

Ion Beam Irradiated ePTFE Remarkably Improving Fibrin Glue and Tissue Adhesion

N. Takahashi, Y. Suzuki, H. Ujiie*, T. Hori* and M. Iwaki

Beam Application Team, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

Fax: 81-048-462-4623, e-mail: noriyosi@postman.riken.go.jp

* Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku, Tokyo 162-8666, Japan

Expanded polytetrafluoroethylene (ePTFE) is used as an artificial dura mater but is often associated with cerebrospinal fluid (CSF) leakage after skull base surgeries, because ePTFE does not adhere well to fibrin glue and tissue. The surface of ePTFE was modified with ion beam irradiation to improve this critical property, and the effects of its biocompatibility were investigated. ePTFE sheets were irradiated with He⁺, Ne⁺, Ar⁺ and Kr⁺ ions with fluences 1x10¹⁴, 5x10¹⁴ and 1x10¹⁵ ions/cm² at an energy of 150keV. Ion beam irradiation induced ca. 5 to 20 μm gaps and spines on the surface of the ePTFE. A dural defect that was surgically created in a rabbit skull was patched with ion beam irradiated ePTFE. CSF leakage was observed in the rabbit covered with un-irradiated ePTFE, however, CSF leakage did not occur in all rabbits covered with ion beam irradiated ePTFE that adhered to surrounding tissues. A histological study indicated that fibroblast-like cells invaded and anchored into the gap of the ion beam irradiated ePTFE. *In vitro* tensile strength and burst tests verified that the adhesiveness of fibrin glue to ePTFE was remarkably enhanced by ion beam irradiation in sealing effects.

Key words: CSF, Biocompatibility, Animal study

1. INTRODUCTION

Cadaveric dura mater was used for a long time to cover dural defects but is known as a transmissible source of Creutzfeldt-Jakob disease [1,2]. Instead of cadaveric dura mater, an expanded polytetrafluoroethylene (ePTFE) sheet has been widely used [3]. ePTFE is a stable polymer, widely used as a prosthesis because of its chemical inertness [4-8]. However, ePTFE as an artificial dura mater is often associated with postoperative leakage of the cerebrospinal fluid (CSF) due to its very low adhesiveness to fibrin glue and surrounding tissue.

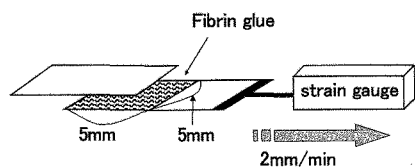
Recently we reported that ion beam irradiation could be used for surface modification of polymers to improve biocompatibility [9-12].

The authors examined the best ion species and irradiation condition in order to improve the shortcomings of ePTFE as an artificial dura mater.

2. MATERIALS AND METHODS

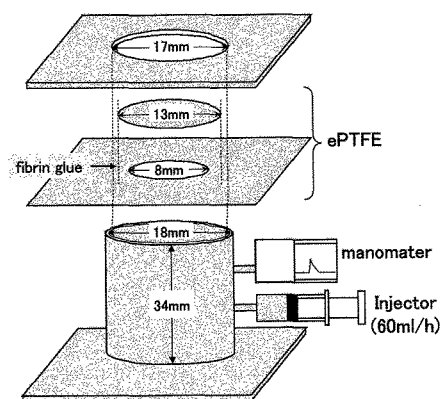
2.1 Ion beam irradiation

The surfaces of ePTFE sheets (DM-0300; W. L. Gore and Associates, United States) were irradiated with 150keV- He⁺, Ne⁺, Ar⁺ and Kr⁺ ions with fluences of 1x10¹⁴, 5x10¹⁴ and 1x10¹⁵ ions/cm². The beam current density was lower than 0.1 μA/cm². Surface and cross-sectional surfaces of ePTFE were examined with scanning electron microscopy (SEM, JSM6330F, JEOL, Japan). To obtain the cross-sections of ion beam irradiated layers, ePTFE was soaked with ethanol and



Tensile strength was measured with a pulling speed of 2 mm/min F.

Fig. 1: Tensile test



Water was constantly injected into the water cell whose top was covered water-tightly with a couple of ePTFE test specimens which that were glued together. The internal pressure was measured.

Fig. 2: Burst test

then freeze-fractured in liquid nitrogen.

2.2 *In vivo* animal study

Small dural defects with a diameter of about 1 to 2 mm were created in a rabbit's skull and patched either with an un-irradiated or an ion beam irradiated ePTFE. Implanted ePTFE sheets were fixed on dura mater and surrounding tissue with fibrin glue (BOLHEAL; KAKETSUKEN, Japan) without suture.

The animals were then killed at the time one week or one month after implantation. CSF leakage and interaction between the specimen and surrounding tissue were observed macroscopically, and the ePTFE was surgically removed en bloc for histological examination. The tissue was fixed in a 10% formalin solution, decalcified with formic acid, and stained with hematoxylin and eosin.

2.3 Fibrin glue adhesion test

The adhesiveness of fibrin glue to ion beam irradiated ePTFE was examined by measuring tensile strength (Fig. 1) and burst pressure (Fig. 2) after ion beam irradiated surfaces of ePTFE sheets were glued together. In the tensile strength test, one of the glued sheets of ion beam irradiated ePTFE was fixed and another sheet was gently pulled at a speed of 2 mm/min until they exfoliated from

each other. Pulling force was measured with a strain gauge. In the burst pressure test, the top of the water cell considered as skull was first covered by the ion beam irradiated ePTFE that had a small hole with a diameter of 8 mm. This small hole was then covered and glued by another ion beam irradiated ePTFE sheet with a diameter of 13 mm. The gentle injection of colored saline into the water cell until the point of leakage was performed as the burst pressure was monitored.

3. RESULTS

3.1 SEM studies of ion beam irradiated ePTFE

Un-irradiated ePTFE has a microporous structure consisting of nodes and fibrils of PTFE (Fig. 3A). Many cracks were created on the surface of ePTFE by all of the ion beam irradiation with a fluence of 1×10^{14} ions/cm² as not in fig.. The depth of the cracks increased with the increasing mass of ions. The number of cracks also increased and cracks became wider and deeper with increasing irradiation fluence in every kind of ion beam irradiation. Ne⁺ (1×10^{15} ions/cm²), Ar⁺ (5×10^{14} and 1×10^{15} ions/cm²) and Kr⁺ (1×10^{14} , 5×10^{14} and 1×10^{15} ions/cm²) ion beam irradiated ePTFE surfaces exhibited numerous spines perpendicular to the surface rather than cracks (Figs. 3 C, D) while He⁺ irradiated ePTFE surfaces with fluences of 1×10^{14} , 5×10^{14} and 1×10^{15}

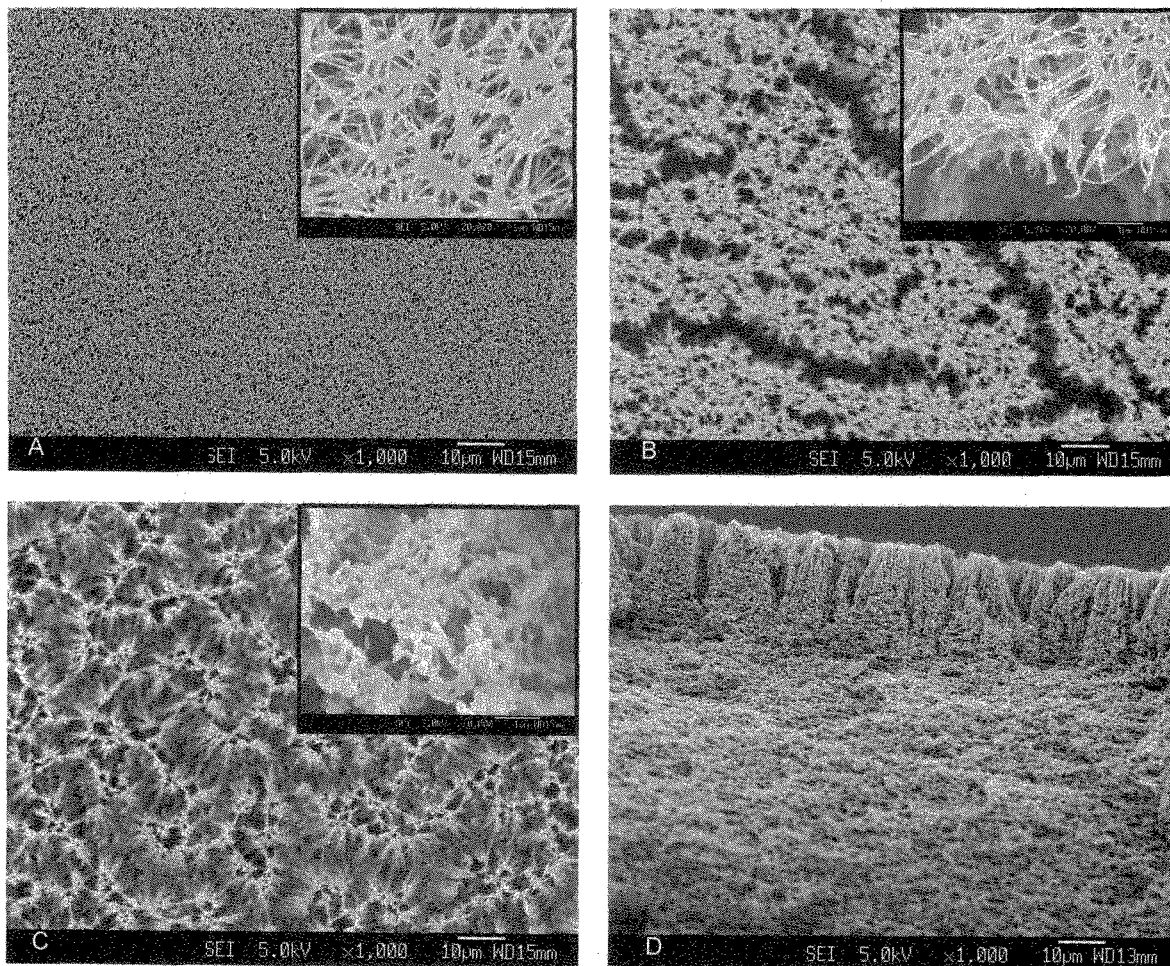


Fig. 3: Scanning electron micrographs of the surface of ion beam irradiated ePTFE

A: Un-irradiated ePTFE surface B: He⁺, 1×10^{15} ions/cm² irradiated ePTFE surface C: Kr⁺, 1×10^{15} ions/cm² irradiated ePTFE surface D: Cross-sectional photograph, Kr⁺, 1×10^{15} ions/cm² irradiated ePTFE

ions/cm² only created cracks (Fig. 3B).

3.2 *In vivo* animal study

During implantation surgery, ion beam irradiated ePTFE sheets were instantly fixed on dura mater with fibrin glue and were watertight. At one week and one month after implantation, CSF leakage was observed in the rabbit with the dural defect patched using un-irradiated ePTFE. However, there was no CSF

leakage in any rabbits patched with ion beam irradiated ePTFE.

In the histological study, cross-sections of implanted ePTFE and surrounding tissue were observed. One week after implantation, fibrin glue continued to adhere to the ion beam irradiated surface of ePTFE. However fibrin glue did not adhere to the un-irradiated surface of ePTFE. One month after implantation, fibrin glue was not observed on either the un-irradiated or irradiated

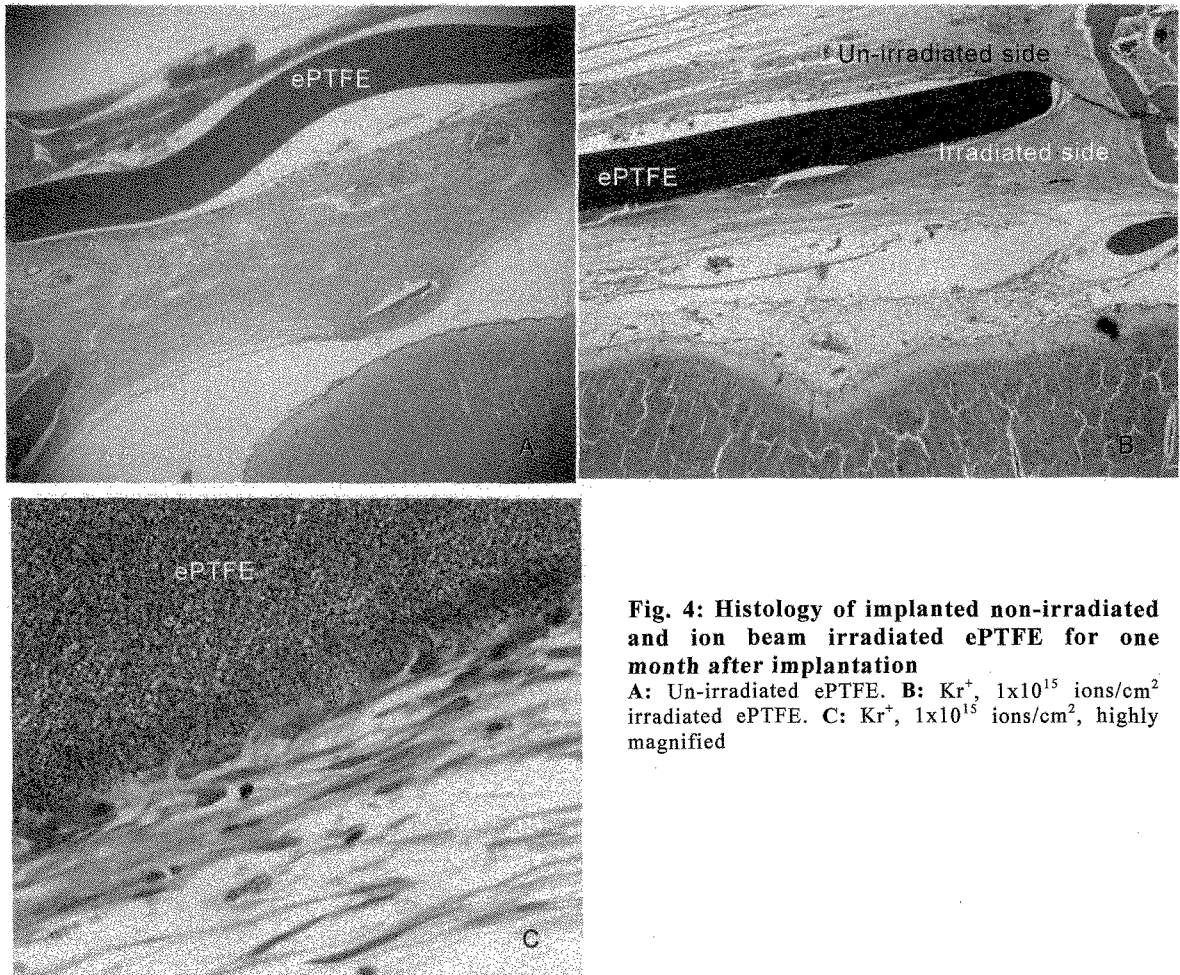


Fig. 4: Histology of implanted non-irradiated and ion beam irradiated ePTFE for one month after implantation

A: Un-irradiated ePTFE. B: Kr⁺, 1x10¹⁵ ions/cm² irradiated ePTFE. C: Kr⁺, 1x10¹⁵ ions/cm², highly magnified

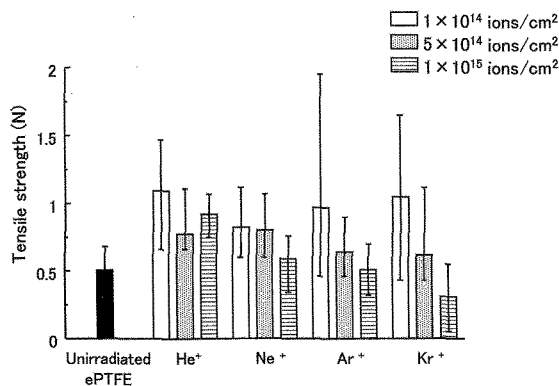


Fig.5: Tensile strength

Error bars indicate minimum and maximum values.

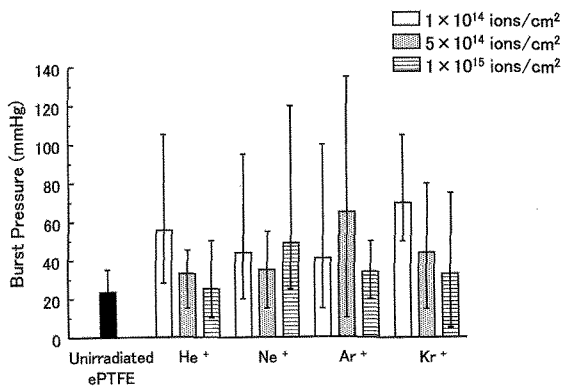


Fig.6: Burst pressure

Error bars indicate minimum and maximum values.

ePTFE. There was a regenerated thin membrane between the implanted ePTFE and the brain. In rabbits patched with un-irradiated ePTFE, there was space around the ePTFE. However in rabbits patched with ion beam irradiated ePTFE, especially that irradiated with Ar^+ and Kr^+ , a regenerated thin membrane that firmly adhered to ion beam irradiated surfaces was observed (Fig. 4B), and fibroblast-like cells invaded and anchored into the gaps between spines (Fig. 4C). The Ar^+ or Kr^+ with 5×10^{14} and 1×10^{15} ions/cm² irradiated ePTFE exhibited excellent healing of the dural defect.

3.3 Fibrin glue adhesion test

The results of fibrin glue tensile strength and burst pressure tests are shown in Figs. 5 and 6. The measured pressures were widely distributed in both tensile strength and burst pressure. This scatter was caused by non-uniform manual application of fibrin glue. Each maximum value was thought to be the best condition of fibrin glue application to test specimens.

Both tensile strength and burst pressure were remarkably enhanced by ion beam irradiation. In the tensile test, four different ions irradiated onto ePTFE at 1×10^{14} ions/cm² exhibited the highest values, 5×10^{14} ions/cm² irradiated ePTFE was second, and the 1×10^{15} irradiated ePTFE had the lowest maximum value. The tensile strength of Ar^+ and Kr^+ ion beam irradiated specimens decreased remarkably as the fluence increased in comparison with He^+ and Ne^+ ion-beam irradiation.

In the burst test, the burst pressure of ion beam irradiated ePTFE tended to decrease as fluence increased except for Ne^+ , and Ar^+ ion beam irradiation. The burst pressure of Ne^+ ion beam irradiated ePTFE possessed minimum value at 5×10^{14} ions/cm² and Ar^+ ion beam irradiated ePTFE possessed maximum value at 5×10^{14} ions/cm².

4. DISCUSSION

In vivo animal studies demonstrated that fibrin glue adhered to ion beam irradiated ePTFE and that CSF leakage was prevented during surgery and post surgery. The histological study also indicated that fibrin glue and regenerated thin membrane adhered to the ion beam irradiated surface of ePTFE. Fibroblast-like cells invaded and anchored into the gap in the ePTFE surface created by ion beam irradiation.

Results indicated that fibrin glue adhesion to ion beam irradiated surfaces of ePTFE prevented the early stage of CSF leakage. One month after implantation, fibrin glue was replaced by the regenerated thin membrane or surrounding connective tissue and the fibroblast-like cells adhered to ePTFE, which prevented later CSF leakage.

The *in vitro* fibrin glue adhesion test also indicated that fibrin glue adhesion to ePTFE surfaces was enhanced by ion beam irradiation.

Fibrin glue infiltrated and anchored into the gap created by the ion beam irradiation, and tensile strength decreased with increasing irradiation fluence. The spines vertical to the ePTFE surfaces become higher and thinner with increasing ion fluence, and the direction of the tension was parallel to the ePTFE surface. This indicated that the tensile strength of ion beam irradiated

ePTFE decreased when ion fluence excessively increased.

The maximum burst pressure of each ion beam irradiated ePTFE did not change linearly with ion fluence. Surface morphology was changed dramatically, and there was critical ion fluence in the formation of cracks and spines. This demonstrates that the shape of spines is closely associated with burst pressure. Spines were induced by Ne^+ beam irradiation over a fluence of 1×10^{15} ions/cm², Ar^+ beam irradiation over a fluence of 5×10^{14} ions/cm² and Kr^+ beam irradiation over a fluence of 5×10^{14} ions/cm². To explain these effects in more detail, further studies are required.

5. CONCLUSIONS

Ion beam irradiated ePTFE is a promising approach for developing artificial dura mater. Its great adhesiveness to fibrin glue and tissue would reduce the risks of CSF leakage, and it can be fixed securely to appropriate surrounding tissue by using ion-beam technology. It is therefore very likely that ion-beam-irradiated ePTFE will be applicable for clinical use.

6. ACKNOWLEDGMENT

This work was partly supported by an Open Research Grant from The Japan Research Promotion Society for Cardiovascular Diseases.

REFERENCES

- [1] J. Prichard, V. Thadani, R. Kalb and E. Manuelidis, *MMWR Morb Mortal Wkly Rep.*, 36, 49-50, 55 (1987).
- [2] V. Thadani, P. L. Penar, J. Partington, R. Kalb, R. Janssen, L. B. Schonberger, C. S. Rabkin and J. W. Prichard, *J. Neurosurg.*, 69, 766-769 (1988).
- [3] S. Yamagata, K. Goto, Y. Oda and H. Kikuchi, *Neurol. Med. Chir.*, 33, 582-585 (1993).
- [4] H. Matsumoto, T. Hasegawa, K. Fuse, M. Yamamoto and M. Saigusa, *Surgery*, 74, 519-523 (1973).
- [5] T. Soyer, M. Lempinen, P. Cooper, L. Norton and B. Eiseman, *Surgery*, 72, 864-872 (1972).
- [6] G. Bhatnagar, S. E. Frenes, G. T. Christakis and B. S. Goldman, *J. Card. Surgery*, 13: 190-193, (1998).
- [7] R. A. Monaghan and S. Meban, *Can. J. Surg.*, 34, 502-505 (1991).
- [8] J. Gottlow, S. Nyman, J. Lindhe, T. Karring and J. Wennström, *J. Clin. Periodontol.*, 13, 604-616 (1986).
- [9] Y. Suzuki, M. Kusakabe, H. Akiba, K. Kusakabe and M. Iwaki, *Nucl. Instrum. Meth. B*, 59-60, 698-704 (1991).
- [10] Y. Suzuki, H. Iwata, A. Nakao, M. Iwaki, M. Kaibara, H. Sasabe, S. Kaneko, H. Nakajima and M. Kusakabe, *Nucl. Instrum. Meth. B*, 127-128, 1019-1022 (1997).
- [11] K. Kurotobi, M. Kaibara, Y. Suzuki, M. Iwaki and H. Nakajima, *Nucl. Instrum. Meth. B*, 175, 791-796 (2001).
- [12] Y. Suzuki, M. Kusakabe, J-S. Lee, M. Kaibara, M. Iwaki and H. Sasabe, *Nucl. Instrum. Meth. B*, 65, 142-147 (1992).

(Received October 9, 2003; Accepted January 20, 2004)