Plasma Irradiation to Poly(acrylic acid) Brushes fabricated on Polystyrene Substrate and its Characterization

Yasushi Sasai1, Akihiro Komatsu1, Shin-ichi Kondo1, Yukinori Yamauchi2, and Masayuki Kuzuya3

1 Laboratory of Pharmaceutical Physical Chemistry, Department of Drug Delivery Technology and Science, Gifu Pharmaceutical University, 1-25-4 Daigaku-Nishi, Gifu 501-1196, Japan
2 Department of Pharmaceutical Physical Chemistry, College of Pharmaceutical Sciences, Matsuyama University, 4-2 Bunkyo-cho, Matsuyama, Ehime 790-8578, Japan
3 Faculty of Human Welfare, Chubu Gakuin University, 2-1 Kirigaoka, Sek-shi, Gifu 501-3993, Japan

sasai@gifu-pu.ac.jp

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1. Introduction

Surface properties of polymeric materials play a crucial role in their application. The surface biocompatibility and antifouling property are of fundamental importance in biomaterials, especially contacting with blood and other body fluids. Various methods have been reported to improve surface properties of polymeric materials for the biomedical applications, which include plasma based approaches such as plasma surface treatment and plasma polymerization [1,2], and polymer-grafting [3,4] methods.

Polystyrene (PS) is one of most common polymers used in biomaterials. However, due to several intrinsic surface properties of PS including hydrophobicity, the biomaterials made of PS are limited in their biomedical applications. We have previously reported that the synthesis of well-defined poly(acrylic acid) (pAAc) brushes on PS substrate by surface-initiated atom transfer radical polymerization (ATRP) of sodium acrylate in aqueous media. [5] Figure 1 shows the reaction scheme for the construction of pAAc-grafted layer on PS substrate using ATRP. The resultant surfaces showed superhydrophilicity, which can be kept for a long period of time. It is well known that protein adsorption onto a substrate strongly depends on the surface wettability and the superhydrophilic surfaces significantly suppress protein adsorption from biological sample. [6]

Several researchers have reported that the surface treatment of pAAc films with argon plasmas results in decarboxylation of poly(acrylic acid). [7,8] These results suggest that the protein adsorption behavior on pAAc-grafted PS substrate can be controlled by plasma surface treatment. In this study, we examined the effects of argon plasma irradiation to the pAAc-grafted PS substrate on the surface hydrophilicity and protein adsorption property for the further biomedical applications.

2. Experimental

2.1. Plasma irradiation to pAAc-grafted PS substrate

A pAAc-grafted PS substrate was prepared by the method reported earlier. [5] The polymerization allowed to proceed at room temperature for 5h. Ar plasma irradiation to pAAc-grafted PS substrate was carried out as follows. The pAAc-grafted PS substrate was placed in the reaction chamber (230 mm long, 45 mm in diameter) to ensure homogeneous exposure to plasma gas. The plasma state was generated by the use of radio-frequency discharge of inductive coupling with five loop antenna at 13.56 MHz with supplied power of 50 W. Flow volume (50 ml/min) and pressure (66.6 Pa (0.5 Torr)) of argon gas were controlled by flow meter and evacuating speed.
2.2. Surface characterization

Chemical bonds in the substrate surface were characterized by measurement of X-ray photoelectron spectroscopy (XPS) (Quantera SXM-GS, ULVAC PHI Inc.) with AlKα radiation. The X-ray source was operated at 10 mA and 20 kV. Peak fitting of the C1s spectra was carried out using three component peaks, a hydrocarbon peak at 284.3 eV, a C-O peak at 286.0 eV, and a carboxylic acid peak at 288.3 eV.

The amount of carboxyl groups in pAAc brushes on PS substrate 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 carboxyl group on solid surface. [9]

The surface hydrophilicity on the substrate was evaluated by measuring the water contact angle (WCA) based on a sessile drop measuring method with a water droplet ca. 0.8 mm in diameter.

2.3. Protein adsorption

A bovine serum for cell culture use was used for protein adsorption test. The sample substrates were incubated in 50 % (v/v) bovine serum solution at 37 °C for 5h and then the surface washed with PBS solution. The amount of adsorbed proteins on sample substrate was determined by bicinechonic acid (BCA) assay kit (Thermo fisher scientific, K.K.) using bovine serum albumin as standard.

3. Results and Discussion

3.1. Plasma irradiation to pAAc-grafted PS substrate

The effect of an argon plasma treatment on the chemical composition of pAAc-grafted layer was investigated. Figure 2 shows the XPS spectra of the C1s region of pAAc-grafted PS substrate before and after Ar plasma-irradiation for 30s. The peak at 288.3 eV, assigned to C=O bond in carboxylic groups of pAAc brushes, almost disappeared by argon plasma irradiation for 30 s. Figure 3 shows the progressive changes in the density of carboxyl groups, determined by XPS and TBO method, as a function of plasma irradiation time. It can be seen that most of the carboxyl groups are removed from the pAAc-grafted PS substrate by argon plasma irradiation for a short time, indicating that argon plasma irradiation can cause the efficient decarboxylation of pAAc brushes. This decarboxylation has been experimentally proved to be caused by vacuum UV radiation contained in plasma. [8]

3.2. Effect of plasma irradiation to pAAc-grafted PS on the protein adsorption property

Surface hydrophilicity of materials is one of the most important factors determining protein adsorption to the surface. Figure 4 shows the effect of argon plasma irradiation on surface hydrophilicity (A) and protein adsorption property (B) of pAAc-grafted PS substrate. The value of WCA on
pAAc-grafted PS was less than 10°, meaning that the surface of PS substrate changed to super-hydrophilic due to the introduction of pAAc-grafted layer.

The plasma irradiation to pAAc-grafted PS caused the significant increase of WCA as shown in Fig. 4(A). However, the WCA was almost leveled off at ca. 40° by plasma irradiation for over 30s under the present plasma conditions. It can be considered that this moderate hydrophilicity on plasma irradiated pAAc-grafted PS results from the removal of carboxyl groups from pAAc grafted layer and the oxidation of plasma-induced radicals. In fact, the fraction of C-O on plasma-irradiated pAAc-grafted PS, determined by deconvolution analysis of the C1s spectra, increased from ca. 6% to ca. 15% by plasma irradiation for 30s and then leveled off by further plasma irradiation.

As can be seen in Fig. 4, the protein adsorption property onto pAAc-grafted PS was closely related with the surface hydrophilicity. The protein adsorption onto pAAc-grafted PS before plasma irradiation was negligible, but after plasma irradiation for over 30 s the amount of adsorbed protein onto the surface from serum solution was significantly increased due to loss of super-hydrophilic nature on the surface. These results show that the simple and short plasma irradiation can dramatically change the surface properties of pAAc-grafted PS and control protein adsorption to the substrate surface.

4. Conclusion

In this study, the effects of argon plasma irradiation on pAAc-grafted PS substrate were investigated. The argon plasma irradiation caused the efficient decarboxylation in pAAc brushes so that the surface hydrophilicity was significantly changed. The protein antifouling properties on pAAc-grafted PS substrate were lost even by short plasma irradiation so that the surface was changed to highly favorable for protein adsorption. These results are useful for controlling the protein adsorption onto pAAc-grafted materials and expected to be used for the development of microarray such as protein and cell array using pAAc-grafted PS as a substrate.

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