

***In-situ* observation of deposition of gold nanoparticles on the amine-functionalized surface by open liquid-AFM**

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Abstract—Gold nanoparticles (AuNPs) are used as fundamental materials in chemical/biological sensor applications, such as the DNA-AuNPs complex system for colorimetric sensor. As a result, the immobilization of AuNPs on target substrates is an important issue. In this study, we investigated the deposition of AuNPs on an amine-derivatized silicon wafer by *in-situ* AFM. Liquid-AFM allows structural changes and reaction kinetics of biomaterials and organic/inorganic nanomaterials in liquid media to be analyzed *in-situ*. The results showed that AuNPs were immobilized on an amine-functionalized silicon wafer within a period of 1.5 hr.

Key words: AFM, Gold Nanoparticles, Self-assembly, Liquid-AFM

INTRODUCTION

AuNPs are the most stable metal clusters and they present fascinating aspects such as their size-related electronic, magnetic and optical properties as well as their potential to applications in catalysis and in biology. A 1D array of AuNPs on a patterned silicon surface [1] and AuNPs-labels for biomaterial detection [2] has been reported. Such chemical or biological sensor systems using AuNPs were based on the deposition of AuNPs on a functionalized surface [3].

AuNPs for immobilization on the surface show different affinities for adhesion, due to the different surface charge as the result of the synthesis conditions employed. AuNPs prepared by the Frens method [4] show good adhesion properties for amine moieties, while the seed method [5] can be used for thiol moieties. Therefore, the type of AuNP for immobilization on a surface can be altered according to the functional moiety of the initial substrate. An investigation of immobilization procedures for AuNPs is an important issue, and the progress of this process could be analyzed with SPR (surface plasmon resonance spectroscopy) or liquid-AFM (atomic force microscopy). SPR is used to analyze an angle shift through a change in the dielectric constant of a substrate [6], while AFM offers direct information such as changes in topographical height and width [7].

To scan a sample in the liquid phase in real-time, commercially applicable liquid cells have been developed by several groups. Closed liquid-cell systems are equipped with a flexible O-ring in a sealed sample holder, but this setup has several disadvantages such as possible O-ring leakage and the entrapment of air bubbles [8]. To overcome these drawbacks, an open liquid-cell has been proposed, to guarantee easy exchange and prevent overflow of the solution [9].

Liquid-AFM permits structural changes in macromolecules or biomolecules in their native environment to be observed, but the solution properties (i.e., kinematic viscosity) have an effect on image resolution. Since the kinematic viscosity of water or methanol is

similar to that of air, no image distortion and blur phenomena appear during image scanning [10]. However, when the scan is performed in fluids with a high kinematic viscosity, it is not possible to obtain non-distorted images. Therefore, to obtain a real-image in the liquid phase, the scan rate should be decreased to zero [10] or be fixed at a specific velocity [11] to reduce the effective force of the cantilever on the fluid and surface. However, the liquid media used in this study is not a simple solvent but a colloidal AuNPs solution, and thus image distortion induced by nanoparticles might appear in the scanned images. To minimize the intrinsic distortion of the apparatus, an independent z scanner was used, which also eliminates the x-z cross coupling problem that is inherent in conventional AFM [12].

In this work, we describe the *in-situ* observation of AuNPs deposition on an amine-functionalized surface using an open liquid-cell system with a glass channel, used in conjunction with a commercial AFM (XE-100, Park System, Korea).

EXPERIMENTAL

AuNPs were prepared by the Frens method [4] as follows. Briefly, a solution of 0.6 ml of 2.5×10^{-4} M tri-sodium citrate was injected into a mixture of 50 ml of a solution containing HAuCl₄. The color of the solution instantly turned reddish brown. The ratio of particle diameter to citrate volume used has been reported by Goodman [13]. Namely, smaller amount of sodium citrate yields larger AuNPs. The 40-50 nm AuNPs prepared was deposited on the amine-functionalized surface.

5 mM APTES solution was prepared in high purity ethanol. APTES [(aminopropyl)triethoxysilane] was deposited on a B-doped p-type Si(100) wafer. Before using the Si wafer, it was treated with piranha solution (H₂SO₄ : H₂O₂ 7 : 3 v/v) at 130 °C for 1 hr, followed by sequentially rinsing with DI water, acetone and ethanol, and drying in a stream of N₂.

To analyze the deposition of AuNPs on the amine-functionalized surface in real-time, an open liquid-AFM was used. As shown in Fig. 1, the probe-arm of the AFM was immersed in the AuNPs so-

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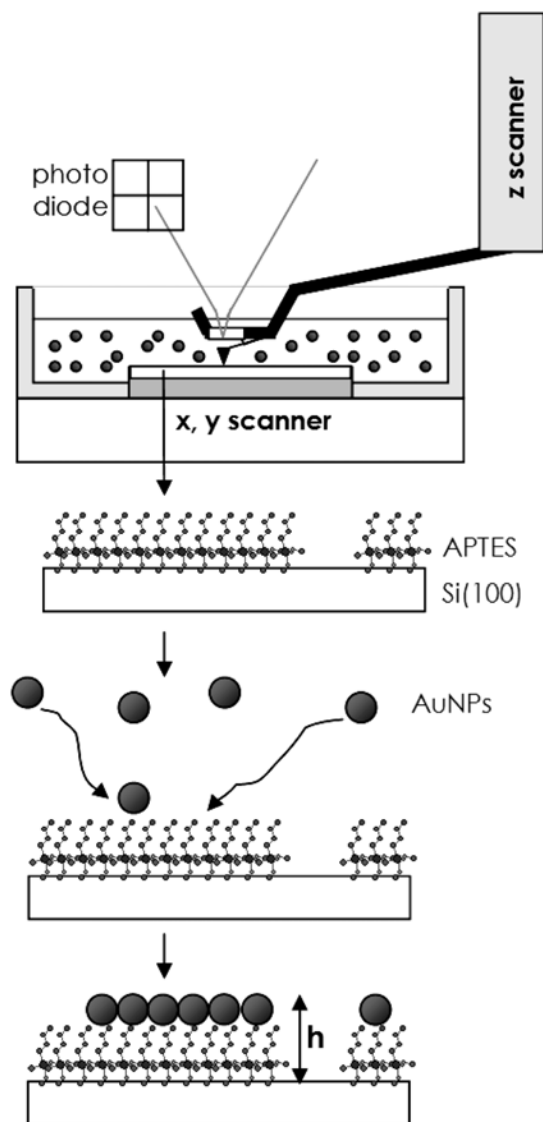


Fig. 1. Scheme for observing the deposition of AuNPs on an amine-functionalized substrate by liquid-AFM.

lution, and topographical images were obtained at intervals of 17 min (0.25 Hz). The cantilever used in this study, which has no affinity for AuNPs, was an NSC36 (Mikromash, Estonia). The particle diameter of the AuNPs was characterized by transmission electron microscopy (TEM) by using a JEM-2000EXII.

RESULTS AND DISCUSSION

The surface deposition of AuNPs was analyzed by open liquid-AFM using AuNPs solutions. The AuNPs prepared were spherical with a size of 40–50 nm (Fig. 2a). The use of higher concentrations of sodium citrate yields smaller particles [13]. When the amount of sodium citrate used is 1.75 ml, AuNPs are produced as 14 nm sized spheres. Large AuNPs used in this study could be effectively used to clearly observe the growth in height with time. Fig. 2b to Fig. 2f show AFM images of an AuNPs-immobilized surface of $10 \times 10 \mu\text{m}^2$ obtained by in-situ observation method at 17 min intervals (1,024 seconds, 0.25 Hz). When the immersing time of the substrate in

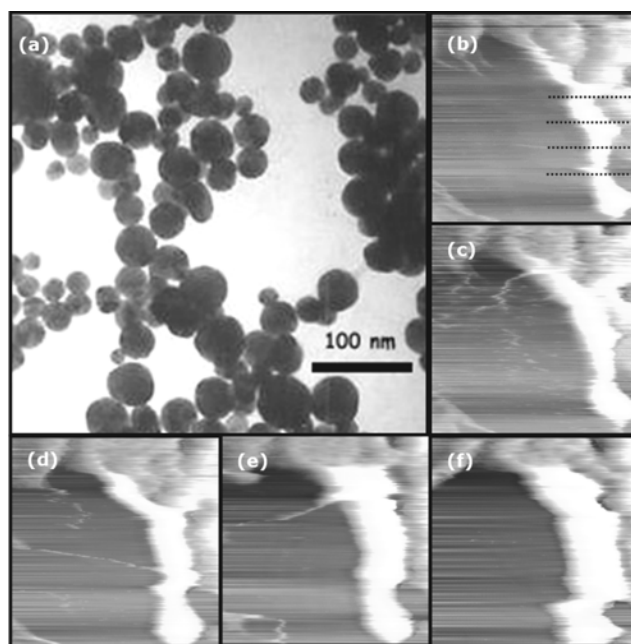


Fig. 2. (a) TEM image of AuNPs, and (b–f) AFM images of AuNPs deposition at (b) 17, (c) 24, (d) 51, (e) 68 and (f) 85 min ($10 \times 10 \mu\text{m}^2$ image).

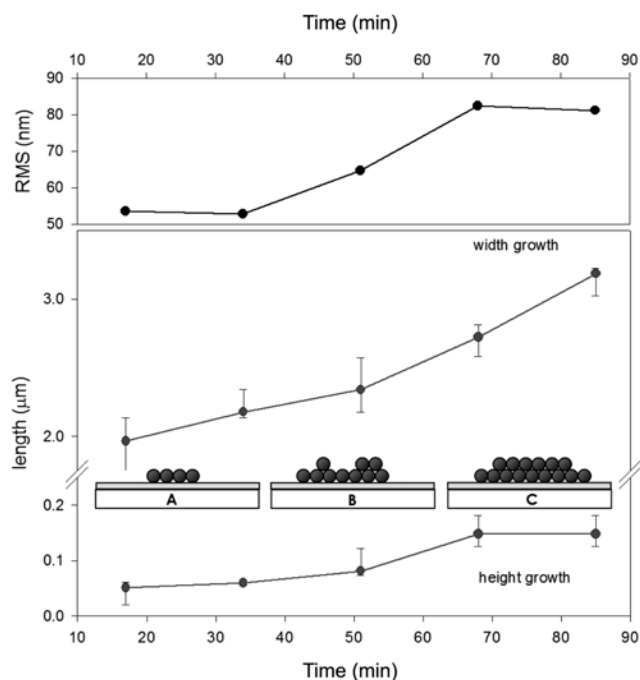


Fig. 3. Changes of the roughness (RMS) and width/height of an AuNPs-deposited surface with deposition time.

the APTES solution to form the self-assembled monolayer (SAM) of APTES is short (ca. 30 min), the SAM shows the expected defect, namely, an organic domain with poor APTES [14]. This method is called stepwise deposition to separate the phase of the mixed SAM.

The white area in Fig. 2b shows that AuNPs are only adsorbed on the covered surface, which was immobilized with amine groups. Therefore, the height change with immobilization time was calcu-

lated from the difference in height between the covered and uncovered surface. The brushed image in Fig. 2 is due to the contradiction of the adsorption rate of AuNPs and the scanning rate.

A change in roughness, width, and height of the AuNPs-deposited surface with deposition time (Fig. 3) was obtained by cross-sectional analyses of 4 lines, as shown in Fig. 2b. The roughness revealed information on morphology change for the entire surface with the deposition time of the colloidal AuNPs. As the immobilization time is increased, the roughness (RMS, root mean square) of the surface was increased with an S-curve, which resembled the general growth curve [15]. It should be noted that AuNPs are immobilized on the amine-functionalized surface as a stack of multilayers not a monolayer with time. The RMS values at 17-34 min and 68-85 min were approximately 55 and 82 nm, respectively, and maintained a similar value for some minutes. This feature of RMS maintenance was also found for height changes.

The height of the deposited AuNPs layer is less changed up to 34 min, but is increased greatly at 68 min. As shown in the inset figure of Fig. 3, AuNPs were deposited as a monolayer to a multilayer with time. It is also possible for multilayer adsorption to occur on one part of a surface while vacant sites are still present on another part. The first layer (~50 nm thickness) is formed by the chemical bonding between an amine group and AuNPs, while the second layer (~110 nm thickness) is formed by physical bonding to the first immobilized layer of the AuNPs. The physically adsorbed AuNPs are easily removed by sonication treatment.

However, the change of width growth was not same as height growth, and showed a linear increase with time. The width growth is slightly faster than height growth due to the difference in force between the adsorbate-surface and the adsorbate-adsorbate. Namely, the interaction force of an AuNPs-amine that is chemically bonded is larger than that of AuNPs-AuNPs with weak electrostatic and physical bonding.

The analysis of height/width growth shows that multilayer coverage is achieved within 1.5 hr of immobilization time, but additional layers are easily removed by post-treatment. In addition, the first deposition of AuNPs was formed within 27 min (pre-setting 10 min+scanning 17 min). Where, the pre-setting time of liquid-AFM in colloidal AuNPs solutions, such as laser focusing and tip approaching steps, required approximately 10 min. Therefore, a monolayer of AuNPs is produced within 1.5 hr. The prepared AuNPs films are applicable to sensor electrodes or tagging substrates for biomaterials.

In collusion, the deposition of colloidal AuNPs on an amine-functionalized surface was analyzed with liquid-AFM in real-time. AuNPs prepared by the Frens method were first deposited on an amine-

film within ca. 30 min, followed by additional deposition on the primarily deposited AuNPs to form AuNPs multilayers. As the immobilization time increased, the roughness of the entire surface and the height growth of a local surface were increased with an S-shaped curve, while the width of the AuNPs deposited area showed a linear increase. The first layer was formed by the chemical bonding of AuNPs-amine groups, while additional layers of AuNPs were bonded by physical force, and were easily removed by sonication treatment. Consequently, a deposition time of about 1.5 hr is sufficient to form a uniform monolayer of AuNPs.

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