Sensors and Actuators B 228 (2016) 401–409

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Ultrasensitive label-free detection of cardiac biomarker myoglobin based on surface-enhanced Raman spectroscopy

Waleed A. El-Said [a,*], Dina M. Fouad [a], Sherif A. El-Safty [b,c]

[a] Department of Chemistry, Faculty of Science, Assiut University, Assiut 71516, Egypt
[b] National Institute for Materials Science (NIMS), 1-2-1 Sengen, Tsukuba-shi, Ibaraki-ken 305-0047, Japan
[c] Graduate School for Advanced Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan

ARTICLE INFO

Article history:
Received 12 September 2015
Received in revised form 1 December 2015
Accepted 11 January 2016
Available online 14 January 2016

Keywords:
Surface-enhanced Raman Spectroscopy
Anisotropic structures
3D Silver nano-pinetree
Cardiac biomarker myoglobin
Rhodamine 6G

ABSTRACT

Acute myocardial infarction (AMI) is the leading cause of mortality worldwide. Myoglobin protein was used as a biomarker for AMI because of its higher sensitivity compared with other biomarkers. Its high sensitivity is attributed to its rapid release in the bloodstream. As such, many researchers have focused on developing label-free biosensors to detect myoglobin levels. This study developed a highly sensitive and label-free myoglobin sensor based on surface-enhanced Raman spectroscopy (SERS). The sensor consisted of new 3D silver anisotropic nano-pinetree array modified indium tin oxide (Ag NPT/ITO) substrates. Moreover, another three Ag nanostructure modified ITO substrates (nanoaggregates, nanorods and nanobranched) were developed to select the highest surface enhanced Raman spectroscopy. Results revealed that Ag NPT/ITO displayed the highest SERS performance compared with other substrates. This finding is attributed to the presence of numerous hotspots, particularly in the junctions between the central rod and side arms. The highly enhanced Raman effect of Ag NPT/ITO substrate was applied to develop an ultrasensitive biosensor for detecting myoglobin as a cardiac biomarker at low concentration levels in solutions (pH 7.4, phosphate buffer) and urine. This biosensor is potentially useful for real sample analysis. The 3D morphology of Ag NPT enhanced the sensitivity performance of the sensor and allowed myoglobin detection over a wide linear range with a detection limit of 10 ng/mL.

1. Introduction

Acute myocardial infarction (AMI) is the main cause of mortality worldwide [1–3]. Different cardiac biomarkers, such as myoglobin, troponins, and creatine kinases (CK-MB), are released into the bloodstream after AMI [4,5]. The World Health Organization (WHO) reported different biomarkers that can diagnose and monitor AMI levels [6]. Therefore, rapid diagnostic methods have been developed to predict susceptibility and assist in patient management [2–4,7]. Myoglobin molecules are released into the blood within 1 h after chest pain because of their small size and reach a maximum level within 2 h, whereas troponins and CK-MB are released after 3 and 6 h, respectively [8]. Myoglobin level increases from 90 pg/mL to >250 ng/mL in 90 min with normal levels of CK-MB and troponins [5]. Thus, myoglobin possesses higher specificity and sensitivity, and is a more accurate AMI biomarker than CK-MB or troponins [9–11]. The current diagnostic techniques of myoglobin, such as radioimmunoassay, chemiluminescent immunoassay, and enzyme immunoassay, have high sensitivity and selectivity; however, they are complicated multistage processes, time consuming, and costly [12]. Therefore, developing a rapid, label-free, and accurate detection method of AMI biomarkers is urgently needed to reduce detection time, decrease cost of patient treatment, and save patient lives.

Fluorescence immunoassay is a powerful method that has been used for detection of AMI biomarkers [13] with high sensitivity, but its labeling process is complicated and time consuming. Therefore, numerous label-free detection methods, such as electrochemical nanowire-based biosensors [14], micro-cantilever biosensors [15,16], and 2D photonic-crystal biosensors [17,18], have been developed. However, these methods showed a limitations in the correlation between the simple handling steps and associating functionality due to the demanded control of (i) the interference between pH values, (ii) solution viscosity, (iii) molecular charges, and (iv) the out-of-plane vibration of the cantilever, respectively [19]. Currently, surface plasmon resonance-based sensor was suc-

* Correspondence author. Fax: +20 88 2342708.
E-mail addresses: waleed@sogang.ac.kr, waleed@au.edu.eg (W.A. El-Said), dinafouad93@hotmail.com (D.M. Fouad), sherif.elsafty@nims.go.jp, sherif@aoni.waseda.jp (S.A. El-Safty).

http://dx.doi.org/10.1016/j.snb.2016.01.041
0925-4005/

cessfully commercialized [20], but it is expensive and limited for small molecule binding assays. Developing a highly sensitive, label-free myoglobin biosensor remains a significant challenge.

Raman spectroscopy provides chemical fingerprints of molecules. However, the low scattering cross-section and fluorescence interference of this method limited its analytical applications. Surface-enhanced Raman scattering (SERS) is a powerful tool to amplify Raman signals by using roughened noble metal (gold (Au), silver (Ag), or copper) nanostructures [21–27]. Thus, this tool is used for highly sensitive and selective quantitative detection of a variety of molecules, including DNA, environmental pollutions, and explosives [28–30].

Noble metal nanoparticles (NPs) are useful in different fields, including environment, medicine, and chemistry; NPs are also applied to create active surfaces to enhance the Raman signals [31–33] based on their unique attractive localized surface plasmon resonance (LSPR) properties [34–36].

Spherical NPs were previously used for Raman enhancement; however, its enhancement factor was relatively low. Therefore, developing new metal nanostructures with large LSPR (i.e., large SERS) has been receiving increasing interest. Many studies have focused on the SERS properties of various Au nanostructures [37–46]. Ag nanostructures possess a broad LSPR region of activity [from blue to near-infrared (NIR)]. Given this characteristic, various Ag nanostructures, such as nanowires, nanorod arrays, Ag-coated NP-based materials, and nanowell and nanopore arrays, have been reported as SERS-active substrates [47–49]. Anisotropic nanostars and nanobranched structures possess higher SERS enhancement factors than other shapes [50–52]. Synthesizing these nanostructures requires the use of surfactants and other reagents, which could remain adsorbed on the nanostructure surface and hence limit their SERS applications. Lithographic technique provides highly organized nanostructures without the use of any surfactants, resulting in high sensitivity and reproducibility [53,54], but this technique is expensive and requires elaborate preparation methods. Hence, a great need exists to develop a low-cost and reliable method for fabrication of patterned or modified metal nanostructures to enhance Raman sensitivity.

In this study, we reported a simple, inexpensive, one-step, and template-free method for the synthesis of a new anisotropic surface composed of Ag nano-pinetree film modified indium tin oxide (Ag NPT/ITO) substrate and the application of the substrate as SERS-active surface. Ag NPT/ITO substrates were prepared based on electrochemical deposition of Ag from aqueous solution of silver nitrate ($AgNO_3$) without adding any surfactants, capping, or aggregating agents. Another three Ag nanostructures/ITO substrates were developed in the presence of different surfactants. Scanning electron microscope (SEM) images revealed a 3D pinetree-shaped morphology with a central rode provided with several vertical protuberances, in addition to nanorods, nanoaggregates, and nanobranched films. UV–vis spectroscopy was also used to investigate the optical properties of Ag NTP/ITO substrate. The Ag NPT/ITO substrate showed two surface plasmon absorption bands in the visible and NIR regions. The activities of the different Ag nanostructures/ITO substrates in SERS applications were evaluated using Rhodamine 6G (R6G) dye. Ag NPT film exhibited good optical properties and induced a strong SERS effect for R6G.

Optimization studies of preparation conditions were performed and discussed in detail. The performance of this new substrate compares favorably with the three Ag nanostructures/ITO substrates (Ag nanoaggregates/ITO, Ag nanorods/ITO, and Ag nanobranched/ITO). The relationships between the morphology of Ag nanostructures and SERS intensity were also discussed. The absence of surfactant agents provided the advantage of producing pure metal nanostructures without using any protective agents that could be adsorbed on the nanostructure surface, which could interfere with the target elements. We also reported the application of Ag NPT/ITO as label-free biosensor for selective detection of myoglobin over a wide concentration range. The Ag NPT/ITO substrate showed a wide working range (from 5×10^{-6} to 10×10^{-9} g/mL), as well as high selectivity and sensitivity with low detection limit of 10×10^{-9} g/mL for myoglobin.

2. Experimental

2.1. Materials

$AgNO_3$, phosphate buffered saline (PBS, pH 7.4, 10 mM) solution, sodium dodecyl sulfate (SDS), and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Polyethylene glycol (PEG; MW = 200) was obtained from Yakuri Pure Chemicals Co. Ltd. (Osaka, Japan). All other chemicals used were of commercial reagent grade.

2.2. Instruments

All electrochemical experiments were studied using a micro-Autolab, a potentiostat/galvanostat instrument connected to a Metrohm 663 VA stand. This three-electrode system was controlled using Autolab Nova software at room temperature. The system consisted of ITO substrate as working electrode, platinum wire as counter electrode, and Ag/AgCl as reference electrode. UV–vis spectra were recorded on a PerkinElmer spectrophotometer. The surface morphologies were analyzed by SEM (ISI DS-130C; Akashi Co., Tokyo, Japan).

2.3. Development of Ag nanostructure modified ITO substrates

ITO-coated glass substrates were cleaned according to our previously reported method [37–39]. ITO substrates (2 cm × 1 cm) were sonicated using 1% Triton X-100 solution for 15 min, deionized water (DIW) and ethanol sequentially, and then by basic piranha solution (1:1:5, H_2O_2:NH_3:H_2O) for 30 min at 80 °C. Afterward, the substrates were cleaned with DIW and dried under a N_2 stream to obtain a clean ITO surface. Ag nanostructure film modified ITO substrates were electrochemically deposited onto ITO substrates. The active area for electrochemical deposition of Ag nanostructures was 1 cm × 1 cm, and a potential of −0.9 V (vs. Ag/AgCl) was applied during the deposition at 25 °C. Ag NPT film was obtained with 1 mM $AgNO_3$ aqueous solution only, whereas nano-branched film was obtained by adding 0.1 mM PEG to the $AgNO_3$ solution. Moreover, Ag nanorod film was formed with 0.1 mM CTAB instead of PEG. The use of 0.1 mM SDS resulted in the synthesis of Ag nanoaggregate structures. All the modified substrates were cleaned by boiling in isopropanol alcohol for 2 min and then rinsing with DIW to remove any PEG residues that could adsorb on the surface.

2.4. Raman measurements

SERS spectra were collected on Raman spectroscopy with a SENTERRA inverted confocal Raman microscope (Bruker Optics Inc., Germany). A CCD camera detection system and OPUS software were employed for data acquisition. Raman spectra were recorded using NIR and green laser emitting lights at wavelengths of 785 and 485 nm, respectively, and a power of 50 mW at the sample. Ten scans of 5 s from 400 cm^{-1} to 1800 cm^{-1} were recorded, and the mean of these scans was used.

All Ag nanostructures/ITO substrates were incubated in a 1 μM R6G aqueous solution for 30 min for SERS measurements. Afterward, the substrates were rinsed with deionized water and then dried at room temperature in the dark for 1 h for the subsequent test. To detect myoglobin by using the developed Ag NPT/ITO