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Cyclodextrin-grafted chitosan hydrogels for controlled drug delivery

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ABSTRACT

A series of β-cyclodextrin-grafted carboxymethyl chitosan hydrogels (CD-*g*-CMCs) were prepared from carboxymethyl chitosan (CMC) and carboxymethyl β-chitosan (CMCD) using a water-soluble carbodiimide as a crosslinker in the presence of *N*-hydroxysuccinimide. Details of the hydrogel structures were determined via FTIR and solid-state NMR spectroscopic analyses. Increasing the feed ratio of CMCD to CMC in the reaction mixture led to an increase in CD grafting within the gel networks comprising CMC; this was confirmed by SEM observations and rheological analysis of the swollen hydrogels. The prepared CD-*g*-CMC hydrogels exhibited absorption properties toward acetylsalicylic acid (ASA, or Aspirin) due to the presence of CD in the structure; the amount of ASA absorbed into the hydrogels was enhanced with an increase in the amount of CD incorporated within the hydrogels. In addition, CD-*g*-CMC hydrogels provided a slower release of the entrapped ASA in comparison to the ASA release profile of a solely CMC-containing hydrogel. From these results, CD-*g*-CMC hydrogels have the potential to function as a biodegradable active material with controlled drug release ability.

1. Introduction

Hydrogels are three-dimensional networks composed of hydrophilic polymers crosslinked through covalent bonds or held together via physical intermolecular interactions [1]. Over the past few decades, methods for administering drugs via hydrogels have gained increasing attention [2,3], as regulating the rate of drug release with a controlled-release mechanism offers numerous advantages over conventional dosage regimens [4]. The use of polysaccharide-based hydrogels as a drug delivery carrier in biomedical and pharmaceutical applications has contributed to resolving relatively complicated biocompatibility problems owing to their non-toxicity, biodegradability, and biocompatibility [5,6].

Chitosan is the partially or fully deacetylated form of one of the most abundant naturally occurring polymers, chitin, which is extracted from the exoskeletons of arthropods, crustaceans, and insects [7]. Chitosan is a cationic polymer that is composed of β-(1 → 4)-linked D-glucosamine (GlcN) residues, where specific GlcN residues within the polymer chain can be replaced by *N*-acetyl-D-glucosamine units. Chitosan is also well known as a promising biomaterial owing to its non-toxicity, antimicrobial properties, and biocompatibility [8,9], and has been extensively studied as a carrier matrix candidate for drug delivery [6,10,11] and gene delivery [12] systems. In addition, chitosan is easily converted to gels [13], membranes [10,11,13], beads [14], nanoparticles [15] and scaffolds [16,17]. Thus it can be adapted for a range of biomedical applications in tissue engineering, wound dressing, cancer drug delivery, and targeting in the area of nanobiotechnology [18–20]. Many approaches have been reported for the preparation of chitosan-based hydrogels, including those based on chemical [6,21,22] as well as physical [23–25] crosslinking methods. Although physical methods have the advantage of crosslink formation without the use of crosslinking agents, they exhibit a disadvantage in the lack of precise control over the quality of chemical properties (including degradation and dissolution) of the obtained gels [26]. On the other hand, chemical crosslinking of hydrogels can be easily performed using small bifunctional molecules such as glutaraldehyde [27] and epichlorohydrin [28] as a crosslinker. The mechanical properties exhibited by chemically crosslinked hydrogels generally exceed those of physically crosslinked hydrogels for biomaterial applications [26]. However, these crosslinkers generally have associated toxicities, and a small amount of residual crosslinker in the obtained hydrogels can be considered as potentially harmful to human health [29].

β-Cyclodextrin (CD) is a cyclic oligosaccharide composed of seven glucopyranosyl units interacting via α-(1 → 4) linkages. CD possesses a hollow truncated cone structure in which the cavity at the center of the molecule is hydrophobic while the outer surface is hydrophilic [30]. This molecular structure is capable of forming inclusion complexes, so-called guest–host compounds, with

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aliphatic and, especially, aromatic molecules [30]. CD is frequently employed in pharmaceutical applications for numerous purposes including improving the bioavailability of drugs [31]. An elegant approach to functional biopolymers is represented by the synthesis of CD conjugates with biocompatible hydrophilic polymers, especially polysaccharides including chitosan; accordingly, many synthetic strategies for CD-grafted polysaccharides can be found in the literature [3,32,33]. However, because most of the methods reported require harmful reagents (such as the crosslinker species) for the preparation of polysaccharide/CD-based hydrogels, alternate methods avoiding harmful reagents are actively sought.

Among the established crosslinking agents, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) is recognized as a virtually non-toxic reagent for the coupling of carboxylates and primary amines to generate the amide bond [17,34,35]; EDC initially reacts with a carboxyl group to form an amine-reactive *O*-acylisourea intermediate, which then reacts with a primary amine to form the amide bond. In the presence of *N*-hydroxysuccinimide (NHS), EDC first couples NHS to the carboxylate species, forming an NHS ester that is considerably more stable than the *O*-acylisourea intermediate, which allows for efficient conjugation to primary amines. Indeed, NHS has been previously used to improve the efficiency of EDC coupling reactions [35]. This coupling method employing the dual EDC–NHS reagent has been used in diverse applications such as amide bond formation in peptide synthesis [36], attaching haptens to carrier proteins to generate immunogens [37], and labeling nucleic acids through their 5′ phosphate groups [38]. Because there is no crosslinking moiety in the coupling product, the EDC–NHS coupling protocol has the significant advantage of biological safety over other commonly used crosslinkers.

In this study, we describe the preparation of smart polymeric hydrogels containing chitosan and cyclodextrin using the EDC–NHS coupling method, and examine their potential use as a carrier matrix for drug delivery systems, which includes an investigation of their drug loading and release behaviors. This new smart hydrogel is obtained using a simple two-step process, as shown in Fig. 1. The first step involves the preparation of carboxymethyl CD (CMCD) and carboxymethyl chitosan (CMC) from CD and chitosan, respectively. The second step is the crosslinking reaction between the carboxyl and primary amine moieties of neighboring CMC species to form a network structure of CMC chains; this is followed by the grafting of CMCD into the network via coupling of the CMC primary amine with the carboxyl group of CMCD. The conjugation reactions between CMC chains (crosslinking) and that between CMC and CD (CD-grafting to CMC) are performed simultaneously using EDC in the presence of NHS, thereby yielding a CD-*g*-CMC hydrogel. A series of CD-*g*-CMC hydrogels differing in the quantity of CD were prepared, and their swelling behaviors were investigated. Additionally, in order to examine the potential of the CD-*g*-CMC hydrogel as a suitable carrier matrix for drug delivery systems, drug adsorption/absorption and release behaviors of the hydrogels were characterized using acetylsalicylic acid (ASA, more commonly known as Aspirin) as a model drug.

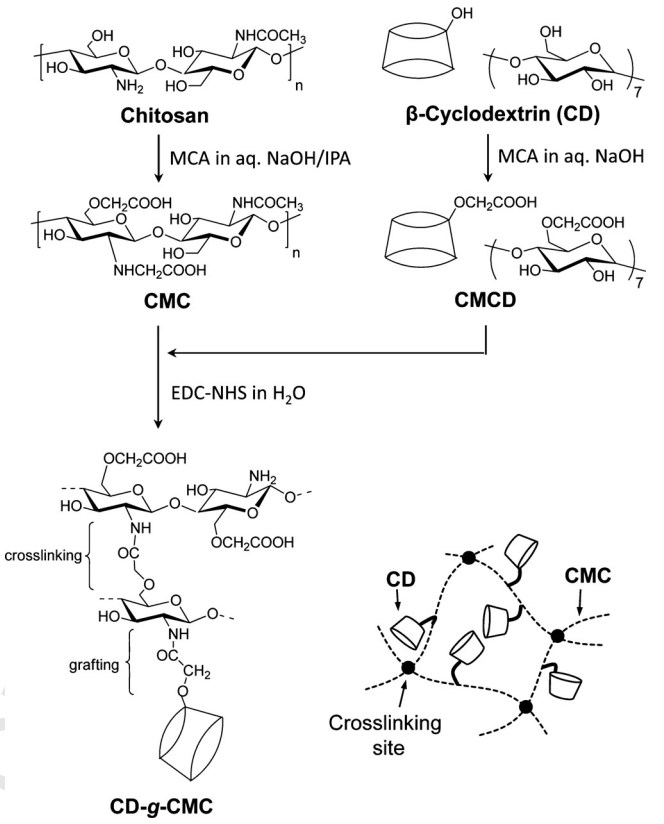

Fig. 1. Scheme for CD-*g*-CMC hydrogel synthesis from chitosan and CD, and a schematic illustration of the structure of the CD-*g*-CMC hydrogel. In this figure, although carboxymethylation at the chitosan C6 hydroxyl group is illustrated, carboxymethylation also can occur at other hydroxyl positions of chitosan as well as CD, which have been omitted from this figure.

2. Experimental

2.1. Materials

Chitosan (Mw; 4.6×10^4, degree of deacetylation; 0.86) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Japan). The chitosan degree of deacetylation was determined via solid-state NMR analysis; details are presented below (Sections 2.3.1 and 3.1). CD (purity 99.4%) was purchased from Wako Pure Chemical Industries, Ltd. (Japan). EDC and NHS were purchased from Sigma–Aldrich Co., Ltd. (USA). All solvents and other chemicals (analytical grade) were purchased from Kanto Chemicals Co., Inc. (Japan).

2.2. Preparation of CD-g-CMC hydrogel

2.2.1. Preparation of CMC

CMC was prepared according to a previously reported method [39]. In brief, chitosan (10 g, 57 mmol for the monomer unit) was added to a mixture of isopropyl alcohol (IPA; 80 mL) and aqueous 40% NaOH (w/v) solution (20 mL) and stirred using a Teflon impeller (300 rpm) at 25 °C for 1 h. To the suspension was added monochloroacetic acid (MCA; 15 g, 170 mmol) dissolved in IPA (20 mL) in five equal portions over a period of 20 min. The mixture was heated with stirring at 50 °C for a further 5 h. The reaction mixture was then neutralized using 4 M HCl solution. After removing the undissolved residue by filtration, the resulting CMC was precipitated by the addition of methanol. The precipitates were washed three times with methanol/water (1:1) and dried under vacuum at 40 °C.

2.2.2. Preparation of CMCD

CMCD was prepared according to a method reported in the literature [39]. Briefly, CD (22.8 g, 20 mmol) was completely dissolved in an aqueous 40% NaOH (w/v) solution (80 mL) containing MCA (3.8 g, 40 mM). The reaction mixture was then heated with stirring at 50 °C for 5 h. After neutralization with aqueous 4 M HCl solution, the obtained product was precipitated by addition of an excess amount of methanol. The precipitates were washed three times with methanol/water (1:1) and dried under vacuum at 40 °C.