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Preparation and biosensing performance of porous-alumina-assisted gold nanostructures on substrates

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Abstract

Gold based nanosize structures such as nanoparticles and nanowires are considered as important building blocks for nanotechnology to be used for construction of sensing micro- and nanodevices because of their excellent optical, electrical and mechanical properties. Here we synthesize via porous-alumina-assisted anodizing and electrodeposition perfect arrays of gold nanodots and nanorods, upright standing, spatially separated and highly aligned on a conductive substrate, which are expected to have exceptional performance in nanoscale electronic, optoelectronics and sensing applications. Biosensing potential of the nanostructured electrodes was assessed by examining electrochemical properties of a protein layer grafted on the electrode for C-reactive protein (CRP) detection using CRP-antibody immobilization method with protein G intermediate layer. The laboratory prototype of immunosensor revealed reproducible, rapid and stable response with a low limit for CRP-antigen detection of 100 $fg \cdot ml^{-1}$

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Keywords: anodizing; porous alumina; electrodeposition; tungsten oxide; gold nanorods; biosensing

1. Introduction

In recent years, much attention has been directed towards the synthesis of anisotropic gold nanostructures such as nanorods, nanoprisms and nanopods due to their tunable optical and electronic properties [1]. Porous-anodic-alumina (PAA)-templated synthesis has received a great deal of attention and experimental approbation, though not yet providing 100% pore-filling efficiency [2]. Moreover, after the PAA is removed, which is needed for most of

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potential applications, the embedded nanorods and especially nanowires often collapse into an entangled mess owing to the interactive forces and the weakness of the Au/substrate interface. The challenges with this technique are to substantially improve the order of pores in a *thin-film* PAA template, to make the pores ideally perforated across the whole sample area, and to grow uniform gold nanostructures spatially separated and not collapsed on a conducting substrate.

Our work to date provides for self-organized arrays of spatially-ordered, either quantum-size dots or vertically aligned intact Au nanocolumns, sized in the range of 80 – 2000 nm, anchored to a conducting layer on a dielectric or semiconductor substrate via a blend of advanced electrochemical approaches applied to sputtered-deposited valve-metal multilayers. Towards potential application of nanoelectrodes developed here, biosensing performance of a PAA-assisted Au nanocolumn array was assessed by examining electrochemical properties of a protein layer grafted on the nanostructured electrode for C-reactive protein (CRP) detection using CRP-antibody immobilization method with an intermediate layer of protein G.

2. Experimental

The initial sample is an aluminium layer over a different valve metal (M), like W, Ti or binary metal alloys and multilayers sputter-deposited onto an oxide-coated Si wafer. First the aluminium layer is converted into nanoporous alumina down to the M-alloy layer by anodizing in an aqueous acid solution, as shown schematically in Fig. 1a. Then, an array of metal-oxide nanosized protrusions forms on the M-alloy due to the local oxidation under the pores. At these sites, the underlayer oxidizes with a large volume change, and metal-oxide nano-hillocks are forced into the alumina barrier layer [3]. The dimensions of the hillocks depend upon the pore size and distribution. Then the sample is re-anodized to a higher potential, so as to achieve the pore-directed growth of longer aspect ratio metal-oxide nanorods via cross-migration of M^{n+}, Al^{3+}, and O^{2-} ions, with the expanding of the pre-existing migration pathways in the alumina barrier layer (Fig. 1b), this being accompanied by field-assisted dissociation of the Al-O bonds in the barrier layer at the alumina/metal interface [3,4]. The presence of mixed or layered metals under the pores is advantageous in that it results in creating specific pathways for cooperative ionic transport, not obvious in the electrochemical properties of each individual metal. Thus, at the end of this stage the film comprises an array of alumina-incorporated metal-oxide nanorods anchored to the metal layer that remains after the anodizing [4].

For the purpose of the present study, the metal-oxide nanorods are to be removed from the pores. This may be achieved either via the field-assisted oxide dissolution inside the pores or by chemical etching in a solution that does not affect the alumina during the treatment (Fig. 1c), for example, as described in the previous work [4].

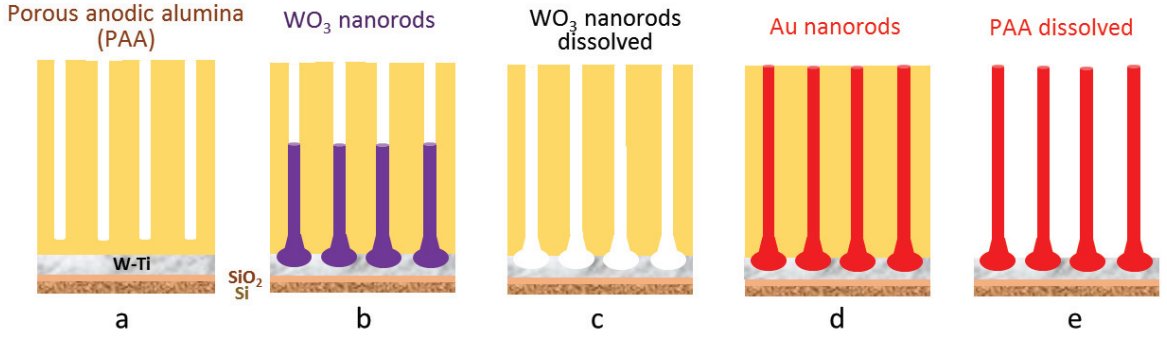

Fig. 1. Schematic diagram showing formation of a PAA-assisted array of Au nanorods: (a) preparation of porous alumina by anodizing aluminium layer in an Al/W-Ti multilayer sputter-deposited onto an oxide-coated Si substrate, (b) anodizing and re-anodizing of the W-Ti underlayer through the alumina nanopores to grow metal-oxide nanorods in the alumina pores, (c) 'cleaning' the pores by selectively removing the metal-oxide nanorods, (d) preparation of Au nanorods *via* the point electrodeposition in the alumina pores, (e) preparation of free-standing Au nanorods by selective dissolution of the alumina overlayer

Either gold nanodots of nanorods are formed in the 'empty' alumina pores via electrodeposition in a buffered $KAu(CN)_2$ bath (pH 6.3) at 50 °C (Fig. 1e). In-situ amplitude-modulated pulse process [3] is used to increase the

homogeneity of deposition and achieve a smooth nucleation and growth of the deposit along the pores, in some cases up to the surface of the PAA film. The length and diameter of gold nanorods may vary, if necessary, in the range of 450-2000 nm and 30-100 nm, respectively.

3. Results

Fig. 2a-c shows SEM views of cross sections of an Al/W-Ti/SiO_2/Si sample processed to stages (c), (d) and (e) in accord with the schematic diagram in Fig. 1. SEMs of Fig. 2d-f show the top views of self-organized array of nanosized pores in the PAA film, the dimples in the underlying metal (W-Ti) and the gold nanorods protruding from the W-Ti layer. A 100% pore filing by gold resulting in an ideal Au nanorod array is appreciated from the present results. The achievement is due to the improved technique for perforating the alumina barrier layer developed here.

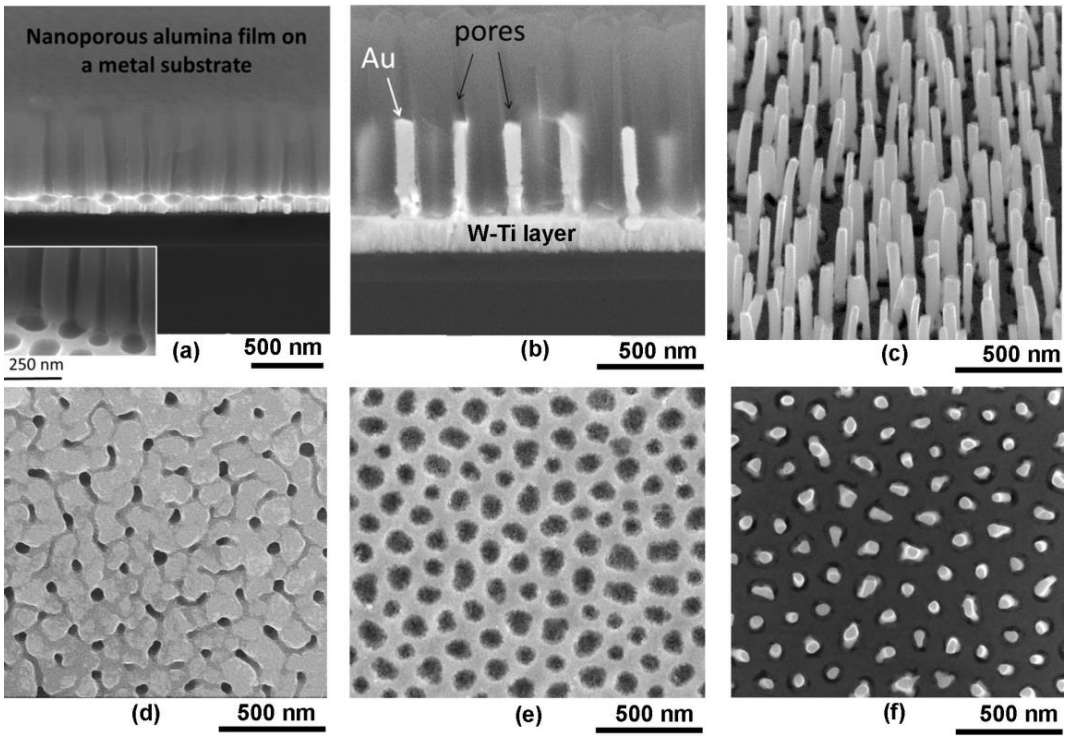

Fig. 2. SEM images showing various stages of PAA-assisted formation of Au nanorods: (a) cross section of a PAA film with perforated barrier layer prepared by anodizing/reanodizing/dissolution of an Al/W-Ti multilayer onto a SiO_2/Si substrate, (b) cross section of Au nanorods prepared via the point electrodeposition in the alumina pores, (c) free-standing Au nanorods derived after selective dissolution of the alumina overlayer (tilted view), (d) the surface of the PAA film shown in (a), (e) the surface of the Ti-W film after dissolution of the PAA overlayer, (f) vertical view of the Au nanorod array.

In the course of this work, we also present a preliminary study showing the potential use of the nanostructured gold electrode for biosensing application. The approach involves estimation of experimentally measured electrochemical properties of protein layer grafted on the gold nanoelectrode for C-reactive protein (CRP) detection. The method is simple and rapid as it requires only one intermediate physisorption step of G protein to orient antibodies for an optimal recognition of CPR [5]. Fig. 3 shows the Nyquist plots demonstrating the stability of the electrode in a phosphate buffered saline with a redox couple, the successful immobilization of protein G, immobilization of anti-CPR, followed by Bovine Serum Albumine as a blocking layer, and a good performance of the electrode to detect low concentrations of CPR-antigen.

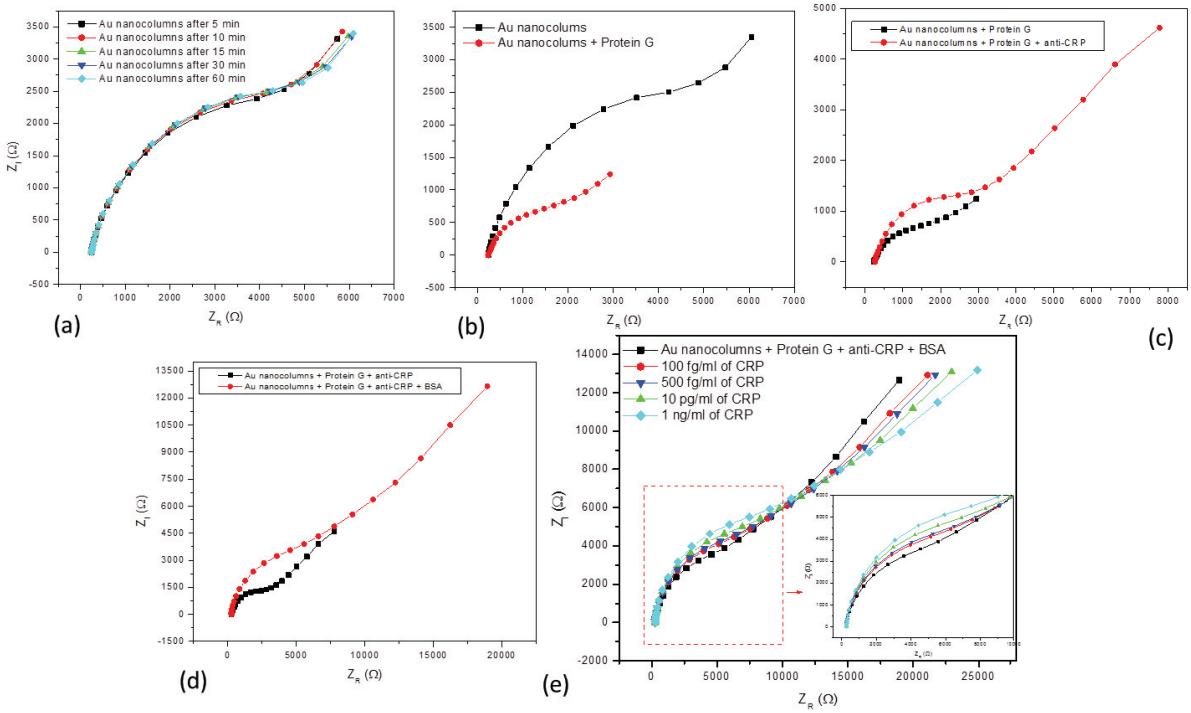

Fig. 3. Nyquist plots showing (a) the stability of the gold nanoarray electrode in a phosphate buffered saline (PBS) containing 140 mM NaCl, 2.7 mM KCl, 0.1 mM Na_2HPO_4, 18 mM KH_2PO_4, pH=7 and the redox couple $K_4Fe(CN)_6^{3-}/K_4Fe(CN)_6^{4-}$ at 5 mM concentration, this being sequentially followed by successful immobilization of (b) Protein G, (c) anti-CRP, (d) Bovine Serum Albumine (BSA) of 0.45 mg L^{-1}) and finally (e) the detection of different concentrations of CRP antigen by the electrode modified up to stage (d).

4. Conclusions

The present findings show that the PAA-assisted arrays of Au nanorods have a potential for use in biosensing applications. Various nanostructured surfaces are now in preparation to fully explore the technological and biosensing advantages of the new electrodes. The effect of electrolyte species, pore size and support film composition on the morphology, electrical and electrochemical properties of the gold nanostructures is being studied, yielding promising results for more applications to catalysts, biosensors and electrical-driven devices.

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