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Cationic micellar nanoparticles for DNA and doxorubicin co-delivery

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ABSTRACT

Cationic micellar nanoparticles for chemotherapeutic drugs and therapeutic gene co-delivery were prepared based on a poly-(*N*-ε-carbobenzyloxy-L-lysine) (PZLL) and dendritic polyamidoamine (PAMAM) block copolymer (PZLL-D3). PZLL-D3 was synthesized by a copper-catalyzed azide alkyne cyclization (click) reaction between α-alkyne-PZLL and azide focal point PAMAM dendrons. Its structure was characterized by ^{1}H NMR and FTIR, and its buffering capability was determined by acid–base titration. MTT, agarose gel electrophoresis and flow cytometry studies showed that PZLL-D3 revealed low in vitro cytotoxicity, strong pDNA condensation ability, protection of pDNA against deoxyribonuclease I degradation and high gene transfection efficiency in 293T and HeLa cells. In addition, the micellar nanoparticles delivered pDNA and anticancer drug doxorubicin (DOX) simultaneously and efficiently to tumor cells, and the DOX loaded nanoparticles showed sustained in vitro release at pH = 7.4 and 5.8.

1. Introduction

In cancer treatment, chemotherapy remains one of the most reliable treatments for many cancers. However, one of the major challenges in chemotherapy is the multi-drug resistance (MDR) by cancer cells caused by the over-expression of drug transporter proteins, especially P-glycoprotein (P-gp) [1]. Many efforts have been made to develop chemomodulators to inhibit MRD, however, the cytotoxicity and side effects of these chemomodulators have limited their clinical use [2–5]. On the other hand, gene therapy has attracted more and more attention as a promising strategy for the treatment of cancer [6,7]. Combination of chemotherapy and gene therapy via the co-delivery of chemotherapeutic drugs and therapeutic gene to tumor cells would achieve synergistic or combining effects. Wang et al. [8] reported that the co-delivery of paclitaxel and interleukin-12-encoded plasmid DNA (pDNA) via cationic core-shell nanoparticles inhibited cancer growth more efficiently than the delivery of either drug or DNA. Wiradharma et al. [9] found that the co-delivery of doxorubicin (DOX) and p53-encoding pDNA by cationic oligopeptide micelles led to a synergistic therapeutic effect in suppressing the proliferation of HepG2 cells. Furthermore, Liu et al. [10] and Prados et al. [11] demonstrated that the combination therapy with anticancer drugs and therapeutic genes could reduce the dose of cytotoxic anticancer drugs without impairing the antitumor efficacy.

In gene therapy, a safe and efficient gene delivery vector or vehicle is crucial. Traditional viral vectors could efficiently deliver gene to target cells, however, they also exhibit severe safety concerns, such as cytotoxicity, immunogenicity and oncogenic effects [12]. On the other hand, nonviral vectors based on cationic liposomes and polymers have attracted increasing interests for their high nucleic acid binding affinity, biocompatibility, ease of modification and large-scale production potential [13,14]. In the past two decades, nonviral vectors have been fabricated based on polyethylenimine (PEI) [15,16], chitosan [17,18], poly (amino ester) [19,20], dendritic polyamidoamine (PAMAM) [21,22], poly (L-lysine) (PLL) [23,24] and their derivates [25,26]. Among those polymeric materials, PLL and dendritic PAMAM are two of the most widely used polymers for nonviral gene vectors [27–33]. PLL is a cationic polypeptide with good biocompatibility, biodegradability and relatively low cytotoxicity, and PLL can self-assemble into protein-like globular structures in aqueous solutions [34]. Poly-(*N*-ε-carbobenzyloxy-L-lysine) (PZLL) is a hydrophobic derivate of PLL, which is widely used as the hydrophobic inner cores of micelles [35–37]. PAMAM dendrimers are monodisperse macromolecules with well-defined architectures [38,39]. PAMAM has primary amine groups

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on their surfaces for nucleic acid condensation and tertiary amine groups in the core to enable the efficient endosomal escape via the proton buffering effect (proton sponge effect) [33]. Despite their distinctive features, both polymers possess drawbacks impeding their applications in gene therapy. PLL does not have good proton buffering capacity for its lack of multi-amine structure and thus leads to a low level of gene transfection expression [40,41]. High-generation PAMAM dendrimers are fairly cytotoxic caused by the large number of primary amine groups and thus limit their applications in vivo [42,43]. By combing PLL derivatives and PAMAM dendrimers, micellar nanoparticles with well-defined core–shell structure, relatively low cytotoxicity, high gene transfer and expression efficiency and high drug loading capacity are expected.

In this paper, cationic micellar nanoparticles for anticancer drugs and gene co-delivery were prepared based on a poly-(*N*-ε-carbobenzyloxy-L-lysine) (PZLL) and dendritic PAMAM block copolymer. The working schematic representation of cationicmicellar nanoparticles is shown in Scheme 1. Their proton buffering capability, DNA condensation ability, protection of pDNA against deoxyribonuclease I degradation, in vitro cytotoxicity and gene transfection efficiency in 293T and 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.

2. Experimental section

2.1. Materials

N-ε-carbobenzyloxy-L-lysine, doxorubicin hydrochloride and triphosgene were purchased from Aladdin Chemical Reagent Co., Ltd. (China). Azido propylamine was purchased from J&K Scientific (China). Methyl acrylate and ethylenediamine were obtained from Sinopharm Group Chemical Reagent 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), poly-L-lysine hydrobromide (molar mass: 1000–5000) and 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 reporter pDNA (plasmid eGFP-N1) was obtained from Invitrogen and was amplified in *Escherichia coli* (*E. coli*). All other reagents were analytical grade and were used as received.

2.2. Synthesis of PZLL-block-dendritic PAMAM copolymers (PZLL-D3)

PZLL-D3 was prepared by copper-catalyzed azide alkyne cyclization reaction of α-alkyne-poly-(*N*-ε-carbobenzyloxy-L-lysine) (α-alkyne-PZLL) and azide focal point PAMAM dendrons of third generation (N_3-D3), as illustrated in Scheme 2.

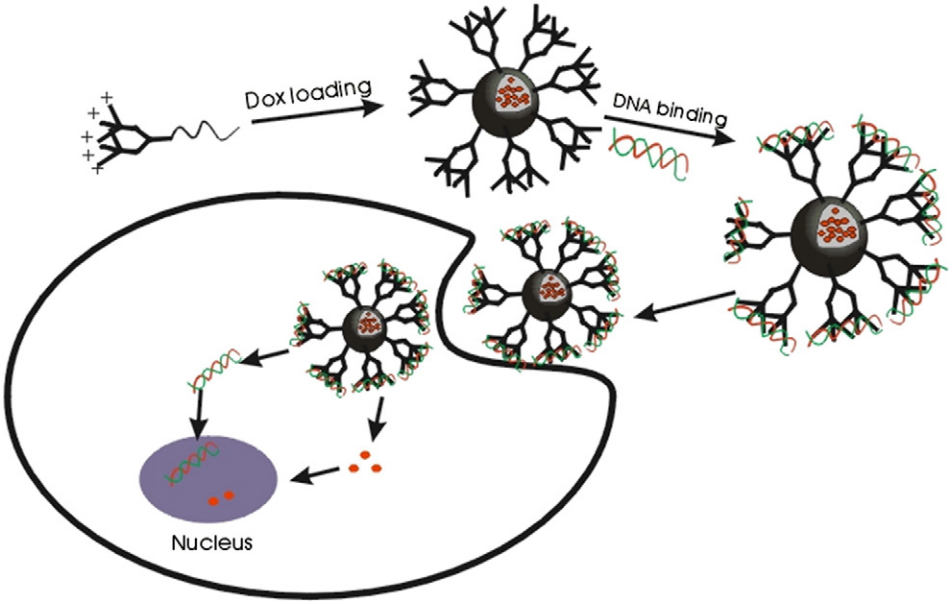
2.2.1. Synthesis of α-alkyne-PZLL

α-Alkyne-PZLL was synthesized following a procedure reported by Gibson et al. [44]. In brief, 5 g *N*-ε-carbobenzyloxy-L-lysine (17.8 mmol) and 3 g triphosgene (10.1 mmol) were dissolved in 100 ml tetrahydrofuran (THF). The solution was stirred for 1 h at 50 °C under a nitrogen atmosphere. After the volatiles were removed in vacuo, the residue was dissolved in ethyl acetate and washed with cold 5% $NaHCO_3$ aqueous solution. The organic layers were collected and dried over anhydrous Na_2SO_4 in a refrigerator at 4 °C. The volatiles were then removed in vacuo and the residue was dried under vacuum. A ε-carbobenzyloxy-L-lysine *N*-carboxyanhydride (Lys (Z)-NCA) white solid with a yield of 75% was obtained.

1.0 g Lys (Z)-NCA and 0.01 g propargylamine were then dissolved in anhydrous dimethylformamide (DMF). The reaction was stirred for 3 days at room temperature under a nitrogen atmosphere. The reaction mixture was precipitated with methanol and an α-alkyne-PZLL white powder was collected by filtration.

2.2.2. Synthesis of N_3-D3

N_3-D3 was synthesized following a procedure described by Deng et al. [45] In brief, azido propylamine (20 mmol) was dissolved in 5 mL methanol and this solution was added dropwise into a solution containing methyl acrylate (80 mmol) and 10 mL methanol. The reaction mixture was stirred vigorously for 24 h at room temperature under nitrogen atmosphere. The volatiles were removed in vacuo, and the residue was dried in vacuo at 35 °C to give the methyl ester-terminated dendron (Dm, m = 0.5). PAMAM dendron (Dm, m = 0.5, 23.6 mmol) was dissolved in 20 mL methanol and this solution was added dropwise to a solution containing ethylenediamine (0.35 mol) and 30 mL methanol. The reaction mixture was stirred vigorously for 48 h at room temperature



Scheme 1. Illustration of the co-delivery of DOX and pDNA into cells by PZLL-D3 cationic micellar nanoparticles.