Characterization of Au and Ag nanoparticle coated silica nanosprings – Toward SERS based diagnostic applications

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ABSTRACT

Silver (AgNP) and gold (AuNP) nanoparticle coated silica nanosprings (NS) are introduced herein as novel materials with high Surface Enhanced Raman Scattering (SERS) activity. Silica nanosprings were coated with nanoparticles using chemical vapor deposition or chemisorption of either pre-synthetized or *in-situ* synthesized nanoparticles. Controlled patterning of NPs on silica nanosprings resulted in small interparticle spacing and concomitant formation of SERS hot spots. Chemisorbed nanoparticles on nanosprings gave rise to significantly higher Raman enhancements than monolayers of chemisorbed nanoparticles on glass cover slips. Enhancement factors as high as 10^8 and 10^9 were obtained from AuNP (20 nm) and AgNP (35 nm) coated nanosprings, respectively.

Keywords: Gold and silver nanoparticles; silica nanosprings; surface enhanced Raman scattering (SERS),

1 INTRODUCTION

Surface Enhanced Raman Spectroscopy (SERS) has attracted much recent attention due to its potential as a single molecule detection technique. This is made possible due to a $>10^6$ enhancement of Raman scattering signals of labeled target molecules that are in close proximity to noble metal surfaces or nanoparticles. A variety of SERS-active materials have been explored, including silver-coated silica nanospheres, multilayers or aggregates of noble metal nanostructures such as spheres, rods and cubes on flat substrates and polymeric fibers [1]. Elongated aggregates of nanoparticles (NP) have also been demonstrated to result in significant enhancement of Raman scattering signals by formation of *hot spots* [2]. Herein, we outline the development of elongated aggregates of metal nanoparticles on silica nanosprings and present the results from SERS characterization studies of these new materials.

2 EXPERIMENTAL

2.1 Preparation and functionalization of silica nanosprings

Preparation: Silica nanosprings (NS), supplied by GoNano Technologies Inc., were grown on an Al foil using the vapor-liquid-solid (VLS) method following a proprietary protocol. Nanosprings (~29 mg) were removed from Al foils using a surgical blade, dispersed in methanol (1 mL), sonicated for 1 hour, and centrifuged (2000 RPM, 1 min). The supernatant was collected to furnish finely structured springs that were used for subsequent experiments. The concentration of NS in methanol was standardized such that the optical scattering intensity obtained from UV-visible absorbance spectrum of NS in methanol at 500 nm equals unity (Fig. 1).
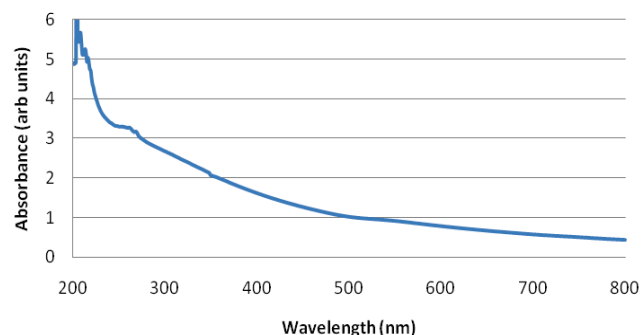

Figure 1 UV-visible absorbance spectrum of nanosprings dissolved in methanol.

Functionalization: nanosprings were centrifuged (6000 RPM, 20 min) and resuspended in ethanol. Aminopropyl triethoxysilane (APTES, 15 µL) was added to 500 µL of NS solution (A_{500} = 1.0) and incubated for 1 hour. A nitrogen atmosphere was used in the microcentrifuge tube to avoid undesirable polymerization of silane molecules upon exposure to air. After 1 hour, APTES was removed by repeated centrifugation and resuspension in ethanol (6000 RPM, 20 min, 3 × 500 µL). Finally, amine-functionalized NS were centrifuged and resuspended in DI water.

2.2 Deposition of AuNP on silica nanosprings

Gold nanoparticles (AuNP) were deposited on silica NS either by chemical vapor deposition techniques, or chemisorption of pre-synthesized AuNPs.

AuNP deposition using PECVD: Gold nanoparticles were deposited onto NS grown on Al foil using plasma enhanced chemical vapor deposition (PECVD) as described elsewhere [3]. Although visual interrogation of the sample revealed a non-uniform color range of the substrate (brown to dark brown), SEM images verified a uniform coating of AuNP all over the NS mat (*vide infra*).

Chemisorption of AuNP: Amine-functionalized NS (10 μL) were added to 1 mL solution of 20 nm AuNP (7×10^{11} NPs, BB International, UK), thouroughly mixed and left for 4 hours. Precipitation of a pink colored substance and a clear supernatant was observed after two hours, which suggests complete binding of AuNP to nanosprings. The sample solution was centrifuged (2000 RPM, 10 min) and final volume was adjusted to 0.1 mL This was confirmed from SEM images (*vide infra*).

2.3 Deposition of AgNP on silica nanosprings

Silver nanoparticles (AgNP) were deposited on nanosprings by chemisorption of either *in situ* generated or pre-synthesized AgNP.

AgNP deposition on NS by DMF reduction of $AgNO_3$: An aqueous solution of $AgNO_3$ (30 μL, 0.1M) and amine functionalized NS solution (10 μL) was added to DMF (3 mL) in a round bottom flask. The solution was stirred and heated at 100°C for 2 hours, at which point the solution was densely gray in color with some AgNP adhering to glass (yellow appearance). The AgNP-coated NS were collected by centrifugation (2000 RPM, 10 min). The resulting clear supernatant (suggesting complete binding of AgNPs to NS) was carefully removed. AgNP coated NS were washed (DI, 3×1 mL) and finally resuspended in DI water.

Chemisorption of citrate reduced AgNP: The following protocol was used for the synthesis of AgNP. An aqueous solution of $AgNO_3$ (34 mg in 200 mL, 1 mM) was heated with continuous stirring until reflux was reached. After 2 minutes, trisodium citrate (103 mg in 10 mL, 35 mM) was quickly added, and the solution was kept boiling for 6 minutes or until a color change from transparent to gray or yellow was observed. The solution was allowed to cool to room temperature and was transferred into 50 mL polypropylene tubes. The absorbance of the resulting solution was $A \sim 2.9$ at $\lambda = 432$ nm. Subsequent chemisorption of AgNP was carried out in a similar fashion as described above for AuNP.

2.4 SEM characterization

Deposition of nanoparticles on NS was confirmed by field emission scanning electron microscopy (FESEM). Samples for FESEM analysis were prepared by drop-coating nanoparticle-functionalized nanospring suspensions (2 μL) on a cover glass slip. The samples were coated with carbon (thermal vapor deposition) to avoid charging of sample area under observation. SEM images were acquired at an accelerating potential of 5 KV and a working distance of 6 mm.

2.5 SERS characterization

SERS analysis of NP-coated NS was carried out using thiophenol as a model Raman label. Thiophenol (2 pmol in 2 μL) in DI water was dropped on physisorbed AuNP or AgNP coated NS on a glass cover slip and incubated for 1 hour in a humid atmosphere to avoid drying of the thiophenol solution on the sample.

SERS spectra were obtained with a confocal Raman microscope (WITec Alpha 300, Ulm, Germany) using an excitation wavelength (λ_{ex}) at 785 nm and incident power of 0.5 mW through a 100× objective (IR) lens. The spot size of incident laser beam was measured as ~1 μm at the highest possible incident power intensity. Scattering signals were collected through the objective lens with an integration time of 1 s. An optical fiber with 100 μm core diameter was used to transmit the signal from microscope to spectrometer. Raman spectra were recorded for shifts between 47 and 3300 cm^{-1} with a resolution of 4.3 cm^{-1}. SERS images were obtained by scanning an area of 10×10 μm^2 of sample with a scan speed of 0.5 μm/s and integration time of 1 s. The scattering intensity values are plotted as an image and represented in a color scale.

3 RESULTS

3.1 Size and shape of nanosprings

The silica nanosprings obtained by the VLS protocol display a heterogeneous distribution of sizes and shapes. The length of these springs varies from 1 to 10 μm, while the width and pitch of springs ranges from 50 nm to 300 nm. These nanostructures are composed of multiple nanowires leading to formation of interstitial defects on their surface. Interstitial defects and heterogeneity in size and shape may help in the formation of nanoparticle aggregates on the surface.

3.2 Surface coverage of NP on NS

Deposition of AuNPs by PECVD or chemisorption of pre-synthesized AuNPs to amine-functionalized NS, resulted in more uniform coverage of NPs on silica nanospring surfaces than observed for corresponding AgNP-coated NS (Fig. 2). The surface of AuNP-coated NS obtained by chemical vapor deposition was particularly uniform with an average particle size of ~20 nm and interparticle spacing (d) of nanoparticles of ~5 nm (Fig 2a). AuNP-coated NS obtained by chemisorption of AuNP on NS, display a larger
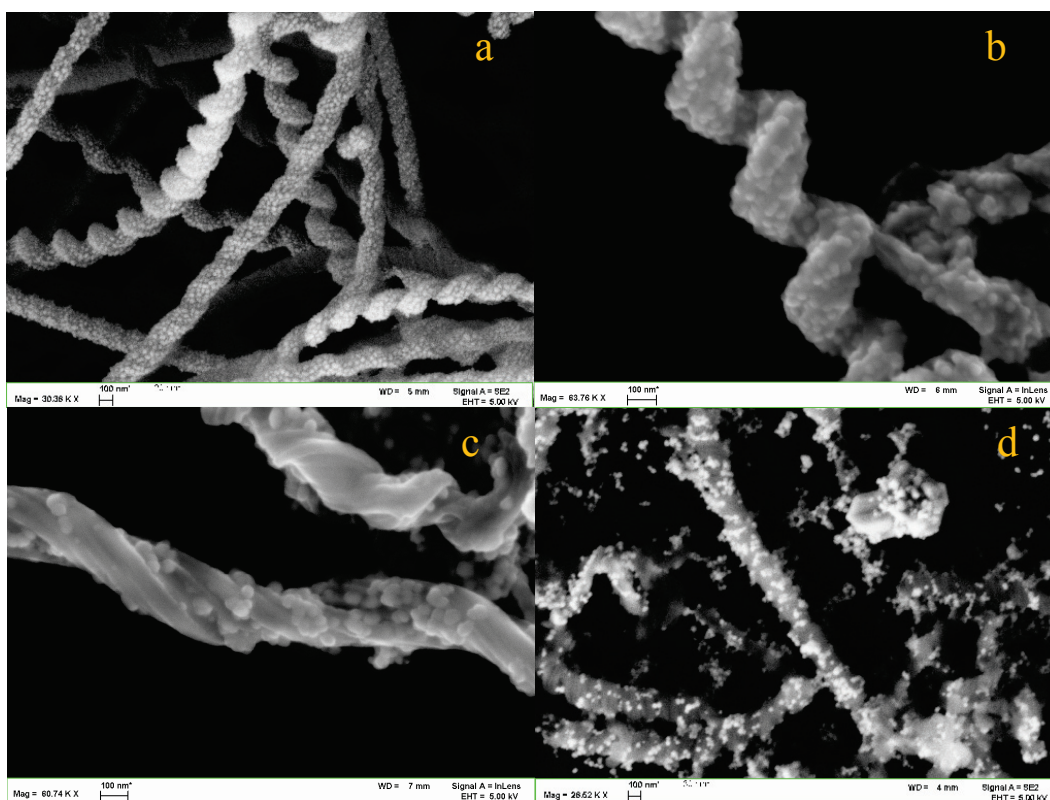
Figure 2: SEM images of nanosprings coated with AuNP using (a) PECVD and (b) chemisorption of citrate reduced AuNP (20 nm) onto amine-functionalized surface; nanosprings coated with AgNP (~35 nm) using (c) chemical reduction of $AgNO_3$ with DMF, or (d) chemisorption of citrate reduced AgNP onto amine-functionalized surface

variation in interparticle spacing (d) with 0 < d < D, where D is the diameter of NP (Fig. 2b).

Deposition of AgNP on NS by DMF reduction resulted in attachment of significant amounts of AgNP on NS. Unlike AuNP-coated NS, however, a non-uniform surface coverage of NP (average diameter ~35nm) was obtained despite the presence of excess $AgNO_3$ (Fig. 2c). In addition, more pronounced nanoparticle aggregation was observed relative to AuNP-coated NS. Interestingly, the presence of aggregates is more pronounced at the structural defects in the NS as shown in Fig 2c. Chemisorption of pre-synthetized AgNP on NS (Fig. 2d) also resulted in aggregation on the spring surface, but again with lower surface coverage than obseved for the corresponding AuNP-coated NS (compare with Fig. 2b).

3.3 SERS response from NP coated NS

SERS responses from AuNP and AgNP-coated NS were evaluated by comparing the Raman scattering intensity of thiophenol bound to NPs. AuNP-coated NS made by the PECVD technique gave rise to lower enhancement in Raman scattering signals than the corresponding nanosprings made via chemisorption of AuNP on to amine-functionalized NS (Fig. 3b and 3d). The size distribution of AuNP on the nanospring surface is similar for the two materials (Fig. 2a and 2b) and therefore unlikely to explain

the differential SERS-responses. Differential interparticle spacing is likely to explain the enhanced Raman response of AuNP-coated NS made by chemisorbtion. Regardless of the underlying mechanism, AuNP-coated NS formed by chemisorption, resulted in the formation of more SERS hot spots and display Raman scattering that is an order of magnitude more intense (Fig 3).

Isolated AgNP are known to give enhanced Raman signals that are two orders of magnitude stronger than corresponding AuNP [1]. Along these lines, our AgNP-coated NS generated by chemisorption of pre-synthesized AgNP display a factor ~10 improvement in Raman signal intensity (Fig. 3a). A lower Raman response was observed for AgNP coated NS generated by chemisorption of *in situ* synthesized AgNP. In fact, these materials display similar SERS responses as AuNP-coated NS formed by chemisorption (Fig. 3b and 3c). This disparity between surface coverage and SERS output is most likely due to the presence of an ensemble of nanoparticles (Fig 2c), where very few particles are optically hot and hence a few surface sites show efficient enhancement [4]. Nonetheless, these preliminary results demonstrate the potential of using silica nanosprings as substrates for arrangement of AuNP or AgNP for SERS applications. In addition, chemisorption allows attachment of larger NPs or nanomaterials of non-spherical shapes, which are known for their improved SERS response (unpublished data).
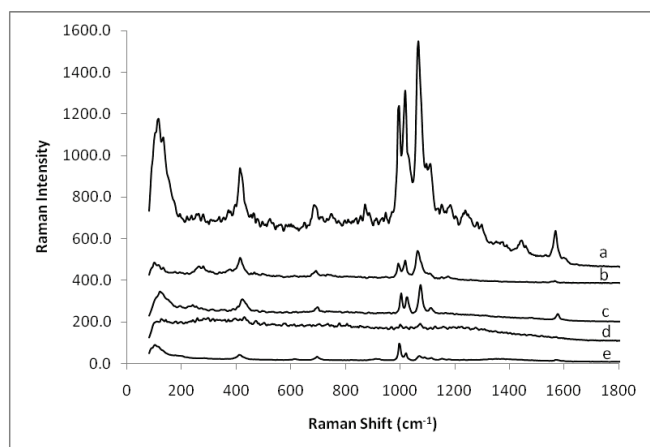
Figure 3: SERS spectra acquired from nanoparticle decorated NS substrates obtained from chemisorption of (a) AgNP, (b) AuNP, (c) AgNP deposited by DMF reduction, (d) AuNP by PECVD and (e) Raman spectrum of free thiophenol in a microwell. Incident power of 66 µW was used for (a) & (b).

3.4 Enhancement Factor (EF) calculations

Enhancement factors (EF) provide an estimate of SERS efficacy of a given substrate. EF for AuNP and AgNP-coated NS obtained by chemisorption of pre-synthesized NP were determined as described by Haynes et al [5]. EF for a given substrate is calculated with respect to Raman scattering signal from free Raman label used for the investigation. EF is defined as the ratio of Raman scattering intensity per Raman label molecule present on the sensor substrate relative to that of free solution. EF at any given Raman shift were determined by the following equation,

$$EF = \frac{I_{Surf} N_{Vol}}{I_{Vol} N_{surf}} \qquad (1)$$

where, I_{surf} is the Raman scatter signal intensity per mW of optical excitation power accumulated in 1s from a Raman active sensor surface; N_{surf} is the total number of Raman labels on the sensor surface under incident laser spot; I_{vol} and N_{vol} are the corresponding intensity and number density of volume of Raman label solution alone in a microwell exposed to laser radiation.

AuNP or AgNP-coated NS (90 µL) were mixed with ethanolic thiophenol solution (10 µL, 100 µM stock solution) and incubated for 1 h to allow thiophenol to bind to NP. The final volume of the sample was reduced to 10 µL by centrifugation (6000 RPM, 10 min). Thiophenol treated Au/AgNP-NS (0.5 µL) were dropped on to a cover glass and allowed to dry for subsequent use in SERS measurement. Incident optical power and integration time were 66 µW and 1 s respectively. Raman spectroscopic measurements of thiophenol were carried out using a microwell plate filled with thiophenol (0.5 µL, 10 M,) and

covered with a glass cover slip. Incident optical power and integration time were 7.2 mW and 5 s respectively.

Table 1 Highest enhancement factor for AgNP or AuNP coated NS treated with thiophenol at different Raman shifts.

Raman shift	1570 cm^{-1}	996 cm^{-1}	413 cm^{-1}
AgNP-NS	5.97×10^9	1.93×10^9	2.23×10^9
AuNP-NS	7.46×10^8	2.57×10^8	7.70×10^8

Raman EFs obtained for AuNP-coated NS and AgNP-coated NS were on the order of 10^8 and 10^9 respectively (Table 1). As reported previously, SERS response obtained from AgNP was almost an order better than that of AuNP. Although EF on the order of 10^{14} may be possible with nanoparticle aggregates [4], AuNP or AgNP coated NS, over an area of 20×20 µm^2, have shown an average EF of 10^7 and 10^8 respectively. Under the same experimental conditions, no detectable SERS response was observed from a monolayer of AuNP and AgNP of 20 nm chemisorbed on glass slide.

4   CONCLUSIONS

AgNP coated nanosprings show promising results for SERS-based diagnostic applications. Higher SERS response was obtained from Au/AgNP functionalized NS compared to glass slide substrates due to higher surface density of Au/AgNP on nanosprings. Further studies to improve SERS response from these substrates by utilizing larger silver nanoparticles are in progress.

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