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Surface modification and cell adhesion of PTFE using ion-beam irradiation

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Abstract:A polytetrafluoroethylene (PTFE) surface is smooth and biologically inert, so that cells cannot attach to it. In this study, we modified a PTFE surface using ion-beam irradiation with various ion fluences to help cell adhesion. To investigate the relationship between the cell-material interaction and physicochemical properties, we observed the irradiated PTFE surface using scanning electron microscopy (SEM), attenuated total reflectance/Fourier transform infrared (ATR/FT-IR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and measured the droplet contact angle. From the SEM observations, micro pores formed on a wrinkled surface at lower fluences, while a large number of protrusions were formed at higher fluences. The contact angle of water decreased from 110° to 90° at a fluence of 1×10^{15} ions/cm^2, and increased again up to 160° at higher fluences. The ATR/FT-IR and XPS analyses showed that >C=O, –OH and –C=C– bonds were introduced on the surfaces irradiated using low fluences, and at higher fluences, the number of these bonds was reduced, but they remained at the top of the protrusions. On culturing L929 cells on the irradiated surfaces, cell adhesion, spreading, and proliferation were observed on all the irradiated surfaces, although the properties were different for each surface. Using these modified surfaces, we fabricated a substratum that could culture single cells.

Key words: ion-beam irradiation, surface modification, PTFE, cell adhesion

1. INTRODUCTION

Polytetrafluoroethylene (PTFE) has a simple linear-chain molecular structure, and is attractive for biomaterials due to its excellent chemical inertness, high thermal stability, and low surface energy [1]. It can be stretched rapidly to create a strong microporous material, known as expanded polytetrafluoroethylene (ePTFE). This material is also nontoxic, leaves no residue, and does not degrade in vivo because of its high chemical stability. ePTFE is widely used for artificial replacement of various soft issues, including blood vessels, and it has been reported that an ePTFE surface irradiated using an ion beam has bioaffinity [2]. ePTFE and PTFE have different surface morphologies after irradiation; an irradiated ePTFE surface has micropores [2], while the morphology of a PTFE surface changes with irradiation fluence [3–6]. To evaluate the bioaffinity of PTFE surfaces irradiated at different fluences, we observed the cell behavior on these surfaces, and investigated the surface properties related to cell adhesion. From the results of our experiments, we proposed a new cell culture substratum using irradiated PTFE surfaces.

2. EXPERIMENTAL

2.1 Surface modification

PTFE sheets (thickness = 0.5 mm, Nichias) were cut into 40 × 40 mm^2 squares, cleaned using ethanol, and dried at room temperature before irradiation. The PTFE samples were irradiated in a vacuum chamber with 40 keV N^+ ions using an ion accelerator (TK–100 ion implanter, RIKEN) using fluences between 1×10^{15} and 1×10^{17} ions/cm^2. The beam current density was approximately 2.3 μA/cm^2, and the irradiated area was 30 × 30 mm^2.

2.2 Surface characterization

After irradiation, the samples were coated with a layer of gold using a plasma coater (SC-701H, Sanyu Denshi Co. Ltd). The surface morphology was observed using a scanning electron microscope (SEM, JSM–6330F, JEOL) using an acceleration voltage of 5 kV. The wettability was evaluated by measuring the contact angle of water dropped onto the irradiated surfaces (Model CA–X, Kyowa Interface Science Co., Ltd.). The chemical bonding of the irradiated surfaces was analyzed using attenuated total reflectance/Fourier transform infrared spectroscopy (ATR/FT-IR, NEXUS 470 FT-IR, Thermo

Scientific). Quantitative information on the chemical bonds present was obtained using X-ray photoelectron spectroscopy (XPS7000, Rigaku) employing Mg Kα X-rays.

2.3 Cell seeding

The PTFE samples were divided into 10 × 10 mm^2 pieces. After immersion in a 70% ethanol solution for a period of 1 h, followed by UV sterilization for a period of 15 min, each piece was placed into a 35 mm polystyrene petri dish (3005, Falcon). Cell adhesion experiments were performed using mouse fibroblast cells (L929). The cells in a medium were seeded in the petri dishes at a cell concentration of 1 × 10^5 cells/ml (2 ml per dish). The medium used was RPMI 1640 (Nissui Pharm. Co.) supplemented with 10% fetal bovine serum (Gibco Lab.). The cells were incubated at 37 °C in a 5% CO_2 atmosphere for periods of 3, 7, and 11 d. After the culturing period, the samples were rinsed using phosphate buffered saline (PBS) solution, and fixed using 5% paraformaldehyde for a period of 1 h. After being transferred to a PBS solution again, the samples were placed in a series of ethanol solutions (30%, 50%, 75%, 90%, and 100%) for a period of 15 min for dehydration. The dehydrated samples were dried overnight in flowing air and coated with a layer of gold for the SEM observations.

3. RESULTS AND DISCUSSION

3.1 Surface modification

SEM images of nonirradiated and irradiated PTFE surfaces are shown in Fig. 1. The surface of the nonirradiated sample is smooth with irregular lines and nanopits, as shown in Figs 1(a) and 1(a′). The manufacturer produced these lines and pits during the fabrication of the PTFE sheets. Numerous micro pores and a wrinkled surface were observed on the sample surface irradiated at a fluence of 1 × 10^{15} ions/cm^2 (Figs 1(b) and 1(b′)). The micro pores were randomly distributed over the surface and the top layers seem to be melted by a beam heating. At a fluence of 1 × 10^{16} ions/cm^2, the micro pores became enlarged and increased in number (Figs 1(c) and 1(c′)). When the fluence reached 1 × 10^{17} ions/cm^2, the surface became covered with small protrusions (Fig. 1(d)). The diameter of the top of the protrusion was about 100 nm and the length was between 40 and 100 μm (Fig. 1(d′)). Not only the evaporation of PTFE but rearrangement and orientation of molecules will be a cause of the protrusion formation and elongation.

Figure 2 shows the contact angle of water for the nonirradiated and irradiated PTFE surfaces. The contact angle of the nonirradiated surface was approximately 110°, and this decreased with increasing fluence, reaching a minimum at 90° for a fluence of 1 × 10^{15} ions/cm^2. The contact angle increased again at higher fluences, reaching a value of 160°.

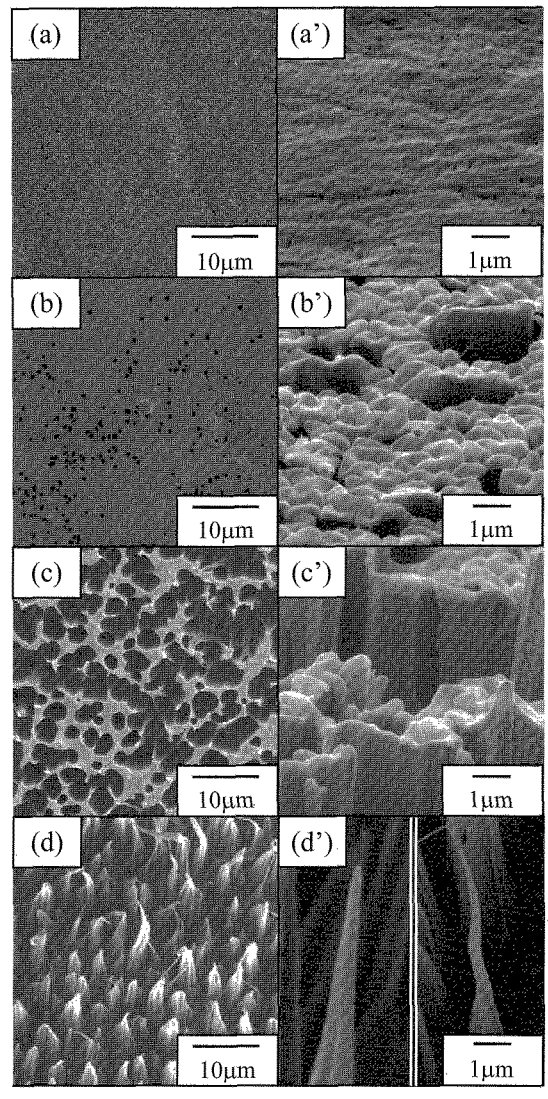

Fig. 1 SEM images of nonirradiated and N^+ irradiated PTFE surfaces. The left-hand column shows plane views and the right-hand column a 30° tilted view: (a) and (a′) none, (b) and (b′) 1 × 10^{15} ions/cm^2, (c) and (c′) 1 × 10^{16} ions/cm^2, (d) and (d′) 1 × 10^{17} ions/cm^2.

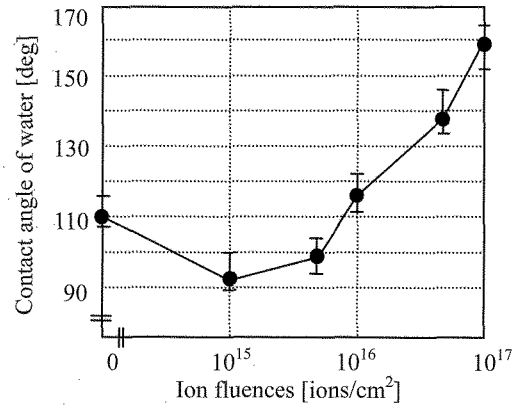

Fig. 2 Contact angle of water for the nonirradiated and N^+ irradiated PTFE surfaces with fluences between 1 × 10^{15} ions/cm^2 and 1 × 10^{17} ions/cm^2.

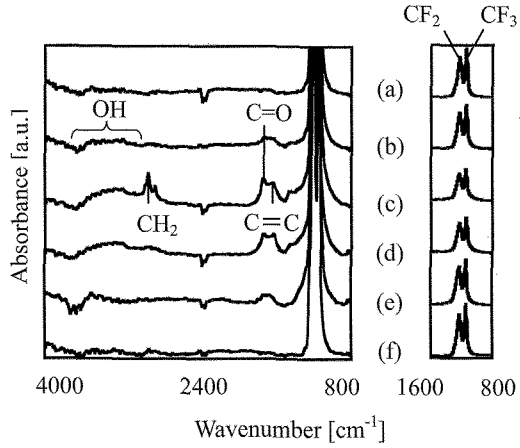

Fig. 3 FT-IR spectra of PTFE surfaces N^+ irradiated with: (a) none, (b) 1×10^{15} ions/cm^2, (c) 5×10^{15} ions/cm^2, (d) 1×10^{16} ions/cm^2, (e) 5×10^{16} ions/cm^2, and (f) 1×10^{17} ions/cm^2.

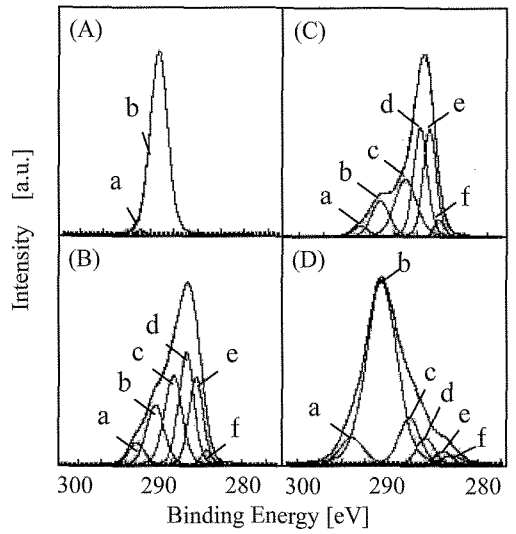

Fig. 4 The C_{1s} XPS spectra of PTFE surfaces N^+ irradiated with: (A) none, (B) 1×10^{15} ions/cm^2, (C) 1×10^{16} ions/cm^2, and (D) 1×10^{17} ions/cm^2. Key: (a) CF_3, (b) CF_2, (c) CFH, (d) >C=O, (e) OH, and (f) CH_2.

The ATR/FT-IR spectra of the PTFE samples are shown in Fig. 3. The spectrum of the nonirradiated PTFE surface showed both a CF_2 band (1205 cm^{-1}) and a CF_3 band (1150 cm^{-1}). At fluences between 1×10^{15} and 1×10^{16} ions/cm^2, a C=C band (1645 cm^{-1}), >C=O band (1720 cm^{-1}), CH_2 bands (2936–2916 cm^{-1} and 1475 cm^{-1}), and an OH band (3600–3200 cm^{-1}) appeared and these increased in intensity with increasing fluence. These functional groups containing oxygen or hydrogen atoms will be formed by a reaction of unstable radicals on the surface with O_2 or H_2O molecules in the air [6]. At a fluence of 1×10^{17} ions/cm^2, these new bands decreased in intensity, and the bands arising from the CF_2 and CF_3 groups became dominant again. With increasing the fluence, the information from the side wall of the pores or protrusions will increase because the ATR crystal deforms the surface.

Quantitative information on the bonds was obtained from analysis of the XPS spectra. Figure 4 shows the C_{1s} XPS spectra of the PTFE surfaces, showing that CF_2 bonds occupied 98% of the nonirradiated surface. On the surfaces irradiated with a fluence of 1×10^{15} and 1×10^{16} ions/cm^2, the percentage of >C=O and –kOH bonds increased to over 50%. On the surface covered with protrusions, the percentage of CF_2 bonds increased again to 70%.

From these results, the change of wettability can be explained as follows. For the sample irradiated with a fluence of 1×10^{15} ions/cm^2, the introduction of >C=O and –OH groups increased the hydrophilicity of the surface, and for the sample irradiated at a fluence of 1×10^{16} ions/cm^2, the contact angle of the water increased due to the roughness of the surface, even though the number of hydrophilic functional groups had increased. At higher fluences, a superhydrophobic surface is formed by protrusions and interspacial air explained by Cassie-Baxter's equation [7].

3.2 Cell adhesion

Figure 5 shows the morphology of the L929 cell adhesion to the sample surfaces after incubation. Adhesion and spreading of the L929 cells was observed on all the sample surfaces, except on the nonirradiated sample surface. The cells spread on the wrinkled surfaces or the tops of the protrusions without invading the pores or intervals between the protrusions. After an incubation period of 11 d the cells grew to form a dense confluent mass, and formed flat layers. This cell behavior is very interesting in that the cells were attached to both the hydrophilic and superhydrophobic surfaces in our experiments. The wettability of a surface is one of the factors influencing cell attachment [8]. However, the experiments were generally performed on flat surfaces, and the change in wettability originated from the surface functional groups. In our case, the cell attachment behavior seemed to be less affected by the wettability, because this was influenced by the surface roughness.

The factors influencing the cell attachment to each of the irradiated surfaces were considered to be as follows. On the one hand, on the surface irradiated at a fluence between 1×10^{15} and 1×10^{16} ions/cm^2, the inducement of the hydrophilic functional groups promoted cell adhesion. On the other hand, on the surface irradiated with a fluence $> 1 \times 10^{16}$ ions/cm^2, the number of >C=O and –OH bonds was very small. We suggest that these bonds are induced only at the top of the protrusions, because the cells spread only on the top of the protrusions.

Based on these results, we fabricated a substratum for isolated cell cultures. The patterning irradiation was performed using a Cu mask with a fluence of 1×10^{16} ions/cm^2, as shown in Fig. 6. The L929 cells spread one by one in each irradiated region (40×40 μm^2), and each irradiated area formed a microcell culture dish. This substratum will be useful for dividing cells without incurring any damage.

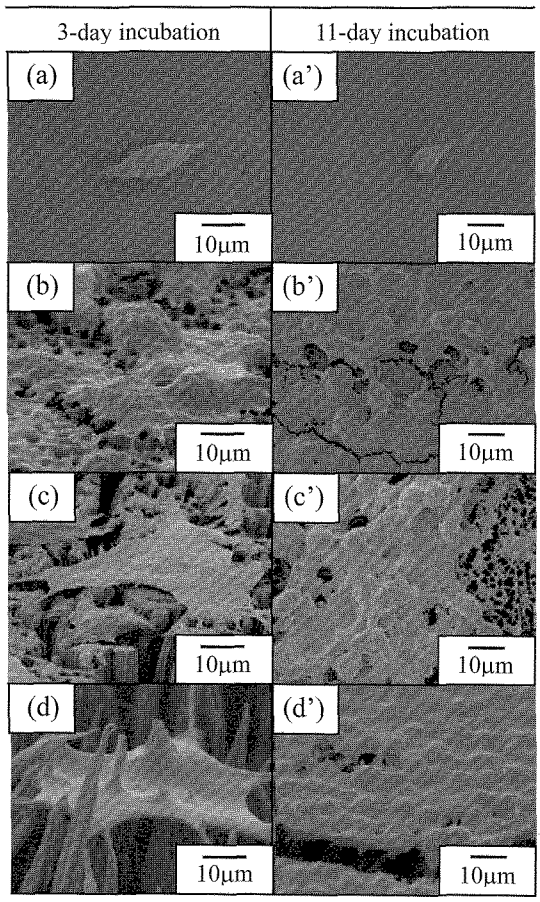

Fig. 5. Morphology of the L929 cell adhesion to the control and N^+ irradiated PTFE surfaces with irradiation of: (a) and (a′) none, (b) and (b′) 1×10^{15} ions/cm^2, (c) and (c′) 1×10^{16} ions/cm^2, and (d) and (d′) 1×10^{17} ions/cm^2.

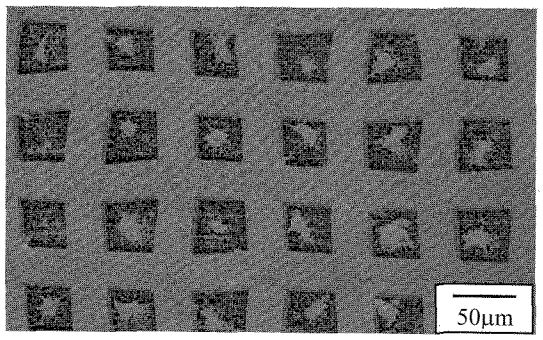

Fig. 6. Morphology of the L929 cell adhesion. Single cells spread on the surface irradiated with 1×10^{16} ions/cm^2. (Only inside the squares was irradiated).

4. CONCLUSIONS

Surface morphology and chemical bonds of ion beam irradiated PTFE were investigated. At a fluence of 1×10^{15} ions/cm^2, the micro pores were formed on the surface. When the fluence increased up to 1×10^{17} ions/cm^2, the surface was covered with micro protrusions. At fluences of between 1×10^{15} and 5×10^{16} ions/cm^2, CH_2, –C=C–, –OH, >C=O bonds were clearly observed. Due to these physical and chemical changes, the water contact angle decreased at a fluence of 1×10^{15} ions/cm^2, them it increased steeply at higher fluences.

We observed that L929 cells adhered and spread on PTFE surfaces at all fluences in contrast to an original surface which suppresses to cell attachment. The factor influencing cell adhesion was the inducement of >C=O, –OH, and –C=C– bonds. At fluences > 1×10^{16} ions/cm^2, cells straddled the protrusions, were attached, and had spread over the top of the surface. The wettability originated from the surface roughness but is not the major factor for cell attachment.

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