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THE MORPHOLOGICAL CHANGES AND ADHESION STRENGTH IN ION BOMBARDED COLLAGEN

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A study has been made of immobilization of collagen onto a substrate resulting from ion bombardment. A coating of type-I collagen was applied to a polystyrene (PS) plate and bombarded with 50-keV He^+ and Ar^+ ions at doses between 1×10^{13} and 1×10^{15} ions/cm^2. The collagen-coated surfaces were mounted on parallel-plate flow chambers, with a flowing shear stress of 2 Pa for 1 hour, in a flow system prepared for tests of collagen adhesion. Morphology observations using transmission-electron microscopy (TEM) and atomic force microscopy (AFM) were performed in order to investigate the mutual relationship between collagen adhesion and morphology.
Key words: ion bombardment, collagen adhesion, morphology, ion beam immobilization

1. INTRODUCTION

Recently, ion implantation into polymer and protein surfaces has been applied in order to improve blood and tissue compatibility [1,2]. On the other hand, immobilization of protein onto substrates has been performed for biocompatible material [3]. In this study, ion-bombardment was performed on a collagen-coated surface with high biocompatibility, to control cell and platelet adhesion. The ultimate aim is to apply this technique to artificial internal organs. It has been shown that cell and platelet adhesion can be controlled by ion-bombardment of polymers, and extra-cellar matrices [4,5].

If exfoliation of collagen from the base material occurs, then the properties of the surface formed by ion-bombardment will be lost. Therefore, immobilization of collagen is important for its application in artificial organs. It has been shown that moderate ion bombardment of collagen-coated polystyrene (PS) causes the cell-adhesion strength of specimens to increase [6]. In this paper, we report immobilization of precoated protein on an artificial material, by ion bombardment, and investigation of the mutual relationship between collagen adhesion and morphology.

2. EXPERIMENT

2-1. Specimens

The substrates used were PS dishes (Corning Co. Ltd). The 0.3% type-I collagen samples (Bovine dermis collagen, KOKEN Co. Ltd, Japan) were coated onto the PS using a spin coater, after which the specimens were dried at 4 °C. The thickness of the collagen layer was about 20 nm. The collagen-coated PS was bombarded with 50-keV He^+ and Ar^+ ions at doses between 1×10^{13} and 1×10^{16} ions/cm^2 using a RIKEN TK-100 ion implanter.

2-2. Quantification of collagen detached under flowing-shear stress

To perform the collagen-adhesion test [6], the collagen-coated samples were placed in a parallel-plate flow chamber in a flow system. Phosphate-buffered saline (PBS (–)) free of Ca^{2+} and Mg^{2+} was pumped from a reservoir through the inlet into the chamber. The temperature of the flow medium was maintained at 37 °C in the reservoir.

A shear stress of 2 Pa was applied to the flow chamber. The shear stress, τ_w (Pa), was calculated using the following equation:

$$\tau_w = 6Q\mu / wh^2,$$

where Q is the volumetric flow-rate (m^3 s^{-1}), and μ is the viscosity of the medium, which was taken as the viscosity of water at 37 °C (6.9×10^{-4} Pa·s) in this calculation. The parameter w is the chamber width (2.56×10^{-2} m), and h is the chamber height (5×10^{-4} m). The shear stress was maintained for 60 min. The rate at which collagen peeled off owing to shear stress was evaluated by comparing the IR spectra before and after the flowing-shear stress. The comparison in the IR spectra was parformed at 1660cm^{-1}, which is attributed to protein amide C = O stretching vibration, and the intensity was higher than other bands such as Amide II (1639 cm^{-1}) and Amide III (1238 cm^{-1}) [7].

2-3. Cross-sectional structure observation by TEM

Using a JEOL 2000 FX electron microscope with energy of 120 keV, cross-sectional TEM observations were performed to observe the structure of the bombarded region. The bombarded specimens were packed in epoxy resin, and 100-nm thick cross-sectional samples were prepared using a Leica ULTRACUT UTC with a diamond knife. Negative staining with 1% uranyl acetate solution was performed on the samples. For the specimens bombarded with He^+ at a dose of 1×10^{15} ions/cm^2, gold was deposited onto the surface of the specimen, and double staining with uranyl acetate and lead was performed because of a difficulty in observing only negative staining.

2-4. Surface morphology observation by AFM

The surface morphology of non-bombarded and bombarded collagen was observed using an AFM, JEOL JSPM-4200. The measurements were carried out at atmospheric pressure, using the non-contact mode. The purpose of this investigation was to understand the relationship between collagen adhesion and the morphology of the collagen. The PS surface is more irregular than the collagen surface. In this experiment, silicon (Si) substrates were used, since Si has a smoother surface.

2-5. Calculation by the Transport of Ions in Matter

The Transport of Ions in Matter (TRIM) is a program that provides a quick calculation of stopping powers and range distributions of ions implanted into surfaces under various conditions, using Monte Carlo simulation. Concentration, Ionization and Recoils of bombarded ions as a function of target depth were calculated by TRIM.

3. RESULTS

3-1. Quantification of collagen detached under flowing shear stress

Fig. 1 shows the IR intensity of collagen before and after the flowing shear stress, and the percentage of collagen remaining on the specimens. For a non-bombarded specimen, 60% of the collagen coated on the PS exfoliates after a laminar flow of 2 Pa for 60 minutes. Most of the collagen was detached from specimens bombarded with He^+ ions at a dose of 1×10^{13} ions/cm^2. The percentage of remaining collagen increased as the dose increased. For specimens bombarded with a dose of 1×10^{15} ions/cm^2, little exfoliation was observed. The percentage of remaining collagen increased as the dose increased for Ar^+ bombarded specimens. In specimens bombarded with Ar^+ at more than 1×10^{14} ions/cm^2, little exfoliation was observed.

3-2. Cross-sectional structure observation by TEM

Fig. 2 shows cross-sectional TEM micrographs of specimens. The thickness of the collagen layer was about 20 nm for the non-bombarded specimen. For the specimen bombarded with He^+ at a dose of 1×10^{13} ions/cm^2, some spaces were observed at the interface between the collagen and the substrate. For the specimen bombarded with He^+ at a dose of 1×10^{15} ions/cm^2, the collagen layer separated.

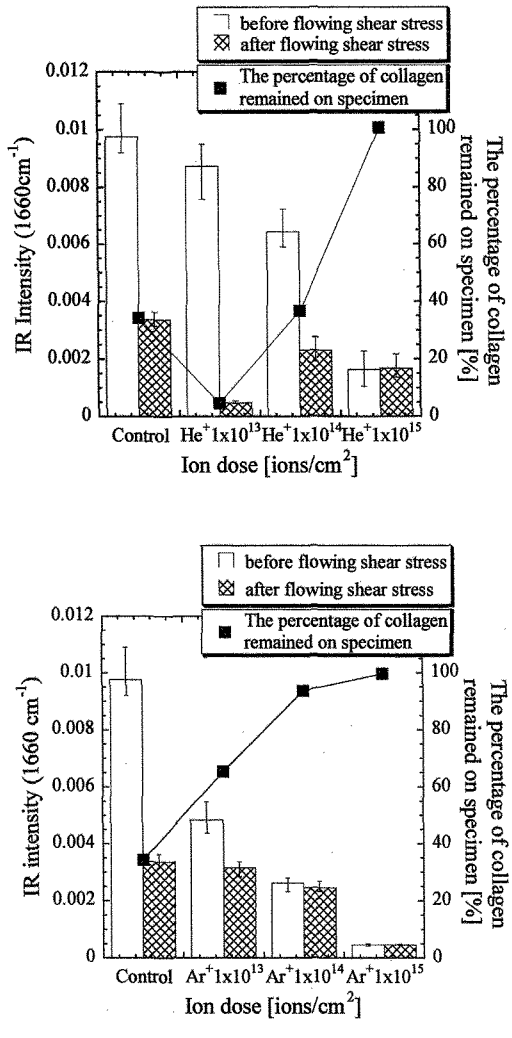

Fig. 1. Collagen before exposure to a flowing-shear stress of 2 Pa, and the percentage of collagen remaining after the stress.

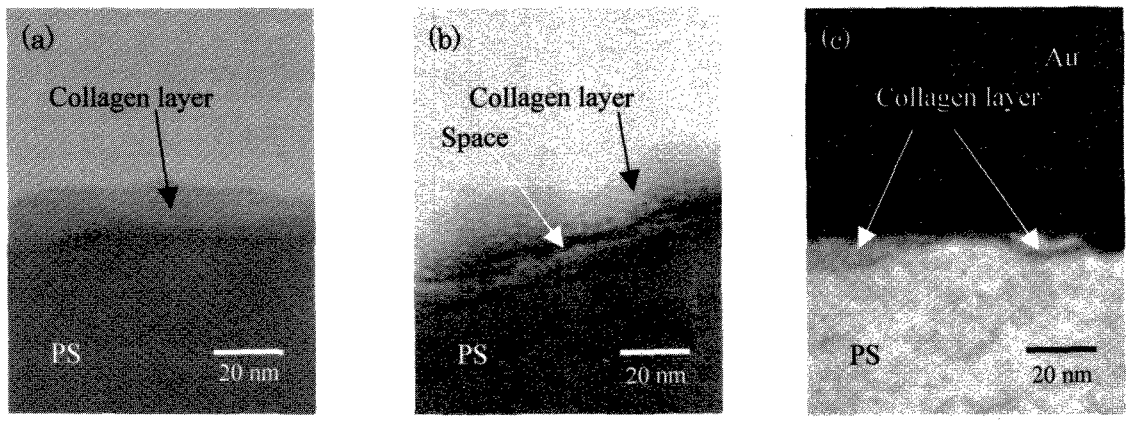

Fig. 2. Cross-sectional TEM micrograph of the specimens: (a) non-bombarded, (b) bombarded with He^+ at a dosage of 1×10^{13} ions/cm^2 and (c) at 1×10^{15} ions/cm^2.

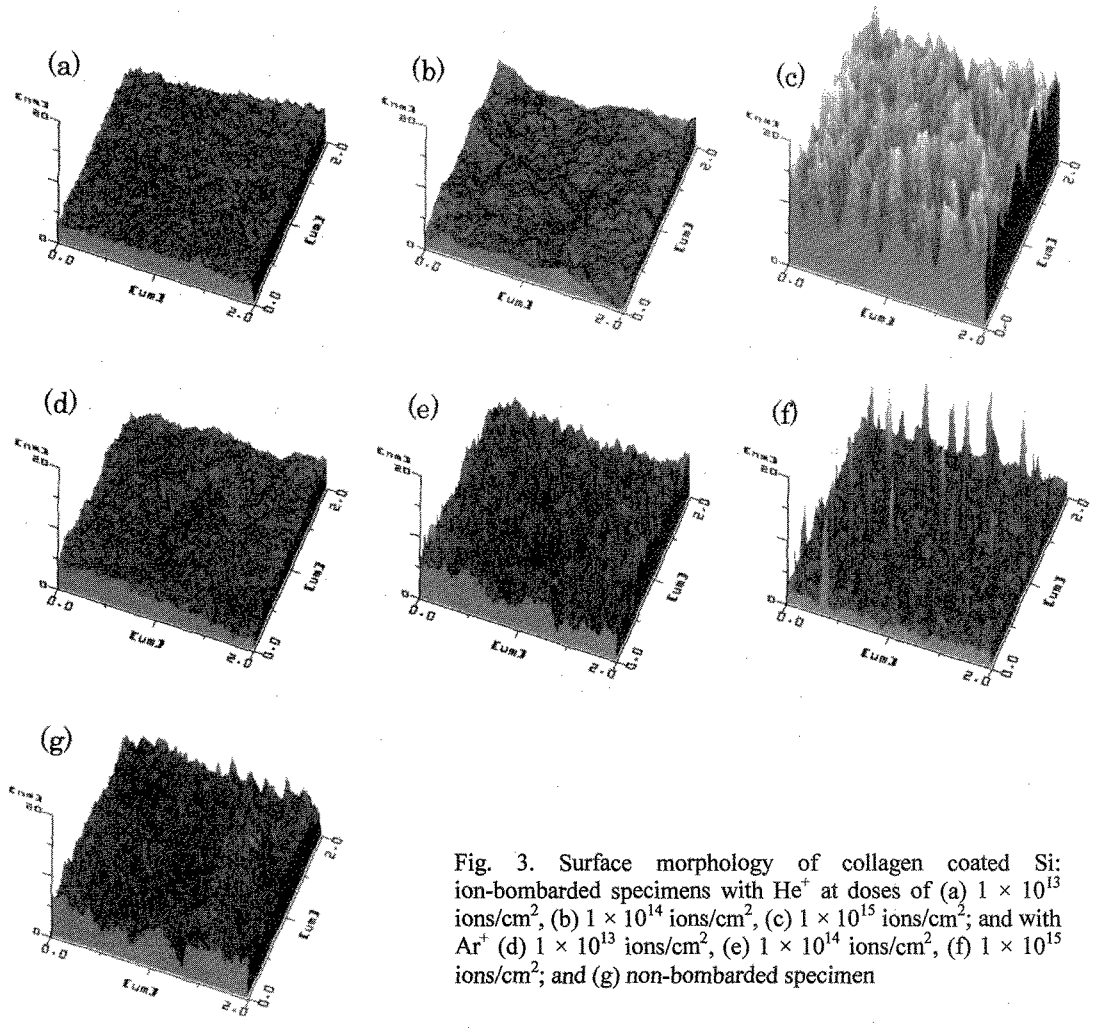

Fig. 3. Surface morphology of collagen coated Si: ion-bombarded specimens with He^+ at doses of (a) 1×10^{13} ions/cm^2, (b) 1×10^{14} ions/cm^2, (c) 1×10^{15} ions/cm^2; and with Ar^+ (d) 1×10^{13} ions/cm^2, (e) 1×10^{14} ions/cm^2, (f) 1×10^{15} ions/cm^2; and (g) non-bombarded specimen

3-3. Surface morphology observation by AFM

Fig. 3 shows the surface morphology of collagen coated Si. The surface roughness of the non-bombarded and bombarded specimens is shown in Fig. 4.

No noticeable changes are observed for the ion-bombarded Si surfaces. The Si surface is relatively flat compared to the collagen-coated Si. Therefore, the surface morphology for ion-bombarded collagen-coated Si was independent of the Si used as the substrate. The surface roughness of collagen-coated Si decreased for the specimen bombarded with He^+ at a dose of 1×10^{13} ions/cm^2. As the dose increased, the surface roughness increased for He^+ ion-bombarded collagen-coated Si. In specimens bombarded with He^+ at more than 5×10^{14} ions/cm^2, roughness decreased. As the dose increased, the surface roughness increased for Ar^+ ion-bombarded collagen-coated Si. In specimens bombarded with Ar^+ at more than 1×10^{15} ions/cm^2, roughness decreased. Studding was observed with a granular structure about 20 nm high in specimens bombarded with He^+ and Ar^+ at more than 5×10^{14} ions/cm^2.

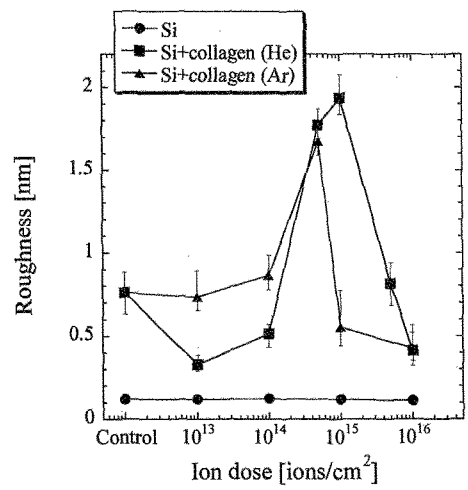

Fig. 4. Roughness of non-bombarded and bombarded collagen surfaces.

3-4. Calculation by the Transport of Ions in Matter

Fig. 5 shows concentration, ionization and recoils calculated for implanted ions as a function of target depth by TRIM. Most of the He^+ and Ar^+ ions penetrated through the collagen layer. With regard to the differences between ion species, there is little energy loss of He^+ ions from recoils. In addition, an Ar^+ ion loses about 500 eV/nm of energy from recoils at the collagen layer. Comparing energy losses from ionization, Ar^+ ions lose twice as much energy as do He^+ ions. In total, Ar^+ ions lose energy 6 times as much as He^+ ions at the collagen layer. Energy loss of Ar^+ ions from recoils decreased at the interface due to the difference between collagen and PS density. Ar^+ ions lose energy 6 times as much as He^+ ions at the interface between collagen and PS.

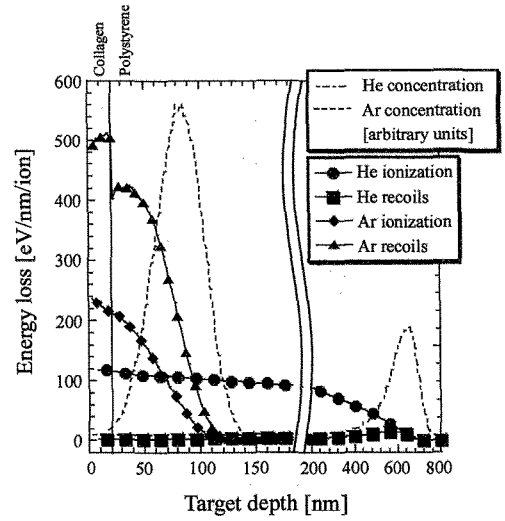

Fig. 5. Concentration, Ionization and Recoils calculation of implanted ions as a function of target depth, by TRIM.

4. DISCUSSION

For specimens bombarded with He^+ at a dose of 1×10^{13} ions/cm^2, it is considered that massive collagen exfoliation occurred because of the formation of spaces in the interface. The formation of these spaces is due to contraction of the collagen as a result of ion bombardment. Since the energy loss of ions is greatest at the surface of the collagen, the strongest contraction of the collagen should appear at the surface. The collagen surface contracts so that it will become flatter. In other words, the thin part of the collagen layer is pulled, and the thick part is compressed. Consequently, an interface space is formed under the thin part of the collagen layer. It is considered that flattening of the surface in the case of Ar^+ bombardment was caused with less than 1×10^{13} ions/cm^2

As the dose increased, the roughness increased in the collagen-coated Si. This is due to the formation of surface undulation following contraction. At a dose of 1×10^{15} ions/cm^2 the collagen layer separates, causing the formation of a collagen colony due to excessive contraction. It is considered that some granular structures about 20 nm high observed by AFM are collagen colonies.

Where the collagen contacted the PS, it is considered that the collagen is immobilized to the PS as a result of generating new bonds at the interface between the collagen and the substrate. Therefore, the collagen adhesion strength increased at higher doses.

An ion dosage of He^+ ions about 10 times that of Ar^+ ions was required to immobilize collagen onto the substrate completely. There is a difference of ion dosage needed to immobilize collagen more than the difference of total energy loss at the interface due to the large energy loss by recoils in Ar bombarded specimen. The great increase in collagen adhesion for the specimens bombarded with Ar^+ ions is probably due to accumulation of displaced atoms at the interface.

There is a difference by ion species in the morphological changes at unit ion dose. The peak for roughness of specimens bombarded with He^+ ions is a dose of 1×10^{15} ions/cm^2. On the other hand, the peak of roughness was at a dose of 5×10^{14} ions/cm^2 when using Ar^+ ions. Collagen contraction of specimens bombarded with Ar^+ ions was progressing more rapidly than with He^+ ions owing to the different ion energy losses.

Collagen adhesion to PS substrate increases with dose monotonously at higher doses. However, in a separate study proceeding in parallel with this one, cell adhesion to collagen-coated PS bombarded with He^+ and Ar^+ ions decreased at a dosage more than 1×10^{15} ions/cm^2. This result is due to the destruction of the ligand recognized by cell in the collagen by ion bombardment at higher doses. Moreover, IR intensity of ion bombarded collagen decreased as the dose increased in this study. This suggests that damage induced by ion-beam irradiation destroyed the collagen structure and ligands. Therefore, excess irradiation is not effective for total cell immobilization.

5. CONCLUSION

Collagen was immobilized to substrate at a dose of 1×10^{14} ions/cm^2 and collagen structure was not changed seriously at this dosage. It is concluded that a dosage in the region of 1×10^{14} ions/cm^2 is suitable to immobilize collagen on the surface of artificial organs.

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