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PAPER

The influence of cyclodextrin modification on cellular uptake and transfection efficiency of polyplexes

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Cyclodextrin-modified polycations have been studied widely due to their low cytotoxicity, low immunogenicity and the ability to form inclusion complexes. However, the influence of CD modification on cellular uptake and transfection efficiency of polyplexes is still unclear. In this research, cyclodextrin-modified polyethylenimines (PEI-CD) with different CD-grafting levels were synthesized, which were named PEI-CD_{15} and PEI-CD_{41}, respectively, according to the CD number per PEI chain. CD modification showed great influence on the DNA condensation ability of the polycation. PEI-CD_{15} could protect DNA completely above N/P ratio of 2. The particle sizes of these polyplexes were about 120 nm. However, PEI-CD_{41} could not protect DNA below N/P of 6, and PEI-CD_{41}/DNA polyplexes were larger than 1 μm, even at N/P ratio of 10. Therefore, this research was mainly focused on PEI-CD_{15}. It was interesting that the PEI-CD_{15}/DNA polyplexes at N/P ratio of 8 and 10 displayed excellent stability in physiological salt conditions, probably due to the hydration shell of CDs. The influence of CD modification on the cellular uptake and transfection efficiency of polyplexes depended on the type of the cells. Uptake inhibition experiments indicated that PEI/DNA polyplexes were internalized by HEK293T cells by both clathrin-mediated endocytosis and caveolae-mediated endocytosis. The route of caveolae-mediated endocytosis was significantly promoted after CD modification. So the cell uptake and transfection efficiency of PEI-CD_{15}/DNA polyplexes were significantly improved for HEK293T cells. However, the uptake and transfection efficiency of PEI-CD_{15}/DNA polyplexes in HepG2 cells was similar to that of PEI/DNA polyplexes, probably due to the lack of endogenous caveolins.

Introduction

The challenge of successful gene therapy relies heavily on the delivery of therapeutic genes into the target cells.[1] Viral vectors have generally been proven to have superior ability to deliver into desired cells and express genes.[2,3] However, their drawbacks including being expensive to produce, immunogenicity and insertional mutagenesis in the host genome limit their wide use.[4] Therefore, in order to overcome the aforementioned problems, much of the effort in non-viral gene delivery systems has been devoted to fabricate suitable delivery systems with high transfection efficiency and minimum cytotoxicity.[5–9]

Non-viral vectors, including cationic lipids and polycations, have recently emerged as viable alternatives due to their lower cost of synthesis, lack of immunogenicity and large nucleic acid loading capacity.[10] Among them, PEI is studied widely due to the so-called proton sponge effect, which shows relatively high transfection efficiency *in vitro*.[11–13] Nevertheless, the non-specific interaction in physiological conditions, polyplex aggregation and inefficient cellular uptake limit its biological efficacy. To avoid these problems, poly(ethylene glycol) (PEG),[14–16] polyanions[17] and saccharides[18] have been introduced into PEI polyplexes by covalent bonds or non-covalent interaction. Cyclodextrins (CDs) are a series of natural oligosaccharides that are composed of D (+)-glucose units.[19] Polycations containing CDs in the main chain, named PCDs, have been studied widely due to their low cytotoxicity and low immunogenicity.[19–21] Davis *et al.* studied β-CD-modified PEI as a gene vector and the results illustrated that the CD-grafting level was correlated with lower transfection and toxicity.[22] However, Pack *et al.* prepared β-CD-modified PEI conjugates with high transfection efficiency.[23] Furthermore, it has been reported that the transfection efficiency of the polycation containing α-CDs was higher than that of polycations lacking α-CD.[24] The difference may be attributed to different CD types and the effect of CDs on cell endocytosis. However, little research has focused on the cell internalization of CD-modified polycation/DNA polyplexes and its effect on transfection efficiency.

The objective of our study is to investigate the influence of CD on the cellular uptake and transfection by different cell lines. Cyclodextrin-modified polyethylenimines (PEI-CD) with

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different CD-grafting levels were synthesized. The proprieties of the polyplexes were characterized, which included physico-chemical properties, cytotoxicity and transfection efficiency. CD-grafting level showed a great influence on the DNA condensation ability of the polycation. Interestingly, it was found that the PEI-CD_{15}/DNA polyplexes at N/P ratios of 8 and 10 displayed excellent stability in physiological salt conditions. The uptake mechanism of PEI-CD_{15}/DNA polyplexes was then preliminarily researched.

Materials and methods

Materials

Branched polyethylenimine (PEI, 25 kDa) was purchased from Sigma-Aldrich. *p*-Toluenesulfonyl chloride and β-cyclodextrin (β-CD) were obtained from Aladdin (Shanghai, China). Deoxyribonucleic acid (DNA, fish sperm, sodium salt) and *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPEs, free acid, high pure grade) were purchased from AMRESCO. Plasmid pEGFP (4733 bp) as transfection reagent was obtained from Clonetech. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Bio Basic Inc. Loading buffer was obtained from TakaRa Biotechnology Co. Ltd. (Dalian, China). Chlorpromazine and Nocdazole were purchased from J&K Scientific Ltd. 0.5×TBE buffer was diluted from 4×TBE buffer (0.36 M tris-boric acid, 8 mM EDTA).

Synthesis and characterization of PEI-CD

Synthesis of 6-deoxy-(*p*-toluenesulfonyl)-β-cyclodextrin (6-*O*-Ts-β-CD). 6-*O*-Ts-β-CD was prepared according to Petter's method with modification.[25] Briefly, β-CD was purified by recrystallization twice in distilled water. 15 g (12.3 mmol) of β-CD was suspended in 125 ml of distilled water. 1.64 g (41 mmol) of sodium hydroxide dissolved in 5 ml of distilled water was added dropwise over 15 min. Once the suspension became homogeneous, it was placed in an ice–water bath and 2.52 g (12.3mmol) of *p*-toluenesulfonyl chloride in 7.5 ml of acetonitrile was added dropwise over 30 min. Then the reaction was carried out at room temperature for 2 h. The remaining precipitate was removed by filtration, and the filtrate refrigerated overnight at 4 °C. The resulting precipitate was washed by water and ethanol twice, respectively, and dried under vacuum. The product was analyzed by ^{1}H NMR (300 MHz, Varian Spectrometer, USA) and ultraviolet-visible spectrophotometry (UV-2550, Shimadzu, Japan).

Synthesis of PEI-CD. PEI-CD was prepared according to the previous study.[22] Briefly, 0.5 g of PEI in 20 ml dimethyl sulfoxide (DMSO) was reacted with 3.8 g of 6-*O*-Ts-β-CD prepared as described above (without any purification). The solution was stirred at 70 °C for 3 d under nitrogen environment. Then, the product was purified by dialysis for 6 d with a MWCO 3500 membrane and lyophilized. The CD grafting level per PEI chain was analyzed by ^{1}H NMR. By changing the molar ratio of PEI to 6-*O*-Ts-β-CD, PEI-CD with different CD-grafting levels was synthesized.

Formulation of polymer/DNA polyplexes. PEI-CD/DNA polyplexes were prepared by adding equal volumes of PEI-CD solution to DNA solution (100 μg ml^{-1} dissolved in 20 mM HEPEs buffer solution) at the appropriate N/P ratio. Then the mixture was vortexed and incubated for 30 min before analysis. The polyplexes at N/P ratio of 4 were named PEI-CD/DNA-4 (*i.e.*, PEI-CD_{15}/DNA-4 for PEI-CD_{15}). PEI/DNA polyplexes were prepared under the same conditions for comparison. All polyplexes were prepared freshly before use.

Characterization of polyplexes

Transmission Electron Microscopy (TEM). TEM was conducted on a transmission electron microscope (JEM-1200EX, NEC, Tokyo, Japan) operated at 80 kV. A drop of the polyplexes prepared as described above was deposited onto a 200-mesh carbon-coated copper grid. The excess aqueous solution was removed by blotting with filter paper after 5 min. In order to obtain enough particles on the grid, the above process was repeated three times.

Particle size and zeta (ζ) potential measurement. Particle size measurement was performed on a 90plus/BI-MAS particle size analyzer (Brookhaven, Holtsville, NY, USA), which detected at a 90° angle and determined at 25 °C. Every measurement was carried out in 4 serial measurements. The ζ-potential was examined by Zetasizer 3000HS (Malvern, UK) at room temperature.

Gel retardation assay. The DNA binding ability of PEI-CD was examined by gel retardation assay. Polyplexes at appropriate N/P ratio containing 300 ng plasmid DNA were mixed with a loading buffer (5 : 1 by volume), loaded to each well of an agarose gel (1% by weight in 0.5× TBE buffer) and subjected to gel electrophoresis at 100 V for 50 min. Then the gel was immersed in ethidium bromide solution (0.5 μg ml^{-1}) for 30 min, visualized and photographed with UV illumination (Gel Doc, Bio-Rad, USA).

The stability of polyplexes in physiological salt conditions. The polyplexes at appropriate N/P ratio were prepared by the aforementioned methods. Then the salt concentration was adjusted to 150 mM. The polyplexes were incubated for different times. The stability profile was detected by dynamic light scattering (DLS).

Cell culture experiments. HEK293T cells (Human embryonic kidney cell line) and HepG2 cells (Human Hepatoblastoma cell line) were cultured with modified DMEM, which was supplemented with 10% FBS (fetal bovine serum) and 1% penicillin–streptomycin. Then, these cells were maintained at 37 °C in a humid atmosphere containing 5% CO_2.

The cellular uptake efficiency and uptake inhibition. The cellular uptake efficiency of polyplexes was performed in HEK293T and HepG2 cells using FITC-labeled DNA which was prepared according to the literature.[26] The cells were seeded in 24-well plate at 1×10^5 cells/well and incubated for an additional 24 h. Before the polyplexes addition, the medium was replaced with 0.5 ml of fresh medium. Polymers complexed with FITC-labeled DNA (2 μg) at the appropriate N/P ratio were prepared as above, exposed to cells for 0.5 h or 4.5 h and incubated at 37 °C in 5% CO_2. At the end of the 0.5 h or 4.5 h incubation, the cells were rinsed three times with PBS (pH = 7.4) in order to remove surface-associated polyplexes, trypsinized and analyzed by flow cytometry. All experiments were performed in triplicate.

For uptake inhibition studies, HEK293T cells were pre-treated with chlorpromazine (CpZ, 45 μM), Nocdazole (Noc, 10 μM)

and a mixture of the two (45 μM CpZ and 10 μM Noc) for 1 h, and then incubated with polyplexes for another 1 h in 5% CO_2. Then the cells were collected and analyzed by flow cytometry. All experiments were performed in triplicate.

Intracellular distribution. HEK293T cells were seeded into confocal imaging dishes at a density of 5×10^4 cells per dish. PEI and PEI-CD_{15} polyplexes at N/P ratio of 10 containing 2 μg FITC-labeled DNA were prepared as described above and exposed for 4.5 h. Then, the cells were washed three times with PBS, fixed for 30 min with 4% (w/v) paraformaldehyde and rinsed three times with PBS. Following incubation with DAPI (2.5 μg ml^{-1}) for 20 min, the cells were washed three times with PBS. The intracellular distribution of polyplexes was analyzed by laser scanning confocal microscope (Leica TSSP5, Germany).

Cell viability assay. The cell viability assay was performed in HEK293T and HepG2 cells. The cells were seeded into 96-well plates at the density of 1×10^4 cells/well and incubated at 37° in 5% CO_2 for 24 h. The medium was then replaced with 100 μl of fresh medium. Polyplexes containing 1 μg DNA were added into the cells and incubated for 36 h or 60 h. At the end of the 36 h or 60 h incubation, the mixture was rinsed with PBS, replaced with fresh medium and 20 μl of MTT solution (5 mg ml^{-1}, dissolved in PBS) and incubated for 4 h at 37 °C. Then the medium was removed, 200 μl of DMSO was added and the mixture was incubated for an additional 15 min at 37 °C. The absorbance at 570 nm was measured by microplate reader (550, Bio-Rad, USA). All experiments were performed in quintuplicate.

Transfection experiment *in vitro*. The transfection efficiency of polyplexes was also performed in both cell types. The cells were plated in 24-well plates (1×10^5 cells/well) and transfected with 2 μg of pEGFP, which complexed with PEI-CD_{15} at various N/P ratios and PEI as a comparison in the presence of 10% FBS. After uptake for 4.5 h, the medium was replaced with 0.5 ml of fresh medium. After 48 h transfection, the cells were observed by fluorescence microscopy and transfection efficiency was detected by flow cytometry. All transfection experiments were performed in triplicate.

Results and discussion

Synthesis and characterization of PEI-CD

PEI-CD was prepared as described by Davis.[22] The product of 6-*O*-Ts-β-CD was analyzed by ^{1}H NMR and ultraviolet-visible spectrophotometry. The ^{1}H NMR spectrum is shown in Fig.1a. The proton peaks of the toluene group appeared at 7.4–7.8 ppm, which was different from that of the CD. The UV spectrum (dissolved in DMSO) of the product showed that the absorption peaks were at 273.6 nm and 262.6 nm (data not shown), which was consistent with the report in the literature.[25] The CD grafting level was calculated based on proton integration according to the ^{1}H NMR spectrum. As shown in Fig. 1b, the CD grafting level was 2.5%, which means that every PEI molecular chain has about 15 CD molecules. So PEI-CD_{15} was synthesized successfully. Similarly, by changing the molar ratio of PEI to 6-*O*-Ts-β-CD, PEI-CD_{41} was also synthesized. The influence of CD grafting level on the DNA condensation ability was then investigated.

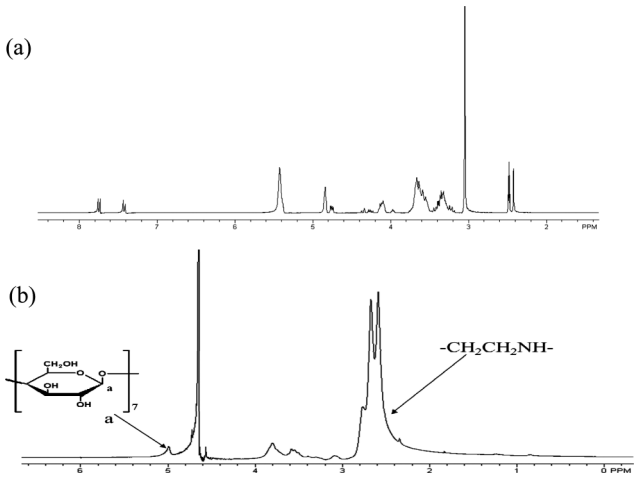

Fig. 1 ^{1}H NMR spectra of 6-*O*-Ts-β-CD (a) and PEI-CD_{15} (b).

Characterization of polyplexes

Polyplexes were formulated by electrostatic interaction when the charged polycation contacted with the negatively charged DNA. For gene delivery, the ability of the polycation to condense DNA into the polyplexes is the primary requirement. Therefore, it is important to carry out characterization of the polyplexes, such as for particle size, ζ-potential and DNA binding ability for gene vectors.

The influence of CD grafting level on the DNA condensation ability was investigated by gel retardation assay. The results are shown in Fig. 2. For PEI-CD_{15}, the migration of DNA was completely retarded when the N/P ratio was above 2. The result indicated that the low CD grafting level did not affect the condensation ability of PEI. However, PEI-CD_{41} with higher CD grafting level could not protect DNA until the N/P ratio increased to 6.

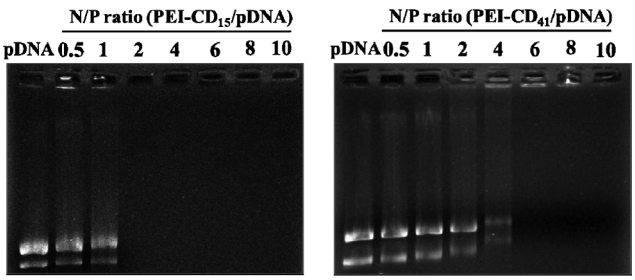

Fig. 2 Agarose gel electrophoresis retardation of PEI-CD/DNA polyplexes.

The particle size of PEI-CD/DNA polyplexes was also investigated. The results are shown in Fig. 3. The size of PEI-CD_{15}/DNA polyplexes decreased with the N/P ratio. At the N/P ratios of 8 and 10, the particle size was about 120 nm. The TEM pictures in Fig. 4 indicate that the PEI-CD_{15}/DNA polyplexes were spherical and had a compacted structure. The particle sizes were consistent with the results determined by DLS. However, PEI-CD_{41}/DNA polyplexes under the same conditions as PEI-CD_{15} had larger particle sizes, and for the N/P ratio of 10 the particles were larger than 1 μm. The reason may be considered from two

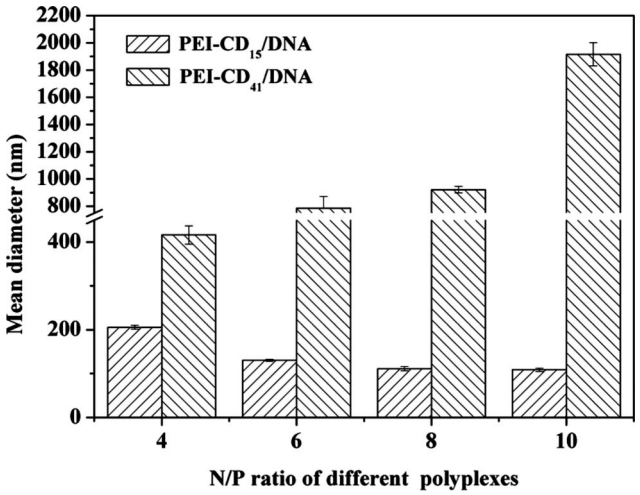

Fig. 3 Particle size of different polyplexes with various N/P ratios. Error bars represent mean ± SD for $n = 3$.

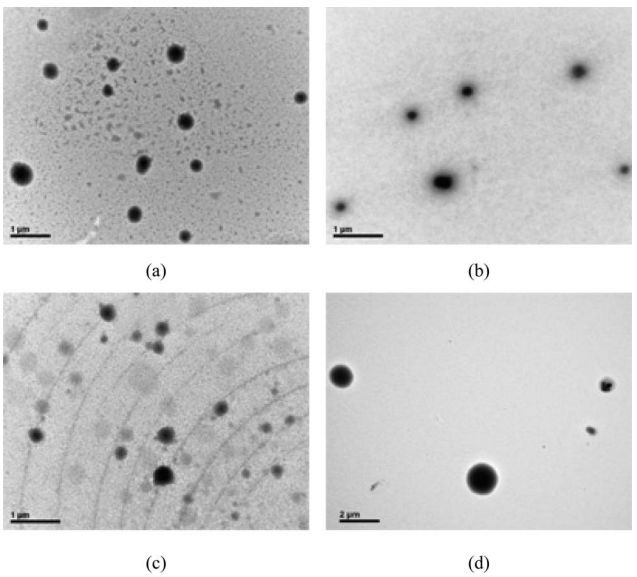

Fig. 4 The TEM images of different polyplexes. PEI-CD_{15}/DNA-6 (a), PEI-CD_{15}/DNA-8 (b); PEI-CD_{15}/DNA-10 (c) and PEI-CD_{41}/DNA-10 (d).

aspects. Firstly, primary amines changed to secondary amines with CD modification. Secondly, the intermolecular hydrogen bonds improved with the CD grafting level, which hindered the electrostatic interaction between the polycation and negatively charged DNA. So the higher CD grafting level led to the poor DNA condensation of the polycation.

A gene delivery vector with high transfection efficiency must have good DNA condensation ability and an appropriate particle size. Based on the above information, the research was mainly focused on PEI-CD_{15}/DNA polyplexes. The ζ-potential of PEI-CD_{15}/DNA polyplexes was further investigated. The ζ-potential of PEI-CD_{15}/DNA-4 was about 16 mV. When N/P ratio increased to above 6, the ζ-potential of PEI-CD_{15}/DNA polyplexes was about 20–25 mV.

The stability of polyplexes in physiological salt conditions

The polyplexes at appropriate N/P ratio were prepared by the aforementioned methods, then the salt concentration was adjusted to 150 mM. The polyplexes were incubated for different times. The stability profile was detected by DLS and the results are shown in Fig. 5. After incubation for 45 min in physiological salt conditions, the PEI/DNA polyplexes were nearly 1 μm. The PEI/DNA polyplexes were unstable and aggregated quickly. The reason for this was that the polycation on one particle bound to another particle by interparticle cross-bridging due to the reduced repulsive barriers.[27] However, the stability of PEI-CD_{15}/DNA polyplexes showed some relationship to N/P ratio. PEI-CD_{15}/DNA-4 and PEI-CD_{15}/DNA-6 polyplexes aggregated quickly, but the PEI-CD_{15}/DNA-8 and PEI-CD_{15}/DNA-10 polyplexes were still about 120 nm. β-CD is a natural oligosaccharide that is composed of 7 D(+)-glucose units. Intermolecular hydrogen bonds existed between the hydroxide groups of the CDs and amino groups of PEI, which limited the movement of the polycations on the shell. On the other hand, the CDs on the shell absorbed water through hydrogen bonding interaction. It was probably due to the hydration shell of CDs that the PEI-CD_{15}/DNA polyplexes at N/P ratio of 8 and 10 displayed excellent stability in physiological salt conditions. However, the CDs on the shell were not sufficient to improve the stability at low N/P ratio.

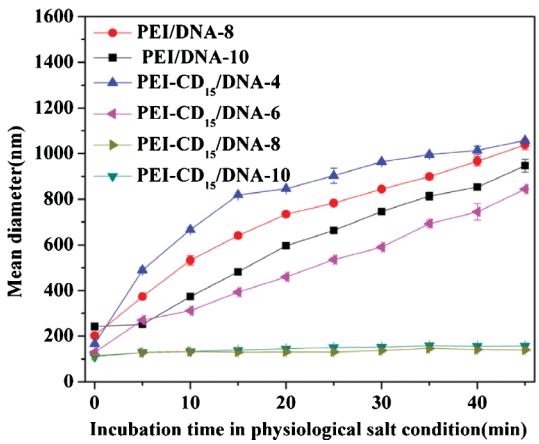

Fig. 5 The stability of PEI-CD_{15}/DNA polyplexes at different N/P ratios in physiological salt conditions detected by DLS (0 min means as-prepared conditions). Error bars represent means ± SD for $n = 3$.

Cellular uptake of polyplexes and uptake inhibition

Endocytosis of polyplexes is one of the barriers that polyplexes must overcome for transfection.[28] Here, different polyplexes with 2 μg FITC-labeled DNA were prepared and then exposed to HEK293T cells or HepG2 cells for a period of time. The cells were rinsed with PBS in order to remove surface-associated polyplexes, trypsinized and analyzed for polyplexes uptake by flow cytometry.

The results are shown in Fig. 6. Naked DNA was not readily internalized by HEK293T and HepG2 cells. The uptake of the polyplexes was significantly better. The modification with CD had some effect on the cellular uptake. The uptake efficiency of PEI-CD_{15}/DNA polyplexes by HEK293T cells was almost 2 or more times that of the PEI/DNA polyplexes. However, the uptake

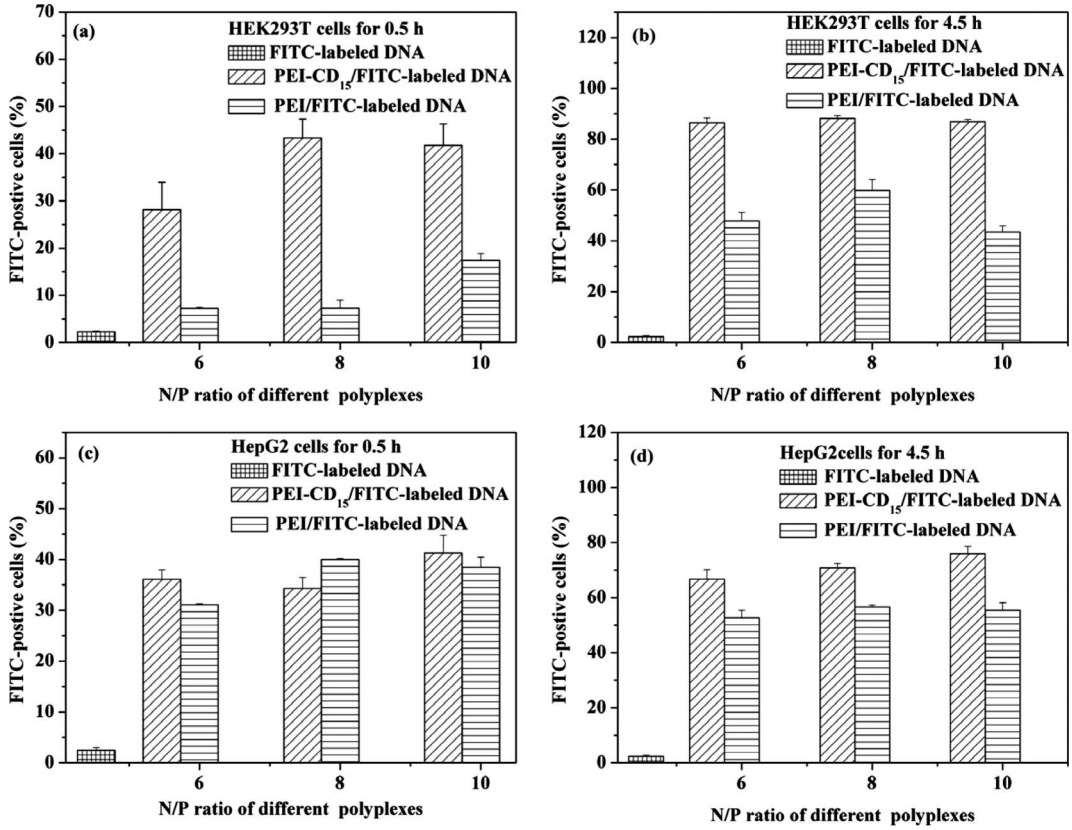

Fig. 6 Cellular uptake efficiency by HEK 293 cells (a, b) and HepG2 cells (c, d) for incubation of 0.5 h (a, c) or 4.5 h (b, d). Error bars represent mean ± SD for $n = 3$.

efficiency of PEI-CD_{15}/DNA polyplexes by HepG2 cells was just slightly improved compared with that of PEI/DNA polyplexes.

In order to investigate the effect of CD on the cell uptake of polyplexes, uptake inhibition of PEI/DNA and PEI-CD_{15}/DNA polyplexes was investigated. Chlorpromazine (CpZ) was the inhibitor of clathrin-mediated endocytosis, and Nocdazole (Noc) was the inhibitor of caveolae-mediated endocytosis. The results are shown in Fig. 7.

When HEK293T cells were pre-treated with CpZ, uptake of PEI/FITC-labeled DNA polyplexes decreased by more than 25%. By contrast, pre-treatment with Noc caused a more than 40% decrease in uptake of PEI polyplexes. Interestingly, the combination of CpZ and Noc pre-treatment caused further decrease. These data showed that HEK293T cells internalized PEI/DNA polyplexes by means of both clathrin-mediated endocytosis and caveolae-mediated endocytosis. However, after pre-treatment with CpZ or Noc alone, the uptake decrease of PEI-CD_{15}/DNA polyplexes was less than 10% and 40%, respectively (Fig. 7b). The combination of the two inhibitors did not cause an obvious further decrease. The combination of above information suggests that the route of

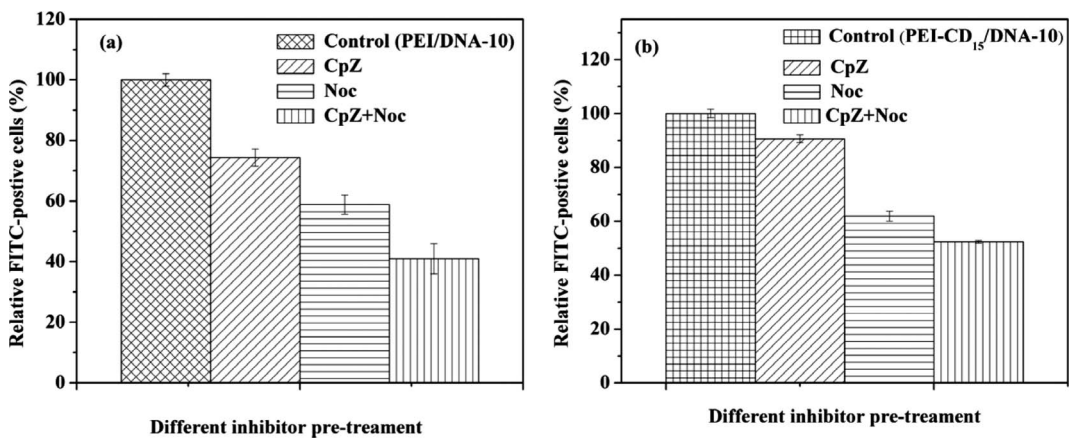

Fig. 7 Cellular uptake inhibition for PEI/DNA (a) and PEI-CD_{15}/DNA (b) polyplexes exposed to HEK293T cells. Error bars represent mean ± SD for $n = 3$.

caveolae-mediated endocytosis was significantly promoted after CD modification. So the cell uptake efficiency of PEI-CD_{15}/DNA polyplexes was obviously improved with HEK293T cells.

The intracellular distribution

The intracellular distribution of polyplexes was observed by confocal microscopy in HEK293T cells. Fluorescence of green dots was from FITC-labeled DNA, and DAPI-stained cell nuclei presented with blue color. As shown in Fig. 8a, just part of the HEK293T cells showed weak green fluorescence of the PEI/DNA polyplexes, and the polyplexes were mainly distributed in cytoplasm. However, PEI-CD_{15}/DNA polyplexes were internalized by most of the HEK293T cells and located in both cytoplasm and nuclei, as shown in Fig. 8b. The fluorescence intensity of PEI-CD_{15} polyplexes was stronger than that of PEI polyplexes, which is consistent with the cellular uptake profile detected by flow cytometry as the means of fluorescence analysis. So it was concluded that the CD modification was favorable for cell uptake and nuclear entry of polyplexes by HEK293T cells, which is believed to be able to enhance transfection efficiency.

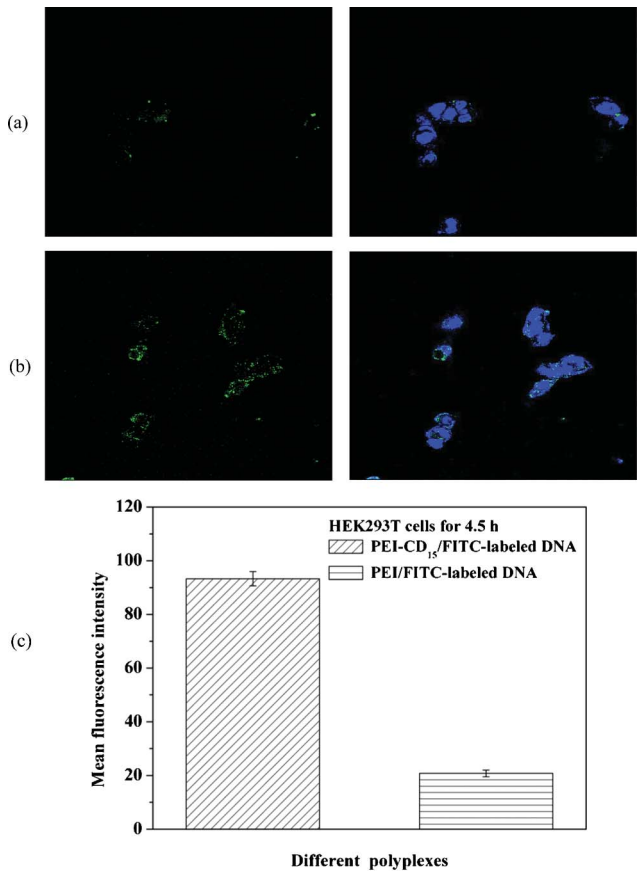

Fig. 8 The intracellular distribution of polyplexes internalized by HEK293T cells by LSCM. FITC-labeled DNA presented as green fluorescence and DAPI-stained nuclei showed blue color. Cells were treated with PEI polyplexes (a), PEI-CD_{15} polyplexes (b); cellular uptake fluorescence detected by flow cytometry (c).

The cytotoxicity of polyplexes

The cytotoxicity of polycation gene vectors is often a result of the density of cationic groups. Herein, the viability of PEI-CD_{15}/DNA polyplexes was evaluated in both HEK293T and HepG2 cells by MTT assay. As shown in Fig. 9, the cytotoxicity of PEI/DNA polyplexes significantly increased with N/P ratio. It was reported that PEI toxicity was due to the cell membrane disruptive behaviour of dense cationic polymers.[29,30] By contrast, the cytotoxicity of PEI-CD_{15}/DNA polyplexes was obviously lower than PEI/DNA polyplexes in both cell types at high N/P ratio, while the distinction was smaller at low N/P ratio.

The amino groups of PEI were modified with CD, and some primary amino groups were converted into secondary amino groups. From the above information, the density of positive charge maybe decreased at pH value of 7.4. Hereby, the lower cytotoxicity of PEI-CD_{15}/DNA polyplexes may be attributed to the decreased charge density. On the other hand, the high cytotoxicity may be due to the higher cellular uptake efficiency. Interestingly, PEI-CD_{15}/DNA polyplexes with higher uptake efficiency, however, have lower cytotoxicity. Therefore the low cytotoxicity is mainly due to the modification by CD.

Transfection experiment *in vitro*

HEK293T and HepG2 cells were chosen for transfection efficiency studies. The *in vitro* transfection experiment was performed in the presence of 10% FBS. pEGFP was used as reporter gene. The polyplexes containing 2 μg pEGFP were prepared as described above. The transfection efficiency of PEI-CD_{15}/DNA polyplexes was compared with PEI/DNA polyplexes. The expression of green protein in the cells after 48 h incubation was determined by flow cytometry analysis. The results are shown in Fig. 10, which indicate that the effect of CD modification on transfection was related to the type of the cells.

The transfection efficiency of PEI-CD_{15} and PEI/DNA polyplexes with HEK293T cells was apparently dependent on N/P ratio. Interestingly, the transfection efficiency of PEI-CD_{15}/DNA polyplexes was higher than that of PEI. The transfection efficiencies of PEI-CD_{15}/DNA polyplexes at N/P ratio of 6, 8 and 10 were almost 1.4-, 1.3- and 2.2-fold higher than that of the PEI polyplexes, respectively. Uptake inhibition results indicated that the route of caveolae-mediated endocytosis was significantly promoted after CD modification. Some literature reports have indicated that caveolae-mediated endocytosis appeared to be slower than the clathrin-mediated endocytosis. However, in some cases the caveolae-mediated endocytosis can enter into the nucleus directly.[28] So this pathway is believed to be beneficial for cell delivery of DNA and might lead to efficient transfection.[31] The improved transfection efficiency of PEI-CD_{15}/DNA polyplexes was probably due to the improved caveolae-mediated endocytosis and nuclear entry by HEK293T cells.

For HepG2 cells, however, the transfection efficiency of PEI-CD_{15}/DNA polyplexes was only slightly higher or even similar to that of PEI/DNA polyplexes. The reason may be attributed to the similar uptake efficiency. Some research has indicated that HepG2 cells lacked endogenous caveolins. Therefore, the cells cannot internalize polyplexes *via* caveolae-mediated endocytosis.[32] Combining the above information, it can be seen that the lack of uptake by caveolae-mediated endocytosis did not cause any further effect on cellular uptake and transfection with HepG2 cells.

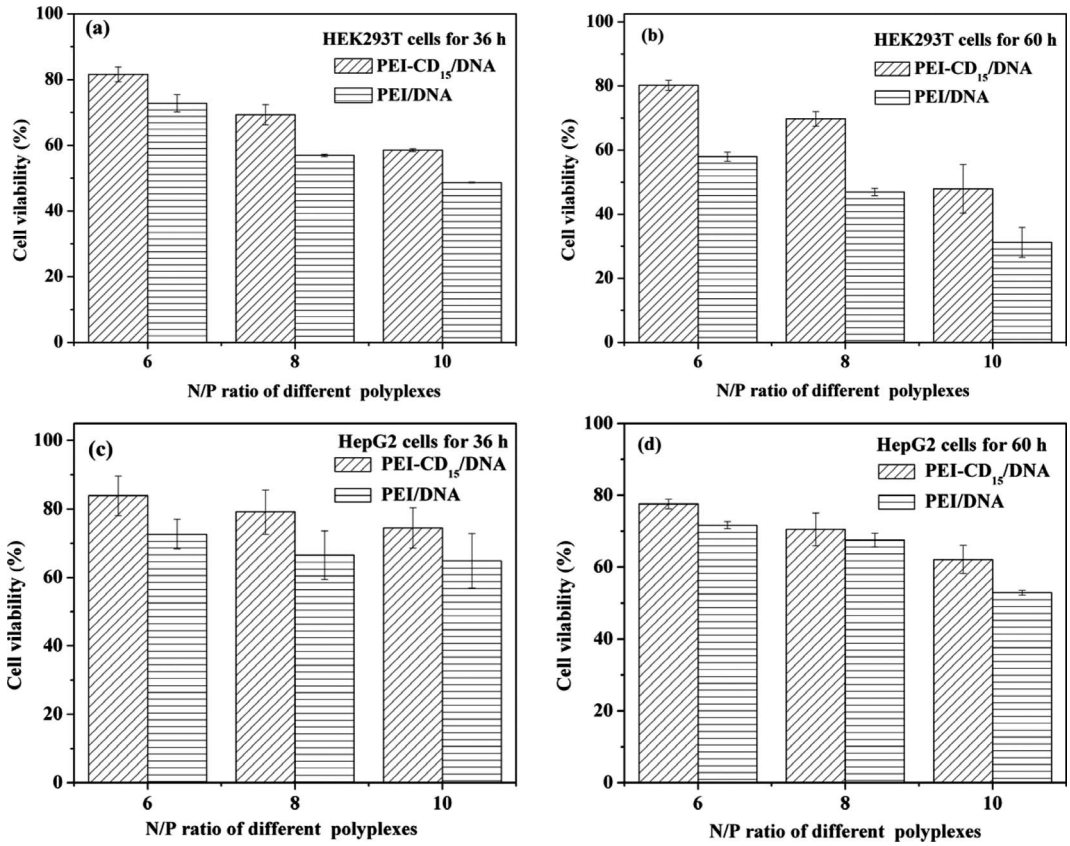

Fig. 9 Cytotoxicity of PEI/DNA and PEI-CD_{15}/DNA polyplexes at various N/P ratios with DNA (1 μg) after incubation for 36 h (a, c) or 60 h (b, d). Error bars represent mean ± SD for $n = 5$.

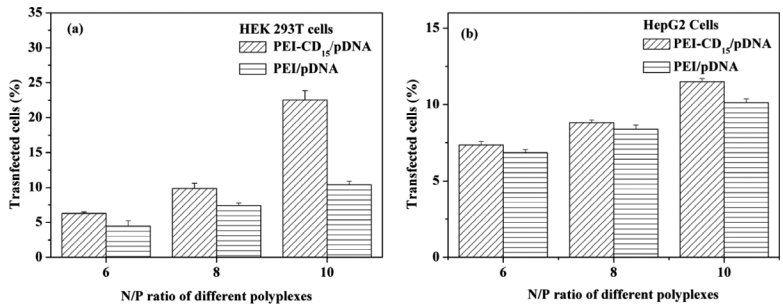

Fig. 10 The transfection efficiency of PEI-CD_{15} and PEI polyplexes with HEK293T cells (a) and HepG2 cells (b) detected by flow cytometry. Error bars represent mean ± SD for $n = 3$.

Conclusions

In this study, PEI-CD_{15} and PEI-CD_{41} were synthesized. It was found that the CD grafting level showed great influence on DNA condensation ability. PEI-CD_{15} could protect DNA completely above an N/P ratio of 2. The polyplexes were about 120 nm in diameter at high N/P ratios. However, PEI-CD_{41} could not protect DNA until the N/P ratio increased to 6, and PEI-CD_{41}/DNA polyplexes have larger size, and are greater than 1 μm at N/P ratio of 10. Therefore, the research was mainly focused on the PEI-CD_{15}. Interestingly, the PEI-CD_{15}/DNA polyplexes displayed excellent stability in physiological salt conditions at N/P ratio of 8 and 10. The cytotoxicity study showed that CD modification reduced the cytotoxicity in both HEK293T and HepG2 cells. Uptake inhibition experiments indicated that PEI/DNA polyplexes were internalized by HEK293T cells by both clathrin-mediated endocytosis and caveolae-mediated endocytosis. The route of caveolae-mediated endocytosis was significantly promoted after CD modification. So the cellular uptake and transfection efficiency of PEI-CD_{15}/DNA polyplexes were significantly improved in HEK293T cells. However, the uptake and transfection efficiency of PEI-CD_{15}/DNA polyplexes in HepG2 cells was similar to that of PEI/DNA polyplexes probably due to the cells' lack of endogenous caveolins. The influence of CD modification at low CD graft degree on the cellular uptake and transfection efficiency of polyplexes depended on the type of the cells.

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