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Codelivery of doxorubicin and p53 by biodegradable micellear carriers based on chitosan derivatives

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Keywords: chitosan, micellear carriers, codelivery

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Abstract

In this work, novel biodegradable cationic micelles were prepared based on poly-(N-ε-carbobenzyloxy-L-lysine) (PZLL) and chitosan (CS) by click reaction, and applied for co-delivery doxorubicin (DOX) and p53 plasmid. The structure of copolymer was characterized by ^{1}H NMR, FTIR. The loading amount of DOX in micelles was 12.8%. Fluorescence spectra confirmed DOX interact π–π stacking with micelles when DOX was encapsulated into the micelles. In particular, its complexation with plasmid DNA was investigated by agarose gel electrophoresis, flow cytometry, zeta potential, and particle size analyses as well as transmission electron microscopy observation. The results showed that the copolymers have strong pDNA condensation ability and protection of pDNA against deoxyribonuclease I degradation. CS-g-PZLL/DOX/p53 nanoparticles showed good gene transfection efficiency in vitro. Fluorescence images and flow cytometry test revealed p53 and DOX could be efficiently transported into Hela tumor cells simultaneously, and the optimum N/P ratio for p53 transfection was 20/1. For co-delivery analysis, the obtained CS-g-PZLL/DOX/p53 complexes showed a better inhibition effect on Hela tumor cells than DOX or p53 used singly.

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1. Introduction

So far, cancer treatment still faces serious challenge. Chemotherapy is one of reliable choices for the treatment of many cancers. However, the treatment of chemotherapy has been limited because of the emergence of multi-drug resistance(MDR), which commonly associates with cancer cell overexpression of drug transporter proteins.[1] A novel approach to address cancer drug resistance is to take the advantage of the co-delivery of anticancer drugs and nucleic acid using multi-functionalized nanocarriers. Co-delivery of the anticancer drugs and nucleic acid to the tumor site could efficiently control the drug transporter proteins. This promising way may sidestep MDR and lead to an improved therapeutic effect.

For the co-delivery of drugs and gene while maintaining their biological functions, there has been an increasing interest in the development of multifunctional polymeric carriers by using polymers [2-4] , liposomes [5-6] dendrimers [7-8], silica [9-10], quantum dots [11]based nanoparticles and so on. As one of the most promising nanocarrier systems, self-assembled cationic polymeric micelles were widely utilized as drug and gene co-delivery systems. Cationic micelles are very effective nano-carriers for the co-deliver of gene and drugs into various cancer cell lines. Shi et al. prepared a series of cationic micelles based on triblock copolymers (MPEG-PCL-g-PEI) to deliver doxorubicin and gene Msurvivin T34A. Their results showed that DOX and gene were successfully co-delivered to the MCF-7 and CT26 cells. By introduction of T34A in combination with doxorubicin, it could greatly reduce systemic toxicity as well as improve the anti-tumor efficiency[12]. Lee et al. designed an amphiphilic copolymer poly{(N-methyldietheneamine sebacate)-co-[(cholesteryl oxocarbonylamido ethyl) methyl bis[13] ammonium bromide] sebacate} [P(MDS-co-CES)] to deliver human TRAIL and paclitaxel simultaneously. They found the co-delivery nanoparticulate system induced synergistic anti-cancer activities with relatively low toxicity in non-cancerous cells[14]. However, these kind of cationic polymers are still associated with problems of biodegradability, biocompatibility and cytotoxicity, which need to be overcome for in vivo application.

In this work, novel cationic micelles based on the polysaccharide and polypeptide were prepared by click chemistry, and applied for co-delivery of doxorubicin (DOX) and

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p53 plasmid. Poly-(N-ε-carbobenzyloxy-L-lysine) (PZLL) is a hydrophobic derivate of polypeptide. Due to their good biocompatibility and biodegradability, PZLL have been widely used as the hydrophobic inner cores of micelles [15-17]. DNA can be bound tightly to the surfaces of the micelles because of the amino groups in chitosan chains and PZLL branch chains. Their proton buffering capability, DNA condensation ability, protection of pDNA against deoxyribonuclease I degradation, in vitro cytotoxicity and gene transfection efficiency into Hela cells were investigated via acid–base titration, agarose gel electrophoresis, MTT, flow cytometry assay and fluorescence microscope. Their drug loading capacity and in vitro release behavior were studied using DOX as a model drug. The co-delivery of an anti-cancer DOX and functional gene (p53 plasmid) into Hela cells was also investigated and discussed in this study.

2. Experimental section

2.1. Materials

N-ε-Carbobenzyloxy-L-lysine, doxorubicin hydrochloride, azido propylamine Phthalic anhydride, hydrazine monohydrate, N-bromosuccinimide(NBS), triphenylphosphine (TPP), copper sulfate, 1-methy-2-pyrrolidinone (NMP) and triphosgene were purchased from Aladdin Chemical Reagent Co., Ltd. (China). Chitosan (Mw = 10 kDa) was purchased from Haidebei Marine Bioengineering Co. Ltd. (China). Propargylamine, Sodium azide (NaN_3, 99%) and sodium ascorbate (99%) were purchased from Alfa Aesar. The Dulbecco's modified Eagle medium (DMEM), trypsin-ethylenediaminetetraacetic acid (Trypsin-EDTA), and fetal bovine serum (FBS) were purchased from Gibco-BRL (Canada). Polyethylenimine (PEI, 25 kDa), 3-[4,5-dimethylthiazol- 2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (U.S.A.). Deoxyribonuclease I (DNaseI) was purchased from Feibo Life Sciences (China). The plasmid p53 was obtained from Invitrogen. All other reagents were analytical grade and were used as received.

2.2. Preparation of CS-g-PZLL copolymers

CS-g-PZLL were prepared by click reaction of *α*-alkyne-poly-(*N*-ε- carbobenzyloxy-L-lysine) (*α*-alkyne-PZLL) and azide focal point chitosan (6-N_3-CS), as shown in Scheme 1.First, α-alkyne-PZLL(Scheme 1A) was synthesized following a procedure reported by Lin [18].

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In brief, 5.0 g N-ε-carbobenzyloxy-L-lysine (17.8 mmol) was reacted with 3g triphosgene (10.1 mmol) by using tetrahydrofuran (THF) as solvent. The reaction time was 1h and reaction temperature was 50 °C. After reaction, solvent was removed in vacuo, the obtained residue was first dissolved in ethyl acetate, then washed with cold 5% $NaHCO_3$ solution. The ethyl acetate layer was collected and dried by anhydrous Na_2SO_4. Ethyl acetate was removed in vacuo and a ε-carbobenzyloxy-L-lysine N-carboxyanhydride (Lys (Z)-NCA) white solid with a yield of 77% was obtained. After that, 1.0 g Lys (Z)-NCA was reacted with 0.01 g propargylamine in anhydrous dimethylformamide (DMF), the reaction time was 3 days. After reaction, methanol was added into solution and white powder deposit was obtained. The chemical structure was confirmed by ^{1}H NMR and FTIR. ^{1}HNMR (400 MHz, D_2O): δ 7.36–7.41 (5H, m -Ph), 5.08 (s, 2H, -OCH_2Ph), 3.95 (s, 2H, -CCH_2NH-), 2.89 (d, 2H, -NHCH_2CH$_2$), 1.7 (t, 2H, -CHCH_2CH$_2$-), 1.24 (m, 2H,-CHCH_2CH$_2$-). IR (KBr, cm^{-1}): 3299, 3053, 2970, 1645, 1544, 1258, 1165, 1013, 539.

Second, 6-N_3-CS (Scheme 1B) was synthesized by the similar method reported by Deng et al[19]. In brief, chitosan and phthalic anhydride was dissolved in DMF and reacted for 8h at 120 °C to obtain N-phthaloyl-chitosan (2), (2) was then reacted with N-bromosuccinimide to obtain 6-bromide-6-deoxy-N-phthaloyl-chitosan (3), after that, (3) was reacted with sodium azide in N-methylpyrrolidone for 8h at 80 °C. After reaction, the solution was filtered and precipitated with ethanol, precipitate was collected and washed by acetone for three times, then dried under a vacuum to get 6-Azido-6-deoxy-Nphthaloyl-chitosan (4) with a yield of 73%.^{1}HNMR (400 MHz, D_2O): δ 3.30-3.90 (m, D-glucosamine unit, H-3, H-4, H-5, H-6, H-60), 3.34-3.78 (2H, -CONHCH_2-), 2.90 (protons next to amines). IR (KBr, cm^{-1}): 3442, 2930, 2114, 1667, 1382, 1071, 1013, 657.

At last, CS-g-PZLL was synthesized by Scheme 1C. α-alkyne-PZLL(3 mmol), 6-N_3-CS (1 mmol) and the catalyst ($CuSO_4 \cdot 5H_2O$/sodium ascorbate, 0.5 mmol/1 mmol) were dissolved in 20.0 mL DMSO/water (5:1, v/v) mixture solution and reacted at 50 °C for 24 h. The mixture was then precipitated with ethanol and purified by dialysis in water for 2 days. After dialysis and lyophilization, the CS-g-PZLL was collected as a brown powder with a yield of 87%. ^{1}HNMR (400 MHz, D_2O): δ 7.36–7.41 (5H, m -Ph), 5.08 (s, 2H, -

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148 OCH_2Ph), δ 3.30-3.90 (m, D-glucosamine unit, H-3, H-4, H-5, H-6, H-60), 3.34-3.78 (2H,
149 -CONHCH_2-), 2.90 (protons next to amines) 1.7 (t, 2H, -CHCH_2CH_2-),1.24 (m, 2H,-
150 CHCH_2CH_2-). IR (KBr, cm^{-1}): 3299, 3053, 2930, 1645, 1382, 1258, 1165, 1013, 539.
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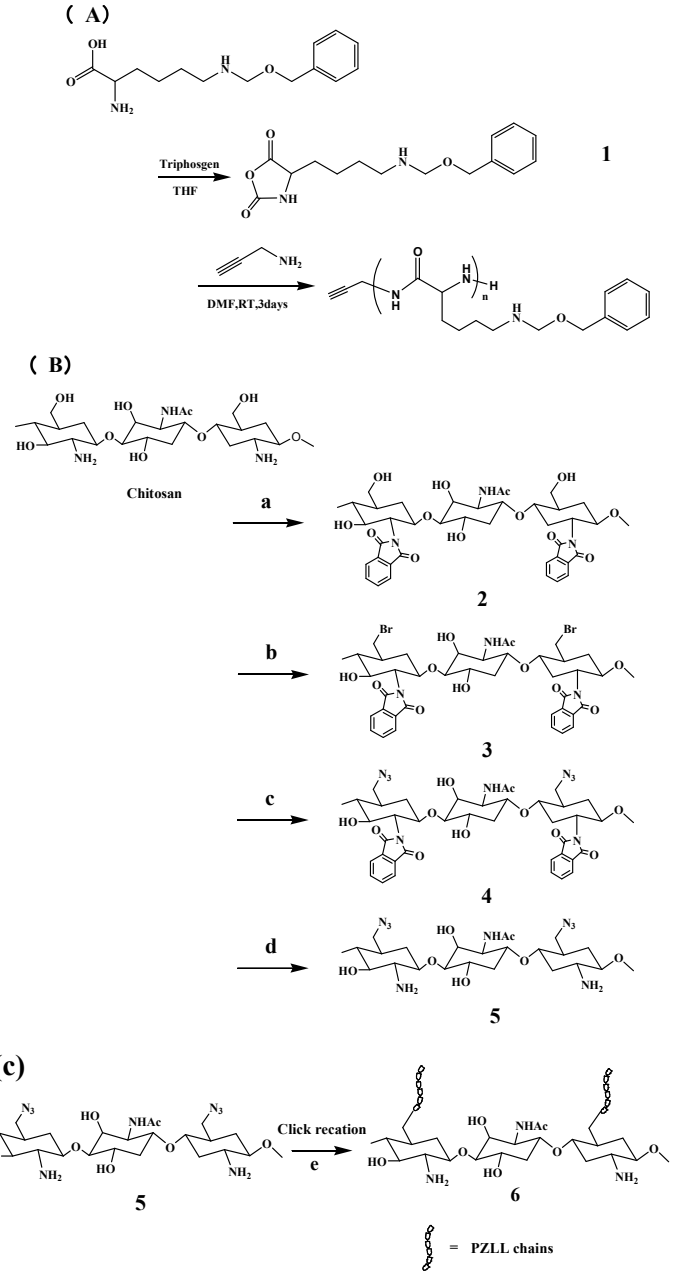

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155 Scheme 1 Synthesis route to CS-g-PZLL

156 Reaction conditions: (a) phthalic anhydride, DMF, 120 °C, 8 h; (b)N-bromosuccinimide,

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triphenylphosphine, NMP, 80 °C, 2 h; (c) sodiumazide, NMP, 80 °C, 4 h; (d) hydrazine monohydrate, water, 100 °C, 10 h; (e)$CuSO_4 \cdot 5H_2O$/sodium ascorbate, DMSO/water, 40 °C, 24 h.

2.3. DOX loading and in vitro release

DOX was loaded by using a dialysis method reported by Lin et al[9]. Briefly, 10mg CS-g-PZLL and 5.0 mg DOX hydrochloride were first dissolved by using 5.0 mL DMSO as solvent, for neutralization of HCl, a drop of triethylamine was then added to the solution. The complete dissolved solution was then transferred to a dialysis bag (MWCO 3000) and subjected to dialysis against distilled water for 48 h. After that, the dialysis solution was filtered through a 0.45 m filter and then lyophilized. To investigate the interactions between CS-g-PZLL and DOX, the obtained CS-g-PZLL/DOX complex was analysised by fluorescence spectra. The excitation wavelength was 330 nm and the fluorescence emission spectra were recorded in the range from 400 to 700 nm. To determine the loading amount of DOX, the obtained DOX/CS-g-PZLL was dissolve in DMSO and analyzed by UV–vis spectrophotometry (TU-1900, China) at 480nm. It was found that the loading amount of DOX was 12.8 %. The loading amount of DOX was calculated according to the following equation:

$$LC=M_1/M_0\times 100\%$$

where M_0 is the weight of micells, M_1 is the weight of the loading DOX.

The release of DOX from CS-g-PZLL was assayed at 37 °C in PBS buffer of pH 5.8 (simulate pH of tumor) and 7.4 (simulate pH of blood plasma). Predetermined amount of the DOX/CS-g-PZLL complexes in 5.0 mL of PBS (pH 5.0 and 7.4) was sealed in a dialysis bag (MWCO=3KDa), then the dialysis bag was submerged in 20 mL of the corresponding buffer. At predetermined time intervals, 2.0 mL of aqueous solution was taken out for drug concentration measurement and replaced by an equal volume of fresh PBS. The released DOX in the incubation buffer was analyzed using a UV–vis spectrophotometry (TU-1900, China) at 480 nm. All measurements were performed in triplicate.

2.4. Plasmid binding

2.4.1. Formation

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For the plasmid binding to the CS-g-PZLL, p53 solution was added to the CS-g-PZLL or DOX loaded CS-g-PZLL solutions at various N/P ratios, then mixed by gentle agitation for 5 s and incubation at 37 ºC for 30 min before used.

2.4.2. Gel electrophoresis

Agarose gel retardation assay was carried out to determine the DNA condensation ability of CS-g-PZLL micellar nanoparticles to p53. CS-g-PZLL/p53 complexes were prepared at various N/P ratios (CS-g-PZLL to p53: 5, 10 and 20). The complexes were mixed with appropriate amounts of loading buffer and incubated for 30 min at room temperature , then loaded onto 1.0 % agarose gel containing GeneGreen (0.1mg·mL-1, Sigma) and electrophoresed with tris-acetate buffer for 30 min at 100 V. The location of DNA in the gel was analyzed using a UV transilluminator and a digital imaging system (Fisher Scientific, PA, USA).

DNase I was added to CS-g-PZLL/p53 complexes (N/P 5, 10 and 20) for examining the protection ability of CS-g-PZLL against DNase degradation. DNase I and complexes were incubated at 37 ℃ for 30 min, after that, EDTA (4.0 mL, 250 mM) and sodium dodecyl sulfate (SDS) solution (4.0 mL,10%, w/v) was added and the mixture was incubated at room temperature for another 1 h. The samples were then electrophoresed on the 1.0 % agarose gel to examine the integrity of DNA.

2.4.3. Size and morphology

The particle size and surface charge of the complexes were determined by a Zeta Potential Analyzer instrument (ZetaPALS, Brookhaven Instruments Corporation, USA). The morphology of the complex was observed by a JEM-2010HR high-resolution transmission electron microscope instrument.

2.4.4. In vitro transfection

For transfection, Hela cells were plated in 24-well plates at 1×10^4 cells/well. Prior to transfection, the cells were washed once with PBS buffer, and the medium in each well was replaced with serum-free media for 12h. After that, Cells were replenished with 10% fetal bovine serum media containing CS-g-PZLL/p53 complexes at different N/P ratios (containing 2.0 μg p53 in each N/P ratio). After 48 h transfection at 37 °C, the cells were observed with a Olympus IX71 fluorescence microscope (Melville, NY, U.S.A.). The transfected cells were washed once with PBS, detached with 0.25% trypsin and collected,

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transfection efficiency was analyzed via flow cytometry quantitatively by scoring the percentage of cells expressing GFP (FACS Aria flow cytometer Germany).

2.5 In vitro Cytotoxicity Assay

In vitro Cytotoxicity assay of CS-g-PZLL and DOX loaded CS-g-PZLL nanoparticles was performed against Hela cells by MTT assay. Five multiple holes were set for every sample. Briefly, Hela cells were respectively cultured onto 96-well plates at a density of 1×10^4 cells/well and incubated in a humidified atmosphere of 5% CO_2 at 37 ºC for 12 h. After that, the growth medium was replaced by 100 μL complete DMEM containing indicated amount of sample and and further incubated for 24 h. Then 10 μL of MTT (0.5 $mg\cdot mL^{-1}$) in PBS solution was added to each well, the cells were incubated for another 4h to form formazan crystals. Finally, the medium was removed and 100 μL of DMSO was added to each well. The optical density values of the samples were measured at 490 nm by using a MRX-Microplate Reader (Thermo, USA). The cells treated with the same amount of PBS were used as control. The relative cell viability was calculated as follow:

Cell viability (%) = [A_{490} (sample) /A_{490} (control)] × 100

Where A_{490} (sample) and A_{490} (control) were obtained in the presence and absence of sample, respectively.

3. Result and discussion

3.1. Synthesis and characterization of CS-g-PZLL

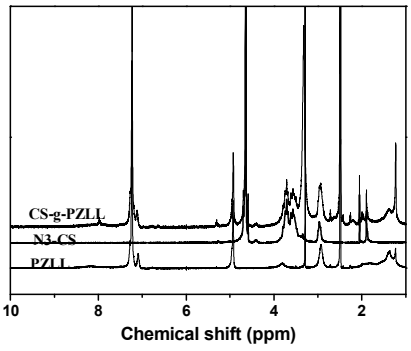

Figure 1. ^{1}H NMR spectra of CS-g-PZLL, N_3-CS and PZLL.

Figure 1 shows ^{1}H NMR spectra of the CS-g-PZLL, 6-N_3-CS, and PZLL. As seen in the spectrum of the CS-g-PZLL, the new peaks at 5.0, 7.0-7.30 ppm showed the presence of the PZLL compared to the spectrum of 6-N_3-CS. Moreover, the peak at 8.1 ppm in the

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spectrum of the CS-g-PZLL indicated the presence of the triazole proton, which could be attributed to the formation of 6-N_3-CS and PZLL by the click reaction.

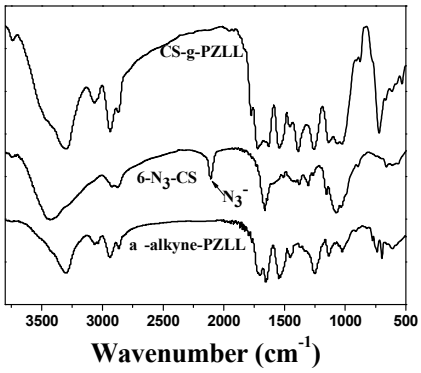

Figure 2. FTIR spectra of CS-g-PZLL, N_3-CS and PZLL.

Figure 2 shows the FTIR spectra of the CS-g-PZLL, N_3-CS and PZLL. As seen in FTIR spectrum of N_3-CS, the characteristic vibration band for azide group at 2110 cm^{-1} demonstrated that azide groups were successfully incorporated in N_3-CS. The new peak at 1450 cm^{-1} appeared in the samples of CS-g-PZLL, which is attributed to the 1,2,3-triazole structure formed during click modification, respectively[20-21]. The FTIR data indicated that the PZLL dendrimer was successfully grafted onto chitosan chains via click chemistry.

3.2. DOX-loaded micelles and in vitro drug release

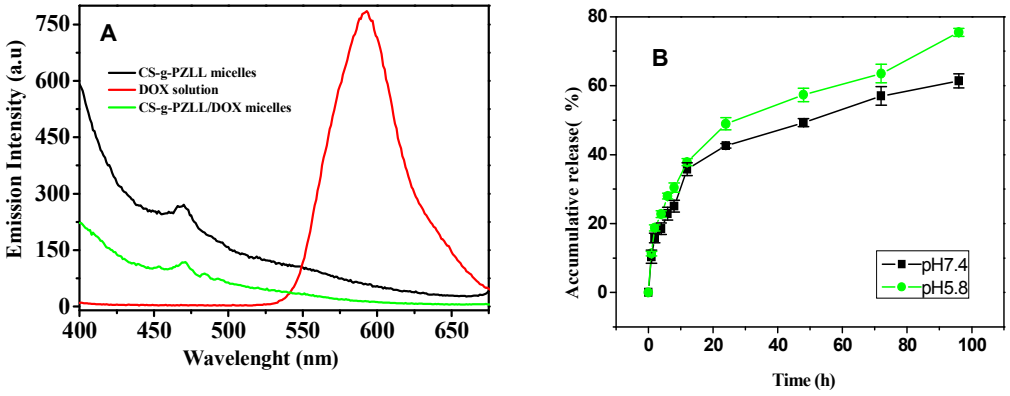

Figure 3 (A) The fluorescence spectra of the micells, DOX solution and complex ($\lambda_{excitation}$=330 nm). (B) In vitro release profiles of the loading DOX from CS-g-PZLL nanoparticles in phosphate buffer (pH 7.4 or 5.8) at 37 °C

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In vitro release of drugs form micells were investigated in pH 7.4 and 5.0 to imitate the pH of blood and tumor site. DOX was chosen as the model drug, and DOX was encapsulated into the hydrophobic core of the micelles. Figure3(A) show the interaction of DOX and micells by fluorescence spectra. It was found that the fluorescence of complex was nearly completely quenched when DOX was encapsulated into the micells, confirming the π–π stacking interaction between DOX and phenyl groups of PZLL chains[22-23]. Moreover, the π–π stacking interaction help to improve high loading amount of DOX. It was found that the loading amount of DOX in micells was 12.8%. As shown in Figure 3(B), release profiles showed sustained release behaviors of DOX at pH 7.4 and pH 5.0. 35.7% and 37.8% of the loaded DOX were released at pH 7.4 and pH 5.0 in the initial 12 h, and the accumulated release reached 61.4% and 75.4% after 96 h, respectively (Figure 3B). It is interesting to find that the release rate and released amount of DOX at pH 5.0 were a little higher than those at pH 7.4, which revealed a promoted drug release behavior at the tumor site. DOX released from micelles mainly by free diffusion. The better solubility of DOX at pH 5.0 than at pH 7.4 promoted the rapid release behavior. Moreover, the pH-responsive property of the copolymers leads to the higher release rate in lower pH. Since tumor shows lower extracellular pH, this observation demonstrated that the CS-g-PZLL micelle might show potential applications as a tumor-targeting drug delivery platform.

3.3.1 Gel electrophoresis

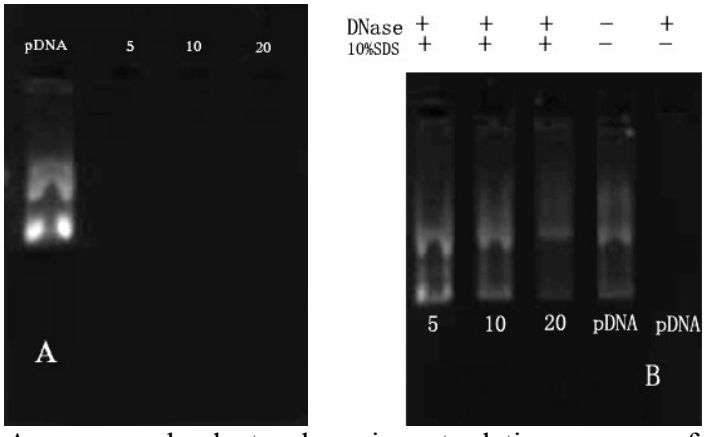

Figure 4. (A) Agarose gel electrophoresis retardation assay of CS-g-PZLL/p53 complexes at different N/P ratios. (B) Protection and release assay of p53. p53 was released by adding 10% SDS to CS-g-PZLL/p53 complexes at different N/P ratios.

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The formation of CS-g-PZLL/p53 complexes was examined by agarose gel electrophoresis assay. As shown in Figure 4A, the migration of pDNA was completely retarded when the MSN-x-G3/pDNA weight ratio exceeded 5. These results indicated that CS-g-PZLL has a strong binding ability to p53. Figure 4B shows the protection effect of CS-g-PZLL against p53 degradation by DNase I. It was found that the naked p53 was completely digested, while CS-g-PZLL/p53 at all N/P ratios (5, 10 and 20) exhibited distinct protective effects against DNase I. These results indicated that the micells formed by CS-g-PZLL copolymer could be used as a co-delivery system for loading hydrophobic drugs and gene. Moreover, the co-delivery system could protect genes against DNase simultaneously.

3.3.2. Size and morphology

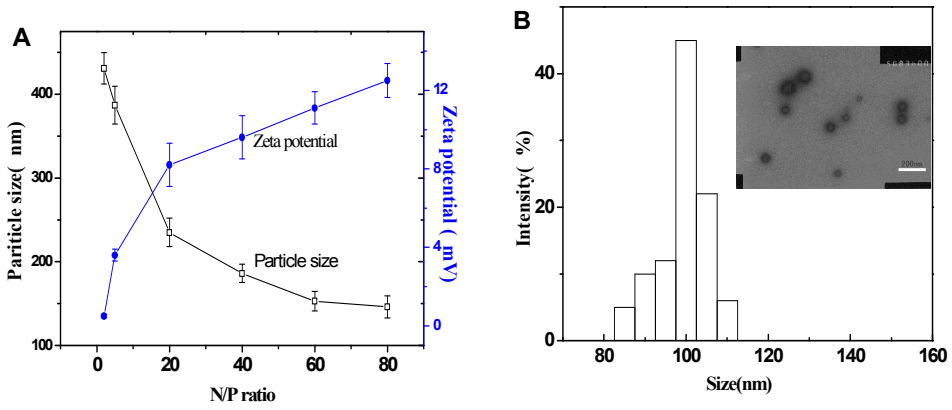

Figure 5. (A) Particle sizes and zeta potentials of CS-g-PZLL/DOX/p53 complexes formed at various N/P ratios. (B) Typical particle size distribution and TEM image of the CS-g-PZLL/DOX/p53 complex formed at an N/P ratio of 20.

The mean particle sizes and zeta potential of CS-g-PZLL/DOX/p53 complexes were investigated by Zeta Potential Analyzer and TEM, as shown in Figure 5. It was found that the zeta potential increased with the N/P ratio of 2-80, and the range was between 0.5-12.5 mV. By contrast, the size of the complexes tended to decrease with the increase of N/P ratio, and remained in the size range from 400 to 150 nm. The TEM photos showed that CS-g-PZLL/DOX/p53 complexes (Figure 5 B) have a spherical shape and compact structure. The complexes had an average diameter of about 100 nm, which was consistent with the results measured by zeta potential test. It is reported that the size range of pDNA containing complexes from 50 to 400 nm was suitable for cellular endocytosis[24]. In this

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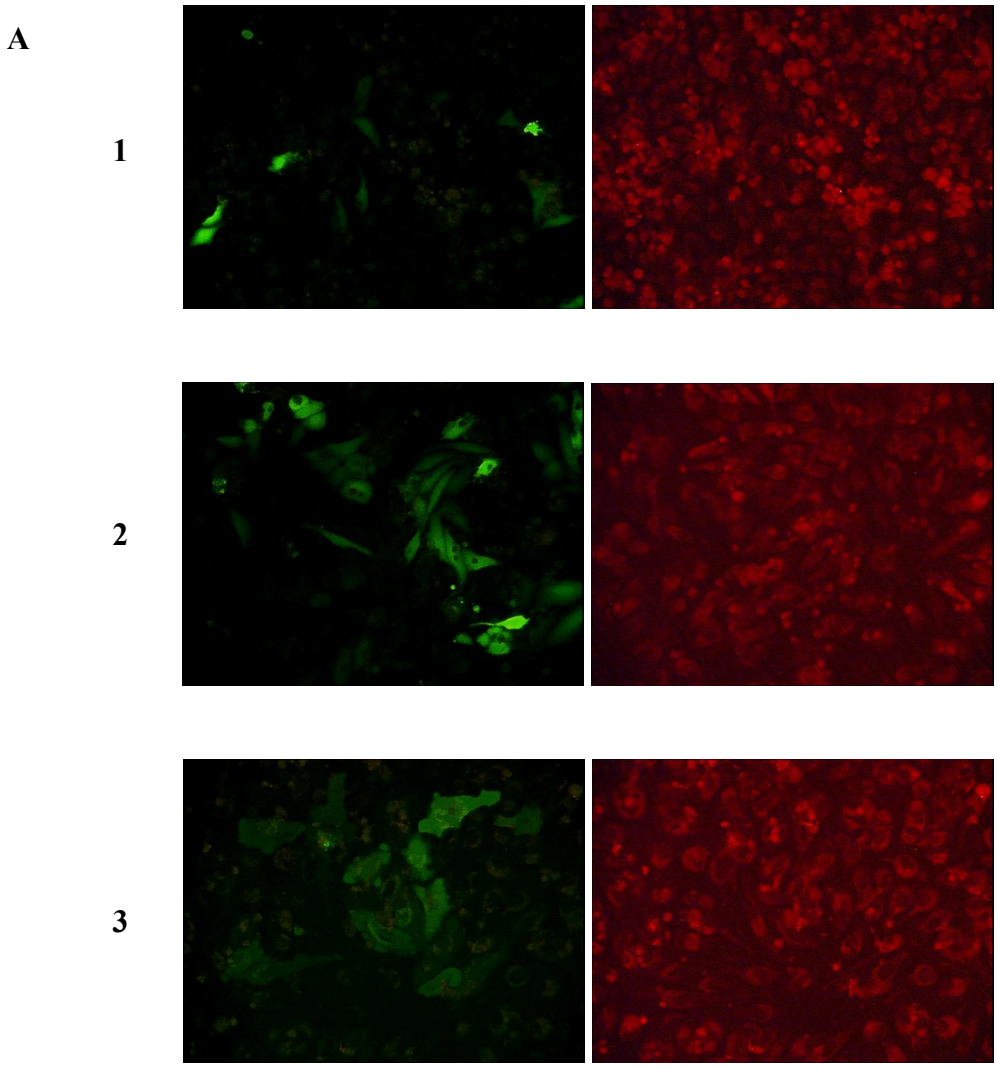
study, the test result of CS-g-PZLL/DOX/p53 could meet the requirement of efficient gene delivery.

3.3.3 Co-delivery and cell viability



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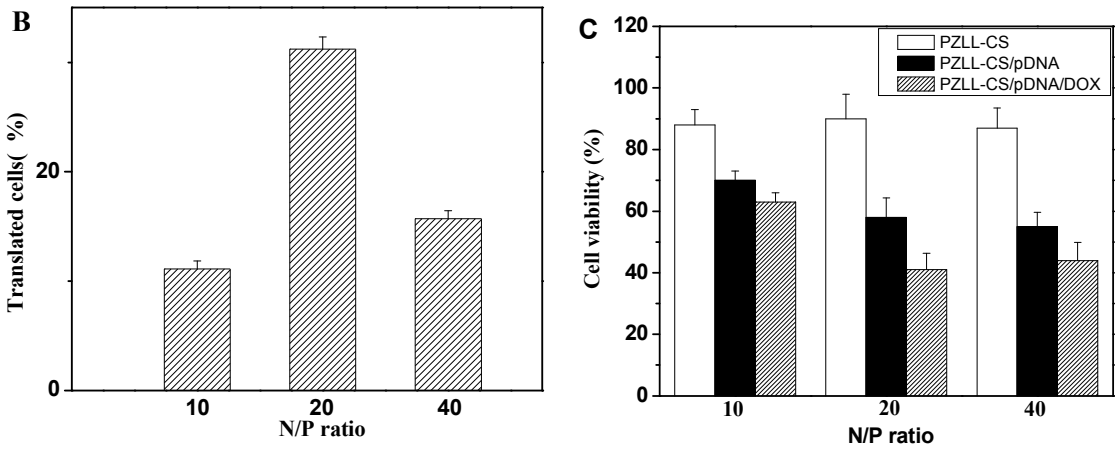

Figure 6. (A)Fluorescence field images of Hela cells transfected by CS-g-PZLL/p53/DOX complexes formed at various N/P ratios(1 N/P=10, 2 N/P=20, 3 N/P=40). (B) Quantitative determination of transfected Hela cells by flow cytometry (C)Hela cells viability treated by CS-g-PZLL, CS-g-PZLL/p53 and CS-g-PZLL/p53/DOX

To investigate the co-delivery ability of CS-g-PZLL, Hela cells was studied by fluorescence microscopy using plasmid eGFP-N1-53 and DOX as model gene and hydrophobic drug. The representative fluorescence images are shown in Figure 6. The green and red fluorescence were both observed in the Hela cells at different N/P ratio, the green fluorescence was from eGFP and the red fluorescence was from DOX (Figure 6 A). The results suggested that both p53 and DOX can be delivered into Hela cells by CS-g-PZLL at different N/P ratio. The intensity of the red fluorescence at different N/P ratios did not show apparent difference. It is suggested that DOX could be efficiently transported into cells by at all N/P ratios. On the other hand, the intensity of the green fluorescence from eGFP was stronger at N/P ratio of 20 than others, indicating that N/P ratio of 20 was the optimum N/P ratio for p53 transfection. This result was consistent with the results of flow cytometry, as shown in figure 5 B. The highest transfected ratio was 31.2% while the N/P ratio was 20. 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 CS-g-PZLL concentration (figure 6 C).

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. For the

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co-delivery of antitumor drugs and genes while maintaining their chemophysical properties and biological functions, a multifunctional carrier which could load gene and drug simultaneously is necessary. In previous studies, cationic polymeric micelles have been widely used as drug and gene co-delivery carriers. Zhu et al.[26] prepared biodegradable cationic micelles based on the self-assembly of PDMAEMA-PCL-PDMAEMA triblock copolymers as siRNA and paclitaxel co-delivery carriers. The results demonstrated that combinatorial delivery of VEGF siRNA and paclitaxel showed an efficient knockdown of VEGF expression. Zheng et al.[27] prepared polypeptide cationic micelles based on poly(ethylene glycol)-*b*-poly(l-lysine)-*b*-poly(L-leucine) (PEG-PLL-PLLeu) triblock copolymers as docetaxel (DTX) and siRNA-Bcl-2 co-delivery vectors. The results showed that the co-delivery of DTX and siRNA-Bcl-2 (siRNA that suppresses the expression of anti-apoptotic Bcl-2 gene) significantly inhibited tumor growth as compared to the individual siRNA or DTX treatment.

To confirm the cell inhibition effect of complexes containing both DOX and p53, we evaluated their cytotoxic effects using a MTT assay. The results showed that CS-g-PZLL was non-toxicity at the concentration of this assay, while the samples containing p53 showed an obvious cytotoxicity, the cell viability was decrease to 70, 58 and 55% at N/P ratio of 10, 20 and 40 (Figure 6C). The further inhibition effect was found by the co-delivery group, the cell viability was decrease to 63, 41 and 44% respectively. The better effect may attribute to that the released DOX could damage DNA; meanwhile, p53 could instigate mRNA to down-regulate protein expression. The result suggested that the co-delivery induce synergistic actions and lead to an effective method for tumor therapy.

4. Conclusion

For the co-delivery of anti-tumor drug and gene to tumor cells, a new cationic micelle consisting of poly-(N-ε-carbobenzyloxy-L-lysine) (PZLL) and chitosan has been synthesized, and used to co-deliver DOX and p53 for cancer therapy. CS-g-PZLL/DOX/p53 nanoparticles showed good gene transfection efficiency in vitro, and could delivered p53 and DOX simultaneously to Hela tumor cells. For co-delivery analysis, the obtained CS-g-PZLL/DOX/p53 complexes showed a better inhibition effect on Hela tumor cells than p53 used singly. Such a cationic micelle deliver

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system may be used as a potential multifunction vector for future cancer therapy applications.

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