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Fabrication of modular multifunctional delivery for antitumor drugs based on host–guest recognition

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ABSTRACT

Herein, learning from the idea of the modular concept widely used in ship building, as a design approach that assembles some subdivided smaller modules to a specific ship, a new modular multifunctional drug delivery (MMDD) with excellent biocompatibility was directly prepared by a flexible host–guest interaction between pH-sensitive benzimidazole-graft-dextran (Dex-BM) and pre-synthesized multifunctional cyclodextrins. In this drug system, pH-sensitive Dex-BM acted as the main case and pre-synthesized multifunctional cyclodextrins were the changeable modules. To verify the feasibility of MMDD in cancer chemotherapy, doxorubicin (DOX) was used as a model drug. *In vitro* drug release experiments indicated that the drug released around 80% from DOX-loaded MMDD at pH 5.3, while approximately 40% of DOX released under the condition of pH 7.4. Moreover, the targeting antitumor activity of DOX-loaded MMDD was investigated in HeLa and HepG2 cells using MTT assays, confocal laser scanning microscopy and flow cytometer, which indicated that the targeted DOX-loaded MMDD provided an efficient drug delivery platform for inhibition of different cancer cells. Meantime, the incorporation of different functional modules into one system was also investigated, simultaneously exhibiting targeting and imaging property. These features suggest that this modular multifunctional drug delivery system can efficiently enhance the inhibition of cellular proliferation *in vitro*, and according to the needs in clinical treatment, some targeting and imaging molecules can be chosen.

1. Introduction

Nanoparticle-based drug delivery systems have been widely developed to improve treatment efficacy and reduce toxic or side effects [1]. These clinically approved nanoparticles for cancer treatments have consistently exhibited achievement in reducing drug toxicity, but their application has always improved clinical efficiency slightly [2]. To overcome this problem, "multifunctional" nanoparticles have already attracted significant interest, to which additional capabilities like targeting and image contrast enhancement are added [3]. However, the additional functionality means complex synthetic steps and high costs. The trade-off between additional functionality and complexity is also a subject of ongoing debate [4].

Recently, many scientists have realized that it is difficult for the mono-functional materials to meet multiple requirements in clinics [5]. Therefore, it is greatly necessary to discover the materials combined with multi-functions [6]. Yet, the application of many multifunctional materials has been limited owing to their complex structures or instabilities. How to fabricate multifunctional materials with simple ways is still regarded as a considerate problem. Zhang and coworkers [7] have designed a novel light-responsive "plug and play" (PnP) polyanionic template as drug delivery system driven by the photo-switchable host–guest interactions between α-cyclodextrin and azobenzene. Functional moieties such as target ligands can be simultaneously loaded and unloaded onto the template using UV irradiation. The PnP template system designed here provides a new enlightenment for designing multifunctional antitumor drug delivery, especially the introduction of supramolecular interactions [8]. Compared with the conventional covalent nanocarriers [9], the nanocarriers based on non-covalent interactions have attracted extensive attention because of their convenient synthesis procedure [10]. The supramolecular interactions [11], especially the host–guest recognition have turned to be more and more significant in drug delivery [12]. Cyclodextrins (CDs) belong to the most important and promising macrocyclic hosts in drug delivery [13], since they are commercially available water-soluble natural products with inexpensive, non-toxic, readily functionalized properties [14]. They

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are able to recognize the thickness, polarity, and chirality of monomeric and polymeric guest molecules [15]. In the past few years, more and more nanocarriers based on host–guest [16] recognition of CDs were reported [17], in which several guest molecules such as azobenzene [18], cholesterol [19], lithocholic acid [20], ferrocene [21] and benzimidazole (BM) [22] were chosen to build drug delivery *via* host–guest complexation [23] with cyclodextrins.

Life often gives us some enlightenment in a scientific research. In order to solve the above mentioned problems of designing multifunctional antitumor drug delivery, we fabricated a new modular multifunctional antitumor drug delivery by host–guest interaction, learning from the idea of the modular concept widely used in ship manufacturing. Modular designing, is a design approach that assembles some subdivided smaller modules to a ship met with a specific requirement. In our previous research, the pH-sensitive interaction between benzimidazole and cyclodextrins at different pH values had been reported [24]. Herein, a series of subdivided multifunctional modules such as targeting module and imaging module were firstly pre-synthesized, and then proper modules were selected and combined to fabricate different delivery systems meeting with different requirements (as shown in Scheme 1). Typically, Dex-BM was synthesized as the pH-sensitive main case, and the pre-synthesized multifunctional cyclodextrins such as cyclodextrins functionalized by folic acid (CD-FA), lactose (CD-LA) and fluorescein isothiocyanate (CD-FITC) acted as changeable modules. For different tumor therapeutic applications, different modules can be chosen and inserted in the main case to fabricate a modular multifunctional drug delivery (MMDD). This highly flexible and multifunctional MMDD with its significant anticancer efficacy toward targeted tumor cells and simple synthesis procedures would provide a new view for designing multifunctional antitumor drug delivery.

2. Experimental section

2.1. Materials

Dextran (Dex, M_n = 40 kDa) was obtained from Sigma–Aldrich. 5-Benzimidazolecarboxylic acid (96%), benzoic acid (99.5%), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl), 4-dimethylaminopyridine (DMAP), *N*-hydroxysuccinimide (NHS), fluorescein isothiocyanate (FITC), folic acid (FA), lactobionic acid (LA), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and polyethylenimine (PEI_{25K}) were purchased from Sigma–Aldrich. NH_2-β-cyclodextrin (98.5%) was bought from Shandong Binzhou Zhiyuan Bio-Technology Co., Ltd. Doxorubicin hydrochloride (DOX·HCl) was purchased from Zhejing Hisun Pharmaceutical Co., Ltd. Dimethyl sulfoxide (DMSO) was dried over calcium hydride (CaH_2) and purified by vacuum distillation with CaH_2. All other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as obtained.

2.2. Characterizations

^{1}H NMR spectrum was recorded on a Bruker AV 400 NMR spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6). FT-IR spectra were recorded on a Bio-Rad Win-IR instrument by using the potassium bromide (KBr) method. Dynamic laser scattering (DLS) measurements were performed on a WyattQELS instrument with a vertically polarized He–Ne laser (DAWN EOS, Wyatt Technology). The scattering angle was fixed at 90°.

2.3. Synthesis of dextran-graft-benzimidazole (Dex-BM)

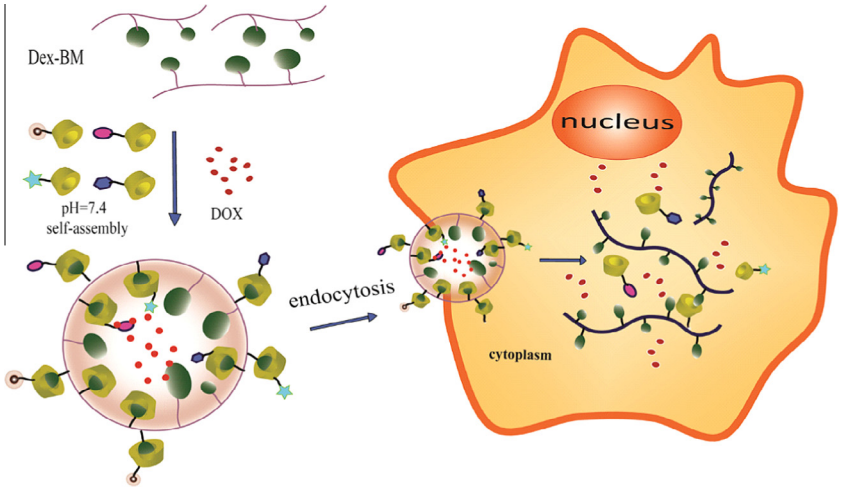
Dex (1 g, 0.025 mmol), 5-benzimidazolecarboxylic acid (0.7 g, 4.32 mmol), EDC·HCl (1.65 g, 8.6 mmol) and DMAP (0.112 g, 0.92 mmol) were dissolved in 40 mL of DMSO in a flask with a magnetic bar. The reaction was allowed to proceed at 20 °C for 72 h. After the reaction completed, the reaction medium was dialyzed (MWCO 7 kDa) for 3 days and the product was collected by lyophilization (Yield: 85%).

2.4. Synthesis of dextran-graft-benzoic acid (Dex-BA)

Dex (1 g, 0.025 mmol), benzoic acid (0.52 g, 4.26 mmol), EDC·HCl (1.52 g, 7.96 mmol) and DMAP (0.07 g, 0.58 mmol) were dissolved in 40 mL of DMSO in a flask with a magnetic bar. The reaction was performed at 20 °C for 72 h. After the reaction completed, the reaction medium was dialyzed (MWCO 7 kDa) for 3 days and the product was collected by lyophilization (Yield: 81%).

2.5. Preparation of folic acid β-cyclodextrin (CD-FA) and lactose β-cyclodextrin (CD-LA)

Functionalized β-cyclodextrin with folic acid (CD-FA) and functionalized β-cyclodextrin with lactose (CD-LA) were synthesized as follows. Folic acid (0.15 g, 0.34 mmol), EDC (0.13 g, 0.68 mmol) and NHS (0.0783 g, 0.68 mmol) was dissolved into 40 mL of DMF under



Scheme 1. Schematic illustration of modular multifunctional delivery systems.

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