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Polysaccharide based bionanocomposite hydrogels reinforced with cellulose nanocrystals: Drug release and biocompatibility analyses

Kummara Madhusudana Rao*, Anuj Kumar, Sung Soo Han*

School of Chemical Engineering, Yeungnam University, 280-Daehak-Ro, Gyeongsan 712-749, South Korea

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ABSTRACT

Cellulose nanocrystals (CNCs) reinforced, bionanocomposite (BNC) has shown great potential in tissue engineering and drug delivery applications due to the low toxicity, low density, and high aspect ratio. In this work, BNC hydrogels reinforced with CNCs were fabricated from xanthan (XG) solutions and chitosan (CS) in presence of green acidifying agent through electrostatic and hydrogen bonding interactions. As developed BNC hydrogels were characterized for complex formation, morphology, and mechanical behavior. The mechanical performance of BNC hydrogels was improved significantly as increased CNC content (from 2 to 10 wt%). 5-Flurouracil as model chemotherapeutic agent was loaded into these BNC hydrogels for evaluating their drug release properties. The BNC hydrogels showed excellent cytocompatability and ability to release of chemotherapeutic agent that shows the suitability to be used in tissue engineering as well as drug delivery applications.

1. Introduction

Polysaccharides are widely distributed in nature derived from renewable resources such as animals, micro-organisms, and plants [1] and may offer a variety of potential biomedical applications [1–3]. In recent years, polysaccharides have attracted much attention as biomedical intelligent hydrogels, because of their biodegradability, biocompatibility, readily available and cost effective nature [2,3]. Among polysaccharides chitosan (CS) has attracted great attention in biomedical applications. CS is a nitrogen-rich polymer composed of randomly distributed β-(1/4)-D-glucosamine and *N*-acetyl-D-glucosamine [4,5]. This semi-crystalline biopolymer has biocompatibility and biodegradability with good aqueous adsorption capabilities, which make CS a good choice for a variety of applications such as wound dressing, tissue engineering and drug delivery [4,6,7]. Generally, biopolymer like CS has poor mechanical properties, which restricts its application in certain fields [8]. Thus, nanofillers have been used in order to improve the mechanical properties without affecting the biocompatibility and their properties [8,9]. Among various kinds of nanofillers, cellulose nanocrystals (CNCs) as polysaccharides have gained importance for making hydrogels, because of good mechanical properties, biocompatibility, commercial availability, nontoxicity, and biodegradability [10]. Recently, hydrogels reinforced with CNCs have received considerable interest for application in tissue engineering as well as drug delivery [10,11].

Xanthan gum (XG) is an extracellular hetero polysaccharide produced by xanthomonas campestris [12]. It consists of D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in a 2:2:1 molar ratio and variable proportions of *O*-acetyl and pyruvyl residues. XG has a cellulosic backbone and a trisaccharide side chain on every second glucose residue in the backbone by α-1,3 linkages composed of mannose (β-1,4)glucuronic acid(β-1,2) mannose [12]. Recently, XG/nanohydroxyapatite based hydrogels have been used for tissue engineering applications osteoblast growth [13]. The blends of XG with other biopolymers such as kappa, and iota-carrageenan, chitosan, and konjac gum have been used for skin tissue engineering applications [14,15]. Recently scaffolds made from xanthan and polypyrrole in combination with external magnetic field offer a new strategy for improved fibroblast cell proliferation [16]. In addition it has good properties for preparation of variety of drug delivery vehicles for controlled drug delivery applications [17].

Polyelectrolyte complexation is well known for preparing hydrogels by mixing of CS solutions to anionic polymer solutions [18–20]. However, the mixing of two polymer solutions leads to fast complexation and appearance of precipitates. As demonstrated in previous reports [21,22], CS particles have simply been mixed with anionic solution followed by the addition of D-(+)-glucuronic acid δ-lactone (GDL), which leads to slow increase viscosity of the solution.

* Corresponding authors.
E-mail addresses: msraochem@yu.ac.kr (K. Madhusudana Rao), sshan3892@gmail.com, sshan@yu.ac.kr (S.S. Han).

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In this case, CS abstracts protons from GDL and XG making polyionic interactions between NH_3^+ groups of CS and COO^- groups of XG. In the view of these literatures, in this study, we were interested to prepare bionanocomposite (BNC) hydrogels from CS and CNCs-XG blends using green acidifying agent. The use of biopolymers and nanofiller like CNCs have advantageous involving biocompatibility, low toxicity, low cost, and simple gelation with anionic XG and CNCs with CS in presence of GDL as green acidifying agent without using any other organic solvent. The obtained BNC hydrogels were characterized by FTIR, XRD, SEM, and compressive properties to confirm the chemical interactions, morphology, crystalline structure and mechanical performance. 5-Flurouracil (5-FU) is a model chemotherapeutic agent was encapsulated into BNC hydrogels in order to evaluate the invitro drug release behavior BNC hydrogels. Finally, BNC hydrogels were evaluated for their cytocompatability on NIH3T3 as model fibroblast cell line for tissue engineering and drug delivery applications.

2. Materials and methods

2.1. Materials

Chitosan (CS, medium molecular weight with an 84% degree of deacetylation), xanthan gum (XG), 5-fluorouracil (5-FU) and D-(+)-glucuronic acid δ-lactone (GDL), were purchased from Aldrich chemicals Co. Cellulose nanocrystals (CNCs) was prepared as per our previously published research work [23]. All chemicals are of analytical grade and used without further purification. Deionized (DI) water was used throughout the experimental procedures.

2.2. Cell culture

NIH3T3 fibroblast cells were cultured using Dulbecco's Modified Eagle Medium (DMEM) contained 10%fetal bovine serum (FBS) and 1% penicillin G-streptomycin (gibco, life technologies, south korea). Cells were maintained in humidified 5% CO_2 incubator at 37 °C.

2.3. Preparation of bionanocomposite hydrogels

CNCs incorporated BNC hydrogels were fabricated as per previous work reported elsewhere with slight modifications [21,22]. Briefly, 0.75 g of XG solution prepared with different amounts of CNCs (0, 2, 5, 10 wt%) in 25 mL of DI water for overnight stirring in order to complete mixing of CNCs and XG. Then, 0.75 g of CS particles were dispersed in CNCs containing XG solution and then sonicated for 30 min followed by stirring continued for 1 day. After complete dispersions of CS granules in XG-CNCs solution, 0.5 g of GDL was added. CNCs incorporated BNC hydrogels were formed and poured into 24 well plates as mould. The formed BNCs hydrogels were frozen at −80 °C and lyophilised for one week at −80 °C. The obtained freeze dried BNC hydrogels were stored in desiccator for further analysis.

2.4. Characterization studies

A Fourier transform infrared (FTIR) spectrum (FTIR; Perkin Elmer, USA) was used for characterization of structural functional groups and their interactions within BNC hydrogels. The crystalline structure of pure CNCs and BNC hydrogels were characterized by X-ray diffraction technique recorded over the angular range 2θ = 10–70 (XRD, Bruker AXS D8 advance, CuKa radiation source (λ = 1.54 Å)) with scanning rate of 5°/min and operating at 40 kV and 30 mA. The surface morphology of BNC hydrogels were characterized by using scanning electron microscopy (SEM; Hitachi S-4800). Before analysis, the samples were coated with platinum with a low deposition rate. Compressive properties of swollen BNC hydrogels (hydrogels were soaked in PBS buffer at pH 7.4) were analysed using a dynamic mechanical analysis (Q800 DMA; TA Instruments, Seoul, South Korea). For this cylindrical shaped swollen BNC hydrogels of 10 mm in diameter and 8–10 mm in heights were used for stress-strain curves under compression mode (preload force 0.05 N at 37 °C with a rate of 0.5 N/min). The measurements were performed in triplicates and average values were reported.

2.5. Swelling studies

The equilibrium swelling ratio (%ESR) was performed for BNC hydrogels in phosphate buffered saline (PBS) at 37 °C. For pH swelling of BNC hydrogels, the hydrogel samples were equilibrated in various pH solutions (2, 4, 5, 6.5 7, 7.4, 10 pH) for 3 days. The %ESR was calculated using following formula.

$$\%ESR = \left(\frac{W_s - W_d}{W_d}\right) \times 100$$

where W_s is the weight of the swollen BNC hydrogel and W_d is the dry weight of BNC hydrogel. The %ESR was measured triplicate for standard deviation errors.

2.6. Drug loading and invitro release studies

5-FU was loaded into BNC hydrogels via equilibrium swelling method. BNC hydrogels were immersed in 10 mL of 5-FU drug solution (65 mg/mL) for 3 days inorder to load maximum amount of drug into the BNC hydrogels. After freeze drying the samples, the drug loaded BNC hydrogels were placed in phosphate buffer medium (PBS) (pH 7.4) for 3 days. The samples were then agitated using an agate mortar and filtered through filter paper. The filtrate was analyzed for drug content by UV–vis spectrophotometry at λ_{max} of 268 nm. The encapsulation efficiency (% EE) was calculated using the following equation.

$$\%EE = \frac{W_t}{W_i} \times 100$$

where W_t is the total amount of drug in the hydrogels and W_i is the total quantity of drug added initially during preparation.

The drug release studies of 5-FU loaded BNC hydrogels were carried out pH 7.4 PBS release media (30 mL) at 37 °C using shaker at a 100 rpm. 3 mL of release media was withdrawn at regular interval time periods and analyzed using UV–vis spectrometry at λ_{max} 268 nm. All measurements were done in triplicate and the values were plotted with the standard deviation errors.

2.7. MTT assay

The cell viability for BNC hydrogels were performed by MTT assay on NIH3T3 cells. 5×10^4 cells/cm^2 cells were seeded on sterilized BNC hydrogels in a 24 well plate and incubated in humidity chamber (5% CO_2 and 37 °C) for 1 and 3 days. A 100 μL MTT solution (5 mg MTT/mL in PBS) was added to each well and incubated with 5% CO_2 at 37 °C for 4 h. After removal of medium, 200 μL of dimethyl sulfoxide (DMSO) was added to each well and incubated in humidity chamber (5% CO_2 and 37 °C) for 15 min. The solution was transferred to a 96-well plate for recording optical density values using microplate reader (Bio-T Instruments, Inc., USA) at a wavelength of 570 nm. The cell viability was calculated by the following equation.

$$\text{Viability} = (OD_{treated}/OD_{control}) \times 100$$