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The Enhancement Effect of Gold Nanoparticles in Drug Delivery and as Biomarkers of Drug-Resistant Cancer Cells

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The enhancement effect of 3-mercaptopropionic acid capped gold nanoparticles (NPs) in drug delivery and as biomarkers of drug-resistant cancer cells has been demonstrated through fluorescence microscopy and electrochemical studies. The results of cell viability experiments and confocal fluorescence microscopy studies illustrate that these functionalized Au NPs could play an important role in efficient drug delivery and biomarking of drug-resistant leukemia K562/ADM cells. This could be explored as a novel strategy to inhibit multidrug resistance in targeted tumor cells and as a sensitive method for the early diagnosis of certain cancers. Our observations also indicate that the interaction between the functionalized Au NPs and biologically active molecules on the surface of leukemia cells may contribute the observed enhancement in cellular drug uptake.

Introduction

Gold nanoparticles (Au NPs), a typical biocompatible nanomaterial, have been widely used in biomedical engineering and bioanalytical applications such as biomedical imaging and biosensors.[1–4] Recently, de La Fuente and Berry reported that Au NPs functionalized with a Tat-protein-derived peptide could pass through nuclear pores to afford potential application in cell imaging and other types of biomedical detection.[5] Au NPs have also been modified with liposome phospholipid bilayers for the control and release of drugs.[6] Biological applications of Au NPs have focused mainly on their use as probes for the detection of oligonucleotides and other biomacromolecules.[7–14] Considering that the early diagnosis of cancer is a big problem in cancer therapy, we report herein our exploration of a convenient and efficient strategy to sensitively mark target cancer cells by combining with functionalized Au NPs.

As cancer is among the most serious and difficult diseases to diagnose and treat, it is a significant challenge to develop effective targeting and detection strategies for the cancerous cells in question and thus to follow their development and proliferation. Although numerous strategies have come to play in managing this disease, many obstacles remain. One such obstacle is drug resistance and multidrug resistance (MDR) of tumor or cancer cells, and the overexpression of P-glycoprotein (P-gp, an integral membrane protein) on the cell surface contributes significantly to drug resistance in anticancer chemotherapy.[9,15–21] It is now known that there are multiple mechanisms involved in the drug resistance of cancer cells. For instance, some drugs may be poorly taken up by mutated tumor cells, or drug-resistance-related proteins in the cell membrane may pump the drug molecules out of the cells. Either process lowers the intracellular drug concentration to below the level effective against tumor or cancer cells. Based on those observations, much effort has been devoted to solving these problems, and generations of drugs have been developed and adopted as efficient alternatives to overcome drug resistance.

With regards to MDR in chemotherapy, the combination of sensitive biomarkers and drug targeting may afford a new strategy toward early diagnosis and efficient treatment of targeted cancers. Therefore, we explored the possibility of adopting 3-mercaptopropionic acid capped Au NPs as a sensitive and efficient way to target cancer cells and to facilitate biomarking and accumulation of the anticancer drug daunorubicin in drug-resistant cancer cells of the human leukemia K562 cell line.

Results and Discussion

Scanning confocal fluorescence microscopy

Gold NPs were initially functionalized with 3-mercaptopropionic acid (MPA), as the thiol groups react to form covalent bonds with the Au surface. TEM characterization of the functionalized Au NPs indicates an average diameter of ~5 nm, as shown in

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Figure 1. Fluorescence microscopy was then used to explore targeted leukemia cells treated with daunorubicin together with Au NPs. Figures 2a–c show typical scanning confocal fluorescence microscopy images of daunorubicin-treated drug-resistant leukemia K562 cells in the absence and presence of Au NPs. For comparison, a typical confocal fluorescence micrograph of normal cells after incubation with daunorubicin in the presence of functionalized Au NPs is also shown (Figure 2d), indicating that the NPs have negligible effect on drug uptake by normal cells. Moreover, it is apparent that the uptake of daunorubicin by the drug-resistant leukemia K562 cells is relatively weak in the absence of Au NPs. Upon addition of the Au NPs, much stronger intracellular fluorescence was observed for the drug-resistant cancer cells. In particular, MPA-capped Au NPs greatly enhance this uptake effect in target cancer cells relative to bare Au NPs (that is, bare Au colloids that were synthesized by citrate reduction). As shown in Figure 2, upon application of the functionalized Au NPs together with daunorubicin, the intracellular fluorescence intensity of daunorubicin in targeted cancer cells was found to be much stronger than that with bare Au NPs or with daunorubicin alone, and the enhanced intracellular fluorescence was mainly located on the cellular membrane. Figure 3 shows fluorescence intensity profiles of daunorubicin-treated cancer cells in the absence and presence of the MPA-capped Au NPs. Because the functionalized Au NP itself does not fluoresce, the relative intracellular fluorescence was generated only by the anticancer drug daunorubicin.

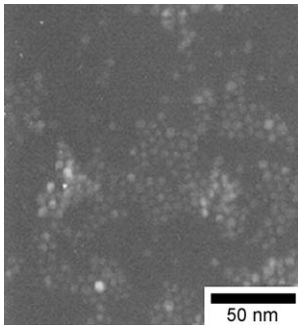


Figure 1. TEM image of MPA-capped Au NPs.

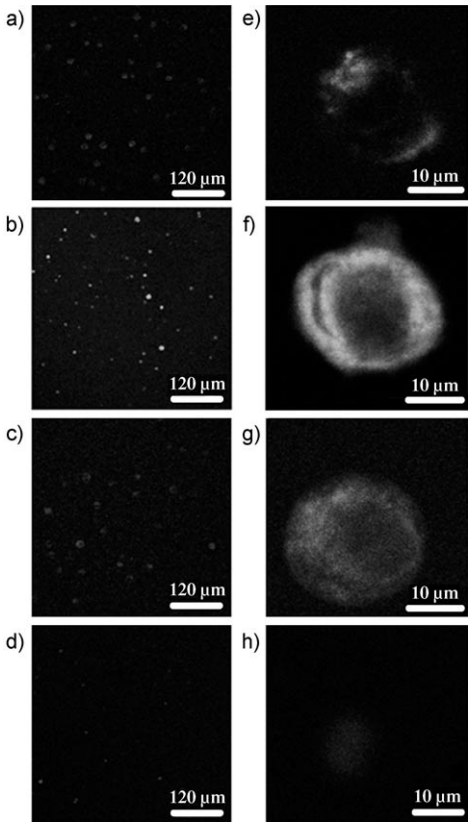


Figure 2. Typical confocal fluorescence micrographs (excitation wavelength: 480 nm) of drug-resistant leukemia K562/ADM cells after incubation with a) daunorubicin alone (2.0×10^{-4} M), b) daunorubicin in the presence of MPA-capped Au NPs (5.0×10^{-8} M), and c) daunorubicin in the presence of bare Au NPs (5.0×10^{-8} M). For comparison, part d) shows a typical confocal fluorescence micrograph of normal cells after incubation with daunorubicin (2.0×10^{-4} M) in the presence of MPA-capped Au NPs (5.0×10^{-8} M). Parts a)–d) show the panoramic images of the target cells; parts e)–h) illustrate typical respective single-cell images from a)–d).

It is well known that nanomaterials could be used in disease diagnostics and therapeutics,[22] and the use of Au NPs has already been reported for gene therapy.[23] Herein, our results indicate that the MPA-capped Au NPs could play an important role in facilitating the targeting and marking of daunorubicin on the plasma membranes of drug-resistant leukemia cells (as shown in Scheme 1). The rationale behind this strategy is to increase the intracellular concentration of the targeted drug by the synergistic effect elicited by the functionalized Au NPs. The results of the confocal fluorescence microscopy study show that bare Au NPs, synthesized by citrate reduction, are much less effective than MPA-capped Au NPs under identical experimental conditions, suggesting that the functional group of the

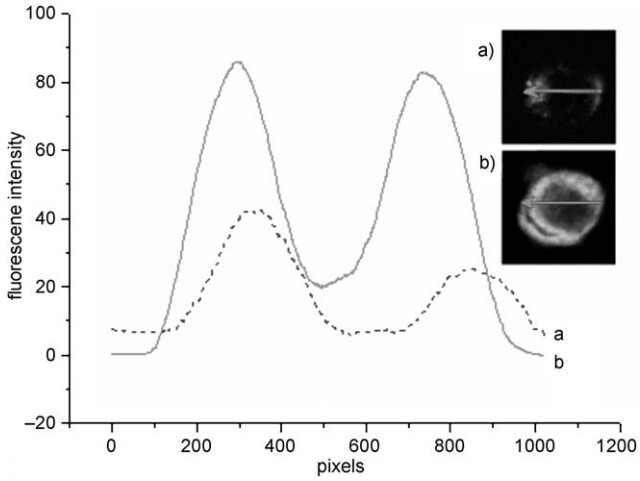
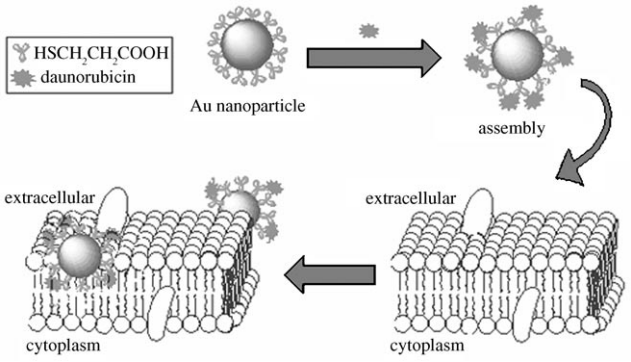


Figure 3. Comparison of the intracellular fluorescence intensity of a single drug-resistant leukemia K562/ADM cell treated with daunorubicin in the a) absence or b) presence of MPA-capped Au NPs (5.0×10^{-8} M); arrows illustrate the location and direction of the sample section taken.



Scheme 1. Proposed process for the synergistic effect of MPA-capped Au NPs on the uptake of daunorubicin by drug-resistant leukemia K562 cells, which could be used as a new sensitive biomarker for the respective drug resistant cancer cells.

modified Au NPs may play a key role in the remarkably enhanced accumulation of anticancer drugs on the membranes of target cells.

Inverted fluorescence and optical microscopy

On the basis of these observations, we further explored the synergistic effect brought about by the MPA-capped Au NPs in drug delivery and biomarking of target cancer cells by using inverted fluorescence and optical microscopy. These studies demonstrate some apparent differences between the extracellular membranes of leukemia cells that result upon the application of daunorubicin alone and together with MPA-capped Au NPs. Figure 4 shows the drug-resistant leukemia cells treated with daunorubicin with or without the MPA-capped Au NPs. As shown in Figure 3, the functionalized Au NPs could remarkably facilitate the drug targeting and uptake of daunorubicin into drug-resistant cancer cells and could thus act as an efficient agent to enhance drug delivery.

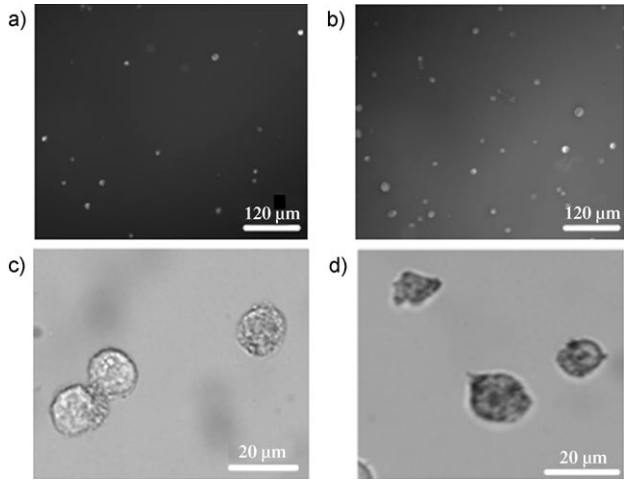


Figure 4. Inverted fluorescence micrographs of drug-resistant leukemia K562/ADM cells after incubation with a) daunorubicin alone and b) in the presence of MPA-capped Au NPs (5.0×10^{-8} M). Parts c) and d) show typical optical micrographs of the systems pictured in parts a) and b), respectively.

MTT assays

The synergistic enhancement of drug uptake in the presence of MPA-capped Au NPs has also been demonstrated in cell viability experiments carried out with MTT assays. As shown in Figure 5, the results of these studies illustrate that the presence of the functionalized Au NPs apparently inhibits the growth of leukemia cells; that is, the growth of target cells treated with daunorubicin in combination with the MPA-capped Au NPs was inhibited much more dramatically than the case of treatment with daunorubicin alone. These observations are consistent with the results of our fluorescence microscopy studies, supporting the remarkable synergistic effect of the MPA-capped Au NPs in facilitating the accumulation of daunorubicin at drug-resistant cancer cells.

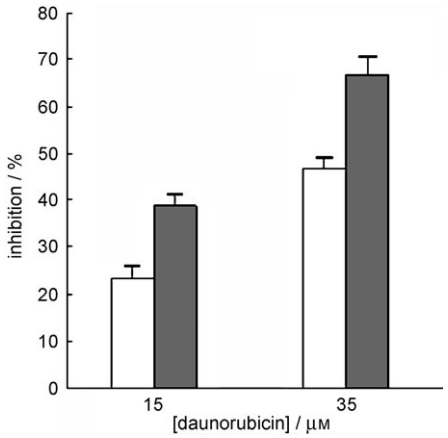


Figure 5. Inhibition of K562/ADM cell-line growth by daunorubicin and the MPA-capped Au NPs as determined by MTT assay. White bars: no NPs; gray bars: with MPA-capped Au NPs (5×10^{-8} M).

Electrochemical studies

Our electrochemical studies provide further evidence for the synergistic effect elicited by the functionalized Au NPs in drug targeting and uptake by targeted cancer cells. The results indicate that the MPA-capped Au NPs alone have little effect on the electrochemical response of daunorubicin under the experimental conditions used. In contrast, a considerable effect was observed upon application of cancer cells to the target system. As shown in Figure 6, the results of the electrochemical study of daunorubicin outside leukemia K562 cells illustrate a remarkable positive shift of the peak potential and a decrease of the peak current after treatment of the drug-resistant leukemia cells with daunorubicin. Moreover, more dramatic changes in the electrochemical response of daunorubicin residue outside leukemia K562 cells was observed upon the addition of functionalized Au NPs to the target system together with daunorubicin. These observations indicate that the presence of NPs results in a much greater accumulation of drug at the cancer cells than occurs in the absence of NPs. A much larger decrease of the peak currents of daunorubicin was observed upon application of the external potentials to the target

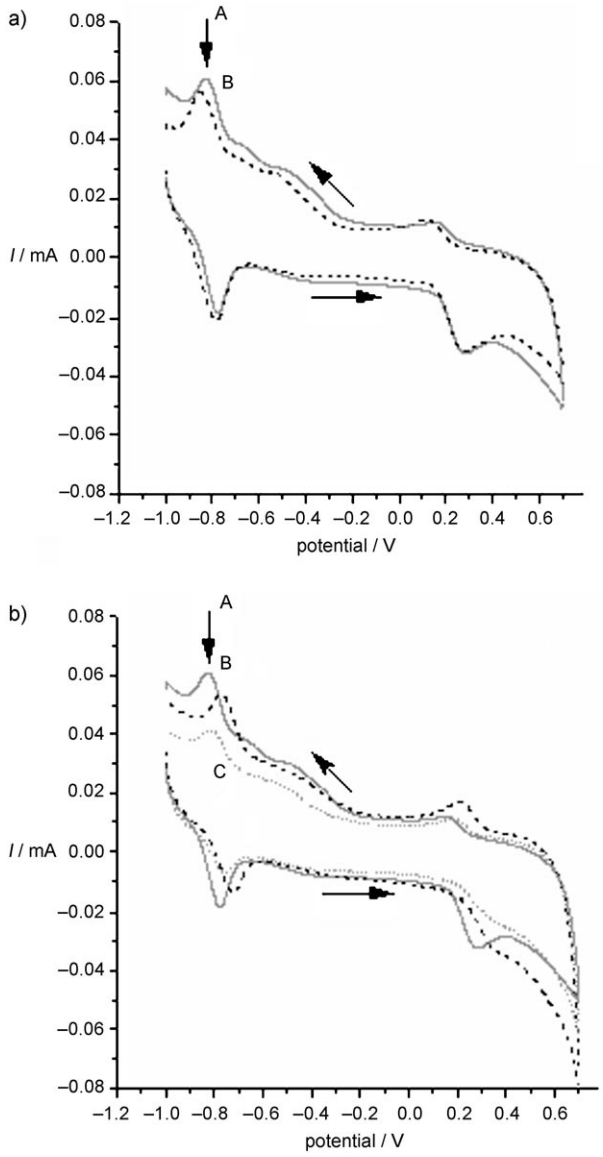


Figure 6. Cyclic voltammograms (scan rate: 100 mV s^{-1}) of daunorubicin alone and residual daunorubicin outside drug-resistant leukemia K562 cells in the absence and presence of the MPA-capped Au NPs. a) Cyclic voltammetry of daunorubicin (2.0×10^{-4} M) (trace A) and of daunorubicin (2.0×10^{-4} M) with the functionalized Au NPs (5.0×10^{-8} M) (trace B); b) Cyclic voltammetry of daunorubicin (2.0×10^{-4} M) (trace A), of residual daunorubicin outside drug-resistant leukemia K562/ADM cells (trace B), and of daunorubicin outside drug-resistant leukemia K562/ADM cells in the presence of the functionalized Au NPs (5.0×10^{-8} M) (trace C).

system, suggesting that daunorubicin is efficiently targeted to and taken up by the drug-resistant leukemia K562 cells in the presence of the MPA-capped Au NPs.

Conclusions

In summary, we have demonstrated that the combination of the anticancer drug daunorubicin with MPA-capped Au NPs could be used as a novel and convenient way to sensitively target and mark drug-resistant cancer cells without complicated procedures. This strategy could be further explored as a new method to inhibit the multidrug resistance of tumor or cancer cells and as a sensitive biomarker for the early diagnosis of cancer.

Experimental Section

Regents: All reagents used were of analytical grade. Buffer solutions were prepared in phosphate buffered saline (PBS, 0.1 M, pH 7.2) with ultrapure water (Milli-Q). Each experiment was repeated at least three times. The functionalized Au NPs were synthesized according to previously published methods,[24] and transmission electron microscopy (TEM) was carried out with a JEM2000EX transmission electron microscope.

Cell culture: Leukemia K562 cells were cultured in a flask in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 mU mL^{-1}), and streptomycin (100 mU mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. Leukemia K562/ADM cells were maintained with adriamycin (1 μg mL^{-1}, Sigma).

Scanning confocal fluorescence microscopy: During the experiments, daunorubicin and solutions of Au NPs were added to the cell culture for incubation in which the final concentration of daunorubicin was 1.0×10^{-4} M and that of Au NPs was 2.0×10^{-8} M. Observations were made with a Leica TCS SP2 instrument after incubating the cells for 0.5 h. In the control experiments, only daunorubicin was added. The freshly prepared cell culture was dropped on a thoroughly cleaned glass plate immediately before measurement. The fluorescence excitation wavelength was 480 nm. In addition, an IX71 inverted fluorescence microscope was used to collect optical and fluorescence images of the target cells under the identical experimental conditions as those used in the confocal fluorescence microscopy studies.

MTT assays: Inhibition of cell growth was measured by MTT assay. Initially, K562/ADM cells in the log phase were placed in a 96-well plate at a concentration of 12×10^4 cells mL^{-1}. The cells were treated with various concentrations of daunorubicin and the MPA-capped Au NPs. Controls were cultivated under the same conditions without the addition of daunorubicin and Au NPs. Each culture was incubated for 48 h in an incubator at 37 °C under an atmosphere containing 5% CO_2. Then 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, 20 μL, 5 mg mL^{-1}, Sigma) was added to the wells. After further incubation for 4 h, the medium in each well culture was carefully removed, followed by addition of 150 μL DMSO into each well. The optical density (OD) was measured at a wavelength of 540 nm. Inhibition of cell growth was expressed as follows:

$$\%\ \text{inhibition} = [1-(A_{tc}-A_b)/(A_{uc}-A_b)]\times 100$$

for which A_{tc} = absorbance of treated cells, A_{uc} = absorbance of untreated cells, and A_b = absorbance of blank.

Electrochemical studies: Cyclic voltammetry was performed on a CHI660b electrochemical workstation to detect the electrochemical behavior of daunorubicin. All measurements were carried out at ambient temperature (22 ± 2 °C) under nitrogen in a three-component electrochemical cell containing a glassy carbon electrode as the working electrode, a Pt wire as the counter electrode, and an Ag wire as the quasi-reference electrode. All potentials are reported versus the Ag wire quasi-reference electrode. We found that the reference potential of a SCE and the Ag wire in phosphate buffer (0.1 M, pH 7.2) have the relationship of $V_{SCE} = V_{Ag\,wire} + 0.07$ V. During the studies, cells were collected and separated from 1.0 mL

medium by centrifugation for 10 min. The sediment was blended with daunorubicin (1.0×10^{-4} M) and a solution of Au NPs (5.0×10^{-8} M) in PBS. In control experiments, only daunorubicin (1.0×10^{-4} M) or Au NPs (5.0×10^{-8} M) were injected into the cell solution.

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Keywords: biomarkers · daunorubicin · drug delivery · drug-resistant cancer cells · gold nanoparticles

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