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Ion Beam Modification of Avidin Coated SiO_2 Substrate for Protein Micropatterning

Kazumasa Sato, Yuji Segawa, Ken-ich Yamakoshi*, Yoshiaki Suzuki** and Tasuku Yotoriyama***

SONY Corporation, Shinagawa-ku, Tokyo 141-0001, Japan
Fax: 81-3-5803-4790, e-mail: Kazumasa.Satoh@jp.sony.com
*Kanazawa University, Kanazawa-shi, Ishikawa 920-1192, Japan
Fax: 81-76-234-4739, e-mail: yamakosi@t.kanazawa-u.ac.jp
**Beam Application Team, Advanced Development and Supporting Center
Riken, Wako-shi, Saitama 351-0198, Japan
Fax: 81-48-462-4623, email: ysuzuki@riken.jp
***Tokyo University of Science, Shinjuku-ku, Tokyo 162-8601, Japan
Fax: 81-3-3260-4772, e-mail: Tasuku.Yotoriyama@jp.sony.com

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^{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 equipped with a CCD camera. 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 in this experimental condition. A fluorescence pattern spread to the outside of the surface of the ion implanted circular domain as a fluence increased. Ion implantation increased contact angle of water and then reached to about 80° at a fluence of 1×10^{15} ions/cm^2. Ion implanted domain were indistinguishable from un-implanted domain by SEM study. FT-IR-ATR results revealed that the amide compounds were decomposed with increasing fluence of implantation, and decomposition was marked at fluence of 1×10^{15} ions/cm^2. In the Raman spectra, two broad features are present in ion implanted avidin-coated SiO_2 at 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.
Key words: avidin, biotin, Cy3, Ion implantation

1. INTRODUCTION

A few million nucleotides may differ from each other in human genome polymorphic sites. Comparative analyses of the polymorphic sites should provide a wealth of information that could help biomedical researchers understand the functions of genes and identify the genes responsible for numerous physiological traits.

Microchip arrays are capable of analyzing hundreds to thousands of different loci simultaneously in a relatively short period of time. Microarrays of oligodeoxyribonucleotides 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].

Ion implantation is a unique method of modifying surface structures and properties of materials. Most research efforts in the field of ion-beam irradiation have concentrated on inorganic materials such as metals, ceramics and semiconductors. 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. We conducted a protein patterning experiment on the protein-coated surfaces by using metal masks. He^+ and Kr^+ ion implantation into avidin-coated substrates 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^{13}, 1×10^{14} and 1×10^{15} ions/cm^2.

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. The substrates were then implanted with He^+ and Kr^+ ion and subsequently used as specimens of ion implanted avidin-coated SiO_2. For He^+ and Kr^+ ion implantation, the accelerating energy was 150 keV and the fluences

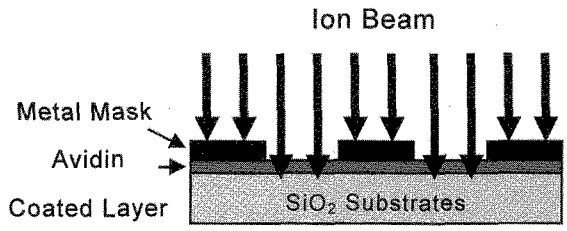

Fig 1. Micro-patterned ion implantation into surface with putting micro-patterned stainless mask.

were 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2. The beam current density was kept below 0.05 μA/cm^2 to prevent the substrates from heating.

The pressure of the target chamber was maintained at a base pressure of 10^{-4} Pa during ion implantation. Micro-patterned ion implantation into surface for fluorescence study was performed with putting micro-patterned stainless mask on the sample surfaces as Fig 1.

2.2 Surface analysis

Micro-patterned ion implanted surfaces were investigated by scanning electronic microscope (SEM, JSM-6330F : JEOL) after sample surface was coated by Au^+ (30 nm) with using quick coater (SANYU DENSI, Japan).

Decomposition and new functional groups in the ion implanted specimens were detected by Fourier transform infrared spectroscopy combined with attenuated total reflectance (FT-IR-ATR, Nicolet, Nexus 470), and Laser Raman spectroscopy (Raman, Jobin Yvon, LabRam). Specimens used were 2×2 cm^2 with ion implantation fluence between 1x10^{13} and 1x10^{16} ions/cm^2.

To investigate the surface wettability, water contact angles were determined using the sessile drop method. Specimens used were 2×2 cm^2 with ion implantation fluence between 1x10^{13} and 1x10^{16} ions/cm^2. The water droplet was about 1.8 μl which was desirable for contact angle measurements to prevent from gravity effects. Each determination was obtained by averaging the results of at least five measurements. Water used to measure was distilled water (Otsuka Seiyaku).

2.3 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 Contact angle

Figure 2 shows contact angle of water for un-implanted and He^+ and Kr^+ ion implanted avidin-coated surfaces as a function of a fluence. The water contact angle of the unimplanted avidin was 39°. The contact angle of He^+ and Kr^+ ion implanted sample with a fluence of 1×10^{13} ions/cm^2 increased to 61.7° and 64.8°. Ion implantation increased contact angle of water and then reached to about 80° at a fluence of 1x10^{15} ions/cm^2.

Ion implantation into biopolymer decomposed original chemical bonds and makes free bond on the surface, functional groups and carbonization. These new structure induced by ion implantation are thought to be main reason for the change of water contact angles. And it is well known that contact angle of water increase with increase in carbon content. From this reason, it was thought that the contact angle of water for ion implanted avidin surface increased owing to decomposition of original chemical bonds and carbonization induced by ion implantation.

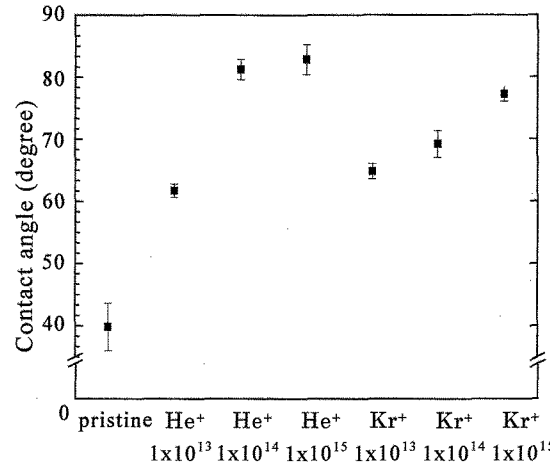

Fig 2. Contact angle of water for un-implanted and He^+ and Kr^+ ion implanted avidin-coated surfaces as a function of a fluence.

3.2 Fluorescence study

Figure 3 shows fluorescence micrographs of Cy3-labeled biotin patterned on the He^+ and Kr^+ ion implanted specimens with fluences of 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. 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. Kr^+ ion implantation significantly affected these phenomena as compared with He^+ ion implantation.

It is expected that the largest cause for the phenomena in this experiment is heat transfer effects of metal mask to the specimen surface during ion implantation. Further studies are required to clarify these effects in more detail.

These results indicated that the most suitable ion implantation fluence was 1×10^{13} ions/cm^2 in this experimental condition. However, it is suggested that ion implantation at a lower fluence seemed to be more probable experimental condition.

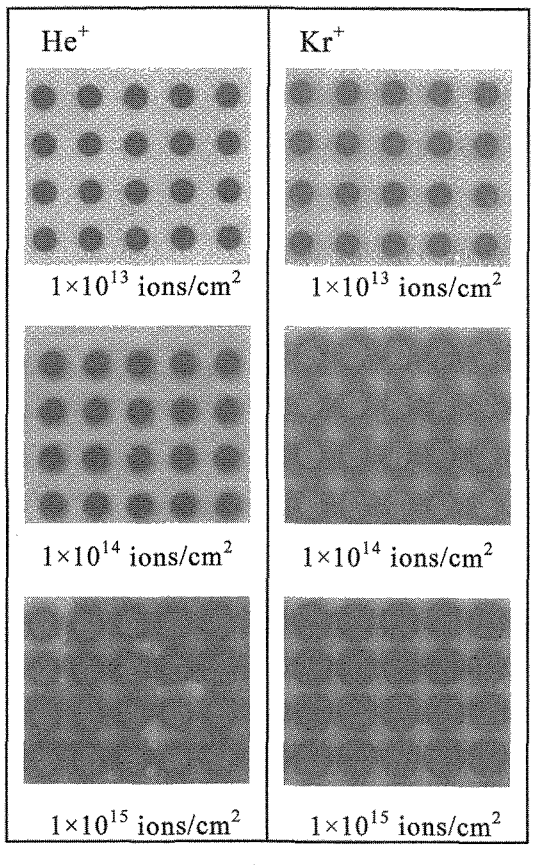

Fig 3. Fluorescence micrographs of Cy3-labeled biotin patterned on He^+ and Kr^+ ion implanted specimens with fluences of 1×10^{13}, 1×10^{14} and 1×10^{15} ions/cm^2.

3.3 SEM observations

Figure 4 shows SEM photographs of He^+ ion-implanted surface with a fluence of 1×10^{13} ions/cm^2. A diameter of ion implanted domain was 80 μm. Ion implanted domain were indistinguishable from un-implanted domain. There were no irregularities between un-implanted and ion implanted domain. The results indicated that fluorescently labeled (Cy3) biotin was not located by depressions.

3.4 FT-IR-ATR study

Figure 5 shows the FT-IR-ATR spectra of un-implanted and ion implanted avidin-coated SiO_2. In the FT-IR-ATR spectra, the absorption of amide I (1600 to 1700 cm^{-1}), and amide II (1500 to 1580 cm^{-1}) decreased with increasing the fluence. The adsorption of C-H deformation vibration (1430-1470 cm^{-1}) attributed to HEPES also decreased with increasing the fluence of implantation.

These FT-IR-ATR results revealed that the amide compounds were decomposed with increasing fluences of implantation, and decomposition was marked at a fluence of 1×10^{15} ions/cm^2.

There are two patterns for forming new structures, one caused by the electronic stopping power and the other by the nuclear stopping power. The electronic stopping powers are related to the energy transfer from energetic ions to the electrons surrounding the nuclei in the sample.

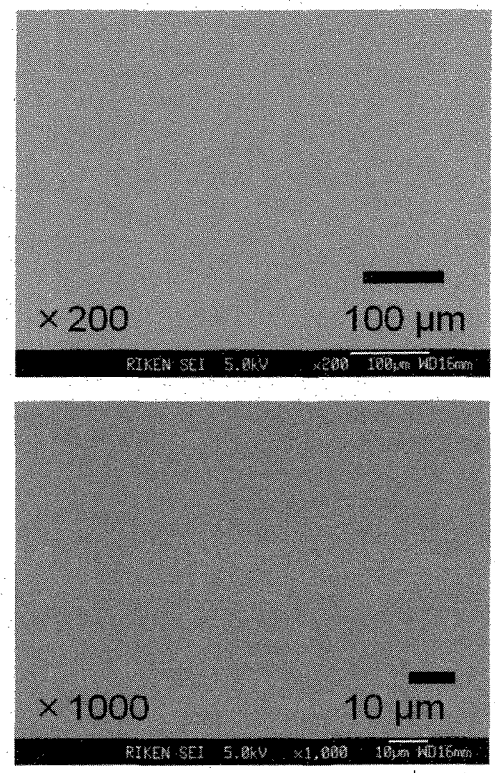

Fig 4. SEM morphology of He^+ ion implanted avidin-coated SiO_2 at a fluence of 1×10^{13} ions/cm^2.

It is thought that He^+ ion implantation decomposed chemical bonds comparing with Kr^+ ion implantation, because He^+ ion implantation possessed mainly the electronic stopping powers and this kind of energy transfer ionized atoms in the sample and induced decomposition of original chemical bonds.

3.3 Raman spectroscopic analysis

Figure 6 shows the Raman spectra of ion implanted avidin-coated SiO_2 at a function of fluence at an energy of 150 keV. In the Raman spectra, two broad features are present in ion implanted avidin-coated SiO_2 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.

The nuclear stopping powers are directly related with the displacement of atoms in the sample. The carbon structure was markedly produced by Kr^+ ion beam irradiation in comparison with He^+ ion irradiation. It is thought that the nuclear stopping power due to Kr^+ ion implantation is main reason for carbonization.

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

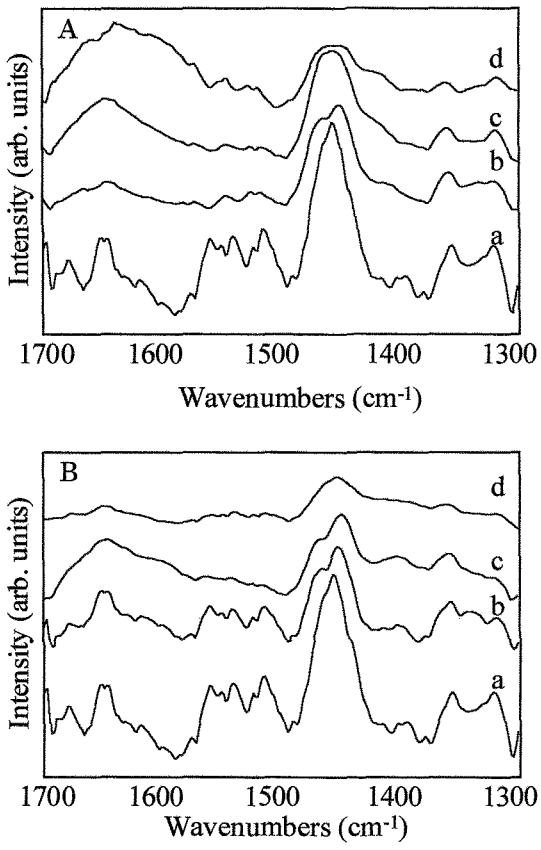

Fig 5. FT-IR-ATR spectra of, He^+ (A) and Kr^+ (B) ion-implanted avidin-coated SiO_2 with fluences of (a) 0, (b) 1×10^{13}, (c) 1×10^{14} and (d) 1×10^{15} ions/cm^2 at an energy of 150 keV.

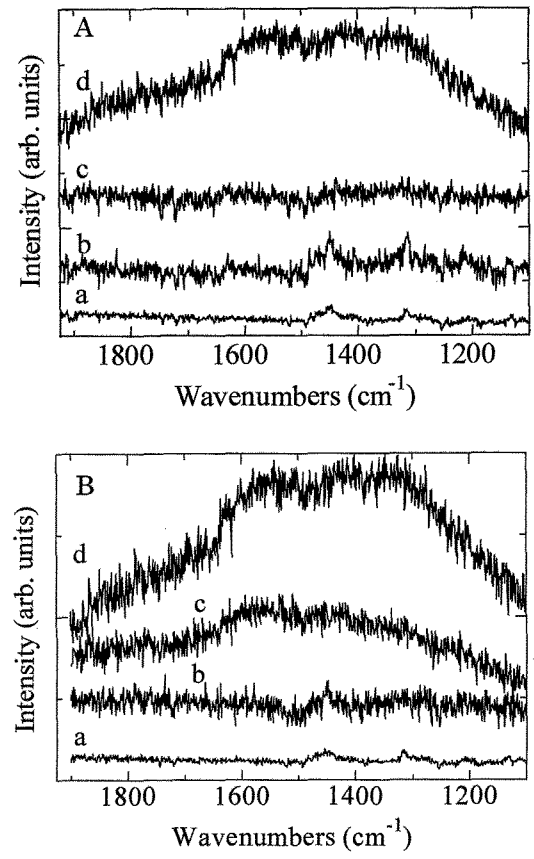

Fig 6. Raman spectra of He^+ (A) and Kr^+ (B) ion-implanted avidin-coated SiO_2 with fluences of (a) 0, (b) 1×10^{13}, (c) 1×10^{14} and (d) 1×10^{15} ions/cm^2 at an energy of 150 keV.

widely applied to other micro-patterned protein chips.

References

[1] G. Yershov, V. Barsky, A. Belgovskiy, E. Kirillov, E. Kreindlin, I. Ivanov, S. Parinov, D. Guschin, A. Drobishev, S. Dubiley and A. Mirzabekov. *Proc. Natl Acad. Sci. USA*, **93**, 4913-4918. (1996).

[2] M. Chee, R. Yang, E. Hubbell, A. Berno, X. C. Huang, D. Stern, J. Winkler, D. J. Lockhart, M. S. Morris and S. P. Fodor. *Science*, **274**, 610-614. (1996).

[3] L. Wodicka, H. Dong, M. Mittmann, M. H. Ho and D. J. Lockhart. *Nature Biotechnol.*, **15**, 1359-1367. (1997).

[4] M. B. Eisen, P. T. Spellman, P. O. Brown and D. Botstein. *Proc. Natl Acad. Sci. USA*, **95**, 14863-14868. (1998).

[5] M. Schena, D. Shalon, H. Heller, A. Chai, P. O. Brown and R. W. Davis. *Proc. Natl Acad. Sci. USA*, **93**, 10614-10619. (1996).

[6] D. Y. Guschin, B. K. Mobarry, D. Proudnikov, D. A. Stahl, B. E. Rittmann and A. D. Mirzabekov. *Appl. Environ. Microbiol.*, **63**, 2397-2402. (1997).

[7] Y. Suzuki, M. Kusakabe, J.-S. Lee, M. Kaibara, M. Iwaki and H. Sasabe, *Nucl. Instrum. Methods Phys. Res.* **B65**, 142-147 (1992).

[8] Y. Suzuki, H. Iwata, A. Nakao, M. Iwaki, H. Sasabe, S. Kaneko, H. Nakajima and M. Kusakabe, *Nucl. Instrum. Methods Phys. Res.* **B127/128**, 1019-1022 (1997).

[9] K. Kurotobi, M. Kaibara, Y. Suzuki, M. Iwaki and H. Nakajima, *Nucl. Instrum. Methods Phys. Res.* **B175/177**, 791-796 (2001).

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