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Tuning the electromagnetic field coupling between nanoporous silver and silver nanoparticles connected by hybridized oligonucleotide

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ABSTRACT

On monolithic nanoporous silver (NPS), via DNA hybridization, we constructed an NPS/DNA-Cy5/silver nanoparticle (Ag NP) sandwich to investigate its SERS effect. In this sandwich, no chemical enhancement contributes to the SERS signal of Cy5. As compared with NPS, the present substrate exhibits particularly strong electromagnetic (EM) field enhancement. At the same Ag NPs surface loading, the SERS intensity decreases exponentially with increasing the length of double-stranded DNA (dsDNA). A larger pore size of NPS leads to weaker EM enhancement within the sandwich, but the relative intensity is not sensitive to the sizes and it is determined by the length of dsDNA.

1. Introduction

Raman scattering technique can be used to probe a molecular structure, but its weak scattering intensity restricts its practical application. Since the discovery of the surface-enhanced Raman scattering (SERS) effect [1,2], Raman spectroscopy has revived. In recent decades, SERS technique has become a powerful tool for the characterization of a molecular structure. Moreover, it has been extended to single molecule detection [3–6].

It is known that the sensitivity of the detection via SERS depends mainly on the nanostructure of metallic substrates (primarily gold, silver, and copper, especially silver). To produce reproducible SERS signals, their morphology should be controlled, periodic and stable [7–12]. Chemical dealloying (etching one or multiple more active elements from an alloy) is a simple and economical technique to prepare nanostructured metal materials [13–18]. The as-prepared nanoporous metal materials has an extremely clean surface, and the pore/ligament size of these materials could be easily tuned, and, moreover, it is quasi-periodic in structure on the micrometer scale. The use of this nanoporous metal as SERS substrate has been reported elsewhere [10,17,19,20].

The enormous enhancement effect of SERS can be explained at present by two distinct mechanisms: chemical enhancement (CE) [21–24] and electromagnetic enhancement (EM) [25–29]. When the probe is immobilized or adsorbed directly on the substrate surface, a charge transfer between the probe and the metallic substrate may occur [22–24]. Compared with CE, EM enhancement, originated from the surface plasmon resonance, is a long-range effect. The intensity of the EM field near the substrate is strong, but it decays at a distance. So the distance dependence study of SERS is very important, especially for hybrid nanostructured substrate [26–30].

In previous works, one observed that loading nanoparticles on nanoporous metal substrate could enhance Raman signal of a probe prominently [17,31]. Utilizing nanoparticles to construct a sandwich structure to study the enhancement mechanism of SERS has been reported elsewhere [32], but using a monolithic nanoporous metal and metal nanoparticles to form EM hot spots has not been tried. To make clear the EM enhancement mechanism of nanoporous substrate and to tune the near-field coupling, we here construct a sandwich nanostructured substrate via DNA hybridization. The distance between silver nanoparticles (Ag NPs) and nanoporous silver (NPS) is tuned by the number of hybridized DNA base pairs. The coupling of the EM between NPS and Ag NP forms a hot spot. The enhancement of the EM field is detected by the probe Cy5 exposed to the field.

2. Experimental

2.1. Materials and chemicals

All thiolated DNAs used in the present experiment were purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (China). The sequences of each probe DNA (pDNA) and its complementary DNA (cDNA) are shown in Table S1 in Supplementary materials. The solutions of each DNA were prepared by dissolving DNA in the TE buffer (10 mM Tris–HCl, 1.0 mM EDTA, pH 7.4).

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The buffer solutions (pH 7.4) used for DNA immobilization was formulated with 10 mM Tris–HCl, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP, Sigma product). For DNA hybridization, the buffer (pH 7.4) was prepared with 10 mM Tris–HCl, 1.0 mM EDTA, and 1.0 M NaCl. The buffer used for washing is 10 mM Tris–HCl (pH 7.4). All aqueous solutions were prepared with triply distilled water (18.25 MΩ cm).

2.2. Immobilization of pDNA on NPS

NPS was prepared by chemical dealloying of Ag–Al alloys according to the procedure reported elsewhere [17]. The solution of pDNA (10^{-6} M, 3 μL) was first activated in the immobilization buffer (its final concentration was 10^{-8} M). After 1 h, the dried NPS (about 0.2 mg) was added. Twenty hours later, the NPS was taken out and rinsed thoroughly with the washing buffer and triply distilled water, respectively.

2.3. Functionalization of Ag NPs

Ag NPs (ca. 30 nm) were prepared according to the literature [33] by reducing $AgNO_3$ (10^{-3} M, 20 mL) with sodium borohydride (3×10^{-3} M, 20 mL). To modify Ag NPs with cDNA, the solution of cDNA (10^{-4} M, 5 μL) was added into Ag NPs suspension (1.9×10^{-10} M, 500 μL). After standing for 20 h, the resulting system was centrifuged at 12 000 rpm for 30 min. The precipitate was washed with the washing buffer, followed by recentrifugation. The resulting precipitate was then dispersed into the hybridization buffer for use. The UV–Vis spectra of bare and functionalized Ag NPs were shown in Figure S1 in Supplementary materials. After functionalization, the absorption peak of Ag NPs shifts from 398 to 404 nm.

2.4. Construction of a sandwich structure

The pDNA modified NPS was put into 1.0 mM 2-mercaptoethanol (ME) for 3 h to obtain a well-aligned DNA monolayer and cover any surface unoccupied by pDNA. The resulting NPS was washed with washing buffer and triply distilled water. After that, the NPS was put into the hybridization buffer that contained cDNA functionalized Ag NPs. The resulting system was incubated at 15 °C (8-mer), 30 °C (14-mer) and 37 °C (20-mer) for 3 h. After hybridization, the hybrid nanostructure was rinsed thoroughly with the washing buffer containing 0.1 M NaCl.

2.5. SEM images

Surface morphologies of NPS and hybrid nanostructures were observed on a JSM-670F field emission scanning electron microscope.

2.6. SERS measurements

SERS spectra were recorded on a Horiba LabRAM HR800 Raman spectrometer equipped with a 25 mW (632.8 nm) He–Ne laser and a charge-coupled device (CCD) detector. The detection slit was set at 100 nm. A 50× objective was used to focus the incident laser beam on the SERS substrate. On the surface of each substrate, four different positions were used to acquire SERS spectra. At each position, duplicate measurements were made. The SERS spectrum given in the text was an averaged one with the relative deviation less than 10%. Prior to each measurement, the spectrum was calibrated with respect to single crystal Si at 520 cm^{-1}.

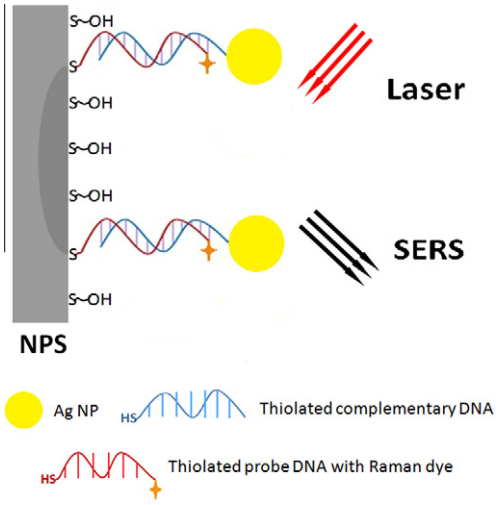

Figure 1. Schematic illustration of the sandwich structure of NPS/DNA-Cy5/Ag NP.

3. Results and discussion

Figure 1 is a scheme of the NPS/DNA-Cy5/Ag NP sandwich structure. pDNA and its cDNA were separately immobilized on NPS and Ag NPs through Ag–S bond self assembly. Prior to DNA hybridization, the surface of NPS was covered uniformly with ME to prevent any nonspecific binding on the substrate [34]. Then the DNA modified NPS and Ag NPs were combined together at different temperature through the hybridization of pDNA and its cDNA. Because Cy5 was labeled at the end of pDNA, when pDNA was hybridized with its cDNA, Cy5 would therefore reside in the EM hot spots formed between the NPS and the Ag NP, and moreover, its distance from Ag NP was kept unchanged. No direct contact of Cy5 with NPS and Ag NP ensured that no CE contributes to the Raman enhancement effect. In addition, a little high concentration of cDNA was used so that cDNAs could be immobilized vertically on Ag NPs surfaces [35], thereby facilitating DNA hybridization and distance control. The SEM images of NPS and its conjugate with Ag NPs were shown in Figure 2. The size of the pores/ligaments of NPS is ca. 100 nm, and that of Ag NPs is ca. 30 nm in diameter. The Ag NPs are well distributed on the NPS, indicating that the sandwich structure was successfully fabricated.

To ensure the SERS intensities of the hybrid nanostructured substrate change only with the length of DNA spacer, i.e., the distance between the NPS and Ag NPs, the densities of pDNA and Ag NP covered on the NPS must be the same. To this end, a lower concentration of pDNA was used to ensure that all the pDNAs were immobilize on the NPS and, moreover, the occupied area of each pDNA on the NPS was close to the projected area of each Ag NP on the NPS. SEM images in Figure 2 show that the density of Ag NPs on the surface of the NPS is approximately the same within the maximum experimental error of 12%.

Figure 3 is the SERS spectrum of Cy5 in the sandwich structure. For comparison, the SERS signal without Ag NPs is also collected and given in Figure S2 in Supplementary materials. Control experiment shows that before hybridization with functionalized Ag NPs, the SERS signal is small, and it changes a little with the pDNA strand length. This is because the pDNA is flexible and cannot keep it upright to the surface. By contrast, the SERS signals in the sandwich structure are much stronger due to the coupling of the EM fields of the NPS and Ag NPs. Moreover, the SERS intensity shows great distance dependence due to the rigidity of double-stranded DNA (dsDNA). Take the peak at 1015 cm^{-1} as an example, its peak