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Nanogel bottom-up gel biomaterials for protein delivery: Photopolymerization of an acryloyl-modified polysaccharide nanogel macromonomer

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ABSTRACT

Polysaccharide nanogels are one of the most attractive carriers for drug delivery systems. Nanogels encapsulate proteins in their hydrated polymer networks, and minimize the denaturation of proteins. In this study, we demonstrated the cross-linking of acryloyl group-modified polysaccharide nanogels via photopolymerization, which allowed the formation of novel hydrogel particles and macrogels. The mechanical properties of the resultant hydrogels depended on the concentrations of the nanogels and the cross-linkers. The most significant property of the nanogel-cross-linked hydrogel was the ability to encapsulate insulin via hydrophobic interactions. After incubation of the hydrogel containing insulin in water, the hydrogel was degraded by hydrolysis, and insulin was gradually released from the hydrogels over a period of 1 week. According to these results, this nanogel-cross-linked hydrogel prepared via photopolymerization has potential for innovative biomaterials.

1. Introduction

A hydrogel is a solid-state material that contains water and has three-dimensional, cross-linked networks of polymer chains. Nanometer-sized hydrogels are called nanogels, and are one of the most attractive carriers for innovative drug delivery systems [1,2]. Various linkages are used to cross-link the polymers in nanogels, including covalent bonds, hydrogen bonds, and electrostatic and hydrophobic interactions. Cholesterol-bearing polysaccharides are one of the most useful types of polymers, because they form stable, monodisperse nanogels with a diameter of approximately 30 nm in water [3]. In this nanogel, the cholesteryl group provides physical cross-linking points through hydrophobic interactions. In comparison with polymeric micro/nanospheres, nanogels can contain a large amount of water, and can incorporate bioactive drugs such as proteins and nucleonic acids within their nanoscale polymer networks. As described in our previous reports, various nanogels have been developed using pullulan, mannan [4], cycloamylose [5–7], cluster dextrin [8], enzymatically synthesized glycogen [9] and hyaluronic acid [10] as polysaccharides, and these nanogels can encapsulate proteins. For instance, the cholesterol-bearing pullulan (CHP) nanogels formed complexes with various proteins such as insulin, bovine serum albumin, α-chymotrypsin, myoglobin, and cytochrome c through hydrophobic interactions [11,12]. In most cases, the nanogel–protein complexes showed high colloidal stability. CHP nanogels and cationic CHP nanogels have been applied in cancer protein vaccines [13] and nasal protein vaccines [14], respectively.

In recent studies, we developed a bottom-up nanogel engineering method, in which nanogels were used as building blocks to control the nanostructure of macrogels or particles. In macro-scale hydrogels, intelligent macrogels, which have designable cross-linking points and nano-domains, are still needed. Double bond-modified CHP nanogels were used as polymerizable units, and nanogel-cross-linked macrogels or particles were prepared via polymerization, with 2-methacryloyloxyethylphosphorylcholine [15,16] and *N*-isopropyl acrylamide [17] as monomers. Multi-branched polyethylene glycols (PEGs) terminated with thiol groups [18,19] can form biodegradable links between nanogels and PEGs via the Michael addition reaction. Cross-linked-nanogel hydrogels are useful as scaffolds in regenerative medicine. We confirmed that effective guided bone regeneration was facilitated by nanogel-cross-linked macrogels [20], and successful bone formation was induced by bone morphogenetic proteins or fibroblast growth factors encapsulated in the macrogels, in vivo [21–24].

In this study, we first demonstrated the cross-linking of acryloyl group-modified CHP (CHPOA) nanogels via, to form novel hydrogel particles or macrogels. The photo-initiation method is effective in

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hydrogel scaffolds for tissue engineering, where macrogels with various shapes are needed, and a faster gelation speed is expected than a speed using the Michael addition reaction. We determined the mechanical properties of the resultant hydrogels prepared using different concentrations of nanogels and cross-linkers, and investigated the interaction behavior with model protein. We selected insulin as a model protein because we confirmed that insulin has strong interaction efficiency to CHP, and it is easy to evaluate release behavior and interaction with CHP by size exclusion chromatography in the previous study [25].

2. Experimental

2.1. Materials

CHP in which pullulan was substituted with 1.2 cholesteryl groups per 100 glucose units was purchased from Nippon Oil and Fat Co. (Tokyo, Japan). The molecular weight of CHP is 1×10^5 g/mol. Di-n-butyltin (IV) dilaurate (DBTDL) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2-acryloyloxyethylisocyanate (AOI) was purchased from Showa Denko Co. (Tokyo, Japan). Irgacure 2959 was kindly provided by Toyotsu Chemiplas Co. (Tokyo, Japan). Polyethylene glycol diacrylate (PEGDA, MW = 1.1×10^4 g/mol) was synthesized as previously reported [26]. Fluorescein isothiocyanate (FITC)-labeled insulin and phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma Aldrich (Tokyo, Japan).

2.2. Synthesis of CHPOA

CHP was used after being dried under vacuum at 70 °C for 3 days. CHP (2.0 g) was dissolved in 100 mL of dry dimethyl sulfoxide (DMSO); this was followed by the addition of DBTDL (580 μL), and AOI (385 μL). The resulting mixture was stirred in dark condition for 24 h at 45 °C (Scheme 1). The reaction solution was dropped into a diethylether/ethanol solution (8/2, v/v), and the isolated solid material was suspended in DMSO and dialyzed against deionized water (MWCO 3500) for 1 week, and was then freeze-dried.

2.3. Preparation of nanogel-integrated or cross-linked hydrogels using photopolymerization

The nanogel-integrated hydrogels and cross-linked hydrogel were prepared using radical photopolymerization in water (Scheme 2). CHPOA (final concentration: 5–40 mg/mL) was swollen in distilled water for 24 h at room temperature. The resulting solution was centrifuged for 30 min at 20,000 *g*, and filtered through two filters with different pore sizes (0.45 and 0.22 μm). Subsequently, PEGDA was added to the CHOPA solution, and irgacure 2959 was then added as an initiator for the radical photopolymerization. The molar ratios for the acryloyl groups in the PEGDA was adjusted to 0–20 mol% of total acryloyl group content in the PEGDA and CHPOA, and the concentration of irgacure 2959 was 3.0 mol% of the total acryloyl group content. After mixing, the solution was polymerized under irradiation with 365 nm-wavelength light for 10 min. The degree of gelation was estimated using the vial-tilting method.

2.3.1. ^{1}H NMR spectroscopy

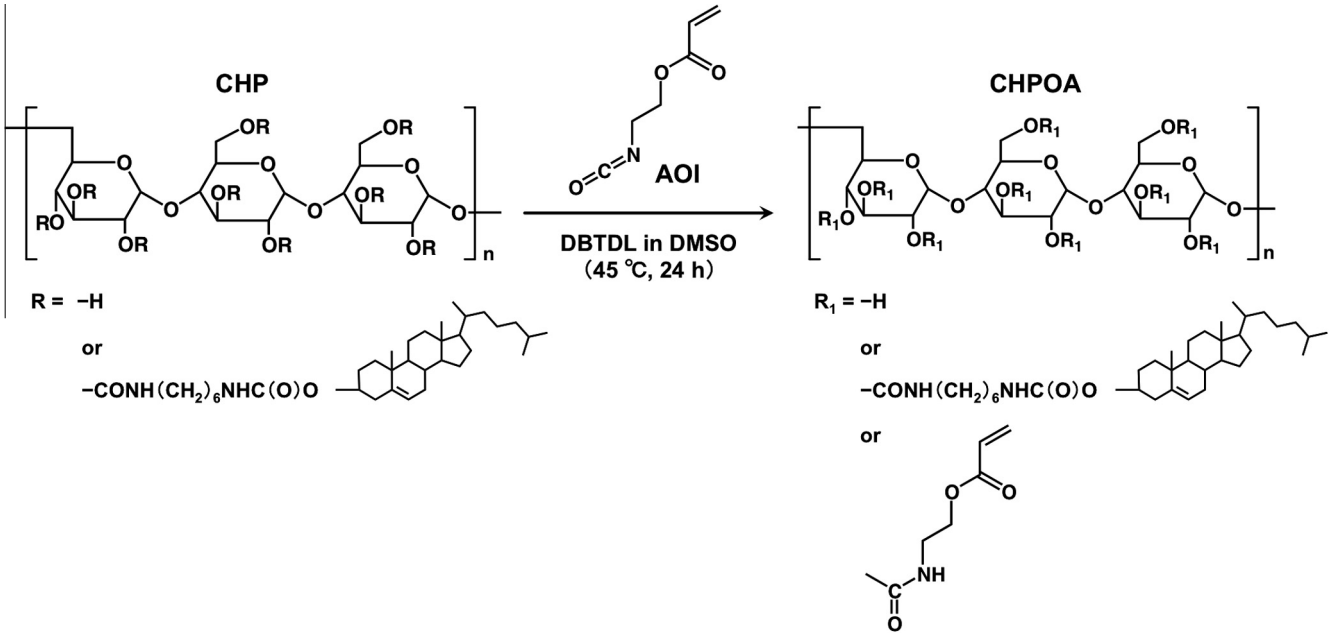
The degree of substitution of the acryloyl groups in the CHPOA or hydrogels prepared using photopolymerization was determined using ^{1}H nuclear magnetic resonance (NMR) spectroscopy (500 MHz, DMSO-d_6/D_2O = 9/1 (v/v)).

2.4. Dynamic light scattering

The CHPOA nanogels and nanogel-integrated hydrogel particles were suspended in PBS. The suspensions were centrifuged (20,000 *g*, 30 min, and 25 °C) and filtered through a Millipore filter (pore size 0.22 μm). The hydrodynamic diameter of the particles in the suspensions was determined using dynamic light scattering (DLS; Zetasizer Nano; Malvern, UK). The scattering angle was maintained at 137°, and the wavelength was set at 633 nm.

2.5. Interaction of FITC-labeled insulin with the nanogel-cross-linked hydrogels

The nanogel-cross-linked hydrogels were prepared as described above. The concentration of CHPOA was 30 mg/mL, and the molar



Scheme 1. Synthetic scheme for CHPOA.