

Surface Observation for Seed-mediated Growth Attachment of Gold Nanoparticles on a Glassy Carbon Substrate

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A seed-mediated growth method for surface modification was applied to the attachment of gold nanoparticles (AuNPs) to glassy carbon (GC) surfaces. By simply immersing a GC plate at first into a seed solution containing 4 nm Au nano-seed particles and then into a growth solution containing HAuCl₄, ascorbic acid and cetyltrimethylammonium bromide, AuNPs could be successfully attached to the GC surface *via* the growth of nanoparticles. A possible control of the size and density of AuNPs on GC was examined by observing surface images with a field-emission scanning electron microscope (FE-SEM) after several preparations with different immersion times. Compared with previous results on the growth of AuNPs on indium tin oxide (ITO) surfaces, it was characteristic that the AuNPs attached to GC surfaces exhibited smaller size and higher density as well as a flatter and non-crystal-like morphology. In addition, for performing the dense attachment of regular nano-sized AuNPs on GC surfaces, immersion for 2 h into the growth solution was sufficient. Longer immersion for 24 h caused an irregular growth of bold Au micro-crystals, while 24 h was necessary in the case of AuNPs on ITO surfaces. Shorter seeding and growth times were found to be effective for a sparse attachment of smaller Au nanoparticles whose size was *ca.* 20 nm. It was clarified that the seed-mediated growth method for surface modification was valid for fabricating a nanointerface composed of AuNPs on GC surfaces.

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Introduction

Metal nanoparticles (NPs) have been attracting active attention as functional units for electroanalysis and bioelectroanalysis, as summarized in some reviews.^{1–3} If we would like to utilize some metal NPs as unique devices in functional electrodes, normally the NPs need to be attached to electrode surfaces. Thus, various combinations and fabrications would be possible for NPs-attached functional electrodes regarding metal NPs, substrate electrode materials and attachment methods. Focusing on the attachment of gold nanoparticles (AuNPs), which is a typical functional nano-metal that has biocompatibility^{1,2} and electrocatalytic functions in some cases, on the surfaces of glassy carbon (GC), which is a typical conducting carbon substrate utilized as an electrode material, several methods have been reported up to now.

One typical method would be electrochemical deposition.^{4–9} Because AuCl₄[–] or Au³⁺ ions can be reduced to Au(0) on GC electrodes, AuNPs were deposited by potential step electrolysis, typically in 0.5 M H₂SO₄.^{4–9} Some modifications of the size and the amount of deposited AuNPs were possible by controlling the amount of AuCl₄[–],^{4,5} the stepped potential⁴ and the deposition time.⁵ AuNPs-electrodeposited GC electrodes were utilized in the analysis of As(III) with anodic stripping voltammetry⁶ and flow analysis.⁷ In addition, Ohsaka and coworkers reported an

interesting crystallographic orientation control of AuNPs by some additives⁸ and the fabrication of ternary self-assembled monolayer on AuNPs deposited on GC.⁹

Another typical method to attach AuNPs to GC surfaces would be to use bridging reagents.^{10–13} In Ref. 10, by using 4-mercaptobenzenediazonium tetrafluoroborate, benzothiol was bonded to GC *via* electro-reductive attachment,¹⁴ and then AuNPs were successfully attached to the terminal thiols. Alternatively, 4-aminobenzoic acid was covalently bonded to GC *via* electro-oxidation, and then 4-aminothiophenol was reacted to form peptide bonding while keeping the thiol terminals.^{11,12} Thus-fabricated AuNPs attached to GC electrodes were applied to the electrochemistry of hemoglobin,¹¹ ascorbic acid and dopamine.¹² A recent paper reported that cysteamine could also be utilized to attach AuNPs to GC *via* a cyclic voltammetric treatment to form covalent GC–N bonding.¹³ In addition to electrochemical and chemical methods, it was reported that the adhesion of AuNPs was possible on electrochemically pretreated GC electrodes.¹⁵ Electroless plating was also utilized to attach AuNPs to the surfaces of GC micro-spheres.¹⁶

While various methods are available at present to attach metal NPs to peculiar conducting surfaces, as mentioned above for the case of AuNPs on GC, the development of facile methods to modify the conducting surface with metal NPs is still desirable. If we can prepare such metal NPs attached electrode surfaces easily, it should be beneficial for practical electroanalytical applications.

Our group has been studying a seed-mediated growth method for surface modification^{17–23} as a new facile strategy to fabricate

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metal NPs attached interfaces. By applying the seed-mediated growth method, which was originally developed for preparing Au nanorods in aqueous solution,^{24,25} to the modification of indium tin oxide (ITO) surfaces mainly, the crystal growth of AuNPs on ITO surfaces could be performed from Au nano-seed particles physically adsorbed on the ITO while keeping adhesion contacts.¹⁷ As one of the advantages of this method in which no bridging reagents were used, the charge-transfer resistance was significantly decreased on AuNPs directly attached ITO electrodes.^{18,19} In addition, because neither bridging reagents nor strong capping reagents, such as thiols, present on the surfaces of AuNPs on ITO, further modifications of AuNPs with myoglobin²⁰ and hemoglobin²¹ were possible to apply to the detection of H₂O₂. As well as AuNPs, it was found that the seed-mediated growth of AgNPs²² and PdNPs²³ was possible on ITO surfaces. As a methodological convenience of the seed-mediated growth method, it should be emphasized that the process is a simple two-step immersion into aqueous solutions at ambient temperature.

However, as for the nano-architecture on conducting substrates, our publications have been limited mainly to those on ITO surfaces. Thus, in the present paper, we would like to show what kind of nano-structured interfaces are formed when we apply the seed-mediated growth method to the above-mentioned representative combination of AuNPs and GC surfaces. As results, as well as the applicability of the proposed method to GC surfaces, some possible controls of the design of nanointerfaces on GC with AuNPs are shown in comparison with the results on ITO surfaces. Such control of the size and density of AuNPs on GC surfaces would be important and useful in electroanalysis and bioelectroanalysis, because it should give unique nanointerfaces for electron-transfer reactions.

Experimental

Apparatus and materials

Scanning electron microscopic (SEM) images were obtained with a field-emission SEM instrument (JSM-7400F, JEOL, Japan). Glassy carbon (GC) plates (10 mm × 10 mm × 1.0 mm) were purchased from BAS Co. Ltd. and used after washing in acetone, ethanol and pure water with sonication, and drying with nitrogen gas. Cetyltrimethylammonium bromide (CTAB) and HAuCl₄·3H₂O were purchased from Aldrich Co. Ltd. Other reagents were obtained from Wako Pure Chemicals Ltd. All solutions were prepared with ultra-pure water obtained from a water-purification system (Millipore WR600A, Yamato Co., Japan).

Attachment and growth of AuNPs on GC surfaces

The detailed procedures used to attach AuNPs onto ITO were described previously.¹⁷ In the present experiment, we simply substituted the immersed material from a piece of ITO to a GC plate. Regarding the actual procedure, briefly, a GC plate was at first immersed for 2 h into a seed solution, which was prepared by adding 0.5 ml of 0.01 M HAuCl₄, 0.5 ml of 0.01 M trisodium citrate, and finally 0.5 ml of 0.1 M NaBH₄ into 18 ml of pure water. Then, after washing and drying, the GC plate on which Au nano-seed particles were physically adsorbed in the first step was immersed for 2 h into a growth solution, which was prepared by adding 2.5 ml of 0.01 M HAuCl₄, 0.5 ml of 0.1 M ascorbic acid and 0.5 ml of 0.1 M NaOH into 90 ml of a 0.1 M CTAB aqueous solution. As mentioned in the following section, the immersion times into the seed solution and the growth solution were changed in order to observe any differences in the growth of AuNPs on GC.

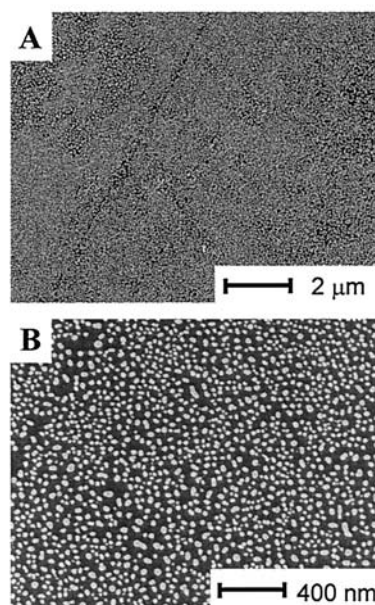


Fig. 1 Typical SEM images of AuNPs attached GC surface prepared with the seed-mediated growth method. Immersion time into the seed solution, 2 h; into the growth solution, 2 h. (A) Low magnification and (B) high magnification images.

Results and Discussion

Attachment of AuNPs on GC surfaces with the seed-mediated growth method

While we typically immersed a piece of ITO into the seed solution for 2 h and into the growth solution for 24 h in our previous work,¹⁷ the attachment of AuNPs on GC surfaces could be performed with relatively short immersion times into the growth solution. Figure 1 shows typical SEM images of AuNPs attached GC prepared by immersing a GC plate first into the seed solution for 2 h, and then into the growth solution for 2 h. As shown in Fig. 1A, the SEM result indicated that AuNPs attached very densely to the GC surface after a seed-mediated growth treatment. Although some minor local changes in the density may be visible in Fig. 1A, the dense coverage with AuNPs would be almost even and homogeneous on the surface. Thus, the applicability of the seed-mediated growth method for modifying GC surfaces with AuNP could be verified.

Figure 1B, a high magnification SEM image, shows that AuNPs were beautifully dispersed without significant gathering to each other. Some larger particles may be the result of the gathering of two or more small nanoparticles, judging from the shapes. However, as a whole, AuNPs would be referred as being well-dispersed on the GC surface shown in Fig. 1B. Concerning the size of AuNPs, larger gathered particles were less than *ca.* 100 nm, and the middle-sized dominant particles were *ca.* 50 nm. Some dot-like AuNPs smaller than 20 nm are also visible in the SEM image in Fig. 1B.

In the seed-mediated growth method, the first step is the physical adsorption of Au nano-seed particles in the seed solution, like similar adsorption that has been reported in the field of catalysis as a precursor method.²⁶ In the present experiment, when we immersed a GC plate directly into the growth solution, *i.e.*, without any seeding treatment, no attachment of Au was observed on the GC surface. Thus, it was verified that the Au nano-seed particles attached to GC surfaces

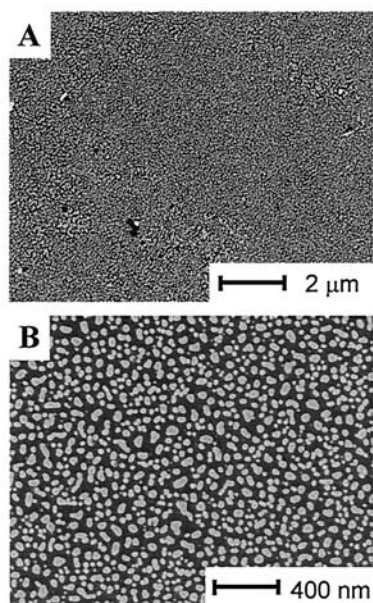


Fig. 2 Typical SEM images of AuNPs attached GC surface prepared with the seed-mediated growth method. Immersion time into the seed solution, 2 h; into the growth solution, 8 h. (A) Low magnification and (B) high magnification images.

by just immersing into the seed solution, and the adhesion was stable enough even after the GC plate was treated in the growth solution. The SEM images in Fig. 1 were observed after the surface was washed by flushing pure water. Thus, the attachment was also stable against such washing, which was similar to the case on ITO surfaces.¹⁷

Regarding the differences from the cases on ITO, the density of the grown AuNPs on the GC surfaces was much higher. This would be a reflection of the dense adsorption of Au nano-seed particles on GC, in spite of the smoother GC surfaces compared with the cases of ITO. Furthermore, the sizes of AuNPs were smaller and the appearance was flatter, while crystal-like growth was observed in the case of AuNPs on ITO.¹⁷ These results may be in relation to each other. That is, because of the dense attachment of Au nano-seed particles, the growth of AuNPs is inferred to have been significantly suppressed compared with the cases on ITO.

Even on ITO, it is known that relatively smaller AuNPs can be attached using different seeding methods.^{27,28} However, from the preset results, it is inferred that the attachment density was essentially different between that on the surfaces of ITO and GC. The change in the immersion time into the growth solution, 2 h for GC and 24 h ITO,¹⁷ would also be a reflection of the differences in the attachment and growth processes.

Effect of the immersion time into the growth solution

On GC surfaces, it was found that a dense attachment of AuNPs was possible with the seed-mediated growth method after the growth treatment for 2 h. To show what happens with a longer growth treatment, Fig. 2 shows typical SEM images of AuNPs attached GC prepared by immersing a GC plate first into the seed solution for 2 h, and then into the growth solution for 8 h.

As a result, the low-magnification SEM image in Fig. 2A shows no significant change from the result of Fig. 1A, except for the formation of some gathered larger particles. Because the dense attachment is not very different between Figs. 1A and 2A, it may be concluded that some extension of the growth treatment

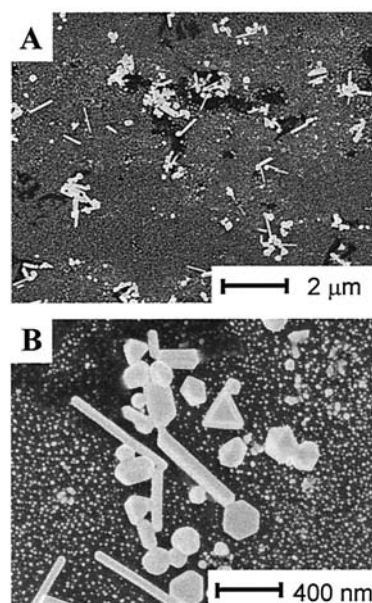


Fig. 3 SEM images of AuNPs attached GC surface prepared with the seed-mediated growth method. Immersion time into the seed solution, 2 h; into the growth solution, 24 h. (A) Low magnification and (B) high magnification images.

gave fewer effects as a whole. However, when we compared a high-magnification image, *i.e.*, Figs. 1B and 2B, the percentage of the gathered or larger AuNPs was apparently increased after growth for 8 h (Fig. 2B). This indicates that the transformation of the AuNPs actually occurred in the growth solution. The process is not fast, but smaller AuNPs are expected to have gradually transferred to larger or gathered AuNPs on the GC surfaces. In spite of the similar dense attachment of AuNPs in Figs. 1A and 2A at a glance, growth control would be important to regulate the size of each AuNP.

Although growth for 2 h was better for preparing densely dispersed AuNPs of relatively regular size on GC surfaces, we tried to observe what happened after leaving the GC substrate for 24 h. Figure 3 shows SEM images of AuNPs attached GC prepared by immersing a GC plate first into the seed solution for 2 h, and then into the growth solution for 24 h. Different from the previous results, the formation of bold micro-crystals, *e.g.*, rods, spheres, triangles, pentagons and hexagons *etc.*, was confirmed in the high magnification SEM image in Fig. 3B. As can be recognized in Fig. 3A, the formation of micro-crystals occurred at local areas, not on the whole surface.

As an interesting feature, in the surrounding areas of the micro-crystals, some removals or disappearances of AuNPs were observed as the dark parts in Fig. 3A. This phenomenon could be explained by assuming that some AuNPs were sacrificed to form bold Au micro-crystals in the growth solution. In the synthesis of AuNPs on some substrates, such ripening processes, *i.e.* Ostwald ripening, were often observed.²⁹ In the present case, some Au ions in the growth solution would be reduced to Au(0) to grow larger AuNPs, but some smaller AuNPs would be oxidized to Au ions to dissolve into the solution.

From the view of crystal growth, the preset results may be interesting because they occurred spontaneously in the solution, *i.e.*, just after leaving and without giving any perturbations. However, the surface in Fig. 3 is no more referred to as being homogeneous. So, under the aims of the present paper, the longer growth time of 24 h should be avoided in the case to

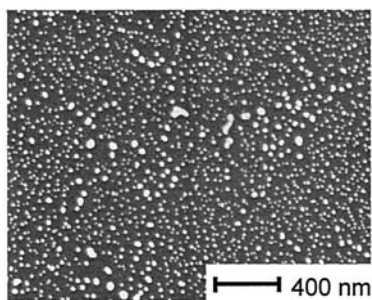


Fig. 4 Typical SEM image of AuNPs attached GC surface prepared with the seed-mediated growth method. Immersion time into the seed solution, 2 h; into the growth solution, 5 min.

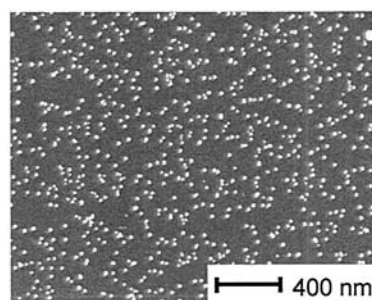


Fig. 5 Typical SEM image of AuNPs attached GC surface prepared with the seed-mediated growth method. Immersion time into the seed solution, 30 min; into the growth solution, 5 min.

modify AuNPs on GC surfaces, while it was a typical condition for ITO.¹⁷

Control of the attached density and size of AuNPs on GC surfaces

Because the dense attachment of AuNPs on GC surfaces was performed with growth for 2 h, we next observed what surface could be prepared with a shorter growth time. Figure 4 shows typical SEM image of AuNPs attached GC prepared by immersing a GC plate first into the seed solution for 2 h and then into the growth solution for 5 min. While some grown AuNPs were observed in this image, smaller AuNPs whose sizes were *ca.* 20 nm became dominant compared with the result shown in Fig. 1B. This result indicates that a certain tuning of the size of AuNPs was possible by reducing the time for the growth treatment.

As the effect of the seeding time, Fig. 5 shows typical SEM image of AuNPs attached GC prepared by immersing a GC plate first into the seed solution for 30 min and then into the growth solution for 5 min. Compared with the result shown in Fig. 4, the density of the attached AuNPs became lower, and the size dispersion became narrower. The size of the dominant nano-dot Au particles was *ca.* 20 nm. From this result, it is considered that a fine tuning of the size and density of AuNPs on GC is possible by controlling the seeding time as well as the growth time.

Conclusion

In the present work, we succeeded to attach AuNPs to GC surfaces using the seed-mediated growth method. The dense attachment of AuNPs, whose typical size was generally *ca.* 50 nm, but ranged from 20 to 100 nm, was possible by simply immersing a GC plate first in the seed solution for 2 h, and then in the growth solution for 2 h. Compared with our previous results in the growth of AuNPs on ITO surfaces, it was characteristic that the AuNPs attached on GC surfaces exhibited smaller size and higher density, as well as a flatter and non-crystal-like morphology.

In the seed-mediated growth method, the first step is the physical adsorption of Au nano-seed particles. Thus, in order to elucidate the reasons for the differences between ITO and GC, further studies, *e.g.*, on the effects of the roughness of the same materials, would be necessary.

Longer growth treatments were found to cause a gathering of AuNPs, and eventually Au micro-crystal growth, while growth for 24 h was necessary in the case of AuNPs on ITO surfaces. Thus, in the case of GC surfaces, the growth time should be

controlled to be less than 2 h to prepare a homogeneous nanointerface composed of AuNPs and GC. In particular, shorter seeding and growth times would be effective to control or fine tune AuNPs having smaller size and lower dispersion density, as shown in Fig. 5.

The attachment for AuNPs to GC surfaces was strong enough for practical uses. Actually, SEM images were observed after washing by flushing pure water. In addition, our trials showed that the AuNPs-attached GC interfaces can be used for electrochemical measurements with no problems, as some results have been already reported.³⁰

As a concluding remark, the present SEM images were obtained on unpolished GC surfaces to show changes in the surface images of AuNPs formed by the seed-mediated growth treatment. In contrast, in an electrochemical analysis, to polish the base electrode, *i.e.*, GC surfaces, are quite normal and important to obtain reproducible responses. However, a high resolution FE-SEM observation showed that polished GC surfaces had some roughness on the nano-scale, while unpolished GC surfaces were quite smooth, as inferred from the background image of Fig. 5. Thus, note that the present results were those obtained for flat surfaces.

In the present work, it was clarified that the seed-mediated growth method for surface modification was valid for fabricating a nanointerface composed of AuNPs on GC surfaces. However, depending on the applications, some more studies, including a pretreatment of GC, might be necessary for practical uses.

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