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Entirely Oligosaccharide–based Supramolecular Amphiphiles Constructed via Host–guest Interactions as Efficient Drug Delivery Platforms

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Entirely oligosaccharide-based supramolecular amphiphiles were constructed via host-guest interactions between ferrocene-terminated acetylated-maltoheptaose (Fc-AcMH) and β-cyclodextrin-terminated four-arm star maltoheptaose (MH_4-β-CD). The amphiphiles could self-assemble to form spherical supramolecular nanoparticles to provide efficient drug delivery platforms. The combination of a pH-sensitive covalent acetal group and the oxidation-sensitive noncovalent host-guest interaction of β-CD and ferrocene provided the obtained fully oligosaccharide-based supramolecular amphiphiles. The structures of these amphiphiles could respond to the intracellular microenvironment.

The biocompatibility and stability of drug-carriers is critical for their utility in living organisms. Drug-carriers with excellent biocompatibility requires incorporating biocompatible materials such as lipids, proteins, and polysaccharoses.[1] Polysaccharides are composed of repeating monosaccharide blocks connectted through glycogen bonds. Polysaccharides can be easily decorated to provide a number of derivatives due to diverse groups on the main backbone. These compounds exhibit well-documented biocompatibility, biodegradability and safety, which are fundamental requirements for medical biomaterials. In addition, the preparation of polysaccharides is simple and widely applied. The generation of synthetic materials from polysaccharides for medical applications, and particularly for drug delivery systems, has drawn considerable attention. Dextran is a widely used polysaccharose in materials, such as those used to construct drug carriers, because it is an analogue of glucose and has distinct features, including broad applicability and nontoxicity.[2] Furthermore, dextran modified with acetals is commonly used to fabricate pH-sensitive drug delivery systems.[3] Maltoheptaose (MH) is an oligosaccharide comprising seven R-1,4-linked glucopyranosyl units and is widely used as a commercially available oligosaccharide. Importantly, maltoheptaose has a well-defined structure and a monod isperse molecular weight, and therefore endows copolymers with a sequential shape.[4] Furthermore, maltoheptaose is easy to prepare and the starting materials are readily available .

Scientists have recently considered supramolecular amphiphiles formed by noncovalent driving forces as novel building blocks for constructing drug delivery systems.[5] In contrast to conventional amphiphiles, supramolecular amphiphiles allow facile fabrication of numerous complex structures and increase the diversity of self-assembled nanostructures,[6] due to the ease of noncovalently attaching various functional groups, thereby avoiding the laborious methods of traditional chemical synthesis.[7] In addition, the structural versatility of noncovalent interactions makes supramolecular amphiphiles stimulus-responsive. These compounds provide an opportunity to combine colloidal and supramolecular chemistry .[8] The application of self-assembled amphiphiles in many research fields is increasing rapidly, for example, as building units to construct organic nanofibers or nanotubes for electrical and medical devices,[9] and for use in drug delivery systems.[10]

To our knowledge, there has been no report of a drug delivery platform based on multi-arm star supramolecular micelle constructed of maltoheptaoses. In the present work, we report a novel class of nanoparticles capable of efficient anticancer drug delivery. These nanoparticles are based on a multi-armed star maltoheptaose and a linear acetylated maltoheptaose which form non-covalently linked block copolymers. The chemical process for fabricating such nanoparticles is thoroughly described in the experimental section and summarized schematically in Scheme 1. The β-CD was modified with maltoheptaoses to form MH_4-β-CD as the hydrophilic block. Ferrocene-terminated acetylated

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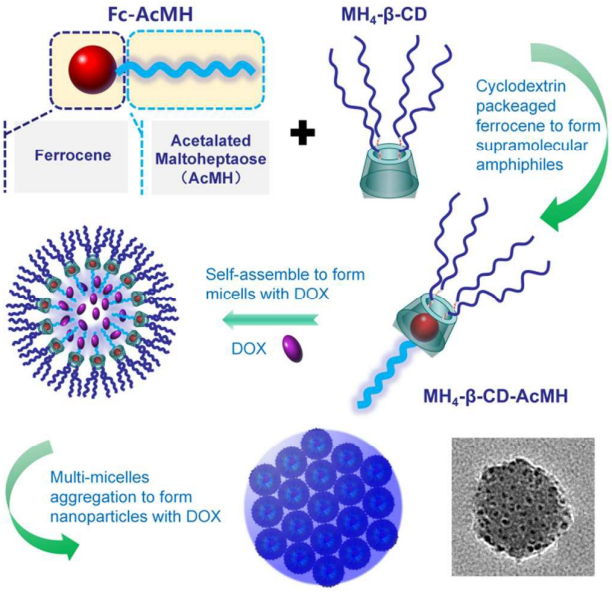
maltoheptaose (Fc-AcMH) was used to fabricate the hydrophobic block. Linkage of the pH-sensitive covalent acetal group and the oxidation-sensitive noncovalent host-guest interaction of β-CD and ferrocene provided completely oligosaccharide-based supramolecular amphiphiles that respond to the intracellular microenvironment. The supramolecular amphiphiles disassembled and released the encapsulated drug, showing promise as a cancer treatment. Moreover, the disassociated supramolecular amphiphiles are nontoxic.



Scheme 1. Schematic illustration of self-assembly of DOX-loaded MH_4-β-CD-AcMH nanoparticles.

The MH_4-β-CD-AcMH supramolecular amphiphile was obtained through a multi-step synthesis process, as shown in Scheme S1-S3, and the details are described in the Supporting Information. In the first step, maltoheptaose, denoted as MH, was obtained by a ring-opening reaction (Scheme S1). This step provided the key saccharide block for our synthetic pathway. The click reaction is ideal for the preparation of block copolymers due to its high chemoselectivity and compatibility with many functional groups such as the hydroxyl groups of MH. Hence, we functionalized maltoheptaose at the distal end with an azide group and with an alkyne group to form MH-N_3 and MH-C≡CH, respectively. All intermediate products and the final product were successfully synthesized, as demonstrated by mass and ^{1}H NMR spectroscopy (ESI-II).

An azide and an alkyne group were also respectively introduced onto β-CD and ferrocene. The direct functionalization of natural polysaccharoses may open a new route towards polysaccharoses. Scheme S2 shows the preparation of β-CD-$(N_3)_7$, obtained by substituting the 6-hydroxyl groups of β-CD with iodine then converting β-CD-$(I)_7$ to β-CD-$(N_3)_7$ via azidation. This β-CD was modified with maltoheptaoses to form MH_4-β-CD via click reaction between MH-C≡C and β-CD-$(N_3)_7$, thereby constructing the hydrophilic block for the target oligosaccharide-based supramolecular amphiphiles. The chemical structures of the intermediate and ultimate molecules were further confirmed by MALDI-TOF MS spectra, ^{1}H NMR, and FT-IR (ESI-II). The results indicated the successful synthesis of the desired MH_4-β-CD comprising four MH-C≡CH units attached to β-CD-$(N_3)_7$ to form a four-armed star MH_4-β-CD.

In the third step, most of the hydroxyl groups of MH-N_3 were protected as acetals, making the modified MH-N_3 hydrophobic in order to enable micelle formation via a self-assembly process. Masking the hydroxyl groups of MH-N_3 as acetals provided both a hydrophobic material readily processed using various self-assembly techniques and a mechanism for introducing pH-sensitivity. Under mildly acidic aqueous conditions, the pendant acetal groups should hydrolyse, unmasking the hydroxyl groups of MH. The complete hydrolysis of AcMH would releas acetone, methanol, and water-soluble MH. Finally, AcMH-N_3 and Fc-C≡CH were used to construct the hydrophobic block of the fully oligosaccharide-based supramolecular amphiphile via a CuACC reaction. ^{1}H NMR and FT-IR spectroscopy (ESI-II) verified the the successful synthesis of Fc-AcMH.

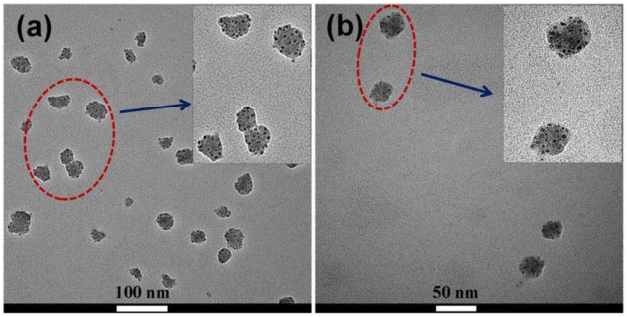


Fig. 1 TEM micrographs of DOX loaded MH_4-β-CD-AcMH nanoparticles. Inset of (a) and (b): HRTEM of the nanoparticles.

Noncovalent linking of the supramolecular amphiphiles (MH_4-β-CD-AcMH) was achieved by mixing hydrophilic Fc-AcMH and hydrophobic MH_4-β-CD in solvent via the host-guest interaction of β-CD and ferrocene. The successful formation of MH_4-β-CD-AcMH was confirmed by the 2D NOESY spectrum (Fig. S15). And the the CMC value was investigated by a widely reported pyrene-probebased fluorescence technique. The CMC value of MH_4-β-CD-AcMH is $1{,}08\times10^{-4}$ mg/ml (Fig. S17). As shown in Fig. 1, the self-assembly of supramolecular amphiphilic block copolymers was confirmed by transmission electron microscopy (TEM). The high-resolution TEM (HRTEM) image (inset of Fig. 1a and b) indicates that the formed nanoparticles have a smooth surface, exhibit minimal aggregation and have an approximately uniform orbicular structure 40 nm on average in diameter. The dark black spots in the centre are Fc moieties, the spectrometry of EDX is displayed in (ESI-II, Fig. S18), showing successful generation of the supramolecular amphiphiles. The average diameter of DOX loaded nanoparticles and nanoparticles are determined by dynamic light scattering (DLS) (ESI-II, Fig. S18).

As shown in Fig. S19, the TEM micrographs of the nanoparticles were taken under both acidic and oxidation-

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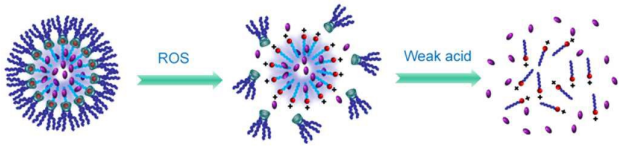
acidic conditions to determine whether these polysaccharide nanocarriers would display pH sensitivity and oxidation sensitivity, respectively, as intended. The presence of an AcMH block at the core of the micelle could facilitate pH-mediated drug-release due to conversion of the AcMH into MH, acetone and methyl alcohol under acidic conditions (Scheme 2). As shown in Fig. S19a, the acetal group was destroyed at pH 4 after 24 hours, leading to disintegration of the nanoparticles. Residual hydrophobic units allowed the formation of smaller nanoparticles With longer incubation times (Fig. S19b), the nanoparticles eventually disintegrated. In general, electrically neutral ferrocene and its derivatives tightly bound to the interior cavity of β-CD, but left the cavity following their transformation into positively charged ions (Fc^+) under oxidative conditions (Scheme 2).[11] As shown in Fig. S19c, oxidative conditions generated by NaClO induced the defluvium of the hydrophiphilic block from the nanoparticles. Driven by hydrophobic forces, the nanoparticles become larger. With time, the nanoparticles completely dissociated, as shown in Fig. S19d. We therefore predicted that DOX would more readily released under oxidation-acidic condition.



Scheme 2. Intracellular microenvironment triggered release from DOX-loaded MH_4-β-CD-AcMH micelle.

The utility of these self-assembled nanoparticles as a potential drug delivery carrier for cancer therapy was confirmed, and DOX was encapsulated into the micelles as a model drug. The release behavior of DOX in vitro was demonstrated at pH 7.4 and pH 4.0, the pH values of normal tissue and lysosomes, respectively. The drug-loaded nanoparticles were incubated in buffer solutions and the released DOX was quantified via UV-visible spectrophotometry. As shown in Fig. 2, approximately 70% of the DOX was released from the MH_4-β-CD-AcMH nanoparticles at pH 4 within 72 hours, which is much higher than the amount of DOX released from the nanoparticles at pH 7.4. Acid-promoted disassembly of MH_4-β-CD-AcMH nanoparticles may release different amounts of drug due to acetal groups in the hydrophobic block. The parallel tendency was obtained by adding 0.1 mM NaClO to bufer solutions with different pH values and incubating for 72 hours. The drug release rate from the nanoparticles in pH 4 buffer containing 0.1 mM NaClO was much higher than that from nanoparticles in pH 7.4 buffer containing 0.1mM NaClO and from the control group with no addictive. This increased release is likely due to the oxidative disassembly of the MH_4-β-CD-AcMH nanoparticles. These nanoparticles were constructed based on oxidative-dependent host-guest interaction between ferrocene and β-CD. The results showed that drug release from MH_4-β-CD-AcMH nanoparticles could be effectively controlled, demonstrating the potential medical application of fully oligosaccharide-based multi-sensitive nanoparticles.

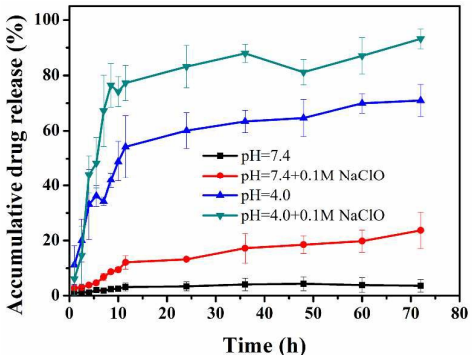


Fig. 2 In vitro DOX release from MH_4-β-CD/Fc-AcMH under various conditions.

It is important to determine if polymeric materials are potentially toxic, which if so, would hinder their application for drug delivery. We evaluated the intracellular cytotoxicity of MH_4-β-CD-AcMH nanoparticles in SW620 cells using the MTT assay. As shown in Fig. 3, cell viability when incubated with MH_4-β-CD-AcMH nanoparticles for 48 hours exceeded 90% at all concentrations up to 191.5 μg/ml, comparable to the concentration of polysaccharides used in drug-loaded nanoparticles. MH_4-β-CD-AcMH is thus essentially non-cytotoxic and could be used as a biocompatible and biodegradable material for drug delivery. The in vitro cellular proliferation inhibition of DOX-loaded MH_4-β-CD-AcMH nanoparticles against SW620 was also evaluated by the MTT assay, using free DOX as a control. The results indicated that endosomal pH and cellular ROS levels promoted rapid release of DOX from the MH_4-β-CD-AcMH nanoparticles, inhibiting cellular proliferation. These results were validated using TEM.

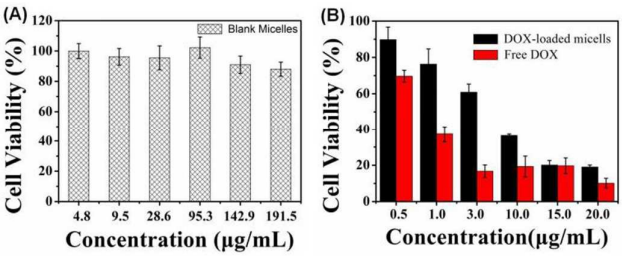


Fig. 3 The cytotoxicity of MH_4-β-CD-AcMH (A) and DOX-loaded MH_4-β-CD-AcMH (B) nanoparticles towards SW620 cells after incubation for 48 h.

The internalization of drug-loaded nanoparticles into SW620 cells was followed by inverted fluorescence microscopy to determine if the drug-loaded nanoparticles could be endocytosed into carcinoma cells, further leading to drug accumulation in the cell. SW620 celles were cultured with DMEM containing DOX-loaded nanoparticles for different lengths of time after staining, the nuclei with 4',6-diamidino-2-phenylindole (DAPI, blue). DOX is a fluorescent molecule and thus its uptake into cells can be directly observed. Fig. 4 shows slight red fluorescence in the cytoplasm following incubation for 0.5 hours, and stronger fluorescence 2 hours later. Red fluorescence was observed in the nuclei of SW620 cells at 6

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hours. These results show that DOX-loaded MH_4-β-CD-AcMH nanoparticles are efficiently taken up by SW620 celles.

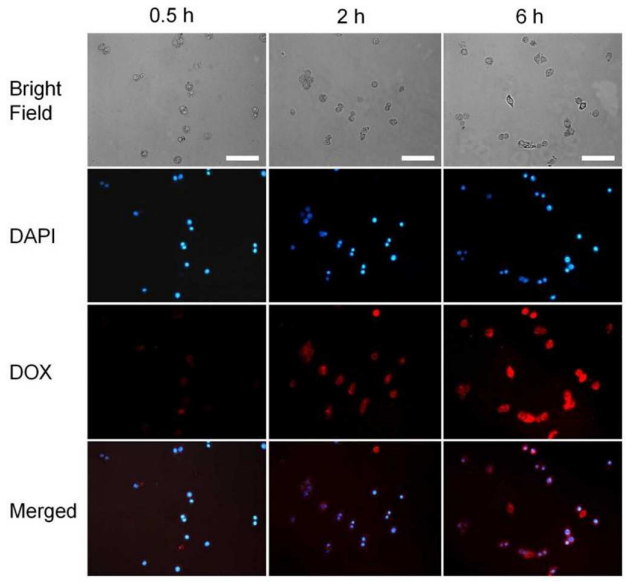

Fig. 4 Representative fluorescence images of SW620 cells incubated with DOX-loaded MH_4-β-CD-AcMH nanoparticles (A) for 0.5 h, (B) for 2h and (c) for 6h. For cell nuclei stained by DAPI (blue), DOX fluorescence in cells (red), and overlays of the two images. Scale bar: 100 μm.

In summary, tumor cells have relatively high concentrations of ROS and are weakly acidic. We therefore designed and prepared multi-sensitive oligosaccharide supramolecular amphiphiles through host-guest recognition between a terminal Fc and β-CD. The supramolecular amphiphiles demonstrated that an oligosaccharide can self-assemble in water to provide well-distributed spherical nanoparticles with an average particle size of 40 nm. The noncovalent β-CD/Fc and acetal groups afforded supramolecular amphiphiles that rapidly released the encapsulated drug in response to acid conditions and NaClO. The properties of the protecting group are critical for tuning the loading and release capacities of the nanocarriers, making it possible to construct a series of supramolecular amphiphiles based on polysaccharides. These novel materials, composed only of amphiphilic ologosaccharides, have great potential for a wide range of uses. Furthermore, their versatile chemical and physical properties, such as biodegradability, biocompatibility, and nontoxicity, make them attractive for further development. The present work therefore provides a new platform for constructing drug delivery and drug release carriers.

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Conflicts of interest

There are no conflicts to declare.

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