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Magnetic hydrogels for levodopa release and cell stimulation triggered by external magnetic field

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ABSTRACT

Magnetic responsive hydrogels composed of alginate (Alg) and xanthan gum (XG), crosslinked with Ca^{2+} ions, were modified by in situ magnetic nanoparticles (MNP) formation. In comparison to magnetic Alg hydrogels, magnetic Alg-XG hydrogels presented superior mechanical and swelling properties, due to the high charge density and molecular weight of XG. The loading efficiency of levodopa (LD), an important antiparkinson drug, in the Alg-XG/MNP hydrogels was the highest (64%), followed by Alg/MNP (56%), Alg-XG (53%) and Alg (28%). A static external magnetic field (EMF) of 0.4 T stimulated the release of LD from Alg-XG/MNP hydrogels achieving 64 ± 6% of the initial loading after 30 h. The viability, proliferation and expression of dopaminergic markers of human neuroblastoma SH-SY5Y cell on the LD loaded magnetic hydrogels were successful, particularly under EMF, which stimulated the release of LD. Overall, the results of this study provided the rational design of magnetic hydrogels for the delivery of drugs, which combined with external magnetic stimulus, might improve cell proliferation and specific differentiation.

1. Introduction

Magnetic responsive polymeric materials are interesting because they can be stimulated by low cost non-invasive static external magnetic field (EMF), enhancing cell proliferation and drug delivery [1–8]. The MNP tend to rotate to align with the EMF, causing mechanical vibrations of the polymeric chains in the matrix and facilitating the drug release [2,5–8], ions transport through membranes [3] or cell signaling [9]. Such magneto-mechanical stimulus on drug release was observed for dopamine from alginate beads [2], mitoxantrone, plasmid DNA and fibroblasts from alginate ferrogels [10], insulin from microcapsules of alginate/chitosan [5], diclofenac sodium from κ-carrageenan and carboxymethyl chitosan beads [6] and cisplatin from starch particles [7]. Cells in contact with magneto sensitive scaffolds might experience forces in nanoscale similar to the mechanical forces that they meet in their natural environment, stretching the cell membrane, activating channels and receptors [11]. For instance, polymer/MNP hybrid scaffolds promoted bone repair and regeneration in the absence [12] and in the presence of static EMF [13–16]. The proliferation of fibroblasts was favored on xanthan/MNP [1] or xanthan/polypyrrole [17] scaffolds exposed to static EMF of 0.4 mT. Magnetic xanthan scaffolds stimulated in vitro neuronal differentiation of embryonic stem cells into sensory neurons [4].

In the present work, we present the preparation and characterization of magnetic hydrogels containing alginate (Alg) and xanthan gum (XG) crosslinked with Ca^{2+} ions and in situ synthesized MNP. Alg and XG are anionic polysaccharides widely applied as drugs and proteins carriers and as scaffolds for cells owing to their non-toxicity, non-immunogenic, biodegradability and biocompatibility [18,19]. They can form gels in the presence of divalent cations, such as Ca^{2+} ions in aqueous media [20–22]. Beads of XG and Alg mixture can be prepared by ionotropic gelation method using Ca^{2+} ions as crosslinking agent, resulting in controlled drug release systems [22,23]. In a recent report, we demonstrated that electrostatic interactions between scaffolds charges and MNP charges might play an important role on the cells magnetic sensitivity, stimulating their proliferation [3]. Considering that XG chains have higher charge density than Alg chains, we hypothesize that magnetic hydrogels prepared with both polysaccharides should (i) provide a more stimulating environment for cell proliferation than the magnetic scaffolds prepared with pure Alg and (ii) be better drug carriers

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than pure magnetic Alg hydrogels. Levopoda, (2S)-2-amino-3-(3,4-dihydroxyphenyl) propionic acid was chosen as drug due to its relevance in the treatment of Parkinson's disease [24]. It is generally administered orally or intravenously in combination with a decarboxylase inhibitor [25] or with carbidopa [26] to prevent the fast metabolism of levodopa (LD) without reaching the brain. The majority of individuals with Parkinson's disease experience motor fluctuations because the neuronal uptake of levodopa as well as storage and release of dopamine become less efficient along the time. Typically these fluctuations result from deficiencies in the delivery of LD due to the poor pharmacokinetic profile [27,28]. Considering the above-mentioned advantages of magneto responsive polymeric materials and the challenges exhibited by the drug itself, we hypothesized that the magnetic Alg/XG hydrogels might be promising materials for drug release devices controlled by static external magnetic field (EMF), which could prolong the therapeutic effect, reducing the number of administrations and stimulate the proliferation of neuronal cells.

2. Materials and methods

2.1. Materials

Alginic acid sodium salt (Alg, Sigma 180947, mannuronate/guluronate ratio = 1.56, M_v from 120000 $gmol^{-1}$ to 190000 $gmol^{-1}$), xanthan gum (XG, M_v 1.1×10^6 g/mol, degree of pyruvyl = 0.38, degree of acetyl = 0.41, Kelco, USA) and levodopa (LD, Sigma PHR1271, M = 197.19 g). Supplementary Information Fig. S1 represents the chemical structures of Alg, XG and LD with its ionization states. LabSynth (Brazil) provided $FeCl_2{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, methanol and ammonium hydroxide. All reagents were of analytical grade and used as received.

2.2. Synthesis of magnetic responsive hydrogels

Magnetic responsive hydrogels were synthesized by a two-steps procedure, as represented in Supplementary Information Fig. S2. Briefly, first aqueous solutions of sodium alginate (Alg) at 1% (w/v) and xanthan gum (XG) at 1% (w/v) were prepared by dissolving each polysaccharide in distilled water. The solutions were homogenized with an IkaTurrax® stirrer at 18,000 rpm for 5 min and kept in refrigerator for two hours to remove air bubbles. Then Alg solution or a mixture of Alg and XG solutions (1:1 in volume) were cast in polypropylene molds (2.7 cm x 4.5 cm × 0.4 mm height) and allowed to dry in an oven at (45 ± 2) °C overnight in order to evaporate the solvent and to form films. The dried polymeric films were removed from the molds and immersed into a 1% (w/v) $CaCl_2$ solution for 15 min for crosslinking at (24 ± 1) °C. The polymeric films were then removed from the $CaCl_2$ solution and rinsed with MilliQ water until conductivity achieved 10 μS/cm. The synthesized films of sodium alginate and sodium alginate with xanthan gum will be hereafter referred to as Alg and Alg-XG, respectively. In the second step, the magnetite particles were synthesized by co-precipitation [1]. The dried polymeric films (~1.0 g) were added to 100 mL solution of Fe(III) and Fe(II) ions at 0.2 mol L^{-1} and 0.1 mol L^{-1}, respectively. Then, the system was kept under mild magnetic stirring at a constant temperature of 60 °C for 15 min. Next, ammonium hydroxide solution at 25% (v/v) was added drop wise until pH raised to 10, while the system was kept at 60 °C for another period of 15 min, under vigorous stirring for the magnetite formation by co-precipitation. After heating the system at 60 °C for another 15 min, the solution was cooled down to (24 ± 1) °C and neutralized to pH ~ 7. The films containing the magnetite particles (MNP) were then washed three times in methanol solution (50% v/v) with the help of a plastic 400–500 μm mesh sieve. Upon rinsing and sifting the system, the magnetic nanoparticles that were not incorporated to the films flowed to the filtrate, as evidenced by the light brown color of filtrate. After three times rinsing with methanol, the filtrate was colorless; indicating that most physically attached magnetic particles had been already removed from the polymeric films by rinsing with methanol. Finally, the films were dried in the oven at 40 °C for 24 h. The polymeric magnetic films will be hereafter referred to as Alg/MNP and Alg-XG/MNP. Noteworthy, pure XG crosslinked with Ca^{2+} ions yielded films with poor stability in water or phosphate buffered saline (PBS) solution.

2.3. Characterization of magnetic films

The morphology of Alg, Alg-XG, Alg/MNP and Alg-XG/MNP samples was investigated using a JEOL Neoscope JCM-5000 microscope. The films were swollen in distilled water, freeze-dried and cryofractured; the cryofracture surfaces were analyzed after gold coating of ~2 nm (sputtering). The surface morphology of freeze-dried films was analyzed by means of atomic force microscopy (AFM) in the air with a PICO SPM-LE (Molecular Imaging) microscope operating in the intermittent contact mode in air at room temperature, using silicon cantilevers with a resonance frequency close to 300 kHz. Images (512 × 512 pixels) processing and calculation of root mean square roughness (rms) values were performed using the Pico Scan® software.

The molecular structure of dried Alg, Alg-XG, Alg/MNP and Alg-XG/MNP samples was analyzed by Fourier transform infrared spectroscopy in the attenuated total reflection mode (FTIR-ATR) (Zn Se crystal, angle of incidence = 45°), using a Perkin Elmer Frontier equipment with resolution of 4 cm^{-1} and in the wavenumber range of 600–4000 cm^{-1}. FTIR spectra of levodopa (LD) were obtained with KBr pellets (5 mg samples per 200 mg KBr, thickness 1 mm). Tensile tests were performed for dried Alg, Alg-XG, Alg/MNP and Alg-XG/MNP samples in a DMA Q800 from TA Instruments, at (24 ± 1) °C and at ramp rate of 2 N/min. All samples were cut as rectangles (30 ± 2 mm x 6 ± 1 mm) with an average thickness of 0.050 ± 0.010 mm. The tensile properties were determined for at least three samples of each composition. One-way analysis of variance (ANOVA) with Tukey post hoc test was used to evaluate the differences of mechanical properties among groups. Values of $p < 0.05$ were considered significantly different.

The swelling behavior of Alg, Alg-XG, Alg//MNP and Alg-XG/MNP films in MilliQ was investigated with a Krüss K100 precision tensiometer (Krüss, Hamburg Germany), in the sorption mode, at 25 °C. For this study, the dried polymeric films were cut as discs of 1.1 ± 0.1 cm diameter and then they were placed inside the sample holder, which is a cylinder (1.1 cm diameter) with porous basis (Supplementary Information Fig. S3a). The sample holder was connected to the tensiometer measuring unit, as detailed in the Supplementary Information Fig. S3b. The swelling ratio% was calculated by:

$$\text{Swelling ratio } \% = \left(\frac{m_t - m_d}{m_d}\right) 100\% \quad (2)$$

Where m_d is the mass of dried polymeric films, m_t is the maximal amount of water absorbed by the polymeric matrix at any time "t". The sorption curves were measured for at least three samples of each composition. One-way analysis of variance (ANOVA) with Tukey post hoc test was used to evaluate the differences of mechanical properties among groups. Values of $p < 0.05$ were considered significantly different.

Inductively coupled plasma optical emission spectrometry (ICP-OES) analyses were performed with a Spectro Smart Analyzer Vision equipment (SPECTRO Analytical Instruments GmbH, Germany) in order to determine the iron content, which allowed calculating Fe_3O_4 in the Alg/MNP and Alg-XG/MNP samples. A