Materials Science and Engineering C 61 (2016) 269–277

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

F-127-PEI co-delivering docetaxel and TFPI-2 plasmid for nasopharyngeal cancer therapy

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ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form 5 December 2015
Accepted 21 December 2015
Available online 24 December 2015

Keywords:
TFPI-2
Docetaxel
Co-delivery
Nasopharyngeal cancer

ABSTRACT

The co-delivery of drug and gene has become the primary strategy in cancer therapy. However, to construct one safe co-delivering system with higher drug loading and gene transfection efficiency for cancer therapy is still challenging. Herein, a novel degradable nanocarriers were synthesized and characterized in this study, which was composed of polyethylenimine (PEI)-linked PEO–PPO–PEO (Pluronic F127), called F127-PEI. Then the nanocarrier was used for hydrophobic docetaxel (DOC) and functional gene (TFPI-2 plasmid) co-delivery to treat nasopharyngeal cancer (NPC). The results indicated that F127-PEI nanocarriers had higher DOC loading amount and possessed good gene delivery effect *in vitro*. For co-delivery analysis, the obtained F127-PEI/DOC/TFPI-2 complexes could induce a more significant apoptosis than DOC or TFPI-2 alone, and decreased invasive capacity of NPC HNE-1 cells more obviously. Moreover, the F127-PEI copolymer exhibited better blood compatibility and lower cytotoxicity compared to PEI-25k by the hemolysis and MTT assays, which suggests a promising potential for NPC therapy.

1. Introduction

Nasopharyngeal carcinoma (NPC) is one of the most common malignant tumors in Southern China [1]. The comprehensive treatment of radiotherapy combined with chemotherapy is the main clinical treatment strategy for NPC nowadays. However, the average 5-year survival rate of advanced NPC patients is still low due to the tumor metastasis, multi-drug resistance and so on [2]. The co-delivery of drug and gene has become the primary strategy in cancer therapy in recent years, because this technique could promote synergistic actions, improve target selectivity and deter the development of drug resistance [3].

For the co-delivery of drug and gene while maintaining their chemophysical properties and biological functions, there has been an increasing interest in the development of multifunctional polymeric carriers, and so some amphiphilic copolymers with cationic character have been prepared [4–5]. For examples, Ma et al. designed a copolymer (PP–PLLD–Arg) consisting of a porphyrin (PP) core and arginine-functionalized poly(L-lysine) dendron (PLLD–Arg) arms and used it to co-deliver docetaxel (DOC) and MMP-9 siRNA plasmid for NPC therapy [6]. We have also prepared a drug and gene co-delivering system (β–CD–PLLD/DOC/TFPI2), which showed a synergistic NPC suppression effect *in vitro* [7]. But DOC loading amount of these nanocarriers were lower and these nanocarriers showed lower gene transfection efficiency than other cationic polymers such as PEI [6–8], which limited their future applications in clinical. So, we wanted to prepare one safe co-delivering system with higher drug loading and gene transfection efficiency for cancer therapy. Poly (ethylene oxide)–poly (propylene oxide)–poly (ethylene oxide), *i.e.*, PEO–PPO–PEO (Pluronic F127) approved by FDA for *in vivo* applications has been widely investigated for its broad-range of therodiagnostic applications in biomedical and pharmaceutical sciences, We chose Pluronic F127 (PF127) as the main body of nanocarrier because it is a copolymer consisting poly (ethylene oxide)–poly (propyleneoxide)–poly (ethylene oxide) blocks, EO_{100}–PO_{65}–EO_{100}. The exterior hydrophilic PEO corona provides an antifouling character to prevent aggregation, protein adsorption, and recognition by the reticuloendothelial system (RES) [9] and the PPO segment of PF127 comprises of a hydrophobic core as a microenvironment for incorporation of hydrophobic anticancer drugs with higher drug loading due to its self-assembly characteristics [10].

PEI and its derivatives are being extensively investigated because of their "proton sponge" effect [11–12]. PEI has an abundance of positive charges that can condense DNA *via* electrostatic interaction into compact complexes that can break through various barriers to the nuclei of target cells [13]. Despite extensive applications, PEI, itself a reagent

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http://dx.doi.org/10.1016/j.msec.2015.12.049
0928-4931/

for nonviral transfection, is cytotoxic. Chemical modification of PEI is required to improve its application as a transfection reagent [14–16]. It has been shown that PEI derivatives obtained by cross-linking PEI with degradable materials have higher transfection efficiency and lower cytotoxicity [17–18]. In light of the above considerations, we wanted to synthesize a novel degradable F127-PEI nanocarrier for solving the problem of efficiency *versus* cytotoxicity.

TFPI2 is the inhibitor of serine protease, it can effectively inhibit proteolytic activity of Matrix metalloproteinases (MMPs) family and inhibit tumor invasion and metastasis [19–22]. DOC, an analog of paclitaxel, is a novel anticancer agent of the taxoid family. The drug was found to promote tubulin assembly in microtubules and to inhibit their depolymerization which can induce apoptosis of tumor cells and inhibit tumor growth, and show a strong anti-tumor effect in cancer chemotherapy [23–24].

For treating NPC, we have designed some nanocarriers such as CD-PLLD, MPEG-MWNTs and Hep-GO to load hydrophobic chemotherapy drugs such as DOC, camptothecin and doxorubicin and deliver functional gene (MMP9 siRNA plasmid) respectively, assay *in vitro* exhibited better anti-tumor effects [7,25–26].

In this paper, to enhance the drug loading amount and gene delivering ability, we attempted to prepare F127-PEI nanocarrier and use it for hydrophobic antitumor drug (DOC) and functional gene (TFPI2 plasmid) co-delivery. For the first time, this kind of material and F127-PEI/DOC/TFPI2 complexes were explored to apply in NPC therapy *in vitro*. Moreover, their biocompatibility *in vivo* was also studied.

2. Experiment section

2.1. Materials

Pluronic F-127 (EO100-PO65-EO100) was purchased from Sigma and used after drying *in vacuo* at 60 °C for 24 h. *p*-Toluenesulfonyl chloride (TsCl) was purchased from Chinese National Chemical Reagents Company. Taxotere® was purchased from Jiangsu Hengrui Medicine Co., Ltd. (Jiangsu, China). 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 TFPI2 mRNA by Invitrogen Corp (Shanghai, China). RP1640 medium, 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 RIOs (Millipore, USA). The human nasopharyngeal carcinoma HNE-1 cells were supplied by the Southern Medical University. BALB/c mice were bought from the laboratory animal center, Southern Medical University. The Institutional Administration Panel for Laboratory Animal Care approved the experimental design.

2.2. Synthesis of F127-PEI

F-127-PEI was synthesized coupled reaction between F-127-sulfanilic acid ester and PEI-25k. Firstly, F-127-sulfanilic acid ester was synthesized according to the reported method [27]. Briefly, 12.3 g (1 mmol) Pluronic F-127 was dissolved in 70 mL anhydrous methylene chloride, then 30 mL pyridine and 1.14 g (6 mmol) TsCl that were added followed. The system was reacted at room temperature for 24 h. After that, the mixture was extracted by 3 mol/L hydrochloric acid and the organic phase was washed with 10 g $NaHCO_3$. After recrystallized by THF/diethyl ether mixture solvents and dried under vacuum, F-127-sulfanilic acid ester was obtained with a yield of 90%.

The obtained F-127-sulfanilic acid ester (1.23 g) was then dissolved in 250 mL DMF mixed with PEI-25k (5.0 g) and the mixture was reacted at 60 °C for 24 h. The product was dialyzed (MWCO = 3500) in water for 3 days and frozen to dry, then F-127-PEI was obtained with a yield of 82%. Its chemical structure was characterized by ^1H NMR analysis.

2.3. DOC loading

For the loading of hydrophobic DOC, 100 mg F127-PEI was first dissolved in distilled water with a concentration of 20 mg/mL, and then 5 mL DMF containing 15 mg DOC was added dropwise to the solution. The mixture was stirred for 6 h in the dark at room temperature. After that, the sample was put into a dialysis bag (MWCO = 3000) and subjected to dialysis against distilled water for 24 h. The drug-loaded complex was obtained by filtering through a 0.45 μm filter and then lyophilized.

To determine the loading amount of DOC, the resultant F127-PEI/DOC complexes were dissolved in CH_3OH, and then analyzed by HPLC. The HPLC analysis of DOC was achieved on a C_{18} column (Waters, USA) with a mobile phase consisting of methanol and purity water (70/30, *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 [28].

2.4. TFPI2 plasmid binding

2.4.1. F127-PEI/TFPI2 formation

For the preparation of TFPI2 plasmid loaded complexes, the TFPI2 and F127-PEI 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 F127-PEI/TFPI2 complexes.

2.4.2. Gel electrophoresis

The binding ability of F127-PEI to TFPI2 was examined by gel electrophoresis. Agarose gel (1.0%, *w*/*v*) containing ethidium bromide (0.25 mg/mL, Sigma) was prepared in TAE buffer (40 mmol/L Trisacetate, 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 suite (Fisher Scientific, PA).

2.5. DOC and TFPI2 co-loaded complexes

2.5.1. F127-PEI/DOC/TFPI2 formation

For the preparation of DOC and TFPI2 co-loaded complexes, the F127-PEI/DOC micelles was firstly prepared with a required concentration, and then TFPI2 plasmid was mixed. The resultant component solutions were then stirred gently for 20 min for the formation of F127-PEI/DOC/TFPI2 complexes. Their particle sizes and zeta-potentials of complexes were determined by a Zeta Potential Analyzer instrument (ZetaPALS, Brookhaven Instruments Corporation, USA). Prior to the measurements, the complexes were incubated at 37 °C for 30 min, and diluted by 0.9 wt.% NaCl solution. The morphological examination of the complex was performed using a JEM-2010HR high-resolution transmission electron microscope (JEOL, JAPAN) after counterstained for 2 min with uranyl acetate.

2.5.2. Drug-release assay in vitro

1 mL of DOC-loaded F127-PEI/DOC/TFPI2, which was enclosed in the dialysis bag (molecular weight cut off = 2000) was immersed in 10 mL of phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.4) containing 10% Tween 80 at 37 °C. At predetermined time points, 5 mL of the medium solution was taken out, and 5 mL of fresh PBS containing Tween 80 was added back to maintain the same solution volume. The amount of released DOC was determined by HPLC (Agilent 1200, USA) analysis. The cumulative amount of released DOC was calculated from a standard calibration curve. All of the release studies were carried out in triplicate.