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Biofunctionalized polymer-lipid supported mesoporous silica nanoparticles for release of chemotherapeutics in multidrug resistant cancer cells

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ABSTRACT

Multidrug resistance (MDR) is a major impediment to the success of cancer chemotherapy. A polymer-lipid supported mesoporous silica nanoparticle (PLS-MSNs) is described here to facilitate intracellular delivery of anticancer drug and enhance the antitumor efficacy against MDR breast cancer cells. By coating MSNs with a synthetic dual-functional polymer-lipid material P123-DOPE, the supported membrane acted as an intact barrier against the escape of encapsulated drugs before reaching the target cells, leading to depolymerization and triggered storm release of loaded irinotecan (CPT-11) in acidic endosomal pH of tumor cells. In addition, P123-DOPE can inhibit breast cancer resistance protein (BCPR) mediated CPT-11 efflux in drug resistant MCF-7/BCRP breast cancer cells, thus acting as a "door blocker". Compared to free CPT-11, PLS-MSNs resulted in a maximum increase in the intracellular CPT-11 concentration (12.9-fold), had 7.1-fold higher cytotoxicity and processed a stronger cell cycle arrest in MCF-7/BCRP cells. Moreover, CPT-11 loaded PLS-MSNs showed high therapeutic performance and low toxicity in BALB/c nude mice bearing drug resistant breast tumors, with an inhibition rate of 81.2% compared to free CPT-11 treatment group. The reported PLS-MSNs provide promising applicability in future preclinical and clinical MDR cancer treatment.

1. Introduction

Multidrug resistance (MDR) has been one of the biggest impediments to the success of cancer chemotherapy and interest is growing in the development of drug delivery systems using nanotechnology to reverse MDR in cancer [1–3]. As one of the most important mechanisms involved in MDR cells, breast cancer resistance protein (BCRP) is capable of effluxing a broad range of anticancer agents, and is over-expressed in breast cancer, leukemia, pancreatic cancer and hepatocellular carcinoma patients [4–8]. Considerable efforts have been devoted to the development of specific small molecules that could potentially inhibit the expression and function of BCRP to increase the intracellular drug levels for the apoptosis of MDR cancer cells [9,10]. However, very little success has been achieved in the approval of small molecule inhibitors for clinic use mainly due to their nonspecific side effects [11]. Therefore, the exploitation of more effective cancer treatment strategies is of great importance in overcoming MDR.

Drug delivery system based on nanoparticles, such as liposomes, lipid nanocapsules, polymer nanoparticles, micelles and mesoporous silica nanoparticles (MSNs) have emerged as innovative and promising protocols for cancer therapeutics [12–15]. It has been reported that nanoparticles can bypass the efflux pump transport and accumulate in cells by passive or active targeting [16,17]. Thus, by applying the nanocarriers, therapeutic drugs can be delivered into MDR cells to increase intracellular drug levels to the concentrations required for induction of cytotoxicity [18,19]. However, several technical difficulties remain to be solved. For example, intrinsic instability of liposomes and other lipid-encapsulated nanoplatforms make them vulnerable to unexpected leakage or premature drug release *in vivo* before reaching the target site, thereby causing systemic toxicity [20]. Additionally, drugs released into the cytoplasm from the nanoparticles would inevitably expose the MDR transporter again, and will also be pumped out of the cells by efflux transporter. These still remain as challenges in MDR treatment.

Recent reports on the design of capped and gated MSN-based systems have shown promise in preventing premature release of

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drug and controlled release of guest molecules from MSNs. For example, inorganic nanoparticles, polymers, and larger supramolecular assemblies have been used as caps to control the opening/closing of MSNs pore entrances [21–23]. Various stimuli, such as temperature, photoirradiation, enzyme and pH have been applied as triggers to release encapsulated drugs [24–27]. Despite these escalating achievements, it still remains a challenge to design a drug delivery system, which can trigger the release of encapsulated drugs in tumor cells and protect the released drugs from the MDR transporter pump effects.

Herein, we introduce a polymer-lipid combined layer coated MSNs (PLS-MSNs) to enhance the antitumor efficacy against MDR cancer cells. Functional polymer-lipid coating material called Pluronic P123 grafted 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (P123-DOPE) was synthesized by combining the drug efflux transporter inhibitor Pluronic block copolymers with pH sensitive phospholipid DOPE. The Pluronic block copolymers are effective inhibitors of P-glycoprotein (P-gp), multidrug resistance protein (MRP), and BCRP, showing the chemosensitization effects in MDR tumors [28–32]. The nanocarrier PLS-MSNs are expected to retain and protect guest molecules before reaching the target cells, and the coated layer can readily disrupt to release antitumor drugs after uptake by cells, thus minimizing toxicity and maximizing effectiveness of drugs. Moreover, functional material P123-DOPE in the coated layer can act as a "door blocker" and prevent drugs from being pumped out of MDR cancer cells (Scheme 1). In this study, chemotherapeutic agent irinotecan (CPT-11), which is a typical BCRP substrate [33], was encapsulated in PLS-MSNs and delivered into drug resistant cancer cells (MCF-7/BCRP). The *in vitro* and intracellular drug release behavior, cellular uptake efficiency, cytotoxicity, BCRP and ATP level in MCF-7/BCRP cells after treatment were extensively evaluated. Antitumor activity in BALB/c nude mice MCF-7/BCRP drug resistance tumor xenograft model was investigated to clarify the biological roles of PLS-MSN in reversion of MDR.

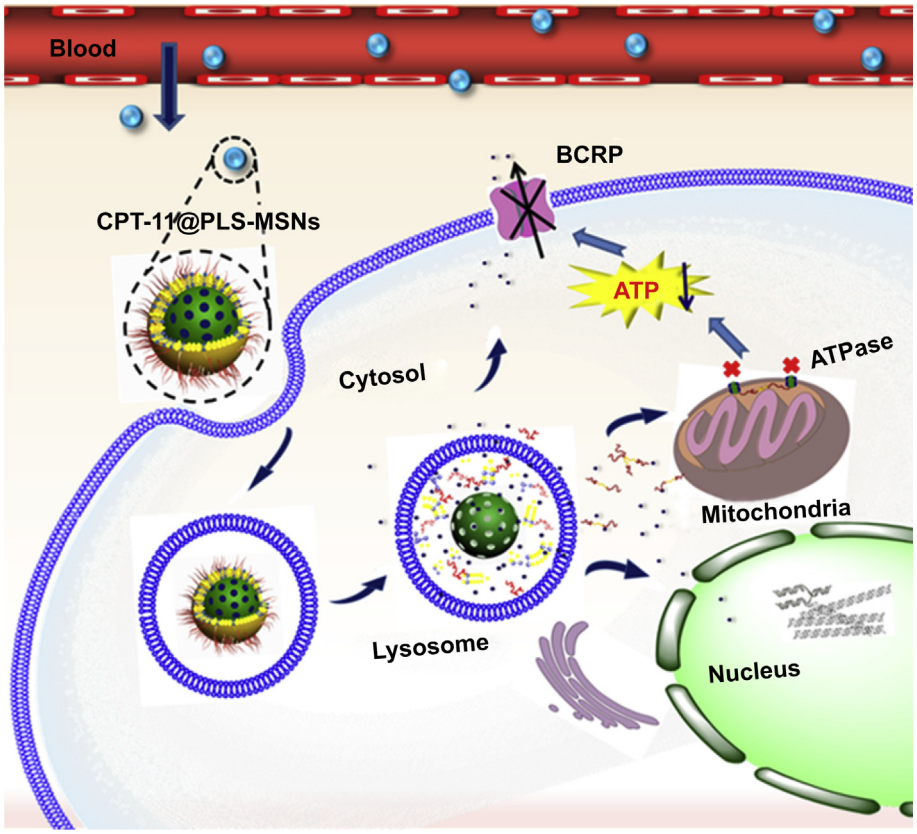
2. Materials and methods

2.1. Materials

Irinotecan hydrochloride (CPT-11, 99.8%) was obtained from Knowshine Pharmachemicals Inc. (Shanghai, China). Tetraethylorthosilicate (TEOS, 98%), cetyltrimethylammonium bromide (CTAB, >99%) and Coomassie brilliant blue G250 for the Bradford protein assay were purchased from Aladdin-chemistry, Co., Ltd (Shanghai, China). 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) were obtained from Q.P. corporation (Tokyo, Japan). Pluronic P123, N,N′-disuccinimidyl carbonate (DSC), pyrene, Lyso Tracker Green DND-26, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DNA-free RNAase and propidium iodide (PI) were from Sigma Aldrich (St. Louis, MO, USA). The ATP assay kit was purchased from Beyotime Institute of Biotechnology (Shanghai, China). RPMI 1640 medium, fetal calf serum (FCS), penicillin/streptomycin, and 1× trypsin–EDTA solution (0.25%, trypsin with 0.53 mM EDTA) were purchased from Life technologies Co. (Grand Island, NY, USA). For all experiments and analyses, water was deionized and filtered with a 0.22 μm pore size polycarbonate syringe filter (Millipore, Billerica, MA). All chemicals were reagent grade and used without further purification or modification.

2.2. Synthesis of P123-DOPE and P123-DSPE

Pluronic P123 (5.2 g, 0.9 mmol) was dissolved in acetonitrile (2 mL) and treated with DSC (0.23 g, 0.9 mmol) and pyridine (0.36 mL, 4.55 mmol) overnight to synthesis P123-SC. The product was precipitated and recrystallized with isopropanol at −20 °C, dried in vacuo. P123-DOPE or P123-DSPE was prepared by adding DOPE



Scheme 1. Schematic illustration of the cooperation between responsive intracellular storm release of CPT-11 and the ability to overcome drug resistance by blocking the efflux transporter. After injection of CPT-11@PLS-MSNs, the nanoparticles accumulated at the tumor site through enhanced permeability and retention effect in tumor blood vessels. The supported membrane leads to ready depolymerization and triggered storm release antitumor drug after internalization into tumor cells. P123-DOPE localized within the mitochondria and acted as a "door blocker" to deplete ATP and inhibit BCPR mediated drug efflux.