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Ion-Beam Modification of Coronary Stent Grafts

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In recent years, the stent graft technology available for endovascular treatment of aortic dissections has undergone tremendous advancement. It is now possible to apply this minimally invasive technique to a wider range of pathology. The aim of this study was to develop anti-thrombogenic coronary stent grafts using ion-beam technology. In vitro platelet adhesion was inhibited on the He^+ ion-irradiated collagen-coated sample with a fluence of $1x10^{13}$ ions/cm^2, but activation occured on the un-implanted collagen surface. Cell attachment was observed both in materials that were irradiated and those that were not. However, more cells tended to adhere on irradiated surfaces than un-irradiated Ti-Ni. In general, inhibition of platelet adhesion and endothelial cell attachment to the surface did not occur in the same samples. However, He^+ ion-irradiated collagen-coated Ti-Ni plates with an ion fluence of $1x10^{13}$ ions/cm^2 simultaneously satisfied these two conditions. The results of this study indicate that ion-beam irradiation into collagen-coated Ti-Ni is a promising approach for developing substrates for coronary stent grafts.
Key words: Platelet adhesion, Cell attachment, Extracellular matrix, Collagen, FT-IR

1. INTRODUCTION

Recently, minimal invasive procedures using stent devices have attracted much attention in the treatment of severe coronary diseases like myocardial infarction. A stent is a medical device that keeps occluded arteries open for an extended period of time by inserting a twisted coil or metal mesh tube into a catheter [1][2]. The process involves inserting the catheter (containing the stent) intravascularly from a large artery such as the femoral artery, and then moving the catheter intravascularly under angiographic monitoring. The stent is then expelled from the catheter in the affected area, expanding it intravenously.

When an occluded coronary artery is opened during stent therapy, the ratio of re-occlusion of the coronary is extremely high (40 to 45 %), despite the short-term preservation of blood flow. This is mainly caused by antithrombogenicity of stent materials that are currently used.

The objective of this work is to fabricate biocompatible stents (Fig. 1) with improved antithrombogenicity, and to promote cell attachment by He^+ ion implantation into the collagen-coated Ti-Ni surface. In this report, we investigate in vitro endothelial cell attachment and in vitro platelet adhesion to the He^+ ion implanted collagen-coated Ti-Ni surfaces, as a function of fluence.

2. EXPERIMENTAL

2.1 Materials and Ion-Beam Irradiation

Ti-Ni alloy metal plates (Daido Special Steel Co., 10 mm × 5 mm × 0.5 mm) were used as a test material (base material). The chemical composition of the alloy metal is shown in Table 1. The base material was heated at 520°C for 5 min, followed by water quenching. The oxidized film was removed by polishing the surface with #120, #600, #800, and #1000 water-resistant abrasive paper, and then by an ultrasonic cleaning wash in ethanol for 15 min (repeated three times for 5 min each). Type I collagen (0.3 vol% solution, Koken Co.) was then coated onto the base material using the 24 hr dip-coat method at 4°C. The plates were then dried at 4°C for 24 hrs. He^+ ion-beam irradiation of the collagen-coated Ti-Ni plates was performed with fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2, at an acceleration energy of 150 keV.

2.2 Platelet Adhesion

Platelet-rich plasma was used to evaluate antithrombogenicity. Platelet-rich plasma was extracted by centrifuging (200 G, 15 mins) 10 ml of fresh blood, to which 1 ml of sodium citrate had been added to inhibit blood coagulation. The residual blood was further centrifuged at 1200 G for 10 min to obtain a platelet-less plasma. The

Table1. Chemical composition of material used.

Alloy	Ni	C	O	Ti
Ni-Ti Alloy	56.03	0.039	0.04	Bal.

Unit:mass %

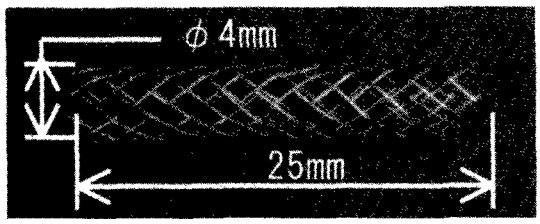

Fig. 1 Coronary stent.

concentration of platelet-rich plasma was adjusted to 10^4 cells/ml by performing a blood count of platelet-rich plasma and platelet-less plasma using an automatic blood counting device (MODEL PC-108, ERMA Co.).

Platelet-rich plasma was incubated in a water bath at 37°C for 5 min, dripped onto the ion-irradiated Ti-Ni alloy plates, and further incubated at 37°C for 5, 10, 20, and 30 mins in an atmosphere of 95% air / 5% CO_2. The samples were then washed twice with PBS(-), fixed with 2 vol% of glutaraldehyde solution, and dehydrated with 50, 70, 90, and 100 vol% of absolute ethanol. The samples were coated with gold in a plasma coater (SG-701, Sanyu Denshi, Japan) and the adhesion and activation of the platelets was then observed with a scanning electron microscope (JED6330F, JEOL, Japan).

2.3 Cell Attachment

Bovine aortic endothelial cells (BAECs) were isolated from the descending aorta using 0.1% collagenase, by a method adapted from Jaffe et al. [3]. The cell suspension was placed on the ion implanted collagen coated Ti-Ni surface in medium (RPMI1640; Nissui Pharmaceutical Co. Japan) supplemented with 10% fetal bovine serum (CFS; Sanko Junyaku Co. Japan). The initial number of cells seeded was 5×10^4 cells/dish. The cells were incubated for two days at 37°C in 5% CO_2 in a humid atmosphere. The extent of cell attachment and spreading were monitored visually with a scanning electron microscope.

2.4 Surface Characterization

Functional group analyses were carried out using FT-IR-ATR (Nexus 470, Thermo Nicolet, USA). The incident angle of light emitted from the ceramic Ge onto the samples was 45°, and the absorbance was obtained as a function of wave number by measuring the intensity of the reflected light. Each spectrum was obtained from at least 128 scans and then averaged at a resolution of 4 cm^{-1} from 4000 to 750 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1 Platelet Adhesion

Figure 2 depicts the in vitro platelet adhesion on non-implanted Ti-Ni and He^+ ion-implanted Ti-Ni surfaces determined using Ca^{2+} re-added platelet-rich-plasma (PRP) for 5 min. In vitro platelet adhesion was activated on the non-implanted Ti-Ni surface and ion implanted Ti-Ni with fluences of $1x10^{13}$, $1x10^{14}$ and $1x10^{15}$ ions/cm^2. These results indicated that platelet adhesion and aggregation were not depressed by ion implantation into the Ti-Ni surfaces.

Figure 3 illustrates the in vitro platelet adhesion on the non-implanted collagen-coated Ti-Ni and He^+ ion-implanted collagen-coated Ti-Ni using Ca^{2+} re-added PRP for 5 min. In vitro platelet adhesion was inhibited on the He^+ ion implanted collagen coated sample with a fluence of $1x10^{13}$ ions/cm^2. However, activation occurred on the non-implanted collagen surface and ion implanted collagen with fluences of $1x10^{14}$ and $1x10^{15}$ ions/cm^2. These results indicated that platelet adhesion and aggregation were decreased by ion implantation with a fluence of $1x10^{13}$ ions/cm^2.

Figure 4 presents the total number of adherent platelets as a ratio of the medical use polystyrene dishes on the collagen-coated Ti-Ni and ion-beam irradiated collagen-coated Ti-Ni per millimeter squared using Ca^{2+} re-added PRP for 5 min. A maximum number of platelets adhered to the collagen-coated Ti-Ni, indicating good thrombogenic property. Fewer platelets adhered on the surface of the ion beam irradiated sample with a fluence of $1x10^{13}$ ions/cm^2. The number of adhered platelets increased with higher ion fluence.

3.2 In Vitro Endothelial Cell Attachment

Figure 5 shows the endothelial cell attachment to non-implanted collagen-coated Ti-Ni and He^+ ion-implanted collagen-coated Ti-Ni with 1 x 10^{13}, 1x 10^{14}, and 1x 10^{15} ions/cm^2. Cell adhesion was observed on non-irradiated and ion-beam irradiated collagen-coated Ti-Ni. Cell attachment decreased with ion fluence.

Collagen-coated Ti-Ni alloy metal irradiated with $1x10^{13}$ ions/cm^2 in particular, as shown in (b), exhibited excellent cell adhesion.

Figure 6 indicates the total number ratio of adherent cells to medical use polystyrene dishes on the Ti-Ni, collagen-coated Ti-Ni and ion-beam irradiated collagen-coated Ti-Ni at an energy of 150 keV as a function of fluence. The cell attachment rate for the collagen-coated Ti-Ni and ion-beam irradiated collagen-coated Ti-N increased in comparison to the non-implanted Ti-Ni. The cell attachment ratio of the ion-beam irradiated collagen-coated Ti-Ni decreased with ion fluence.

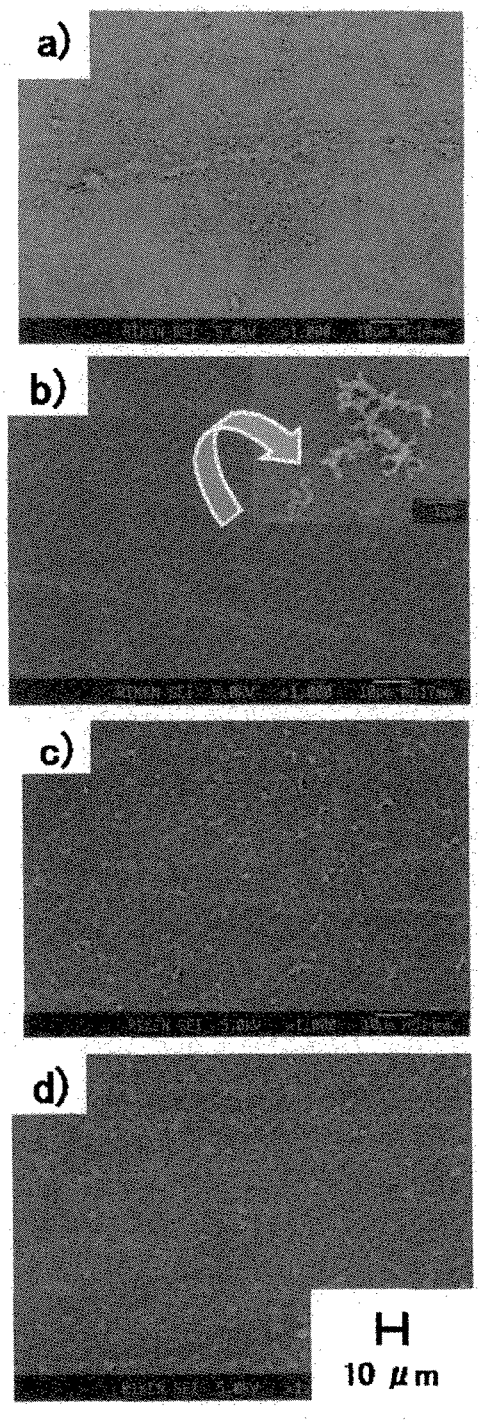

Fig.2 SEM photographs of platelets adhesion after contacting with $Ca2^{+}$ re-added PRP for 5 minuets to He^{+} ion implanted Ti-Ni alloy at an energy of 150 keV with fluences of a):non-implanted, b):$1x10^{13}$, c): $1x10^{14}$ and d):$1x10^{15}$ ions/cm^{2}.

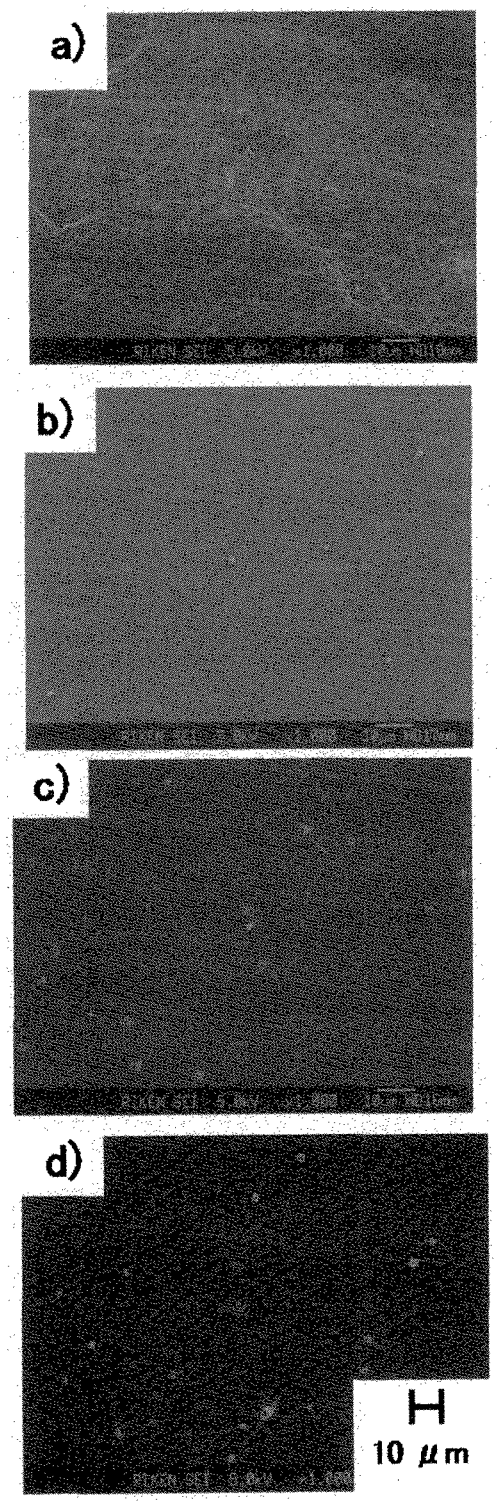

Fig.3 SEM photographs of platelets adhesion after contacting with Ca^{2+} re-added PRP for 5 minuets to He^{+} ion implanted collagen-coated Ti-Ni alloy at an energy of 150 keV with fluences of a):non-implanted, b):$1x10^{13}$, c): $1x10^{14}$ and d):$1x10^{15}$ ions/cm^{2}.

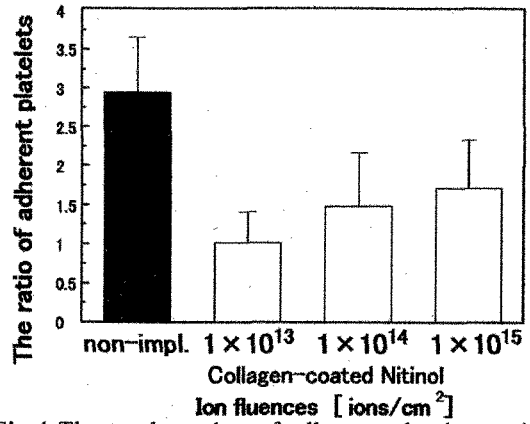

Fig.4 The total number of adherent platelets ratio to medical use polystyrene dishes on the collagen-coated Ti-Ni and ion beam irradiated collagen-coated Ti-N at an energy of 150 keV as a function of fluence.

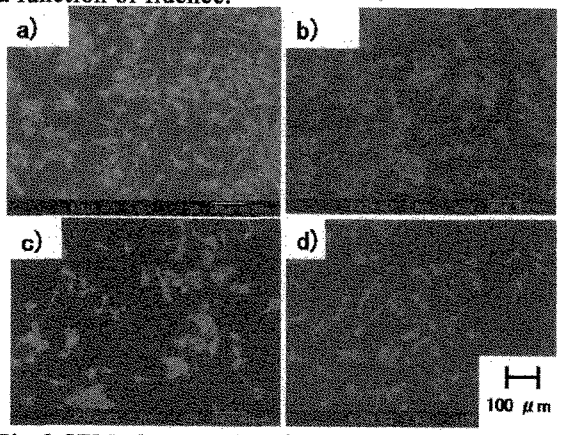

Fig.5 SEM photographs of attached bovine aortic endothelial cells after incubating for 48 hours to He^+ ion implanted collagen-coated Ti-Ni at an energy of 150 keV with fluences of a):non-implanted, b):$1x10^{13}$, c):$1x10^{14}$, and d):$1x10^{15}$ions/cm^2.

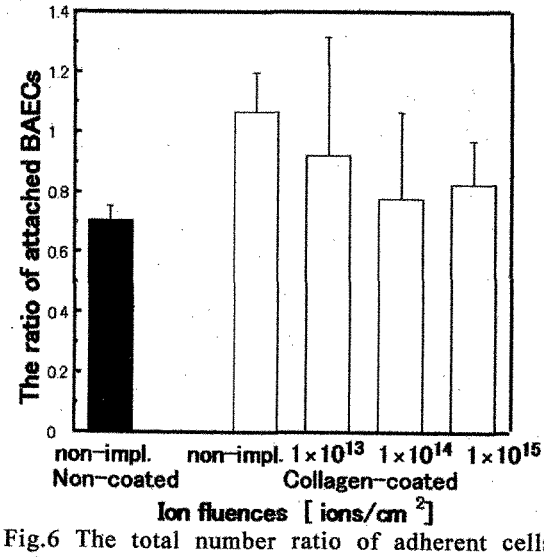

Fig.6 The total number ratio of adherent cells ratio to medical use polystyrene dishes on the Ti-Ni, collagen-coated Ti-Ni and ion beam irradiated collagen-coated Ti-Ni at an energy of 150 keV as a function of fluence.

Figure 7 shows the FT-IR-ATR spectra of un-irradiated and ion-irradiated collagen -coated Ti-Ni at irradiation fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2 at an energy of 150 keV. In the FT-IR-ATR spectra, the absorptions of amide I (1600 to -1700 cm^{-1}), amide II (1500 to -1580 cm^{-1}), amide A (3325 to -3330 cm^{-1}) and amide B (3080 cm^{-1}) [4][5] decreased with increasing the fluence of irradiation. These FT-IR-ATR results revealed that the amide compounds decomposed with increase increasing the fluences of irradiation, and decomposition was marked at an irradiation fluence of 1×10^{15} ions/cm^2.

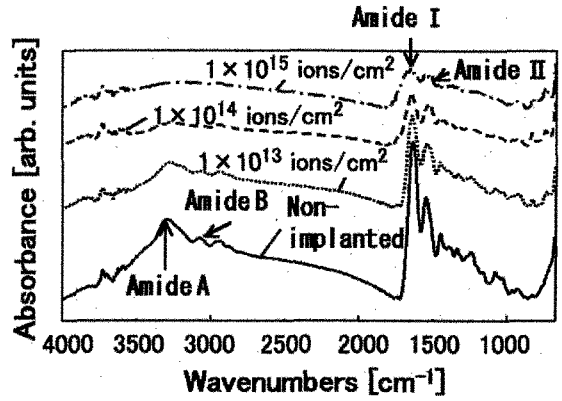

Fig. 7 FT-IR-ATR spectra of un-irradiated and ion-irradiated collagen-coated Ti-Ni at irradiation fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2 at an energy of 150 keV.

4. CONCLUSION

Platelets adhesion and aggregation on He^+ ion-implanted collagen-coated Ti-Ni were depressed by ion implantation with a fluence of $1x10^{13}$ions/cm^2. The cell attachment rate of collagen-coated Ti-Ni and ion beam irradiated collagen-coated Ti-N increased in compared with non-implanted Ti-Ni. Cell attachment ratio of ion beam irradiated collagen-coated Ti-Ni decreased with ion fluence. FT-IR-ATR results revealed that the amide compounds decomposed with increase increasing the fluences of irradiation, and decomposition was marked at an irradiation fluence of 1×10^{15} ions/cm^2.

Consequently, He^+ ion-beam irradiation into collagen is one of the most effective methods to improve antithrombogenecity of medical devices, such as coronary stents.

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