Fabrication of Hydrophilic Polymer Brushes on Polystyrene Substrate by Plasma-based Surface Functionalization

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1. Introduction
Polystyrene (PS) is one of most common polymers used in biomaterials because of its optical transparency, good mechanical properties and low production cost, etc. However, due to several intrinsic surface properties of PS including hydrophobicity, the biomaterials made of PS are limited in their biomedical applications. For example, the nonspecific adsorption of biological molecules including proteins on the surface is often observed. In order to improve surface property of PS for biomedical use, plasma treatment has often been used to introduce new oxygen- and/or nitrogen-containing groups on the PS surface without affecting the bulk properties. However, the surfaces obtained by simple plasma treatment have been shown to be most unstable with respect to both washing and aging. As the other methods, covalent immobilization and grafting of hydrophilic polymer such as poly(ethylene glycol) (PEG) and dextran have been examined for reducing nonspecific protein adsorption onto hydrophobic polymer substrate. On the other hand, surfaces covered with bioinert polymer such as PEG may not be enough for some application due to absence of functional groups for further modification. In fact, the development of biosensor requires controlled immobilization of bioactive molecules for specific detection of a target molecule on excellent anti-fouling substrate surface.12

In this communication, we report the synthesis of well-defined poly(sodium acrylate) (pSA) brushes on PS substrate by surface-initiated atom transfer radical polymerization (ATRP) of sodium acrylate (SA) in aqueous media. To introduce the ATRP initiator on PS substrate, the reaction of plasma irradiated PS substrate with N-bromo-succinimide (NBS) was examined for surface bromonation of PS substrate, based on the assumption that alkene structures formed by plasma-induced cross-link reaction on PS substrate are easily brominated by NBS. pSA is water-soluble polymer at physiological pH so that the synthesis of pSA brushes on PS surface would change the hydrophobic PS surface into highly hydrophilic surface. In this work, we examined the effect of experimental conditions on the formation of pSA brushes and surface properties of pSA-grafted PS surface.

2. Experimental
2.1. Plasma irradiation to PS substrate
A commercial polystyrene (PS) petri dish (35 mm in diameter) was used as PS substrate. It was cleaned by ultrasonication in methanol and dried in vacuo at room temperature. Ar plasma irradiation to the washed PS petri dish was carried out using the essentially same apparatus as that reported

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earlier. [13] The plasma state was generated by the use of radio-frequency discharge of inductive coupling with five loop antenna at 13.56 MHz with the prescribed power. Flow volume (50 ml/min) and pressure (66.6 Pa (0.5 Torr)) of argon gas were controlled by flow meter and evacuating speed. The sample was placed in the reaction chamber (230 mm long, 45 mm in diameter) to ensure homogeneous exposure to plasma gas.

2.2. Fabrication of poly(sodium acrylate) brushes on plasma-irradiated PS by ATRP

Figure 1 shows the reaction scheme for ATRP of sodium acrylate (SA) from plasma-irradiated PS substrate. The plasma-irradiated PS surface was brominated with N-bromosuccinimide (NBS). Briefly, 0.12 M NBS in 50% tert-butanol aqueous solution was added into plasma-irradiated PS petri dish and incubated at 25 °C for 24 h. After washing with distilled water under ultrasonication, the surface-brominated PS petri-dish thus prepared was dried in vacuo and then served as an initiator for surface-initiated atom transfer radical polymerization (ATRP) of SA. A solution consisting of [SA]/[CuBr]/[CuBr2]/[N,N,N',N",N"-pentamethyldiethylenetriamine (PMDETA)] feed ratio of 50 (5 M) : 1 : 0.2 : 1.2 in milli-Q water was added into the surface-brominated PS petri-dish under a nitrogen atmosphere and incubated at room temperature for a prescribed period of time. The poly(sodium acrylate) (pSA)-grafted PS (pSA-g-PS) thus prepared was then washed with distilled water under ultrasonication and incubated in distilled water overnight at room temperature.

2.3 Surface characterization

The density of carboxylate ion group on PS surface was determined according to the method reported by Sano et al., based on the assumption that Toluidine Blue O (TBO) was complexed to equivalent moles of carboxylate ion group on solid surface. [14] Chemical bonds in the substrate surface were characterized by measurement of X-ray photo- electron spectroscopy (XPS) (ESCA-3400, Kratos Analytical Ltd., Japan) with MgKα radiation. The x-ray source was operated at 12 mA and 20 kV. The surface hydrophilicity on the substrate was evaluated by measuring the water contact angle based on a sessile drop measuring method with a water droplet ca. 1 mm in diameter.

3. Results and Discussion

XPS measurement was carried out to confirm the bromination of plasma-irradiated PS surface by NBS. Figure 2 shows the survey scan spectra of non-treated PS, Ar plasma-irradiated PS and surface brominated PS (Br-PS), and Table 1 shows the atomic concentration on each surface. After plasma-irradiation, the oxygen concentration on PS surface significantly increased due to the oxidation of plasma-induced surface radicals. In addition, the new bromine peak at ca. 71 eV was observed in Br-PS. These results indicate that the plasma-irradiated PS is successfully brominated with NBS under the present experimental conditions. Separately, we investigated the possibility of bromination of non-treated PS by NBS, but it did not occur on non-treated PS surface. (date not shown) Thus, the surface modification of PS substrate by plasma-irradiation is essential for the subsequent surface bromination of PS substrate with NBS.

The ATRP of SA from surface brominated PS was conducted in aqueous solution at room temperature. Figure 3 shows the C 1s XPS spectra of pSA-g-PS surface, indicating the formation of pSA-grafted layer on PS substrate by the present
method because new peak assigned to C=O bond appears on pSA-g-PS. One of noteworthy results in Fig. 3 was that same spectra were observed in pSA-g-PS surfaces prepared by different polymerization time (Fig. 3 (B) and (C)). This is considered to result from fact that XPS spectra reflect the elemental compositions of upper most layers. To confirm that the polymerization of SA was started from the bromo groups on Br-PS, we also attempted the ATRP of SA from non-treated PS substrate, but the polymerization of SA did not occur. (data not shown) Figure 4 shows the effect of ATRP time on the density of carboxylate ion group on pSA-g-PS. The density of carboxylate ion group increased with increasing ATRP time, indicating that the length of pSA-chain can be well controlled by ATRP time. These results suggest that the chain growth of pSA from Br-PS is well-defined process. Therefore, the result in Fig. 3, that C1s XPS spectra observed in surfaces prepared by different polymerization time are same, means that the both surfaces are evenly covered with pSA-grafted layer even though the thickness of grafted layer are different from both surfaces.

To evaluate the surface hydrophilicity, the water contact angle (WCA) was measured at various pH. (Table 2) At physiological pH (pH 7.4), the hydrophobic PS surface (WCA > 90°) was changed to superhydrophilic surface (WCA < 10°) by grafting pSA chain by the present method. This superhydrophilicity on pSA-g-PS was kept for a long period of time. (data not shown) The observed pH dependence of water-contact angle on pSA-g-PS results from the protonation of carboxylate ion in the pSA-grafted layer at acidic condition, indicating that superhydrophilicity on pSA-g-PS stems from pSA-grafted layer.

4. Conclusion
The surface-brominated PS (Br-PS) was prepared by bromination of Ar plasma-irradiated PS with NBS and it was used as ATRP initiator. The hydrophilic pSA-grafted layer was successfully synthesized on hydrophobic PS substrate.
The surface properties of pSA-g-PS is considered to be of great advantage in preventing non-specific protein adsorption. The present method, thus, should be useful for the surface modification of hydrophobic PS for further biomedical applications.

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References

Table 2  Effect of pH-Values on Static Water Contact Angle of Plasma-irradiated PS and pSA-g-PS Surface

<table>
<thead>
<tr>
<th>sample</th>
<th>water contact angle (degree)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 2.2</td>
</tr>
<tr>
<td>plasma irradiated PS</td>
<td>30.8 ± 1.0</td>
</tr>
<tr>
<td>pSA-g-PS (ATRP: 0.5 h)</td>
<td>31.8 ± 2.8</td>
</tr>
<tr>
<td>pSA-g-PS (ATRP for 2 h)</td>
<td>43.2 ± 3.6</td>
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</tbody>
</table>

* The data represents the mean ± S.D. (n > 3)