

HIGH SENSITIVE SERS IMMUNO SENSOR BASED ON GOLD-SHELLED AND CORRUGATED POLYSTYRENE NANOBEADS FOR IN-VIVO TRACKING IN CELLS

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ABSTRACT

To increase the hot-spot area on a surface enhanced Raman scattering (SERS) nanoparticle for the increment of overall signal intensity in average has become an important task these years. Therefore, this paper proposes a reliable method for the fabrication of gold-shelled corrugated polystyrene nanobeads (GS-CPSBs) to increase the hot-spot area for SERS, and compares the SERS ability of gold nanobowls with two sizes and a GS-CPSB. The results show that GS-CPSBs can carry out the highest enhancement ability among all and have a great biocompatibility with living cells for being a SERS-active biosensor.

KEYWORDS: polystyrene bead (PSB), plasma etching, SERS, carboxyl groups, nanobowl

INTRODUCTION

SERS nanostructures possessing large surface-to-volume ratio of hot-spot area have been considered a potential technique to detect single (or very rare) molecule(s) for biological applications. The various radius sizes of the nanobowl structures with sub-10nm edge were reported by simulations and experiments [1-2]. However, the hot-spot area was still too small (~1%). Therefore, Xie, J. *et.al.* [3] reported a method to synthesize nanogold-particles with nano-gaps for further SERS enhancement, giving an order more hot-spot area and higher enhancement efficiency, compared to that of nanobowls and smooth nanogold spheres with a similar size. On the other hand, Luke Lee's group also reported a nanocoral structure to obtain many sub-10nm gaps on a polystyrene bead by using oxygen plasma [4]. However, the possible cause of the surface-roughness was attributed to non-uniformly etching, in which not only the mechanism was not fully understood, but also the structure size/shape might not be easily tuned. Indeed, the increment of surface roughness will contribute significantly on SERS from many prior excellent works [4]; however, non-uniformity of the secondary structures on nanoparticles can deliberate the effectiveness and efficiency for SERS. Therefore, this study provides a controllable way to engineer carboxylic PSBs surface under argon/oxygen plasma by using the carboxyl groups as nano-masks, thus size-controllable nanopillars can be easily formed on the beads for SERS [5].

THEORY

Electromagnetic resonances occur near the metal surface, usually silver or gold, so large localized electric fields (E) can be generated at the sharp corner or edge [1-2]. Besides, the electric field decreases very quickly from the sharp edge to the broad center [1-2]. As a result, the SERS enhancement ability, proportional to E^4 , dramatically decays while the position is not at the sharpest point. Therefore, a nanoparticle possessing many sub-10nm structures can provide a great improvement of overall Raman intensity for an efficient enhancement of SERS-active nanoparticles [3-4]. For the plasma etching method, we take advantages of an easy available commercial product of carboxyl modified polystyrene beads to obtain corrugated surfaces by a selectively plasma etching phenomenon between polystyrene and carboxyl groups. While increasing the ratio of oxygen gas during the plasma etching process, the etching rate increases and the etching direction changes from vertical to be more horizontal etching.

EXPERIMENTAL

The polystyrene beads for GS-PSBs were polystyrene beads without carboxyl groups from Duke Scientific Corporation, and the carboxylic polystyrene beads for GS-CPSBs were from Bangs Laboratories, Inc. The fabrication process of smooth and corrugated gold-shelled nano-PSBs (GS-PSBs and GS-CPSBs) is in Figure 1. Figure 1(a) shows the process to use polystyrene beads as a sacrificial material for the fabrication of GS-PSBs or gold nanobowls that were embedded in a PDMS. In Figure 1(b), the polystyrene beads were first etched by Ar plasma to form corrugated surface, and then coated with a thin Au/Ti layer and embedded in a PDMS. To detect a fluorescent image of R6G solution near the surface of gold nanobowls (500nm inner diameter), a 100-fold magnification objective with N.A. 1.3 was used (see Figure 1c). For SERS observation (see Figure 1d), GS-PSBs and GS-CPSBs embedded in a PDMS were put on a 170 μ m coverslip and excited by a 532nm laser. To filter out the laser intensity, a notch filter (532nm \pm 1%, OD>3) (NT46-565, Edmund Optics Inc., USA) was put in front of a spectrometer (Acton SP-2150i, Princeton Instrument Inc., USA) with a 150mm focal distant, 600 lines/mm, and 500nm blaze and an electron multiplying charge coupled device (EM CCD) (PhotoMAX 512B, Princeton Instruments Inc., USA). To incubate HeLa cells on the GS-CPSBs, the Au/Ti coated corrugated polystyrene beads, which were not embedded in a PDMS, was exposed under UV light for 15min to sterilize the surface. Then immerse the substrate with GS-CPSBs into a DMEM medium containing HeLa cells for 6hr incubation to settle down cells on the substrate and observe the bright field images. After 20hr incubation, a 532nm laser was used as a light source with a filter cube (U-MNIB2, OLYMPUS) to observe the fluorescent emission light from the half part of the polystyrene beads without metal coating.

RESULTS AND DISCUSSION

The SEM images of the gold nanobowl particles are shown in Figure 1(e)-(f). The polystyrene beads were removed and the Au/Ti shells were left to form Au/Ti nanobowls. A carboxyl polystyrene bead was treated by a mixture gas of Ar 10sccm and O₂ 1sccm to form corrugated surface with nano-pillar sizes of ~20nm (Figure 1g). Another carboxyl polystyrene bead was etched by pure O₂ plasma to form a nano-pillar size of ~12nm (Figure 1h). The other one was etched by pure Ar plasma to form a nano-pillar size of ~30nm on the surface of the polystyrene bead (Figure 1i). The SEM picture in Figure 1(j) depicts the particles with 30nm nanopillars deposited with ~25nm Au/Ti. After the fabrication, PDMS was used to capture these nanoparticles for the fluorescent measurement and SERS observation (Figure 1(c)-(d)).

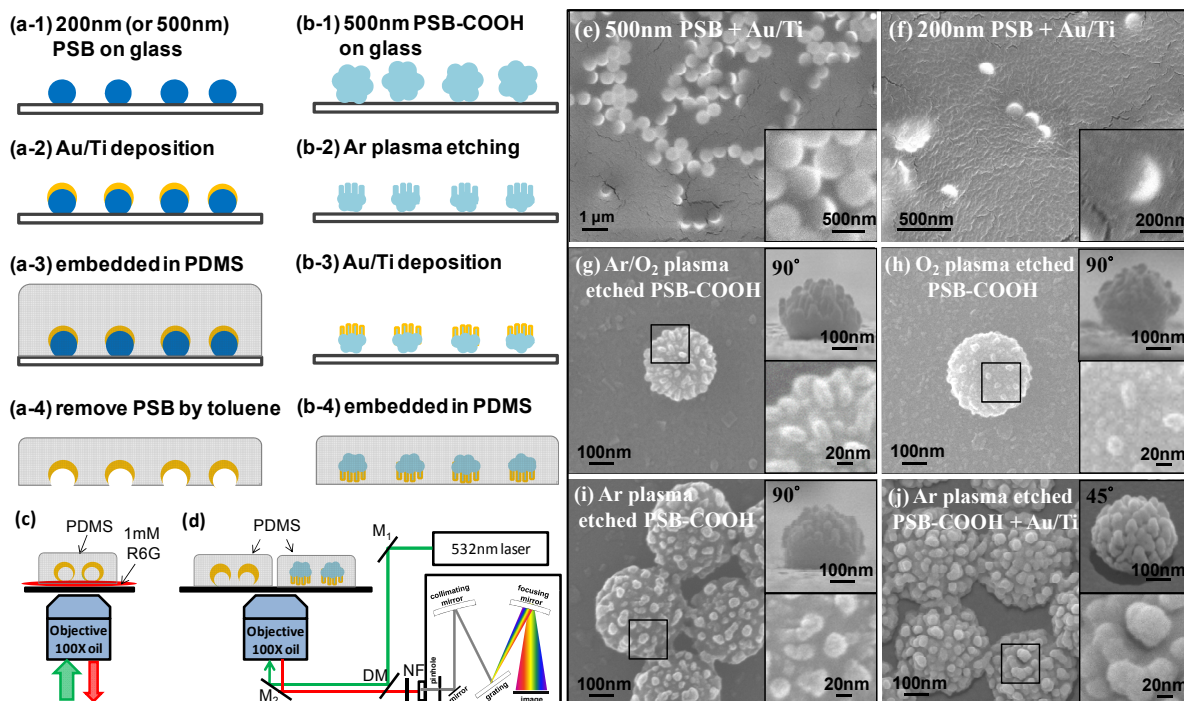


Figure 1. Fabrication processes of the (a) GS-PSBs and (b) GS-CPSBs structures, and the observation setup for (c) fluorescence observation, and (d) surface enhance anti-stoke Raman scattering measurement. (M_1M_2 : mirror. DM: dichroic mirror. NF: notch filter.) The SEM images of (e) Au/Ti nanobowls with 500nm inner diameter, (f) Au/Ti nanobowls with 200nm inner diameter, (g) Ar:O₂=10:1 plasma etched CPSBs with ~20nm nanopillars, (h) O₂ plasma etched CPSBs with ~12nm nanopillars, (i) Ar plasma etched CPSBs with ~30nm nanopillars (j) Au/Ti deposition of the CPSB (GS-CPSBs) in (i).

Figure 2(c) shows the cross-section intensity of 1mM R6G fluorescent dye enhanced by a nanobowl with a 500nm hole (top view in Figure 2(a)-(b)). The maximum enhancement appears in the sharp ring, or the hot-spot, of the nanobowl and the maximum quenching in the center of the hole, in agreement with previous studies [1-2]. The SERS results of these three structures in Figure 1((j), (e), and (f)) were listed in Figure 2((d), (e), and (f)), which represent the anti-stoke Raman signal of GS-CPSBs (350nm), Au-nanobowls (500nm), and Au-nanobowls (200nm), respectively. The GS-CPSBs show the highest anti-stoke Raman intensity among all, but several factors should be considered to fairly compare the enhancement ability. For examples, the particle sizes and numbers of those three samples should be also considered due to their contributions to total intensity. To normalize the factor of particle numbers, the enhancement factor E_f is defined as the formula in Figure 2. We counted the peak intensity by first smoothing the raw data, and then choosing the maximum number to be the intensity, I_{Au-PSB} , as well as calculating the intensity of the control group of only PDMS without any particles, $I_{w/o PSB}$. Thus the E_f can be obtained as an index to understand the SERS enhancement ability of a single particle for those three samples. The results in Figure 2(d)-(g) demonstrate the GS-CPSBs (350nm) with 30nm-nanopillars consist of a highest E_f value, ~2.4, among all and a comparable signal enhancement with that of Au-nanobowls (500nm). However, as the size of nanoparticle decreases, the SERS signal decreases dramatically [3]. Therefore, the GS-CPSBs possess the best enhancement ability while considering that the size of GS-CPSBs (350nm) is smaller than Au-nanobowls (500nm).

To demonstrate the biocompatibility of the GS-CPSBs for living cells, we delivered GS-CPSBs into HeLa cells by culturing cells on the GS-CPSBs sample for 2 days (Figure 3(b)-(c)). After 20hr incubation, the GS-CPSBs entered into the cell membrane by endocytosis. Then not only the fluorescence of GS-CPSBs can be seen in Figure 3(c), but also the particle can be tracked under a confocal microscope to monitor the position of the fluorescence emitted from a GS-CPSB. After two days incubation, the health condition of HeLa cells was still good as those on the Petri dish.

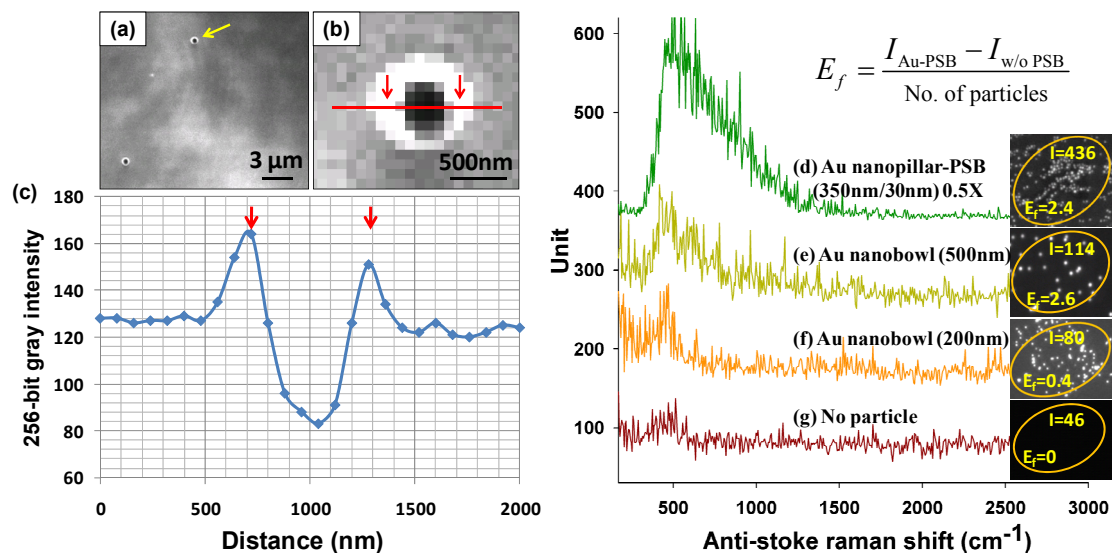


Figure 2. The fluorescence observation of Au nanobowl images and surface enhanced anti-stoke Raman scattering spectra of Au nanoparticles embedded in PDMS. (a)(b) R6G fluorescence image enhanced by a nanobowl particle with a 500nm inner hole in diameter. (c) The cross section intensity in (b). Anti-Stoke SERS spectra of (d) 350nm GS-CPSB with 30nm pillar size (the intensity is drawn as the half of the original intensity), (e) GS-PSBs with 500nm inner diameter, (f) GS-PSBs with 200nm inner diameter, and (g) Only PDMS w/o any particles. The orange ellipse shows the region of a 532nm laser spot in the images. “I” represents the anti-stoke Raman peak intensity of the 488 cm^{-1} . The enhancement factor E_f represents the spectrum intensity per nanoparticle.

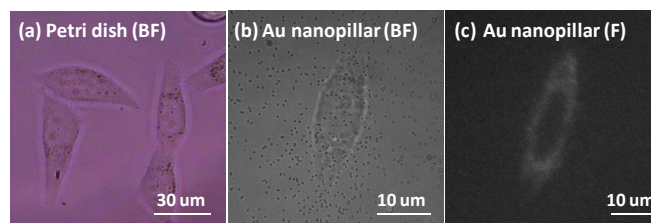


Figure 3. HeLa cells on (a) a Petri dish and (b) a gold film with GS-CPSBs after 6hr incubation. (c) The fluorescence image of HeLa cells on GS-CPSBs excited by a 532nm laser after 20hr incubation. BF: bright field image. F: fluorescence image.

CONCLUSION

In this paper, we have demonstrated a plasma etching method for the facile fabrication of corrugated polystyrene beads. While changing the ratio of argon and oxygen gases, the nano-pillars can be tuned as different sizes. This GS-CPSBs compared to gold nanobowls have much more hot-spot sites on a single bead. Therefore, the enhancement ability of the GS-CPSBs is better than gold nanobowls.

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