Transactions of the Materials Research Society of Japan 33[4] 1031-1034 (2008)

Ion Beam Modification of PTFE Fiber for Improving Cell and Tissue Compatibility

Takanori Uchida, Hiroshi Toida*, Rena Ujiie*, Yoshiaki Suzuki*, Hiroshi Ujiie**, Tomokatsu Hori**, and Shizutoshi Ando***
KAKETSUKEN, Kumamoto-shi, Kumamoto 860-8568, Japan
Fax: 81-96-345-1345, E-mail: uchida-ta@kaketsuken.or.jp
*AD&S, RIKEN, Wako-shi, Saitama, 351-0198 Japan
**Tokyo Women's Medical University, Shinjuku-ku, Tokyo 162-8666, Japan
***Faculty of Engineering, Tokyo University of Science, Tokyo 102-0073, Japan

The surface of PTFE fiber was modified with ion beam irradiation to improve this critical property, and the effects of its biocompatibility were investigated. PTFE fibers were irradiated with Ar^+ ions with a fluence of 5×10^{14} ions/cm^2 at energy of 150 keV. L929 fibroblasts cell was used for in vitro cell culture. Japanese white rabbits were used in this study. Ion–implanted PTFE fiber was implanted subcutaneously on the carotid arteries after administering anesthesia. Non-implanted PTFE fiber and ion-implanted PTFE fiber were used for the test specimens. These specimens were wrapped around the rabbit's carotid arteries. Both of the in vitro and in vivo studies demonstrated that ion-implanted PTFE fiber exhibits remarkably greater adhesion and spreading of living cells than non-implanted.
Key words: fibroblast cell, in vivo study, FT-IR-ATR, Raman spectroscopy

1. INTRODUCTION

Ion implantation has proven to be a useful technique for improving the surface properties of materials [1-2]. In recent years, ion implantation has been used for the surface modification of polymers to improve blood compatibility [3-5], tissue compatibility [6], and the clinical application of this technique [7-10].

Major features of expanded polytetra- fluoroethylene (ePTFE) as a biomaterial are its high flexibility, strength, durability, and excellent biological inertness. In clinical medicine, ePTFE is now widely used as prostheses such as vascular graft, dural substitute, pericardial substitute, peritoneal patch, barrier membrane for guided tissue regeneration, and suture material. ePTFE is used as an artificial dura mater but is often associated with cerebrospinal fluid (CSF) leakage after skull base surgeries, because ePTFE dose not adhere well to fibrin glue and tissue. PTFE (polytetrafluoroethylene) fiber is also often used for brain surgery.

The adhesion, growth, and proliferation of endothelial cells in cell culture are anchorage-dependent. It is supposed that the adhesion of endothelial cells is mainly influenced by the nature of the substrate. Many investigations have been introduced focusing on the development of biocompatible materials, and the improvement of cell attachment to such surfaces.

PTFE is a polymer which consists of carbon and fluorine. PTFE has a long, straight carbon back bone to which the fluorine atoms are bonded. Expanded polytetrafluoroethylene (ePTFE) is a porous material obtained by thermo-mechanically expanding polytetrafluoroethylene (PTFE), which is generally known to the public by DuPont's brand name "Teflon". PTFE and ePTFE possess outstanding properties, such as excellent chemical and biological inertness, wide temperature range stability, and excellent dielectric properties. Therefore, PTFE and ePTFE are widely used in industrial and medical fields. However, PTFE has a highly hydrophobic surface that limits cell adhesion. This study concerns fabrication of new cell adhesive materials by ion implantation into PTFE fiber.

An in vitro and in vivo study of ion-implanted PTFE fiber were performed using rabbits to develop cell adhesive artificial materials in relation to surface characteristics.

2. EXPERIMENTAL

2.1 Ion Implantation

The specimens were PTFE fiber (Boston Scientific). Ar^+ (5×10^{14} ions/cm^2) ion were implanted into PTFE at an energy of 150 keV at room temperature. The beam-current density was lower than 0.05 μ A/cm^2 to prevent temperature rising.

2.2 In Vitro Cell Attachment Study

L929 fibroblasts cells were used for in vitro cell culture. This cell suspension was placed on the ion-implanted PTFE in medium (RPMI1640; Nissui Pharmaceutical Co.) supplemented with 10% fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY). The initial number of cells seeded was 2.5×10^4 cells/ml. The cells were incubated for eight days at 37℃ in 5% CO_2 in a humid atmosphere. The extent of cell attachment and

spreading was determined visually with an optical microscope equipped with phase contrast objectives and a camera.

2.3 In Vivo Animal Study

Thirteen Japanese white rabbits weighing 3 to 4.5 kg were used in this study. Ion–implanted PTFE fiber was implanted subcutaneously on the carotid arteries after administering anesthesia of sodium pentobarbital. Non-implanted PTFE fiber and ion-implanted PTFE fiber were used for the test specimens. These specimens were wrapped around the rabbit's carotid arteries as depicted in Fig. 1. All the procedures were conducted under sterile conditions.

The animals were then killed by an intravenous overdose of KCl at different times, and the specimens were surgically removed for histopathological examination. The tissue was fixed in a 10% formalin solution, decalcified with formic acid, and stained with hematoxylin and eosin.

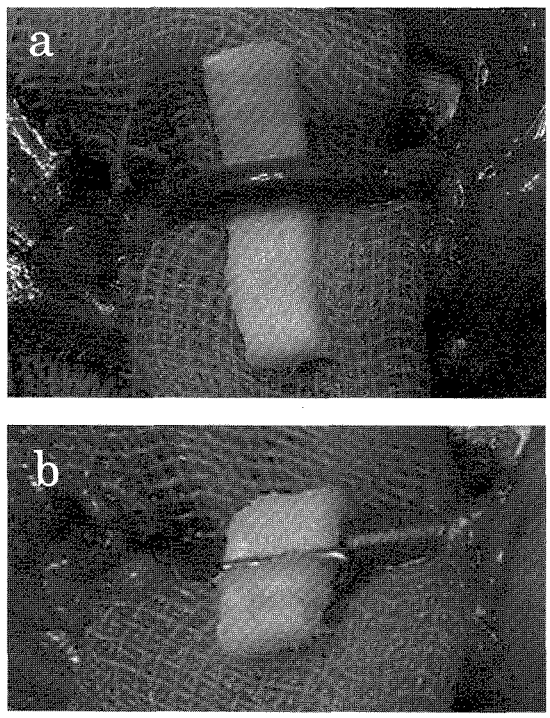

Fig. 1 In vivo tissue adhesive study

2.4 Physico-Chemical Properties

Ion-implanted and non-implanted PTFE fiber were coated with gold in a plasma coater (SG-701, Sanyu Denshi, Japan). Microphotographs of field emission scanning electron microscopy (SEM) were obtained at a 5 kV acceleration voltage (JSM6330F, JEOL, Japan). Laser raman spectroscopy (Raman, Jobin Yvon, LabRam) and fourier transformed infrared spectroscopy analyses (in attenuated total reflectance mode, FT-IR-ATR) of Ar^+-implanted and non-implanted PTFE surfaces were performed with an FT-IR-ATR spectrometer (NEXUS470, Nicolet, France). The analyses were conducted with an internal reflection element (Ge, 45° incident angle), 128 scans, and a resolution of 4 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1 SEM Observations

The SEM micrographs of non-implanted (a) and Ar^+ ion-implanted PTFE fiber with a fluence of 5×10^{14} ions/cm^2 (b) were displayed in Fig. 2. The morphology of Ar^+ ion-implanted PTFE fiber became thinner because of radiation effects in comparison with non-implanted PTFE fiber.

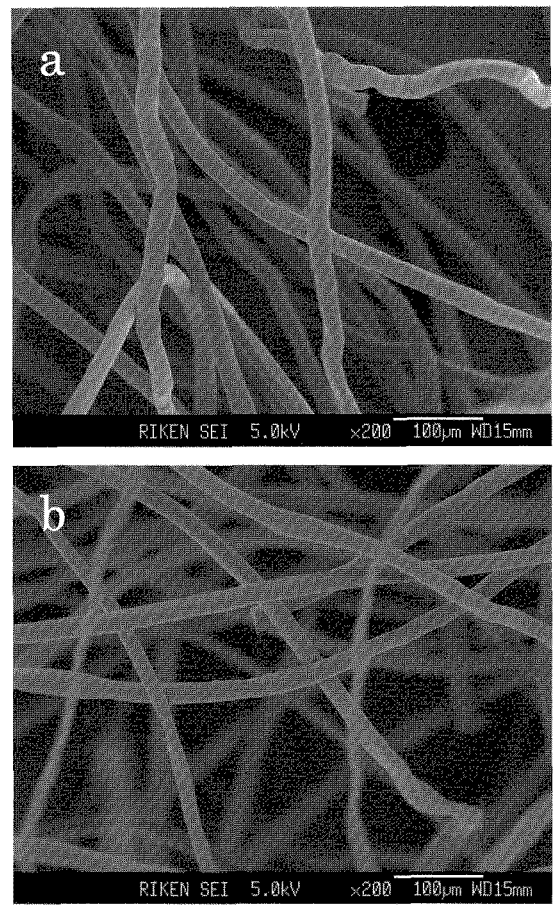

Fig.2 SEM photographs of non-implanted PTFE fiber (a) and Ar^+ ion implanted PTFE fiber (b).

3.2 In Vitro Cell Attachment Study

Figure 3 illustrates SEM images of fibroblast cell attachment to 5×10^{14} ions/cm^2-Ar^+ ions/cm^2 ion-beam implanted PTFE fiber, after an incubation of 8 days. The seeded cells recognized the surface of ion-irradiated PTFE fiber and attached to the ion-beam implanted domain. Cell attachment to the Ar^+ ion-implanted PTFE fiber further improved in performance as compared with non-implanted PTFE fiber.

3.3 In Vivo Animal Study

Figure 4 presents the histology of non-implanted (a) and Ar^+ ion-implanted PTFE fiber surfaces (b) 30 days after implantation. The circum-vascular tissue contacting the ion-implanted PTFE fiber surface was composed of

tissue in growth fibroblasts. A histological examination of the ion-implanted PTFE fiber after implantation exhibited excellent attachment in contact with the wall of the carotid artery.

On the other hands, fibroblast-like cells invaded and anchored into the space between PTFE fiber and the wall of the carotid artery.

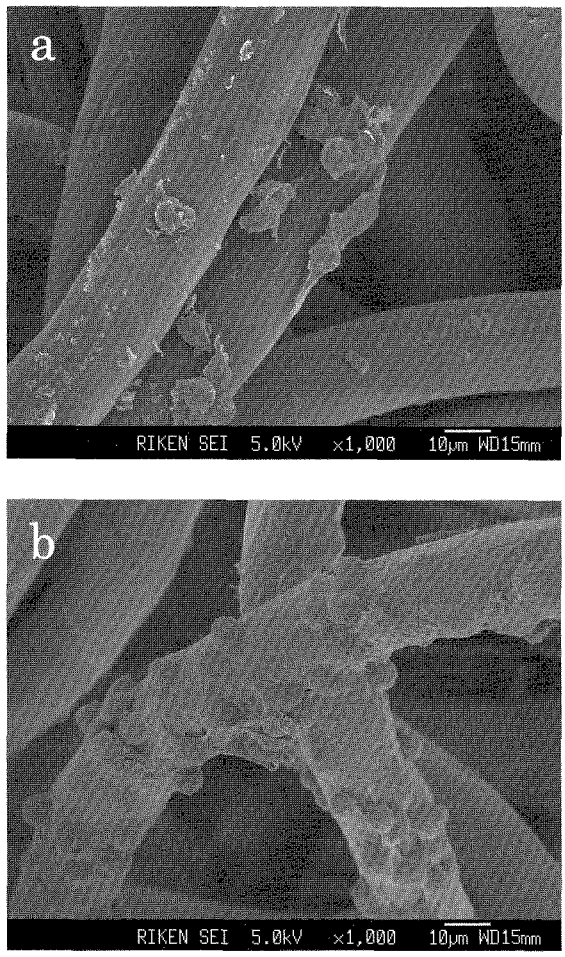

Fig.3 SEM photographs of fibroblast cell attachment to non-implanted and Ar^+-implanted PTFE fiber after 8 days.

3.4 Raman spectroscopic analysis

Figure 5 shows the Raman spectra of non-implanted and ion-implanted PTFE fiber with fluence of 1×10^{14}, 5×10^{14}, 1×10^{15} ions/cm^2 at an energy of 150 keV. No differences exist between un-implanted and ion-implanted PTFE fiber at irradiation fluences of 1×10^{14} ions/cm^2 as noted in the figure. In the Raman spectra, peaks at 735 and 1384 cm^{-1} are assigned to symmetric stretching modes of CF_2 and C–C, respectively, and the peaks at 287 and 382 cm^{-1} are assigned to CF_2 twisting and CF_2 bending modes, respectively. Overtone or combination vibration modes are found at 1215 and 1295 cm^{-1}. These Raman spectroscopic study revealed that the C-F and C-C decomposed with increasing fluences of irradiation, and decomposition was marked at an irradiation fluence over 5×10^{14} ions/cm^2.

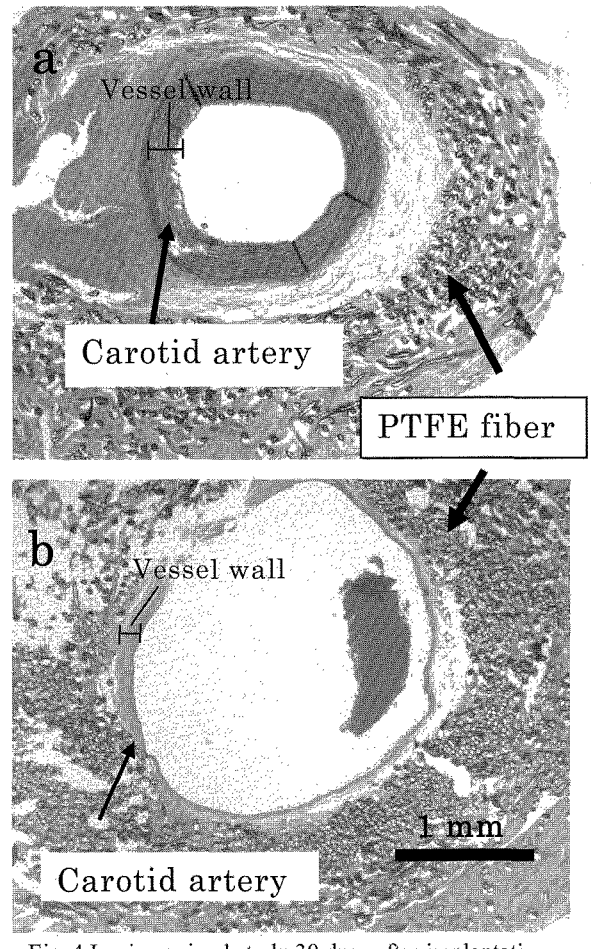

Fig.4 In vivo animal study 30 days after implantation. Histological Examination: Non-implanted PTFE fiber (a) and ion-implanted PTFE fiber (b) Ar^+, 150 keV, 5×10^{14} ions/cm^2.

3.5. FT- IR- ATR study

The surface of ion-implanted PTFE fiber was analyzed by FT-IR-ATR to study new functional groups and bond scission. Figure 6 illustrates the IR spectra of non-implanted (a) and Ar^+ ion-implanted (b) PTFE fiber. The strong bands of the CF_2 appear in the region of 1100-1300 cm^{-1}. The peaks of 1150 cm^{-1} and 1205 cm^{-1} are assigned as C-F bond. FT-IR-ATR study indicates that ion implantation destroyed the chemical structure of the surface layer of PTFE (CF_2 band). We observed the characteristic band of ion-implanted PFTE fiber at 1680 cm^{-1} - 1620 cm^{-1} (C=C bonds).

4. CONCLUSIONS

In vitro cell attachment to Ar^+ ion-implanted PTFE fiber demonstrated improved performance compared with non ion-implanted PTFE fiber. A histological examination of the ion-implanted PTFE fiber after implantation revealed excellent attachment in contact with wall of carotid artery. Our previous study already reported that the concentration of carbon atoms increased with the increase of fluence and the concentration of oxygen showed the same behavior. In summary, chemical characteristics of polymer may influence the host-tissue reaction [10]. With

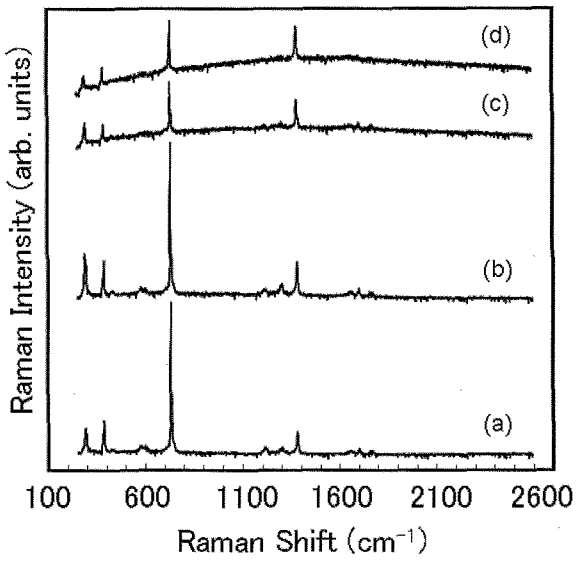

Fig.5 Raman spectra of non-implanted (a), Ar^+ ion-implanted with fluence of 1×10^{14} (b), 5×10^{14} (c), and 1×10^{15} ions/cm^2 (d) ion implanted PTFE fiber.

ion-implanted PTFE fiber, C=C radical induced by ion-implantation were major factor influencing cell invasion.

The ion implantation into PTFE fiber is a promising approach for developing artificial wrapping materials to prevent the rupture of brain aneurysms.

Therefore, it is very likely that ion-implanted PTFE fiber is applicable for clinical use.

RERERENCES

[1] P. Sioshansi, *Nucl Insrum Meth* B, **24-5**, 767-770 (1987).

[2] G. Marletta. *Nucl Insrum Meth*, **B46 (1-4)**,295-305 (1990).

[3] Y. Suzuki, M. Kusakabe, H. Akiba, K. Kusakabe, and M. Iwaki. *Nucl Instrum Meth B*, **B59/60**, 698-704 (1991).

[4] Y. Suzuki, H. Iwata, A. Nakao, M. Iwaki, M. Kaibara, H. Sasabe, S. Kaneko, H. Nakajima, and M. Kusakabe. *Nucl Instrum Meth B*, **127**, 1019-1022 (1997).

[5] K. Kurotobi, M. Kaibara, Y. Suzuki, M. Iwaki, and H. Nakajima. *Nucl Instrum Meth B*, **B175**, 791-796 (2001).

[6] Y. Suzuki, M. Kusakabe, J-S Lee, M. Kaibara, M. Iwaki, and H. Sasabe. *Nucl Instrum Meth B*, **B65**, 142-147 (1992).

[7] Y. Murayama, Y.Suzuki, F. Vinuela, M. Kaibara, K. Kurotobi, M. Iwaki, and T. Abe. *Am J Neuroradiol*, **20**, 1986-1991 (1999).

[8] Y. Murayama, F. Vinuela, Y. Suzuki, Y. Akiba, A. Ulihoa, G. Duckwiler, Y. Gobin, H. Vinters, M. Iwaki, and T. Abe T. *Am J Neuroradiol*, **20**, 1992-1999 (1999).

[9] M. Kaibara, H. Iwata, H. Wada, Y. Kawamoto, M. Iwaki, and Y. Suzuki. *J Biomed Mat Res*, **31**, 429-435 (1996).

[10] Y. Suzuki, M. Iwaki, S. Tani, G. Oohashi and M. Kamio, *Nucl. Instrum. Methods Phys. Res.* **B206**, 538-542 (2003).

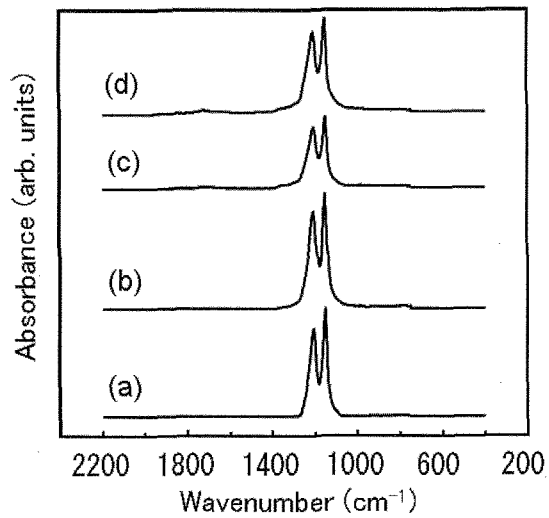

Fig.6 FT-IR-ATR spectra of non-implanted (a), Ar^+ ion-implanted with fluence of 1×10^{14} (b) , 5×10^{14} (c), and 1×10^{15} ions/cm^2 (d) ion implanted PTFE fiber.

(Recieved January 21, 2008 ; Accepted June 30, 2008)