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Polysaccharide-based magnetically responsive polyelectrolyte hydrogels for tissue engineering applications

Kummara Madhusudana Rao[a,b,*], Anuj Kumar[a,b], Sung Soo Han[a,b,*]

[a] School of Chemical Engineering, Yeungnam University, 280 Daehak-Ro, Gyeongsan 38541, South Korea
[b] Department of Nano, Medical and Polymer Materials, Yeungnam University, 280 Daehak-Ro, Gyeongsan 38541, South Korea

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ABSTRACT

Polysaccharide-based bionanocomposite hydrogels with functional nanomaterials were used in biomedical applications. Self-organization of xanthan gum and chitosan in the presence of iron oxide magnetic nanoparticles (Fe_3O_4 MNPs) allowed us to form magnetically responsive polyelectrolyte complex hydrogels (MPECHs) via *insitu* ionic complexation using D-(+)-glucuronic acid δ-lactone as a green acidifying agent. Characterization confirmed the successful formation of (and structural interactions within) the MPECH and good porous structure. The rheological behavior and compressive properties of the PECH and MPECH were measured. The results indicated that the incorporation of Fe_3O_4 MNPs into the PECH greatly improved mechanical properties and storage modulus (G'). *In vitro* cell culture of NIH3T3 fibroblasts on MPECHs showed improvements in cell proliferation and adhesion in an external magnetic field relative to the pristine PECH. The results showed that the newly developed MPECH could potentially be used as a magnetically stimulated system in tissue engineering applications.

1. Introduction

Polysaccharide-based bionanocomposite hydrogels (BNCHs) are hybrid materials that can be prepared by incorporation of nanofillers, such as graphene oxide, clay materials, and other inorganic nanoparticles into natural biopolymers [1–3]. These BNCHs show an improved biological response due to the presence of nanofillers because of low toxicity, biodegradability, low cost and are easily available from natural renewable sources [4]. In addition, these BNCHs can effectively mimic the extracellular matrix and thus enhance cell growth [5]. Among polysaccharides, chitosan (CS) has been extensively used as a natural polymer for production of hydrogels and their composites in biomedical fields. CS is a nitrogen-rich polymer composed of randomly distributed β-(1/4)-D-glucosamine and *N*-acetyl-D-glucosamine. It has excellent biocompatibility, biodegradability, and nonantigenicity with aqueous adsorption capabilities. The structural characteristics of CS are similar to those of glycosaminoglycans and show good attachment of CS to cell membranes with lower immunogenicity [6]. Therefore, CS is a promising material for fabrication of scaffolds for various applications in tissue engineering. Nonetheless, the disadvantages of a pure CS scaffold are mechanical weakness and an insufficient biological response, which limit its practical applications in tissue engineering. To overcome these drawbacks, combinations of CS with natural and/or synthetic polymers with or without nanofillers have been tested in tissue regeneration applications to enhance cell adhesion and proliferation capacity [7,8]. Compared to conventional hydrogels, polyelectrolyte complex hydrogels (PECHs) are more advantageous for tissue engineering applications. Because these gels contain counter ions located within the polymer network, they can respond to electrically induced chemomechanical contraction as in biological responses [9]. Polyelectrolyte complexation is a well-known phenomenon useful for preparation of hydrogels using two oppositely charged polymers [10,11]. In general, the cationic charge of CS allows for hydrogel formation with anionic natural polymers via polyelectrolyte complexation. It should be noted that the obtained hydrogels yield a good biological response in tissue engineering applications. Therefore, PECHs have shown a considerable potential as excellent matrices for cellular adhesion, proliferation, and differentiation as artificial tissues for tissue regeneration [12,13]. Xanthan gum (XG) is another important anionic extracellular heteropolysaccharide (produced by *Xanthomonas campestris*) [14]. XG has proven to be an excellent matrix for several tissue engineering applications such as bone, cartilage, and skin. Such matrices can congeal *in situ* within seconds,

* Corresponding authors at: School of Chemical Engineering, Yeungnam University, 280 Daehak-Ro, Gyeongsan 38541, South Korea.
E-mail addresses: msraochem@gmail.com (K.M. Rao), sshan@yu.ac.kr (S.S. Han).

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retaining large quantities of water, and thus can provide an environment similar to that of a natural extracellular matrix. XG-based hydrogels composed of other natural polymers and nanohydroxyapatite have been used for tissue engineering applications [15,16]. In addition, the combination of XG with electroactive polypyrrole has been applied to external magnetic field (EMF) tissue engineering tasks for improvement of fibroblast proliferation [17]. Kumar et al. [18] developed a XG-based hybrid scaffold system for cell adhesion and proliferation of preosteoblast cells; it can potentially be employed in low-load-bearing bone tissue engineering applications.

Recently, CS-and-XG-based PECHs were prepared by a green method using D-(+)-glucuronic acid δ-lactone (GDL) as an acidifying agent in an aqueous environment to avoid fast complexation and precipitation [4,19,20]. In this system, GDL acts as an acid (H^+)-generating agent in the aqueous solution, promotes the solubility of CS, and produces cation amino groups that can interact with anionic XG *in situ* via complexation. Rao et al. [4] prepared silver nanoparticles (Ag-NPs) in the PECHs composed of CS and XG. In that study, the Ag-NP-containing PECHs showed good compatibility with (and good adhesion to) NIH3T3 fibroblasts [4]. In another study, mechanically improved BNCHs were developed by incorporation of cellulose nanocrystals into a CS-and-XG-based PECH system and showed a good potential for drug delivery as well as tissue engineering applications [19].

In tissue engineering, interfacial interactions between cells and scaffolds are important for successful tissue regeneration. Therefore, a scaffold can be activated during cell colonization, thus restructuring its architecture according to the different mechanical and anatomical characteristics and the different maturation phases of the tissue. As previously discussed, mechanical properties promote cellular differentiation into bone, cartilage, muscle, and connective tissues [21]. Recently, Fe_3O_4 magnetic nanoparticles (MNPs) were introduced into tissue engineering applications [22,23]. The Fe_3O_4 MNP-incorporating three-dimensional (3D) scaffolds can stimulate cellular interactions in a magnetic field. These MNPs can improve cell adhesion, proliferation, and even differentiation under the influence of an external magnetic field. The development of magnetically responsive hydrogels using green methods is more advantageous than synthetic methods because of the better control of cellular behavior, such as adhesion and proliferation for tissue engineering constructs controlled by magnetic fields. Bringing together polysaccharides and MNPs tends to be a relevant strategy for developing scaffolds from magnetically responsive biocompatible natural renewable materials. The present study explored incorporation of Fe_3O_4 MNPs into a PECH system formed by electrostatic interactions between cationic CS and anionic XG in a facile and green procedure. The newly developed magnetically responsive PECH system (MPECH) was applied to magnetic stimulation of adhesion and proliferation of NIH3T3 cells as a model fibroblast cell line in an external magnetic field. Therefore, the proposed facile ecofriendly method for preparation of biocompatible polysaccharides based on magnetically responsive polyelectrolyte hydrogels opens up promising perspectives for novel functional platforms useful in skin, cartilage, muscle, and connective tissue engineering applications.

2. Experimental

2.1. Materials

CS of medium molecular weight with an 84%–86% degree of deacetylation, XG, GDL (≥99.0% in purity), $FeCl_3{\cdot}6H_2O$, and $FeCl_2{\cdot}4H_2O$ were purchased from Sigma-Aldrich (St Louis, MO, USA). All the chemicals were used as received without further purification. Fe_3O_4 MNPs were prepared by the chemical precipitation method as per previously reported procedure [24]. Sterilized double-distilled water (DDW) was used throughout the experiment.

2.2. Fabrication of an MPECH

A Fe_3O_4 MNP-containing MPECH was prepared using self-organization during polyelectrolyte complexation of XG and CS using GDL as an acidifying agent as described elsewhere with some modifications [4,19,20]. In the present study, 0.1 wt% Fe_3O_4 MNPs were used with 1.5 wt% xanthan and 1.5 wt% CS polymers in 50 mL of sterilized DDW. Briefly, Fe_3O_4 MNPs were completely dispersed in 50 mL of sterilized DDW by sonication for 15 min. Then, XG was added to this Fe_3O_4 MNPs dispersion with shaking, and this incubation was continued overnight to complete dissolution of the XG polymer. Next, CS granules were dispersed in the Fe_3O_4-XG solution to prepare a polymer solution (3.0 wt% total concentration) followed by sonication for 30 min and then shaking overnight. Finally, GDL (1 g) was added after complete dispersion of CS granules in the magnetic XG solution. The CS polymer chains self-organized within the magnetic XG solution via formation of an electrostatic complex followed by hydrogen-bonding interactions with magnetic XG. The resulting hydrogels were freeze-dried (at −80 °C) for 5 day and stored in a desiccator for further characterization and cell experiments.

2.3. Characterizations

The size and morphology of Fe_3O_4 MNPs were analyzed by high-resolution transmission electron microscopy (HR-TEM, JEOL JEM-2010). A drop of MNPs dispersed in ethanol was deposited on the surface of a copper grid and then dried under a lamp. Attenuated total reflectance with Fourier transform infrared spectroscopy (ATR-FTIR) (FTIR, PerkinElmer, USA) was carried out for functional group characterization of CS, XG, Fe_3O_4 MNPs, the PECH, and MPECH. Thermogravimetric analysis (TGA, SQT600) was conducted for evaluation of all the samples (CS, XG, Fe_3O_4 MNPs, PECH, and MPECH) at a heating rate of 10 °C min^{-1} in a nitrogen atmosphere (30–800 °C). For morphological analysis of PECH and MPECH, we used scanning electron microscopy (SEM, Hitachi S-4800). Before the analysis, we freeze-dried PECH and MPECH samples and coated them with a fine platinum layer at a low deposition rate using an ion sputter coater. The crystalline nature of XG, Fe_3O_4 MNPs, PECH, and MPECH was characterized by X-ray diffraction analysis (XRD, 2θ= 10°–70°, Bruker AXS D8 Advance, CuK_a radiation source (λ= 0.1.54 nm)) at a scanning rate of 5°/min and operating at 40 kV and 30 mA. The rheological properties of the PECH and MPECH were determined using an Anton Paar Physica MCR 302 rheometer in dynamic oscillatory shear mode at 30 °C (a parallel plate diameter of 25 mm and a gap of 15 mm using a frequency sweep from 0.1 to 100 Hz at 0.01% shear strain). Compressive properties of cylinder-shaped swollen PECH and MPECH (8 mm in diameter and 5 mm in height) were evaluated by dynamic mechanical analysis (Q800 DMA; TA Instruments, Seoul, South Korea) in compression mode (preload force 0.05 N at 37 °C at a rate of 3 N/min).

2.4. Equilibrium swelling assays

These assays of PECH and MPECH were conducted in phosphate-buffered saline (PBS) at 37 °C ± 0.5 °C during 3-day incubation as per previously reported method [19]. The percentage equilibrium