Transactions of the Materials Research Society of Japan 28 [2] 499-502 (2003)

Improvement of Blood Compatibility of Titanium with Helium Ion-Beam Irradiation

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Excellent blood compatibility is required for blood-contacting biomaterials such as guide wires, stents, and artificial heart valves used for treating cardiovascular disease. However, blood compatibility of metallic biomaterials is currently insufficient. In this study, we irradiated an He^+ ion-beam into collagen-coated pure titanium at an energy of 150 keV with fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2. Platelet adhesion and bovine aortic endothelial cell (BAECs) attachment to specimens were observed using scanning electron microscope (SEM). Changes in the surface composition and structures of specimens with ion-beam irradiation were also characterized using X-ray photoelectron spectroscopy (XPS), Auger electron spectroscopy (AES), laser-Raman spectroscopy (Raman) and Fourier transform infrared spectroscopy with attenuated total reflectance (FT-IR-ATR). We found that the platelet adhesion decreased and the attachment of endothelial cells increased when an ion-beam with a fluence of 1×10^{13} ions/cm^2 was irradiated onto collagen-coated titanium compared with pure titanium. Irradiation of He^+ ion-beam onto a collagen-coated surface is thus a promising approach for developing medical devices using titanium or metallic materials.
Keywords: Platelet, Endothelial cell, Collagen, XPS, AES, FT-IR-ATR

1. INTRODUCTION

Certain kinds of biomaterials will contact blood when initially implanted into the human body. For blood-contacting metallic materials, such as stents or artificial heart valves, two important factors should be considered, suitable mechanical properties and blood compatibility. Today, various types of blood-contacting metallic materials have been developed and applied clinically. Titanium is a well-known blood-contacting metallic material widely used in medicine. Blood-contacting metallic materials, including titanium, have good mechanical properties but have a main problem of thrombogenecity.

Our laboratory reported that polymer surfaces with blood compatibility and tissue compatibility can be controlled at the same time by using ion-beam modification of an extra-cellular matrix [1].

This paper investigates the blood compatibility of collagen-coated titanium modified with ion-beam irradiation.

2. EXPERIMENTAL

2.1 Collagen coating and ion-beam irradiation

The specimens used were pure titanium, un-irradiated collagen-coated titanium, and He^+ ion-beam irradiated collagen-coated titanium at fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2.

Commercially pure titanium plates were used. Table 1 presents the chemical compositions of the materials. The plates were polished on one side to a mirror finish and then cleaned by an ultrasonic rinse in acetone.

The titanium sample with a mirror-like surface was used as a specimen of pure Ti in the experiments. The substrates of the pure titanium with mirror-like surface were immersed in Type I collagen (CELLGEN, bovine dermis collagen, KOKEN, Japan) for two hours. After surplus collagen was removed, the substrates were dried under ambient cool conditions (4 ℃). The substrates were then irradiated with He^+ ion-beam irradiation and subsequently used as specimens of ion-irradiated collagen-coated Ti in the experiments. During He^+ ion-beam irradiation, the accelerating energy was 150 keV and the fluences were 1×10^{13}, 1×10^{14}, 1×10^{15} ions/cm^2. The beam current density was kept below 0.5 μ A/cm^2 to prevent the substrates from heating.

Table I
Chemical Compositions of Commercial Pure Titanium

Composition (Mass %)						
Fe	Ni	Cr	Al	Cu	Mn	Ti
< 0.05	< 0.02	< 0.01	< 0.005	< 0.001	< 0.002	99.5

2.2 In vitro platelet adhesion experiment

The quantity and morphology of adherent platelets were evaluated as parameters for blood compatibility of specimens in separating platelets from other blood components.

Human blood from a healthy volunteer was drawn into a syringe filled with 1 ml of 3.8% sodium citrate solution used as an anticoagulant at a ratio of 9 parts blood to 1 part citrate. Platelet-rich plasma (PRP; 1×10^5 platelets/μ l) was obtained from the citrated blood. Ca^{2+} free platelet adhesion experiments were performed with the prepared PRP. In performing Ca^{2+} re-added platelet adhesion experiments, 79.3 μ l of 0.25 M $CaCl_2$ aq was added to 1 ml PRP of the same cell density. The freshly prepared PRP was added to specimens and incubated at 37℃ for 5 min in a static system. Those specimens were then rinsed, fixed with 2% glutaraldehyde, dehydrated, and observed with a scanning electron microscope (SEM, JEOL JSM-6330F, Japan).

2.3 In vitro endothelial cell attachment

Bovine aortic endothelial cells (BAECs) were cultured in medium (RPMI 1640, Nissui Pharmaceutical Co.) supplemented with 10% fetal bovine serum (FBS, Sanko Junyaku Co., Japan). The cells were used for the experiments. Initially, 5×10^4 cells/ml was seeded into specimens. The cells were incubated for 24 hours at 37℃ in 5% CO_2 in a humid atmosphere. After 24 hours incubation, the specimens were rinsed, fixed with 2% glutaraldehyde, dehydrated, and observed with a scanning electron microscope.

2.4 Surface analysis

Decomposition and new functional groups in the ion-beam irradiated specimens were detected by Fourier transform infrared spectroscopy combined with attenuated total reflectance (FT-IR-ATR, Nicolet, Nexus 470), Laser Raman spectroscopy (Raman, Jobin Yvon, LabRam), and X-ray photoelectron spectroscopy (XPS, SSI-SSX100). In the XPS analyses, all binding energies are relative to the Fermi level, and all spectra were excited with the monochromatized Al Kα line [2]. The depth profiles of elements in specimens with collagen coating were obtained by Auger electron spectroscopy (AES, JEOL, JAMP-7100) in combination with argon-ion-sputter etching. The vacuum level of the analyzing chamber was 10^{-7} to 10^{-8} Pa. The accelerating voltage of the primary electrons for the auger electron excitation was 10 kV. The electron probe diameter was 1.2 μ m, and the current was 5×10^{-7} A [3].

3. RESULTS AND DISCUSSION

3.1 In vitro platelet adhesion

Figure 1 shows the total number of adherent platelets on the specimens per millimeter squared using Ca^{2+} free PRP. Figure 2 depicts the SEM morphology of adherent platelets spread on pure Ti and collagen-coated Ti at an irradiation fluence of 1×10^{13} ions/cm^2 using Ca^{2+} re-added PRP.

Fewer platelets adhered on the surfaces of pure Ti and collagen-coated Ti at an irradiation fluence of 1×10^{13} ions/cm^2 than on other specimens. Comparing the two specimens, the adherent platelets on the surface of pure Ti were spread more than those on the surface of collagen-coated Ti at an irradiation fluence of 1×10^{13} ions/cm^2. Results indicate that platelet adhesion was depressed on the surface of collagen-coated Ti at an irradiation fluence of 1×10^{13} ions/cm^2 compared with pure Ti.

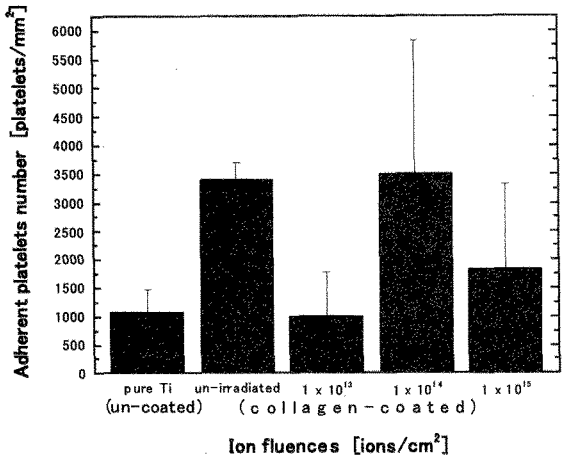

Fig.1. The total number of adherent platelets per mm^2 on pure Ti, un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2 after contact with Ca^{2+} free PRP for 5 min.

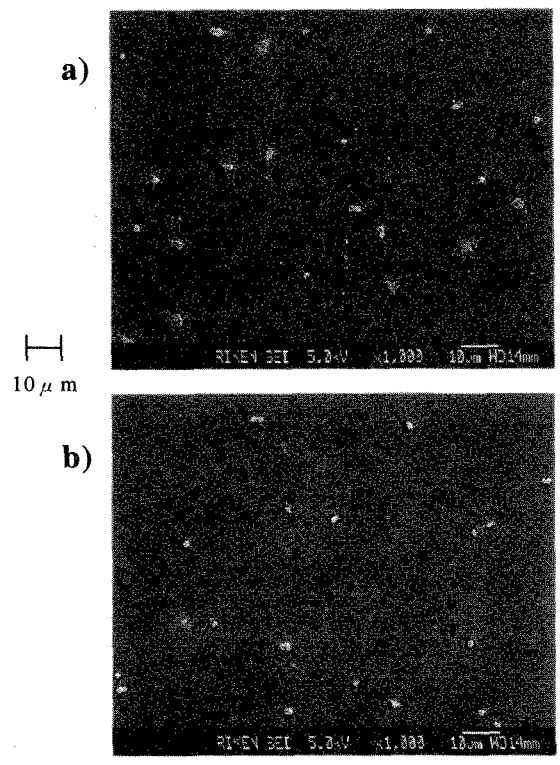

Fig.2. SEM morphology of adherent platelets on a) pure Ti and b) ion-irradiated collagen-coated Ti at a fluence of 1×10^{13} ions/cm^2 after contact with Ca^{2+} re-added PRP for 5 min.

3.2 In vitro endothelial cell attachment

Figure3 shows the total number of endothelial cells (BAECs) attached on the specimens per millimeter squared after incubation for 24 hours. The endothelial cells' attachment to collagen-coated Ti at an irradiation fluence of 1×10^{13} ions/cm^2 was improved compared with other specimens.

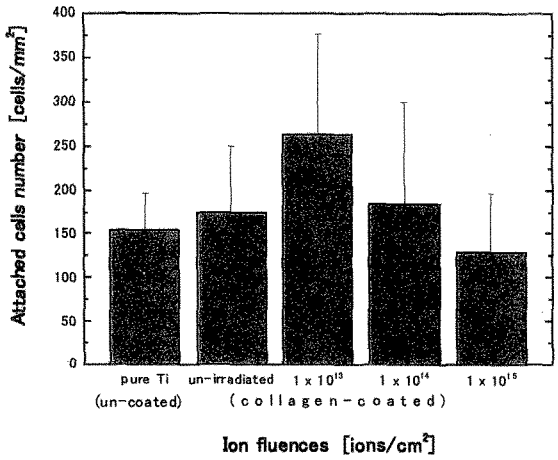

Fig.3. The total number of BAECs per mm^2 attached on pure Ti, un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2 after incubation for 24 hours.

3.3 Raman spectroscopic analysis

Figure 4 shows the Raman spectra of ion-irradiated collagen-coated Ti at an irradiation fluence of 1×10^{15} ions/cm^2 at an energy of 150 keV. In the Raman spectra, two broad features are present in ion-irradiated collagen-coated Ti at an irradiation fluence of 1×10^{15} ions/cm^2: one at 1330 cm^{-1} from disordered graphitic carbon, and the other at 1540 cm^{-1} from amorphous carbon that included sp^1, sp^2 and sp^3 bonded carbon [4, 5]. On the other hand, any features don't exist between un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14} ions/cm^2 that are not indicated in the figure.

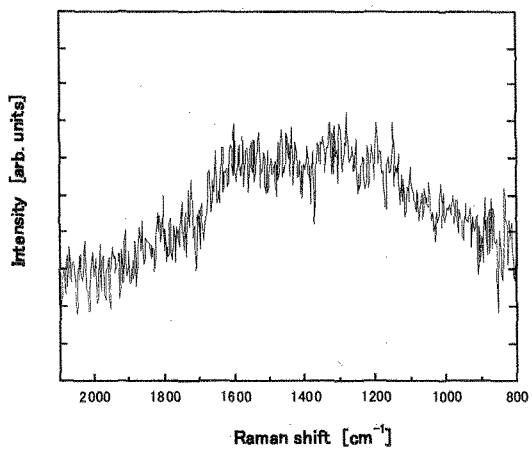

Fig.4. Raman spectra of ion-irradiated collagen-coated Ti at an irradiation fluence of 1×10^{15} ions/cm^2 at an energy of 150 keV.

3.4 FT-IR-ATR analysis

Figure 5 shows the FT-IR-ATR spectra of un-irradiated and ion-irradiated collagen-coated Ti 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}) [6, 7] decreased with increasing the fluence of irradiation. These FT-IR-ATR results revealed that the amide compounds decomposed with increasing fluences of irradiation, and decomposition was marked at an irradiation fluence of 1×10^{15} ions/cm^2.

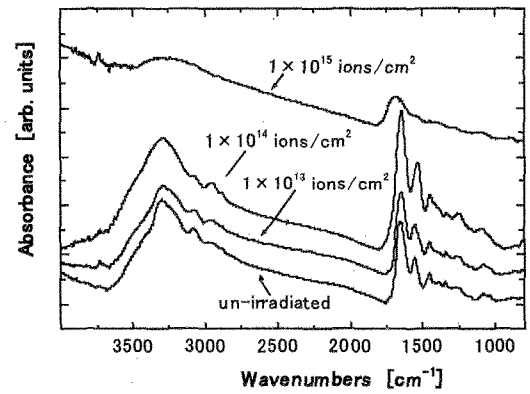

Fig.5. FT-IR-ATR spectra of un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2 at an energy of 150 keV.

3.5 X-ray photoelectron spectroscopy

Figures 6 and 7 illustrate the fraction of elements obtained from C_{1s} and N_{1s} XPS spectra of un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2 at an energy of 150 keV. In carbon compounds obtained from the C_{1s} XPS spectra, both C-O and –COO, which are the components of collagen, decreased and C-C increased with increasing fluences of irradiation. Of the nitride compounds obtained from the N_{1s} XPS spectra, $-NH_3^+$, which is a component of collagen, decreased and $-NH_2$ increased with increasing irradiation fluences.

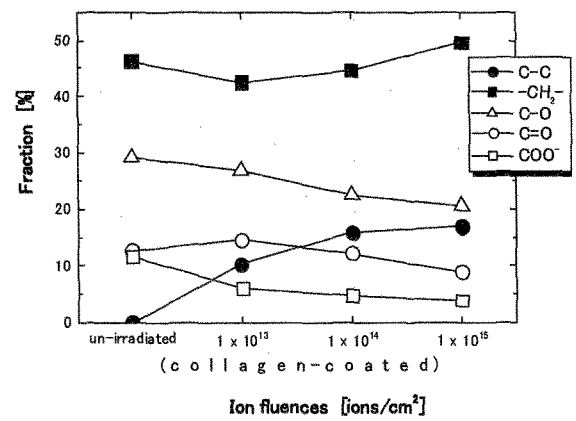

Fig.6 Fractions of elements obtained from C1s XPS spectra of un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2 at an energy of 150 keV.

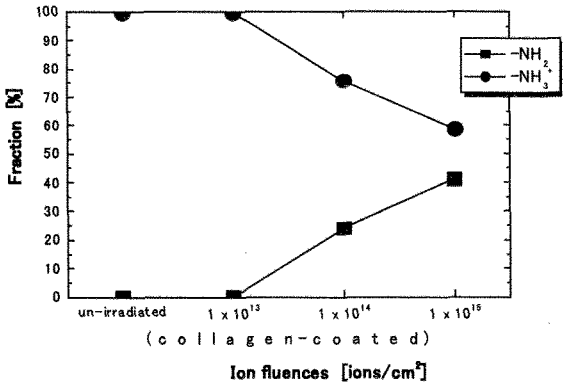

Fig.7 Fractions of elements obtained from N 1s XPS spectra of un-irradiated and ion-irradiated collagen-coated Ti at irradiation fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2 at an energy of 150 keV.

3.6 Auger electron spectroscopy

Figure 8 depicts the transition of amounts of carbon, that are contained in un-irradiated and ion-irradiated collagen-coated Ti, in relation to fluences of irradiation at an energy of 150 keV obtained from AES study. In Fig. 8, the amount of Ti indicates the distance from the collagen-coated surface to the interior part of the collagen-coated Ti. Accordingly, the increase of the amount of Ti indicates the more interior part of the substrate. There is no difference in carbon content between un-irradiated and ion-irradiated collagen-coated Ti at an irradiation fluence of 1×10^{13} ions/cm^2. Conversely, the amount of carbon increased the interior of ion-irradiated collagen-coated titanium at irradiation fluences of 1×10^{14} and 1×10^{15} ions/cm^2. The results indicate that carbon, the main element of collagen, was embedded from coated collagen layer into the titanium substrates by the effects of elastic and inelastic collision induced by ion-beam irradiation. This suggests that atomic mixing effects were produced at the interface between the coated collagen and titanium by He^+ ion-beam irradiation.

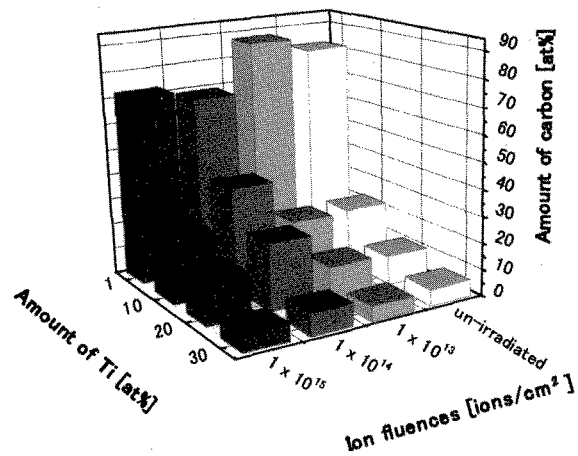

Fig.8. the amounts of carbon and titanium in collagen-coated Ti of un-irradiated and ion-irradiated substrate (irradiation fluences: 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2) in relation to fluences of irradiation at an energy of 150 keV obtained from AES study.

4. CONCLUSIONS

The results of in vitro study indicated that He^+ ion-beam irradiation at a fluence of 1×10^{13} ions/cm^2 at an energy of 150 keV into collagen-coated titanium both strongly inhibited platelet adhesion and increased endothelial cell attachment compared to pure titanium.

Collagen consists of many kinds of amino acids and also includes ligands that correspond to platelet adhesion and cell attachment. He^+ ion-beam irradiation breaks the ligands and forms chemical compounds [8].

XPS study revealed that the amino compounds still remain and that carbonization was produced by 1×10^{13} ions/cm^2-irradiation into collagen-coated Ti. This physio-chemical feature might contribute to the depression of platelet adhesion and the increase of cell attachment.

Antithrombogenecity is an essential factor in blood-contacting materials. The antithrombogenecity can be controlled by depressing platelet adhesion or by covering the surface with endothelial cells. In conclusion, platelet adhesion and cell attachment to the surfaces of metallic materials can be controlled by collagen coating followed by He^+ ion-beam irradiation into the collagen-coated layer on metallic surfaces. Consequently, He^+ ion-beam irradiation into collagen is one of the most effective methods to improve antithrombogenecity of medical devices, such as stents and artificial heart valves.

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(Received December 21, 2002; Accepted March 26, 2003)