Interaction between antitumor drug and silver nanoparticles: combined fluorescence and surface enhanced Raman scattering study

Jing Yang (杨晶)¹, Hong Wang (王华)², Zhuyuan Wang (王家元)¹, Xuebin Tan (谈学斌)¹, Chunyuan Song (宋春原)¹, Ruohu Zhang (张若虎)¹, Jin Li (李健)¹, and Yiping Cui (崔平)∗¹

¹ Advanced Photonics Center, Southeast University, Nanjing 210096, China
² Clinical Laboratory of the First Affiliated Hospital of Nanjing Medical University, Nanjing 210006, China
*E-mail: cyp@seu.edu.cn
Received July 14, 2009

Optical methods and MTT method are used to characterize the antiproliferation effect of antitumor drug 9-aminoacridine (9AA) with and without silver nanoparticles. Intracellular surface enhanced Raman scattering (SERS) spectra and fluorescent spectra of 9AA indicate the form of 9AA adsorbed on the surface of silver nanoparticles. Although both silver nanoparticles and antitumor drug can inhibit the growth of Hela cells, silver nanoparticles can slow down the antiproliferation effect on Hela cells at low concentration of antitumor drugs. Our experimental results suggest that silver nanoparticles may serve as slow-release drug carriers, which is important in antitumor drug delivery.

OCIS codes: 160.4236, 290.5860, 180.1790.
doi: 10.3788/COL20090710.0894.

In the field of drug therapy, materials of drug carriers are propitious to control the speed of drug to release in an effective range and last for a long time, in which case patients could suffer less from taking medicine for many times. Currently, drug delivery materials are focused on polymers micelle drug carriers[1], polysaccharides[2], proteins[3], peptides[4], lipids, and hydrogels[5,6], etc. Though great progress has been made on the design and synthesis of smart (stimuli-sensitive) drug carriers as well as the detection methods, there are few direct methods for monitoring the delivery process and providing spectra information in living biosystems. Instead, the physical and chemical properties of those drug carriers are usually measured before being applied to biosystems, or only the proliferation of the cells are observed to characterize the efficiency of the drug carriers.

Nanocarriers have been extensively investigated to facilitate the intracellular delivery of therapeutics[7,8]. Here, we use silver nanoparticles as the antitumor drug release materials. Compared with other drug release materials, the unique surface plasmonic properties of the silver particles make it possible to observe the drug release process in living cells by surface enhanced Raman scattering (SERS) method. As is well known, the usually weak Raman signals can be remarkably enhanced by several orders of magnitude when Raman scattering takes place on molecules at the surface or in the very close vicinity to noble metal nanostructures[9,10]. In recent years, SERS has become a promising tool for monitoring in vitro or in vivo biological processes[11-14]. The intercellular SERS signals provide direct information of the interactions between antitumor drug, silver nanoparticles, and living cells.

The antitumor drug 9-aminoacridine (9AA) is chosen as the drug model in the experiments. Acridine drugs are important intercalant molecules with mutagenic, antitumor, and antibacterial properties[15]. Up to now, 9AA has been successfully employed as antibacterial, mutagenic[16,17], and antitumor drug[18,19]. As an antitumor drug, 9AA exhibits antiproliferation effect on cancer cells. The acridine core intercalates DNA base pairs and then the formed drug-DNA complex blocks the activity of topoisomerase Π, leading to a catalytic inhibition of the enzyme, an induction of apoptosis, and then a programmed cell death[20]. However, it is interesting that the antiproliferation effect of 9AA on Hela living cells can be slowed down at the presence of silver nanoparticles. We analyze such a phenomenon considering the intracellular interaction between 9AA and the surface of silver particles using combined SERS spectra and fluorescence spectra. Our experimental results suggest that silver nanoparticles may hold the potential for slow-release drug carriers.

Silver nanoparticles were synthesized by the method of Lee[21]. In brief, 18-mg AgNO₃ dissolved in 100-mL deionized water was heated to boiling. 2 ml of 1% trisodium citrate was added in the solution under intense stir. After 1-h boiling, the silver nanoparticles turned into gray color. The as-prepared colloid was stored in sealed and dark condition for future use.

Hela cells were purchased from Nanjing KeyGen Biotech Co., Ltd., and cultured in medium (DMEM) under a humidified atmosphere (5% CO₂ plus 95% air) at 37 ºC. Media were supplemented with 10% heat-inactivated newborn calf serum (Hangzhou Every Green Organism Engineering Materials Co., Ltd.), and 1% penicillin-streptomycin. For fluorescence and SERS experiment, Hela cells were seeded into tissue culture dishes and incubated for 24 h. The culture medium was replaced by the one mixed with 9AA-adsorbed silver nanoparticle dispersion (3:1 in volume ratio) and incubated for 7 h. For fluorescence images of the 9AA stained Hela cells, 10 µL of 1-nmol/L 9AA solution was added into the culture medium and incubated for 1 h.

was added, a mountain-like absorption band belonging to the sorption spectrum when 10-3 mol/L of 9AA hydrochloride may induce the aggregation of nanoparticles formed upon the addition of 9AA solution, the new absorption band exhibiting a red shift with an intensity increase. The chloride ions of 9AA, nanoparticles, and the mixture on cell proliferation were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. 50 µL of MTT solution (MTT buffer to dilution buffer 1:4) was added to each well and the plates were incubated for 4 h. The reaction was terminated by adding 150 µL DMSO (Sigma) after removing the supernatant medium. When the purple formazan crystals were resolved by DMSO, the absorbance of the wells at 490 nm was measured with a microplate reader (BIO-RAD Model 680). Cells incubated in the absence of drugs and silver nanoparticles were used as controls. For each experiment, Hela cells were washed with phosphate buffered saline (PBS) for three times before use.

Absorption spectra were measured with a Shimadzu UV-3600 PC spectrophotometer. For absorption measurement of silver particles, 1-mL silver colloid was diluted to 3-mL with deionized water to prevent the saturation of absorption. For absorption measurement of the mixture of silver particles and 9AA, 1-mmol/L 9AA solution was added to 3 mL of the above diluted silver colloids with different volumes of 3, 5, 8, 10, 20, and 100 µL, respectively. Fluorescence spectra were obtained using Edinburgh FLS900 instruments. Intracellular fluorescence images were taken with an Olympus confocal scanning microscope. In the experiments, the excitation wavelength was selected at 458 nm. An oil immersion objective (60 ×) was used to focus the excitation beam. For SERS measurements, holographic notch filters were used to remove the Rayleigh scattering light and the Raman scatter light was directed to a spectrograph equipped with a Newton303i charge-coupled device (CCD) detector. A 10 × microscope objective was used to focus the excitation beam (argon ion laser, 514.5 nm with a power of 1.3 mW) to the sample surface.

As shown in Fig. 1, 9AA exhibits unique optical properties as having the symmetric ultraviolet (UV) absorption and fluorescent emission spectra. The spectrum of pure silver nanoparticles shows a single absorption peak at 420 nm due to the nanoparticle plasmon resonance, as shown in Fig. 2. The addition of 9AA to silver nanoparticles caused a significant decrease of the absorption peak while a new broad absorption band appeared at longer wavelength regions. The new band occurring in the near-infrared (NIR) region is well known to arise from the aggregation of nanoparticles formed upon the addition of the adsorbing molecules[22]. With the continuous addition of 9AA solution, the new absorption band exhibited a red shift with an intensity increase. The chloride ions of 9AA hydrochloride may induce the aggregation of silver nanoparticles which may result in large clusters. One thing needs to be mentioned is that a small band appeared and stacked on the band of silver colloid absorption spectrum when 10-µL 9AA solution was added into the silver colloid solution. It indicates that the 9AA molecules have already occupied all the surface sites by forming a monolayer. When more 9AA solution (12 µL) was added, a mountain-like absorption band belonging to 9AA absorption was obviously observed, indicating that the redundant 9AA molecules may have escaped from the silver surfaces. The concentration of 9AA at the turning point was 3.3 × 10−3 mmol/L.

To monitor the dynamic process of 9AA being added to the silver solution, fluorescence spectra were taken with different added amounts of 9AA. At the beginning, the fluorescence intensity of 9AA slowly increased linearly with the amount of added 9AA solution. In Fig. 3, it can be seen that when 10-µL 9AA solution was added, the fluorescence intensity increased suddenly and then increased linearly with the added 9AA amount. The above phenomena could be explained as follows. At the beginning, 9AA molecules continuously adsorbed onto the surface of silver nanoparticles. Since the fluorescences of most 9AA molecules were quenched due to the charge transfer to the metal surfaces, the fluorescence intensity of the whole mixture solution kept at a low level. Then with the further increase of the added amount of 9AA, they began to form a monolayer until the turning point was reached. Finally, after the surface sites of nanoparticles were occupied completely, the further added 9AA molecules started to dissociate into the mixture solution, and they were unable to be quenched by silver nanoparticles any more. That is to say, the dissociated molecules made a great contribution to the fluorescence intensity increase after the turning point. By curve fitting, the turning point was found to be 9.9 µL and the concentration of the 9AA was about 3.3 × 10−3 mmol/L. Thus, the fluorescence spectra measurement result proved a similar process of the interaction between 9AA molecules and
attributed to δ1255-cm−1 vibrations coupled to exocyclic δing incorporated into living cells, the 1607-cm−1 SERS intensities of three bands decreased after 9AA be-
the SERS spectra of 9AA in silver colloid solution, the living Hela cells are shown in Fig. 5(b). Compared with
The obtained SERS spectra from 9AA incorporated in
cess in living cells at the presence of silver nanoparticles , control the release of drugs inside the living cells.

details are needed to explain how silver nanoparticles
be deduced that when mixed with silver nanoparticles,
some 9AA molecules could not move freely inside cells
but adsorb on the surfaces of silver nanoparticles. More
details are needed to explain how silver nanoparticles
control the release of drugs inside the living cells.

For a better understanding of the 9AA releasing pro-
cess in living cells at the presence of silver nanoparticles, intracellular 9AA SERS spectra were obtained and com-
pared with those in solutions. Rivas et al. have reported
that 9AA is adsorbed on the silver surface under two
different forms: strongly attached 9AA characterized by
imino form and weakly attached 9AA by amino form[23].
The obtained SERS spectra from 9AA incorporated in
living Hela cells are shown in Fig. 5(b). Compared with
the SERS spectra of 9AA in silver colloid solution, the
SERS intensities of three bands decreased after 9AA be-
ing incorporated into living cells, the 1607-cm−1 band
attributed to δ(NH2) motions, 1560-cm−1 ring stretching
vibrations coupled to exocyclic δ(NH2) motions, and the
1255-cm−1 band contributed by γ(C-NH2) vibrations.

This demonstrated that the amino vibrations were weak-
ened when the 9AA-absorbed silver nanoparticles en-
tered into living Hela cells. To explain the SERS spectra
changes of 9AA after being incorporated into living cells, two hypotheses are made. One is the change of pH en-
vironment, which will cause the tautomer form changes
of 9AA molecules. The other is the decrease of the num-
ber of 9AA molecules with amino group. It is known
that 9AA molecules adsorb to the silver surface through
unprotonated imino form under alkaline and neutral pH
condition, while through a protonated amino form under
acid pH condition[24]. Considering that the pH value of
the original prepared 9AA functionalized silver colloid
is 10.04 but the pH environment of Hela cells is neutral
except lysosomes (below 6), it could be deduced that
the SERS decrease of amino band is not attributed to
the change of pH value. So the decrease of amino vibra-
tion bands of intracellular SERS spectra could be due
to the decrease of the amino formed 9AA molecules on
the silver surfaces. That is to say, the spectral change of
SERS spectra of 9AA under intracellular conditions may
be caused by parts of the 9AA molecules breaking away
from the surfaces of the silver nanoparticles due to the
weakened bonding ability of amino groups. The escaping
9AA molecules may diffuse to the cytoplasm and exert
the antitumor effect. All the above experimental results
indicate that silver nanoparticles play an important role
in controlling the drug release in biosystems.

As an antitumor drug, 9AA has a proliferation inhibi-
tion effect to Hela cells, and the survival rate decreases
with the accumulated concentration of 9AA. As shown
in Fig. 6, silver nanoparticles themselves can inhibit
the cells at 89%. Besides, silver nanoparticles change the
behaviors of the drug in living Hela cells. More interest-
ingly, we find that the silver particles play different roles
for the 9AA treated Hela cells when the 9AA concen-
tration changes. For cells incubated with 9AA at high
concentrations, silver nanoparticles increase the inhibi-
tion effect on proliferation. However, for those incubated
with 9AA at low concentrations, silver nanoparticles re-
duce the inhibition effect on proliferation.

The fluorescence, absorption, and SERS spectra may
give an answer to the above phenomenon. The inter-
action between 9AA molecules and silver nanoparticles
plays a key role for the inhibition effect on proliferation
of Hela cells at different 9AA concentrations. At low concen-
trations (below 3.3 ×10−3 mmol/L), 9AA molecules

silver nanoparticles as compared with that obtained by
absorption spectroscopy.

The intense fluorescence of 9AA makes it possible to
detect its distribution in Hela cells by fluorescence im-
ages. As shown in Fig. 4, 9AA molecules were mainly
located in the cytoplasm, especially in the endoplasmic
reticulum. Only a fraction of 9AA molecules entered the

cell nucleus. However, in the presence of silver nanoparti-
cles, 9AA molecules were unable to have the same cellular
distribution as in the pure 9AA stained cells. It could
be deduced that when mixed with silver nanoparticles,
some 9AA molecules could not move freely inside cells
but adsorb on the surfaces of silver nanoparticles. More
details are needed to explain how silver nanoparticles
control the release of drugs inside the living cells.

For a better understanding of the 9AA releasing pro-
cess in living cells at the presence of silver nanoparticles, intracellular 9AA SERS spectra were obtained and com-
pared with those in solutions. Rivas et al. have reported
that 9AA is adsorbed on the silver surface under two
different forms: strongly attached 9AA characterized by
imino form and weakly attached 9AA by amino form[23].
The obtained SERS spectra from 9AA incorporated in
living Hela cells are shown in Fig. 5(b). Compared with
the SERS spectra of 9AA in silver colloid solution, the
SERS intensities of three bands decreased after 9AA be-
ing incorporated into living cells, the 1607-cm−1 band
attributed to δ(NH2) motions, 1560-cm−1 ring stretching
vibrations coupled to exocyclic δ(NH2) motions, and the
1255-cm−1 band contributed by γ(C-NH2) vibrations.

This demonstrated that the amino vibrations were weak-
ened when the 9AA-absorbed silver nanoparticles en-
tered into living Hela cells. To explain the SERS spectra
changes of 9AA after being incorporated into living cells, two hypotheses are made. One is the change of pH en-
vironment, which will cause the tautomer form changes
of 9AA molecules. The other is the decrease of the num-
ber of 9AA molecules with amino group. It is known
that 9AA molecules adsorb to the silver surface through
unprotonated imino form under alkaline and neutral pH
condition, while through a protonated amino form under
acid pH condition[24]. Considering that the pH value of
the original prepared 9AA functionalized silver colloid
is 10.04 but the pH environment of Hela cells is neutral
except lysosomes (below 6), it could be deduced that
the SERS decrease of amino band is not attributed to
the change of pH value. So the decrease of amino vibra-
tion bands of intracellular SERS spectra could be due
to the decrease of the amino formed 9AA molecules on
the silver surfaces. That is to say, the spectral change of
SERS spectra of 9AA under intracellular conditions may
be caused by parts of the 9AA molecules breaking away
from the surfaces of the silver nanoparticles due to the
weakened bonding ability of amino groups. The escaping
9AA molecules may diffuse to the cytoplasm and exert
the antitumor effect. All the above experimental results
indicate that silver nanoparticles play an important role
in controlling the drug release in biosystems.

As an antitumor drug, 9AA has a proliferation inhibi-
tion effect to Hela cells, and the survival rate decreases
with the accumulated concentration of 9AA. As shown
in Fig. 6, silver nanoparticles themselves can inhibit
the cells at 89%. Besides, silver nanoparticles change the
behaviors of the drug in living Hela cells. More interest-
ingly, we find that the silver particles play different roles
for the 9AA treated Hela cells when the 9AA concen-
tration changes. For cells incubated with 9AA at high
concentrations, silver nanoparticles increase the inhibi-
tion effect on proliferation. However, for those incubated
with 9AA at low concentrations, silver nanoparticles re-
duce the inhibition effect on proliferation.

The fluorescence, absorption, and SERS spectra may
give an answer to the above phenomenon. The inter-
action between 9AA molecules and silver nanoparticles
plays a key role for the inhibition effect on proliferation
of Hela cells at different 9AA concentrations. At low concen-
trations (below 3.3 ×10−3 mmol/L), 9AA molecules


is not enough to occupy all the surface sites. The interaction between 9AA molecules and silver particles is so strong that most of the 9AA molecules with imino groups are unable to escape from the surfaces of silver nanoparticles. So the adsorbed imino formed 9AA molecules cannot exert the drug effect as much as those in free. As a result, the survival rate of the cells is higher than those incubated with pure 9AA at the same concentration. On the contrary, in the case of cells incubated with 9AA at high concentrations (above $3.3 \times 10^{-5}$ mmol/L), 9AA molecules keep adsorbing on the surfaces of silver nanoparticles until a monolayer is formed. The redundant 9AA molecules and the adsorbed 9AA molecules through amino groups will escape from the silver surfaces and diffuse to the cytoplasm, leading to an increased inhibition effect on proliferation. Besides, a high concentration of 9AA will induce the silver nanoparticles to form large aggregations having a great injury to the cellular membrane, which may also contribute to the increased inhibition on the proliferation at high 9AA concentration. So the survival rate of Hela cells treated with the silver and 9AA complex at high 9AA concentrations is lower than those incubated with pure 9AA at the same concentration.

In conclusion, we have investigated the intracellular interaction between antitumor drug 9AA and silver nanoparticles using the combined SERS spectra and fluorescence spectra. We have observed different antiproliferation effects of 9AA on Hela cells with and without silver nanoparticles. It is found that silver nanoparticles can be used to control the release of 9AA in living cells. The reason may be that silver nanoparticles can hold the surrounding 9AA molecules to its surface until a monolayer is formed. The way 9AA adsorbing on the silver surfaces plays an important role in their delivery effect in living cells. Compared with other drug release methods such as using polymers or silica nanoparticles, the way using silver nanoparticles has the advantage of providing more spectral information, such as SERS spectra. By optimizing the surface modification, silver nanoparticles may hold great potential in smart drug carrier applications.

This work was supported by the National Natural Science Foundation of China (Nos. 60708024 and 60877024).

References