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# Efficient gene transfection in the neurotypic cells by star-shaped polymer consisting of β-cyclodextrin core and poly(amidoamine) dendron arms

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ABSTRACT

In order to develop the effective vectors that had high gene transfection capability and low cytotoxicity in the neuronal cells, we tested the star-shaped polymer consisting of β-cyclodextrin core and poly(amidoamine) (PAMAM) dendron arms [β-CD-$(D_3)_7$] as the vector to transfect the human neuroblastoma SH-SY5Y cells. The physicochemical properties of the β-CD-$(D_3)_7$/plasmid DNA (pDNA) complexes were characterized by using gel electrophoresis, dynamic light scattering, transmission electron microscopy and zeta-potential experiments. Among the human neuroblastoma SH-SY5Y cells, β-CD-$(D_3)_7$/pDNA complex demonstrated a lower toxicity compared to those of PAMAM ($G=4$, with an ethylenediamine core)/pDNA complex. When the N/P ratio was over 20, it was observed that PAMAM had a faster increment in toxicity compared to β-CD-$(D_3)_7$. Fluorescent image, confocal microscopy image and flow cytometry showed that β-CD-$(D_3)_7$/pDNA complexes had significantly higher transgene activity than that of PAMAM/pDNA complexes. For example, the transfection efficiency was 20% and 7.5% for β-CD-$(D_3)_7$/pDNA and PAMAM/pDNA complexes, respectively. These results indicated that β-CD-$(D_3)_7$ might be a promising candidate for neurotypic cells gene delivery with the characteristics of good biocompatibility, relatively high gene transfection capability and potential *in vivo* gene delivery ability.


## 1. Introduction

Neurological diseases were a class of acute or chronic diseases, such as Parkinson's disease (PD) and multiple sclerosis (MS), which characterized by neuronal injury or neuronal loss (Liu et al., 2011). Gene therapy was a new therapeutic strategy for these diseases (Gresch et al., 2004; Suk, Suh, Lai, & Hanes, 2007). Compared with the high transfection efficiency of the viral vectors in the neuronal cells, the nonviral vectors were considerably safer and easy to produce, and they possessed potential cell targeting property of the large gene-carrying capacity (Kealy et al., 2009; Rejman et al., 2010). However, many researchers observed that the neurons and neurotypic cells were difficult to transfect with the nonviral transfection reagents (*i.e.* Lipofectamine 2000, ExGen 500 and TurboFectin 8.0) (Martin-Montanez, Lopez-Tellez, Acevedo, Pavia, & Khan, 2010) and some newly developed nonviral vectors (*i.e.* 1,5-dihexadecyl N-arginyl-L-glutamate lipoplexes and $NH_2$ functionalized CdSe/ZnS quantum dot-doped $SiO_2$ nanoparticles) (Bardi et al., 2010; Obata et al., 2010). Neuronal cells had special properties, such as their polarized nature and the elongated morphology of neuronal projections, which could result in barriers to uptake that were unique to such cells. Suk and coworkers also demonstrated that the neurotypic cells endocytosed significantly few nanocomplexes, suggesting cell entry of gene vectors may be a critical barrier to efficient gene delivery into neurotypic cells (Suk et al., 2006). Because the human neuroblastoma SH-SY5Y cell line possessed many biochemical and functional properties of neurons, and also it was easy to culture, which had been widely used as the model of neurons since the early 1980s. This SH-SY5Y cell line also showed very low levels of gene transfection just as neurons with a maximum value ranging from 2.5 to 12.5% (Martin-Montanez et al., 2010; Suk et al., 2006).

Recently, poly(amidoamine) (PAMAM) dendrimer had been widely used as nucleic acid carriers to the neuronal cells, which

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had well-defined architectures, precise molecular weights, and multivalent functionalization sites (Eichman, Bielinska, Kukowska-Latallo, & Baker, 2000; Kim et al., 2010). It had been postulated that PAMAM dendrimers had a high buffer capacity, owing to protonable amine groups. This characteristic enabled PAMAM dendrimers to act as a weak base and retard degradation caused by acidification within the endosome–lysosome (Haensler & Szoka, 1993). A reduction in pH might also lead to polymeric swelling within the endosome, thus disrupting the membrane barrier of the organelle and promoting DNA and/or complex release (Demeneix et al., 1998). Several studies have demonstrated successful delivery of plasmid DNA (pDNA) or small interfering RNA (siRNA) into using PAMAM dendrimers or their derivatives. Such as, Kim ID and coworkers showed that PAMAM esters allow siRNA delivery to a primary culture of mixed cortical cells containing neurons and glia, achieving an 80% reduction in the target protein (Kim et al., 2010). Kim et al. (2006) reported that the synthesis of arginine grafted G4-PAMAM dendrimers exhibited a transfection efficiency of 35–40% in primary cortical neurons. However, a higher generation of the polycationic dendrimers was generally demanded for better nucleic acid binding ability and ultimate transfection efficiency, and this will lead to practical problems of high cytotoxicity (Jevprasesphant et al., 2003).

It was known that cyclodextrins (CDs) had low immunogenicity and toxicity in animals and humans (Davis & Brewster, 2004; Uekama, Hirayama & Irie, 1998) due to their excellent biocompatibility, and could enhance the absorption and resistance to nucleases through binding and interacting with oligonucleotides (Cryan, Holohan, Donohue, Darcy, & O'Driscoll, 2004). Most recently, we reported the synthesis of cationic star-shaped polymers consisting of β-CD core and PAMAM dendron arms [β-CD-$(D_3)_7$], which had a higher siRNA transfection capability and a lower cytotoxicity than those of PAMAM dendrimers in fibroblast cells (Deng et al., 2011). This study focused on the transfection capability of β-CD-$(D_3)_7$/plasmid DNA (pDNA) complexes in the neurotypic cells (SH-SY5Y cells). Here we analyzed the physicochemical properties of β-CD-$(D_3)_7$/pDNA complexes. In addition, we found that β-CD-$(D_3)_7$ showed much lower cytotoxicity and higher transfection efficiency investigated than those of PAMAM ($G=4$) with an ethylenediamine core in the SH-SY5Y cells.

## 2. Materials and methods

### 2.1. Materials and reagents

Most reagents were of the best grade available and were purchased from common suppliers. Star-shaped polymer β-CD-$(D_3)_7$ was synthesized in our laboratory as previously described (Deng et al., 2011). Polyamidoamine (PAMAM) dendrimer ($G=4$) with an ethylenediamine core (EDA dendrimer) was purchased from Sigma (St. Louis, MO). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO, USA). pEGFP-N3 vector was purchased from BD Biosciences Clontech (Palo Alto, CA, USA). Cell culture media and fetal bovine sera (FBS) were purchased from Invitrogen Corporation (Carlsbad, CA, USA).

### 2.2. Preparation of plasmids

The reporter plasmid expressing green fluorescent protein (pEGFP-N3) was used in this study. The vector was amplified in *Escherichia coli* and was purified according to the manufacturer's instructions (QIAGEN, CA, USA). The quantity and quality of the purified pEGFP-N3 was assessed by measuring its optical density at 260 nm and 280 nm, and by electrophoresis in 1% agarose gel, respectively. The purified pEGFP-N3 was kept in aliquots at a concentration of 1 μg/μl.

### 2.3. Preparation of β-CD-$(D_3)_7$/pDNA complexes

The complexes were prepared at various charge ratios by mixing equal volumes of β-CD-$(D_3)_7$ with pEGFP-N3 in phosphate buffered saline (PBS). The charge ratio, N/P, was calculated as a ratio of the number of primary amines in the polymer to the number of anionic phosphate groups in the pEGFP-N3. The samples were then vortexed and incubated at 37 °C for 30 min to ensure the complex formation. The complexes were prepared under a final pEGFP-N3 concentration of $4 \times 10^{-8}$ mol/L of 0.5 mL solution and varying polymer concentrations to reach the desired charge ratios.

### 2.4. Gel retardation assay

In order to confirm the pDNA condensation ability of the β-CD-$(D_3)_7$, electrophoresis was performed. Complex formation was induced at various N/P ratios from 0 to 30 in a final volume of 6× agarose gel loading dye mixture (*i.e.* 10 μL). After the incubation for 15 min at room temperature, pEGFP-N3 binding was studied by assaying for agarose gel retardation. The complexes were loaded onto the 0.9% agarose gels with ethidium bromide (0.1 μg/mL) and ran with Tris–acetate (TAE) buffer at 100 V for 40 min. pDNA retardation was revealed by irradiation with UV light.

### 2.5. Particle size and z-potential measurements

The particle size and *z*-potential of the complexes in aqueous system were measured at 25 °C using a ZetaPALS (Brookhaven Instruments Corporation, USA). According to the N/P ratio, various concentrations of polymer solutions were added to pEGFP-N3 solution in equivalent volume to form the β-CD-$(D_3)_7$/pDNA complexes. The sample systems were allowed to incubate for 30 min at 37 °C.

### 2.6. Transmission electron microscopy observation

The morphology of the β-CD-$(D_3)_7$/pDNA complexes at the N/P ratio of 10 was observed using transmission electron microscopy (TEM) (JEM2010, Japan). Briefly, one drop of β-CD-$(D_3)_7$/pDNA complex solution was placed on a copper grid and incubated for 60 s. The sample was negatively stained with 2% (w/v) uranyl acetate solution for 60 s. Excess liquid was removed by blotting with filter paper.

### 2.7. Cell culture

Human neuroblastoma SH-SY5Y cells and human embryonic kidney 293 cells (used for a comparative study) were obtained from American Type Culture Collection (ATCC) and maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penn/strep, Invitrogen Corporation) in a humidified atmosphere of 5% $CO_2$ at 37 °C. When the cell confluence of 90% was reached, they were trypsinized and subcultured.

### 2.8. MTT assay

Two kinds of cells were grown in 96-well plates at an initial density of 6000 cells/well. 24 h later, the transfection was performed using the complexes of various N/P ratios (from 10 to 40) in 150 μL of serum-free growth medium with 150 ng of pDNA/well. Four hours later, the transfection media were changed with fresh and complete DMEM culture media. After transfected

for 24 h, 150 μL of serum-free growth medium was replaced into the wells, and 20 μL of MTT solution (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide, 5 mg/mL) were added. The cells were then incubated for another 4 h before 100 μL of DMSO was added. After gentle agitation for 5 min, the absorbance at 570 nm of each well was recorded with the Tecan's Infinite M200 microplate reader (Infinite M200, TECAN, Switzerland). Each concentration was replicated in 3 wells. Percentage viability of the cells was calculated as the ratio of mean absorbance of triplicate readings with respect to mean absorbance of control wells: cell viability = $(I_{sample}/I_{control}) \times 100$.

### 2.9. Transfection efficiency assay by fluorescence and confocal microscopy

Two kinds of cells ($1 \times 10^5$) were seeded in 24-well plates a day before transfection in complete media. They were incubated until the cells reached about 70% confluence, and then the media was removed and replaced with the complexes of various N/P ratios (from 10 to 40). The cells were incubated with the complexes in serum-free or 10% serum-containing culture medium for 4 h at 37 °C. Then the medium was replaced with fresh and complete DMEM culture media. The cells were further incubated for 24 h at 37 °C and were observed under the fluorescence microscope (Nikon, Tokyo, Japan) equipped with the appropriate filter. The similar steps were performed in the 35-mm culture dishes. After 24 h transfected, the cells were observed under the Radiance 2100 confocal laser scanning microscope (BioRad, CA) with the appropriate filter. The fluorescent images were captured and recorded.

### 2.10. Flow cytometry

For flow cytometry, 293 and SH-SY5Y cells were seeded in 6-well plates and incubated for being transfected. Transfection was performed as above with 4 μg pEGFP-N3 plasmid each well and the complexes of various N/P ratios (from 10 to 40). 24 h after transfection, cells were washed with PBS and detached with 0.25% trypsin/EDTA. Then, cells were resuspended in 500 μL of 1× Hank's balanced salt solution (HBSS, pH 7.4). Transfection efficiency was evaluated by scoring the percentage of cells expressing green fluorescence protein using a FACS AriaTM System from Becton-Dickinson (San Joes, CA). The experiments were performed in triplicates and 30,000 cells were counted in each experiment.

## 3. Results

### 3.1. Formation and characterization of β-CD-$(D_3)_7$/pDNA complexes

To confirm the complex formation, we tested the retardation of pDNA mobility by agarose gel electrophoresis. Complex formation was induced at various N/P ratios from 0 to 30 in this study (Fig. 1). Compared with the naked pDNA control, the β-CD-$(D_3)_7$ showed the capability of pDNA condensation as less pDNA run into the gel. From Fig. 1, we found that at N/P = 10 or above, all the complexes showed complete retardation of pDNA motion, indicating the full pDNA complexation.

Fig. 2 showed the mean particle size and *z*-potential of β-CD-$(D_3)_7$/pDNA complexes at various N/P ratios. From Fig. 2a, it was found that the particle sizes of the complexes decreased with the increase of N/P ratio from 1 to 30 and then remained in the 100–200 nm range with further increase of N/P ratio to 30. From Fig. 2b, it was found that all complexes were positively charged and the z-potential increased with the increase of N/P ratio from 1 to 30. The morphology of β-CD-$(D_3)_7$/pDNA complexes at the N/P ratio of 10 were investigated by TEM. As shown in Fig. 2c, the complexes were found to have a spherical shape and compacted structure. Moreover, the TEM image demonstrated that the size of the complex no more than 200 nm, which was consistent with the result measured by ZetaPALS (Fig. 2a).

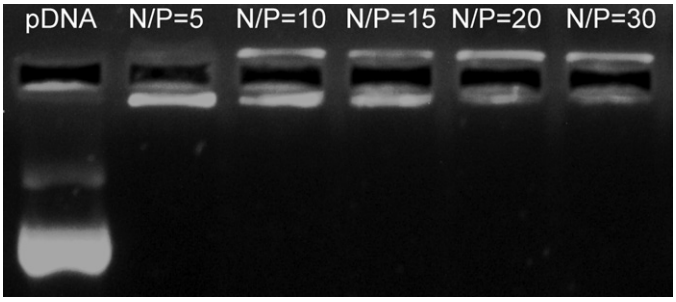


**Fig. 1.** Agarose gel electrophoresis retardation assay of β-CD-$(D_3)_7$/pDNA complexes at various N/P ratios.

### 3.2. Cell viability

All of the polymers and complexes exhibited minimal toxicity at low N/P ratios no more than 10, with the cell cultures maintained high viability over 80% (Fig. 3). Among the two kinds of cell lines, β-CD-$(D_3)_7$ and β-CD-$(D_3)_7$/pDNA complex demonstrated a lower toxicity compared to those of PAMAM and PAMAM/pDNA complex, when the N/P ratio was over 20. And at high N/P ratios, it was observed that PAMAM had a faster increment in toxicity compared to β-CD-$(D_3)_7$. For example, at the same high concentration of 42 mg/mL in the SY5Y cells, the cell viability was found to be 58% for β-CD-$(D_3)_7$ and 44% for PAMAM, respectively. The similar results in both cell lines confirmed the toxicity of β-CD-$(D_3)_7$ and β-CD-$(D_3)_7$/pDNA complex was lower than that of PAMAM and PAMAM/pDNA complex when the N/P ratio was over 20.

### 3.3. Transfection efficiency

The transfection efficiency and the transgene expression level of the complexes were visualized by observation of EGFP positive cells using the fluorescence microscope and the confocal microscopy. For SH-SY5Y cells, the highest transfection efficiency and the strongest fluorescent density were shown by the confocal microscopy when cells were transfected with β-CD-$(D_3)_7$/pDNA complex at the N/P ratio of 20 (Fig. 4). Comparably, lower transfection efficiency and weaker fluorescent density were displayed when the cells were transfected with PAMAM/pDNA complex at the N/P ratio of 20 (Fig. 4). But there was no higher transgene expression level shown when the cells were transfected with β-CD-$(D_3)_7$/pDNA complexes at the N/P ratio of 10 or over 30, compared to PAMAM/pDNA complex at the N/P ratio of 20. The similar results were obtained from HEK-293 cells.

The results of flow cytometry assay confirmed the highest transfection efficiency was shown by the confocal microscopy when cells were transfected with β-CD-$(D_3)_7$/pDNA complex at the N/P ratio of 20. Our results showed that the ratio of EGFP-positive cells were 45% and 23% when HEK 293 cells were transfected with β-CD-$(D_3)_7$/pDNA and PAMAM/pDNA complexes, respectively (Fig. 6a). For SH-SY5Y cells, the transfection efficiency were 19% and 7% for β-CD-$(D_3)_7$/pDNA and PAMAM/pDNA complexes, respectively (Fig. 6b).

At the same time, we also tested the effect of the serum on the transfection efficiency. Higher fluorescent density was displayed when the cells were incubated with the presence of 10% serum-containing culture medium (Fig. 5). And the transfection efficiency was further quantified by flow cytometry assay. Our flow

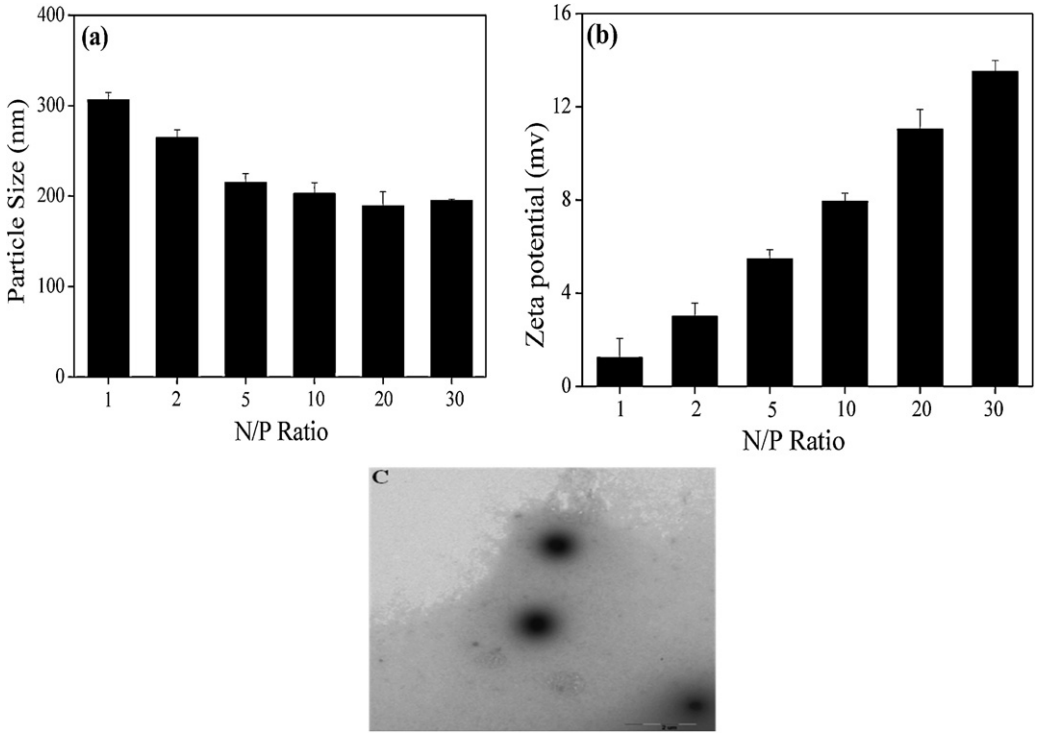


**Fig. 2.** The particle size (a) and *z*-potential (b) of β-CD-$(D_3)_7$/pDNA complexes formed at various N/P ratios. Typical TEM image (c) of the β-CD-$(D_3)_7$/pDNA complexes formed at the N/P ratio of 20.

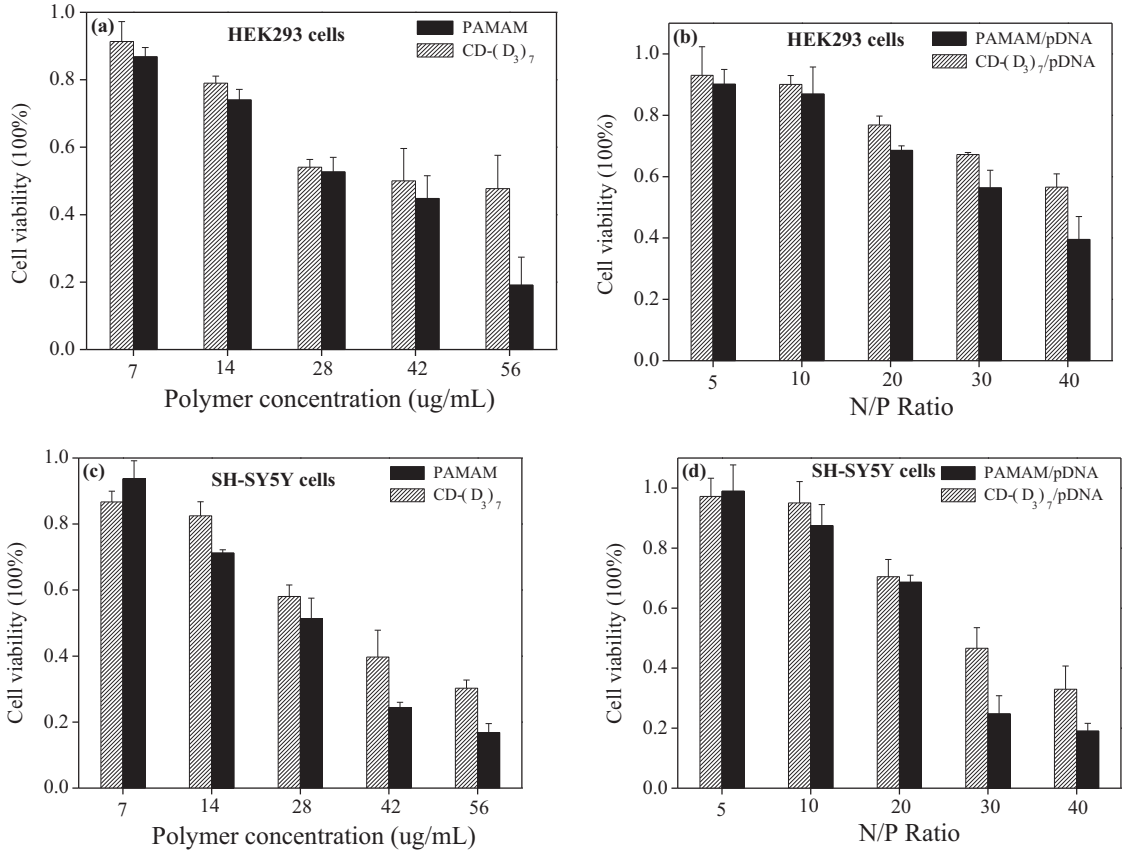


**Fig. 3.** Cytotoxicity induced by the polymers at a variety of concentrations in (a) HEK 293 cells and (c) SH-SY5Y cells (mean ± SD, $n = 3$). Cytotoxicity induced after transfection of β-CD-$(D_3)_7$/pDNA and PAMAM/pDNA complexes into (b) HEK 293 cells and (d) SH-SY5Y cells (mean ± SD, $n = 3$).

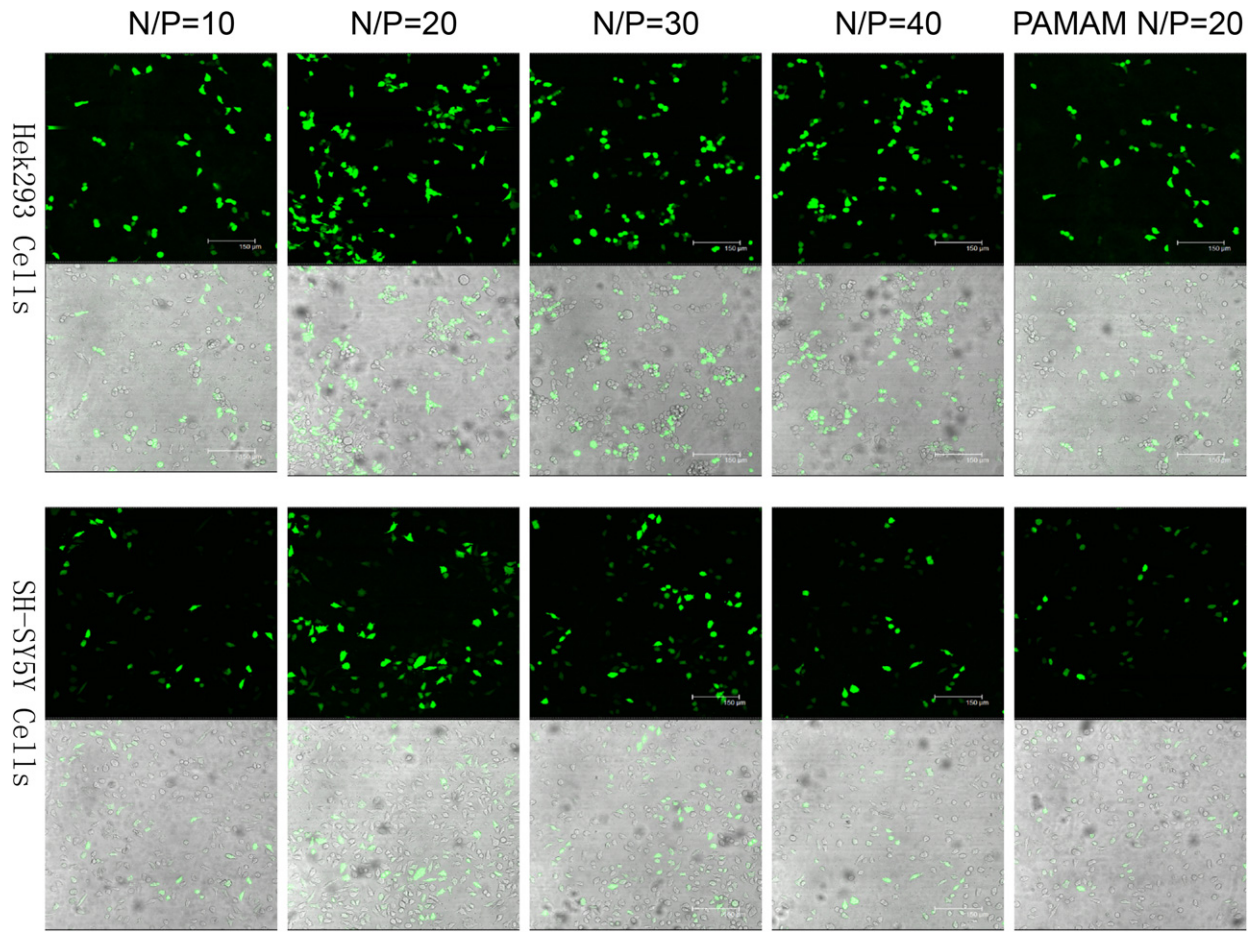


**Fig. 4.** Typical fluorescence images of the HEK 293 cells and SH-SY5Y cells transfected by β-CD-$(D_3)_7$/pDNA complexes formed at different N/P ratios (10, 20, 30 and 40) and PAMAM/pDNA complexes formed at the N/P ratio of 20 by confocal microscopy (magnification: 100×).

cytometry assay results showed that the ratio of EGFP-positive cells were 48% and 44% when HEK 293 cells were transfected with β-CD-$(D_3)_7$/pDNA complexes with and without the presence of 10% serum-containing culture medium, respectively (Fig. 6a). For SH-SY5Y cells, the transfection efficiency were 22% and 19% for β-CD-$(D_3)_7$/pDNA complexes with and without the presence of 10% serum-containing culture medium, respectively (Fig. 6b).

## 4. Discussion

The delivery of therapeutic materials such as DNA or siRNA had the potential to treat neurological disease and spinal cord injury. While neuron was a notoriously difficult cell type to transfect, both *in vitro* and *in vivo* (Lo, Singhal, Torchilin, & Abbott, 2001; Washbourne & McAllister, 2002). Consequently, the SH-SY5Y cell line had been widely used in experimental neurological studies, including analysis of neuronal differentiation, metabolism, and function related to neurodegenerative and neuroadaptive processes, neurotoxicity, and neuroprotection (Xie, Hu, & Li, 2010). In addition, SH-SY5Y cells possessed the capability of proliferating in culture for long periods without contamination, a prerequisite for the development of an *in vitro* cell model (Biedler, Helson, & Spengler, 1973).

Dendrimers had been shown to cross-cell barriers at sufficient rates to act as potential carrier/delivery systems (Wiwattanapatapee, Carreno-Gomez, Malik, & Duncan, 2000). It had also been shown that PAMAM dendrimer nanocarriers could be used to enhance the transport of genetic material across the membrane of neurotypic cells (Posadas et al., 2009). Enhancement of gene transfer had been observed when PAMAM structures were modified with less polar moieties such as PEG groups and cyclodextrins (CDs) groups. When PEG was located on the periphery of the dendrimer, non-specific adsorption to the cell membrane and subsequent ingestion by endocytosis rather than an electrostatic interaction could be observed in cationic dendrimers (Kannan et al., 2004). Davis et al. and others had shown that polymers containing CD units could complex adamantane moieties functionalize with CDs and cell-type specific target moieties to discourage nanoparticle flocculation and encourage tissue-specific nucleic acid delivery (Bellocq, Pun, Jensen, & Davis, 2003; Hwang, Bellocq, & Davis, 2001; Reineke & Davis, 2003). In this study, cationic star-shaped polymer consisting of β-CD core and PAMAM dendron arms [β-CD-$(D_3)_7$], had been used to transfect pDNA into SH-SY5Y cells. The star-shaped polymer provided biocompatibility, protection from serum proteins, and could be functionalized with a targeting ligand.

Before the transfection, we confirmed the pDNA condensation ability of the β-CD-$(D_3)_7$ by electrophoresis. Complex formation was induced at various N/P ratios from 0 to 30 in this study (Fig. 1). Compared with the naked pDNA control, we found that at N/P = 10 or above, all the complexes showed complete retardation of pDNA motion, indicating the full pDNA complexation. This confirmed the β-CD-$(D_3)_7$ had a good pDNA condensation capability due to the relatively high density of primary amino group.

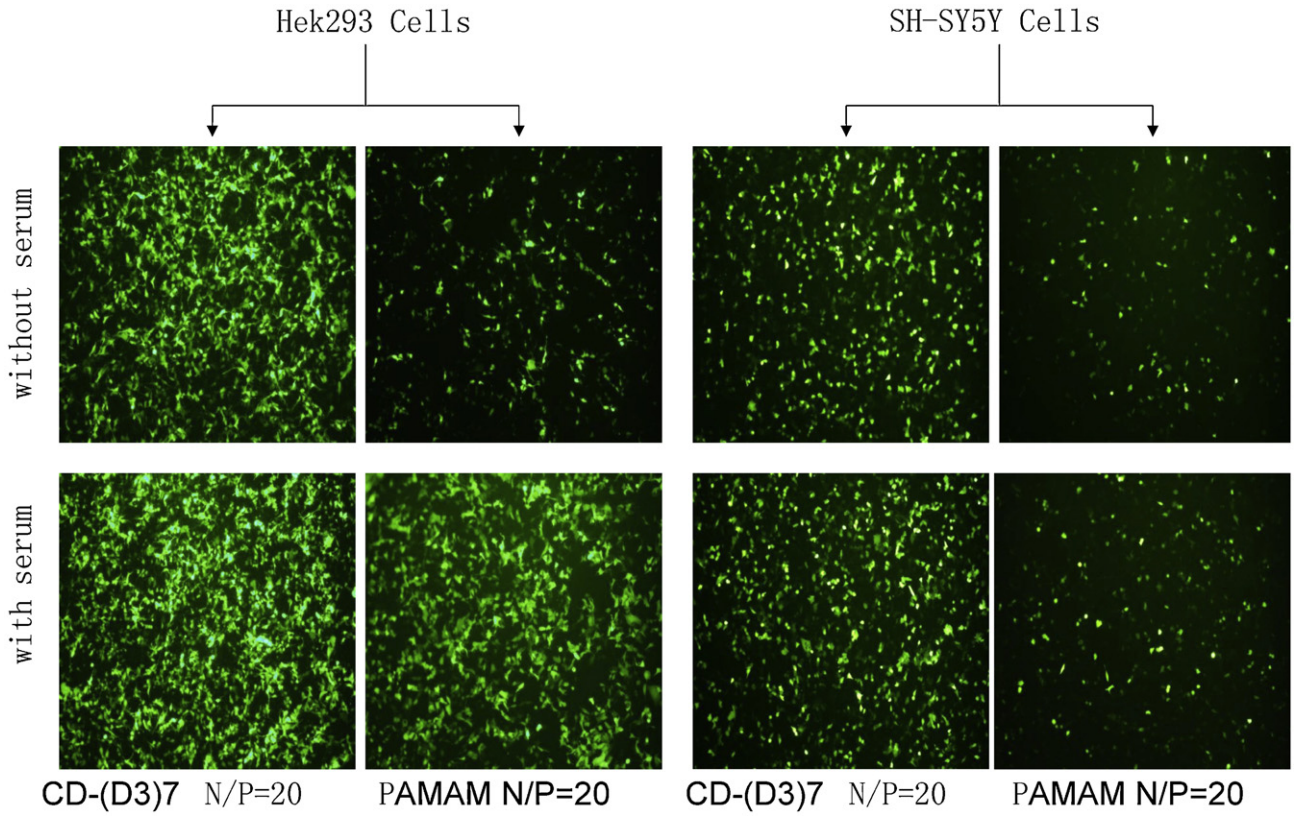


**Fig. 5.** Typical fluorescence images of the HEK 293 cells and SH-SY5Y cells transfected by β-CD-$(D_3)_7$/pDNA complexes and PAMAM/pDNA complexes formed at the N/P ratio of 20, with or without serum.

Appropriate size and surface charge were important to ensure the uptake of the polymer/pDNA complexes by cells (Ren, Jiang, Pan, & Mao, 2010). From Fig. 2a, it was found that the particle sizes of the complexes decreased with the increase of N/P ratio and remained in the 100–200 nm range. From Fig. 2b, it was found that all complexes were positively charged and the z-potential increased with the increase of N/P ratio. As shown in Fig. 2c, the complexes were found to have a spherical shape and compacted structure. Moreover, the TEM image demonstrated that the size of the complex no more than 200 nm, which was consistent with the result measured by ZetaPALS (Fig. 2a). Therefore, we confirmed that β-CD-$(D_3)_7$ could form the nanoparticles with pDNA and the average diameters of the complexes were within the size requirements for efficient cellular endocytosis (Sharma, Schwarzbauer, & Moghe, 2011).

In order to evaluate the potential toxicity of β-CD-$(D_3)_7$, the viability of two kind of cell lines was tested in the presence of β-CD-$(D_3)_7$ and β-CD-$(D_3)_7$/pDNA complexes at various N/P ratios. For a comparative study, commercially available PAMAM dendrimer ($G=4$) with an ethylenediamine core was used as the controls, which has similar number of amino with that of β-CD-$(D_3)_7$. The results in both cell lines confirmed the toxicity of β-CD-$(D_3)_7$ and β-CD-$(D_3)_7$/pDNA complex was lower than that of PAMAM and PAMAM/pDNA complex when the N/P ratio was over 20, which would become an advantage when they were used for gene delivery.

The transfection capability and transgene expression level were the most important features of gene carriers. For a basic experiment, because of the cell line presented great transfection potential and high-yield production, 293 cells were usually vulnerable to conventional nonviral transfection agents and were used as control cells in this work. Based on the results of EGFP-emitted fluorescence, it was found that higher transfection capability and higher viability were shown when transfected the SY5Y cells and 293 cells with β-CD-$(D_3)_7$/pDNA complex than those of PAMAM/pDNA complex at the N/P ratio of 20 by the confocal microscopy. While at a lower N/P ratio and as well a value over 20, the fluorescent spots were markedly reduced (Fig. 4). The results obtained from flow cytometry assay were consistent with the transfection efficiency assay (Fig. 6). With an increase of N/P ratios, both the transfection capability of β-CD-$(D_3)_7$/pDNA complex and the fluorescence density decreased (data not shown).

The presence of serum proteins, however, was unavoidable *in vivo*, and was advantageous *in vitro* because it increased cell viability and reduced transfection-associated cytotoxicity (Dai & Liu, 2011; Konopka, Overlid, Nagaraj, & Duzgunes, 2006). Serum usually had a negative impact on the transfection efficiency of cationic liposomes. Serum was also shown to inhibit transfection capability of the complexes depending on the composition, size and charge of the complexes (Yang & Huang, 1997). The interactions of cationic polymers with serum may serve as a predictive model for the *in vivo* efficiency of a cationic polymer. Therefore, there was an intense effort to develop lipoplexes and polyplexes that efficiently transfer genes *in vivo* and in serum (Dai & Liu, 2011; Eliyahu et al., 2007). Our results showed a slight increase of the transfection capability of both complexes when the two kinds of cells were incubated with the presence of 10% serum-containing culture medium (Fig. 6). This confirmed that the serum had little effect on the transfection capability of β-CD-$(D_3)_7$/pDNA complex.

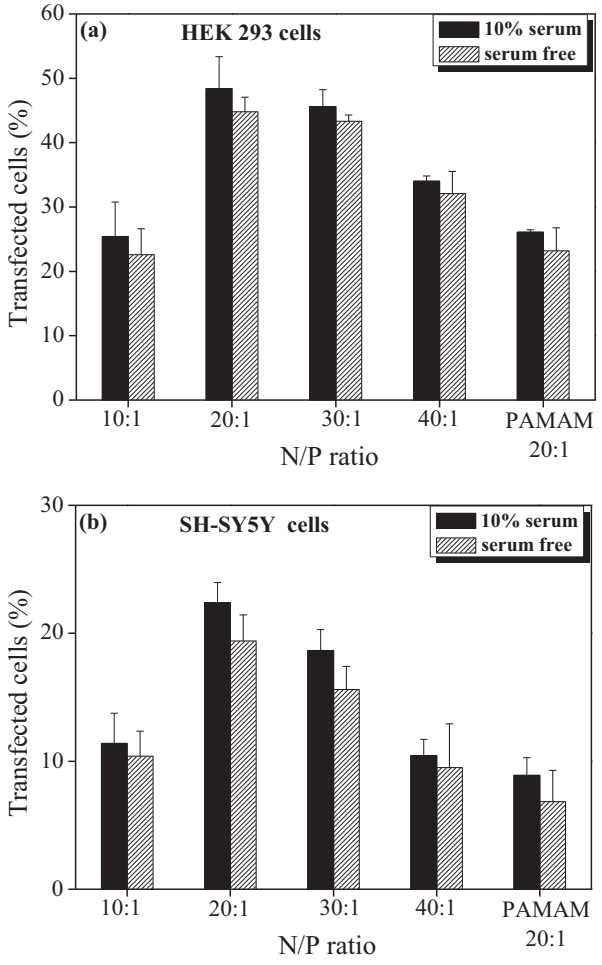


**Fig. 6.** Transfection efficiency of β-CD-$(D_3)_7$/pDNA complexes and PAMAM/pDNA complexes by FACS analysis in (a) HEK 293 cells and (b) SH-SY5Y cells (mean ± SD, $n = 3$). Cells transfected by β-CD-$(D_3)_7$/pDNA complexes formed at different N/P ratios (10, 20, 30 and 40) and PAMAM/pDNA complexes formed at the N/P ratio of 20.

## 5. Conclusion

In conclusion, star-shaped polymer β-CD-$(D_3)_7$ exhibited a low cytotoxicity and efficient pDNA transfection capability in SH-SY5Y cells. It is expected that such a star-shaped polymer may be used as a new nonviral gene delivery vector for gene therapy in the neuronal cells. The studies on its *in vivo* toxicity and gene delivery efficacy should be further conducted in different animal models.

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