we conclude that PMSi and PMBSSi coatings are permanent coatings.

3.3 Anti bio-adsorption coating

Albumin is a typical kind of proteins in blood. Without PMSi or PMBSSi coating, the albumin was strongly adsorbed to the quartz and glass surface, yielding bright and homogeneous fluorescence emission even at a short exposure time of 1/5.5s (Fig.6 A). In contrast, with 0.3wt% and 0.03wt% PMBSSi coating (Fig.6 B, C, E), nearly no fluorescence emission was observed under exposure time of 1/5.5s. Even

though under a long exposure time of 1s, only very low fluorescence emission can be observed on 0.03wt% coating plates (Fig 6. D, F), while fluorescence images of 0.3wt% coating had no significant difference between 1s exposure and 1/5.5s exposure. These results indicate that with PMSi or PMBSSi coating, the protein (albumin) adsorption on quartz or glass can be significantly suppressed even by a low concentration of 0.03wt% polymer solution coating.

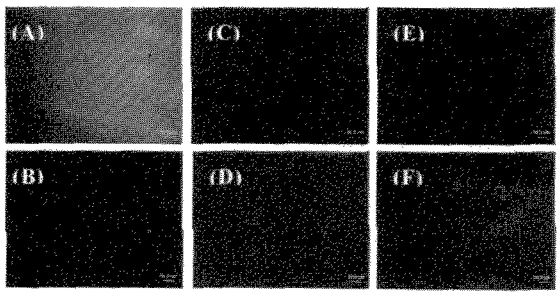

Fig 6 Fluorescence images of glass and quartz surface coated or not coated with PMBSSi after incubating with FITC-Albumin. All the images was observed with exposure time of 1/5.5s except of (D), (F) with exposure time of 1s. (A) bared glass; (B) glass coated by 0.3wt% PMBSSi . (C), (D) glass coated by 0.03wt%PMBSSi; (E), (F) quartz coated by 0.03wt%. All the scale bars are 50 μm

Our previous work [2] showed that sticking density of the human lymphocytes on the capillary coated with the 0.03wt% PMSi was drastically decreased with comparing that on bared quartz. Although the mechanism of cell adhesion is complex, it is generally believed that cell adhesions to the surface are induced by the protein adsorptions. That is to say, if the protein adsorptions are suppressed, the cell adhesions would also be decreased. Thus, the results of anti protein adsorption of PMBSSi coating would give us some positive evidences of property of PMBSSi on anti cell adhesion.

3.4 Coating with minus ξ-potential

As expressed in introduction, initially, the PMBSSi was designed to adjust the surface ξ-potential for achieving high pressure EOF pump with low voltage. The determination showed that ξ-potentials of 0.3wt % PMBSSi and 0.3wt% PMSi coated quartz plates were -24.2±2.5mV and near 0 mV(in pH7), respectively. Consequently, with PMBSSi coating, minus ξ-potential can be achieved in contrasting with PMSi coating. Our previous work [2] using a serials of MPC polymer coating to evaluate performance of a cell separation chip actuated by EOF pump [18] indicated that the MPC polymers with negatively charged would be preferred for improving the cell separation rate. Thus, based on the study we conclude that PMBSSi would be such kind of negatively charged MPC polymer for constructing permanent interface in cell separation chip actuated by EOF pump, which not only can suppress the biological adsorptions, but also can improve the performance of EOF pump and cell separation rate at the same time.

4. CONCLUSION

In this paper, two biomembrane mimic phosphorylcholine polymers (MPC polymers), PMSi and PMBSSi were designed and synthesized for coating the inner surface of quartz/glass cell separation chip. Their coating effects on the quartz and glass surfaces were investigated systematically. As a result, both PMSi and PMBSSi coatings can construct permanent biomebrane-mimic interfaces with properties of suppressing biological component adsorptions on the quartz/glass substrate. In contrast of PMSi coating, PMBSSi coating can create such kind of surface with ξ-potential at the same time further, which will be more potentially applied to the systems actuated by EOF pump, because the pressure of EOF pump increases with the surface ξ-potential increasing.

5. ACKNOWLEDGMENTS

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