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# In vitro study of drug accumulation in cancer cells via specific association with CdS nanoparticles

Jingyuan Li,[a,b] Chunhui Wu,[a] Feng Gao,[c] Renyun Zhang,[a] Gang Lv,[a] Degang Fu,[a] Baoan Chen[c] and Xuemei Wang[a,b,*]

[a]*State Key Lab of Bioelectronics (Chien-Shiung WU Laboratory), Southeast University, Nanjing 210096, PR China*
[b]*Department of Chemistry and Chemical Engineering, Southeast University, Nanjing 210096, PR China*
[c]*School of Clinical Medical, Southeast University, Nanjing 210096, PR China*

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**Abstract**—We report a novel approach to enhance the efficient accumulation and utilization of anticancer drug daunorubicin on cancer cells through the combination with CdS nanoparticles. Our observations using confocal fluorescence scanning microscopy as well as electrochemical analysis methods demonstrate that CdS nanoparticles can readily bind with daunorubicin on the external membrane of the targeted cells and facilitate the uptake of drug molecules in the human leukemia K562 cells. Besides, our results also indicate that the competitive binding of CdS nanoparticles with accompanying anticancer drug to the membrane of leukemia K562 cells could efficiently prevent the drug release by the drug-sensitive and drug-resistant leukemia cells and thus inhibit the possible multidrug resistance of cancer cells, which could be further utilized to improve the future drug efficiency in respective tumor chemotherapies.


The efficient cure of cancers is still a hot topic in the relative biomedical areas involving in the disease diagnostics and treatments as well as patient care. The detection and identification of pathogens are often painstaking in the clinic due to the low abundance of diseased cells in sputum, blood, and other clinical samples. Besides, the multidrug resistance is another major obstacle in cancer therapies. Nevertheless, the new drug design and selection technology offer the efficient alternatives for the respective cancer diagnostics and tumor target treatments.[1–7] Some reports have demonstrated that the drug coated polymer nanospheres and nanoparticles could efficiently increase the intracellular anticancer drug delivery.[8–10] Recently, researchers have paid much attention to the use of nanoparticles as a 'solid phase' surface for biosensing or early cancer diagnostics since nanoparticles could present a versatile scaffold for the recognition of biomolecular surface.[10–13] Thus, in this report, we have explored the possibility to utilize nanoparticle-based receptors to recognize biomacromolecules or modulate some important biological process by merging the biomolecules and cadmium sulfide (CdS) nanoparticles through the specific interaction of anticancer drug, daunorubicin, and nanoparticles to the target leukemia K562 cell lines.

Cadmium sulfide nanoparticles (CdS NPs), typical semiconductor quantum dots (QDs), have been widely used in molecular recognition, nanoscale biosensors, cellular labeling, deep-tissue imaging, etc.[14–18] Some water-soluble, biocompatible semiconductor quantum dots (QDs) micelles have been reported recently, which could be utilized as the good probes for investigating intracellular transport and other cellular signaling pathways in living cells.[18–21] In our recent study, CdS NPs were synthesized according to the sol–gel method,[22] which are superfluous of $Cd^{2+}$ on the nanoparticle surface with the diameter of about 5 nm. These CdS NPs were introduced to the biomolecular recognition and the drug binding process pertaining to the biopolymer DNA/DNA bases, which demonstrated that CdS NPs could remarkably enhance the selectivity and binding affinity of the respective biomolecular recognition process.[23] Recently, some studies have illustrated that in live cells, some cellular component and membrane proteins

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* Corresponding author. Tel.: +86 25 83792177; fax: +86 25 83619983; e-mail: xuewang@seu.edu.cn

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(such as nucleus proteins, cytokeratin, serotonin transport proteins, P-glycoprotein, and others) could be labeled with QDs.[18,21,24–26] Since P-glycoprotein plays an important role in mediating the drug absorption to cancer cell (like a mediator of multidrug resistance),[24–28] it is possible to further utilize the respective nanoparticles to merge with the biomolecules to inhibit the drug resistance of target cancer cells. Considering the unique properties of CdS nanoparticle as well as the broad interest in its potential application in biology, in this study we have explored to utilize CdS NPs to efficiently improve the drug uptake of daunorubicin (DNR), one of the most important anticancer drugs in the clinic, into the target cancer cells (including the human lymphoid–leukemia cell line (K562/B.W) and its adriamycin-resistant subline (K562/ADM) cells). Our observations indicate that CdS NPs could obviously facilitate the uptake of DNR into the respective drug-sensitive and drug-resistant leukemia cells, suggesting that the interaction between CdS NPs and biologically active molecules could provide a new strategy to inhibit the drug resistance in cancer chemotherapy.

Initially, the confocal fluorescence scanning microscopy was utilized to image the intracellular fluorescence resulting from the uptake of DNR in different kinds of leukemia cells. Figure 1 illustrates the typical images of the cellular confocal fluorescence microscopy of the uptake of DNR by drug-sensitive leukemia K562 cells in the absence and presence of CdS NPs. As shown in Figure 1, it is obvious that after incubating the drug-sensitive leukemia cells with the drug, DNR molecules can approach and enter into the cell so that the cell's image could be observed through the relative fluorescence of absorbed DNR. Besides, it was found that the fluorescence intensity of the drug-sensitive leukemia cells (K562/B.W) was much stronger than that of the drug-resistant cells (K562/ADM) under the identical experimental conditions (see Supplementary data), which is consistent with the cell's intrinsic characteristics.

While CdS NPs were introduced into the respective drug-sensitive leukemia cells (K562/B.W), the fluorescence intensity of the drugs accumulated in the cells became significantly stronger than that without CdS NPs. Moreover, the intracellular fluorescence was observed to become stronger with the increase of the accumulation time of CdS NPs with DNR on cancer cells (shown in Fig. 2). After incubating the leukemia cells with DNR for about 1 h, the intracellular drug concentration may reach the highest level so that the strongest intracellular fluorescence could be detected at this time. The similar observations were also observed for the drug-resistant K562/ADM cells. Since CdS NP itself has almost no effect on fluorescence emission spectroscopy of DNR under the related experimental conditions (see Supplementary data), the above observations indicate that the specific association of CdS NPs with biologically active molecules could efficiently prevent the drug release by the drug-resistant and -sensitive cells so that the drug uptake in the targeted cells could be remarkably enhanced in the presence of CdS NPs. Additionally, in the control experiment for normal human cells, the intracellular fluorescence could not be observed by using the confocal fluorescence scanning microscopy even though the normal cells were treated by daunorubicin (DNR) together with CdS NPs under the identical experimental condition as that of respective leukemia K562 cells, indicating that these nanoparticles have almost no effect to the normal cells under our experimental condition.

Meanwhile, the microscopic studies of the respective cells in different experimental systems also demonstrate the apparent distinguished difference of the extracellular membrane when CdS NPs were introduced into the target leukemia K562 cell systems (see Supplementary data). Hence it is clear from the above studies that CdS NPs could facilitate the drug accumulation of DNR on both drug-resistant and drug-sensitive leukemia K562 cells.

Scheme 1 illustrates the possible synergistic effect of CdS NPs on drug uptake of leukemia K562 cells. Since CdS NPs used in this study are superfluous of $Cd^{2+}$ on the nanoparticle surface, the electrostatic interaction as well as other possible cooperation binding forces between the superfluous of $Cd^{2+}$ on the surface of CdS NPs and the respective cell membrane make it readily for CdS NPs to approach to the cancer cells. Besides, because CdS NPs

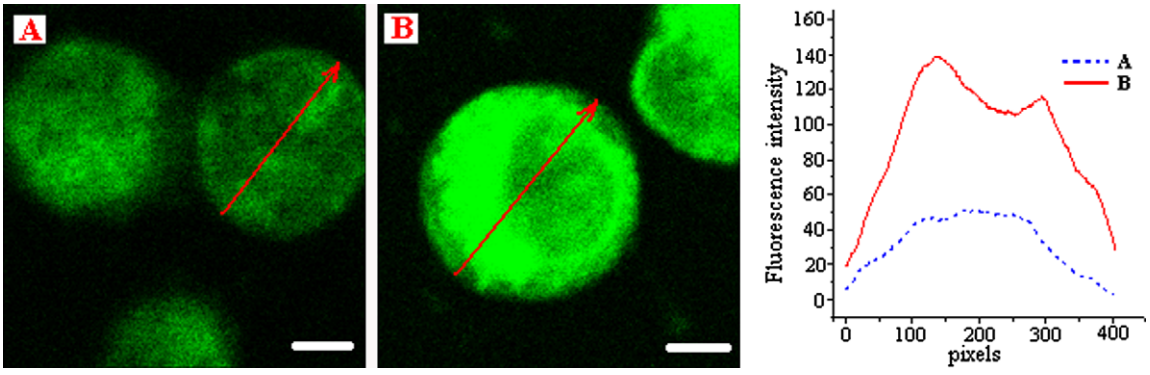



**Figure 1.** Confocal fluorescence microscopy (left) and respective fluorescence intensity curves (right) of K562/B.W cells after incubating with DNR in the absence and presence of CdS NPs. (A) Daunorubicin treated K562/B.W cells with free CdS NPs; (B) daunorubicin treated K562/B.W cells together with CdS NPs. The scale bar represents 4 μm. The excitation wavelength is 480 nm. All images were obtained after incubating the cells for 1 h.

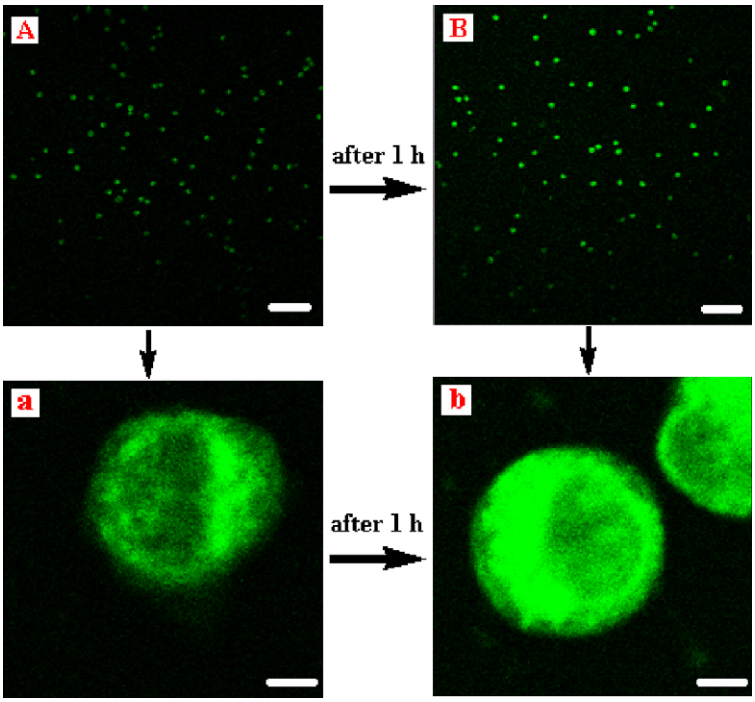
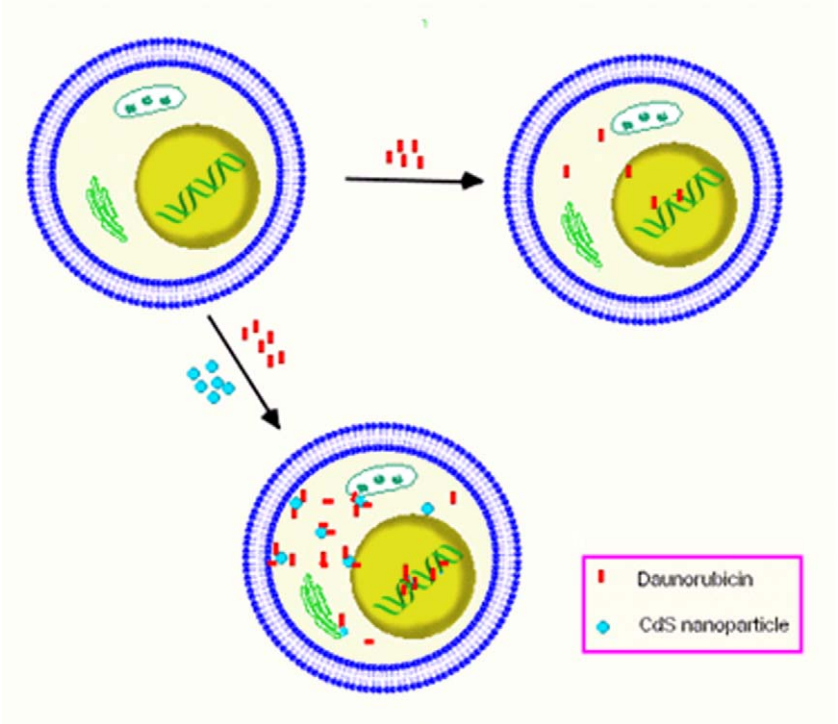



**Figure 2.** Confocal fluorescence microscopy of K562/B.W cells after incubating with daunorubicin in the presence of CdS NPs at different incubation times. (A) Fluorescence image of leukemia K562/B.W cells after incubating with daunorubicin in the presence of CdS NPs for about 20 min; (B) fluorescence image of leukemia K562/B.W cells after incubating with daunorubicin in the presence of CdS NPs for 1 h, where the scale bar represents 100 μm. ((a) and (b) represents the fluorescence image of a single cell from (A) and (B) system, respectively. The scale bar represent 4 μm.) The excitation wavelength is 480 nm.




**Scheme 1.** Schematic drawing of the synergistic effect of CdS nanoparticles on drug (daunorubicin) uptake of leukemia K562 cells.

here were fabricated with water-soluble organic compounds as capping reagents (mercaptopropionic acid, negatively charged species in physiological condition), the formation of an ion pair between the negatively charged species and the positively charged DNR may also contribute to an increased diffusion of DNR across

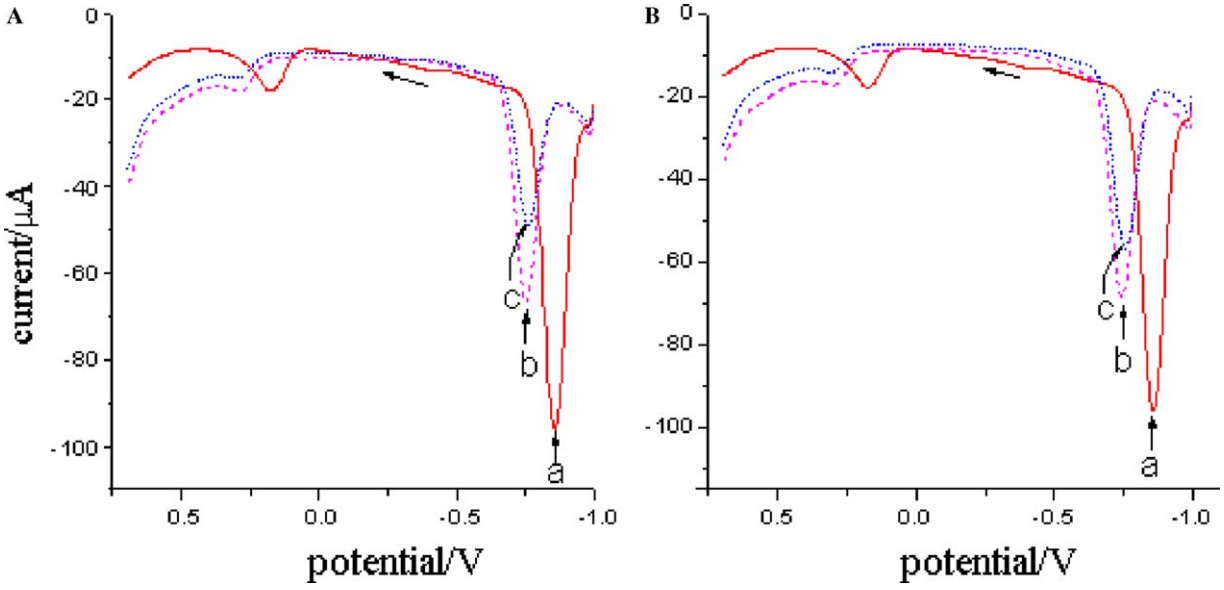



**Figure 3.** Differential pulse voltammetry (DPV) of daunorubicin (DNR) residue outside cells in the absence and presence of CdS NPs ($2.0 \times 10^{-6}$ M): (A) (a) DPV of DNR ($1.0 \times 10^{-4}$ M), (b) DPV of DNR residue outside K562/B.W with free CdS NPs, and (c) DPV of DNR residue outside K562/B.W with CdS NPs; (B) (a) DPV of DNR ($1.0 \times 10^{-4}$ M), (b) DPV of DNR residue outside K562/ADM with free CdS NPs, and (c) DPV of DNR residue outside K562/ADM with CdS NPs. Pulse amplitude: 0.05 V. Pulse width: 0.05 s. Pulse period: 0.1 s.

the plasma membrane. Moreover, for the drug-resistant leukemia K562 cells, the relative multidrug resistance (MDR) is mainly due to the over-expression of the membrane P-glycoprotein, which is capable of extruding various generally positively charged drugs out of the cell, thus the competitive binding of CdS NPs with accompanying anticancer drug to the cell surface may affect the activity of P-glycoprotein and other proteins and facilitate the accumulation of more DNR molecules in target cells. Therefore, considering those described above, it appears that in the presence of CdS NPs, the enhanced binding affinity of the DNR molecules to the cell membrane of the leukemia K562 cells makes it possible for the efficient accumulation of DNR in cancer cells, which could further lead to an effective drug concentration level in both drug-resistant and drug-sensitive leukemia K562 cells. Furthermore, for drug-resistant leukemia K562 cells, CdS NPs may also play as an inhibitor of P-glycoprotein to efficiently inhibit the respective multidrug resistance (MDR) of cancer cells.

Moreover, our electrochemical studies also afford additional evidence for the respective binding behavior of DNR to leukemia K562 cells when combining with CdS NPs. First, the cyclic voltammetry (CV) of daunorubicin with both kinds of cells was explored (see Supplementary data), which illustrates remarkable positive shifts of the peak potentials and considerable decrease of the peak currents after treated with the relative cells, suggesting the efficient uptake of DNR into the cells upon application of the external potentials to the related systems. Figure 3 illustrates the differential pulse voltammetry (DPV) of DNR residue outside cells in the absence and presence of CdS NPs. As shown in Figure 3A, for the drug-sensitive leukemia cells (K562/B.W), the peak current of DNR remarkably decreases after treated with DNR, accompanying with significantly positive shift of the peak potential (ca. 100 mV). In comparison, while CdS NPs were introduced together with DNR into the target system, it is noted that compared to that without CdS NPs, the relative peak current of DNR had a much more remarkable decrease under similar experimental conditions. Meanwhile, the similar phenomenon was also observed for the system of the drug-resistant K562/ADM cells. As shown in Figure 3B, when CdS NPs were introduced into the related target system of K562/ADM cells, the peak current of DNR was found to have a more considerable decrease in the presence of nanoparticles than that without nanoparticles. Since CdS nanoparticle itself has little affect to the electrochemical behavior of DNR under similar experimental conditions (see Supplementary data), the results of our electrochemical studies are consistent with those of our previous fluorescence studies, suggesting that much more DNR molecules have been absorbed into the respective cells when combined with CdS NPs.

In summary, it is clear from the above studies that the presence of CdS NPs could efficiently prevent the drug release by the drug-sensitive and -resistant leukemia cells so that CdS NPs could facilitate the accumulation of DNR on external membrane of leukemia cells and finally improve the relative drug delivery efficiency in target cancer cells. These observations demonstrate that the specific interactions between CdS NPs and biologically active molecules could provide a new sensitive way for the early cancer diagnostics or as a new strategy to inhibit the relative multidrug resistance (MDR) in cancer chemotherapy.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.069.

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