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Assembling of stimuli-responsive tumor targeting polypyrrole nanotubes drug carrier system for controlled release

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ABSTRACT

A stimuli-responsive polypyrrole (PPy) nanotubes drug carrier system has been designed to deliver anticancer drugs to tumor cells in a targeted and controlled manner. The PPy nanotubes drug carrier was fabricated by a template method. The nanotubes surface was functionalized with cleavable acylhydrazone and disulfide bonds by attaching thiolated β-cyclodextrin (β-CD). The solubilizing poly(ethylene glycol) polymer (PEG), attached with an adamantane (Ad) entity at one end and a folate (FA) entity at the other end, was introduced onto the nanotubes surface via β-cyclodextrin-adamantane interaction. The synthesized FA-PEG-Ad-β-CD-PPy showed excellent biocompatibility and low cytotoxicity for two cell lines. Doxorubicin (Dox) loaded FA-PEG-Ad-β-CD-PPy nanotubes showed a triggered in vitro drug release behavior in the presence of acidic media and reducing agents. The folate-mediated endocytosis and intracellular release of Dox-loaded nanoparticles were confirmed by fluorescence microscopy and cell viability evaluations. In the in vitro study, Dox loaded within the nanoparticles showed enhanced selectivity for cancerous cells and reduced cytotoxicity for normal cells compared to free Dox. The PPy based targeted drug vehicle shows excellent promise for drug delivery.

1. Introduction

Cancer is a disease associated with uncontrolled proliferation and diffused invasion of tumor cells, and it is a leading cause of death in people [1]. Chemotherapy has been the main treatment option for several decades, however, conventional chemotherapeutic agents often cannot differentiate between tumor cells and normal ones, thereby providing high dose to the tumors and resulting in undesired side effects [2]. Although macromolecules and nanoparticles can selectively accumulate into the tumor vascular wall on account of leaky vasculature and impaired lymphatic, which is known as the enhanced permeability and retention (EPR) effect [3], it is also not enough to effectively fight cancer cells. Therefore, it is essential to develop active targeting and controlled release nano-sized drug carriers to provide precise drug targeting and release at tumor sites [4].

On the one hand, folate is an essential component of cell metabolism, DNA synthesis and repair, therefore the rapidly growing tumor cells need enough folate to maintain DNA synthesis. For this reason, folate receptors (FR), a function of binding folate, are overexpressed on many tumor cells, especially on metastatic tumor cell membranes, while they are expressed in low amounts on normal cell membranes [5–7]. On account of this, folate-based conjugates typically show high binding affinities for FR [8,9]. Therefore, FA grafted nanoparticles can target tumor tissues by the high affinity ligand-receptor interactions.

On the other hand, a general route to develop multifunctional drug delivery is urgent. Over the past years, a number of organic/inorganic nanocarriers have been developed for loading drugs with release manners of temperature [10], pH change [11], and redox[12]. One of the heavily investigated strategies depends on temperature. Most temperature-sensitive drug carriers are based on diblock [13,14], triblock [15] and multiblock [16] copolymer consisting of hydrophilic segment and hydrophobic segment with temperature responsiveness. Then, the micellization with hydrophobic cores and hydrophilic coronas is constructed by the aid of aqueous solubilization of hydrophobic compounds [17], and drugs is inclined to enrich into the hydrophobic core. These micelles should be very stable under a low critical micelle concentration and can release the contents held in the core when exposed to thermal stimulus. Moreover, multifunctional platforms with the combination of dual-respond have attracted tremendous attention. Among the various types of dual-responsive systems, pH- and thermo-responsive cancer drug delivery has been developed rapidly due to the acidic nature of tumor cell and noninvasive infrared irradiation. Thus, a series of pH/thermo-sensitvie copolymers drug delivery, including a thermo-responsive section and a pH-responsive moiety, have been intensively investigated [18–20]. More efforts on tri-responsive nanocarriers are also gaining increasing attention [17,21].

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In addition, nanoparticles have been widely used in drug delivery applications due to their large surface-to-volume ratio and simple preparation. In particular, hollow nanotubes have received considerable attention due to their ability to accommodate guest molecules in their distinctive inner hollow space, as well as ease of chemical modification of their open orifice and internal/external surface [22,23]. Silicon nanotubes [24], carbon nanotubes [25], and peptide nanotubes [26] are some of the most active research areas in the field of nanotubes biomaterials. Similarly, polypyrrole, a compound initially isolated from bone oil, is extensively used for several biomedical applications such as stimulating nerve growth, revival of bone cells and DNA adsorption due to its excellent biocompatibility [27]. PPy nanoparticles have been employed as drug carriers, however, PPy nanotubes have not yet been reported due to the challenges to transforming spherical dimension into tubular dimensional. Therefore, a versatile and simple method needs to be developed to prepare the PPy nanotubes. Common methods including electrospinning, self-assembly, coprecipitation and others have been proposed to the large scale synthesize of nanofibers instead of nanotubes [28]. Among the different nanotubes preparation methods, a simple approach to fabricate polymer nanotubes by template method has been proposed based on careful control of the size, shape and composition. Therefore, the PPy nanotubes can likely be achieved by the template method, and function as a promising and versatile drug carrier candidate due to its ability to fit other nanoparticles into its hollow cavity [29]. Moreover, pure PPy nanotubes cannot be multifunctional through chemical grafting methods with rigidity in the molecular chain and high levels of conjugation. The amino, carboxyl and halohydrocarbon groups have been selectively introduced onto PPy surface to improve its functionality [30]. Despite these promising benefits, there are problems with available stealth delivery, active drug release and favorable crossing of biobarriers. Polyethylene glycol (PEG) is commonly used as a solubilizing agent to improve the bioavailability and stability of drug carriers [31]. Therefore, nanoparticles are coupled with PEG to form the hydrophilic shell to stealth transport. Beyond these basic features of nanoparticle design, a suitable approach to control drug release by means of a molecular switch is also required. Correspondingly, the extracellular environment is significantly acidic (pH ≤ 6.5) in tumor tissues, while the pH of endosomes and lysosomes in cancer cells can be as low as 4.5–5.5, and slightly alkaline in blood and normal tissues (pH 7.4). Schiff bases are generally quite stable in alkaline medium, but can be degraded in acidic medium (pH ≤ 6.5) [32]. Therefore, a Schiff base can serve as a sensitive pH-responsive linker with the advantages of mild reaction and high yield [33,34]. In addition, nanoparticles with multiple responsive release have also been developed by combining two or more stimuli responses to multicomponent formulation [35]. The concentration of reducing glutathione (GSH) is higher (1–10 mM) in intracellular environment than in extracellular environment (20–40 μM) and blood plasma (0.002–0.2 mM) [36,37]. More importantly, the intracellular GSH concentration in tumor cells is higher than that in normal cells. The disulfide bond can be formed by the oxidation (with O_2 or H_2O_2) of two thiol groups, while it can be cleaved under reducing conditions and stable in normal physiological conditions [38]. Therefore, the Schiff base can be used in combination with disulfide bond to enable a synergistic effect. To achieve efficient drug entrapment, β-CD is a suitable choice, as it has hydrophobic internal cavity and hydrophilic external surface [39]. Furthermore, β-CD possesses cyclic oligosaccharide which can form inclusion complexes with guest molecules, providing a more convenient approach to assemble the polymer nanotubes [40].

In this study, a novel stimuli-responsive polymer nanotubes drug carrier system was fabricated to achieve tumor targeting and stimuli-responsive drug release based on modular approach. As illustrated in Fig. 1, Dox was loaded in the PPy nanotubes scaffold, whose surface was linked with β-CD through disulfide bond and acylhydrazone bond to form the domain scaffold. The PEG and FA molecules were further incorporated into the main unit. Once all the functional domains were integrated into a single nanoassembly system, the PEG protection layer of nanoparticles can improve stability and dispersibility in aqueous medium. The FA moiety acts as a guidance system to target specific cancer cells through ligand-receptor affinity. The drug could be released quickly from the nanotubes upon removal of the β-CD gatekeeper due to the cleavage of acylhydrazone linker and disulfide linker in the presence of acidic pH and high GSH concentration inside tumor cells. In brief, this multicomponent formulation was prepared involving appropriate synthesis procedures.

2. Experimental methods

2.1. Materials

The following chemicals and reagents were purchased from Energy Chemical Co. (Shanghai, China) and used as received: Pyrrole, 1,2-dichloroethane, NH4F, ethylene glycol, $FeCl_3$, Polyethylene glycol (PEG, Mw = 4 kD), β-cyclodextrin,p-toluenesulfonyl chloride,triethylamine, potassium phthalimide, hydrazine hydrate, tetrabutylammonium bromide (TBAB), methyl 3-mercaptopropionate, 1-(3-(dimethylamino) propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), 1-adamantane carboxylic acid, folate, triphenyl phosphine (Ph_3P), sodium methoxide, iodine, thiourea, 4-hydroxybenzaldehyde, and dithiothreitol (DTT). Doxorubicin hydrochloride (Dox•HCl) was purchased from Maya Reagent (China). Nanopure water (Millipore, USA) was used in all experiments.

2.2. Charaterizations

^{1}H NMR spectra were recorded on Ascend 400 M and 500 M spectrometers (Bruker, Germany) using deuterated solvents. UV–Vis measurements were performed on UV 2450 spectrometer (Shimadzu, Japan). Infrared absorption spectra were recorded by a FTIR spectrometer (Nicolet, USA) using KBr-pellet method. Thermogravimetry analysis (TGA) was performed by using a Perkin-Elmer TGA-7 recorded from 100 to 900 °C with a heating rate of 10 °C/min under nitrogen. High-resolution transmission electron microscope (HR-TEM) images were obtained on JEOL 2100 F (JEOL, Japan) with an accelerating voltage of 200 kV. The TEM samples were prepared by pipetting a drop of nanotubes ethanol suspension on a super-thin carbon film. The hydrodynamic diameters and zeta potential were measured by Zetasizer Nano ZS (Malvern, UK).

2.3. Preparation of PPy nanotubes

The TiO_2 nanotubes array template [41] was soaked into 0.2 M $FeCl_3$ for 36 h in order for Fe^{3+} to adsorb into the inner pores of the TiO_2 template. Then, the template was placed in a vacuum drying oven and dried at 60 °C for 24 h. Then, the pyrrole monomer was added into the sealed reaction vessel at the ratio of 150 μl for each TiO_2 template. The reaction vessel was placed under vacuum to reach 0.01 Pa and heated to 180 °C for 1.5 h, namely chemical vapor deposition (CVD). After polymerization of the pyrrole molecules, PPy nanotubes were obtained by etching the TiO_2 nanotubes array template with 5 wt% HF solution, isolating by centrifugation and washing with ethanol several times to remove HF thoroughly. The other two N-halohydrocarbon monomers, 1-(2-chloroethyl)pyrrole (Py-Cl) and 1-(3-bromopropyl) pyrrole (Py-Br) [42,43](Supporting information), were polymerized and prepared into nanotubes in the same manner (Fig. 2).

2.4. The assembly of FA-PEG-Ad-SS-C=N-PPy

2.4.1. Synthesis of PPy-ArCHO

The assembly strategy of FA-PEG-Ad-SS-C=N-PPy is illustrated in Fig. 3. PPy-Cl (0.2 g) was dispersed in anhydrous DMF (10 ml). Then 4-hydroxybenzaldehyde (0.305 g, 2.5 mmol), K_2CO_3 (0.518 g, 3.75 mmol)