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Research paper

Tailored periodic Si nanopillar based architectures as highly sensitive universal SERS biosensing platform

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ABSTRACT

We report a skeleton key platform for surface enhanced Raman spectroscopy (SERS) based biosensor, utilizing ordered arrays of Si nanopillars (SiNPLs) with plasmonic silver nanoparticles (AgNPs). The optimized SiNPLs based SERS (SiNPLs-SERS) sensor exhibited high enhancement factor (EF) of 2.4×10^8 for thiophenol with sensitivity down to 10^{-13} M of R6G molecules. The ordered array of SiNPLs stabilizes the distribution of AgNPs along with the light trapping properties, which resulted in high EF and excellent reproducibility. The uniformity in the arrangement of AgNPs makes a single SiNPLs-SERS substrate to work for all types of biomolecules such as positively and negatively charged proteins, hydrophobic proteins, cells and dyes, etc. The experiments conducted on differently charged proteins, amyloid beta (the protein responsible for alzheimers), *E. coli* cells, healthy and malaria infected RBCs provide a proof of concept for employing universal SiNPLs-SERS substrate for trace biomolecule detection. The FDTD simulations substantiate the superior performance of the sensor achieved by the tremendous increase in the hotspot distribution compared to the bare Si sensor.

1. Introduction

Surface enhanced Raman spectroscopy (SERS) is a widely used vibrational spectroscopic technique for potential applications in various fields such as environmental monitoring, chemical and biosensing, biomedical detection, etc. [1–6]. In the recent years, extensive research interest has been focused towards the chemical and biosensing by exploiting the localized surface plasmon resonance (LSPR) on the surface of noble metal nanoparticles [7–10]. The photo-induced electromagnetic field enhancement in the vicinity of metal nanoparticle due to LSPR leads to ultrasensitive probing by SERS [11–13]. An ideal SERS substrate should have (i) high enhancement factor (ii) uniformity in nanoparticle distribution (iii) reproducibility and reusability [14]. The sensitivity of the SERS substrate mainly depends on the enhancement factor, which can be achieved by controlling the size (40–70 nm), interparticle distance (less than 10 nm) and choosing a laser frequency close to the LSPR of the substrate [15–18]. The uniformity of the substrate demands orderly arranged plasmonic nanoparticles on the substrate. The lack of uniformity leads to non-reproducible enhancement and the maximum deviation in the enhancement factor is preferably less than 20% for a good SERS substrate [14].

The plasmonic behavior of noble metal nanoparticles with various sizes and shapes has been widely utilized in two forms: (i) individual and randomly oriented colloidal nanoparticle aggregates (ii) ordered nanostructure arrays [19–21]. The colloidal nanoparticles are generally dispersed in water and possess charged capping agents around them. This makes the detection of oppositely charged and hydrophobic proteins very difficult through SERS. In addition, the difficulty in controlling the localized hot spots and irreproducibility of colloidal nanoparticles have led the researchers to move ahead with the fabrication of heterogeneous solid sensors [22,23]. In the solid sensors, the nanoparticles are arranged or decorated on a micro/nano engineered surface to achieve large area uniform hot spot distributions. There are various techniques for the fabrication of heterogeneous SERS sensors such as reactive ion etching, focused ion beam lithography, E beam lithography, wet etching, etc. [24–27]. Among these methods wet etching is a simple, cost effective fabrication process for achieving uniform nanostructures in large area. Gupta et al. have reported silicon micro-pyramid based SERS sensor, fabricated by wet chemical route with enhance-

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ment factor 10^6-10^7 for multianalyte detection [28]. However, they have used Si micropyramids as a platform for growing AgNPs, which reduce surface area and number of hot spots in comparison to nanopyramids. Moreover, the non-uniform sized aperiodic micropyramids bring down the reproducibility of the substrate.

Recently, Si nanowires/pillars decorated with metal nanoparticles as SERS sensors are drawing great deal of interest because of their huge surface to volume ratio, unique light trapping properties and high compatibility with the existing silicon technology [29–31]. In the literature, Caldwell et al. have presented Au capped Si nanopillar SERS substrates fabricated using e-beam lithography and RIE etching [32]. The number of hotspots in this case will be less, since the Au is deposited only on the top of the nanopillars (not on the sidewalls). There is a substantial light penetration inside the nanopillars which goes untapped when no nanoparticles are there on the sides. This substrate has shown SERS enhancements for 633 and 785 nm lasers, but did not give any enhancement for 532 nm laser. Gartia et al. have conducted SERS experiments made up of silver coated silica nanopillars [33]. They have fabricated the SERS substrates using high cost laser interference lithography and E-beam evaporation. Moreover, the enhancement was shown only on benzothiophenol analytes and 785 nm laser. In addition, the reported enhancement factor varies from 10^6 to 10^8. The universality and the simplicity of our approach and large enhancement in wide range is because of our preparation of our substrates. Schmidt et al. have reported the metal deposited leaning Si nanopillars as SERS substrates [34]. They have fabricated using e-beam evaporation and reactive ion etching. Their SERS studies are limited for 785 nm wavelength laser and thiophenol. All of them have used high cost instrumentation for the fabrication of SERS substrates. None of them have reported a detailed study on the biosensing using these SERS substrates. Moreover, in literature, most of them have used randomly oriented Si nanowires/pillars for fabricating SERS sensors, which results in irreproducible SERS sensing due to the difficulty in controlling the nanogaps (less than 10 nm) between the nanoparticles. Intuitively, three dimensional nanostructures, which contain highly ordered periodic Si nanowire/pillar arrays with plasmonic nanoparticles can improve the enhancement factor and reproducibility to a great extent. The efficient utilization of these sensors in biomolecule detection can fulfill the widespread applications in the biomedical field. SERS based bio analysis is a label free technique used to identify and characterize microorganisms, living cells, proteins and tissues [35–37]. Siddantha et al. have reported the SERS from oppositely charged proteins using GaN based hybrid SERS substrate for biosensing. This substrate showed relatively low enhancement factor of $\sim 10^5$ and other biomolecules such as cells, microorganism, etc. have not been studied using this substrate [38]. Very recently, Chen et al. have studied the malaria infected RBC using SERS using Ag nanorod substrates [39] Another example for SERS based biosensor is the detection of water-borne pathogens through *E. coli* cells by Zhou et al. [36]. The morphology and nature of the SERS substrate plays a vital role in determining the vibrational modes for biomolecules. Therefore, a single (universal) SERS substrate with high enhancement factor and reproducibility, which works for all types of biomolecules become indispensable.

Herein, we present a simple strategy to fabricate an ultrasensitive, highly reproducible and versatile SERS biosensor based on periodic array of Si nanopillars decorated with AgNPs (SiNPLs-SERS) in a controlled manner. The periodic array of SiNPLs stabilizes the distribution of AgNPs along with the light trapping properties, which result in high enhancement factor ($\sim 10^8$) and excellent reproducibility with a lower detection limit of 10^{-13} M of R6G molecules. The universality of this substrate was verified by the experiments conducted on both positively and negatively charged proteins, hydrophobic proteins (amyloid beta, which is responsible for alzehemiers), normal RBCs and malaria infected RBCs, *E. coli* cells and fluorescent dyes. Further, the near field intensity in the vicinity of AgNPs on the SiNPLs is demonstrated and compared with the bare Si sensor using finite difference time domain (FDTD) simulations.

2. Experimental details

The SiNPL arrays were fabricated using nanosphere lithography and metal assisted chemical etching. Spin coating was used to get a monolayer of polystyrene nanosphere (PS) on the Si wafers. The PS nanospeheres were self assembled in hexagonally close packed arrangement by spin coating and O_2 plasma etching has been performed to separate the PS nanospheres. Gold film of thickness 8–10 nm was coated on the top of PS nanospheres using sputtering. The PS nanospheres were removed after gold coating by sonication using CH_2Cl_2. This results in the formation of nanoporous gold template on the Si wafer. The Si wafers with nanoporous gold template were placed in an etching solution containing HF (40%)/H_2O_2 (30%) and ethanol in the volume ratio 3:1:1, respectively to achieve periodic array of SiNPLs. The SiNPL arrays were deposited with varying thickness of Ag films using magnetron sputtering system. As deposited samples were vacuum annealed at 400 °C for 5 h to achieve the silver nanoparticles of different average diameters. The detailed information about the fabrication of SiNPLs can be found elsewhere [40].

The morphology studied by field emission scanning electron microscopy (FESEM, Carl Zeiss). The absorptance spectra and plasmon modes are observed by UV–vis-NIR spectroscopy (PerkinElmer, Lambda 950). Raman spectrometer with excitation source 532 nm from a diode pumped frequency doubled Nd: YAG solid state laser (PhotopSuwtech Inc., GDLM-5015L) and DILOR-JOBIN-YVON-SPEX with excitation source 633 nm (Model Labram) for He-Ne 20 mW laser beam was used for SERS characterization. LabRam HR Evolution 800, equipped with Horiba iHR 800 monochromator and a Peltier-cooled CCD was used for 785 nm wavelength SERS experiments. The 785 nm, NIR solid state laser made by Horiba was used. For obtaining SERS spectra, 10 μL of the analyte solution of thiophenol and other analytes was dropped on the sensor and allowed to dry. SERS spectra were then collected from three different locations and averaged.

3. Results and discussion

It has been already demonstrated in our previous publications that the vertically aligned SiNPLs with controlled diameter, period and uniformity can be formed using nanosphere lithography and metal assisted chemical etching [40]. The fabricated SiNPLs have size of 70 nm and periodicity of 100 nm. In order to exploit the additional surface area and the three dimensional space provided by the SiNPLs for SERS applications, AgNPs of different average sizes are decorated on the SiNPLs. Fig. 1(a) and (b) shows the FESEM cross-sectional and the top view images of the SiNPLs-SERS sensor with 45 s silver deposition with DC power supply of 5 W. The optimization details of the SiNPLs-SERS sensors are given in Supplementary information Fig. S1. The size distribution of the AgNPs is shown in Fig. 1(c). The corresponding UV–vis spectrum, provided in inset of Fig. 1(c), represents a dipole plasmon mode at 402 nm. The SERS substrates should be excited at its LSPR peak to ensure the effective surface enhanced Raman scattering. Since the UV–vis absorption peak is observed at 400–500 nm, Raman spectrometer with laser source of excitation wavelength $\lambda = 532$ nm has been chosen for the experiments. The enhancement factor (EF) of the optimized SiNPLs-SERS sensor calculated using thiophenol (PhSH) as a model analyte is shown in Fig. 1(d). The highest EF of 2.4×10^8