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In situ synthesis of polysaccharide nanoparticles via polyion complex of carboxymethyl cellulose and chitosan

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ABSTRACT

Biocompatible polymer–magnetite hybrid nanoparticles were prepared by means of *in situ* synthesis of magnetite within polysaccharide hydrogel nanoparticles. Hydrogel nanoparticles were first fabricated by blending high-molecular-weight carboxymethyl cellulose as an anionic polymer, and low-molecular-weight chitosan as a cationic polymer to form polyion complexes (CC particles). These polyion complexes were then chemically crosslinked using genipin, a bio-based cross-linker, to form stable nanoparticles having a semi-IPN structure (CCG particles). Magnetite was lastly synthesized within CCG particles by the coprecipitation method to obtain polymer–magnetite hybrid nanoparticles (CCGM particles). The formations of CC, CCG and CCGM particles were mainly observed by transmittance, absorbance of genipin and TEM, respectively, and their hydrodynamic diameters and zeta-potentials were analyzed. It was confirmed that the hydrodynamic diameters and the zeta-potentials of these particles were significantly influenced by pH of the suspension, which was attributed to the charges of polymers. The diameters of CCGM particles were smaller than 200 nm at any pH conditions, suggesting the possibility to apply them as drug delivery carriers. CCGM particles exhibited the responsiveness to a magnetic field in addition to their high dispersion stability, indicating their potential to be utilized as a biomaterial for hyperthermia.

1. Introduction

Polysaccharides are naturally derived polymers with storage and structural functions, which are one of the main constituents in biological systems such as the glycocalyx and the extracellular matrices. Polysaccharides and their derivatives are commonly used for applications in food, biomedical and environmental fields because they possess good biocompatibility, biodegradability and low toxicity. Due to their high hydrophilicity, they have often been utilized in the form of hydrogels, which are soft materials that have capacity to hold a large amount of water to be swollen. Hydrogels are fabricated by physical or chemical cross-linking of water-soluble polysaccharides [1,2]. These hydrogels are expected to be utilized as biomaterials due to their similarity to extracellular matrices. In particular, hydrogel particles show a drastic volume change superior to bulk hydrogels, and a controlled release of functional materials in response to external circumstances such as temperature, pH and ionic strength. Polysaccharide-based hydrogel particles have been investigated by several researchers [3–5]. However, applications of hydrogel particles as drug delivery and release carriers were limited because it is difficult to produce nano-sized particles. Therefore, it is important to find a way to produce nanoparticles from polysaccharides with convenient and biocompatible methods.

Among a number of types of hydrogel particles, an interpenetrating polymer network (IPN), which is comprised of an interlaced network of two polymers, has received much attention in recent years. The IPN is one of attractive materials because of having high swelling properties and good stability of a three-dimensional structure [6]. Here, we focused on semi-IPN, where one component of polymers is cross-linked leaving the other in the linear form. There have been some reports based on the formation of semi-IPN particles through crosslinking of constituent polysaccharides [7,8]. For example, semi-IPN particles with diameters of 250–550 μm were prepared from gelatin and carboxymethyl cellulose [7]. These particles were not nano-sized, but possessed good functionalities as drug delivery carriers. In order to downsize polyion complexes, we intended to carry out *in situ* synthesis of polysaccharide nanoparticles through the polyion complex of naturally derived anionic polymer, carboxyl methyl cellulose (CMC) with a high molecular weight and cationic polymer, chitosan (CHI) with a low molecular weight. CHI is a natural polysaccharide carrying positive charges, and its low toxicity, biodegradability and mucoadhesive property are advantageous to a candidate for the drug delivery carrier and the artificial organs. We assumed that low-molecular-weight CHI would suppress the entanglement and diminish the size of polymer

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nuclei, leading to the production of particles with small diameters. Furthermore, to strengthen the polyion complex, chemical crosslinking of the amino groups of CHI was carried out to produce a semi-IPN, which is formed by interpenetration of linear CMC into cross-linked CHI. Since commonly used crosslinking agents, such as glutaraldehyde, are often disinfectant and toxic, we used genipin, gardenia fruit extract, as a highly safe and biocompatible cross-linker.

Magnetic nanoparticles have a high potential as carriers for separation, targeting, imaging and hyperthermia in biomedical fields. In hyperthermia, nanoparticles are accumulated in a target site and an alternating-current magnetic field is applied to increase temperature of the target site, it is possible to diminish the activity of cancer cells due to their high sensitivity to heat [9–11]. In order to apply magnetite for such applications, it is required to provide carriers with the sufficient dispersion stability and high responsiveness to a magnetic field. To satisfy these requirements, magnetite has been encapsulated within particles consisting of synthetic polymers, polysaccharides, liposomes and inorganic materials [12,13]. Here, as a preliminary approach, synthesis of magnetite within the gel particles was carried out in a co-precipitation method and the ability of polymer–magnetite hybrid nanoparticles to respond to the magnetic field was examined. The dispersion stability of these hybrid nanoparticles was then studied. Also, the effect of pH of dispersions on the hydrodynamic diameters and zeta-potentials of the obtained particles were analyzed to examine the unique characteristics of the particles.

2. Experimental

2.1. Materials

Carboxymethyl cellulose sodium salt (M.W. = 3000–300,000, degree of substitution (D.S.) = 0.7–0.8), genipin, sodium chloride (NaCl) and sodium nitrite ($NaNO_2$) were purchased from Wako (Tokyo, Japan). Three kinds of chitosan, CHI-1, CHI-2 and CHI-3, were used with different molecular weights and the degree of deacetylation (D.D.). CHI-1 and CHI-3 were purchased from Wako (Tokyo, Japan), and CHI-2 was purchased from YSK (Shizuoka, Japan). The molecular weights and the DD of CHI used in this study were M_n = 3000, D.D. = 0.50 (CHI-1) [14], M_n = 8100, D.D. = 0.84 (CHI-2) [15] and M_n = 34,800, D.D. = 0.80 (CHI-3) [16,17]. Iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), aqueous solutions containing hydrochloric acid (HCl) (0.01, 0.10, 1.0 and 6.0 mol/L) and 28 wt% ammonium aqueous solution (NH_3) were purchased from Junsei (Tokyo, Japan). Aqueous solutions containing sodium hydroxide (NaOH) (0.01, 0.10 and 1.0 mol/L) was purchased from Wako (Tokyo, Japan).

2.2. Synthesis of CC particles

The general procedure for the preparation of CMC–CHI particles (CC particles) was carried out as follows. CMC and CHI aqueous solutions (1.0 wt%) were first prepared by dissolving CMC and CHI in distilled water, respectively, and they were adjusted to pH 4.2 using 10 mM HCl aq. and 10 mM NaCl aq. The CMC solution (0.125 g) was then diluted by adding distilled water (0.25 g). To the diluted CMC solution was added the CHI solution (0.125 g) with a constant rate of 1.0 mL/min using a peristaltic pump (SMP-23S, EYELA, Tokyo, Japan) with stirring. The solution of the mixture was then stirred for 30 s and stood for 24 h.

The hydrodynamic diameters of the particles in distilled water were measured by dynamic light scattering using a laser particle analyzer system (PAR-3s, Otsuka Electric Co., Osaka, Japan). The gelation was analyzed using an X-ray diffractometer (XRD, RAD-C, Rigaku, Tokyo, Japan). The particles were observed using a field-emission scanning electron microscope (FE-SEM S-4700, Hitachi, Tokyo, Japan). Zeta-potentials of particles were measured in 10 mM NaCl aqueous solution (Zeecom, Microtec Co. Ltd., Chiba, Japan). Transmittance of particle suspensions was measured by using Bio Spec-1600 (Shimadzu, Tokyo, Japan).

2.3. Synthesis of CCG particles

To a suspension of obtained CC particles, which was prepared by the mixture of the CMC solution (1.0 wt%, 0.125 g), the CHI solution (1.0 wt%, 0.125 g) and distilled water (0.125 g), was added 1.0 wt% genipin solution in ethanol (0.125 g) with a constant rate of 1.0 mL/min with stirring. After mixing, the particle suspension was stirred for 30 s and stood for 24 h. The cross-linking reaction of amino groups of CHI with genipin as a cross-linker was analyzed by measuring infrared (IR) spectroscopy of genipin. Resultant CCG particles were freeze-dried, embedded within KBr pellets and analyzed using FT-IR spectrometer (FTS-60A, Bio-Rad, Cambridge, MA). The reaction rate of genipin was analyzed by measuring absorption with using Bio Spec-1600 (Shimadzu, Tokyo, Japan).

2.4. Synthesis of CCGM particles

The general procedure of *in situ* synthesis of iron oxide within gel particles was carried out as follows. A suspension of CCG particles, which consisted of CMC (0.25 wt%), CHI (0.25 wt%) and genipin (0.25 wt%) with a total weight of 10.0 g, and an aqueous solution of $FeSO_4{\cdot}7H_2O$ (1.2 wt%, 10.0 g) were added to the four-neck round bottom flask. The mixture was stirred at a speed of 200 rpm for 4 h under nitrogen atmosphere to absorb iron ions within the CCG particles, and then the particles were purified by centrifugation at 5000 rpm for 20 min. Distilled water was added to the particles and then nitrogen gas was bubbled to purge oxygen for 30 min with stirring at a speed of 200 rpm. $NaNO_2$ aqueous solution (1.74 mol/L, 1.0 mL) was added to the particle suspension (20.0 g) by using a syringe and stirred for 15 min, followed by the addition of NH_3 aqueous solution (28 wt%, 2.4 mL) and the stirring for 15 min. The obtained particles (CCGM particles) were purified by dialysis against distilled water for 3 days and ultrafiltration. The particles were observed by transmission electron microscopy (TEM) (HU-12AF, Hitachi Ltd., Tokyo, Japan). To explore the magnetic response of CCGM particles, magnet of NdFeB was attached to a side of a vial containing CCGM particles and allowed to stand for 24 h.

3. Results and discussion

3.1. Preparation of CC particles via a polyion complex

There have been reports on the preparation of gel particles by the polyion complex formation of chitosan and cellulose [5,18–23]. Especially, it is expected that particles with diameters below 200 nm will possess great potentials for the utilization as biomedical tools due to their applicability to EPR effect [24]. Therefore, we explored appropriate methods to prepare size-controlled CC particles with sufficient strength and high functionality.

The polymer–magnetite hybrid nanoparticles were prepared in three steps as the synthetic route shown in Scheme 1. CC particles were first prepared via the polyion complex formation of anionic CMC and cationic CHI. It is required for the production of the polyion complex that both carboxyl groups of CMC and amino groups of CHI are ionized and these polymer chains are extended in the solution to increase the reactivity of each polymer via the electrostatic attraction. Considering that pK_a values of the carboxyl groups in CMC and the amino groups in CHI are 3.9 and 6.5, respectively, the viscosity of polymer solutions was measured to find a pH condition