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Anticancer drug-based multifunctional nanogels through self-assembly of dextran–curcumin conjugates toward cancer theranostics

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ABSTRACT

Curcumin (CCM) has been received much attention in cancer theranostics because CCM exhibits both anticancer activity and strong fluorescence available for bio-imaging. However, CCM has never been utilized in clinical mainly due to its extremely low water solubility and its low cellular uptake into cancer cells. We fabricated novel CCM-based biodegradable nanoparticles through self-assembly of amphiphilic dextran–CCM conjugates. Significantly high CCM loading contents in the nanoparticles and the high water solubility were achieved. Importantly, the dextran–CCMs nanoparticles were effectively delivered into HeLa cells and exhibited strong fluorescence available for live-cell imaging, although the nanoparticles were not delivered into normal cells. Thus, the dextran–CCMs nanoparticles could be a promising for creation of novel CCM-based cancer theranostics with high efficacy.

Polymer-based nanoparticles are large and fast growing fields, and such nanoparticles have been applied as delivery carriers for therapeutic agents including anticancer drugs and bio-imaging agents, especially for cancer therapy and diagnostics.[1,2] Recently, a new field combining both therapy and diagnostics, called theranostics, has been generated for cancer treatment.[3] One of the main therapeutic systems in cancer theranostics is imaging-guided drug delivery. The therapeutic system usually employs polymer nanoparticles for their achievement. Polymer nanoparticles for cancer theranostics must be both nano depot and nano carriers for therapeutic and diagnostic agents.[4] Common imaging components utilized for cancer theranostics include photoluminescent mostly fluorescent materials, while common therapeutic components are anticancer drugs.[2] Nanoparticles consisting of amphiphilic copolymers, such as micelles, polymersomes, and nanogels with hydrophobic domains, are commonly used in cancer theranostics.[5] In these cases, both fluorescent materials and anticancer drugs should be loaded in hydrophobic domains of nanoparticles with enough high amounts. However, there are inherent difficulties in achieving enough high drugs and imaging materials loading per nanoparticle because of the limited capacity of hydrophobic domains in nanoparticles for loading.[5]

Recently, curcumin (CCM), naturally-occurring hydrophobic molecule derived from turmeric, has been received much attention in cancer theranostics because CCM induces apoptosis to various kinds of cancer cells[6,7] with a safe manner to healthy cells and exhibits strong fluorescence as biocompatible probes available for bio-imaging.[8,9] Thus, CCM can be applied to our concept described above as anticancer drug, imaging material, as well as building block to create nanoparticle for cancer theranostics. However, CCM has never been utilized in clinical mainly due to two problems. The first problem is poor aqueous solubility. CCM is hydrophobic molecule, and thus the maximum water solubility is about 30 nm, whereas CCM induces apoptosis to cancer cells with an IC_{50} 10–75 μM.[10] The second problem is low cellular uptake. CCM deeply inserts into cell membrane, anchored by hydrogen bonding to the phosphate group and hydrophobic interaction with fatty acyl group of lipid, and thus only a few parts of CCM translocate into cytoplasm and nucleus whereat it works.[11] Thus, intracellular delivery of intact CCM to cancer cells is big issue to achieve clinical application of CCM in cancer theranostics. Herein, we fabricated novel CCM-based polymer nanoparticles by self-assembly approach. Importantly, the obtained CCM-based nanoparticles exhibited not only cell penetrating property in cancer cell-selective manner, but also strong fluorescence in cancer cells enough for visualizing. Therefore, we describe the synthesis of dextran–CCM conjugates, fabrication of their self-assembled nanogels, its fluorescence properties, and the cell penetrating properties. This study is the first example in theranostics studies

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to overcome water-insoluble problems of useful anticancer and fluorescence agents through utilization of polymeric self-assembly.

Ulbrich et al. has reported that hydrophilic polymer-hydrophobic drugs conjugates self-assembled into nanoparticles in aqueous media via hydrophobic interactions among the drug molecules.[12] Inspired by this paper, we synthesized amphiphilic polymers composed of hydrophobic CCMs and hydrophilic dextran to fabricate CCM-based nanoparticles. Dextran–CCMs conjugates were synthesized through coupling reaction of activated dextran and CCMs (Fig. 1). Dextran–CCMs conjugates are expected to self-assemble into nanoparticles through hydrophobic interactions between CCM side-chains attached to the dextran in aqueous environment (Fig. 1). In this molecular systems, hydrophobic/hydrophilic balance of amphiphilic dextran–CCMs conjugates would affect the water solubility and their self-assembly behavior to form nanoparticles. Therefore, we synthesized a series of dextran–CCMs conjugates with different CCM contents by varying feed molar ratios of CCM to dextran in the synthesis process. Purification of the obtained products were carried out by precipitation technique using excess amount of methanol as poor solvent for the obtained dextran–CCMs conjugates and as good solvent for uncoupled CCM, CDI, and DMAP. Fig. S1 (Supplemental data) shows gel permeation chromatography (GPC) profiles of the dextran–CCMs conjugates. The dextran–CCMs conjugate each shows unimodal peak with a reasonably narrow molecular weight distribution, indicating the complete removal of uncoupled CCM and the reaction byproducts from the obtained dextran–CCMs conjugates. The conjugation of CCMs to dextran was also confirmed by ^{1}H NMR measured in DMSO-d_6 as good solvent for dextran–CCMs conjugates (Fig. S2, Supplemental data). UV–vis spectra of the dextran–CCMs conjugates measured in DMSO were shown in Fig. 2a. We used the absorbance at 434 nm to determine the average number of CCM side-chains conjugated to dextran. The characteristics of the obtained dextran–CCMs conjugates were summarized in Table 1. We used the abbreviations for dextran–CCMs conjugates as DC*x* (*x* means numbers of CCM per dextran molecule). DC3, DC16, and DC30 were synthesized and these polymers were used following experiments.

It has been reported that amphiphilic polymers composed of polysaccharides main-chain and hydrophobic side-chains, such as cholesterol-conjugated pullulan, form hydrogel-like nanoparticle (nanogel) with polysaccharide skeleton and hydrophobic multi cores in dilute aqueous solutions.[13,14] Self-assembly of the dextran–CCMs conjugates was accomplished by direct dispersion of the polymers in water. Tyndall phenomena were observed for all the aqueous solutions (data not shown), meaning the presence of colloidal particles in these solutions. These results clearly show that amphiphilic dextran–CCMs conjugates self-assembled into nanoparticles in aqueous solutions. So, the colloidal particles were analyzed by DLS measurement. DC16 and DC30 nanoparticles showed a unimodal size distribution peak, but the distribution peak of DC3 was bimodal, as shown in Table 1. The mean diameter of colloidal particles were ranged from approximately 30 nm to 220 nm depending on the number of conjugated CCM, indicating that the number of conjugated CCM is one of the critical factors to determine their size.

Typically, loading contents of anticancer drugs per polymeric nanocarrier for cancer theranostics are less than 10 wt %.[15] The CCM content (wt %) in per DC3, DC16, and DC30 were 2.7 wt %, 12.8 wt %, and 21.6 wt %, respectively. This result means that DC16 and DC30 nanoparticles load relatively higher amounts of anticancer drugs as well as imaging agents as compared with those of typical nanoparticles reported, because CCM possess activities as both anticancer drugs and imaging agents. It is well known that CCM exhibits anticancer activity and enough strong fluorescence when the concentration of CCM in solutions is reached up to

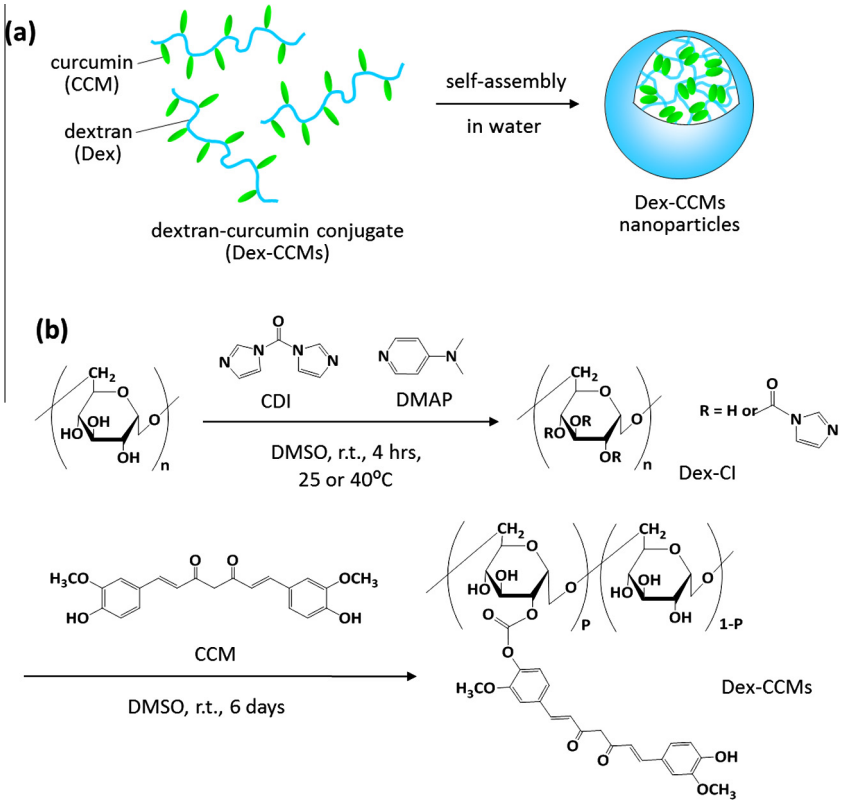

Figure 1. Schematic illustration of the formation of dextran–CCMs nanoparticles. (a) Self-assembled dextran–CCMs nanoparticles via intermolecular hydrophobic interactions between CCM side-chains. (b) Synthesis of dextran–CCMs conjugates.