Au Coating of Carbon Nanofiber-Tipped SPM Probes for Immobilization of Thiolated Biomolecules

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Abstract. An argon-ion-induced carbon nanofiber (CNF) is expected to be an ideal nanocarbon for developing probes for scanning probe microscopes (SPM) owing to its superior physical properties and structures. To use this pristine CNF as a biofunctionalized SPM probe, its surface needs to be further modified with probing molecules that have specific biochemical affinities toward biological targets. In this study, using a carbon-coated Si wafer as a substitute for a CNF-tipped SPM probe, we investigated ion sputtering for the formation of an Au film on a carbon surface, followed by thermal annealing for making the Au-crystal orientation preferable for the chemisorption of thiolated biomolecules. Finally, we confirmed the biofunctionality of the immobilized thiolated oligonucleotide probe on the annealed Au film in order to hybridize it with the complementary oligonucleotide target. The hybrids were detected by fluorescence emitted from streptavidin-conjugated quantum dots tagged to the biotin molecules. From these results, it was shown that Au coating, thermal annealing, and the subsequent immobilization of thiolated biomolecules can be used for the development of new biofunctional CNF-tipped SPM probes.

1. Introduction

Tanemura et al. demonstrated that argon ion (Ar⁺) bombardment of both bulk carbon [1] and carbon-coated substrates [2] induced growth of conical protrusions, and carbon nanofibers (CNFs) grew on the cone tips without the presence of any catalysts, even at room temperature. The CNFs created by Ar⁺ bombardment were one-dimensional and amorphous nanocarbon materials without a hollow structure, having a high aspect ratio and nanoscale tip radius of curvature [2]. Considering their physical properties and structures, the CNFs, along with carbon nanotubes (CNTs), were expected to be an ideal material for use in the imaging probe of a scanning probe microscope (SPM) [3]. Several methods have been developed for fixing a single CNT on the apex of an SPM tip, which require manual or electrophoretical manipulation and additional post-processing [4-6]. These methods are considered to be time consuming and costly, and are not suitable for mass production of commercial CNT-tipped SPM probes. On the other hand, by bombarding Ar⁺ to carbon-coated silicon (Si) cantilevers, batch growth of a linear-shaped single CNF with a typical diameter of 16-30 nm on each tip of the Si cantilevers has been achieved on a wafer scale [7, 8]. The CNF-tipped SPM probes have been mass-produced and supplied by Olympus Co. for practical use in nanotechnology. Recently,
researches in nanobiotechnology need sensing and manipulating techniques using SPMs equipped with cantilevers containing biofunctionalized needlelike tips (nanoneedle) [9]. CNFs are considered to be superior to CNTs as a basal nanoneedle for developing biofunctionalized SPM probes because of their superior mass productivity compared with that of CNTs, as mentioned above. In the present study, using a carbon-coated Si wafer as a substitute for a CNF-tipped SPM probe, we examined ion sputtering to form an Au film on carbon-coated surfaces, thermal annealing to make the Au-crystal orientation preferable for chemisorption of thiolated biomolecules, and the immobilization of thiolated oligonucleotide probes on the annealed Au film.

2. Materials and Methods

2.1. Preparation of carbon-coated Si wafers
Prior to carbon vapor deposition, Si (100) wafers (5 × 5 mm²; Shin-Etsu Chemical Co.) were cleaned by exposing them for 30 min at 10⁻³ Pa to 172-nm vacuum UV (VUV) radiation (λ = 172 nm, 10 mW/cm²) from an excimer lamp (UER20-172V; Ushio Inc.) with a distance of 20 mm between the lamp window and the sample surfaces. A carbon layer of about 40 nm thickness was formed on the surface of the Si wafers by carbon vapour deposition using a carbon coating unit (Hitachi High-Technologies Co.). During the deposition process, the vacuum of the chamber was maintained below 7 Pa and the current was held at 20 A.

2.2. Au coating on carbon-coated Si wafers
A carbon-coated Si wafer was then coated with an Au film at a rate of about 6 nm s⁻¹ by ion sputtering using an ion sputter coater (E-1020; Hitachi High-Technologies Co.) at a discharge current of 15 mA.

2.3. Thermal annealing of the Au film on the carbon-coated Si wafer
The Au film deposited on the carbon-coated Si wafer was annealed in air at 350°C for 1 h with a lamp heater (MILA-3000; ULVAC-RIKO Co., Japan). The crystal orientations of the Au films as-deposited and after annealing on the carbon-coated Si wafer were examined by X-ray diffraction (XRD) (MXP-1; Bruker AXS K.K.) with CuKα anode radiation generated at 40 kV and 20 mA. The crystal orientations were determined by comparing the relative intensities of Au (111) and Au (200) peaks. The surfaces of the Au films as-deposited and after annealing were observed by atomic force microscopy (AFM) (SPA400; SII Nanotechnology) and field emission scanning electron microscopy (FE-SEM) (S-4300; Hitachi High-Technologies Co.).

2.4. Immobilization of thiolated biomolecules
The annealed samples were incubated in 1 µM HS-(CH₂)$_n$-(dT)$_{30}$ for 2 h, washed with TE-1 M NaCl buffer three times, incubated in 1 mM 6-mercapto-1-hexanol for 1 h, washed with TE-1 M NaCl buffer thrice, rinsed with milli-Q water, and then dried in air. To confirm the biofunctionality of the immobilized HS-(CH₂)$_n$-(dT)$_{30}$ on the annealed Au film deposited on the carbon-coated Si wafer, samples irradiation by the masked 172-nm vacuum UV (VUV) were incubated in 0.1 µM 5'-biotin-labeled A$_{30}$ at 0°C for 1 h, and then washed with TE-0.5 M NaCl buffer three times. The hybrids formed between (dT)$_{30}$ and 5'-biotin-labeled A$_{30}$ were detected by tagging streptavidin-conjugated quantum dots (Q-dot 655) to the biotin. Fluorescence (Em: 655 nm) from Q-dot 655 coupled to the biotin hydrazide immobilized on the sample surface was detected using an epifluorescence microscope (BX51; Olympus Co.) equipped with a charge-coupled-device (CCD) camera detector (DP70; Olympus Co.), and a U-MWG2 filter set (excitation filter: 510-550 nm, emission filter: 590 nm; Olympus Co.).

3. Results and Discussion
Kitazawa et al. demonstrated that Ar⁺-induced CNFs possess excellent mechanical properties similar to those of high quality CNT probes [10] and electrical properties superior to conventional electroconductive metal-coated Si probes [11]. Because of these excellent physical properties and
mass productivity, the CNF-tipped SPM probe can be practically used with high resolution in nanotechnology. To use the CNF-tipped SPM probe as a biofunctionalized nanoneedle equipped in SPMs for sensing and manipulating living cells and biomolecules in life science and medical fields, the surface of the pristine CNF must be further modified with probe molecules having specific biochemical affinities toward these targets. One of the most promising approaches for immobilizing probing molecules is to coat CNFs with a thin layer of Au, and then subsequently functionalize the Au-coated CNFs with probing molecules having thiol terminal groups by forming self-assembled monolayers (SAMs) through the chemisorption of thiols on the Au surfaces. In the present study, conditions for Au coating and thermal annealing were examined initially using carbon-coated Si wafers as a substitute for the CNF-tipped SPM probe. An Au film was deposited by ion sputtering on the surface of a carbon-coated Si wafer with a carbon film of about 40 nm in thickness.

Figure 1. AFM images of Au films (A) as-deposited (thickness of 15 nm) and (B) after annealing at 350°C for 1 h.

The Au film deposited for 2.5 min had a thickness of about 15 nm and a surface roughness of about 1.2 nm (Fig. 1A). The deposited Au films with thicknesses ranging from 15 nm to 120 nm on the carbon-coated Si wafers were then annealed in air at 350°C for 1 h. As a result of the thermal annealing, growth of Au polycrystals was observed in the Au film by AFM, with more polycrystals seen in the thicker samples (Fig. 1B). The crystal orientations of the Au films as-deposited and after

Figure 2. XRD patterns of Au films (A) as-deposited (thickness of 15 nm) and (B) after annealing at 350°C for 1 h.

Figure 3. FE-SEM images of Au films (A) as-deposited (thickness of 15 nm) and after annealing at 350°C for 1 h with thickness values of (B) 15 nm, (C) 30 nm, (D) 60 nm, and (E) 120 nm.
thermal annealing were examined by XRD. As shown in Fig. 2, Au (111) orientation appeared to be predominant in the thermally annealed Au film, which is considered to be preferable for forming SAMs of probing molecules having thiol terminal groups.

As shown in Fig. 3, more pinholes were observed in the thinner films after thermal annealing, which might be caused by both the accelerated growth of the Au-crystalline particles at high temperatures and a weak affinity between Au and carbon. An oligonucleotide probe, HS-(CH$_2$)$_6$-(dT)$_{30}$, was then immobilized via thiol and Au binding. The biofunctionality of the immobilized HS-(CH$_2$)$_6$-(dT)$_{30}$ was confirmed by epifluorescence microscopic observation of the hybrids formed with a complementary target oligonucleotide (Fig. 4). From these results, Au coating and subsequent immobilization of thiolated biomolecules were shown to be suitable for developing new biofunctional SPM probes composed of CNFs.

4. Conclusions

Based on the results of Au film deposition on a carbon-coated Si wafer by ion sputtering, it was determined that deposition can occur with negligible increase in the surface roughness for Au films up to 120 nm in thickness. It was also found that thermal annealing enhances the growth of Au-crystalline particles, making Au (111) orientation predominant in the annealed Au film. Considering more pinholes were observed after thermal annealing in the thinner Au films on a carbon-coated Si wafer, it is necessary to improve the low binding affinity between Au and carbon, for example, by forming a buffer metal layer between them. The biofunctionality of immobilized HS-(CH$_2$)$_6$-(dT)$_{30}$ on the carbon-coated Si wafer was confirmed by epifluorescence microscopic observation of the hybrid formed with a complementary target oligonucleotide, A$_{30}$. Therefore, Au coating and subsequent immobilization of thiolated biomolecules were shown to be suitable for developing new biofunctional SPM probes composed of CNFs.

Acknowledgments

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Figure 4. Epifluorescence microscopic observation of the hybrids formed between (dT)$_{30}$ and 5’-biotin-labeled A$_{30}$ (red fluorescence). In advance to the hybridization, the immobilized HS-(CH$_2$)$_6$-(dT)$_{30}$ probes on the Au film were locally removed by masked VUV photooxidation.