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Design and construction of polymerized-chitosan coated Fe_3O_4 magnetic nanoparticles and its application for hydrophobic drug delivery

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ABSTRACT

In this study, a novel hydrogel, chitosan (CS) crosslinked carboxymethyl-β-cyclodextrin (CM-β-CD) polymer modified Fe_3O_4 magnetic nanoparticles was synthesized for delivering hydrophobic anticancer drug 5-fluorouracil (CS-CDpoly-MNPs). Carboxymethyl-β-cyclodextrin being grafted on the Fe_3O_4 nanoparticles (CDpoly-MNPs) contributed to an enhancement of adsorption capacities because of the inclusion abilities of its hydrophobic cavity with insoluble anticancer drugs through host–guest interactions. Experimental results indicated that the amounts of crosslinking agent and bonding times played a crucial role in determining morphology features of the hybrid nanocarriers. The nanocarriers exhibited a high loading efficiency (44.7 ± 1.8%) with a high saturation magnetization of 43.8 emu/g. UV–Vis spectroscopy results showed that anticancer drug 5-fluorouracil (5-Fu) could be successfully included into the cavities of the covalently linked CDpoly-MNPs. Moreover, the free carboxymethyl groups could enhance the bonding interactions between the covalently linked CDpoly-MNPs and anticancer drugs. In vitro release studies revealed that the release behaviors of CS-CDpoly-MNPs carriers were pH dependent and demonstrated a swelling and diffusion controlled release. A lower pH value led to swelling effect and electrostatic repulsion contributing to the protonation amine impact of NH_3^+, and thus resulted in a higher release rate of 5-Fu. The mechanism of 5-Fu encapsulated into the magnetic chitosan nanoparticles was tentatively proposed.

1. Introduction

Nanostructured organic/inorganic hybrid materials have recently gained significant attention for their potential applications in biological fields, including protein/DNA bioseparation, disease diagnostics and cancer therapy [1–3]. Among various hybrid nanomaterials, targeted nano-scaled drug delivery systems have received tremendous attention over the past decades due to their applications as targeted carriers of therapeutics and/or diagnostic agents to diseased tissues, which can effectively improve the therapeutic efficiency and minimize the side effects of medicine on normal cells and tissues [4]. Among the different types of nanoparticles, superparamagnetic iron-oxide nanocomposites (SPIONs) are considered to be the promising candidates in cancer therapy due to their superparamagnetic behavior and surface-modification properties. At present, magnetic nanoparticles have been widely used in the field of biology and medicine, such as bioseparation, protein and enzyme immobilization, immunoassay, drug delivery and magnetic resonance imaging (MRI) [5–7].

Currently, a great challenge that researchers have to confront with is the effective clinical application of hydrophobic agents, because of the poor solubility of most chemotherapeutic drugs for cancer treatment in aqueous media [8]. For example, the low bioavailability after administration of these agents was due to the insufficient drug doses at the lesion site [9]. To cope with the challenges, abundant attempts have been made to deliver hydrophobic anticancer agents to the targeting sites as much as possible. At present, the most popular approach to improve the bioavailability of insoluble anticancer drugs is to deliver via carriers, such as self-assembling peptide for ellipticine, magnetic iron oxide nanotubes for paclitaxel, and polymeric carrier for doxorubicin [10,11]. Thus, as drug delivery applications, magnetic nanoparticles (MNPs) must be preprocessed with substances that assure their stability, biodegradability, and non-toxicity in the physiological medium in order to achieve the combination properties of high magnetic saturation, and biocompatibility with interactive functionality on the surface [12]. The surfaces of these particles could decorate with some

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surfactants or polymers by depositing a few atomic layers of biocompatible polymers. The polymer coating not only leads to the creation of more hydrophilic nanostructures, but also provides a variety of surface functional groups to bind drug molecules, inhibit aggregation, and increase stability [13].

Chitosan, as a multifunctional natural polymer containing large numbers of active amino groups together with hydroxyl groups in the backbone, can interact with mucins and open the tight junctions between epithelial cells, which are in favor of the drug transport. The chitosan-based delivery systems have shown great potential to deliver nucleic acids, therapeutic proteins, vaccinations and so on [14,15]. Nevertheless, apart from the evident advantages of chitosan, one important demerit as drug carrier is its hydrophilicity, which gives rise to its poor loading capacity for hydrophobic therapeutic agents [16]. Herein, researchers have made great efforts to improve the loading ability of chitosan for hydrophobic drugs [17]. Among them, magnetic nanoparticles modified by cyclodextrins (CDs) and then coupled with chitosan have attracted substantial attention.

It is widely known that doughnut-shaped β-cyclodextrin (β-CD) can form inclusion complexes with a wide variety of organic compounds in its hydrophobic cavity through host–guest interactions, because of unique structure features with a hydrophilic exterior and the sufficient internal hydrophobic cavity to host water-insoluble drug molecules [18,19]. The formation of these complexes has been attributed to the weak interactions such as hydrophobic effect, van der Waals interactions, and hydrogen bonding. As a drug carrier, β-CD not only facilitates solubilization, stabilization, and transport of hydrophobic drugs, but also minimizes the unwanted side effects associated with the corresponding drugs. It has been suggested that CDs and their derivatives might undergo degradation in the colon by vast microflora, which can break CDs and their derivatives into small saccharides to be absorbed in the large intestine [20]. This molecular encapsulation ability is widely utilized in many industrial products, technology and analytical methods [21].

Several studies have been directed toward constructing MNPs–cyclodextrin, CS–cyclodextrin or MNPs–CS nanocomposites to form new drug carriers. Banerjee and Chen [22] reported the designing of magnetic nanocarrier functionalized with cyclodextrin (CD)–citrate–gum arabic as a novel nanocarrier for hydrophobic drug delivery. The inclusion and the release experiments of ketoprofen demonstrated that this system could be a very promising vehicle for the administration of hydrophobic drugs. Lv et al. [23] explored the beta-cyclodextrin (β-CD)-conjugated magnetic Fe_3O_4 colloidal nanocrystal clusters for the loading and release of hydrophobic molecule. The developed magnetic hybrid nanomaterial can markedly increase the loading capacity of ANS owing to the formation of β-CD/ANS inclusion complexes. In a word, chitosan and β-cyclodextrin derivatives are attractive carriers since they possess both the mucoadhesive property of chitosan and the hydrophobic cavities of β-cyclodextrins. However, a simultaneous combination of magnetic nanoparticles, cyclodextrin and CS into a novel drug carrier system is rarely reported. Anirudhan et al. [12] investigated the chitosan crosslinked β-cyclodextrin grafted silylated magnetic nanoparticle (CTSCD-g-SilylMNP) as a drug delivery of Indomethacin (IND). The magnetic nanoparticles (MNP) should be precoated with SiO_2 and then modified with amino functional groups, followed by β-cyclodextrin which was grafted onto the surface of a silylated magnetic nanoparticle. Thus, the fabrication was fussy in a whole. In the present work, we demonstrate a facile route to synthesis multifunctional drug delivery system. The carboxymethyl-β-cyclodextrin conjugated with magnetic nanoparticles was realized via in situ one-step synthesis. Therefore, the magnetic target properties of the Fe_3O_4 nanoparticles and biological functionality of chitosan together with β-cyclodextrins with satisfactory loading capacity are highly desirable on the targeting way to applying insoluble anticancer drugs for the clinic.

During the several decades, an antineoplastic hydrophobic agent, 5-fluorouracil (5-Fu), has been widely used in the therapy of different malignant tumor types such as cancer of the stomach, liver, and intestine. Because of the short plasma half-life of 10–20 min, high dose (e.g. 400–600 mg/m_2) has to be administered weekly to reach a therapeutic drug level [24]. This requires us to make continuous infusion schedules of 5-Fu in order to maintain an effective concentration in vivo. Thus, 5-Fu based on the grafting carboxymethyl-β-cyclodextrin molecules on the magnetic nanoparticles as drug delivery system appears to offer a comparable efficacy while providing a more convenient schedule. During the complexes formation with drug molecules, no covalent bonds exist between the CDs and its guest, so the complexation can be considered as a dynamic process. The drug molecules included within the CD cavity may therefore be dissociated upon dilution, displaced by a more suitable guest or transferred to a matrix for which it has a higher affinity, such as a biological membrane.

For selective delivery of nanoparticle device, the challenge lies in successfully combining therapeutic and targeting functionalities along with biodegradation capability into one single nano-system. Thus, with an effort to improve the drug loading capacity and the merit of cyclodextrin derivative, carboxymethyl-β-cyclodextrin (CM-β-CD) polymer was synthesized and used to graft on the surface of Fe_3O_4 nanoparticles. The ligands on the crosslinked CM-dextrin polymers were predominant carboxyl groups, along with hydroxyl groups. These polymer grafted magnetic nanoparticles (CDpoly-MNPs) used were easily separable and recyclable. Meanwhile, based on the nontoxic and biocompatible natural polysaccharide of chitosan, a facile approach was used to prepare polymerized chitosan-coated Fe_3O_4 drug-loaded nanoparticles (CS-CDpoly-MNPs) via the emulsion chemical cross-linking method. The coating processes, particle characteristics (shape, size, and surface), loading and releasing of 5-fluorouracil from CS-CDpoly-MNP composites were characterized by various measurement techniques. The sizes and loading efficiency of these novel magnetic nanoparticles can be controlled by adjusting the contents of cross-linking agent, reaction times or chitosan/CDpoly-MNP weight ratios of the synthesis system. Quantitative measurements of the loading and release processes of 5-Fu were carried out using a spectroscopic method. Finally, the possible mechanism in drug delivery of 5-Fu will be discussed as well. The use of CS-CDpoly-MNPs as the carrier might be a promising strategy for the dosing of hydrophobic drugs.

2. Materials and methods

2.1. Materials and characteristics

$FeCl_3 \cdot 6H_2O$ (≥99.0%), $FeSO_4 \cdot 7H_2O$ (≥99.0%), CH_3COOH (≥99.5%), NaOH (≥96.0%), glutaraldehyde (25%, v/v, aqueous solution), paraffin, Span-80, 5-Fluorouracil (5-Fu), β-Cyclodextrin (99%), chloroacetic acid (99%), $Na_2HPO_4 \cdot 2H_2O$, $NaH_2PO_4 \cdot 2H_2O$ and chitosan (degree of deacetylation 95%) with medium molecular weight were all purchased from Shanghai Chemical Reagents Company (China) and used without further treatment. All the reactants and solvents were of analytical grade.

2.2. Synthesis of CM-β-CD polymer

CM-β-CD polymer was prepared following the procedure of literature [25], with the detailed description as follows: a mixture of 10 g β-CD and 9.3 g NaOH in 37 mL of water was treated with 27 mL of 16.3% monochloroacetic acid solution at 50 °C for 5 h. Then the reaction mixture was cooled to room temperature, and pH was adjusted (6–7) using hydrochloric acid (2 M). The obtained neutral solution was then poured to superfluous methanol solvent and white precipitates were produced. The resultant product was purified by precipitation with methanol several times until no AgCl precipitation was detected when $AgNO_3$ solution was added to the aqueous solution of the product. The solid precipitation was then filtered and dried under vacuum to give carboxymethyl-β-CD (CM-β-CD). IR (KBr): $\nu(cm^{-1})$: 3389 (–OH),