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# Nerve Cell Attachment Properties on Spin-coated Polystyrene Modified by Carbon Negative-ion Implantation

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The effects of the carbon negative-ion implanted into the spin-coated polystyrene (PS) through the pattering mask slits of 50-μm width on the nerve cell attachment properties were studied. Contact angles of the PS implanted at the same conditions without the pattering mask were measured by water drop and air bubble. The implantation conditions were various ion doses from $1x10^{14}$ - $3x10^{16}$ ions/cm$^2$ at various ion energies from 5 - 20 keV. After 2-day *in vitro* culture of the nerve-like cell of rat adrenal pheochromocytoma (PC12h) on the implanted films as well as on type–I collagen-coated dish as a reference, the phase contrast micrographs show that the cells attached only on the stripe regions implanted with order of $10^{15}$ ions/cm$^2$. The best condition for cell attachment is around 2 - 3 ($x10^{15}$) ions/cm$^2$ of ion doses corresponding to the low contact angle at this region, which is about 63° after 48 h dipping in the de-ionized water. As implanted at low dose, no cell attached. At high dose, mostly cells attached on the implanted regions, some attached on the unimplanted regions, as well as cells are abnormal in shape and large size.

Key words: negative ion implantation, cell attachment, polystyrene, contact angle

## 1. INTRODUCTION

The modifications of polymeric surface for the biocompatible improvement by using ion implantation have been investigated [1-6]. The negative ion implantation techniques have been used to modify the polymeric surface for the improvement of nerve-cell attachment because of the advantage of charge-up free [7-9]. The improved-attachment of cells on the polymeric surfaces modified by the carbon negative ion implantation is related to the modified physical surface property such as the hydrophilicity [3-4]. The simple way to evaluate this modified surface property is contact angle of a pure de-ionized water (DIW). Generally, the low contact angle corresponds to the high hydrophilic surface and to high possible attachment of cells. The dependences of contact angle in DIW on the dose and energy implantation, on the circumstance and on the measuring time were reported [3].

In this present, the nerve cell attachment properties and the contact angle of water at various circumstances on the spin-coated polystyrene films modified by the carbon negative-ion implantation are investigated.

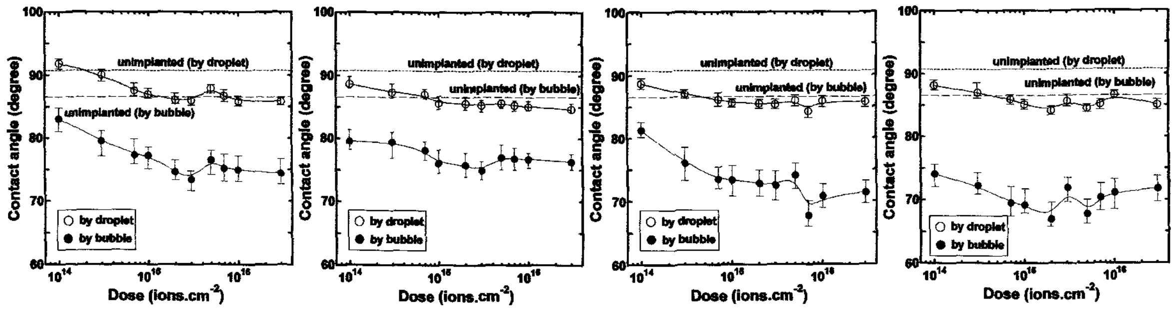
## 2. EXPERIMENT

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) [10, 11] were mass-separated and transported to an implantation chamber. The carbon negative-ion beam of 11.28 mm in diameter was implanted to the films at various ion energies from 5 to 20 keV and various doses from 0.1, 0.3, 0.7, 1, 2, 3, 5, 7, 10, and 30 (x $10^{15}$) ions/cm$^2$ with a current density less than 400 nA/cm$^2$ under residual gas pressures less than 6 x $10^{-4}$ Pa. For the physical surface property evaluation, the wettability 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, 24 and 48 h at 37°C. The air bubble method was done by putting a small bubble less than 1-mm diameter on the sample during dipping in the de-ionized water. For observation of the nerve-cell attachment on the modified films the samples were implanted through a pattering mask of many slit apertures 50-μm width and 70-μm spacing, and each C-implanted sample was then fixed with a 35-mm dish (Non-treated polystyrene dish, Corning). After 2-day dry all fixed dishes were 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 (PC12h) about $3.7x10^5$ cells/ml were cultured on the sample dishes 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 on the modified surfaces were observed by phase contrast microscope (CK2, Olympus).

## 3. RESULTS AND DISCUSSION

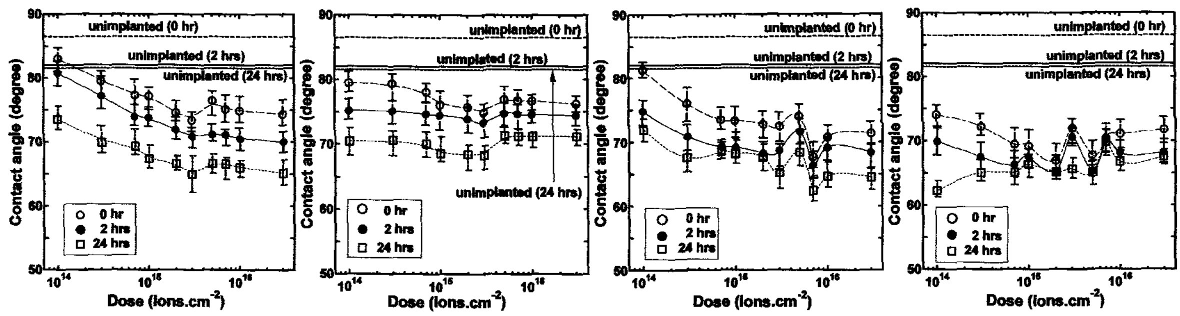
### 3.1 Contact angle

The contact angles of DIW on the C-implanted surfaces of polystyrene as the function of ion dose at various energies from 5-20 keV measured by the water drop and air bubble method after the implantation within 2 h are shown in Fig. 1.




(a) C/PS at 5 keV (b) C/PS at 10 keV (c) C/PS at 15 keV (d) C/PS at 20 keV

Fig. 1. Contact angle measured by water drop and air bubble method on C-implanted polystyrene films (C/PS) as a function of the ion dose at: (a) 5; (b) 10; (c) 15 and (d) 20 keV.




(a) C/PS at 5 keV (b) C/PS at 10 keV (c) C/PS at 15 keV (d) C/PS at 20 keV

Fig. 2. Contact angle measured by air bubble method at 0, 2, 24 h on C-implanted polystyrene films as a function of the ion dose at: (a) 5; (b) 10; (c) 15 and (d) 20 keV.

As previous work, the carbon negative ions implanted cut the C-C bond on the polymeric surface and penetrated into the bulk material [3-4]. Then 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 induce the defect or to form the functional group of OH and C-O on the implanted surface resulting in gradually change in contact angle on the implanted surface. That means the change in angle of the implanted polystyrene should depend on the dose and energy implantation condition.

At low energy implantation such as 5 and 10 keV as shown in Figs. 1(a) and 1(b), the contact angles were decreased by increasing in the ion dose from 91° to the lowest angle of about 85° (for water drop method) and from 86° to that of about 74° (for air bubble method) at the $3\text{x}10^{15}$ ions/cm$^2$. After this ion dose, the angle increased with small degrees before became saturate at the value around 86° for water drop method and at the value around 75° for air bubble method. While at high energy implantation such as 15 and 20 keV as shown in Figs. 1(c) and 1(d), the angles were also decreased by increasing in the ion dose from 91° to the lowest angle of about 84° (for water drop method) and from 86° to that of about 68° (for air bubble method) at the $7\text{x}10^{15}$ and $5\text{x}10^{15}$ ions/cm$^2$ for 15- and 20-keV implantation, respectively.

The time dependence of the contact angles after 0-, 2-, and 24 h dipping in the DIW on the C-implanted surfaces of polystyrene implanted as the function of ion dose at various energies from 5-20 keV are also shown in Fig. 2. After dip all implanted polystyrene films in DIW, the angle rapidly decreased for 3°-5° within first 2 h and then gradually decreased for the dipping time from 2 to 24 h, until to reach the saturation.

For all energy implantations at the same ion dose of $3\text{x}10^{15}$ ions/cm$^2$, insignificant decrease of contact angles after dipping in DIW for 0, 2, 24 and 48 h occurred when increase in the ion energy as shown in Fig. 3.

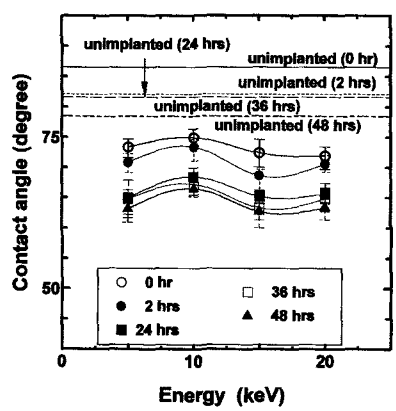



Fig. 3. Contact angle on C-implanted polystyrene films as a function of the ion energy at $3\text{x}10^{15}$ ions/cm$^2$ after 0, 2, 24, and 48 h dipping in DIW

From Fig. 3 after 24 h dipping in DIW, the contact angles obtained from all energy implantation became saturate and after 48 h dipping they saturate at around 63°.

3.2 Nerve-cell attachment

Based on phase-contrast optical micrograph, for all energy implantation at low dose with the order of $10^{14}$ ions/cm$^2$ the negligible cells attached on the surface as shown in Fig. 4, typical phase contrast micrograph of PC12h cells cultured for 2 days on the C-implanted polystyrene films at 10-keV and $1\text{x}10^{14}$-ions/cm$^2$ implantation.

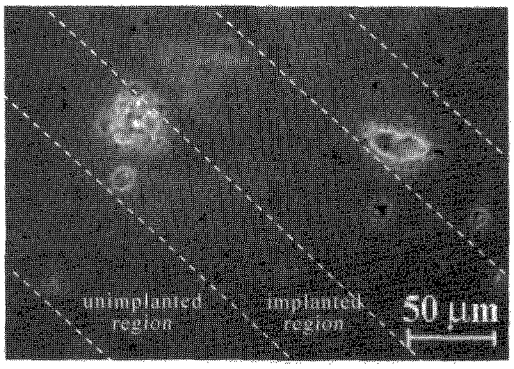
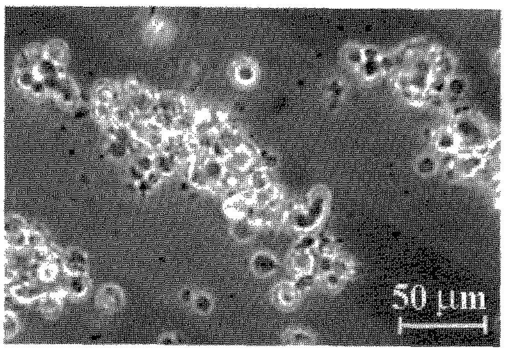



Fig. 4. Phase contrast micrograph of PC12h cells after 2 days culture on the films implanted at 10 keV and $1\text{x}10^{14}$ ions/cm$^2$

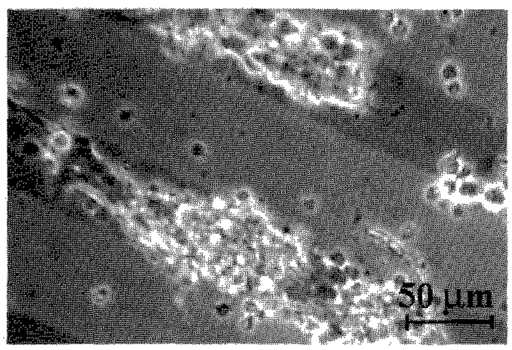
Although, at low dose implantation the stripe of implanted region can not been seen, we can confirm that the negligible cells attached only on the implanted surface, which is the narrow region between the dotted lines, from a small lowering of contact angle value at the implanted region. The attachment of nerve cells at low dose implantation is not good, and the cells detached from the implanted surface when only the movements of culture medium were happened.

Typical phase contrast micrographs of PC12h cells cultured for 2 days on the C-implanted films at various energies from 5-20 keV with $3\text{x}10^{15}$ ions/cm$^2$, which corresponding to the optimum lowering value of the contact angle, are shown in Fig. 5. At this dose implantation the PC12h cells clearly attached on the implanted region, where is the dark narrow stripe region of 50-µm width. As similar to implantation at $3\text{x}10^{15}$ ions/cm$^2$, at implantation with the order dose of $10^{15}$ ions/cm$^2$ the cells also clearly attached on implanted region, corresponding to a very low contact angle at these implanted regions. In case of high energy implantation of 15 and 20 keV, the cells also well attached on the polystyrene films at the dose implantation that give the lowest contact angle, but the number of cells on this implanted region is less than that of cells on the implanted region with around 2-3 ($\text{x}10^{15}$) ions/cm$^2$ (data not shown). However, the numbers of cells on the sample implanted at $3\text{x}10^{15}$ ions/cm$^2$ with high energies of 15-20 keV are smaller than that of 5-10 keV.

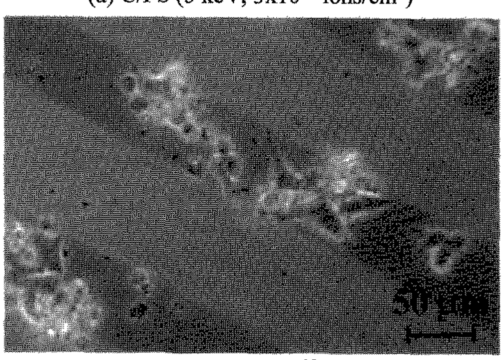
The abnormal observations of cell attachment were seen on the films implanted at high dose implantation both with low and high energy as shown in Fig. 6.



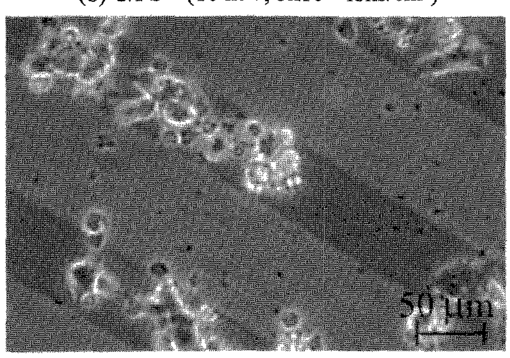

(a) C/PS (5 keV, $3\text{x}10^{15}$ ions/cm$^2$)




(b) C/PS (10 keV, $3\text{x}10^{15}$ ions/cm$^2$)

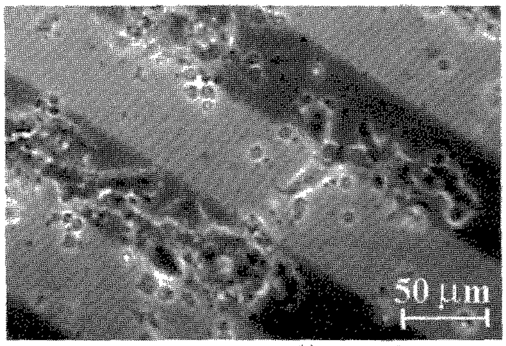


(c) C/PS (15 keV, $3\text{x}10^{15}$ ions/cm$^2$)

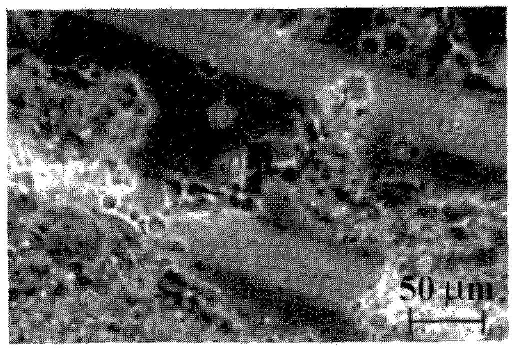



(d) C/PS (20 keV, $3\text{x}10^{15}$ ions/cm$^2$)

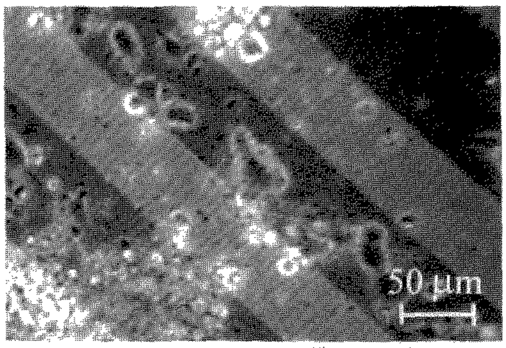
Fig. 5. Phase contrast micrograph of PC12h cells after 2 days culture on the films implanted by $3\text{x}10^{15}$ ions/cm$^2$ at: (a) 5, (b) 10, (c) 15 and (d) 20 keV.



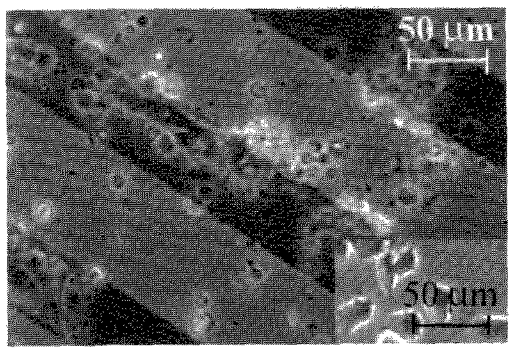

(a) C/PS (5 keV, $3\text{x}10^{16}$ ions/cm$^2$)




(b) C/PS (10 keV, $3\text{x}10^{16}$ ions/cm$^2$)




(c) C/PS (15 keV, $3\text{x}10^{16}$ ions/cm$^2$)




(d) C/PS (20 keV, $3\text{x}10^{16}$ ions/cm$^2$)

Fig. 6. Phase contrast micrograph of PC12h cells after 2 days culture on the films implanted by $3\text{x}10^{16}$ ions/cm$^2$ at: (a) 5, 10, 15 and (b) 20 keV.

At high dose implantation, the selective attachments of nerve cells were not clear since the attachments to both of implanted and unimplanted regions were observed. Almost all nerve cells attached on the implanted region. In addition, the shape and size of cells look different and larger than that of cells cultured in the reference dish, as shown in the small picture on the right hand of Fig. 6(d).

The effect of the carbon negative ion implantation at high ion dose is unclear because only the evaluation of the physical surface property by the contact angle measurement can not explain this effect.

## 4. CONCLUSION

Nerve-cell attachment on the spin-coated polystyrene modified by carbon negative-ion implantation with the various ion doses and energies were investigated. All implanted surfaces of polystyrene films were modified to be hydrophilic as the lowering of contact angles. Especially, the angles on the polystyrene films implanted at $3\text{x}10^{15}$-ions/cm$^2$ dose decrease from 86 to about 63° after 48 h dipping in DIW. After 2-day culture of the PC12h cells, the suitable doses for the selective nerve-cell-attachment property of all energy implantations are obtained with order dose of $10^{15}$ ions/cm$^2$ that correspond to the low contact angle value of about 69° after 24 h dipping in DIW. The best condition for selective nerve-cell-attachment property at these energy implantations is at 2-3 ($\text{x}10^{15}$) ions/cm$^2$. At 5-10 keV, the best condition is at the dose with the lowest contact angle. However, the best condition for this property at 15-20 keV does not be obtained at the dose in spite of their lowest contact angle.

## 5. REFERENCES

[1] H. Tsuji, H. Sato, T. Baba, S. Ikemura, Y. Gotoh, and J. Ishikawa, *Nucl. Instr. and Meth.*, **B 166/167,** 815-19 (2000).

[2] H. Tsuji, M. Izukawa, R. Ikeguchi, R. Kakinoki, H. Sato, Y. Gotoh, and J. Ishikawa, *Nucl. Instr. Meth.*, **B 206**, 507-11 (2003).

[3] H. Tsuji, H. Sasaki, Y. Utsumi, H. Sato, Y. Gotoh, and J. Ishikawa, *Surf. Coat. Tech.*, **158–159**, 620-23 (2002).

[4] H. Tsuji, H. Sasaki, H. Sato, Y. Gotoh, and J. Ishikawa, *Nucl. Instr. and Meth.*, **B 191**, 815-19 (2002).

[5] H. Tsuji, M. Izukawa, Y. Utakawa, R. Ikeguchi, R. Kakinoki, H. Sato, Y. Gotoh, and J. Ishikawa, *Trans. Mater. Res. Soc. Japan*, **29 [2]**, 575-80 (2004).

[6] H. Tsuji, P. Sommani, T. Muto, Y. Utagawa, S. Sakai, H. Sato, Y. Gotoh, and J. Ishikawa, *Nucl. Instr. and Meth.*, **B 237**, 459-64 (2005).

[7] H. Tsuji, Y. Toyota, J. Ishikawa, S. Sakai, Y. Okayama, and S. Nagumo, "Ion Implantation Technology-94", Eds. by S. Coffa, G. Ferla, and R. Priole, Elsevier, New York (1995) pp. 612-15.

[8] H. Tsuji, J. Ishikawa, S. Ikeda, and Y. Gotoh, *Nucl. Instr. and Meth.*, **B 127/128**, 278-81 (1997).

[9] H. Tsuji, Y. Gotoh, and J. Ishikawa, *Nucl. Instr. and Meth.*, **B 141**, 645-51 (1998).

[10] J. Ishikawa and H. Tsuji, *Nucl. Instr. and Meth.*, **B 74**, 118-22 (1993).

[11] H. Tsuji, T. Taya, J. Ishikawa, and T. Takagi, "Proceedings of the International Ion Engineering Congress, ISIAT'83 & IPAT'83", Vol. 1, Ed. by T. Takagi, Institute of Electrical Engineers of Japan, Kyoto (1983) pp. 141-46.

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