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Thermoresponsive supramolecular micellar drug delivery system based on star-linear pseudo-block polymer consisting of β-cyclodextrin-poly (N-isopropylacrylamide) and adamantyl-poly(ethylene glycol)

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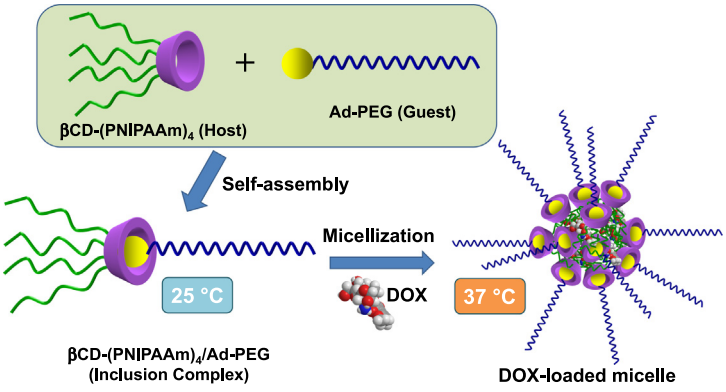
GRAPHICAL ABSTRACT

Illustration of the formation of βCD-$(PNIPAAm)_4$/Ad-PEG supramolecular pseudo-block copolymer via host-guest interactions, followed by the thermoresponsive micellization while encapsulating drug molecules into the core, forming drug-loaded micelles for drug delivery and overcoming drug resistance.



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ABSTRACT

Chemotherapy is facing several limitations such as low water solubility of anticancer drugs and multidrug resistance (MDR) in cancer cells. To overcome these limitations, a thermoresponsive micellar drug delivery system formed by a non-covalently connected supramolecular block polymer was developed. The system is based on the host-guest interaction between a well-defined β-cyclodextrin (β-CD) based poly(N-isopropylacrylamide) star host polymer and an adamantyl-containing poly(ethylene glycol) (Ad-PEG) guest polymer. The structures of the host and guest polymers were characterized by ^{1}H and ^{13}C NMR, GPC and FTIR. Subsequently, they formed a pseudo-block copolymer via inclusion complexation between β-CD core and adamantyl-moiety, which was confirmed by 2D NMR. The thermoresponsive micellization of the copolymer was investigated by UV–vis spectroscopy, DLS and TEM. At 37 °C, the copolymer at a concentration of 0.2 mg/mL in PBS formed micelles with a hydrodynamic diameter of ca. 282 nm. The anticancer drug, doxorubicin (DOX), was successfully loaded into the core of the micelles with a loading level of 6% and loading efficiency of 17%. The blank polymer micelles showed good biocompatibility in cell cytotoxicity studies.

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Moreover, the DOX-loaded micelles demonstrated superior therapeutic effects in AT3B-1-N (MDR−) and AT3B-1 (MDR+) cell lines as compared to free DOX control, overcoming MDR in cancer cells.

1. Introduction

Cancer remains one of the leading causes of death worldwide, with over 10 million new cases each year [1]. Chemotherapy, currently the most effective treatment for metastatic cancers, is still impeded by several limitations, such as low water solubility of the anticancer drugs and multidrug resistance (MDR) in cancer cells [2,3]. MDR is the ability of the cancer cells to become resistant to different drugs simultaneously. Therefore, there is also an urgent need to reverse or circumvent the multidrug resistance to improve chemotherapy [3,4].

One strategy of MDR reversal is to encapsulate anticancer drugs in carriers that deliver them to cancer cells. Polymeric micelles with dense cores of hydrophobic blocks surrounded by diffuse hydrophilic shells (coronas) have attracted much interest and various systems based on polymeric micelles have been developed to deliver drugs such as doxorubicin (DOX) [5,6]. The hydrophobic core is able to interact physically or chemically with therapeutic drugs [7] while the hydrophilic shell interacts with the solvent, providing stability in aqueous solutions [8]. Recently, there has been increasing interest to form micelles by using non-conventional method [9,10]. These "non-covalently connected micelles (NCCMs)" were constructed by physical interactions, such as host-guest inclusion complexations [11] between the core and the shell, instead of chemical bonds in conventional micelles. One example is the formation of pseudo-block copolymer based on the host-guest inclusion complexation between β-cyclodextrin-based polymers and adamantyl-containing polymers [11]. The NCCMs that are constructed by physical interactions are advantageous as it allows separate modifications and easy combinations of various components. It makes it easier to tune and optimize the systems' properties and to screen for suitable formulations for the desired applications.

Cyclodextrins (CDs) are a series of cyclic oligosaccharides that are composed of 6, 7, or 8 D(+)-glucose units linked by α-1,4-linkages, named α-, β-, or γ-CD, respectively [12,13]. CDs are popularly known to have a hydrophobic interior and hydrophilic exterior. As they are able to form supramolecular inclusion complexes by fitting various molecules (guests) into their cavities (host), CDs have been widely applied in pharmacy and biomedical engineering [12,14–19]. One extensively studied complexation is between adamantyl (Ad) derivatives and β-CD [20–24]. They form very strong inclusion complexes, with association constant $K_a \approx 5 \times 10^4\ M^{-1}$ at room temperature, due to the close fit of Ad into β-CD cavity [25].

Recently, stimuli-responsive polymers have become very attractive and used to form micelles or other drug delivery systems that are sensitive to environmental changes [26]. Poly(N-isopropylacrylamide) (PNIPAAm) is one of the most popular and extensively-studied thermoresponsive polymers. It has a lower critical solution temperature (LCST) at 32 °C in water [27]. PNIPAAm is water soluble below its LCST, and undergoes a reversible, abrupt coil-to-globule phase transition above its LCST [28–30]. Because of this unique property, PNIPAAm and its copolymers have been studied and analysed as "intelligent" systems for their potential in biomedical applications, such as for drug delivery, biosensor, bioaffinity separation and cell and enzyme immobilization [31–34]. There has been increasing interest and studies to take advantage of the thermoresponsive properties of PNIPAAm to develop nanocarriers for drug delivery applications. Among them, one of the most popular nanocarriers studied is the micelles formed by copolymers containing poly(ethylene glycol) (PEG) and PNIPAAm for drug delivery [35–37]. PEG serves as the hydrophilic shell for stabilizing the micelle while PNIPAAm functions as the hydrophobic core for drug loading.

Herein, we report a thermoresponsive NCCMs consisting of a hydrophobic PNIPAAm core and hydrophilic PEG corona for drug delivery applications. A well-defined β-CD-based PNIPAAm star host polymer with a degree of polymerization of 21 per arm (βCD-$(N_{21})_4$) and adamantyl-containing poly(ethylene glycol) (Ad-PEG) guest polymer were synthesized. They formed pseudo-block copolymers via inclusion complexation between β-CD core of the host and adamantyl-moiety of the guest. The temperature-induced micellization and its ability for drug loading were extensively investigated. The NCCMs are expected to effectively deliver the anticancer drug doxorubicin (DOX) to cancer cells. The enhanced therapeutic effects and effectiveness in overcoming MDR by the NCCMs were also evaluated in cell cytotoxicity studies.

2. Experimental section

2.1. Materials

β-Cyclodextrin (β-CD, ⩾98.0%) was purchased from Tokyo Chemical Industry Co. Ltd (TCI) and dried at 100 °C for 24 h under vacuum before use. N-Isopropylacrylamide (NIPAAm, >98.0%, TCI) was purified by recrystallization from hexane and dried at 100 °C under vacuum before use. Copper(I) bromide (CuBr, ⩾98.0%), 2-Bromo-isobutyric bromide (98.0%), and tris[2-(dimethylamino)ethyl]amine (Me_6-TREN, 97%) were purchased from Sigma-Aldrich. 1-adamantaneacetic acid (98%), Poly(ethylene glycol) with average molecular weight of 8000 Da (PEG-8k) were purchased from Sigma-Aldrich. 4-Dimethylaminopyridine (DMAP, >99.0%) and N, N′-Dicyclohexylcarbodiimide (DCC, >98.0%) were purchased from TCI. Doxorubicin hydrochloride (DOX·HCl, 98.4%) was purchased from Pharmacia and Upjohn. Tetrahydrofuran (THF, >99%) and *n*-hexane (95%) were purchased from Tedia and diethyl ether (⩾99.5%) was purchased from QReC. DMSO-d_6 and D_2O used as solvents in the NMR measurements were obtained from Aldrich. All other reagents were used as received without further purification.

2.2. Synthesis of β-CD-PNIPAAm star polymer (βCD-$(PNIPAAm)_4$) via ATRP method

β-CD based macroinitiator with four Br initiation sites (βCD-4Br) and β-CD-PNIPAAm 4-arm star polymer (βCD-$(PNIPAAm)_4$) were synthesized according to our previous report [11,38]. The feed ratio of [NIPAAm]:[CD-Br]:[CuBr]:[Me_6-TREN] was increased to 45:1:1:1.5 to obtain star polymer with higher degree of polymerization per arm, where [NIPAAm], [CD-Br], [CuBr] and [Me_6-TREN] represented concentrations of NIPAAm monomers, initiation sites of the macroinitiator, CuBr and Me_6-TREN, respectively. In brief, macroinitiator βCD-4Br (69 mg, 0.04 mmol) and NIPAAm (815 mg, 7.20 mmol) were added into a dry flask. The flask was sealed with a septum. Me_6-TREN (55 mg, 0.24 mmol) in 2 mL methanol was added with a syringe. The reaction mixture was stirred at room temperature and purged with dry nitrogen for 50 min