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Magnetic field-responsive release of transforming growth factor beta 1 from heparin-modified alginate ferrogels

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ABSTRACT

Stimuli-responsive polymeric systems have been widely used for various drug delivery and tissue engineering applications. Magnetic stimulation can be also exploited to regulate the release of pharmaceutical drugs, growth factors, and cells from hydrogels in a controlled manner, on-demand. In the present study, alginate ferrogels containing iron oxide nanoparticles were fabricated via ionic cross-linking, and their various characteristics were investigated. The deformation of the ferrogels was dependent on the polymer concentration, calcium concentration, iron oxide concentration, and strength of magnetic field. To modulate the release of transforming growth factor beta 1 (TGF-β1) under magnetic stimulation, alginate was chemically modified with heparin, as TGF-β1 has a heparin-binding domain. Alginate was first modified with ethylenediamine, and heparin was then conjugated to the ethylenediamine-modified alginate via carbodiimide chemistry. Conjugation of heparin to alginate was confirmed by infrared spectroscopy and proton nuclear magnetic resonance spectroscopy. Sustained release of TGF-β1 from alginate-*g*-heparin ferrogels was achieved, and application of a magnetic field to the ferrogels regulated TGF-β1 release, resultantly enhancing chondrogenic differentiation of ATDC5 cells, which were used as a model chondrogenic cell line. Alginate-based ferrogels that release drugs in a controlled manner may therefore be useful in many biomedical applications.

1. Introduction

Hydrogels contain large amounts of water and are typically prepared from polymeric materials by either physical or chemical cross-linking. Hydrogels are excellent biomaterials for biomedical applications, including drug delivery, as they can mimic many aspects of extracellular matrices of living tissues (Kopeček, 2007; Soppimath et al., 2002). Drugs are, however, conventionally released from hydrogels by diffusion, and their release rate is typically high when the drug molecules are especially water-soluble. Hydrogels with predetermined release rates and prolonged delivery time periods of drugs can be exploited to effectively provide therapeutic benefits to patients (Gupta, Vermani, & Garg, 2002; Huang & Brazel, 2001; Qiu & Park, 2001; Zhang, Wu, & Chu, 2004). Recently, stimuli-sensitive hydrogels that can respond to external environmental changes such as pH, temperature, redox potential, ionic strength, and light have been reported that can provide active control of drug delivery (Lee & Nguyen, 2013; Lendlein & Shastri, 2010). Ferrogels represent one such stimuli-responsive system, which responds to external magnetic stimulation and has attracted much attention as a platform for cell and drug delivery devices (Gonzalez et al., 2014). Ferrogels typically consist of polymeric hydrogels embedded with iron oxide nanoparticles, and their volumes change under magnetic fields, enabling on-demand release of cells and drugs from the gels (Qin et al., 2009). The repetitive application of magnetic fields to ferrogels moves iron oxide nanoparticles and deforms the gels, thereby squeezing out the loaded drug (Satarkar & Hilt, 2008).

Alginate was chosen as a base material for manufacturing ferrogels, as it has been widely used in many biomedical applications, due to its excellent biocompatibility (Rowley, Madlambayan, & Mooney, 1999). Alginate is a natural biopolymer obtained from brown algae, and is composed of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues (Gombotz & Wee, 2012). An alginate solution can form hydrogels in the presence of divalent cations such as calcium ions by cross-linking the blocks of G-residues present in the alginate backbone. Alginate hydrogels cross-linked with calcium sulfate have frequently been used as delivery vehicles for cells and drugs (Kuo & Ma, 2001; Rowley et al., 1999). The continuous release of growth factors enhances successful tissue regeneration in tissue engineering approaches (Jeon, Powell, Solorio, Krebs, &

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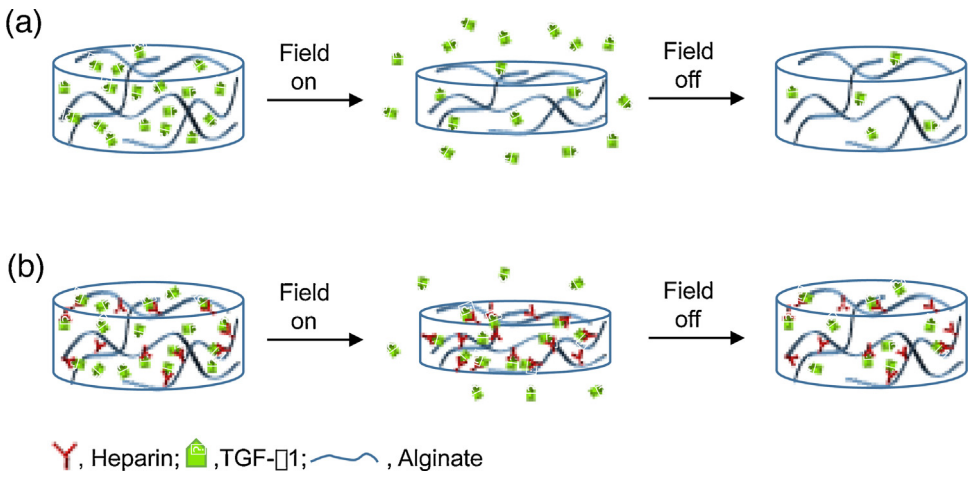

Fig. 1. Schematic representation of TGF-β1 release from (a) alginate ferrogel and (b) alginate-*g*-heparin ferrogel upon the application of a magnetic field. Iron oxide nanoparticles are not illustrated here.

Alsberg, 2011). Therefore, the sustained and actively controlled release of growth factors from alginate gels may be critical when the gels are designed to be utilized in tissue engineering applications. However, the release of hydrophilic drugs, including growth factors, from alginate gels is generally fast and often uncontrollable, which may be undesirable for long-term uses.

One interesting approach to controlling the release rate of growth factors from hydrogels is the introduction of heparin into the gels via chemical conjugation (Jeon et al., 2011). Heparin has the highest electronegative charge among biological molecules, which facilitates its binding to many proteins and has been used to provide controlled release of growth factors (Wu et al., 2011). Transforming growth factor beta 1 (TGF-β1) was chosen as a model growth factor in this study due to its utility in various clinical indications (Roberts, Mccune, & Spore, 1992) and its reversible binding to heparin. Human TGF-β1 and TGF-β2 bind to heparin, but TGF-β3 does not (Rider, 2006). TGF-β1 (25 kDa) is an important chondrogenic growth factor (Han et al., 2005), and has the ability to accelerate the chondrogenic differentiation of progenitor cells and enhance chondrocyte proliferation. In addition, the extracellular matrix of cartilage is synthesized in response to TGF-β1 (Holland et al., 2007).

In this study, we hypothesized that heparin-grafted alginate (alginate-*g*-heparin) ferrogels could be prepared by ionic cross-linking, and that TGF-β1 release from the ferrogels could be actively regulated by the application of magnetic stimulation (Fig. 1). Alginate-*g*-heparin was synthesized by conjugation of ethylenediamine to the alginate backbone, followed by reaction with heparin via carbodiimide chemistry. Various characteristics of alginate-based ferrogels, including their mechanical properties and deformation depending on experimental parameters, were investigated. The release behavior of TGF-β1 from alginate-*g*-heparin ferrogels was monitored *in vitro* under stimulation with magnetic fields, and the bioactivity of the released TGF-β1 was tested using chondrocytes.

2. Experimental

2.1. Materials

Sodium alginate (number-average molecular weight = 26.5 kDa; M/G = 6.6) was purchased from Alfa Aesar (Ward Hill, MA, USA). Iron oxide nanoparticles (Fe_3O_4; mean diameter = 10–15 nm) were purchased from SkySpring Nanomaterials (Houston, TX, USA). Heparin sodium salt (molecular weight = 17 kDa), ethylenediamine, calcium sulfate, 2-(*N*-morpholino)ethanesulfonic acid (MES), 1-ethyl-3-(dimethylaminopropyl) carbodiimide (EDC), and ethylenediaminetetraacetic acid tetrasodium salt (EDTA) were purchased from Sigma (St. Louis, MO, USA). Dulbecco's phosphate buffered saline (DPBS) containing calcium and magnesium ions, Dulbecco's modified Eagle's medium (DMEM), and Ham's F-12 medium (DMEM/F-12) were purchased from Gibco (Grand Island, NY, USA). *N*-Hydroxysulfosuccinimide sodium salt (sulfo-NHS) was purchased from Thermo Scientific (Pittsburgh, PA, USA). Transforming growth factor beta 1 (TGF-β1) and a TGF-β1 ELISA kit were purchased from BioLegend (San Diego, CA, USA).

2.2. Synthesis of alginate-g-heparin

Heparin-grafted alginate (alginate-*g*-heparin) was synthesized by carbodiimide chemistry. Alginate (1 g) was dissolved in MES buffer (0.1 M, pH 6.5). EDC (27.4 mg), sulfo-NHS (48.4 mg), and ethylenediamine (60 μl) were added to the alginate solution and mixed vigorously for 16 h at room temperature. Once the reaction had completed, the mixture was placed into a dialysis bag and dialyzed against distillated water for 4 days. After dialysis, the solution was lyophilized. Alginate modified with ethylenediamine (0.33 mg) was dissolved in MES buffer, and heparin (10 mg) dissolved in MES buffer was added to the solution (MES buffer, pH 6.0) in the presence of EDC (11 mg) and sulfo-NHS (13 mg) with vigorous stirring for 16 h at room temperature. After dialysis against distilled water, the solution was lyophilized. Synthesis of alginate-*g*-heparin was confirmed by Fourier transform infrared spectroscopy (TENSOR^{TM}-27, Bruker Optics; Billerica, MA, USA). Powdered sample was mixed with potassium bromide and the mixture was compressed to form disks. The average acquisition was 64 scans (resolution, 4 cm^{-1}) at a rate of 4 mm/s over a wavenumber range of 1000–1800 cm^{-1}. A ^1H NMR spectrometer (Avance-500, Bruker; Karlsruhe, Germany) was also used to confirm conjugation between alginate and heparin (D_2O, 10 mg/ml). The heparin content in the conjugate was determined by elemental analysis (Thermo Finnigan Flash, San Jose, CA, USA).

2.3. Fabrication of alginate-based ferrogels

Alginate-based ferrogels were fabricated by ionic cross-linking of an alginate solution containing iron oxide nanoparticles using calcium sulfate. In brief, alginate was dissolved in distilled water and iron oxide nanoparticles were dispersed in the alginate solution using a vortex mixer. A mixture of an alginate solution and iron oxide nanoparticles was cross-linked with calcium sulfate (17 mM)