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Surface Analysis of Ion Implanted Avidin Coated SiO_2

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Protein-coated surfaces were modified using an ion beam for fabrication of protein chips and biosensors. The substrates used were un-implanted avidin-coated SiO_2, and He^+ and Kr^+ ion implanted avidin-coated SiO_2 at fluences of 1×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2. Fluorescently labeled (Cy3) biotin was dropped on ion implanted avidin-coated specimens for 30 min. Cy3-biotin patterning images were obtained with microscope. Fluorescently labeled biotin was found to bind only to un-implanted area. Fluorescence study indicated that the most suitable ion implantation fluence was 1×10^{13} ions/cm^2 to produce micro-patterned protein chips. Surface morphology and roughness of the Kr^+ ion implanted surfaces became wave-shape and surface roughness became smooth as compared with non-implanted avidin surface. XPS study indicated that amorphous carbon was strongly induced and original structure was destroyed by ion beam irradiation and new functional group was produced as a result of recombination.
Key words: avidin, biotin, Cy3, ion implantation, AFM, XPS

1. INTRODUCTION

Microchip arrays are capable of analyzing hundreds to thousands of different loci simultaneously in a relatively short period of time. Microarrays of oligo-deoxyribonucleotides and DNA immobilized on filters or glass have been effective for parallel hybridization analysis of a large number of DNA and RNA sequences to identify genetic mutations and gene polymorphisms [1,2], gene expression [3-5], and to detect different microorganisms [6].

In many applications, the sensitivity obtained with simple antigen-antibody interactions may not be sufficient. In such cases, avidin-biotin and streptavidin-biotin systems are particularly useful as a bridging or sandwich system in association with antigen-antibody interactions. Biotin molecules can be coupled easily to either antigens or antibodies. Because, only one conjugate preparation is required for many different assays, the avidin-biotin system can be attractive for use in immunological procedures.

In recent years, ion-beam irradiation into polymers was investigated with ion implantation being applied to modify the surfaces of polymers to improve their compatibility with blood and tissue [7-9]. Protein-coated surfaces were modified using ion beam for the fabrication of protein chips and biosensors [10]. We conducted a protein patterning experiment on the protein-coated surfaces by using metal masks. He^+ and Kr^+ ion implantation into avidin-coated substrates and surface analysis were performed.

2. EXPERIMENTAL

2.1 Avidin coating and ion implantation

The substrates used were un-implanted avidin-coated SiO_2, and He^+ and Kr^+ ion implanted avidin-coated SiO_2 at fluences of 1×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2 at an energy of 150 keV. The substrates of SiO_2 on Si wafer were immersed in 0.1 mg/ml avidin solution (Wako Jyunyaku, Japan) for one hour. After surplus avidin was removed by 100 mM 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) buffer, the substrates were dried under ambient room condition. A full coating avidin layer was confirmed by fluorescently labeled (Cy3) biotin study. After coated with avidin, the substrates were implanted with He^+ and Kr^+ ion and subsequently used as specimens of ion implanted avidin-coated SiO_2. The beam current density was kept below 0.05 μA/cm^2 to prevent the substrates from heating. Micro-patterned ion implantation into surface for fluorescence study was performed with putting micro-patterned stainless mask on the sample surfaces.

2.2 AFM observation

The surface morphology of non-implanted and implantted surface was observed using an AFM (MFP-3D: Asylum Research, U.S.A) in the air. Scan area were 50 and 2.5 μm squares and using the non-contact mode with silicon cantilevers.

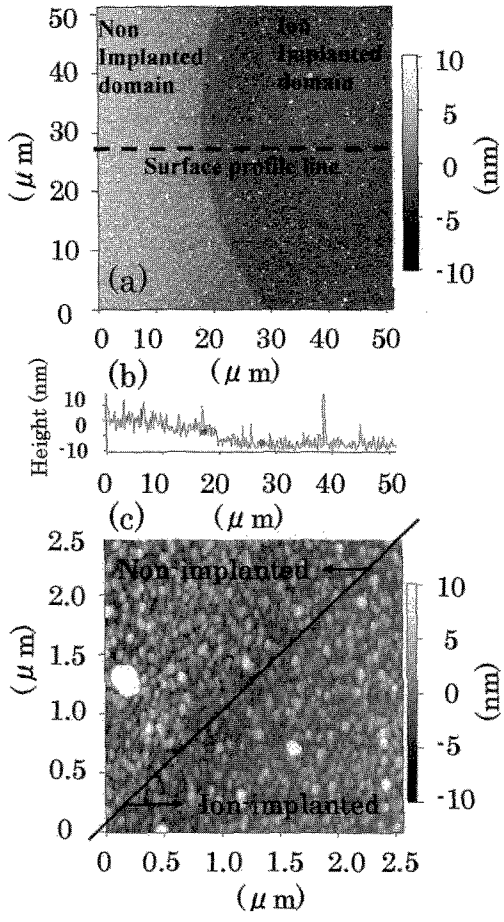

Fig. 1. Surface morphology (a), (c) and surface profile (b) of Kr^+ ion-implanted avidin surface with a fluence of 1×10^{14} ions/cm^2.

2.3 XPS analysis

The XPS measurements were carried out using a VG ESCALAB 250 spectrometer (Thermo Electron Co.) employing monochromatic X-ray AlK (1486.6 eV, 200W) radiation. Masking of the samples using copper tape with an electron shower was preformed to avoid any charge-up. The overview spectra were taken between 0 and 1000 eV with an energy step of 1.0 eV, while the detailed spectra of the peaks of interest (C_{1s} and N_{1s}) were recorded with an energy step of 0.1 eV. Band deconvolution was performed to separate the multiple components observed. The band shape used was a mixture of Gaussian and Lorentz functions (L/G = 0.3).

2.4 Fluorescence study

Fluorescently labeled (Cy3) biotin was dropped on ion implanted avidin-coated specimens for 30 min in the dark and washed with HEPES - 0.5% poly (oxyethylene) sorbitan monolaulate (Tween 20) solution. Cy3-biotin patterning images were obtained with microscope equipped with a CCD camera.

3 RESULTS AND DISCUSSION

3.1 AFM

Figure 1 shows that surface morphology of Kr^+ ion-implanted avidin surface with a fluence of 1×10^{14} ions/cm^2. The reported size of the avidin single molecule

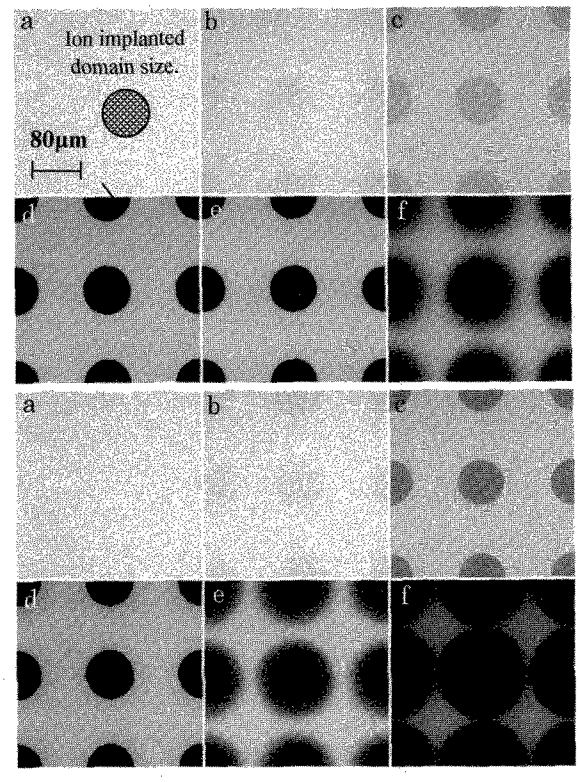

Fig. 2. Fluorescence micrographs of Cy3-labeled biotin patterned on the (a) non-implanted, He^+ (upper) and Kr^+ ion (below) implanted specimens with fluences of (b) 1×10^{11}, (c) 1×10^{12}, (d) 1×10^{13}, (e) 1×10^{14} and (f) 1×10^{15} ions/cm^2.

was about 5 nm. Surface morphology of the non-implanted surfaces showed a state of aggregated avidin, and surface roughness became smooth by Kr^+ ion implantation. The avidin surface was depressed by ion implantation.

3.2 Fluorescence study

Figure 2 shows fluorescence micrographs of Cy3-labeled biotin patterned on the non-implanted, He^+ and Kr^+ ion implanted specimens with fluences of 1×10^{11}, 1×10^{12}, 1×10^{13}, 1×10^{14}, 1×10^{15} ions/cm^2. A diameter of ion implanted domain was 80 μm using a stainless mask on specimens. Fluorescently labeled biotin was found to bind only to un-implanted area. Biotin receptors of avidin on the substrate were begun to destroy from $1x10^{12}$ ions/cm^2 ion-implantation. It is obvious that there is a clear distinction between the un-implanted region and ion-implanted region with a fluence of 1×10^{13} ions/cm^2. A fluorescence pattern spread to the outside of the surface of the ion implanted circular domain as a fluence increased owing to heat transfer effects [10]. Kr^+ ion implantation significantly affected these phenomena as compared with He^+ ion implantation. These results indicated that the most suitable ion implantation fluence was 1×10^{13} ions/cm^2. It is expected that ion implantation broke the binding site that corresponds to avidin-biotin bonding.

3.3 XPS study

Figure 3 presents the C_{1s}, N_{1s} and O_{1s} spectra of non-implanted avidin and He^+ and Kr^+ ion-implanted

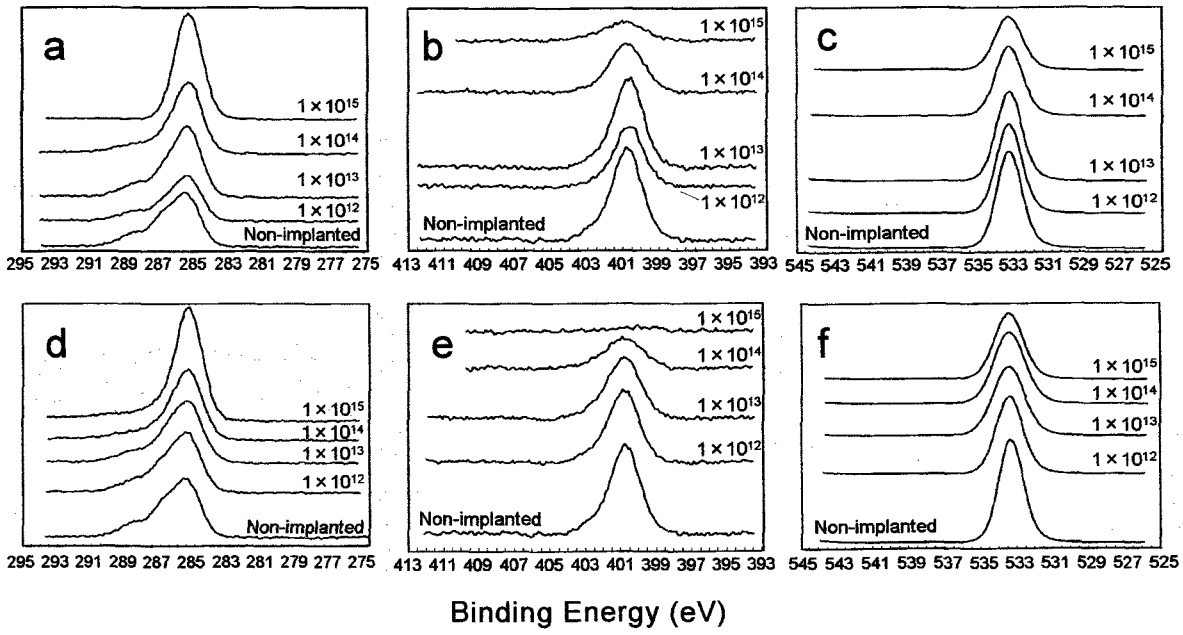

Fig. 3. C_{1s} (a, d), N_{1s} (b, e) and O_{1s} spectra (c, f) of non-implanted avidin, He^+ (upper) and Kr^+ ion-implanted (below) avidin-coated Si with fluences of 1×10^{12}, 1×10^{13}, 1×10^{14}, and 1×10^{15} ions/cm^2

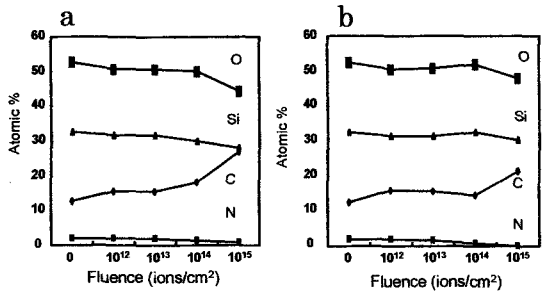

Fig. 4. Atomic % of oxigen, carbon, silicon and nitrogen of non-implanted, He^+ (a) and Kr^+ (b) ion-implanted avidin.

avidin-coated Si with fluences of 1×10^{12}, 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2. Figure 4 illustrates atomic concentration of oxigen, carbon, silicon and nitrogen of non-implanted, He^+ and Kr^+ ion-implanted avidin. Oxigen, silicon and nitrogen decreased monotonically with ion fluence. Carbon increased with ion fluence.

Figure 5 shows the C_{1s} and N_{1s} bands deconvolution of non-irradiated the He^+ and Kr^+ ion implanted avidin at 150 keV with a fluence of 1×10^{15} ions/cm^2. The C_{1s} spectrum of the non-implanted avidin contains four peaks at 285, 286, 287 and 289 eV; these are assigned to the C-C group, C-NH_2 group, C-OH group and C-OOH. After ion implantation, new components appeared at 284 eV ; these were assigned to the amorphous carbon (a-C). The N_{1s} spectrum of the non-implanted avidin contains two peaks at 401 and 402 eV; these are assigned to the NH_2 group, NH_3^+ group. After ion implantation, increased components appeared at 402 eV; these was assigned to NH_3^+ or nitrogen oxide.

Figure 6 illustrates area ratio of C-C group, C-NH_2 group, C-OH group, C-OOH, NH_2 group, NH_3^+ group or

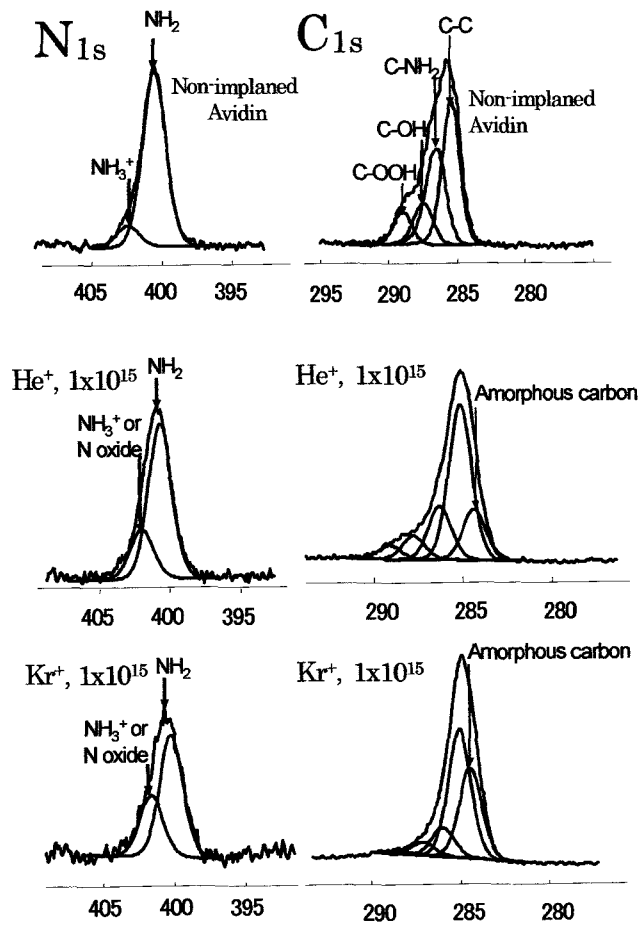

Fig. 5. The band deconvolution of non-implanted, He^+ and Kr^+ ion implanted avidin at 150 keV with a fluence of 1×10^{15} ions/cm^2.

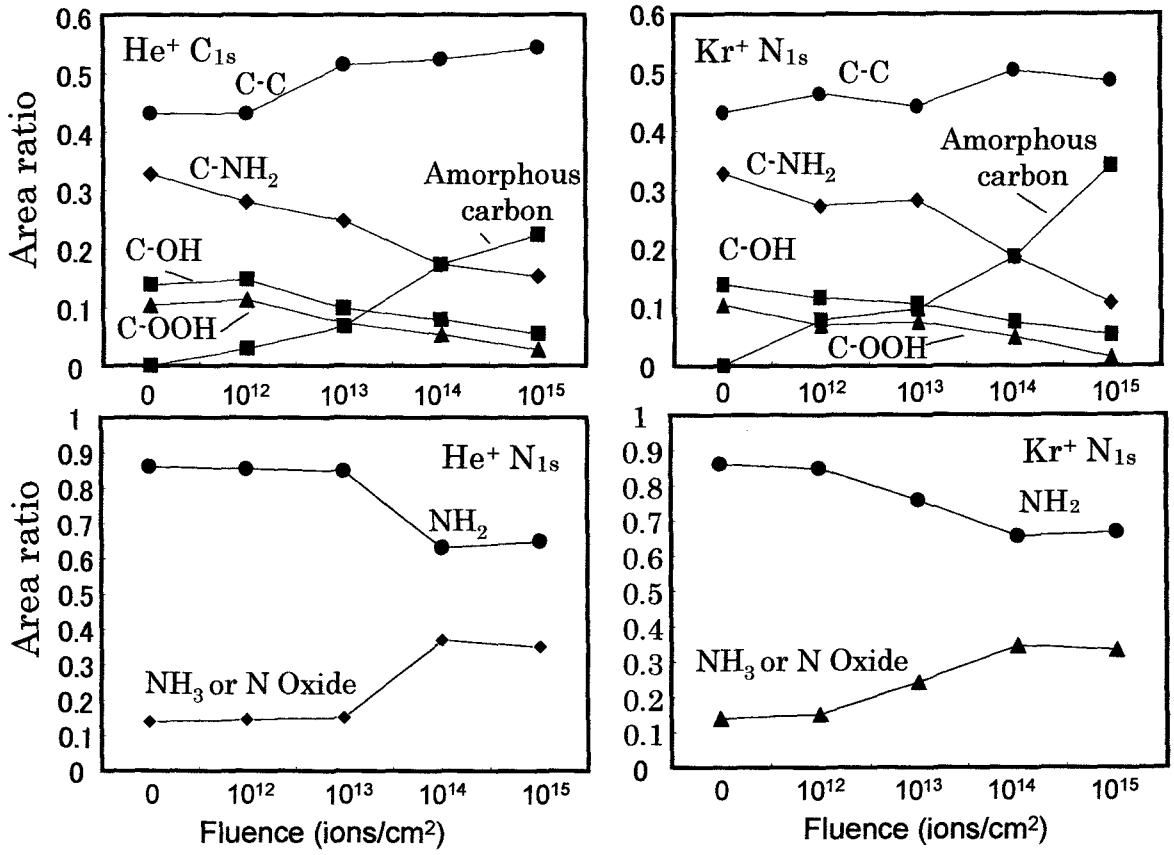

Fig. 6. Area ratio of C-C group, C-NH_2 group, C-OH group, C-OOH, NH_2 group, NH_3^+ group or nitrogen oxide of non-implanted, He^+ and Kr^+ ion-implanted avidin as a function of fluence.

nitrogen oxide of non-implanted He^+ and Kr^+ ion-implanted avidin as a result of XPS study. C-NH_2, C-OH and C-OOH decreased monotonically with ion fluence. The ion-beam generated C-C bond and amorphous carbon increased with ion fluence. This result indicated that amorphous carbon was produced by 150 keV- He^+ and Kr^+ ion implantation.

NH_2 decreased with ion fluence. NH_3^+ or nitrogen oxide was produced by ion implantation increased with ion fluence. These results indicated that ion implantation broke the original chemical bond (NH_2) to form NH_3^+ or nitrogen oxide.

These result indicated that amorpous carbon was strongly induced and original structure was destroyed by ion beam irradiation and new functional group was produced as a result of recombination.

4. CONCLUSIONS

Micro-patterned avidin surface should have utility in a wide variety of applications including DNA, antibody array and biochips. Micro-patterned avidin surface was obtained by ion implantation. Fluorescently labeled biotin was found to bind only to un-implanted area, because ion implantation broke original chemical bond in avidin. Micro-patterned avidin surface can be obtained by ion implantation. The technique should be widely applied to other micro-patterned protein chips.

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