Thin Solid Films 518 (2009) 634–637

Contents lists available at ScienceDirect

Thin Solid Films

journal homepage: www.elsevier.com/locate/tsf

Nanoscaled redox active protein adsorption on Au-dot arrays: An electrochemical scanning probe microscopic investigation for application in nano-biodevices

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ARTICLE INFO

Available online 8 August 2009

Keywords:
Azurin
Au-dots
Electrochemical scanning tunneling microscopy
Conduction
Nanobiochip

ABSTRACT

Highly dense and uniform protein dot arrays on Au-nanodots using size controllable method were fabricated on indium tin oxide (ITO) substrate in order to develop an electrochemical nanobiochip. Cysteine modified azurin was directly immobilized on the fabricated Au-nanodots without any linker materials. Atomic force microscopy was used for characterizing Au-dots formed on ITO substrate. Electrochemical scanning tunneling microscopy (ECSTM) revealed the monolayer formation with an *in situ* cyclic voltammetry to observe redox behaviour of both bare Au-dots and protein immobilized Au-dots. *I–V* characteristics were obtained on both bare Au-dots and protein immobilized Au-dots structured on ITO conductive electrodes.

1. Introduction

In recent years, much effort have been devoted for the development and investigation of organic molecules and biomolecules as electron-conductive materials for applications to nanoelectronics and sensors [1,2]. Biomolecules such as proteins have great promise in this connection, as constitute nano-sized building blocks carry out high specific reactions, and self assemble, which opens the prospect of nanoscale surface patterning as an alternative to lithographic process [3]. One-way to fabricate highly-ordered monolayers of Bio/organic materials for application in nanoelectronics a nano level pattern is needed. However, it is so difficult to generate nano patterns with well-established ordering using the character of the molecules itself. So another alternative is to develop nanostructure materials in order to form well-ordered monolayer structures of biomolecules [4,5].

Recently, fabrication of nanodot structure of various materials has attracted intense interest in nano-technological applications. These dot structures were demonstrated by several groups as an efficient method for controlled positioning of biomolecules on these nano-structures. To develop these kind of nanostructures many techniques have been widely used such as e-beam lithography [6], self-assembled epitaxial growth based on Stranski–Kranstanov (S–K) mode [7], and template systhesis using anodic porous alumina membrane as a template [8,9]. Among these techniques, E-beam lithography is the most common technique in developing these structures but this technique has some drawbacks such as low throughput due to its long exposure time and high cost equipment.

In order to make commerical application of nanodots, a method that could easily produce nanostructred films with defect free and highly ordered nanodot arrays over a large area has to be developed. So among these techniques template-synthesis method using anodic porous alumina membrane as a template for the fabrication of nanodot structured materials is widely used [8,12]. Because this method is advantageous it is easy to control the dot-size, uniformity of the dot shape and highly ordered nanodot arrays over a large area.

Hence, in this article, we fabricated gold (Au) nanodot arrays on ITO substrate using template-synthesis method. Cysteine modified azurin was directly immobilized on the Au-dot arrays and scanning probe microscopy experiments were carried out to assess the morphological, electrochemical and electrical properties of the azurin molecules. The results underline the crucial role of biomolecules in the development of nanoscale electronic devices.

2. Experimental details

2.1. Fabrication of Au-dots on ITO substrate

Aluminum oxide layer was prepared from an alumium foil (99.99%,100 μm thickness) by a two-step anodization method which has been reported elsewhere [10–12]. An aluminum foil was achieved by the pretreatment of electro-polishing in a mixed solution of perchloric acid and ethanol (1:4 in volume) for 60 s. First anodization of Al electrode was performed by applying a constant DC voltage of 40 V in 0.3 M oxalic acid solution of 3 °C for 8 h. After the first anodization, the generated anodic aluminum oxide (AAO) was removed by immersing the sample in a 60 °C solution composed of a mixture of phosphoric acid

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doi:10.1016/j.tsf.2009.07.053

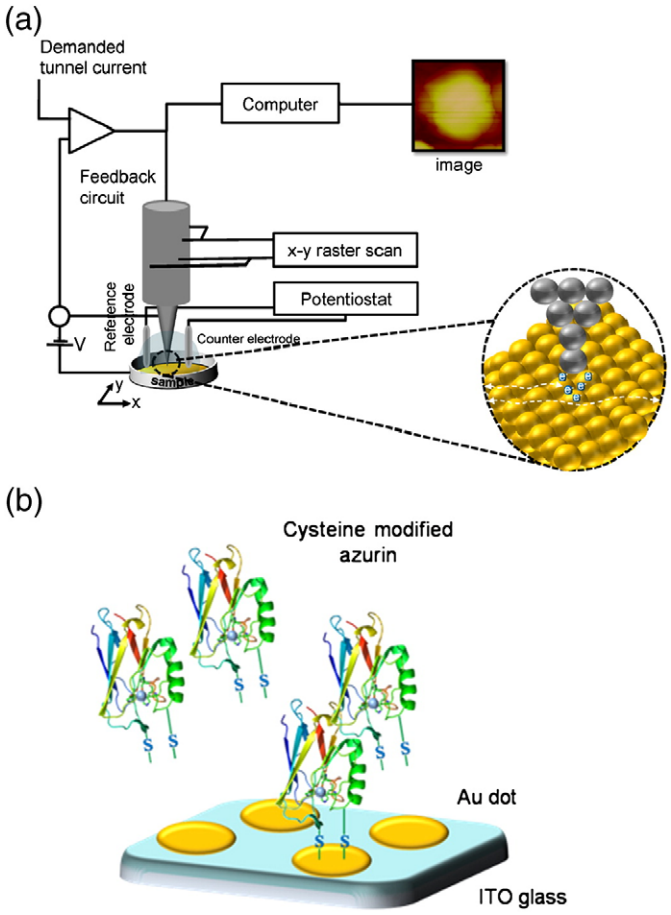

Fig. 1. Schematic representation of (a) ECSTM experimental set-up and (b) immobilization of cysteine modified azurin onto Au-nanodot.

(1.8 wt.%) and chromic acid (2 wt.%). Then the second anodization was carried out under the identical conditions for 5 min. The thickness of AAO formed by anodization for 5 min is below 500 nm. After the second anodization, the remaining aluminum substrate was removed in a saturated $HgCl_2$ solution. After the lift-off process, the barrier layer at the bottom of the AAO was uniformly etched out in an aqueous 5 wt.% phosphoric acid at 30 °C sequentially. In this process, to remove thoroughly the barrier layer of AAO it is very important to fabricate the AAO mask with through-hole. Then the AAO mask with through-hole was placed on an ITO substrate for the fabrication of Au-nanodots. Then pure gold was deposited by thermal evaporation at a vacuum pressure of 1×10^{-6}Torr. After Au deposition, the AAO mask was dissolved in 1 M NaOH solution for several minutes and then rinsed with distilled water. The Au-nanodots formed on ITO substrate were revealed after the removal of the AAO mask.

2.2. Azurin sample preparation

Azurin was recombined with cysteine residues by site-directed mutagenesis [13]. A cysteine modified azurin was immobilized on the patterned Au-dots by strong affinity between thiol of cysteine and Au-dot. The protein sample was prepared by adding an amount of 20 µl of recombinant azurin of optimal concentration 0.1 mg/ml [14–17] in180 µl of HEPES buffer solution of concentration of 10 mM of pH 7.4. An amount of 6 µl of prepared azurin solution was deposited on the gold dots for 3 h and then treated with 0.05% polyoxyethylene sorbitan monolaurate solution for 2 h. Finally the dots were cleaned with DI water and dried under nitrogen gas.

2.3. Morphological studies on Au-dots

The morphology of the gold dot arrays fabricated on an ITO substrate was investigated by atomic force microscopy (AFM)(Nanoscope IV/Multimode, Digital Instruments) equipped with a 50 µm scanner operated in tapping mode. The probes with 1–100 Ω cm phosphorous (n) doped (Si) tip with a spring constant of 20–80 N/m, having resonant frequencies between 262 and 307 kHz were used.

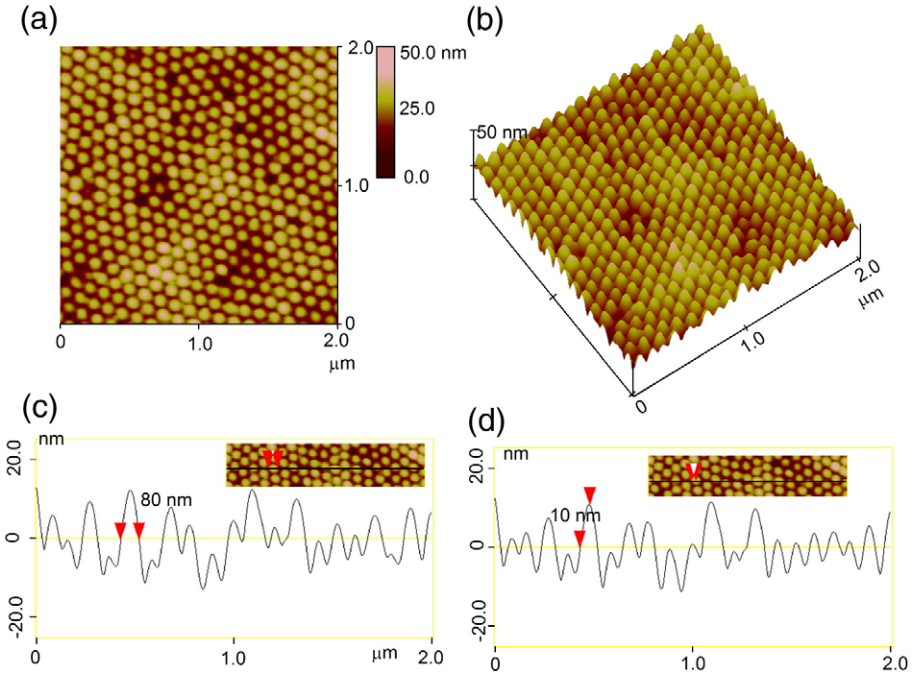

Fig. 2. (a) TM-AFM images (2×2 µm) of fabricated Au-dots (b) the 3D profile of the dots on ITO substrate. (c, d) Are the height and width profile of the patterned dots, the image has been recorded with a scan rate of 1 Hz.