Star-shaped cyclodextrin-poly(l-lysine) derivative co-delivering docetaxel and MMP-9 siRNA plasmid in cancer therapy

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A B S T R A C T

A new cyclodextrin derivative (CD-PLLD) consisting of a β-cyclodextrin core and poly(l-lysine) dendron arms was prepared by the click conjugation of per-6-azido-b-cyclodextrin with propargyl focal point poly(l-lysine) dendron of third generation, and then used for docetaxel (DOC) and the best siRNA plasmid targeting MMP-9 (pMR3) co-delivery. Different from commonly used amphiphilic copolymers with cationic character, the as obtained cyclodextrin derivative may be used directly for the combinatorial delivery of nucleic acid and lipophilic anticancer drugs without a complicated micellization process. It was found that CD-PLLD/pMR3 nanocomplex showed a good gene transfection efficiency in vitro, and could mediate the reduce of MMP-9 protein in HNE-1 cells. Moreover, the star-shaped copolymer exhibited better blood compatibility and lower cytotoxicity compared to PEI-25k in the hemolysis and MTT assays, which should be encouraged in nasopharyngeal cancer therapy.

1. Introduction

The co-delivery of drug and gene has become the primary strategy in cancer and other disease therapy in recent years, because this technique could promote synergistic actions, improve target selectivity and deter the development of drug resistance [1]. For the co-delivery of drugs and genes while maintaining their chemophysical properties and biological functions, there has been an increasing interest in the development of multifunctional polymeric micelles. In this context, cationic micelles have been self-assembled from some amphiphilic copolymers with cationic character [2–5]. For example, Zheng et al. synthesized the amphiphilic triblock copolymer poly(ethylene glycol)-b-poly(l-lysine)-b-poly(l-leucine) polypeptide to co-deliver docetaxel and siRNA-Bcl-2 to overcome drug resistance in MCF-7 cells [6]. Liu et al. conjugated doxorubicin to PEG-modified PEI, and then used it to deliver pEGFP to MCF-7 cells, which showed a good co-delivery effect [7]. Han et al. designed an amphiphilic peptide with pH-responsibility for gene and drug delivery, and its unique structure endowed the peptide/DNA complexes with well endosome escaping ability [8].

In these micelles carriers, hydrophobic anticancer drugs were incorporated into the hydrophobic cores, and plasmid DNA or siRNA was bound to the hydrophilic shells with cationic character. For the preparation of these micelles, however, these self-assembly processes are usually difficult to control, which was not easy to obtain the stable and uniform complexes. Moreover, micelles was not stable in blood circulation in vivo, the disassembly of micelles may result in the drug emission [9–12].

Recently, the star-shaped copolymers consisting of a cyclodextrin (CD) core and cationic arms have sparked much attention, because this kind of copolymers could co-load hydrophobic drugs and gene simultaneously and form the stable complexes in vivo [13–18]. Our previous work has synthesized a cyclodextrin derivative (CD-PLLD) consisting of a cyclodextrin core and poly(l-lysine) dendron arms [19]. The CD core could interact with hydrophobic model drug and cationic arms could bind pEGFP respectively. Different from the above mentioned amphiphilic copolymers, it was found that such a conjugate could be used directly for the drug or gene delivery respectively without a complicated micellization process. In this paper, we attempted to co-deliver hydrophobic...
antitumor drug (docetaxel, DOC) and functional gene (MMP-9 siRNA plasmid) into HNE-1 cells, and explored its application in nasopharyngeal cancer therapy. Moreover, its in vivo biocompatibility was also studied.

2. Experiment section

2.1. Materials

CD-PLLD was synthesized according to our previous report [19]. Docetaxel (DOC) and PEI (25 kDa) were purchased from Sigma and used without further purification. A pcDNA3 plasmid was used for construction of vectors expressing small interfering RNA (siRNA) for MMP-9 by Invitrogen Corp (Shanghai). Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyltetrazolium bromide (MTT), Propidium iodide (PI) and Dulbecco’s phosphate buffered saline (PBS) were purchased from Invitrogen Corp. Ultrapure water was obtained from Millipore ROIs. The human nasopharyngeal carcinoma HNE-1 cells were supplied by Southern Medical University.

2.2. DOC loading

For the loading of hydrophobic DOC, 50 mg CD-PLLD was first dissolved in distilled water with a concentration of 10 mg/mL, and then 5 mL DMF containing 15 mg DOC was added dropwise to the solution. The mixture was stirred for 4 h in the dark at room temperature. After that, the sample was put into a dialysis bag (MWCO 5000) and stirred to dialysis against distilled water for 24 h. The drug-loaded complex was obtained by filtered through a 0.45 μm filter and then lyophilized. To determine the loading amount of DOC, the resultant CD-PLLD/DOC complexes were dissolved in CH3OH, and then analyzed by HPLC. The HPLC analysis of DOC was achieved on a C18 column (Waters, USA) with a mobile phase consisting of methanol (50%) and distilled water (50%) (v/v) at a flow rate of 1.0 mL/min. The effluents were monitored at 227 nm and quantified by comparing the peak areas with the standard curve [20]. It was found that the loading amount was 13.2 μg/mg.

2.3. MMP-9 siRNA plasmid binding

2.3.1. Formation

The best siRNA plasmid targeting MMP-9 (pMR3) was screened out by RT-PCR and Western-blot, the screening result was shown in Supporting Information. For the complexation of CD-PLLD with pMR3, the pMR3 and CD-PLLD were firstly dissolved in distilled water to make aqueous solutions with appropriate concentrations, respectively. The resultant component solutions were then mixed at room temperature, and stirred gently for 15 min for the formation of CD-PLLD/pMR3 complexes.

2.3.2. Gel electrophoresis

The binding ability of CD-PLLD to pMR3 was examined by gel electrophoresis. Agarose gel (1%, w/v) containing ethidium bromide (0.25 mg/mL Sigma) was prepared in TAE buffer (40 mmol/L trisacetic, 1 mmol/L EDTA). After incubation for 15 min at room temperature, all samples were separated by electrophoresis on the agarose gel at 70 V for 30 min. Visualization and image capture was accomplished using a UV-transilluminator under a Kodak EDAS 290 digital imaging system (Fisher Scientific, PA). For the study on protection against DNase degradation, DNase I (10 units, 2 μL) was added to 1 μg of naked pMR3 or CD-PLLD/pMR3 complexes (N/P = 10, 20 and 40) and incubated at 37 °C while shaking at 100 rpm for 30 min. Subsequently, EDTA (4 μL, 250 mm) and sodium dodecyl sulfate (SDS) solution (4 μL, 10%, w/v) was added and the mixture was incubated at room temperature for another 1 h. The samples were then loaded onto the gel and electrophoresed to examine the integrity of DNA [18].

2.3.3. Size and morphology

Their particle sizes and zeta potentials of complexes were determined by a Zeta PALS, Brookhaven Instruments Corporation, USA). Prior to the measurements, the complexes were incubated at 37 °C for 30 min. The morphological examination of the complex was performed using a JEM-2010HR high-resolution transmission electron microscope after counterstained for 2 min with uranyl acetate.

2.4. In vitro transfection

HNE-1 cells were selected for studying the in vitro gene transfection of the CD-PLLD/MMP-9 complexes. Before transfection, HNE-1 cells were seeded at a density of 1 × 10⁴ cells per well onto 12-well tissue culture plates in complete DMEM (Dulbecco’s Modified Eagle Medium) culture medium, and then incubated in a humidified 5% CO₂ atmosphere at 37 °C. Freshly prepared CD-PLLD/pMR3 (N/P = 10, 20, and 40 respectively) complexes in serum-free DMEM were added. The pH3 in each well was fixed at 3.0 μg. After 6 h incubation, the formulations were removed and 500 μL of fresh DMEM culture medium was added. The experiments of studying gene transfection were continued to 42 h, and then the cells were analyzed for green fluorescence protein (GFP) expression with a fluorescence microscope (Nikon-2000U, Japan). The cells treated with PEI/pMR3 (N/P = 10) were set as the control groups. After the cells were digested by trypsinase (0.05% w/v in PBS), the transfection percent (positive cell percent) were calculated by dividing the number of fluorescent cells by the number of total cells in a certain area of a well. The transfection efficiency was recorded by a flow cytometer (Accuri C6).

2.5. mRNA and protein expression

HNE-1 cells (5 × 10⁴) were seeded in 6-well plates and incubated at 37 °C in 5% CO₂ for 24 h to reach 70% confluence. Various formulations (Blank CD-PLLD, DOC and PEI plasmid complexes at the ratio of N/P = 20, 40 and 40 respectively) were added and incubated with the cells for 48 h (for mRNA isolation and protein extraction). The cellular levels of MMP-9 mRNA and protein were assessed using RT-PCR and Western blot, respectively.

In RT-PCR analysis, total RNA from transfected cells was isolated using the AmbisPrep Multiresource total RNA Miniprep Kit (Axygen, USA) according to the protocol of manufacturer. 1 mg of total RNA was transferred into cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan). Thereafter, 2 μL of cDNA was subjected to RT-PCR analysis targeting MMP-9 and β-actin using Premix Taq Version 2.0 (Takara, Japan). PCR parameters consisted of 35 cycles of PCR (denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 30 s). The PCR products were run on 2% agarose gel with Ethidium bromide and visualized in Gel Doc™ XR+ imaging system (Bio-Rad). Relative gene expression values were determined using Quality One Software. Primers used in RT-PCR for MMP-9 and β-actin are:

MMP-9-forward 5′-GACAAGAGAGGGCCCGACG-3′
MMP-9-reverse 5′-ATGGTACCATGGACATCTGC-3′
β-actin-forward 5′-GCGGATATGGCTGTCGAC-3′
β-actin-reverse 5′-TGGAAGCTGAGCCGAGG-3′

In Western blot analysis, transfected cells were washed twice with cold PBS, and then resuspended in 100 μL of lysis buffer (50 mm Tris–HCl, pH = 7.4, 150 mm NaCl, 1% Triton X-100, 10% glycerol, 1.5 mm MgCl₂, 1 mm EDTA) freshly supplemented with Roche’s Protease Inhibitor PMSE Tablets. The cell lysates were incubated on ice for 30 min and vortexed every 5 min. The lysates were then clarified by centrifugation for 10 min at 12,000 rpm at 4 °C. The supernatant was loaded in loading buffer for 10 min. Total protein (20 μL) was separated (at 120 V for 40 min) on 12% PAGE-SDS gels and then transferred (at 300 mA for 40 min) to PVDF membranes (Bio-Rad). After incubation in 5% BSA (Merck, Germany) in phosphate buffered saline with Tween-20 (PBST, pH 7.2) for 1 h. The membranes were incubated in 5% BSA in PBST with MMP-9 antibodies (1:1000) over night. After incubation in 5% BSA in PBST with goat anti rabbit IgG-HRP antibody (1:5000) for 60 min, bands were visualized using the ECL system (Pierce). Relative gene expression values were determined using ImageJ Software.

2.6. Cellular toxicity

HNE-1 cells were cultured onto a 96-well plate (1 × 10⁴ cells/well) in complete DMEM (with high glucose and 10% fetal bovine serum supplemented) in a humidified atmosphere of 5% CO₂ at 37 °C. After 2 h, the growth medium was replaced with 200 μL complete DMEM culture medium that contained the desired amount of samples (CD-PLLD, CD-PLLD/DOC (DOC: 0.066 μg/well), CD-PLLD/pMR3 (N/P = 20, 40; pMR3: 0.5 μg/well)) and CD-PLLD/DOC/pMR3 (N/P = 20, 40; DOC: 0.066 μg/well; pMR3: 0.5 μg/well)) respectively. Five multiple holes were set for every sample. The cells treated with the same amount of PBS were used as a control group. The cells were incubated for another 48 h and the cell viability was assayed by adding 20 μL of MTT (Sigma) PBS solution (5 mg/mL). After incubation at 37 °C for another 4 h, the formed crystals were dissolved in 150 μL of DMSO. The absorbance that correlated with the number of viable cells in each well was measured by an MRX-Microplate Reader at a test wavelength of 490 nm.

2.7. apoptosis assay

HNE-1 cells seeded on the 24-well plates were treated with CD-PLLD, CD-PLLD/DOC, CD-PLLD/pMR3 and CD-PLLD/DOC/pMR3 (DOC concentration of 0.264 μg and pMR3 concentration of 2 μg/well) at 37 °C for 48 h. Cells without treatment were used as control. At the end of incubation, all cells were trypsinized, collected and resuspended in 200 μL of binding buffer. Thereafter, 5 μL of annexin V-FITC and 10 μL of PI were added and mixed for 15 min in the dark. The stained cells were analyzed using a flow cytometer.

2.8. Cell invasion assay

For invasion assays, the cells were plated into 6-well dishes in triplicate at high density. The cells were serum starved for 12 h before performing the assay, and BD BioCoat Matrigel Invasion Chambers were used for invasion assays. After starvation, cells were trypsinized and resuspended in 200 μL of binding buffer. Thereafter, 5 μL of annexin V-FITC and 10 μL of PI were added and mixed for 15 min in the dark. The stained cells were analyzed using a flow cytometer.
was used as an attractant. Cells were incubated for 24 h at 37 °C. Invading cells on the lower surface of the membranes were stained with 0.3% crystal violet stain and counted manually.

2.9. Biocompatibility

2.9.1. Cell viability

HNE-1 cells were cultured onto a 96-well plate (1 × 10⁴ cells/well) in complete DMEM (with high glucose and 10% fetal bovine serum supplemented) in a humidified atmosphere of 5% CO₂ at 37 °C. After 24 h, the growth medium was replaced with 200 µL complete DMEM culture medium that contained the desired amount of CD-PLLD or PEI respectively. Five multiple holes were set for every sample. The cells treated with the same amount of PBS were used as a control group. The cells were incubated for another 48 h, and the cell viability was assayed by adding 20 µL of MTT (Sigma) PBS solution (5 mg/mL). After incubation at 37 °C for another 4 h, the formed crystals were dissolved in 150 µL of DMSO. The absorbance that correlated with the number of viable cells in each well was measured by an MRX-Microplate Reader at a test wavelength of 490 nm.

2.9.2. Blood compatibility

The blood compatibility of CD-PLLD was evaluated by its hemolysis assay. For each sample, its hemolytic potential was tested according to the method reported by O’Leary and Guess [21]. Human blood (0.1 mL) anticoagulated with citrate was added to 5 mL of PBS containing the samples with different amounts in test tubes. Separate positive (100% hemolysis induced by replacing the PBS with 5 mL of 0.1% Na₂CO₃ solution) and negative (0% hemolysis, PBS with no material added) controls were also set up. Each set of experiments was carried out for three times. All the test tubes containing the samples and the control were incubated for 1 h at 37 °C. After the incubation, the tubes were centrifuged at 500 rpm for 5 min. The percentage hemolysis was calculated by measuring the optical density (OD) of the supernatant solution at 545 nm in a UV–vis spectrophotometer as per the following formula:

\[
\text{Hemolysis} = \frac{OD_{sample} - OD_{negative}}{OD_{positive} - OD_{negative}} \times 100\%
\]

Scheme 1. Chemical structure of CD-PLLD.

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Hemolysis (%) = \frac{\text{OD of the test sample} - \text{OD of negative control}}{\text{OD of positive control}} \times 100\%

2.9.3. In vivo toxicity

The CD-PLLD (500 mg/kg mouse) was dissolved in PBS and injected into 7 female BALB/c mice (4-week old, 18 ± 2 g) through tail vein, and physiological saline was used as control reagent. After 7 days, all animals were sacrificed, and the liver, heart, brain, spleen and kidney were separated, washed twice with PBS and fixed in 4% formaldehyde for histological examination.

2.10. Statistical analysis

Comparison between groups was analyzed by the one-tailed Student’s t-test using statistical software SPSS 11.5. All data are presented as means ± S.D. Differences were considered to be statistically significant when the P values were less than 0.05.

3. Results and discussion

3.1. CD-PLLD

Our previous work has synthesized the star-shaped poly[1-lysine] dendrons-β-cyclodextrin polymer (CD-PLLD) [19], and its chemical structure was shown in Scheme 1.

3.2. DOC loading and pMR3 binding

Due to the hydrophobic cavity of CD core and hydrophilic cationic dendron arms, CD-PLLD was amphiphilic and has a potential application in hydrophobic drugs delivery. In this paper, DOC was loaded in CD-PLLD using a dialysis method, and the loading amount was 13.2 μg/mg by a HPLC analysis.

Moreover, CD-PLLD could also electrostatically interact with pMR3 in aqueous solution due to the cationic arms, and then result in the formation of CD-PLLD/pMR3 complexes. The ability for CD-PLLD to bind pMR3 was examined by their electrophoretic mobility on an agarose gel. As shown in Fig. 1A, at low N/P ratios (lower than 2), CD-PLLD could not bind pMR3 entirely and the migratory MMP-9 could be observed. When the N/P ratio was equal to or higher than 5, the used CD-PLLD could retard completely the electrophoretic mobility of pMR3, indicating a strong binding ability to pMR3. The protection effect of complexes against DNA degradation by DNase I was shown in Fig. 1B. It was found that the naked pMR3 was completely digested, while CD-PLLD/pMR3 at all N/P ratios (10, 20 and 40) exhibited distinct protective effects against DNase I. These results indicated that CD-PLLD could not only load hydrophobic drugs, but also bind and protect genes against DNase, which showed a potential application in drug and gene co-delivery.

It is reported that the positive surface charge and proper particle size of cationic polymers/DNA complexes were important for efficient gene delivery, and cells typically uptake particles ranging from about 50 to several hundred nanometers [22], so it was expected that CD-PLLD could form the compact co-loading nanoparticles with DOC and pMR3, and the average diameters of the complexes were within the size requirements for efficient cellular endocytosis. Fig. 2A gave the sizes and zeta potentials of the co-loading CD-PLLD/DOC/pMR3 complexes at various N/P ratios. It was found that the particle size of the complexes decreased obviously with the increase of N/P ratio from 10 to 80, and remained in the range of 150–200 nm. The zeta potential of the complexes increased a little with the increase of N/P ratio from 10 to 40, and all complexes were positively charged. These results indicated that CD-PLLD could form stable and compact complexes with DOC and pMR3 when N/P ratio was high than 10. Moreover, TEM observation was used to characterize further the complex formation. For the CD-PLLD/DOC/pMR3 complex formed at the N/P ratio of 20, its particle size distribution and TEM image was shown in Fig. 2B. It was seen that the particle size of CD-PLLD/DOC/pMR3 complexes showed a good monodispersity and had a compact spherical shape with a size of 125 nm or so.
3.3. In vitro transfection

The gene transfection efficiencies of CD-PLLD/pMR3 complexes at different N/P ratios (10, 20 and 40, respectively) were evaluated by in vitro gene transfection assay using HNE-1 cells. For a comparative study, the PEI/pMR3 complex was also investigated at an N/P ratio of 10, at which PEI could achieve the highest level of transfection efficiency [23]. Fig. 3A and B showed the transfection results and fluorescence images of the transfected HNE-1 cells in these cases. It was found that PEI showed a high transfection efficiency and about 47.8% HNE-1 cells were transfected. Although CD-PLLD showed a reduced transfection efficiency compared with PEI, it also had a receivable gene transfection ability, especially at an N/P ratio of 20, at which more than 26% cells were transfected. Moreover, the transfected cells at the N/P ratio of 40 were slightly less than those at the N/P ratio of 20. This result may be caused by the slightly reduced tolerance of HeLa cells under the high CD-PLLD concentration [24], although the MTT results from Fig. 7 showed that CD-PLLD was non-toxic at the high concentrations.

To study further the transfection of CD-PLLD/pMR3 complexes to HNE-1 cells, RT-PCR and Western blot analysis was carried out to detect MMP-9 mRNA and protein expression. From the result of Fig. 4A, it was found that after sequence-specific MMP-9 gene silencing by MMP-9 complexes, the MMP-9 mRNA expression level was reduced obviously. There was no clear knockdown efficiency between control and blank CD-PLLD, while PEI mediated an obvious reduction of MMP-9 mRNA. Moreover, the samples treated with CD-PLLD/pMR3 also showed an obvious inhibition of MMP-9 mRNA expression. At an N/P ratio of 20, the MMP-9 mRNA expression reduced about 50% compared with PBS control. A reduction in MMP-9 mRNA was subsequently accompanied by decreased MMP-9 protein expression (Fig. 4B and C), as determined by Western blot analyses of MMP-9 protein in the cell lysates 48 h after transfection. It was found that black CD-PLLD could not induce low expression of MMP-9 protein, while the samples treated with pMR3 showed the obvious reduction of MMP-9 protein expression. From these results, it was confirmed that the CD-PLLD could deliver pMR3 effectively into HNE-1 cells. Although showed a slightly

Fig. 3. Quantitative determination (A) and result histogram (B) of transfected HEN-1 cells by flow cytometry. 1: PEI, N/P = 10; 2–4: CD-PLLD with N/P = 10, 20 and 40 respectively.

Fig. 4. (A) Expression of MMP-9 mRNA determined by quantitative real-time PCR. (B) Representative MMP-9 protein expression determined by Western blot analysis. (C) Analysis of light intensities of MMP-9 protein expression as the ratio of MMP-9 to β-actin from Western blot results. 1: CD-PLLD; 2: CD-PLLD/pMR3 (N/P = 40); 3: CD-PLLD/pMR3 (N/P = 20); 4: PEI/pMR3 (N/P = 10). PBS was set as the control.
higher MMP-9 protein expression than PEI/MMP-9, the CD-PLLD was considered as a promising gene carrier yet.

3.4. Co-delivery and in vitro therapy

To confirm the cell inhibition effect of complexes containing both DOC and pMR3, we evaluated their cytotoxic effects using a MTT assay. Fig. 5A show that CD-PLLD was non-toxicity at the concentration of this assay, while the samples containing DOC or pMR3 showed an obvious cytotoxicity. For the sample of co-delivering DOC and pMR3, the cell viability reduced further. The better inhibition of co-delivery to HNE-1 cells may be that the released DOC could damage DNA and meanwhile pMR3 could instigate mRNA to down-regulate protein expression. From the result we could know that the co-delivery may be an impactful method for tumor therapy.

To examine whether the co-delivered DOC and pMR3 could induce cell apoptosis effectively, the percentage of cell apoptosis treated with various formulation of DOC or pMR3 was determined by flow cytometer. Annexin V-FITC staining in conjunction with PI can distinguish early apoptosis from late apoptosis or living cells from necrotic cells [25]. As shown in Fig. 5B and C, after incubation with CD-PLLD for 48 h, HNE-1 cells displayed limited apoptosis compared with control group, which was consistent with the observed results of cytotoxicity analysis, demonstrating the non-toxicity of CD-PLLD. Cells treated with DOC or pMR3 exhibited the obvious increase of early and late cell apoptosis. The percentage of apoptosis (including early and late apoptosis) of CD-PLLD/DOC treated cells was 13.8%. Similarly, CD-PLLD/pMR3 caused 40.7% of cell apoptosis. For co-delivering DOC and pMR3, the apoptosis percentage reached up to 55.5%. These results revealed that pMR3 combined with DOC significantly enhanced the cell apoptosis, in
other words, the co-delivering strategy is a promising method in cancer therapy.

To determine whether introducing DOC and pMR3 renders HNE-1 cells less invasion, a transwell invasion assay was conducted on black CD-PLLD, CD-PLLD/DOC, CD-PLLD/pMR3 and CD-PLLD/DOC/pMR3. From Fig. 6, it was found that CD-PLLD/DOC/pMR3 significantly decreased cells invasion than the group of DOC or pMR3 used only. The number of tumor cells, which were able to invade through matrigel in CD-PLLD/DOC/pMR3 treated group was only 45% for DOC and 48% for pMR3 treated cells. CD-PLLD/DOC/pMR3 significantly decreased invasive capacity of HNE-1 cells.

3.5. Biocompatibility

The cytotoxicity of CD-PLLD was evaluated on HNE-1 cells by an MTT assay. Fig. 7A gave the cell viabilities result of the HNE-1 cells cultured in the media treated with different CD-PLLD or PEI concentrations. As seen, CD-PLLD had an obviously lower toxicity than
PEI. The cell viability was higher than 90% even if CD-PLLD concentration reached as high as 500 μg/mL. Contrarily, the viability of HNE-1 cells treated with 500 μg/mL PEI was lower than 50%.

The instability of delivery vehicles in the blood was considered as one of the serious limitations in the therapeutic of cationic polymers [26]. The nonspecific interactions of cationic polymers with blood components could severely diminish the half-life and targetability of complexes. The blood compatibility of CD-PLLD was assessed by spectrophotometric measurement of hemoglobin release from erythrocytes after polymer treatment. Fig. 7B showed the percentage hemolysis of blood in contact with CD-PLLD or PEI with different concentrations. It was found that PEI caused serious hemolysis in a concentration-dependent manner as a result of the erythrocyte membrane disruption, while CD-PLLD showed a much better blood compatibility. When the CD-PLLD concentration was up to 500 μg/mL, it showed non-hemolytic with the extent of hemolysis lower than the permissible level of 5%.

In vivo toxicity studies are essential to prove the safety of any polymers used as gene delivery. Herin, a histological analysis of organs was performed to determine whether CD-PLLD caused tissue damage, inflammation, or lesions. As shown in Fig. 8, histologically, no visible difference was observed compared to the control (top row). The in vivo toxicity of polymers is influenced by the chemical structures, size, exposure duration, biodistribution, location, metabolism as well as the nature of the surface and terminal groups. The toxicity of CD-PLLD also depends on its type, molecular weight and generation [27]. The non-observed toxicity of star-shaped CD-PLLD could be attributed to its lower molecular weight (less than 5 KDa) and the characteristic of molecular structure, such as the biodegradability and carrying a positive charge only on the surface. The biodegradability of CD-PLLD can also promote its elimination from organism and thereby enhance the in vivo biocompatibility.

4. Conclusion

For the co-delivery of hydrophobic drug and functional gene to tumor cells, a star-shaped copolymer (CD-PLLD) consisting of a β-cyclodextrin (CD) core and poly(lysine) dendron (PLLD) arms has been synthesized, and used to co-deliver DOX and pMR3 for nasopharyngeal cancer therapy. The CD-PLLD showed a good gene delivery ability in vitro, and could mediate a significant reduce of MMP-9 mRNA and protein in HNE-1 cells. For co-delivery assay, the CD-PLLD/DOC/pMR3 could induce a more significant apoptosis than DOX or pMR3 used only, and decreased invasive capacity of HNE-1 cells. Moreover, the star-shaped copolymer exhibited lower toxicity compared to PEI-25K. Therefore, CD-PLLD is a promising non-toxic drug and gene delivery system, which has a potential application in nasopharyngeal cancer therapy.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2014.01.040.