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Mechanical Adhesive Strength, XPS and AES Studies of Ion Beam Immobilized Collagen

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He^+ ion implantation into collagen (Type I)-coated substrates was performed to develop hybrid small-diameter artificial vascular grafts. These surfaces improved their antithrombogenicity by reducing platelet adhesion and promoting cell attachment. Ion implantation into collagen-coated substrates induced surface modification and immobilization of collagen. Mechanical adhesive strength of immobilized collagen in an arterial blood flow plays an important part in a body. In this study, we investigated mechanical adhesive strength and performed XPS and AES studies of collagen immobilized by ion implantation as a function of fluence. Polystyrene and titanium plates were used as substrates. He^+ ion implantation into collagen-coated substrates was performed at an energy of 150 keV with fluences between 1×10^{13} and 1×10^{15} ions/cm^2. The results indicated that the mechanical adhesive strength of He^+ ion-implanted collagen with a fluence of $1x10^{14}$ ions/cm^2 was improved dramatically, and an element of collagen was detected at an inner surface layer of substrates. We concluded that improvement of mechanical adhesive strength of immobilized collagen was caused by ion-beam mixing effects.

Keywords: SAICAS, Endothelial cell, Vascular graft, Biocompatibility

1. INTRODUCTION

The adhesion, growth, and proliferation of cells in a cell culture are anchor-dependent. The adhesion of cells is considered to be primarily influenced by the nature of the substrate [1], [2], [3]. The chemical characteristics of the surface of a material are changed when ion implantation is performed [4]. Therefore, alteration and manipulation of the thrombogenicity and control of the cell-attachment properties of a material surface are possible and could be advantageous [5]. An anti-thrombogenic surface and a cell adhesive surface cannot exist together in artificial surfaces. Our laboratory has developed polymer surfaces with blood compatibility and tissue compatibility by using 150 keV-He^+ ion-beam modification of type I collagen and applied it to small-diameter artificial grafts.

We then implanted those grafts into dog carotid arteries. During this animal study, endothelium at the graft surface was observed. Our studies of cell attachment strength demonstrated the importance of ion implantation in maintaining and strengthening the adhesive contact between collagen and the substrate [6]. Other important properties are adhesion to substrates and resistance against blood flow. The objective of our present work is to investigate the effects of He^+ ion beam immobilization on the collagen-substrate interfacial strength and binding mechanism.

2. MATERIALS AND METHODS

The substrates we used were polystyrene (PS) and titanium plates. We performed collagen coating, and ion implantation under the following four conditions: a) collagen (CELLGEN, Bovine dermis collagen, KOKEN Co. Japan) coating, b) Ne^+ ion-implantation into the substrate at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 as a pre-treatment of collagen coating and collagen coating, c) collagen coating, and He^+ ion-implantation into the substrate at an energy of 150 keV with fluences between $1x10^{13}$ and 1×10^{15} ions/cm^2, d) Ne^+ ion-implantation into the substrate at an energy of 150 keV with a fluence of 1×10^{15} ions/cm^2 as a pre-treatment and collagen coating, and He^+ ion-implantation into the substrate at an energy of 150 keV with a fluence of 1×10^{14} ions/cm^2. A schematic diagram of the four conditions is illustrated in Fig.1. The ion beam current density was kept below 0.5 μ A/cm^2 to prevent the substrates from heating.

The cutting and peeling instrument for measuring mechanical parameters and preparing the analysing surface was the "Surface and Interfacial Cutting Analysis System" (SAICAS) CN-20 (Daipla Wintes) [7]. This system is for measuring shear strength, peel strength, and degree of adhesion of coated collagen and ion-beam-immobilized collagen.

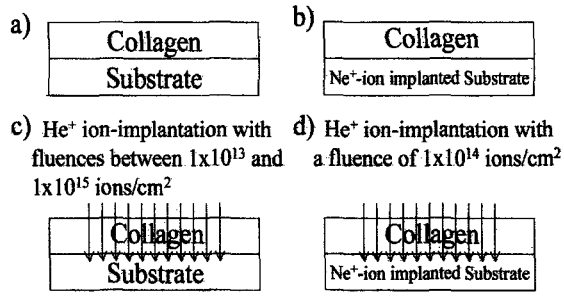

Fig.1. Schematic diagram of the four conditions.

The chemical composition of ion-implanted surfaces was evaluated by x-ray photoelectron spectroscopy (XPS, SSI-SSX100), and Auger electron spectroscopy (AES, JEOL, JAMP-7100) analysis. In the XPS analyses, all biding energies are relative to the Fermi level, and all spectra were excited with the monochromatized Al Kα line. The depth profiles of specimen elements 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 size was about 1.2 μ m in diameter, and the current was 5 $\times 10^{-7}$ A.

2. RESULTS AND DISCUSSION

3.1 Mechanical adhesive strength

Figure 2 illustrates the horizontal force and the cutting depth of the (a) Collagen-coated PS, (b) Collagen-coated Ne^+-ion pre-implanted PS, (c) He^+ ion-implanted collagen-coated PS, and (d) He^+ ion-implanted collagen-coated Ne^+-ion pre-implanted collagen-coated PS.

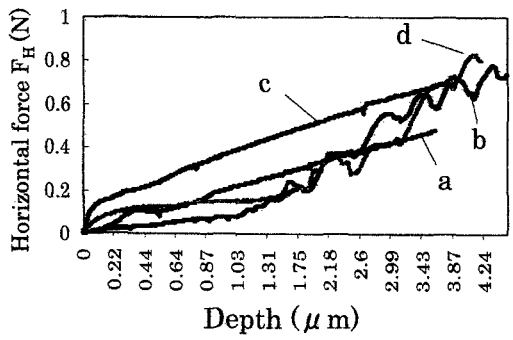

Fig.2 Horizontal force and cutting depth.

The shear strength τ at the cutting stage can be described as

$$\tau = F_H/2wdcot\,\phi$$

where F_H is the horizontal force, w is the width of the cutting knife (1 mm), and ϕ is the shear angle (45 degrees).

Using atomic force microscopic study, we estimated the thickness of the collagen-coated layer to be about 400 nm, and, using TRIM code, the simulation results of mean projected range of implanted He^+ ions was found to be 810 nm. He^+ ions implanted at an energy of 150 keV penetrated the coated collagen layer and reached to the substrate polystyrene layer.

Figure 3 depicts shear strength of regions near the surfaces (< 0.4 μ m) of the test samples. Differences in shear strength are evident. Pre-Ne^+ ion implantation decreased shear strength between collagen and the substrate. The shear strength was maximum in the He^+ ion implanted collagen-coated polystyrene with $1x10^{14}$ ions/cm^2 and the value of 190 Mpa..

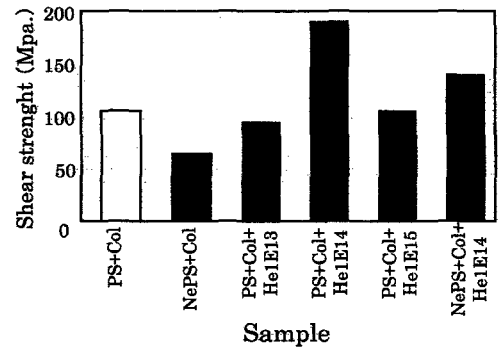

Fig. 3. Shear strength data from regions near the surfaces (< 0.4 μ m). PS+Col: Collagen-coated polystyrene. NePS+Col: Collagen-coated Ne^+ ion pre-implanted polystyrene. PS+Col+ He1E13: He^+ ion-implanted collagen-coated polystyrene with a fluence of 1×10^{13} ions/cm^2. PS+Col+ He1E14: He^+ ion implanted collagen-coated-polystyrene with 1×10^{14} ions/cm^2. PS+Col+ He1E15: He^+ ion-implanted collagen-coated polystyrene with $1x10^{15}$ ions/cm^2. NePS+Col+ He1E14: He^+ ions with a fluence of 1×10^{14} ions/cm^2 implanted into collagen-coated Ne^+ ion pre-implanted polystyrene.

3.2 XPS study

Figure 4 presents the C1s spectra of non-implanted collagen and He^+ ion-implanted collagen-coated titanium with fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2. The C1s spectrum of non-implanted collagen contained four peaks originating from COO^-, C=O, C-O and CH2. However, the C1s spectra of He^+ ion-implanted collagen contained a new peak originating from the C-C bond.

Figure 5 depicts the N1s spectra of non-implanted collagen and He^+ ion-implanted collagen-coated titanium with fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2. The N1s spectrum of non-implanted collagen contained a peak originating from NH_3^+. The N1s spectra of He^+ ion-implanted collagen over a fluence of 1×10^{14} ions/cm^2 contained a new peak originating from NH_2.

Figure 6 illustrates Atomic % of COO^-, C=O, C-O, CH2 and C-C of non-implanted and He^+ ion-implanted collagen as a result of XPS study. COO^- and C-O decreased monotonically with ion fluence. CH2 first decreased, then increased with ion fluence. The ion-beam generated C-C bond increased with ion fluence. This result indicated that amorphous carbon was produced by 150 keV-He^+ ion implantation.

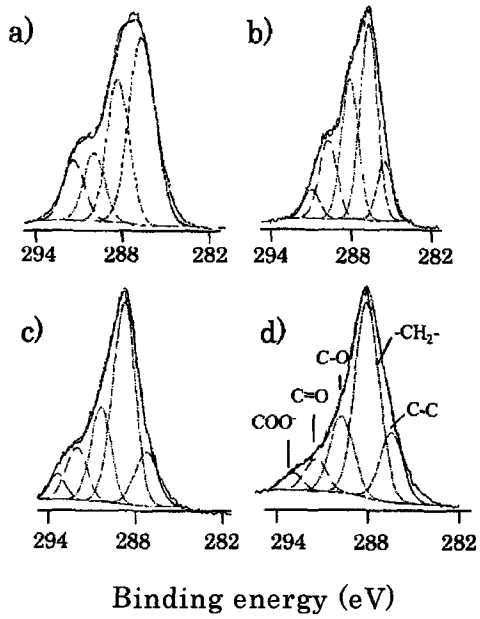

Fig.4 C1s spectra of (a) non-implanted Collagen and He^+ ion-implanted collagen-coated titanium with fluences of (b) 1×10^{13}, (c) 1×10^{14}, and (d) 1×10^{15} ions/cm^2.

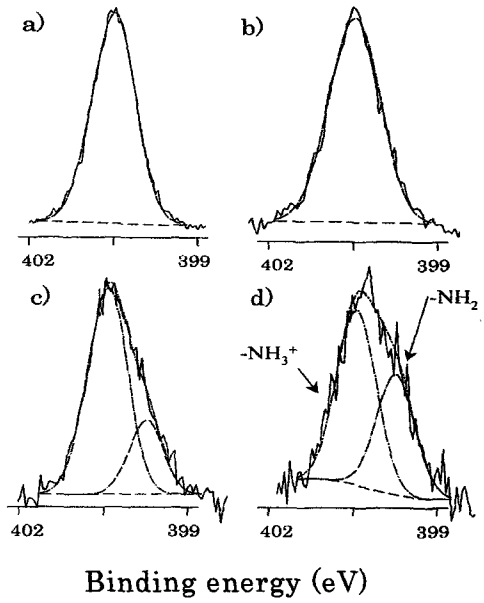

Fig.5. N1s spectra of (a) non-implanted Collagen and He^+ ion-implanted collagen-coated titanium with fluences of (b) 1×10^{13}, (c) 1×10^{14}, and (d) 1×10^{15} ions/cm^2.

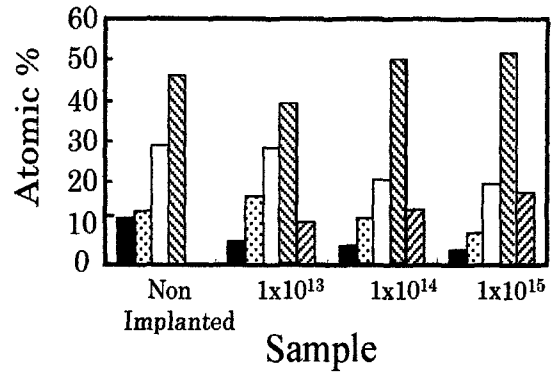

Fig.6. Atomic % of COO^- (black), C=O (dotted), C-O (white), CH2 (negative slope oblique line), and C-C (positive slope oblique line) of non-implanted and He^+ ion-implanted collagen.

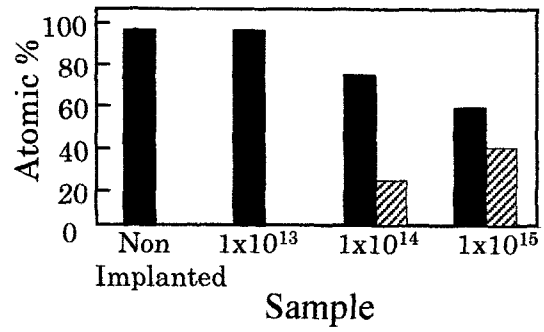

Fig.7. Atomic % of $NH3^+$ (black), and NH_2 (positive slope oblique line) of non-implanted and He^+ ion-implanted collagen.

Figure 7 illustrates the Atomic % of $NH3^+$ and NH_2 of non-implanted and He^+ ion-implanted collagen as a result of our XPS study. $NH3^+$ decreased with ion fluence. NH_2 was produced by He^+ ion implantation over a fluence of 1×10^{14} ions/cm^2 and increased with ion fluence. These results indicated that He^+ ion implantation broke the original chemical bond ($NH3^+$) to form NH_2.

3.3 AES study

Figure 8 plots depth profiles obtained by AES measurement of the non-implanted and He^+ ion-implanted collagen coated titanium with fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2. Clearly, carbon exists at the collagen-coated layer, and titanium exists at titanium substrates. It is worthwhile to note that carbon concentration increased with fluence in the titanium substrates.

Figure 9 illustrates the carbon concentration of the non-implanted and He^+ ion-implanted collagen-coated titanium with fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2 at the titanium substrates layer (titanium concentration = 20 atomic %).There is little difference in carbon content between non-implanted collagen-coated titanium and He^+ ion-implanted collagen-coated titanium with a fluence of 1×10^{13} ions/cm^2.

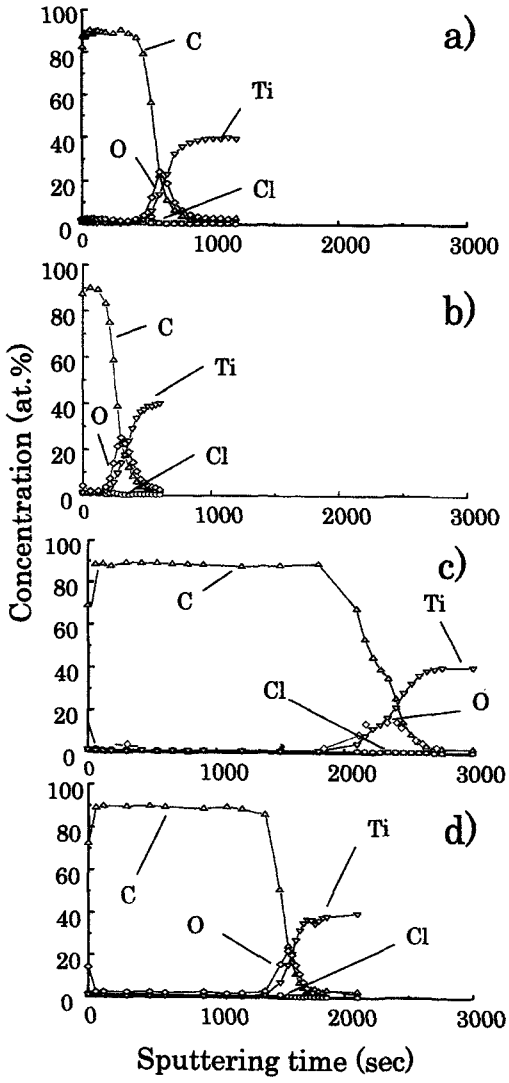

Fig.8. Depth profiles obtained by AES measurement of the (a) non-implanted and He^+ ion-implanted collagen-coated titanium with fluences of (b) 1×10^{13}, (c) 1×10^{14}, and (d) 1×10^{15} ions/cm^2.

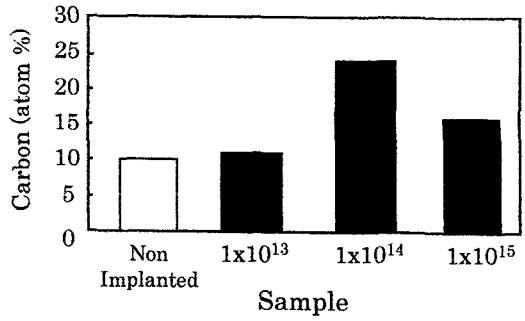

Fig.9. Carbon concentration of the non-implanted and He^+ ion-implanted collagen-coated titanium with fluences of 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2 at the titanium substrate layer (titanium concentration = 20 atomic %).

However, the amount of carbon increased inside the titanium substrate of ion-implanted collagen-coated titanium with fluences of 1×10^{14} and 1×10^{15} ions/cm^2. The results indicate that the carbon, which is the main element of collagen, was embedded into the titanium substrates by the elastic and inelastic collisions induced by He^+ ion implantation. Therefore, we suggest that the atomic mixing effects were produced at the interface between the coated collagen and titanium by 150 keV- He^+ ion implantation over a fluence of 1×10^{14} ions/cm^2.

3. CONCLUSIONS

We have studied the effects of He^+ ion-beam immobilization on the collagen-substrate interfacial strength and binding mechanism. The shear strength was maximum in He^+ ion-implanted collagen-coated polystyrene with 1×10^{14} ions/cm^2 and a value of 190 Mpa.. He^+ ion implantation with a fluence of $1x10^{14}$ ions/cm^2 increased the amount of carbon inside the substrate.

We concluded that the atomic mixing effects were produced at the interface between the coated collagen and substrates by He^+ ion implantation with a fluence of 1×10^{14} ions/cm^2 at an energy of 150 keV. Mechanical adhesive strength between collagen and substrate increased dramatically due to the atomic mixing induced by He^+ ion implantation.

References

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