Sensors & Actuators: B. Chemical 270 (2018) 500–507

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Direct laser scribing of AgNPs@RGO biochip as a reusable SERS sensor for DNA detection

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ARTICLE INFO

Keywords:
Biochips
SERS
Laser scribing
Graphene
Silver nanoparticles

ABSTRACT

The combination of surface-enhanced Raman spectroscopy (SERS) technology with microfluidics makes it possible to diagnose genetic disease through label-free on-chip DNA detection. However, open problems including the integration of SERS substrate with microfluidic devices, controllable trapping and releasing of target molecules are still challenging. Here we demonstrate a facile laser scribing method to fabricate silver nanoparticles (AgNPs) and graphene oxide (GO) based biochips as a reusable SERS sensor for DNA detection. Programmable laser scribing of the AgNPs@GO composite film enables direct patterning of sensitive SERS channels that consist of graphene supported AgNPs by exfoliating the composites into hierarchical porous structures. Integrating the SERS-active patterns with a microfluidic chip forms a biochip for allowing SERS detection of DNA. The noncovalent interactions between DNA and graphene mediated controllable trapping and releasing of DNA sequences, enabling efficient on-chip SERS detection and the regeneration of the biochip. The simple, green and cost-effective fabrication of the SERS-active biochips reveals great potential for biomolecular sensing and genetic engineering applications.

1. Introduction

DNA analysis [1–4] that enables diagnosis of genetic disease is of great importance to molecular biology [5]. Currently, to get effective genetic structure information, various optical- [2,6,7], chemical- [8,9], electrical- [1,10], and colorimetric [11] strategies have been employed in gene detection. Generally, polymerase chain reaction (PCR) [12] and fluorescent detection [7,13] are common procedures in DNA analysis, in which the collection of specific DNA sequences enables quantitatively detecting the hybridization of the fluorophore-containing DNA, and thus the sequencing information of target DNA can be obtained. However, the intricate PCR procedure that consists of pretreatment, amplification and detection is not compatible with high-throughput on-chip DNA detection, because PCR generally requires precise and repeated temperature alteration; and thus a certain length of time is needed to complete the repeated replication of a segment of DNA. In this regard, novel DNA detection strategy that permits high-throughput on-chip analysis is highly desired.

Surface-enhanced Raman spectroscopy (SERS) features label-free fingerprint Raman spectra of analytes with ultra-high sensitivity by taking advantages of the vibrational modes of molecules [14,15]. The enhancement mechanism of SERS has been generally ascribed to the highly enhanced electromagnetic field on the metal nanostructures due to the excitation of localized surface plasmon resonance (LSPR) and the enhanced chemical interaction between target molecules and SERS substrates, in which the strong amplified electromagnetic field is dominant since it could reach an enhancement up to 10^{10} [16,17]. Recently, SERS enabled lab-on-a-chip (LoC) systems emerge as a promising approach to label-free DNA detection [4,17–19], since SERS technology permits highly sensitive detection of trace DNA sequences with fingerprint information [4,20–23]. Especially, the combination of SERS substrates with microfluidic devices [24,25] enables high-throughput on-chip analysis with low sample consumption, high sensitivity and specificity to target molecules, revealing great potential for gene diagnosis. As typical examples, Zhao et al. fabricated an on-chip SERS system with silver nanoparticles (AgNPs)/Si nanopillar array embedded in microfluidic channels [26,27], exhibiting an excellent performance in detecting double strand DNA. Luke P. Lee et al. reported a high-density Ag nanoparticle film, which successfully tuned the particle spacing with controllable SERS detection [23]. Besides, on-chip

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https://doi.org/10.1016/j.snb.2018.05.043
Received 30 January 2018; Received in revised form 8 May 2018; Accepted 9 May 2018

SERS detection has been achieved based on various SERS enabled LoC platforms, such as microfluidic channels equipped with roughed-Au layers [28,29], aluminum nanocrystals [30], silver nanoplates [31], and nanogaps-rich gold nanomaterial [32,33].

Despite the aforementioned advances in recent years, on-chip SERS detection of DNA still suffers from series of problems. First, it is technically challenging to integrate efficient SERS substrates within a microfluidic chip, since the traditional strategies for producing SERS substrates are 2D technologies that are not compatible with non-planar microfluidic channels; second, owning to the low Reynolds number, flowing fluids in micro-channels become laminar. A key consequence is target biomolecules do not necessarily interact with the SERS substrates through diffusion, thus high-efficiency trapping strategy is essential for sensitive DNA detection [34]; third, most of the SERS chips suffer from poor reusability [35], residual biomolecules on the SERS substrate would cause cross contamination, interfering the next experiments. In this regard, the development of reusable SERS-active bio-chips that enable controllable trapping and releasing of biomolecules is still a challenging task.

We report here a facial laser scribing [36,37] of AgNPs and graphene oxide (GO) composites (AgNPs@GO) for the fabrication of SERS-active biochips towards DNA detection. AgNPs@GO composites were prepared by UV irradiation induced photoreduction of $Ag(NH_3)^+$ and GO in aqueous solution [38]. Programmable laser scribing of the AgNPs@GO composite film enables direct writing arbitrarily shaped patterns that consist of AgNPs and reduced GO (AgNPs@RGO), since the laser treated region has been exfoliated into hierarchical porous structures. By transferring the channel-shaped AgNPs@RGO patterns to a pre-designed PDMS-based microfluidic chip through general stamping method, a SERS-active biochip has been successfully developed. The laser exfoliated RGO foam not only serves as nanoporous scaffold for AgNPs [38,39], but also acts as an active substrate that shows strong interaction with target biomolecules such as DNA sequences [6,13,34]. The closely packed AgNPs on graphene sheets can electromagnetically enhance the Raman signal [40–42] due to coupling of localized surface plasmon resonances of adjacent nanoparticles [17,31,43]. Thus the AgNPs@RGO biochip can work as a SERS sensor for on-chip DNA detection. The noncovalent interaction [13,34] between DNA and graphene can be employed to selective trapping and releasing of target DNA sequences, which not only promotes the sensitivity of this SERS sensor, but also makes it reusable [44,45]. Direct laser scribing of AgNPs@RGO-based SERS sensor reveals great potential for developing sensitive, portable and reusable biochips.

2. Experiments

2.1. Preparation of AgNPs@GO composite material

AgNPs@GO composite materials were fabricated by one step photoreduction method. $NH_3{\cdot}H_2O$ (Sigma-Aldrich Co.) was slowly dropped into 10 mL of $AgNO_3$ solution (Sigma-Aldrich Co., 1 mM) under magnetic stirring (800 rpm) until the precipitates disappeared. The suitable amount of ammonia was added to form clear $Ag(NH_3)_2^+$ first. The obtained $Ag(NH_3)_2OH$ was mixed with 10 mL of GO solution (4 mg/mL), which was prepared by a modified Hummer's method. The resulting solution was irradiated under UV-light (500 W, Philips, QVF135) for 10 min, under stirring. The AgNPs@GO solution was formed and washed with deionized water for 3 times. Then the composite materials were re-dissolved in 20 mL of deionized water.

2.2. Laser scribing of AgNPs@GO film

The laser-scribe approach, which belongs to continuous laser direct writing method, is based on a 780 nm focused near-infrared laser (200 mW) within a DVD drive. The DVD can be located and written repeatedly by Nero StartSmart Essentials, and it could be used to realize a large-area, fast and maskless reduction of GO. Only 20 min were required for the entire patterning of the front cover of a DVD disc. In our experiment, the AgNPs@GO composite solution was directly cast onto DVD disc. After drying under ambient condition, the stack and flat films were formed on the disc with an average thickness of 2 μm. Then the disc was inserted into the laser-scribe DVD drive. The pre-programmed channel patterns could be directly "written" onto the composite film, forming foam-like AgNPs@RGO structures.

2.3. Fabrication of the SERS-active biochip

Our biochip is composed of a PDMS (Sylgard 184 Silicone Elastomer, Dow Corning Corporation) microfluidic channel and a flat PDMS substrate integrated with the same AgNPs@RGO channel patterns. The AgNPs@RGO based channel pattern prepared by laser scribing was selectively transferred to the PDMS channel, and the magnified SEM image showed that the transferred substrate retained the loose and exfoliated morphology (Fig. S1). Meanwhile, the region without laser-scribe treatment could not be lifted-off due to the compact stack of graphene layers. The bare PDMS surface facilitated the follow-up sealing with channels. After exposure to air plasma, tight covalent bonds were formed between two conformal layers, ultimately making an enclosed SERS-active biochip. Through this simple procedure, a flexible AgNPs@RGO biochip was obtained.

2.4. Electric field simulation of the AgNPs array on graphene sheet

The electric field distribution of the AgNPs on graphene sheet was simulated by the finite difference time domain method (FDTD). The AgNPs@RGO composite material was simplified into two-dimensional case. The AgNPs were adjusted with an average diameter of nearly 5 nm, and the nanogaps between particles were set to be ~2 nm. Localized surface plasmon resonance was excited by 532 nm wavelength light excitation around AgNPs. The LSPR was coupled with one another between two adjacent particles; thereby the electromagnetic field could be enhanced by 200.

2.5. Characterizations

The SEM images of the samples were measured using a JEOL JSM-7500F field emission scanning electron microscope. X-ray photoelectron spectroscopy (XPS) was performed using an ESCALAB 250 spectrometer (Thermo Fisher Scientific, USA). XRD data were recorded on a Rigaku D/Max-2550 diffractometer with Cu Kα radiation (λ = 0.15418 nm). Surface enhanced Raman spectra were measured on a JOBIN YVON T64000 equipped with a liquid-nitrogen-cooled argon ion laser at 532 nm (Spectra-Physics Stabilite 2017) as an excitation source. The laser power was ~30 μW on the samples, and the average spot size was 1 μm in diameter using a long-working distance 50× objective. The ssDNAs were purchased from Takara Bio Incorporated (Otsu, Shuga, Japan). The samples were dissolved in phosphate buffer (1 nM). SERS spectra were collected with 5 s exposure time, 3 accumulations. CLSM images were obtained using an LEXT 3D measuring laser microscope (OLS4100).

3. Results and discussion

To develop a facile strategy to fabricate a SERS-active DNA biochip, a laser scribing technology [36] was employed to make AgNPs@RGO SERS substrate. Fig. 1a shows the fabrication procedure of the AgNPs@RGO biochip. First, AgNPs@GO composite material was prepared through one-step UV-photoreduction of an aqueous mixture of silver-ammonia and GO [38]. Herein, to prevent uncontrollable chemical reduction of both GO and silver ions, a suitable amount of ammonia was added to form clear $Ag(NH_3)_2OH$ solution. The formation mechanism of AgNPs on GO sheet under UV irradiation is illustrated in