

Cell adhesion behaviors on polyethylene terephthalate surface modified by surface-wave plasma-initiated graft polymerization

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ABSTRACT

Hydrophilic modification of polyethylene terephthalate (PET) was successfully achieved by surface wave plasma treatment followed by graft polymerization with acrylic acid (AA) monomer. The graft reaction was confirmed by water contact angle measurements and X-ray photoelectron spectroscopy (XPS). 3T3 fibroblast cells were cultured on the AA-modified PET surface. The protein adsorption behaviors were also investigated using an optical waveguide spectroscopy.

Keywords: Poly(ethylene terephthalate) (PET), Surface modification, surface-wave plasma, hydrophilicity, cell adhesion

1 INTRODUCTION

Polyethylene terephthalate (PET) film is widely used in a variety of applications such as bottles for beverages and containers because it has some excellent material properties, such as a high melting point and high tensile strength; other characteristics include very good barrier properties, crease resistance, solvent resistance, and resistance to fatigue. In addition, because of its biocompatibility and good mechanical properties, PET has found new developments in the field of medical devices, such as surgical suture material, tendon and ligament replacement material [1], and drug/cell delivery system [2]. However, one main issue for biomedical applications of PET concerns low surface hydrophilicity. This affects wettability, biocompatibility, adhesion, and various other surface treatments. Among them, biocompatibility is very important for biomedical applications of PET film, since it greatly affects on proteins adsorption and cell cultures. Thus, controlling the hydrophilicity of the PET surface is crucial for the biomedical applications. In order to impart hydrophilicity to the PET surface, many methods have been developed and used commercially. Among them, plasma treatment is a promising means of enhancing the hydrophilicity of the polymer surface [3–5], because it can activate the molecular layer of the surface without affecting the bulk of the polymer. Moreover, Plasma treatment also leaves active

sites on the surfaces, which are subject to the post-reactions. [6]. However, the surface modification by the plasma process faces two complications. One is damage of the polymers by plasma irradiation [7]; this induces polymer chain scission, thereby contaminating the polymer surfaces. Another is the decrease in the surface hydrophilicity with time evolution due to surface reorientation [8]. In order to overcome these problems when using plasma treatments, it is necessary to introduce organic molecules with hydrophilic functional groups without causing surface damage. Surface-wave plasma (SWP) is a promising method for this purpose, because it allows treatment of polymer surfaces on a large scale at a low electron temperature [9]. In addition, plasma-initiated graft polymerization can attach functional groups or long alkyl chains to the polymer surfaces. In this study, we report the surface modification of the PET film to impart hydrophilicity through plasma-initiated graft polymerization using surface-wave plasma (SWP) and the cell adhesion on the hydrophilically modified PET surface.

2 EXPERIMENTAL PROCEDURES

The PET surface was modified by SWP excited by microwave radiation. The SWP system consisted of a waveguide attached to a microwave generator, a coaxial waveguide, and a stainless steel chamber. A curved reflective plate was attached to the waveguide to efficiently introduce microwaves to the coaxial waveguide. The top of the chamber was sealed with a quartz plate 12 mm in thickness and 14 cm in diameter. The coaxial waveguide was connected vertically to the quartz plate as the dielectric. The generated microwave passed through the waveguide and reached the quartz plate, leading to the formation of the plasma. The cleaned PET substrates were placed at the center of the substrate stage in the chamber. The distance between the quartz plate and substrate stage was kept constant at 5 cm. The chamber was evacuated to 0.5 Pa with a rotary pump prior to surface modification and Ar gas was subsequently introduced. The total pressure in the chamber was changed from 20 to 100 Pa. A microwave power of 200 W was applied to generate plasma for 5 min using a 2.45 GHz generator. The PET surface was

irradiated with Ar plasma that generated radicals on the polymer surface [10]. The polymer surface were then exposed to acrylic acid (AA) monomer in the vapor phase. The gas pressures of AA monomer were 2000 Pa and was kept constant for 60 min, leading to the graft polymerization of the monomer. After exposure to the gas, the AA monomer was introduced on the PET surface. The AA-introduced PET surface was ultrasonically cleaned for 10 min in ultrapure water and ethanol in that order.

Topographic images of PET surfaces before and after plasma irradiation were acquired with an atomic force microscope (AFM) in the tapping mode. The scanning area was a square 10 μm on one side. The hydrophilicity of the polymer surface was characterized using a static water contact angle measurement. A 2 μL water droplet was placed on the sample surfaces. The chemical bonding states and chemical composition of the polymer surfaces were analyzed using XPS. The $\text{MgK}\alpha$ X-ray source was operated at 10 mA and 12 kV.

To characterize the cell adhesion of the modified PET surface, mouse fibroblast cells (NIH-3T3) were cultured onto both untreated and modified PET surfaces by immersing them into the culture medium (DMEM, pH: 7.0) in a humidified atmosphere containing 5.0% CO_2 at 37.0°C for 3 days. The initial cell seeding number was 5000 cells/ cm^2 . The cultured cells were observed with optical and phase-contrast microscopes at intervals of 12 h and were counted using a blood cell counting chamber.

The protein adsorption behaviors to the AA-modified substrates was probed using an optical waveguide spectroscopy. In this case, a quartz with a size of 2cm X 5cm was used as substrate. Prior to the modification of the AA monomer, the quartz substrate was cleaned photochemically using vacuum ultraviolet (VUV) light with a wavelength of 172 nm. AA monomer was then introduced on the cleaned quartz substrate by SWP under same plasma conditions for PET substrate. To investigate the protein adsorption amount on the AA-modified quartz, a mixed solution containing Dulbecco's phosphate-Buffered Salines (D-PBS), and human plasma fibrinogen (HPF) or human serum albumin (HAS) was flowed onto the AA-modified quartz substrate at a flow rate of 10 $\mu\text{L}/\text{min}$. The concentrations of HPF and HAS in the mixed solution were varied from 5 to 50 μM and from 50 to 500 μM , respectively.

3 RESULTS AND DISCUSSION

First, we investigated the effect of the Ar plasma treatments on the changes in the wettability and damage of the PET surface. Figure 1 (a) shows the water contact angles of the PET surface modified at different Ar gas pressures. The water contact angle of the untreated PET surface was estimated to be 76.9°, while those modified by Ar plasma at gas pressures from 20 to 100 Pa ranged from 34° to 37°. The Ar plasma treatments greatly improved the hydrophilicity of the PET surface. However, the

hydrophilicity of the modified PET surface decreased over a period of 70 h after the plasma treatments, which could have been caused by surface reorientation of the PET film. It should be noted that Ar gas pressures have no effect on the chemical properties of the modified PET film surface. Figure 1 (b) shows the effects of the plasma irradiation time on the water contact angles of the PET surface modified by Ar plasma at 20 Pa. The hydrophilicity of the PET surface was greatly improved by the Ar plasma treatment for 60 s. The hydrophilicity of the modified PET film remained almost constant with treatment times longer than 60 s. The decrease in the hydrophilic degree with time evolution after the treatment of the modified PET surfaces remained almost the same, independent of the plasma treatment time.

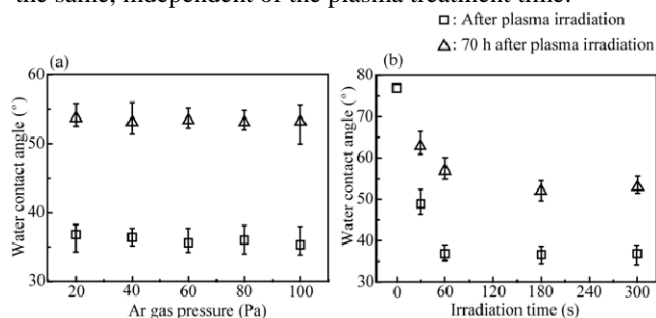


Figure 1: (a) Water contact angles of the PET surface modified at different Ar gas pressures. (b) Effect of plasma irradiation time on water contact angles of the PET surface modified by Ar plasma at 20 Pa.

AFM study revealed that no change in the topography was observed on the PET surface before and after Ar plasma irradiation, and the R_{rms} of the PET surface before and after Ar plasma treatment were estimated to be 1.2 and 0.7 nm, respectively. This indicated that Ar plasma treatments using SWP did not etch the PET surface. Thus, we were successful in altering the hydrophilicity of the PET surface without any physical damage. However, there was still a lack of permanence of the hydrophilicity of the PET surface modified by Ar plasma.

Plasma-initiated graft polymerization was carried out to enhance the permanence of the hydrophilicity on the PET surface. Figure 2 shows a time evolution of the water contact angles of the PET surface modified by (i) Ar plasma treatment and (ii) plasma-initiated graft polymerization using hydrophilic AA monomer. The water contact angles of the PET surface after Ar plasma treatment and Ar plasma followed by hydrophilic AA modification were 35° and 23°, respectively, indicating that plasma-initiated graft polymerization slightly improved the hydrophilicity. In addition, there was a noticeable difference between the treatments in the permanence of the hydrophilicity. The water contact angles of the Ar plasma-treated PET surface increased over time as a result of the instability of hydrophilic groups, such as $-\text{OH}$ and $-\text{COOH}$ groups, on the polymer surface. In contrast, those on the PET surface after Ar plasma followed by hydrophilic AA modification remained constant at approximately 20°, thereby showing stable hydrophilicity.

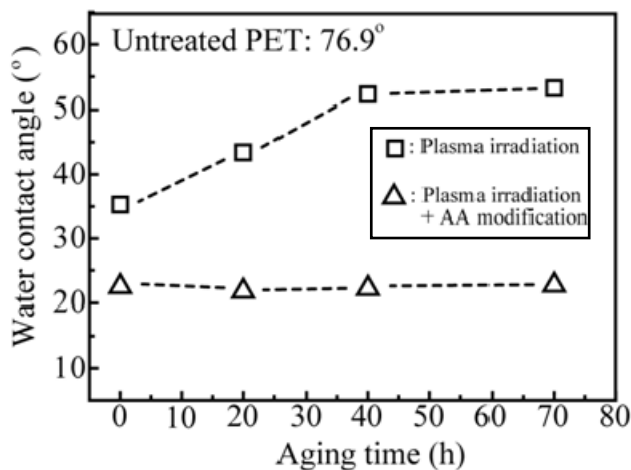


Figure 2: Time evolution of water contact angles of the PET surface modified by Ar plasma treatment and plasma-initiated graft polymerization using hydrophilic AA monomer.

To probe the chemical bonding states of the PET surface, XPS measurements were performed. Figure 3 shows XPS C 1s spectra obtained from (a) the untreated PET surface, (b) the PET surface after Ar plasma treatment, and (c) the PET surface after Ar plasma followed by hydrophilic AA modification. The C 1s spectrum of the untreated PET surface (Figure 3(a)) is deconvoluted into three peaks corresponding to carbon atoms of the benzene rings unbonded to the ester group (peak C1 at 284.7 eV), carbon atoms singly bonded to oxygen (peak C2 at 286.5 eV), and ester carbon atoms (peak C3 at 289.1 eV) [11–20]. The relative component concentrations determined from these peak areas were 58.9% for C1, 21.9% for C2, and 19.2% for C3. However, the C 1s spectrum (Figure 3(b)) reveals that the PET surface was chemically altered by the Ar plasma treatment. The relative component concentrations determined from these peak areas were 54.8% for C1, 34.1% for C2, and 11.1% for C3. The C1–C3 peaks of the untreated PET were broadened by the Ar plasma treatment, indicating that each peak includes more than one unique species. These species are ascribed to be formed through a chemical reaction of the polymer chains with activated ion species and radicals. The broadening of C1 and C2 peaks has been associated with the destruction of aromatic rings in PET [17,18]. These peaks are assumed to include signals from polar groups such as $-C-C=O$, $-C-COO$, or $-C-C-O$ [15,17]. The C 1s spectrum (Figure 3(c)) reveals that the intensity of the C2 and C3 peaks originating from polyacrylic acid has become stronger than those of the plasma-treated PET surface. This indicates that many AA monomers were adsorbed onto the PET surface and then polymerized by plasma-initiated radicals. Thus, polyacrylic acid was successfully grafted onto the PET surface, resulting in increased hydrophilicity. The modified PET surfaces maintained stable hydrophilicity for 70 h because two functional groups in polyacrylic acid (the $-COOH$ group and the hydrocarbon chain) strongly affect the surface properties.

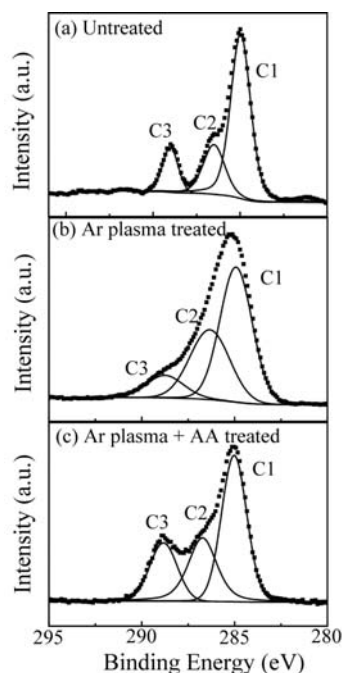


Figure 3: XPS C 1s spectra obtained from (a) the untreated PET surface, (b) the PET surface after Ar plasma, and (c) the PET surface after Ar plasma followed by hydrophilic AA modification.

To study cell adhesion of the AA-modified PET substrate, 3T3 fibroblast cells were cultured on the untreated and AA-modified PET surface. Figure 4 shows the number of cells cultured on the untreated, Ar plasma treated and AA-modified PET surface and the phase-contrast microscopic images for the cell growth behavior at 12 (Fig. 4a-4c) and 48 h (Fig. 4d-4f) after the cell culture. Initial cell growth was depressed on the AA-modified PET surface compared with the untreated and Ar plasma-treated PET surfaces. Phase-contrast microscopic images showed that the 3T3-fibroblast cells did not adhere well to the AA-modified surface. The number of cells cultured for 12 h correlated to some extent with the hydrophilicity of the PET surface. The cells increased as the water contact angles of the PET surface increased. After the cells were cultured for 48 h, the cell numbers increased on all PET surfaces, i.e., untreated, Ar plasma-treated, and AA-treated PET surfaces. The growth rate remained almost constant on all PET surfaces. This indicates that the number of cultured cells might depend on the cell adhesion behavior after cell seeding.

The HPF and HAS adsorption behaviors to the AA-modified quartz substrates with time evolution were investigated using an optical waveguide spectroscopy (OWG). The absorbance spectra with time evolution for HPF and HAS adsorption to quartz substrate before ((a) and (c)) and after ((b) and (d)) AA modification were shown in Figure 5. At the middle concentrations (HPF: 20 μ M, HAS: 100 and 200 μ M), the adsorption rates of the two proteins to the AA-modified quartz substrate were higher than those of the untreated quartz substrate. This could be

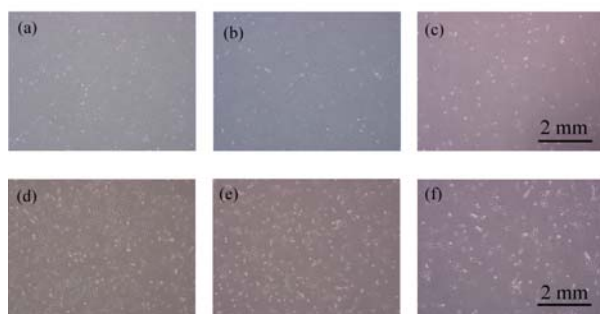
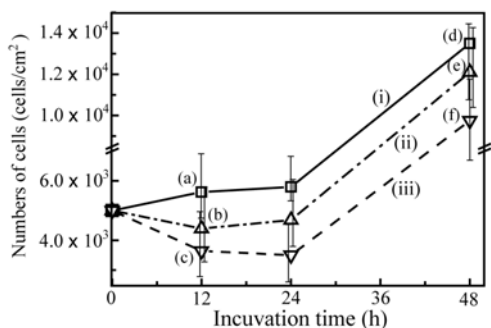


Figure 4: Numbers of cells cultured on the Ar plasma or AA-modified PET surface and the phase-contrast microscopic images for the behaviors of the cell growth at (a-c) 12 and (d-f) 48 h after the cell culture. (i) untreated PET surface. (ii) Ar plasma-treated PET surface. (iii) AA-modified PET surface.

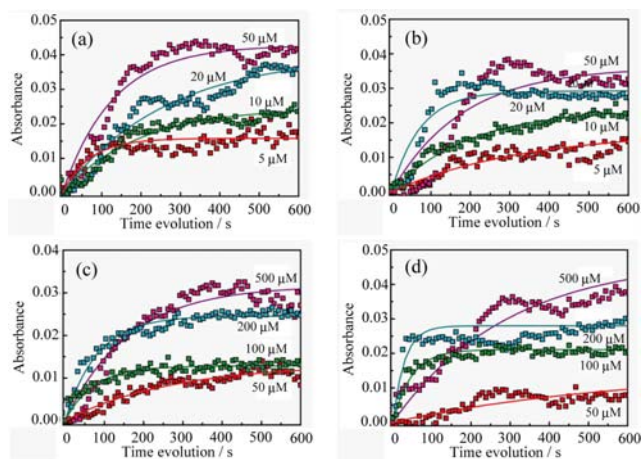


Figure 5: Absorbance spectra with time evolution of HPF ((a) and (b)) and HAS ((c) and (d)) adsorption to quartz substrate before ((a) and (c)) and after ((b) and (d)) AA modification.

due to that the AA-modified quartz substrate that has many polar functional groups such as $-\text{COOH}$ groups electrostatically interacted with the proteins which has many $-\text{NH}_2$ and $-\text{COOH}$ groups on the surfaces. In contrast, at high concentration, the adsorption rates of the proteins to the quartz substrates before and after AA modification hardly changed.

4 CONCLUSIONS

We successfully produced stable hydrophilicity on the PET surface through plasma-initiated graft polymerization using a SWP process. The hydrophilicity of the PET surface modified with hydrophilic AA monomer was maintained for 70 h. 3T3 fibroblast cells were cultured on the modified PET surface. The protein adsorption behaviors were also investigated using an optical waveguide spectroscopy. We believe that our surface modification method would provide a way to control hydrophilicity and bioactivity of various polymer surfaces.

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