

Biocompatible and stable core/shell drug nanocarrier with high surface-enhanced Raman scattering activity

Xuebin Tan (谈学斌)¹, Zhuyuan Wang (王著元)¹, Hong Wang (王宏)², Jing Yang (杨晶)¹, Jin Li (李锦)¹, Chunyuan Song (宋春元)¹, Ruohu Zhang (张若虎)¹, and Yiping Cui (崔一平)^{1*}

¹Advanced Photonics Center, Southeast University, Nanjing 210096, China

²Clinical Laboratory Center of the First Affiliated Hospital of Nanjing Medical University, Nanjing 210011, China

*E-mail: cyp@seu.edu.cn

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A novel drug carrier based on SiO₂-coated silver nanoparticle aggregates and antitumor drug is successfully synthesized. The surface-enhanced Raman scattering (SERS) spectra of the antitumor drug in living cells are obtained. By using silver nano-aggregates as SERS substrates instead of dispersed silver particles, a great improvement of SERS signal intensity is achieved. It is found that the chemical stability of the drug carrier can also be increased with the existence of SiO₂ shell. The adsorbing effect between antitumor drug 9-aminoacridine (9AA) and silver particles is investigated to optimize the SERS signal. The core/shell structure of the drug carrier is characterized by ultraviolet-visible (UV-Vis) spectroscopy and transmission electron microscopy (TEM) pictures. The experimental results show that the drug carrier offers biocompatibility, stability, and high SERS activity, holding the potential for realizing the intracellular drug tracing.

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Surface-enhanced Raman scattering (SERS) is of great interest due to its potential application of detecting samples in biology^[1–5]. Compared with fluorescence spectra, SERS spectra own the advantages as narrow, separable, spectral linewidths and fingerprints^[6]. In order to obtain intense SERS signals in tissues or cells, surface modifications of the SERS probe are needed to improve the stability and biocompatibility in aggressive conditions. Recently, silica coated nanoparticles have appeared as attractive alternatives for their reproducibility and stability. Doering *et al.* have reported a silica-coated SERS-based nanoparticle structure^[7,8]. The results show that the robust SERS signal can be obtained from the Raman dye embedded in the silica shell and the silica-coated SERS probes are more stable than the uncoated ones^[7].

The 9-aminoacridine (9AA) has been employed as antibacterial, mutagenic^[9], and antitumor drug^[10,11]. It is interesting that the physic-chemical properties of these drugs are related to their ability to undergo a tautomeric equilibrium between two forms: amino and imino. Researches show that 9AA can adsorb on the surface of silver. The mechanism of this kind of adsorption is considered as the existence of different binding sites on metal surface. The active adsorption sites existing on the Ag colloid can be responsible for the chemical reactions observed in many molecules adsorbed on these kinds of metal surfaces^[12]. It is also reported that under the increase of Cl⁻ ion concentration, the ability of adsorption of 9AA on the metal surface is enhanced. In this way, the fluorescence signal of 9AA is quenched and the SERS signal increases as a consequence of the charge transfer from 9AA to metal.

Although it was reported that SiO₂-coated single metal nanoparticles can be used as SERS enhancement substrate for dyes molecules with the groups such as organothiol, isothiocyanate, or multiple sulfur atoms, the aggregation of metal nanoparticles may bring a larger

enhancement factor of SERS. Recently, Brown *et al.* indicated that by controlling the aggregation condition of Ag and Au nanoparticles, stable dye-tagged, silica coated metal nanoparticles could be synthesized and the SERS spectra of the label were also obtained^[13,14]. The advantage of the aggregated Ag or Au nanoparticles is the greater enhancement of SERS signal offered by more hop existed hot-spots^[15,16]. Huang *et al.* developed a kind of SERS probe (NAEBs) by encapsulating the dye-induced aggregation of Au nanoparticles with a protective outer silica shell and demonstrated the potential of NAEBs as Raman tags for biodetection^[17]. Herein, we propose a kind of drug carrier with high SERS activity, in which silver aggregates are used as SERS substrate instead of dispersed silver nanoparticles. It is found that compared with single Ag nanoparticles, the nanoparticle aggregation supplies more stable SERS intensity. Extinction spectra and transmission electron microscopy (TEM) pictures are used to characterize the formation of the core/shell structure process. High signal-to-noise ratio (SNR) SERS spectra of 9AA in the capsulated drug carrier are obtained in living Hela cells. The results show the potential of the probe for being used as an alternative way to design drug carrier nanodevices.

The materials (3-mercaptopropyl) trimethoxysilane (MPTMS), rhodamine 6G (R6G), and 9-aminoacridine hydrochloride (9AA-HCl) were purchased from Alfa Aesar. Sodium silica solution was purchased from Sigma-Aldrich. Silver nitrate was obtained from Shanghai Shenbo Chemical Co., Ltd. Trisodium citrate dehydrate was purchased from Shanghai Heiwei Chemical Co., Ltd. The water used in the experiments was ultrapure deionized water.

Ag nanoparticles were synthesized based on the method of Lee *et al.*^[18] Briefly, AgNO₃ solution (10 mmol/L, 50 mL) in 500-mL distilled water were brought to boiling under stirring. Then 1% sodium citrate solution (10 mL)

was added. The mixture was kept on boiling for about 1 h under stirring. The prepared Ag nanoparticles were greenish yellow and had extinction maximum at 429 nm.

The SiO₂-coated 9AA-HCl tagged nanoparticles were prepared as follows. 15-mL as-prepared Ag colloid was mixed with 9AA-HCl solution (1 mmol/L, 15 μ L) under stirring. The final concentration of the 9AA molecule was 1 μ mol/L. After being stirred for 5 min, NaCl solution (100 μ L, 0.5 mol/L) was added to induce the aggregation of the 9AA-tagged Ag nanoparticles. MPTMS (10 μ L, 2.63 μ mol/L) was added to the above solution and stirred for 15 min. Finally, 3-mL sodium solution (pH10.8) was added to the mixture and stirred for 42 h under room temperature.

Ultraviolet-visible (UV-Vis) spectra were measured by using a Shimadzu UV-3600 PC spectrophotometer with quartz cuvettes of 1-cm path length. The cell images were recorded by confocal microscopy (Olympus Fluo View 1000) with a 10 \times microscope objective. SERS spectra were recorded at 632.8-nm excitation (2.36 mW at the sample) and detected via confocal microscopy system, and the Rayleigh scattering light was removed by a holographic notch filter. The Raman scattering light was directed to an Andor Shamrock spectrograph equipped with a Newton 303i charge-coupled device (CCD). A Sartorius PP-15 pH meter was employed for all pH measurements, using a standard electrode with a three-buffer calibration (typically pH4.0, 7.0, and 11.0). The TEM (JEOL 2100) measurements were performed at 200 kV. The fluorescence intensity of 9AA was collected by Edinburgh FLS900 instruments.

Hela cells were purchased from Nanjing KeyGen Biotech. Co., Ltd. and cultured in medium (diethylene glycol dimethyl ether, DMEM) under a humidified atmosphere (5% CO₂ plus 95% air) at 37. Media were supplemented with 10% heat-inactivated newborn calf serum (Hangzhou Every Green Organism Engineering Materials Co., Ltd.), and 1% penicillin-streptomycin (Nanjing KeyGen Biotech. Co., Ltd.). The as-prepared SERS probes were added into the cell plates with a volume rate of 1:3 of the culture medium and incubated for 0.5, 1, 1.5, 2, and 3 h, respectively. Before SERS measurements, the cell plates were washed with phosphate buffer solution (PBS) for three times.

The principal steps of preparation of the SiO₂-coated, 9AA-tagged Ag nanoparticles is depicted in Fig. 1. The surface of Ag nanoparticles was first adsorbed by 9AA molecules through electrostatic adsorption. Then an appropriate amount of NaCl solution was added to the 9AA-tagged Ag nanoparticles to induce the aggregation of Ag nanoparticles for optimizing the SERS signal of the probe. Here the amount of NaCl solution was critical for the SERS performance of the probe. Insufficient concentration of NaCl will cause a low enhancement of SERS signal due to the poor aggregation condition, while excessive amount of NaCl will destroy the stability of the Ag colloid system and lead to a nonreversible aggregation. After the aggregation phase of the Ag nanoparticles was formed, MPTMS molecules were introduced to displace some of the 9AA molecules attached on the surface of Ag. During the experiments, it was demonstrated that too much amount of MPTMS will cause the decrease of

SERS intensity due to the declination of 9AA molecules on the Ag surface. So a careful calculation was needed to make sure that there was enough MPTMS molecules on the surface of metal to serve as brocks of growth of SiO₂ shell while not sacrificing the SERS signal. After the MPTMS molecules adsorbed on the Ag surface, the activated sodium solution (pH10.8) was added to the mixture. After 42-h stirring, a SiO₂ shell (3–5 nm thick) was formed. The main difference between the coated and uncoated SERS probes is their chemical stability. Results show that after being coated with SiO₂, the probe can sustain in 0.1-mol/L NaCl solution while remaining its property.

Figure 2 shows the UV-Vis spectra of raw Ag colloids and SiO₂-coated SERS probe after being filtrated with filter (0.22 μ m). The plasmon resonance peak of the raw Ag colloids was around 429 nm. To obtain large enhancements of SERS, Ag nanoparticles were aggregated by using NaCl solution as aggregation agent. With the increase of NaCl, a decrease of the plasmon resonance peak and an increase in the band after 630 nm, which resulted from the aggregation of Ag nanoparticles, were found. During the aggregation procedure, the color of the Ag colloids changes from greenish yellow to dark grey.

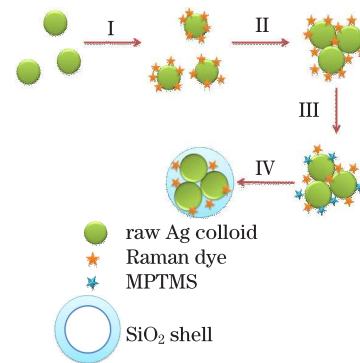


Fig. 1. Preparation of SiO₂-coated 9AA-tagged Ag nanoparticles. (I) Addition of Raman reporter (9AA) to raw Ag nanoparticles, (II) addition of NaCl solution (100 μ L, 0.5 mol/L) to induce the aggregation of 9AA-tagged Ag nanoparticles, (III) addition of MPTMS (10 μ L, 2.63 μ mol/L) to the mixture, (IV) addition of 3-mL activated sodium solution (pH10.8) to the Ag nanoparticles with a 42-h stirring to form a SiO₂ shell.

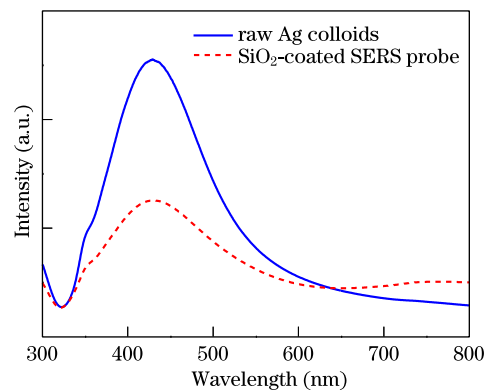


Fig. 2. Extinction spectra of Ag nanoparticles and SiO₂-coated 9AA-tagged Ag nanoparticles.

The excessive amount of NaCl will cause large aggregation, which may result in particle precipitation.

The size and morphology of the SiO₂-coated 9AA-tagged SERS probe were characterized by TEM pictures. In Fig. 3, it is observed that Ag nanoparticles were aggregated before the formation of SiO₂ shell. Meanwhile, a SiO₂ shell with a thickness of 3–5 nm can be found around the Ag nanoparticles. To make the probe deliver into living cell easy, the SiO₂ shell here was controlled in an appropriate thickness so that the probe was too large to enter the living cells, while the shell was also thick enough to maintain the chemical stability of the probe in aggressive conditions. The SiO₂ shell was also used to control the aggregation procedure here for the formation of nano-aggregates with 2–5 Ag nanoparticles^[17]. The large aggregates may be removed by low speed centrifugation or filtering. It was also found that some dye may penetrate the thin SiO₂ shell gradually to the surrounding solution, which may have potential applications in drug release.

To examine the chemical stability of SiO₂-coated 9AA-tagged SERS probe, we compare the extinction spectra of Ag colloids and SiO₂-coated SERS probe upon the same added amount of NaCl (0.1 mol/L). As depicted in Fig. 4, it is found that after the addition of the NaCl solution, the intensity of the extinction peak of the Ag colloids drops obviously and the color of the Ag colloid changes from greenish yellow to dark yellow. Meanwhile, the extinction intensity in the red wavelength region increases a little. The changes of extinction spectra indicate the aggregation of the Ag colloids. However, with the same amount of the added NaCl solution, the SERS intensity of the probe only decreases a little, and in the red wavelength region, there is almost no change, which indicates that the SERS probe with a SiO₂ shell is more stable than the raw Ag nanoparticles due to the protection function of SiO₂ shell.

In order to study the adsorbing effects of 9AA and MPTMS towards the surface of Ag nanoparticles, we measured the fluorescence and SERS intensities of 9AA and Ag with/without the existence of MPTMS. Figure 5 shows that by adding MPTMS to the 9AA saturated Ag nanoparticles, the fluorescence of 9AA increases and the SERS intensity declines obviously, which means that some 9AA molecules are removed from the surface of Ag and then contribute to the enhancement of fluorescence in solution. In addition, due to the reduction of the 9AA amount on the surface of Ag, the SERS intensity decreases. This demonstrates that the adsorbing effects between MPTMS and Ag nanoparticles are stronger than that between 9AA and Ag nanoparticles. The strong conjugate adsorbing ability of thiol group of MPTMS towards Ag surface will cause the attached 9AA molecules on the Ag surface to be replaced by MPTMS.

Figure 6 shows the SERS spectra of SiO₂-coated 9AA-tagged SERS probe in solution and Hela cell. Since the extinction peak of 9AA is at 400 nm, it can be seen that under non-resonance excitation (632.8 nm), 9AA owns high SERS intensity. This prevents photobleaching or phototoxicity and results in a high stability of the label^[19]. Before the SERS spectra in Hela cell being measured, the probes were incubated in living Hela cell for 2.5 h. In order to reduce the influence of background

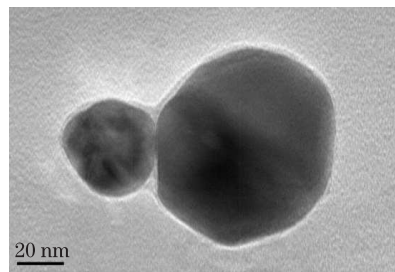


Fig. 3. TEM image of SiO₂-coated 9AA-tagged SERS probe.

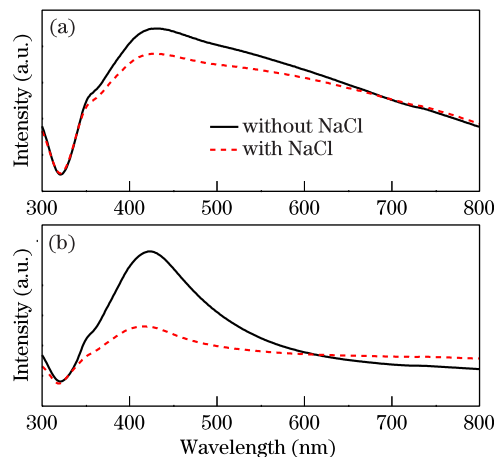


Fig. 4. UV-Vis spectra of (a) SiO₂-coated probe and (b) raw Ag nanoparticles upon addition of 0.1-mol/L NaCl solution.

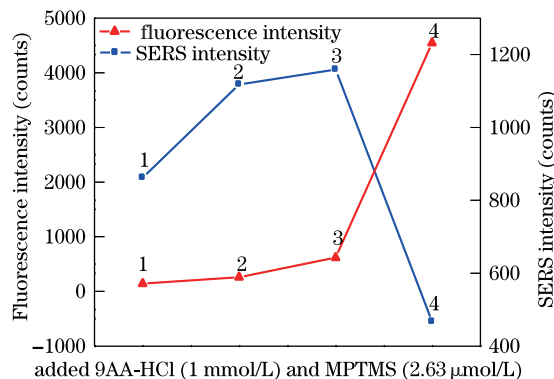


Fig. 5. SERS and fluorescence spectra of various amounts of 9AA-HCl and MPTMS in 1-mL Ag nanoparticles. (1) 15- μ L 9AA-HCl, (2) 30- μ L 9AA-HCl, (3) 35- μ L 9AA-HCl, (4) 35- μ L 9AA-HCl and 10- μ L MPTMS. The SERS intensity (1371cm^{-1} ref.) was measured under 632.8-nm excitation (2.36 mW). The fluorescence intensity was recorded under the 360 nm.

of SERS, PBS buffer was used to clean the probe deposited on the bottom of the tissue culture dish. It can be seen that the bands at 1128, 1163, 1267, 1367, 1454, 1497, 1558, and 1586cm^{-1} decrease in Hela cells while the band at 1233cm^{-1} increases. The reason for the changes in spectra may be the drug effects of 9AA towards Hela cell, which will lead to the transformation of structure of 9AA molecules or changing the adsorbing methods between 9AA and Ag surface for the chemical environment difference in and out the living cells. It is also illustrated that there is a decrease of the total SNR

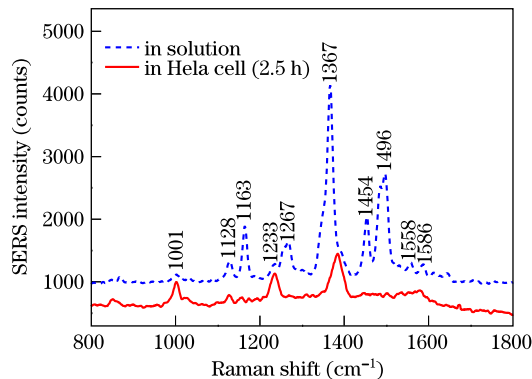


Fig. 6. SERS spectra of SiO₂-coated SERS probe in solution and in HeLa cell. The Raman dye is 9AA. The excitation wavelength is 632.8 nm (2.36 mW) with 60-s integration time and 100- μ m slit width.

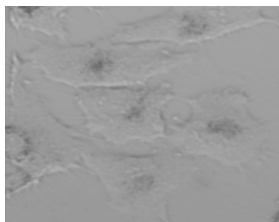


Fig. 7. Transmission image of SiO₂-coated SERS probe incubated in HeLa cells for 1.5 h.

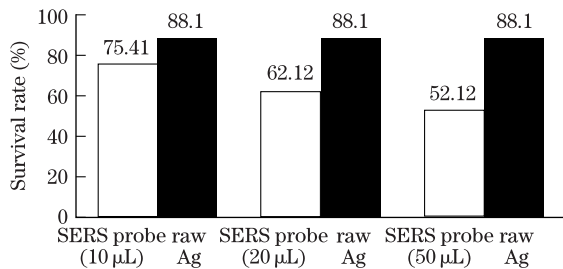


Fig. 8. Survival rate of HeLa cells incubated with SERS probe and raw Ag nanoparticles (20 μ L).

level of the SERS spectra compared with that in solution. This is because the distribution and concentration of SERS probe in HeLa cells rely on the modification of the surface and size of the probe. Gold and silver nanoparticles are both common SERS enhancing materials. However, the biocompatibility of silver substrates is not as good as that of gold substrates. So it is necessary to modify the surface of Ag nanoparticles to make them more biocompatible and nontoxic. The results show that with Raman labels such as R6G, the SiO₂-coated probe can also obtain robust and sensitive SERS spectra in living HeLa cells. The morphology of HeLa cells cultured with SiO₂-coated SERS probe is shown in Fig. 7. After the SERS probe is incubated in living HeLa cells for 1.5 h, the SERS probe can be found inside the living HeLa cells, which indicates that SiO₂-coated SERS probe may not cause too much damage to the cell activity. Meanwhile, the normal morphology of the living HeLa cell also demonstrates the biocompatibility of this kind of SERS probe.

In order to study the drug release ability of 9AA in

living HeLa cells and its drug effects to the cancer cell, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was utilized to measure the cell proliferation. Since raw Ag nanoparticles may suppress the proliferation of HeLa cells, Fig. 8 describes the survival rate of HeLa cells upon different amounts of SiO₂-coated 9AA-tagged probe and Ag nanoparticles. It can be seen that as the amount of probe increases from 10 to 50 μ L, the survival rate drops, which may be due to the accumulation of 9AA in the culture medium.

In conclusion, the core/shell structure of SERS active drug carrier is demonstrated based on aggregated Ag nanoparticles. We study the SERS performance of such silica-coated 9AA-tagged SERS probe in living HeLa cells. It is found that by using the aggregated Ag nanoparticles instead of the dispersed ones, much stronger SERS signal can be obtained. Besides, compared with the uncoated SERS probe, the silica-coated SERS probe has more biocompatibility and stability, showing its potential application in drug tracing at cellular level. The release ability of 9AA from the probe to the surrounding solution demonstrates the value of such nanostructure serving as an antitumor drug carrier.

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