Relation Between Hydrophilicity and Cell Culturing on Polystyrene Petri Dish Modified by Ion-Assisted Reaction

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ABSTRACT: Polystyrene Petri dishes were modified by an ion-assisted reaction to supply a suitable surface for culturing cells. Wettability was measured by a contact anglometer after surface modification of polystyrene. Contact angles of water on the polystyrene were not reduced much by Ar^+ ion irradiation only, but dropped rapidly to a minimum of 19°, when polystyrene surface was modified by Ar^+ ion irradiation with flowing oxygen gas. X-ray photoelectron spectroscopy analyses showed that hydrophilic groups were formed on the surface of polystyrene by a chemical reaction between unstable chains induced by the ion irradiation and the blown oxygen gas. Newly formed hydrophilic groups were identified as C—O, —(C=O)— and —(C=O)—O— bonds. The influence of the ion beam modification in growth of the rat pheochromocytoma cells was investigated. The results showed exclusively preferential cell growth in the polystyrene Petri dish that was treated by the ion-assisted reaction. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 73: 41–46, 1999

INTRODUCTION

Polymers have been widely used as biomaterials due to durability, low production cost, and easy shapability. Among the polymers, polystyrene (PS) has commonly been used as a disposable culture dish because, besides these properties, it is optically transparent in visible range, and nontoxic. PS Petri dishes have been widely used for culturing animal cells. However, surface of the PS Petri dish is hydrophobic, and does not provide a suitable surface for animal cell growth. To facilitate animal cell growth on the PS Petri dishes, the surface of the Petri dishes has been treated by either γ-irradiation,[1] chemical modification,[2] or plasma discharge[3] to produce hydrophilic groups such as ether, carbonyl, and carboxylic groups. Many research groups have examined the relationship between cell growth and surface hydrophilicity.[4,5] They found that tissue cells more easily grow on hydrophilic surfaces than on hydrophobic surfaces, because tissue cells more easily adhere to hydrophilic surface than to a hydrophobic one.

To modify the surface of hydrophobic polymer to a hydrophilic one, many methods like plasma, corona discharge, rf sputtering, ultraviolet light, etc., have been developed, and wettability of polymers to water has just slightly been improved by the above-described methods. Wróbel et al.[6] and Fakes et al.[7] explained the improved wettability by changes of the surface morphology by micropore and formation of hydrophilic groups by oxygenation. Recently, the ion-assisted reaction (IAR) method was developed by Koh et al.,[8] and it showed excellent results to

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change the polymer surface to a hydrophilic one.[9–11] Polymers such as polycarbonate (PC: [—C_6H_4—$C(CH_3)_2C_6H_4$—OCO_2—]$_n$), polyethylene terephthalate [PET: (—$OCH_2CH_2O_2C$ C_6H_4—CO—)$_n$], and polymethyl methacrylate (PMMA: [—$CH_2C(CH_3)$(CO_2CH_3)—]$_n$), which were modified by the IAR, became wettable (wetting angles were lower than 10°). Koh et al. have also surveyed wettability improvement mechanisms with PC and found that wettability was not greatly dependent on the surface morphology but on the amount of hydrophilic groups such as C—O, C═O, and (C═O)—O formed by a chemical reaction between the unstable chains generated by ion irradiation and the blown oxygen gas.[8] Another advantage of using IAR is that it is a suitable process for mass production. Generating the large area ion beam is relatively easy comparing with high energy (10 s ~ 100 keV) ion beam, and the time required to the modification is relatively short.

In this article, PS ([—$CH_2CH(C_6H_5)$]$_n$) was modified by Ar^+ irradiation in an O_2 environment to form hydrophilic groups, and the effect on cell growth was investigated. Wettability of water on PS was examined by measuring the contact angle of sessile drops on PS at various Ar^+ ion doses with and without flowing oxygen gas, and the chemical reaction on a surface of polymer was identified by X-ray photoelectron spectroscopy (XPS). To elucidate the relation between the hydrophilic surface and tissue cell growth, PC12 cells were cultured on the surface of the modified PS Petri dishes, and cell density was counted.

EXPERIMENTAL PROCEDURES

To examine wettability of water on the PS Petri dish, commercially available sterilized PS Petri dishes (Green Cross medical industry Co. Ltd.: bacterialogical grade) were cut into 10×10 mm^2. For culturing animal cells, the inside of the PS Petri dishes (5-cm diameter) were modified. All of these samples were not cleaned before and after IAR treatment because the Petri dishes could be contaminated by the cleaning process and the surface contamination could affect the results of culturing cell. A 5-cm diameter cold-hollow cathode ion source was employed to generate the Ar^+ ion. The Ar^+ ions were irradiated onto the PS surface at room temperature with or without flowing oxygen gas (99.99%), whose flow rate was changed from 0 to 8 mL/min. An applied ion beam potential of Ar^+ ions was varied from 0.6 to 1.2 keV. The ion beam current was controlled by a discharge current and was measured by a Faraday cup biased −30 V to prevent secondary electrons from entering it. The amount of ions was changed from 5×10^{14} to 1×10^{17} ions/cm^2 at a fixed ion beam current.

Changes of the contact angles were measured by a contact anglometer (ERMA, Goniometer type), and the values of contact angles were taken as average values of four drops at different places on five different PS samples fabricated at same modification conditions. The measured wetting value was the static contact angle, and the test liquids were triple distilled water and formamide.

XPS was performed to examine the chemical environment on the modified polymer surfaces, using Surface Science Instrument 2803-S spectrometer, which had a base pressure of 2×10^{-10} Torr. XPS data were obtained with monochromatic Al K_α line ($h\nu$ = 1486.6 eV). A concentric hemispherical analyzer, which has an energy resolution of 0.48 eV, was used as an energy analyzer. Surface cleaning by Ar^+ ion sputtering was not performed because the surface chemical states could be changed due to the sputtering process.

The wettable PS Petri dish surfaces modified by the IAR were investigated by growing rat pheochromocytoma (PC12) cells on them. The reason the PC12 cell was chosen is that it adheres very poorly to the surface of plastic dishes, thus showing difficulties of growing on the PS Petri dishes without any treatment. Six of each type of Petri dishes were prepared to compare the extent of growth of the PC12 cell between the IAR-treated and nontreated groups of the PS Petri dish. The PC12 cells were seeded in RPMI 1640 media, which contains 5% fetal bovine serum (FBS) and 10% horse serum (HS), and cultured in the incubator with 5% CO^2. The growth of PC12 cells was compared by counting the number of cells from each group every 24 h for 6 days. Counting the number of cells were performed as follows: the media were removed by aspiration, and cells were rinsed with phosphate-buffered saline (PBS) pH 7.3. The cells were detached from the PS Petri dishes by incubating with trypsin solution (GIBCO) for 5 min at 37°C. Cell suspensions were pipetted several times to obtain single cells to count. Then the cell suspensions were diluted with media, and the number of cells were counted by an optical microscope using a hematocytometer.

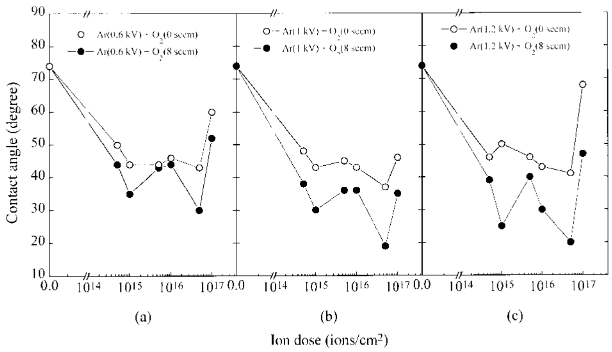

Figure 1 Changes of contact angles of water on PS as a function of Ar^+ ion dose with flowing oxygen gas at ion energy of (a) 0.6 kV, (b) 1 kV, and (c) 1.2 kV.

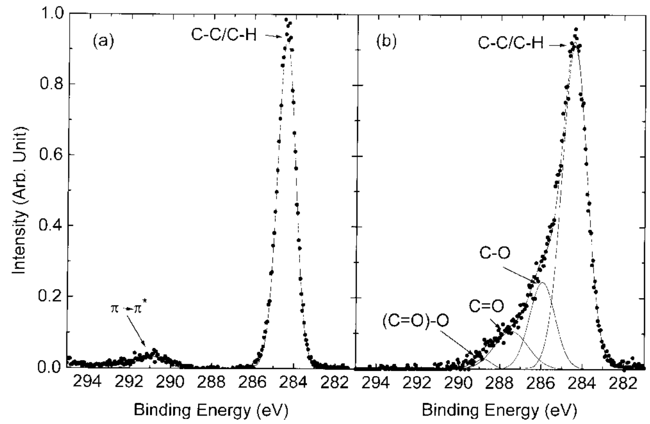

Figure 2 C1s spectra of untreated (a) PS and (b) PS irradiated by 1 kV Ar^+ ion with a dose of 5×10^{16} ions/cm^2 and an oxygen gas flow rate of 8 mL/min.

RESULTS AND DISCUSSION

Figure 1(a), (b), and (c) show the changes of the contact angles of water on PS as a function of irradiating ion dose with and without blowing oxygen gas at the acceleration voltage of 0.6, 1.0, and 1.2 kV, respectively. As shown in Figure 1(b), the contact angles of water on the PS samples irradiated by Ar^+ ions without blowing oxygen gas were reduced as much as 30–40° from those of the untreated samples. The effect of blown oxygen gas was also surveyed. The contact angle minima of water on PS at a fixed ion potential with different oxygen flow rates were represented at Table I. The contact angles of PS irradiated with Ar^+ ions were reduced as the oxygen gas flow rate increased. In this experiment, the changes of wettabilities do not mainly depend on the ion energy, but on the ion dose and the oxygen gas flow rate. As shown in Figure 1(b), the minimum contact angle was 19° at the ion dose of 5×10^{16} ions/cm^2 and the oxygen gas flow rate of 8 mL/min, which is lower than that of sample irradiated without oxygen gas under the same ion dose. When the ion dose exceeded 1×10^{17} ions/cm^2, an increment of the contact angle was observed due to carbonization. Therefore, it can be said that the introduction of oxygen gas makes the contact angle decrease considerably because of some chemical reaction between the oxygen gas and the surface of the PS.

Table I Contact Angle Minima of Water on PS at Fixed Ion Potential

	Applied Ion Beam Potential		
Oxygen Gas Flow Rate	0.6 kV	1.0 kV	1.2 kV
0 mL/min	43	37	41
4 mL/min	36	36	34
6 mL/min	36	26	25
8 mL/min	30	19	20

XPS was performed to investigate the chemical reaction between the oxygen gas and the irradiated surface of PS. Figure 2 shows the C1s core level spectra of (a) untreated PS, and (b) modified PS, which was irradiated at an Ar^+ ion dose of 5×10^{16} ions/cm^2 with an oxygen flow rate of 8 mL/min and an applied acceleration potential of 1 kV. Each XPS spectrum was normalized, and background was subtracted. In the case of untreated PS, the C—C peak and/or the C—H peak located at 284.6 eV appeared, and other peaks due to the $\pi \rightarrow \pi^*$ transition are observed at 291.0 eV. In the case of sample treated by Ar^+ ion irradiation in an oxygen environment, the intensity of the C—C peak (284.6 eV) decreased and that of the —(C—O)— peak (286.0 eV), —(C═O)— peak (287.4 eV), and —(C═O)—O— peak (288.8 eV) increased respectively, as shown in Figure 2(b), while the peak assigned to the $\pi \rightarrow \pi^*$ transition (291.0 eV) disappeared, i.e., the phenyl ring structure was destroyed by ion beam irradiation. From this result, it could be explained that the C—C or C—H chains in the polymer are broken by energetic ion bombardment, and the induced unstable chains react with the blown oxygen. It could be suggested that the change of the contact angle is due to the formation of new bonds, especially —C—O—, —(C═O)—, and —(C═O)—O— bond by a chemical reaction of unstable chains and the blown oxygen.

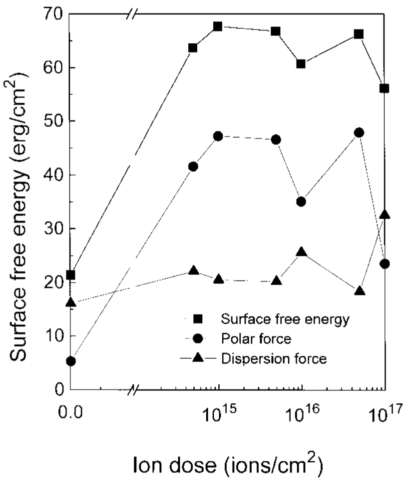

Figure 3 Change of surface-free energy of PS as a function of ion dose with an oxygen flow rate of 8 mL/min and an applied acceleration voltage of 1 kV.

These results agree with those of our previous works where we modified PMMA, PC, and PET with 1 keV energy Ar^+ ion irradiation in an oxygen environment and identified the formation of hydrophilic groups.[8–11] A significant increase of the O1s spectra is also observed in the PS irradiated in the O_2 environment. Theoretically, the PS does not contain oxygen, and, in our sample, less than 1% oxygen was detected in a bare sample because of additive or impurities from the PS. After IAR treatment, the concentration of oxygen increased up to 27%. The increased oxygen concentration indicates that the blown oxygen participated in the chemical reaction to form the hydrophilic group on the surface of the polymers. From this result, it can be confirmed that the IAR is the effective method to form hydrophilic groups on the polymer surface.

It is well known that surface-free energy is closely related to the wettability, and consists of a dispersion force and a polar force. By using Owen's method,[12] the surface energies of the untreated PS and the modified PS were calculated. Figure 3 shows the changes of surface-free energy for PS as a function of ion dose with an oxygen flow rate of 8 mL/min and an applied acceleration voltage of 1 kV. This figure shows that the dispersion force slightly increased with the ion dose, while the polar force was significantly increased. Before the ion irradiation, the value of the polar force was measured to be lower than 10 erg/cm^2. After Ar^+ irradiation with an oxygen flow rate of 8 mL/min, the value of the polar force was measured to be more than 40 erg/cm^2, and the surface-free energy was measured to be more than 60 erg/cm^2. Callen et al.[4] modified polystyrene by remote plasma and ultraviolet (UV)-ozone. Their XPS results show that the concentration of oxygen increased up to 37% by remote plasma and UV-ozone, respectively, but the oxygen concentration reduced to 15% in both methods after ultrasonic washing of the sample. They also measured the surface-free energy of UV-ozone–treated samples, and maximum surface free energy was measured to be 34.8 erg/cm^2. They also surveyed the relationship between the oxygen concentration and the surface-free energy and they reported that, although the surface-free energy was closely related to the oxygen concentration at the initial stage, the surface-free energy is not exactly proportional to the oxygen concentration. In the IAR experiments, the oxygen concentration was measured to be 27% on the PS surface, and the surface-free energy was measured to be around 60 erg/cm^2, which is a much higher value than that of PS treated by UV-ozone. It is apparent that the introduction of oxygen gas effectively increases the polar force in the surface energy by the dipole–dipole interaction of hydrogen bond between the newly formed hydrophilic group and the water.[8] From this result, a large decrease of the contact angle can be explained directly by means of a dominant increase of the polar force in the surface-free energy of the modified polymer.

Although it is well known that oxygen-containing components, such as ethers, hydroxyls, carbonyls, carboxylic acids, etc., increase the polar force, the degree of their contribution to the increment of the polar force is not clear. Although the amount of oxygen-containing components on the surface of the PS treated by the IAR is lower or equal to that by UV-ozone treatment, IAR treated PS shows more effective results to reduce wetting angle.

Figure 4 shows changes of the wetting angle as a function of keeping time in dry air condition (open symbols) and in deionized water (solid symbols). The samples that were modified at 1 kV with oxygen flow of 8 mL/min. were selected for examination. The samples that were kept in water were dried by dry N_2 gas for cleaning the adsorbed impurities on the modified surface, and kept in air for 4 min before measuring the wetting angles. As shown in Figure 4, the wetting angle of PS kept in water were not changed with keeping time, and this means that the functional groups formed by the IAR do not dissolve into the water. However, the wetting angles after exposure in the air were recovered to high wetting angle value,

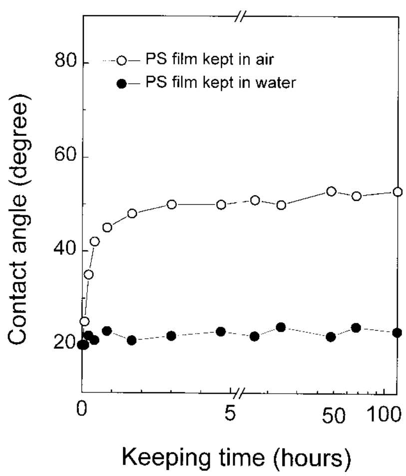

Figure 4 Wetting angle changes as a function of keeping time in dry air condition (open symbols) and in deionized water (solid symbols).

and the recovered wetting angles were returned to the low wetting angles again when the samples were kept in water after 2 days. As shown in Figure 3, wetting angles are inversely proportional to the surface-free energy, and the increment of the surface free energy is not due to the dispersion force but due to the polar force, i.e., IAR scarcely affects the dispersion force. For the same reasons, the wetting angle recovery is due to decrements of the polar force, not due to the dispersion force. This result could represent that wetting angle recovery is due to a chain rotation in polymer chains.[13,14] In the case of the films kept in water, the wetting angles were maintained by continuous interaction between the water and polar group in the chains, while, in cases of films exposed to air, such an interaction did not take place, and chain rotation took place to reduce the surface-free energy. In the case of PS film modified by conventional methods such as γ-ray irradiation,[1] UV-ozone,[4] $\sim$ 100 keV energy ion beam,[15] glow discharge,[16] etc., these recovery phenomenon did not take place because the hydrophilic groups formed by the above-listed methods were washed out, while, as mentioned above, PS films modified by IAR do not show an increase of wetting angle after washing, i.e., the hydrophilic groups formed by IAR does not dissolve in water.

Figure 5 shows the growth curves of PC12 cells cultured in PS Petri dishes (a) nontreated (■), (b) treated by IAR with a dose of 1×10^{15} ions/cm^2 (○), and (c) treated by IAR with dose of 5×10^{16} ions/cm^2 (●). In the case of IAR treatment, the oxygen flow rate and the applied acceleration voltage were fixed at 8 mL/min and 1 kV, respectively. Initially 2.5×10^5 cells were seeded in each Petri dishes (5 cm in diameter). For the group of nontreated Petri dishes (a), after 1 day of seeding most of the cells were not attached to the surface of the dishes, and only a few cells survived, while, for both groups of IAR-treated Petri dishes (b and c), most of the cells were able to attach to the dishes and survived. The reduction of cell number after 1 day means either the number of cells in day 0 is oversaturated or not every cell is healthy enough to attach to the surface of the dishes, and those phenomena are common for culturing animal cells. The number of PC12 cells in Petri dishes treated by IAR with the doses of 5×10^{16} ions/cm^2 and 1×10^{15} ions/cm^2 increased up to 2.0 $\times 10^5$ and 1.4×10^5 after 2 days, and up to 1.6 $\times 10^5$ cells/cm^2 and 1.9×10^5 cells/cm^2 after 3 days, respectively. After the number of viable cells reached a maximum value, the population of the cells decreased in both of the treated groups, which might be due to a depletion of media and a contact inhibition.

Figure 6 shows the microscopic images of PC12 cells grown on Petri dishes after 2 days. Panel (a) represents the images of the cells on the nontreated Petri dishes, and panel (b) represents those on the IAR treated with dose of 5 $\times 10^{16}$/cm^2. As shown in Figure 6, a distinct difference in cell growth was observed, which might be dependent on the surface hydrophilicity. PS Petri dishes modified by the IAR showed exclusively preferential culturing properties of

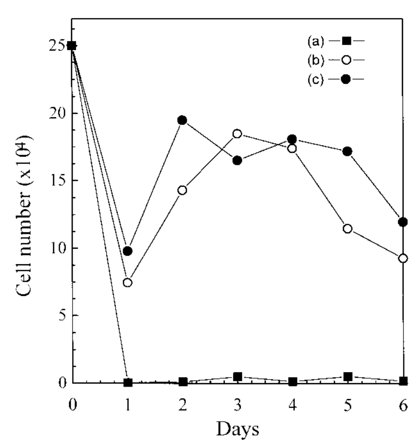

Figure 5 Growth curves of PC12 cells cultured on a PS Petri dish (a) nontreated, (b) treated by IAR with a dose of 1×10^{15} ions/cm^2, and (c) treated by IAR with a dose of 5×10^{16} ions/cm^2. IAR treatment was performed with an oxygen gas flow rate of 8 mL/min and an applied acceleration potential of 1 kV.

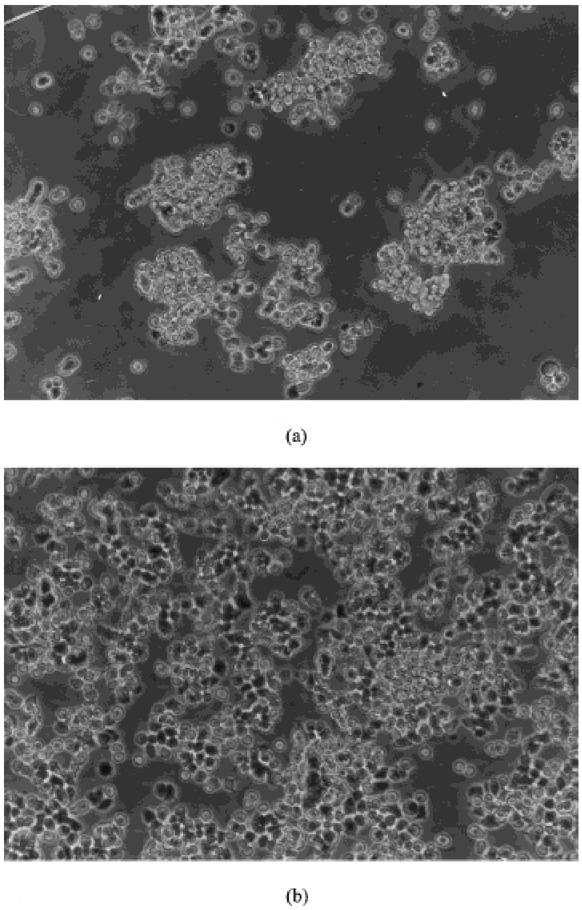

Figure 6 Optical microscopic images of PC12 cells 2 days after seeding: (a) nontreated, and (b) IAR treated Petri dishes modified by 1 kV Ar^+ ion with a dose of $5 \times 10^{16}/cm^2$ and an oxygen flow rate of 8 mL/min.

PC12 cells. From the above results, we can conclude that the IAR treatment is a very effective method to control the hydrophilicity of the surface of various polymers, and this technique can be applied in the field of other tissue cultures, such as animal cell cultures, which require hydrophilic surfaces.

CONCLUSION

Wettability of water on PS was improved by Ar^+ ion irradiation with flowing oxygen gas. The contact angle was not largely reduced by Ar^+ ion irradiation without flowing oxygen gas, but significantly decreased when the surface of PS modified by Ar^+ ion irradiated with flowing oxygen gas. The minimum contact angle of PS was 19°. On the basis of XPS analysis, it was observed that hydrophilic groups was formed by a chemical reaction between unstable chains induced by the ion irradiation and the oxygen gas, and were identified as —(C—O)—, —(C═O)—, and —(C═O)—O— groups. Consequently, the enhanced wettability of water on PS modified by the Ar^+ ion irradiation with oxygen gas resulted from the formation of hydrophilic groups on the surface of polymers. Cell growth curves show that hydrophilicity of the Petri dish is very important for growth of PC12 cells, and this IAR technique could be applied to modify the PS Petri dishes for culturing other types of animal cells.

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