Journal of Biomedical Optics

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Abstract. Gold nanoparticles are delivered into living cells by transient electroporation method to obtain intracellular surface-enhanced Raman spectroscopy (SERS). The subcellular localization of gold nanoparticles is characterized by transmission electron microscopy, and the forming large gold nanoaggregates are mostly found in the cytoplasm. The SERS detection of cells indicates that this kind of gold nanostructures induces a high signal enhancement of cellular chemical compositions, in addition to less cellular toxicity than that of silver nanoparticles. These results demonstrate that rapid incorporation of gold nanoparticles by electroporation into cells has great potential applications in the studies of cell biology and biomedicine. [DOI: 10.1117/1.JBO.20.5.051005]

Keywords: gold nanoparticles; surface-enhanced Raman spectroscopy; electroporation; cell analysis.

Paper 140419SSPR received Jun. 30, 2014; accepted for publication Oct. 21, 2014; published online Nov. 11, 2014.

1 Introduction

Raman spectroscopy, which provides molecular and structural information of biological samples, is playing an increasingly vital role in biomedical analysis in recent years. In particular, because of its narrow Raman bands and low water influence, Raman spectroscopy is suitable for characterizing the compositions of complex biochemical mixtures.[1] Raman scattering is an extremely weak effect since the typical Raman cross section is between 10^{-31} and 10^{-29} cm^2 per molecule.[2] However, with the emergence of surface-enhanced Raman scattering (SERS) spectroscopy, Raman signals can be enhanced by many orders of magnitude (10^2 to 10^{14} times).[3,4]

"Passive uptake" is one of the most common methods used for delivering silver or gold nanoparticles into cells.[5–7] However, this approach is time-consuming in that cells need to be incubated with nanoparticles for several hours or even more, and the amount of nanoparticles delivered into the cells is highly dependent upon the physical dimension of the nanoparticles.[8] To address these issues, we have successfully delivered silver nanoparticles into the cells through electroporation, which is much faster and more efficient than the "passive uptake" method.[9] Notably, however, gold nanoparticles are preferred over silver nanoparticles in many biomedical applications because of their favorable physical and chemical properties and biocompatibility.[10,11] Near-infrared (NIR) is also the desired excitation range for Raman detection of cells because the lower-energy photons reduce the fluorescence background in biological samples and decrease the risk of cell degeneration.[11,12] Meanwhile, gold nanoparticles have comparably good SERS enhancement factors as silver nanoparticles when NIR excitation is applied.[12] In this study, we explore the use of gold nanoparticles and NIR laser excitation for SERS application in the biochemical analysis of single-living cells and deliver gold nanoparticles by transient electroporation.

2 Materials and Methods

2.1 *Preparation of Metallic Nanoparticles*

Gold and silver nanoparticles were synthesized according to the protocols reported by Grabar et al.[13] and Munro et al.,[14] respectively. A Perkin–Elmer Lambda 950 spectrophotometer (Waltham, Massachusetts) and a Hitachi model H-800 transmission electron microscope (with an accelerating voltage of 200 kV) were used to characterize the prepared gold and silver nanoparticles.

2.2 *Cell Culture*

Human hepatoma carcinoma cells (HepG2 cell line) were obtained from the Fujian Provincial Cancer Hospital. The cells were grown as monolayers in Dulbecco's modified eagle medium (DMEM) supplemented with 100 IU/mL penicillin/streptomycin and 10% fetal calf serum.

2.3 *Cell Electroporation*

Cell electroporation was carried out according to our previous studies.[9,15] In brief, cells were first harvested and resuspended in the medium with a density of 1×10^6 cells/mL. Next, 400-μL cell suspension and 100-μL gold nanoparticles were mixed under 4°C in a 0.4-cm cuvette. After incubation for 10 min, the electroporation was processed using rectangular electric pulses with voltage of 375 V/cm, 1-ms duration time for two times utilizing a BTX-ECM 830 Electroporator (Bio-Rad Laboratories Ltd., Shanghai, China). Prior to Raman measurement, cells were washed exhaustively with phosphate buffer solution (PBS) by centrifuging at 1,000 rpm for 5 min to remove

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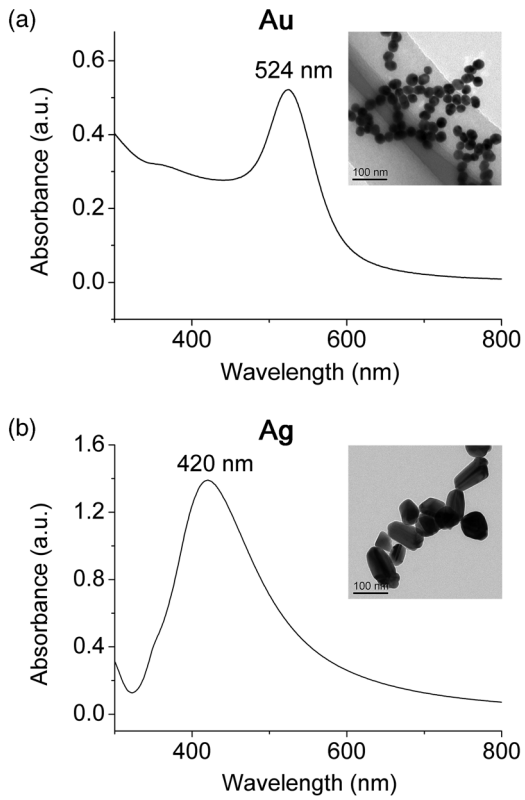

Fig. 1 The UV-Vis absorption spectra of (a) gold nanoparticles (Au) and (b) silver nanoparticles (Ag) in water. The inserted figures show the transmission electron micrographs of gold nanoparticles and silver nanoparticles, respectively. Scale bars: 100 nm.

the nanoparticles, which were possibly attached to the cell exterior surface. The process was repeated several times. Ultimately, the living cells suspended in PBS were dropped and thinly spread on a stainless steel substrate, and the SERS measurements were performed immediately in order to maintain the activity of each cell.

2.4 *SERS Detection*

A Renishaw Raman microscope (InVia system) and a 785-nm diode laser were used for the SERS measurement. Typically, the incident laser power was attenuated to 1 to 3 mW and the laser focus was enlarged up to 200% by adjustment of the pinhole size. The enlarging the laser beam (~20-μm size) was focused on the center of single-living cell (15 to 20-μm size). In this case, the SERS spectrum of each living cell with good quality can be acquired by using a 50× objective. The fresh cells were redropped for each measurement in the range of 560 to $1,730\ \mathrm{cm}^{-1}$ with a spectral resolution of $2\ \mathrm{cm}^{-1}$, each Raman spectra were acquired with a 10-s integration time.

The software package of WIRE 2.0 (Renishaw) was employed for spectral acquisition and analysis. An automated algorithm, which was kindly offered by the BC Cancer Research Centre for autofluorescence background removal, was applied to the measured raw data to extract pure Raman spectra.[16] Then, each corrected SERS spectrum was normalized by the integrated area under the curve from 560 to $1,730\ \mathrm{cm}^{-1}$.

3 Results and Discussion

Figure 1(a) is the UV-Vis absorption spectrum of gold nanoparticles with an absorption peak at 524 nm. The transmission electron microscopy (TEM) photograph [inserted in Fig. 1(a)] of the gold nanoparticles shows the particle sizes follow a normal distribution with a mean diameter of 43 nm and standard deviation of 6 nm. The absorption peak of the silver nanoparticles is around 420 nm, and the TEM photograph reveals that the silver nanoparticles have a size distribution centered at about 60 nm in mean diameter [Fig. 1(b)].

Figure 2(a) shows the procedure for delivering gold nanoparticles into a cell by electroporation. Gold nanoparticles (red dots) are first mixed with cells in DMEM medium, and then there are a number of gold nanoparticles around the periphery of each of cells. The cell membrane acts as a barrier to the free diffusion of gold nanoparticles between the cytoplasm and the external medium. When the cells are treated by an electrical impulse, pores appear rapidly in the membrane, making cells highly permeable. During the effective pore open time, impermeable gold nanoparticles absorbed on or close to the membrane can freely diffuse through these openings into the cell interior. Once the electrical impulse fades, the membrane recovers its integrity and acts normally as the barrier again. Finally, gold nanoparticles are successfully trapped inside the cell.

Figure 2(b)-1 shows the TEM photograph of cell internalization of gold nanoparticles delivered by electroporation. Most of gold nanoaggregates locate in the cytoplasm; the magnification image [Fig. 2(b)-2] also indicates that the gold nanoparticles form a bigger nanoaggregate size in 300 nm or so, where the gaps between the spheroids can be vanishingly

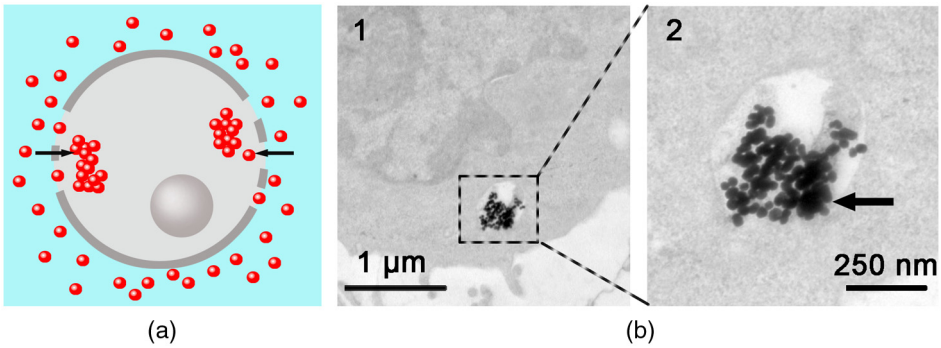

Fig. 2 (a) Schematic diagram of the procedure for delivering of gold nanoparticles (as indicated by red dots) into a cell by electroporation. (b) Transmission electron micrograph of gold nanoparticles in a HepG2 cell delivered by electroporation, and the rectangle area is the one with higher magnification. Scale bars: 250 nm.

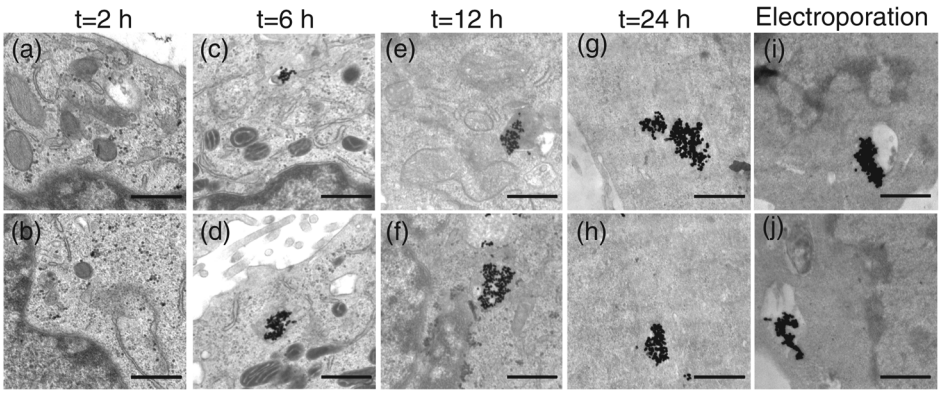

Fig. 3 (a)–(h) Parallel transmission electron micrographs of intracellular gold nanoparticles delivered by "passive uptake" (2, 6, 12, and 24-h incubation). (i) and (j) Parallel transmission electron micrographs of intracellular gold nanoparticles delivered by transient electroporation. Scale bars: 500 nm.

small in some area. There is a compelling evidence that these aggregated nanoparticles contain hot spots and can provide stronger SERS enhancement than individual particles.[17–19]

Figure 3 shows the parallel TEM photographs of the distribution of the intracellular gold nanoparticles, which were delivered by "passive uptake" (2, 6, 12, and 24-h incubation) and electroporation method, respectively. As shown in Figs. 3(a) and 3(b), gold nanoparticles could be observed over the entire cell after 2-h incubation, but mostly they are dimers and trimers. This indicates that it is hard for the clusters of nanoparticles to enter cells through "passive uptake." At $t = 6$ h [Figs. 3(c) and 3(d)], the clusters of gold nanoparticles begin to appear with irregular outlines. This aggregation inside cells is due to the interaction of the nanoparticles with components in the cellular environment.[6] With the increasing incubation times, the gold nanoaggregates significantly grow larger [Figs. 3(e)–3(h)], and the largest gold nanoaggregates are developed in lysosomes at 24 h in Figs. 3(g) and 3(h). In the electroporation experiment [Figs. 3(i) and 3(j)], the gold nanoaggregates can form as large as those developed through "passive uptake" by 24-h incubation in a very short time (within 1 min). Specially, the interparticle distance between gold nanoparticles delivered by electroporation method is closer than that transported by "passive uptake" (24-h incubation). In consideration of the distance dependence of plasmonic coupling, the closer interparticle distance in the assemblies of nanoparticles will achieve greater SERS signal enhancement;[20,21] therefore, gold nanoparticles aggregation developed by electroporation method may be more sensitive and effective in detecting intracellular components.

Figures 4(a) and 4(b) are the normalized mean SERS spectra of the individual HepG2 cells treated with silver nanoparticles and gold nanoparticles, respectively. The shaded areas represent the standard deviations of the means. In both figures, curve 1 is the cells subjected to the transient electroporation ($n = 15$), and curve 2 is the cells incubated with nanoparticles for 24 h ($n = 15$). Table 1 summarizes the tentative assignment of the observed SERS bands.[2,22–31] As shown in Fig. 4(a), the cells being treated with silver nanoparticles through electroporation (less than 1 min) and "passive uptake" experiment (24-h incubation) have significant differences in SERS spectral shapes, such as the appearance of 1,054 and $1,698\text{-cm}^{-1}$ bands and a decrease in the intensity of 1,002 and 832-cm^{-1} peaks for "passive uptake" [Fig. 4(a), curve 2]. Other peak positions include 662, 799, 1,340, and $1,416\ \text{cm}^{-1}$ for electroporation experiment shift to 668, 804, 1,344, and $1,397\ \text{cm}^{-1}$ for "passive uptake." These spectral changes may be related to the cellular toxicity caused by incubating with silver nanoparticles for a very long time (24 h). It has been demonstrated that the silver species release Ag^{+} ions and then interact with the thiol groups in proteins, affecting the replication of deoxyribonucleic acid.[32,33] However, the cells being delivered gold nanoparticles by these two methods have similar SERS spectral profiles [Fig. 4(b)], except for the band at $1,371\ \text{cm}^{-1}$. The appearance of this peak is expected to be the result of higher signal enhancement, which is consistent with phenomena observed in Figs. 3(g)–3(j).

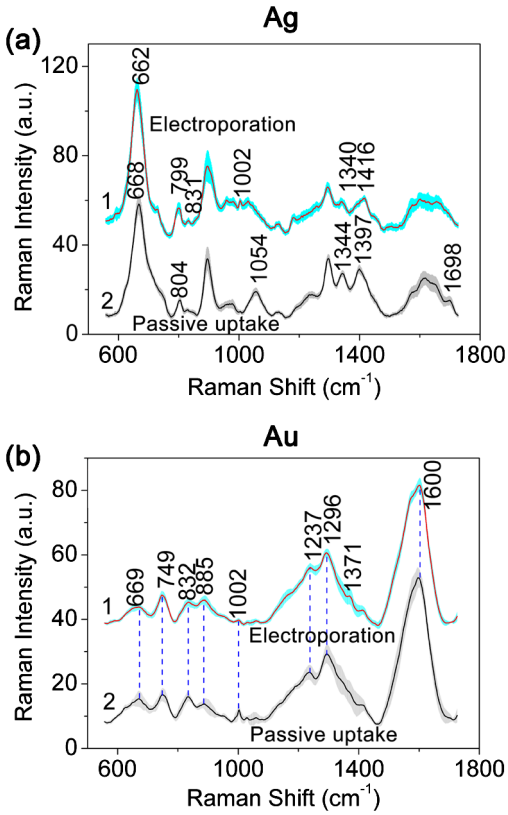

Fig. 4 (a) Mean whole-cell surface-enhanced Raman scattering (SERS) spectra of the HepG2 cells ($n = 15$) with silver nanoparticles (Ag) delivered by (1) transient electroporation and (2) "passive uptake" (24-h incubation). (b) Mean whole-cell SERS spectra of the HepG2 cells ($n = 15$) with gold nanoparticles (Au) delivered by (1) transient electroporation and (2) "passive uptake" (24-h incubation).

Table 1 Tentative assignment of the observed surface-enhanced Raman scattering (SERS) bands.

Raman shift (cm^{-1})	Tentative assignment[a]
662	ν (C—S)
669	T, G, ν (C—S)
749	T
799	Tyr, ν (C—S—C)
804	Tyr
832	Tyr
885	Tyr
1,002	Phe, p: ν (C—C)
1,054	ν (C—O), p: ν (C—N)
1,237	amide III
1,296	amide III
1,340	G, p: def (C—H)
1,344	p: def (C—H)
1,371	p: def (C—H)
1,416	aspartic and glutamic acids, ν (C—O)
1,600	Phe, Tyr

[a]p, protein; ν, stretching; def, deformation; Tyr, tyrosine; Phe, phenylalanine; T, thymine; G, guanine.

4 Conclusion

To summarize, we have used the electroporation method for rapid delivery of gold nanoparticles into living HepG2 cells to achieve intracellular SERS spectra. Without decreasing the SERS enhancement, this method is much faster and more efficient than "passive uptake" in delivering gold nanoparticles into living cells. Furthermore, by comparison with silver nanoparticles, gold nanoparticles have better biological compatibility but less cellular toxicity. The results demonstrate that ultrasensitive gold nanoparticles—based SERS spectroscopy would allow to monitor very slight changes in chemical composition of living cells, which are very promising for developing a potential diagnosis method for cancer detection.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 11274065, 61210016, 61178090, 61308113, 61178083, 81301253, and 61335011), Natural Science Foundation of Fujian Province (Nos. 2012J01254 and 2013J01225), the Science and Technology Project of Fujian Province (No. WKJ-FJ-01), and Program for Changjiang Scholars and Innovative Research Team in University (No. IRT1115). Peng Lu, Jing Wang, and Jinyong Lin designed the experiments, performed electroporation and Raman measurement, and wrote the paper together. Nenrong Liu and Zufang Huang performed statistical analyses. Haishan Zeng and Buhong Li contributed to manuscript proofreading. Juqiang Lin and Rong Chen managed the project. All authors participated in the discussion.

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