Effect of C− implantation on Nerve-Cell Attachment to Polystyrene Films*

Piyanuch SOMMANI*1, Hiroshi TSUJI*1, Tsuyoshi KITAMURA*1, Mitsutaka HATTORI*1, Tetsuya YAMADA*1, Hiroko SATO*1, Yasuhiro GOTOH*1 and Junzo ISHIKAWA*1

*1Department of Electronic Science and Engineering, Graduate School of Engineering, Kyoto University, Kyoto (Received November 10, 2005, Accepted October 13, 2007)

The surfaces of the polystyrene films spin-coated on glass were modified by carbon negative-ion implantation with various ion doses from $1 \times 10^{14}$ to $3 \times 10^{15}$ ions/cm$^2$ at 5 and 10 keV. The implantation conditions with and without a patterning mask were for investigation of the cell-attachment properties and for evaluation of surface physical properties of contact angle, respectively. The contact angles of modified surface were investigated by pure water drop and air bubble method. The lowest angle value of the implanted films at 5 and 10 keV were approximately $72^\circ$ at $3 \times 10^{15}$ ions/cm$^2$ after dipping in the de-ionized water for 2 hours. The lowering of contact angles on C-implanted surfaces when increase the ion dose is due to formation of the OH and C−O bonds. Nerve-cell-attachment properties of modified surface were investigated by the nerve-like cell of rat adrenal pheochromocytoma (PC12h) in vitro. After 2 days culture of the PC12h cells, no cells attached on the polystyrene films implanted with low ion dose from $1 \times 10^{14}$ to $3 \times 10^{14}$ ions/cm$^2$. On the polystyrene films implanted with the dose order of $10^{14}$ ions/cm$^2$, the cells selectively attached only on the implanted region. Whereas on the surfaces implanted with high dose such as $1 \times 10^{14}$ and $3 \times 10^{15}$ ions/cm$^2$ mostly cells attached on the implanted region, and some attached on the unimplanted region, as well as cells were abnormal in shape and large size. Therefore, the suitable dose implantation for the selective-attachment of nerve-cells on the polystyrene films implanted at 5 and 10 keV were obtained around the dose order of $10^{15}$ ions/cm$^2$, and the best condition for the selective attachment properties was at $3 \times 10^{15}$ ions/cm$^2$ corresponding to the lowest contact angle.

1. Introduction

Many researchers investigated about the biocompatibility of polymeric surfaces for the biomedical field by using ion implantation1–7). The negative-ion implantation techniques with low ion energy have been used to modify the polymeric surfaces for the improvement of nerve-cell attachment because of the advantage of charge-up free8–10). The implantation with negative-ion such as silver2) and carbon1,3–7) presented the achievement of this improvement. However, the heavy metal elements such as silver can be harmful to the living body. The carbon element, which is lighter and is more familiar to cells than silver element, will be harmless for the living body. Thus, the implantation with the carbon negative-ion is better to use in the biomedical field. Since the difference in weights of both elements may affect to each suitable implantation condition for the improved-attachments of nerve-cells on the modified surface, the researches concerning to the effect of the carbon negative-ion implantation on the attachment properties are then necessary. The improved-attachments of cells on the polymeric surfaces of polystyrene, silicone rubber, and poly-lactic acid modified by using the carbon negative-ion implantation were reported5,9). Their attachments are related to the physical surface property such as the hydrophilicity. Generally, the attachment of each cell line concerns to the extracellular matrix of many proteins absorbed on the surfaces. Such proteins are absorbed on the hydrophilic surface, which the contact angle is in the range of $40^\circ$–$80^\circ$11). In case of polystyrene and silicone rubber, as the ion dose and implantation energy increased, the contact angle decreased10). The circumstance dependence of the contact angle in de-ionized water (DIW) was also reported. After implantation, the relaxation or stabilization of the ion-implanted polymeric surface gradually occurs with time dependence by rearrangement and adsorption of the environmental oxygen atoms to form the functional groups of OH and C−O on the implanted surface resulting in gradually change in contact angle. The contact angle depends on many factors such as the ion-implanted condition, the measuring period after implantation, and the reserve circumstance. The well-known method for contact angle measurement is water droplet. However, in cell culture, the culture surface is used in aqueous condition, that affects the change in the angle. So, the angles in aqueous state should be also considered. In this work, the effects of the carbon negative-ion implantation at 5 and 10 keV on the selective-nerve-cell attachment to the spin-coated polystyrene films and the contact angle of water at various circumstances were investigated.

2. Experiment

Spin-coated polystyrene films on glass (7% of polystyrene in Toluene, PS Nacalai tesque, Inc., Japan) were implanted by carbon negative ions for surface modification. Carbon negative ions produced in a cesium sputter-
type heavy negative-ion source (NIABNIS)\textsuperscript{12,13} were mass-separated and transported to an implantation chamber. The carbon negative-ion beam of 11.28 mm in diameter was implanted to polystyrene films at the energies of 5 and 10 keV and various doses from 0.1, 0.3, 0.7, 1, 2, 3, 5, 7, 10, and 30 (\(\times 10^{15}\)) ions/cm\(^2\) with current density lower than 400 nA/cm\(^2\) under residual gas pressures lower than \(6 \times 10^{-4}\) Pa. For evaluation of the surface physical properties, the wettability of the surface is considered to strongly relate to the cell affinity, and it can be simply evaluated by contact angle measurement. The contact angle of pure water de-ionized by a filter was measured by water drop method as implanted and by air bubble method after dipping in pure water for 0, 2 and 24 hours at 37°C. For investigation of the nerve-cell attachment, the samples were implanted through a patterning mask of many slit apertures 50–\(\mu\)m width and 70–\(\mu\)m spacing in the area of \(5 \times 8\) mm\(^2\). After each C-implanted sample fixed with a 35-mm dish (Non-treated polystyrene dish, Corning) was dried for 2 days, it was sterilized by 70% ethanol, rinsed three times with the sterilized DIW and rinsed once with the phosphate buffered saline (PBS) before cell culture. Nerve-like cells of rat adrenal pheochromocytoma (PC12 h) with \(3 \times 10^{5}\) cells/ml were cultured on the modified surfaces in Dulbecco’s modified Eagle’s medium (DMEM, Nissui, Japan) containing 5% heat-inactivated horse serum (HS, Biomedicals, USA) and 5% fetal bovine serum (FBS, Bio-Wittker, USA), sodium hydrogen carbonate (1.8 mg/ml, Wako, Japan) with antibiotic of penicillin G and streptomycin for 2 days under 5% CO\(_2\) at 37°C in incubator, as well as on type-I collagen-coated dish as a reference. Then, their cell-attachment properties were observed by phase contrast microscope (CK2, Olympus).

3. Results and Discussion

3.1 Contact angle

The contact angles of DIW on the C-implanted surfaces of polystyrene implanted at 5 keV are shown in Fig. 1, which (a) is the angle measured by the water drop and air bubble method after the implantation within 2 hours, and (b) is the angle measured by the air bubble method after dipping in DIW for 0, 2, 24 hours. From Fig. 1(a) as the ion dose increased the contact angle on the polystyrene surface decreased from 91° to 86° at lowest in the case of the water drop method and from 86° to 73° in the case of the air bubble method. The lowest angle was at the \(3 \times 10^{15}\) ions/cm\(^2\). The angle of the higher dose-implanted samples became saturated around 85° by water drop method. As time dependence of contact angle after dipping in DIW for 24 hours at \(3 \times 10^{15}\)-ions/cm\(^2\) dose, the angle decreased from 75° to 68° and it was saturated at 66° after dipping in DIW for 48 hours (data not shown). Therefore, the lowering of contact angle shows the modification of polystyrene-film surface from hydrophobicity to hydrophilicity by carbon negative-ion implantation. Results from both measurement methods show the same lowering tendency of the contact angle as the function of ion dose. Especially, by the air bubble method shows that the decreasing in contact angle depends on the dipping time in DIW, and the lowering of angle became saturated after 48-hour dip in DIW. From both Figs. 1 and 2, there are differences in ion dose at 5

![Fig. 1](image-url)
Fig. 2  Contact angle of C-implanted spin-coated polystyrene as a function of the ion dose at 10 keV of ion energy by: (a) water drop and air bubble method and (b) air bubble method at 0, 2, 24 hours.

Fig. 3  Phase-contrast optical micrographs of PC12h cells cultured on the implanted region, where is corresponded to the narrow region between the dotted lines, of the polystyrene surfaces implanted by $1 \times 10^{14}$ ions/cm$^2$ at: (a) 5 keV and (b) 10 keV.

and 10 keV. The decreasing in contact angle of 5 keV at low dose such as $1 \times 10^{14}$ ions/cm$^2$ was less than that of 10 keV. Moreover, the angle of 5 keV decreased and became saturated, while that of 10 keV decreased and increased as a little at high dose such as $5 \times 10^{15}$–$3 \times 10^{16}$ ions/cm$^2$ before saturated. By the measured angle values, the considerably good conditions with hydrophilic property for high possible cell-attachment properties should be 2–30 $(\times 10^{15})$ and 0.1–30 $(\times 10^{15})$ ions/cm$^2$ for 5 and 10 keV, respectively. The best condition was also expected around $3 \times 10^{15}$ ions/cm$^2$.

3.2 Nerve-cell attachment

Phase-contrast optical micrographs of PC12h cells cultured for 2 days on the C-implanted polystyrene films at 5 and 10 keV with the ion dose of $1 \times 10^{14}$ ions/cm$^2$ are shown in Figs. 3(a) and 3(b), respectively. At very low dose implantation, the implanted area could not be clearly seen. Since a present of lowering contact angle on the C-implanted surface the areas where cells attached should be the implanted region. Less than 30% of the seeded cells attached on the implanted region, where is the narrow region between the dotted lines. A number of cells were floated and died by the non-attachment on the surface because of a little decrease in contact angle to 73° and 71° after 24-hour dip in DIW for 5- and 10-keV implantation, respectively. Although, the angle for 10 keV at low dose is nearly the angle at the medium dose, the cell attachment at this dose was not good.

Micrographs for the C-implanted polystyrene films at 5 and 10 keV with $3 \times 10^{15}$ ions/cm$^2$, where the best condition for selective cell attachment expected by the lowest angle, are shown in Figs. 4(a) and 4(b), respectively. More than 80% of the seeded cells clearly attached on the implanted region, where is the narrow dark stripe region of 50-μm width. As similar to implantation at $3 \times 10^{15}$ ions/cm$^2$, more than 75% of seeded cells clearly attached on the implanted region with over the dose range of 0.7–7 $(\times 10^{15})$ ions/cm$^2$ and with that of 1–5 $(\times 10^{15})$ ions/cm$^2$ for implantation at 5 and 10 keV, respectively. These dose ranges correspond to the lowering of contact angle to 69°, which is in the suitable range for the adsorption of the adhesive protein for cell attachment$^{11}$.

Figs. 5(a) and 5(b) are the micrographs of the PC12h cells cultured on the C-implanted polystyrene films with $3 \times 10^{16}$ ions/cm$^2$ dose at 5 and 10 keV, respectively. Attachment of nerve cells was not clear because of the unselective attachment of cells on both of the surfaces. Although most of cells attached on the implanted region,
Fig. 4 Phase-contrast optical micrographs of PC12h cells cultured on the implanted region, where is the dark stripe region, of the polystyrene surfaces implanted by $3 \times 10^{15}$ ions/cm$^2$ at: (a) 5 keV and (b) 10 keV.

Fig. 5 Phase-contrast optical micrographs of PC12h cells cultured on the implanted region, where is the dark stripe region, of the polystyrene surfaces implanted by $3 \times 10^{16}$ ions/cm$^2$ at: (a) 5 keV and (b) 10 keV.

some of them also attached on the unimplanted region. Moreover, the shape and size of cells cultured on the C-implanted surfaces at $3 \times 10^{16}$ ions/cm$^2$ looked different from those of cells cultured on the surface implanted at lower dose or on the reference dish. However, the effect of the carbon negative-ion implantation at high ion dose is unclear because evaluation of the physical surface property only from the contact angle measurement could not show.

4. Conclusion

The effects of the carbon negative-ion implantation at 5- and 10-keV energy on the nerve-cell attachment to polystyrene films spin-coated on glass were reported. The surface of polystyrene was improved to be hydrophilic as the lowering of contact angles. After 2 days culture of the PC12h cells, the suitable doses for the selective nerve-cell-attachment properties at 5- and 10-keV implantation were obtained over the dose ranges of 0.7–7 ($\times 10^{15}$) and 1–5 ($\times 10^{15}$) ions/cm$^2$, respectively. Those dose ranges correspond to the low contact angle value of about 69°. The best condition for nerve-cell-attachment property at 5- and 10-keV implantation was at $3 \times 10^{15}$ ions/cm$^2$ with the lowest value of contact angle.

Reference