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The enhancement of transfection efficiency of cationic liposomes by didodecyldimethylammonium bromide coated gold nanoparticles

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ABSTRACT

The development of transfection enhancement of liposomes with attributes of high stability and easy handling in gene therapy is challenging. In this study, we report didodecyldimethylammonium bromide (DDAB, a cationic lipid) coated gold nanoparticles (DDAB-AuNPs), which can enhance the transfection efficiency generated by two kinds of commercially available cationic liposomes: Lipotap and DOTAP. It showed that DDAB-AuNPs at the optimal concentrations could produce more than 2 times increase when measuring the number of cells expressed green fluorescent protein and 48-fold increase for luciferase levels after transfection, respectively. The electrophoretic mobility shift assay (EMSA) and confocal laser scanning microscopy (CLSM) experiments showed that more DNA molecules binding to the lipoplexes after adding DDAB-AuNPs. In addition, the flow cytometry (FCM) results indicated that DDAB-AuNPs increased cellular uptake efficiency of DNA molecules, which might account for the enhancement of transfection efficiency. It has also been found that the DDAB-AuNPs could decrease the cytotoxicity of liposomes to the cells.

1. Introduction

Gene therapy is the approach that can cure some of the human diseases using nucleic acids as therapeutic agents. In many cases, it needs vectors or vehicles for carrying therapeutic genes to target cells [1]. To date, nonviral vectors have gained more attention in terms of safety, simplicity and large load capability in gene delivery compared to viral vectors [2–6]. As one of the nonviral vectors, cationic liposome is approved to be one of the most promising nonviral vectors in gene therapy [7–9]. However, the efficiency of cationic liposome needs to be promoted before their being widely used in clinical applications [5].

The question is then, how to increase the transfection efficiency for a certain cationic liposome? Screening out the rate-limiting steps involved during the gene delivery process and overcoming the obstacles embedded in them may be the current challenge jobs [3]. As a result, some kinds of molecules, such as synthetic compounds and biomolecules were introduced to the liposome-based gene transfer systems [10,11]. With the advance of nanotechnology, nanoparticles have been recently used in potentiating the transfection efficiency of nonviral vectors through increasing the cellular uptake efficiency. Silica and silicalite nanoparticles were used to increase the transfection efficiency of polyethylenimine (PEI) via increasing the concentration of DNA–vector complexes at the cell surface [12,13]. Magnetic nanoparticles were also widely used as transfection enhancer elements in liposome or PEI based gene delivery systems, in which the diffusion barrier of the vector/DNA complexes to the target cells was broken by magnetic force [14–16]. Gold nanoparticles have advantages in terms of biocompatibility, noncytotoxicity, non-immunogenicity [17]. Positively colloidal gold nanoparticles [18] and functionalized ones [19–22] have been directly used as new transfection agents. However, few studies have been conducted on applying gold nanoparticles, which by themselves do not possess transfection ability, can be served as transfection enhancers for the commonly used transfection agents.

Didodecyldimethylammonium bromide (DDAB, a cationic lipid) coated gold nanoparticles (DDAB-AuNPs) have better stability when associated with DNA than DDAB alone [23]. However, DDAB-AuNPs did not show any transfection activity in our earlier research. Accidentally, we found that DDAB-AuNPs could enhance the transfection efficiency of liposomes in a high degree. Therefore, we made a detailed investigation on the influence of DDAB-AuNPs

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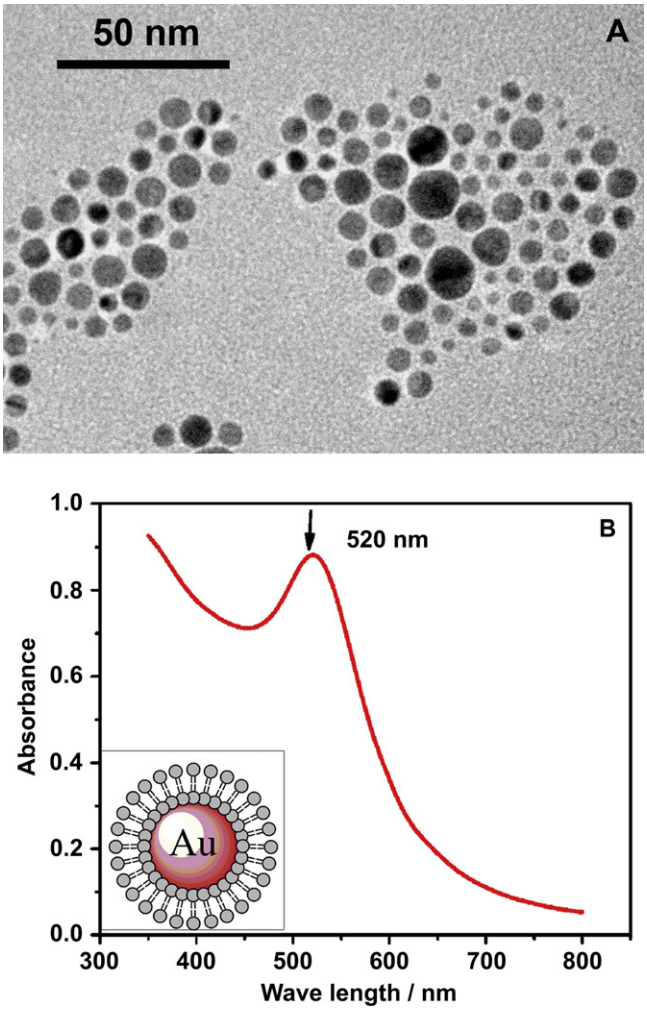


Fig. 1. Transmission electron micrograph (TEM) of DDAB-AuNPs (A). UV–vis absorption spectrum of the purified DDAB-AuNPs (B) and the schematic photograph of DDAB-AuNPs structure (insert).

to the transfection efficiency of Lipotap and DOTAP (two commercial cationic liposomes) in this study. We also investigated the interaction of DDAB-AuNPs with lipoplexes by electrophoretic mobility shift assay (EMSA) and confocal laser scanning microscopy (CLSM). The mechanism studied by flow cytometry (FCM) showed that the increased uptake of DNA into the cells accounted for the enhancement of transfection efficiency. The cytotoxicity of new transfection system was studied by MTT (3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide) assay. Owing to the properties of the ready synthesis, high stability, excellent performance in promoting the transfection efficiency and reducing cytotoxicity of liposome system, DDAB-AuNPs are expected to serve as transfection enhancer elements for cationic liposomes in gene therapy in the future.

2. Materials and methods

2.1. Materials

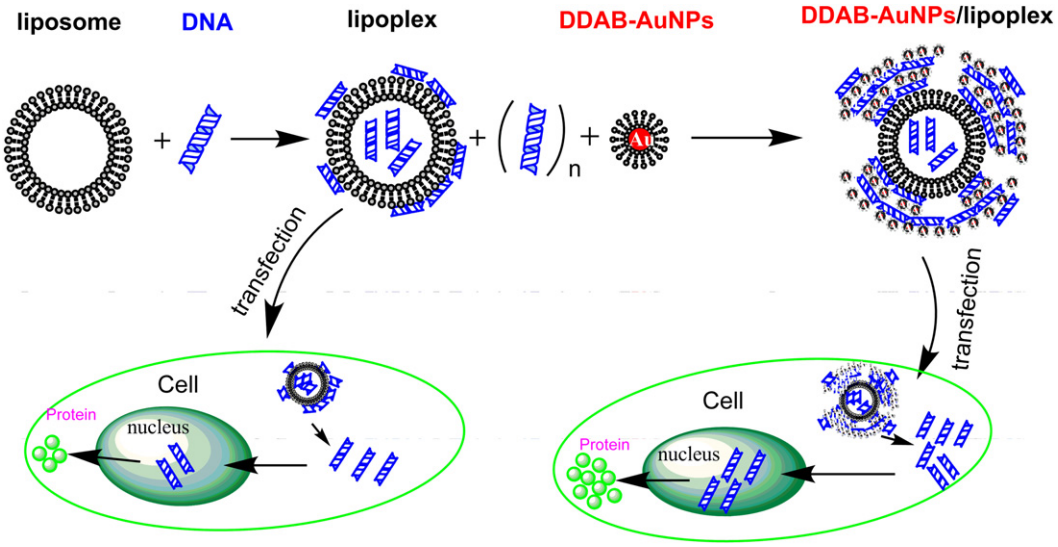
$HAuCl_4$, $NaBH_4$, DDAB and MTT were obtained from Sigma–Aldrich (USA). Dulbecco's modified eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (USA). Trypsin was obtained from AMRESCO (USA). Lipotap and DOTAP transfection reagent were purchased from Beyotime Company (Jiangsu, China) and Applygen Technologies Inc (Beijing, China), respectively. GeneFinder™ was obtained from Bio-v Company (China). PEGFP-C1 (4.9 kb, Clontech, Mountain View, CA, USA) and pGL3-Control (5.3 kb, Promega Corp. USA) were purified by midipreps DNA purification system obtained from Promega. HEK 293 cells were obtained from Kunming Institute of Zoology, Chinese Academy of Sciences. BCA protein assay kit was purchased from Pierce (France). Fluorescein isothiocyanate (FITC) labeled DNA (FITC-5′-ATGGTGAGCAAGGGCGAGGAGCTGTTCAC-3′) is synthesized by TaKaRa Biotechnology (Dalian, China) Co., Ltd. Deionized water was purified by Milli-Q purification system (Millipore). All of the reagents were used as received without further purification.

2.2. Synthesis and characterization of DDAB-AuNPs

DDAB-AuNPs were synthesized according to the reported method [23,24]. DDAB-AuNPs were condensed and purified by centrifugation at 14,000 rpm for 30 min for three times. The DDAB-AuNPs samples were characterized by UV–vis–NIR spectrophotometer (CARY 500) and Hitachi H-8100 transmission electron microscope operating with an accelerating voltage of 200 kV.

2.3. Cell culture and transfection experiment

HEK 293 cells were maintained in DMEM medium with 10% FBS at 37 °C in a humidified incubator (5% CO_2). 10,000 cells per well were seeded in 96-well cluster plate the day before transfection. According to the user manual for the two



Scheme 1. Schematic representation of the formation of DDAB-AuNPs/lipoplex complexes and the mechanism involved in the transfection enhancement. There are more exogenous proteins expressed for more DNA molecules are internalized for each delivery event during the transfection mediated by DDAB-AuNPs/lipoplex compared to lipoplex alone.

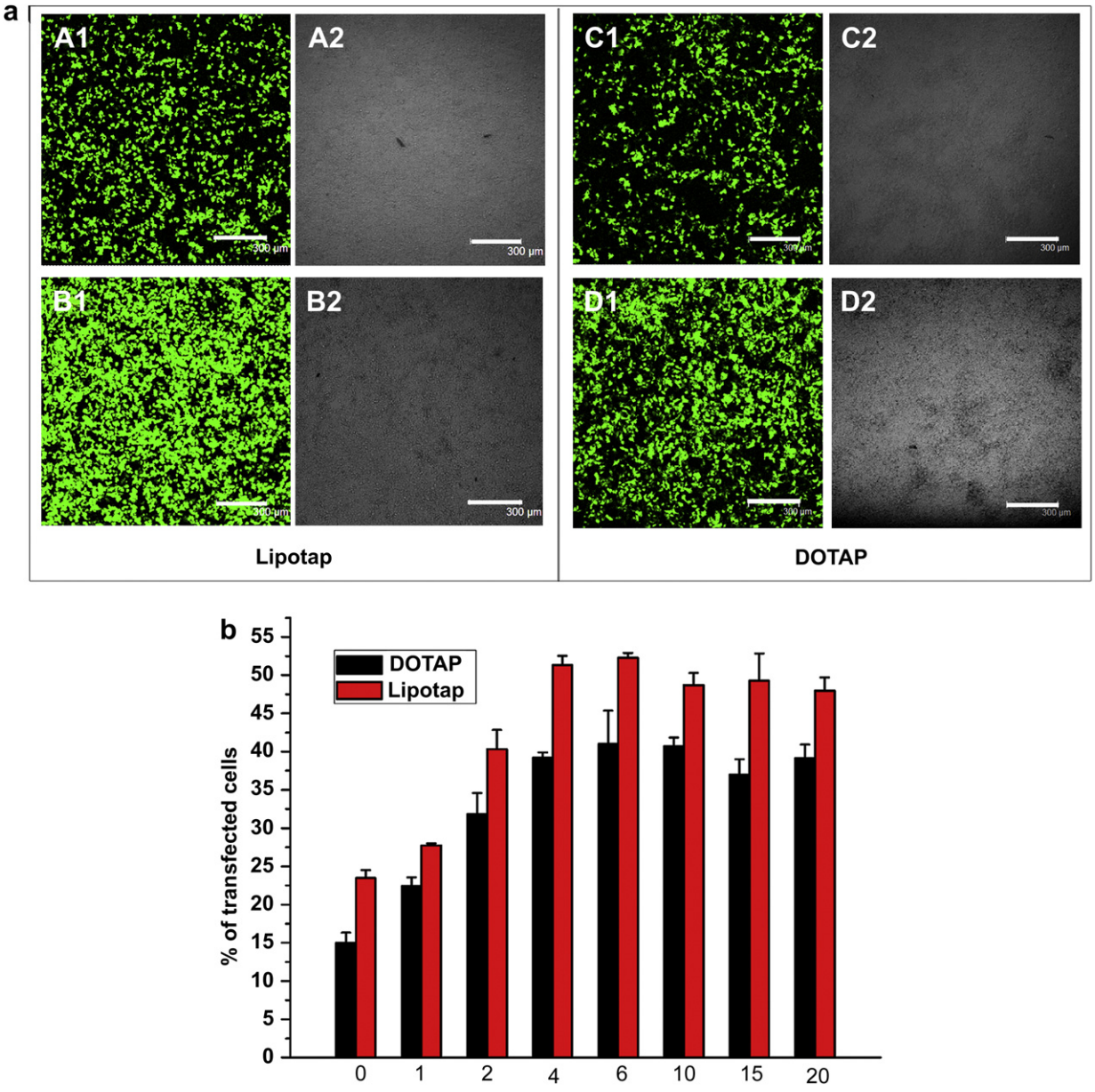


Fig. 2. The influence of DDAB-AuNPs on the transfection efficiency of Lipotap and DOTAP. The selected confocal microscopic images of EGFP fluorescence (A1, B1, C1, D1) and bright field (A2, B2, C2, D2) of HEK 293 cells were shown with 300 μm scale bars. The cells in plates A and C, were transfected with Liposome/pEGFP at the optical ratio. The cells in plates B and D were transfected with Liposome/pEGFP/DDAB-AuNPs (6 nM). The percentage of fluorescent cells in each sample measured by FCM was also shown (b), in which HEK 293 cells were transfected with Liposome/pEGFP/DDAB-AuNPs (0, 1, 2, 4, 6, 10, 15, and 20 nM).

liposomes, the culture in each well was changed by fresh medium 2 h before transfection. Then, 1 μL Lipotap and 100 ng plasmid DNA (0.5 μL DOTAP and 200 ng DNA) for each well were mixed in a tube and incubated at room temperature for 15 min. After that, various amounts of DDAB-AuNPs (with the final concentrations of 0, 1, 2, 4, 6, 10, 15 and 20 nM in cell culture) were added to the above tube and mixed sufficiently. Then the mixture was added to the cell medium and the cells grew in the incubator for 4 h before the culture was changed by fresh medium. After another 48 h of growth, the transfection efficiency was measured by FCM (FACSAria, BD Biosciences) and luciferase assay. The photographs of cells after transfected by liposome/pEGFP were taken using a confocal laser scanning fluorescence microscope (CLSM, Leica TCS SP2). In the cellular uptake assay by FCM, the cells were detached by trypsin–EDTA (0.25% trypsin, 0.53 mM EDTA) from the culture plate and resuspended in ice-cold PBS. The fluorescence of EGFP was measured with 488 nm excitation, and the data were analyzed by the BD FACSDiVa software.

2.4. Luciferase assay

The assay was carried out according to the protocol of luciferase assay kit from Promega and the details were same to our reported method [25]. Luciferase activity was normalized to the protein content measured by BCA assay (Pierce) of each sample.

2.5. Complexes formation detected by EMSA and CLSM

The interaction of the liposome, DNA and DDAB-AuNPs was firstly analyzed by EMSA using a 1.0% w/v agarose gel. During the investigation, 100 ng DNA and 1 μL Lipotap (200 ng DNA and 0.5 μL DOTAP) were first mixed in tubes and incubated at room temperature for 15 min. After incubation, various quantities of DDAB-AuNPs (with final concentration of 0, 4, 6, 10 and 20 nM used for the transfection test) were added to each tube and then the mixtures were loaded into the gel in turn. The gel was allowed to run for 45 min at 90 V. Afterward, the gel was incubated in 3× GenFinder™ solution for 2 h for visualization. Lastly, the gel was photographed under UV light using a fluorescence imaging system (Vilber Lourmat, Marne laVallee, France).

The morphology of the complexes for transfection was then visualized by CLSM. Briefly, FITC-DNA, lipoplex or lipoplex/DDAB-AuNPs (6 nM) were prepared (same to EMSA test) and dipped on the glass bottom dish (MatTek, Ashland, MA) separately and photographed by CLSM with 488 nm excitation.

2.6. Cellular uptake assay

Cellular uptake of the DNA with or without DDAB-AuNPs was measured by FCM. It was recorded by counting the cells that had internalized FITC-DNA. In this assay,

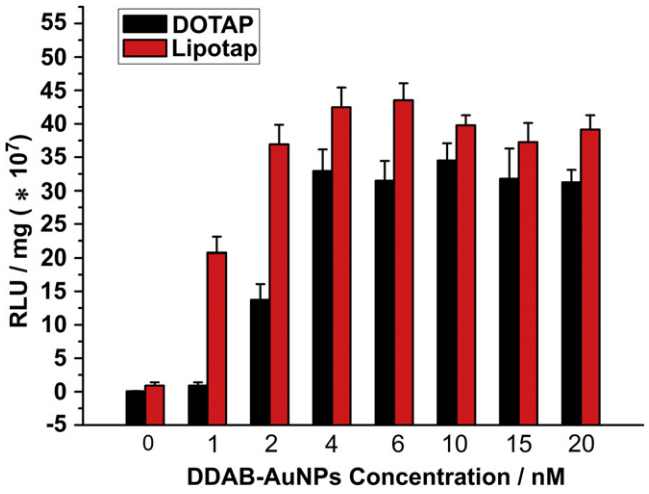


Fig. 3. The influence of DDAB-AuNPs on the transfection efficiency of Lipotap and DOTAP measured by luciferase assay. HEK 293 cells were transfected with Liposome/pGL3/DDAB-AuNPs (0, 1, 2, 4, 6, 10, 15, and 20 nM). The intensity of the chemiluminescence was normalized to the amount of protein. Data were shown as mean ± S.D. ($n = 4$).

100 ng FITC-DNA was mixed with 1 μL Lipotap (or 200 ng FITC-DNA with 0.5 μL DOTAP) and incubated at room temperature for 15 min before DDAB-AuNPs (at the same concentrations using in the transfection test) were added. The negative control was added neither DDAB-AuNPs nor any liposomes. Then the prepared mixtures were added to the each cell culture in turn. After incubation for 4 h, the cells were trypsinized and washed with PBS for 3 times. The fluorescence intensity was analyzed by FCM with 488 nm excitation. The fluorescence of 10,000 single cells was measured and the mean ± S.D. (standard deviation) of three experiments was calculated.

2.7. MTT assays

Cytotoxicity of the DDAB-AuNPs/lipoplex used in our study was evaluated by MTT assays. Briefly, HEK 293 cells were seeded to a 96-well culture plate and the cells would come to about 50% confluence after 24 h of culture. The media were changed by fresh ones, and the mixtures of lipoplex containing different concentrations of DDAB-AuNPs were added to the wells. The cells of positive control were only incubated with equal DMEM (10% FBS) medium and the cell viability was set as 100%. All of the cells were allowed to grow for 24 h before 10 μL MTT (5 mg/mL) was added to each well. Then, the cells were incubated at 37 °C for an additional 4 h until the purple precipitates were visible. The medium was replaced by 100 μL DMSO and the cell plate was vibrated for 15 min at room temperature to dissolve the crystals formed by the living cells. Finally, the absorption at 490 nm of each well was measured by an EL808 ultramicroplate reader (Bio-TEK Instrument, Inc., Winooski, VT, USA). The relative cell viability was recorded and shown.

3. Results and discussion

3.1. Preparation of and characterization of DDAB-AuNPs

DDAB was coated onto the Au nanoparticles by reducing $HAuCl_4$ with $NaBH_4$ in lipid vesicle solution. TEM image in Fig. 1A exhibited the morphology of as-synthesized DDAB-AuNPs, which was significantly dispersed with an average diameter of 9 ± 2 nm in the aqueous medium. Fig. 1B showed the UV–vis spectrum of the as-prepared DDAB-AuNPs, the absorption peak at 520 nm presented the excitation of surface plasmon vibrations of the gold nanoparticles. The structure of DDAB-AuNPs has been characterized in our previous work by the dynamic light scattering and AFM (atomic force microscope), which confirmed the existence of the single DDAB bilayer on the surface of AuNPs (Scheme 1) [24].

The concentration of the original prepared AuNPs was calculated as 10 nM (6.02×10^{12} particles/ml) [23]. After being condensation, the DDAB-AuNPs solution was ready for the transfection study with the final concentration of 1.0 μM. The preparation for DDAB-AuNPs is very easy and they can be synthesized in every laboratory for its needlessness of any complicated instruments. It is also worth mentioning that the DDAB-AuNPs are so stable that they can be kept at room temperature for more than six months without any aggregation in the solution.

3.2. Transfection efficiency enhancement induced by DDAB-AuNPs

It is known that more DNA molecules available to the cells can be got if we increase the total concentration of DNA for the transfection, but the liposomes are always toxic to the cells and effective only at certain liposome–DNA stoichiometries [12]. So, before studying the influence of DDAB-AuNPs on the transfection efficiency of liposome, we screened out the proper quantity for plasmid DNA with the highest efficiency by fixing the volumes of Lipotap (1 μL) and DOTAP (0.5 μL). Then we used the optimal quantity of DNA for the two transfection systems (data not shown). Fig. 2 showed the impact of DDAB-AuNPs on transfection efficiency investigated by both FCM and CLSM. Cells expressing EGFP in the samples transfected with Lipotap/DNA and DOTAP/DNA were 23.5% and 15% to the total cells, respectively (Fig. 2b). There were more cells in each well expressed EGFP when DDAB-AuNPs were added. DDAB-AuNPs, at the final concentration of about 4 or 6 nM, generated the highest transfection enhancement for both of the liposomes. The transfection efficiency increased to be 52.3% and 41% with 6 nM DDAB-AuNPs for the samples transfected with Lipotap and DOTAP, respectively. There was more than 2 times in

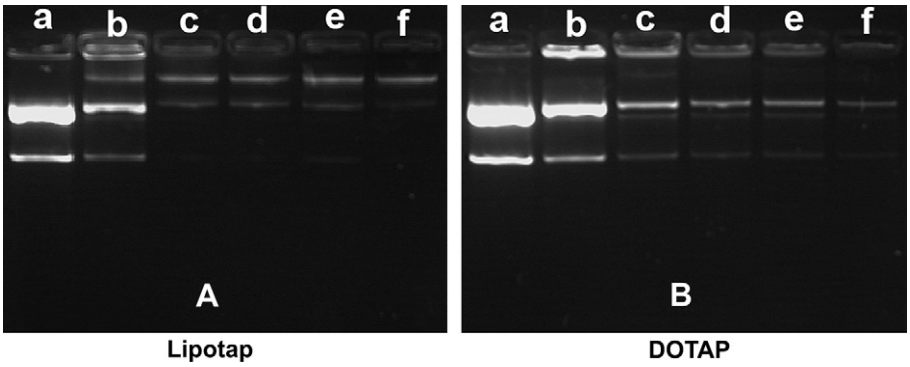


Fig. 4. Agarose gel electrophoresis images for measuring the interaction of lipoplex with DDAB-AuNPs at different concentrations. Panel A and B presented the results for Lipotap and DOTAP, respectively. The first well in the gel was loaded with pEGFP only (lane a). Lanes b to f were loaded with lipoplex accompany with DDAB-AuNPs at concentrations of 0, 4, 6, 10 and 20 nM sequencely.

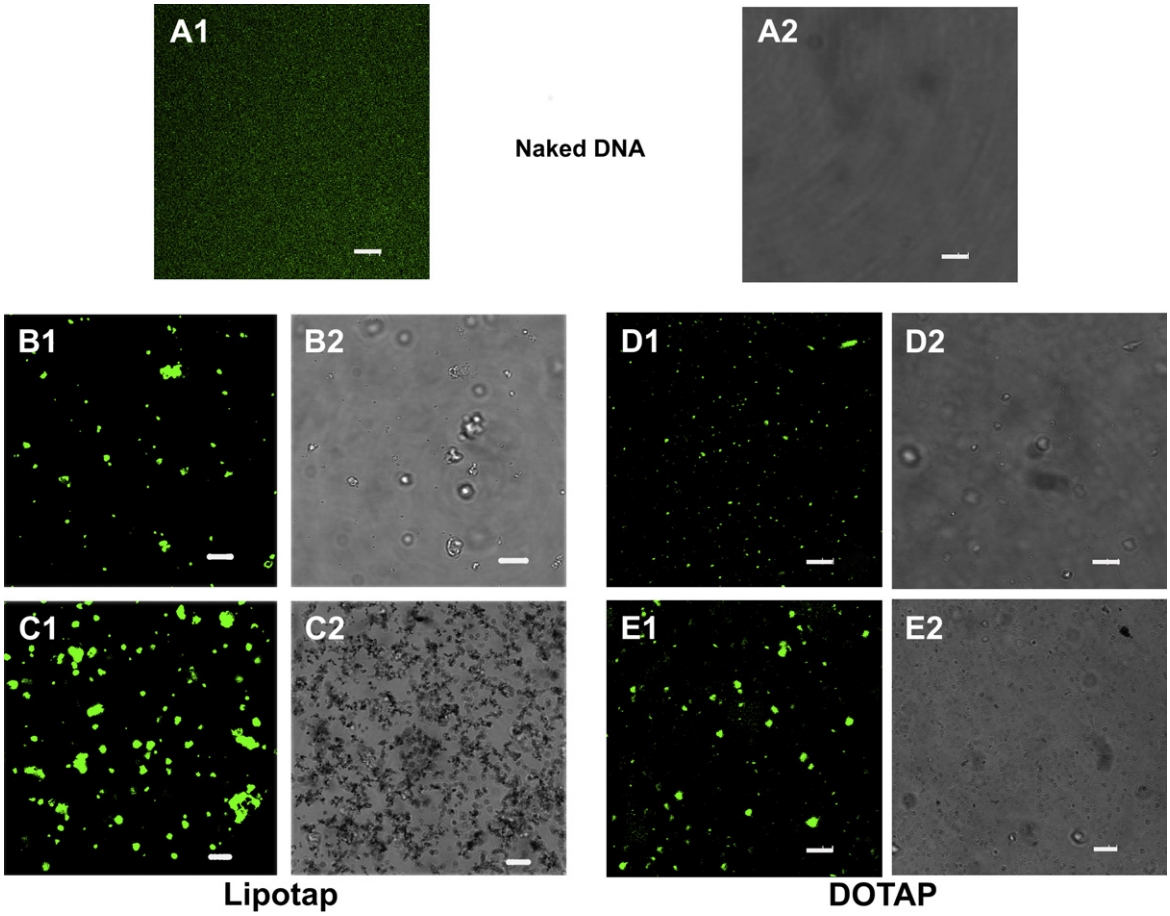


Fig. 5. CLSM images for investigating the interaction of DDAB-AuNPs with lipoplex. Several fluorescence and corresponding bright field images were shown. Panel A represented the images of FITC-DNA. Panel B and D represented the images of Lipotap/FITC-DNA and DOTAP/FITC-DNA complexes, respectively. Panel C and E showed the complexes of 6 nM DDAB-AuNPs with Lipotap/FITC-DNA and DOTAP/FITC-DNA, respectively. The bars indicated 5 μm.

enhancement for each sample. The selected photographs taken from CLSM were shown in Fig. 2a. In comparison with the samples which were only transfected with lipoplexes, there were more cells expressing EGFP when 6 nM DDAB-AuNPs were added before the transfection.

The enhancement in transgene expression level produced by DDAB-AuNPs was measured by the luciferase assay. The similar trends of enhancement were obtained in this assay. As shown in Fig. 3, with the addition of DDAB-AuNPs the transfection efficiency increased accordingly. When 6 nM DDAB-AuNPs were added, the relative luciferase unit (RLU) of the samples transfected by DOTAP and Lipotap increased from 6.90×10^5 and 8.89×10^6 to 3.15×10^8 and 4.35×10^8, respectively. It implied that DDAB-AuNPs brought about more than 456 and 48 times increase in gene expression for DOTAP and Lipotap mediated transfection, respectively. From the above measurements, it could get the conclusion that DDAB-AuNPs could be served as a transfection enhancer for both Lipotap and DOTAP. In addition, the enhancement trend for transfection became very weak when further increasing the concentrations of DDAB-AuNPs to 10, 15 and 20 nM. Therefore, DDAB-AuNPs of 4–6 nM are enough for Lipotap and DOTAP mediated transfection systems in this investigation.

3.3. The detection of interaction between DDAB-AuNPs and lipoplexes

EMSA was firstly employed to test the interaction of DDAB-AuNPs with lipoplex. The amount of plasmid DNA and liposome used throughout for this assay was the same as that used in the transfection experiment. Various amounts of DDAB-AuNPs were added to the lipoplex solution and then analyzed by agarose gel electrophoresis (Fig. 4). As shown in lane a, without liposome and DDAB-AuNPs, the supercoiled and relaxed circular plasmids moved towards the positively electrode as usual. With the addition of Lipotap or DOTAP (lane b), the migration of plasmids was slightly affected and only part of the plasmids allowed to come out from the wells compared to the naked plasmid DNA (lane a). It is known that the migrations of DNA molecules will be inhibited if negative charged of DNA molecules are completely neutralized by liposome or the newly formed lipoplexes are too big to enter the agarose gel. When DDAB-AuNPs with the concentration of 4 nM were added, there were more DNA molecules prevented from entering the agarose gel (lane c) in comparison with lane b. This is because that the as-prepared DDAB-AuNPs were positively-charged and could interact with residual DNA or lipoplexes those possessing a net negative charge. Therefore much less free DNA could migrate into the gel in these lanes. With further addition of DDAB-AuNPs to the final concentration of 6 nM, there was only a little more DNA molecules were trapped in the well (gel B, lane d). Additionally increased DDAB-AuNPs to concentrations of 10, 15 and 20 nM did not further affect the migration of the DNA in both gels. Thus, 6 nM DDAB-AuNPs might be enough for the further condensation of DNA molecules in these systems. The results were in line with the transfection test, extra DDAB-AuNPs with the concentrations of 10, 15 and 20 nM did not contribute more to the enhancement of transfection.

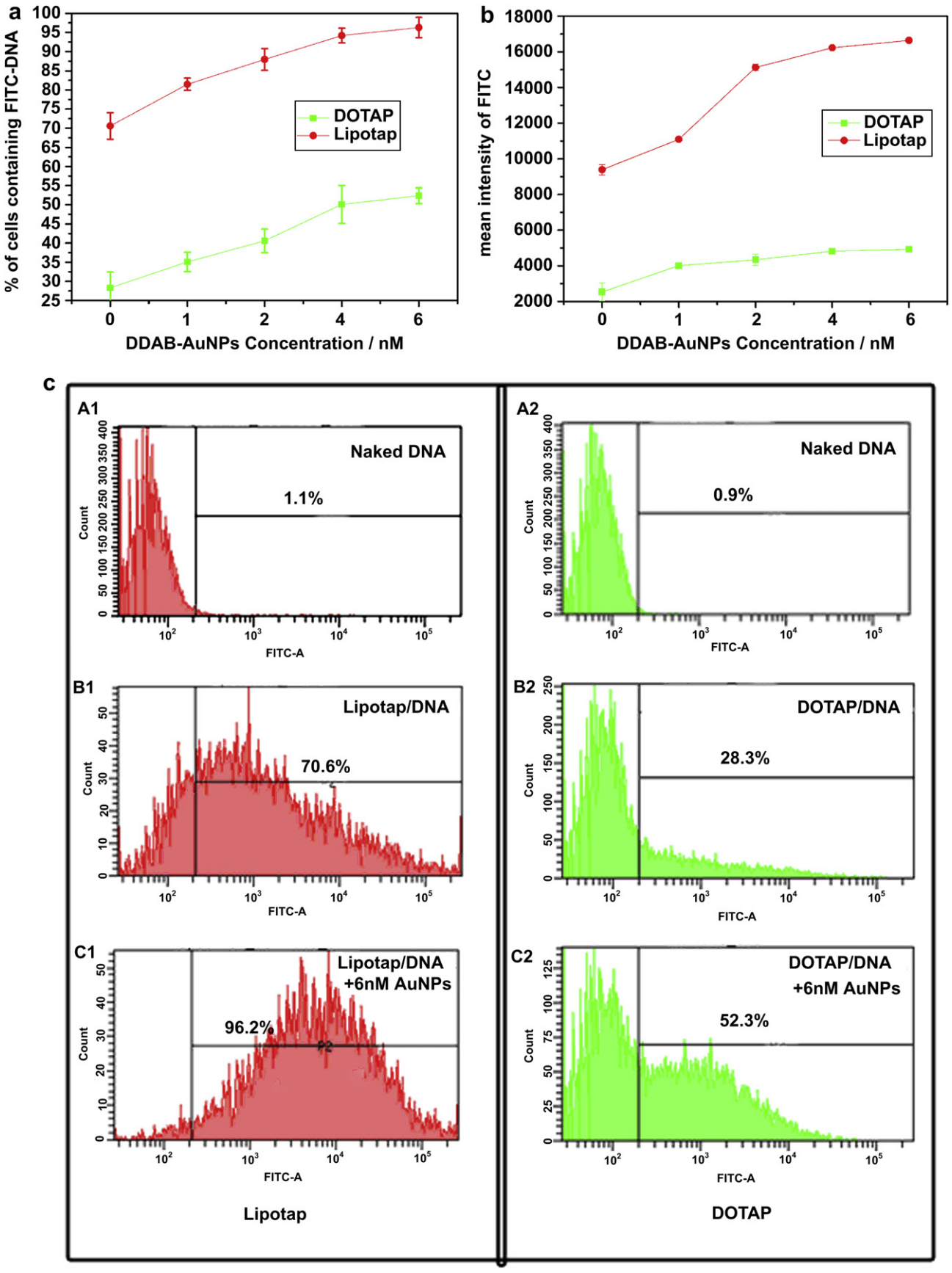


Fig. 6. The cellular uptake assay measured by FCM. (a) Measurement of the number of the cells containing the FITC-DNA after being incubated with lipoplex and DDAB-AuNPs with the final concentrations of 0, 1, 2, 4 and 6 nM. The red and green lines represented the cells treated by Lipotap and DOTAP complexes, respectively. All the data were shown as mean ± S.D. ($n = 3$). (b) The average intensity of FITC in above cells. (c) Selected FCM images for the above assay. The cells were incubated with naked FITC-DNA, lipoplex or lipoplex/DDAB-AuNPs (6 nM).

3.4. The association of DDAB-AuNPs with lipoplex investigated by CLSM

With the development of imaging technology, there have been more studies on the morphology of transfection reagents interacted with DNA before and during transfection [26,27]. We have also measured the interaction of DDAB-AuNPs with lipoplexes in a visible manner by CLSM, in which FITC-DNA was in place of pEGFP. The fluorescent images of DNA, lipoplex or lipoplex/DDAB-AuNPs (6 nM) were shown in Fig. 5. As shown in Fig. 5 A1, FITC-labeled DNA molecules were very dispersive and few aggregates could be found. When Lipotap (B1) or DOTAP (D1) was added, many visible complexes occurred. It indicated the formation of lipoplexes resulted from electrostatic interaction between negatively charged DNA and positively-charged amine headgroup of the cationic liposome [28]. Two liposomes containing various contents and quantities of cationic lipids, which resulted in difference in packing densities for FITC-DNA, so the sizes for the two kinds of lipoplexes were not equal. Fig. 5 C1 and E1 showed the images of Lipotap/DNA and DOTAP/DNA complexes with the addition of 6 nM DDAB-AuNPs, respectively. The green dots with bigger size compared to lipoplexes indicated that more FITC-DNA molecules were loaded onto the both Lipotap/DNA and DOTAP/DNA complexes.

3.5. DNA uptake measurement

It is generally accepted that the lipoplex is taken up by cells mainly through endocytosis and cellular uptake represents one of the rate-limiting steps to liposome-mediated transfection [29,30]. In this report, the cellular uptake of DNA was quantified to explore the mechanism involved in DDAB-AuNPs induced enhancement in transfection. Cellular uptake was assessed by counting the cells that internalized FITC-DNA and measuring the mean intensity of FITC in the cells by FCM. As shown in Fig. 6, when being transfected with lipoplex alone, the cells containing FITC-DNA were about 70.6% (Lipotap) and 28.3% (DOTAP) to the total cell invents. With the addition of DDAB-AuNPs, the numbers of cells containing FITC-DNA were increased for both of the two transfection systems. When 6 nM DDAB-AuNPs were incorporated into the systems, the cells containing FITC-DNA increased to be 96.3% and 52.5%, respectively. In the mean time, the mean intensity of FITC from 9391 to 16,644 and 2527 to 4926 for Lipotap and DOTAP mediated transfection, respectively.

The addition of DDAB-AuNPs to the lipoplexes increased the amount of accessible amines, which could facilitate their interaction with the negatively charged cell membrane and accelerate the cellular uptake [31]. Similar to the silica [12] and magnetic [16] nanoparticles, DDAB-AuNPs might also increase DNA concentration at the cell surface through elevated sedimentation rate, which would result in increasing cellular uptake. During the process of DDAB-AuNPs associating with lipoplexes, more DNA could be uploaded by each complex, which would contribute to the gene delivery efficiency to target cells. Therefore, the increased cellular uptake might be a main contributing factor of enhanced transfection efficiency for the liposomes. However, further increasing the concentration of DDAB-AuNPs to 10–20 nM did not promote significantly in DNA uptake (data not shown), the likely cause for this was that redundant DDAB-AuNPs could not further associate with lipoplexes and would not assist the internalization of DNA. Additionally, the excrescent nanoparticles might compete with DDAB-AuNPs/lipoplex in entering the cells to some degree, which could resulted in slightly decreasing of DNA cellular uptake and even the transfection efficiency of the systems.

3.6. Cytotoxicity of the DDAB-AuNPs used in the transfection

The cytotoxicity of the DDAB-AuNPs/lipoplex on the cell proliferation used in our system was investigated by MTT assay. Fig. 7 showed the data of cell viability of the HEK 293 cells after 24 h of incubation with the transfection complexes. Non-transfected cells were severed as the control and the cell viability of which were set as 100%. It was found that the lipoplex for the transfection had some toxicity to the cells and the cell viabilities were about 69% and 60% for Lipotap and DOTAP, respectively. In the presence of DDAB-AuNPs, the cell viabilities increased to be more than 80% for both of the transfection systems. With addition of DDAB-AuNPs at the concentration of 6 nM, the cell viabilities for Lipotap and DOTAP were increased to 91% and 85%, respectively.

It is known that AuNPs have good biocompatibility and low cytotoxicity [32] and we have also found that the gold core in DDAB-AuNPs can decrease the cytotoxicity of DDAB [23]. The lower cytotoxicity with additional DDAB-AuNPs in our systems might be due to the increased stability of the complexes for gene transfection. The plasmid DNA in the DDAB-AuNPs/lipoplex was less likely to be detached and degraded before and after entering the cells, so less toxicity will be introduced [22]. It could conclude that, DDAB-AuNPs/lipoplex-based transfection system has higher transfection efficiency and lower cytotoxicity.

3.7. Mechanism for the improved transfection efficiency

Scheme 1 showed the formation process of DDAB-AuNPs/lipoplex and the mechanism of transfection enhancement of our newly formed transfection system. Liposome and DNA firstly forms lipoplexes through electrostatic interaction, at which time some DNA molecules are still left in the system. With the addition of DDAB-AuNPs, a charge-mediated layer-by-layer self-assembly process induced by positively-charged DDAB-AuNPs occurs. The AuNPs interact with DNA on lipoplexes also through electrostatic interaction, and then extra DNA molecules in the transfection mixture will associate with the positively-charged amine on the surface of DDAB-AuNPs. When increasing the quantity of DDAB-AuNPs, DNA packaging density to the lipoplexes increases accordingly until the system comes to equilibrium and no more DNA molecules can be loaded onto the complexes (6 nM DDAB-AuNPs in

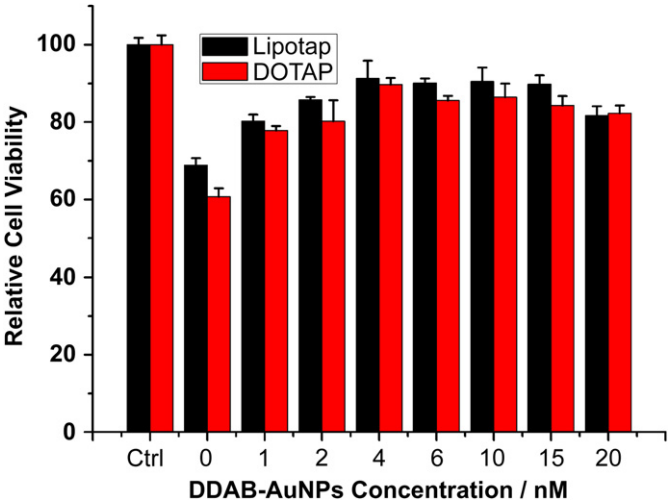


Fig. 7. Cytotoxicity of the DDAB-AuNPs/lipoplex on HEK 293 cells studied by MTT assays ($n = 4$, mean ± S.D.). The result showed the relative cell viability of the cells exposed to lipoplex accompany with DDAB-AuNPs with the final concentrations of 0, 1, 2, 4, 6, 10, 15 and 20 nM. The viability of positive control that treated with same volume of water was taken as 100%.

our experiment). The mechanism involved in DDAB-AuNPs induced enhancement of transfection efficiency after the formation of vector/DNA complexes might be as follows: (I) The addition of DDAB-AuNPs could increase the gravity of lipoplexes and help to increase DNA concentration on the surface of the cells. (II) DDAB-AuNPs incorporated liposome-based transfection system might minimize the surface negative charges of lipoplexes and help connect lipoplexes to cell membrane with a slighter negative charge [22]. (III) More DNA molecules are internalized for each delivery event during the transfection mediated by DDAB-AuNPs/lipoplex compared to lipoplex alone.

4. Conclusion

In summary, we prepared DDAB-coated gold nanoparticles with the function of promoting the transfection efficiency of liposomes. The results from EMSA and CLSM showed that DDAB-AuNPs could associate with lipoplexes and condense more DNA molecules to the complexes. Cellular internalization of DNA, one of the rate-limiting steps for lipoplex, measured by FCM indicated that DDAB-AuNPs enhanced the cellular uptake efficiency of DNA. The MTT assay results suggested that newly formed DDAB-AuNPs/lipoplex had lower cytotoxicity than lipoplex. In general, the lipoplex transfection system incorporated with DDAB-AuNPs has higher transfection efficiency and lower cytotoxicity, which has the potential of being used in clinical application of gene therapy in the future.

Acknowledgments

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Appendix

Figures with essential color discrimination. Most of the figures in this article have parts that are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.11.027.

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