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Magnetic hyaluronate hydrogels: preparation and characterization

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ABSTRACT

A novel soft way of hyaluronate (HyA) based magnetic hydrogel preparation was revealed. Magnetite nanoparticles (MNPs) were prepared by co-precipitation. Since the naked MNPs cannot be dispersed homogenously in HyA-gel, their surface was modified with natural and biocompatible chondroitin-sulfate-A (CSA) to obtain CSA-coated MNPs (CSA@MNPs). The aggregation state of MNPs and that loaded with increasing amount of CSA up to 1 mmol/g was measured by dynamic light scattering at pH ~ 6. Only CSA@MNP with $\geq$ 0.2 mmol/g CSA content was suitable for magnetic HyA-gel preparation. Rheological studies showed that the presence of CSA@MNP with up to 2 g/L did not affect the hydrogel's rheological behavior significantly. The results suggest that the HyA-based magnetic hydrogels may be promising formulations for future biomedical applications, e.g. as intra-articular injections in the treatment of osteoarthritis.

1. Introduction

Hydrogels are in the focus of scientific interest because of their tuneable mechanical, chemical and biocompatible properties [1,2]. These materials have been widely used in tissue engineering and drug-delivery [1,3–5]. Magnetic hydrogels have also been produced by filling hydrogels with magnetic particles (e.g. maghemite, magnetite, cobalt-ferrite) [1], this procedure makes them more suitable for certain biomedical applications, such as drug-delivery [1,6,7], enzyme immobilization [1,8–10] or cancer therapy by the combination of controlled drug release and magnetic hyperthermia [1,11]. Magnetic hydrogels have been prepared by three, fundamentally different methods: blending, *in situ* precipitation and grafting-onto methods [1]. Innumerable magnetic hydrogels have been synthesized, e.g. superparamagnetic magnetite nanoparticles (MNPs) in kappa-carrageenan nanospheres [12], *in situ* synthesized MNPs in chitosan hydrogel [13] and magnetic hydrogel of chitosan and MNPs for a potential cancer treatment [14]. Some hyaluronate (HyA) based magnetic hydrogels have been also prepared, e.g. hybrid magnetic hydrogels for controlled drug-delivery prepared from (3-aminopropyl)trimethoxysilane-functionalized magnetite or cobalt-ferrite bound chemically to HyA by N-hydroxysuccinimide and N-(3-dimethylaminopropyl)-N-ethyl-carbodiimidehydrochloride [15]. Injectable *in situ* forming hydrogel has been synthesized from oleic acid-coated iron oxide nanoparticles dispersed in toluene and hyaluronic acid modified with hydrazide and thiol groups (HA-hy-SH) for MRI contrast agent [16]. The giant HyA molecules flocculate MNPs and loose aggregates are formed, which can be prevented only by the pre-modification of either the MNP's surface [15,17] or the chemical structure of HyA [18] or by the combination of the previous two [16,19]. Usually harmful chemicals were used during these preparations. In our laboratory, several types of core-shell magnetite nanoparticles have been produced [20–22] in the absence of risky chemicals by using poly(acrylic acid) (PAA) [23], poly(acrylic acid-co-maleic acid) (PAM) [24] and chondroitin-sulfate-A (CSA) [25] as coating agents to modify the MNP surface.

The osteoarthritis (also called degenerative joint disease) is a common form of arthritis, which causes health problems for millions of people [26]. There are many reasons potentially responsible for the osteoarthritis, such as genetic factors, increased stress (i.e., hard physical work, sport, overweight) and decreased viscoelasticity of the intra-articular fluid. The latter can be caused by the reduced amount of hyaluronate (HyA) in the synovial fluid [27]. The supplement of synovial fluid by hyaluronate-containing intra-articular injection is a common treatment for osteoarthritis [26–30]. The typical composition of these gels (for example Hyalgan, Ostenil-Tendon, SportVis, Synvisc, Synocrom) is an isotonic solution (150 mM NaCl, pH ~7.3 adjusted by buffer) containing 1% of sodium hyaluronate. There are several problems with this treatment, for example the rapid enzymatic degradation of HyA within the joints [31], the pain and the risk of infection during the injection procedure. Furthermore, during movement and under the weight of the patient's body, the synovial fluid could be

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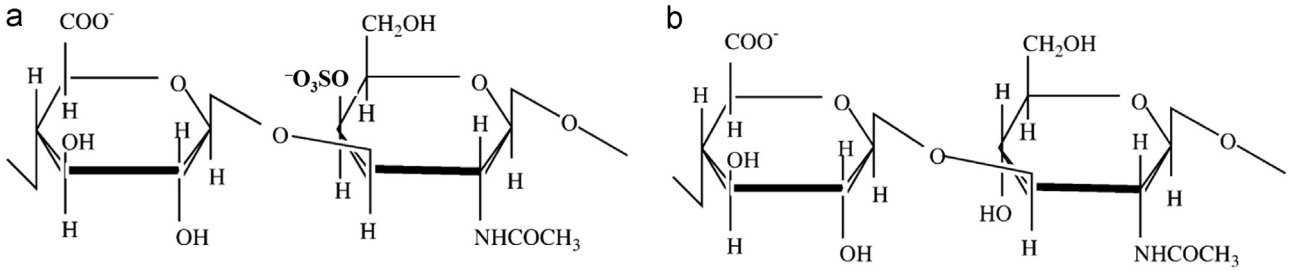

Fig. 1. The repeating units of (a) chondroitin-sulfate-A (CSA) and (b) hyaluronic acid (HyA) [41].

squeezed outside the joints reducing the efficiency of the intra-articular treatments. Our approach is to overcome the latter defect by using magnetic hydrogel and its flowability that can be regulated by an implanted permanent magnet.

The aim of our research was to prepare HyA-based magnetic hydrogel samples in a soft way using biocompatible chemicals, which may be potentially used as an intra-articular injection in the future. The chemical structures of hyaluronate and biocompatible chondroitin-sulfate-A are very similar (Fig. 1), so it is presumable that the surface of CSA-coated superparamagnetic magnetite nanoparticles (CSA@MNPs) is compatible with the HyA-gel. We intend to prepare stable MNPs and CSA-coated MNPs, and to test their stability and aggregation in dilute HyA-gels. Finally, we plan to prepare HyA-based magnetic hydrogels and to test the effect of increasing CSA@MNP concentration on the rheological properties.

2. Materials and methods

2.1. Materials

The magnetite was prepared by co-precipitation [32–36]. The $FeCl_2$, $FeCl_3$ and NaOH for magnetite synthesis were analytical grade reagents obtained from Molar, Hungary. The precipitate was purified carefully through washing and dialysis. It was stored at pH ~3 and 4 °C as stable sol. The resulting iron-oxide was identified as magnetite, based on the characteristic black color, strong magnetism and the X-ray diffraction pattern (JCPDS database [37]). The mean diameter of the MNPs, determined by Scherrer equation and transmission electron microscopy, was ~10 nm [21].

The chondroitin-sulfate-A (CSA) was purchased from Sigma-Aldrich as sodium-salt, and the sodium-salt of hyaluronic acid (HyA) was kindly provided by the Department of Pharmaceutical Technology (University of Szeged, Hungary). One repeating unit of CSA (M=503 Da) contains one –COOH and one $–SO_3H$ group (Fig. 1a) and that of HyA (M=379 Da) contains one –COOH group (Fig. 1b). The strongly acidic sulfate groups ($–SO_3^-$) in CSA are fully deprotonated at a wide pH-range [38,39]. However, the –COOH groups in CSA and HyA have pH-dependent dissociation ($pK_{\beta\text{-glucuronic acid}}$ ~2.9) [40] but, even so, these groups are fully deprotonated ($–COO^-$) at pH ~6.3 applied in our experiments. The notations "CSA" and "HyA" are used in this article for sodium-salt regardless of the actual degree of dissociation of the carboxylic groups. The amount of CSA and HyA is expressed through the mole of repeating units, which equals to the number of dissociable –COOH groups.

The CSA-coated magnetite nanoparticles (CSA@MNPs) were prepared at pH=6.3 ± 0.3 and 10 mM NaCl, the MNP-content was 20 g/L, the CSA-loading was 0.2, 0.4 and 1.0 mmol –COOH/g MNP and the adsorption time was one day. These amounts of CSA completely coat MNPs and provide the high colloidal stability of the CSA@MNP particles [25].

The HyA-gel was prepared by the dissolution of hyaluronic acid in ultra pure water under one day long continuous stirring. The HyA-content of the stock-gel was 11 mg/mL and the pH was ~5.8. Thereafter, the calculated amounts of the naked MNPs or CSA@MNPs at 0.4 mmol/g CSA-loading were added to 9 mL of the previously prepared HyA-gel under vigorous magnetic stirring and the total volume was adjusted to 10 mL using 10 mM NaCl solutions (pH=6.3 ± 0.3). Stirring was continued for five minutes. The final HyA-content of the hydrogels was 10 mg/mL similar to that in commercial HyA-based intra-articular injections (for example Synocrom). The magnetite-content of the magnetic hydrogels was 2.0 g/L for the naked MNPs and 0.0, 0.2 and 2.0 g/L for CSA@MNPs at pH ~6.0 and ~1 mM NaCl. The HyA-gels were stored in closed vials at 4 °C.

NaCl, HCl and NaOH, analytical grade products of Molar (Hungary), were used to adjust the pH and salt concentration in all experiments. Ultra pure water from a Milli-Q RG water purification system (Millipore) was used. All measurements were performed at 25 ± 0.1 °C.

2.2. Particle size determination

For characterization of the aggregation state of magnetic nanoparticles in diluted HyA-systems, the Z average particle hydrodynamic diameter of bare magnetite and of CSA-coated nanoparticles was determined at 25 ± 0.1 °C using dynamic light scattering (DLS) method, an apparatus Nano ZS (Malvern) with a 4 mW He−Ne laser source (λ=633 nm) operating in back-scattering mode at an angle of 173°. The dispersions contained 100 mg/L of magnetite to get an optimal intensity of ~10^5 counts per second. Prior to the measurements, the samples were homogenized in an ultrasonic bath for 10 s, after which 2 min relaxation was allowed. For evaluation, second- or third-order cumulant fit of the autocorrelation functions was used, depending on the degree of polydispersity. The influence of the added HyA amounts (0.0–2.5 mmol HyA/g magnetite) was determined at pH=6.3 ± 0.3 and 10 mM NaCl in case of MNPs and CSA@MNPs. The CSA-loadings were 0.2, 0.4, and 1.0 mmol CSA/g magnetite [25].

2.3. Electrokinetic potential measurements

Electrophoretic mobilities of the naked magnetite and CSA@MNP dispersions were measured at 25 ± 0.1 °C in a Nano ZS (Malvern) apparatus using disposable zeta cells (DTS 1060). The zeta-standard of Malvern (−55 ± 5 mV) was used for calibration. The Smoluchowski equation was applied to convert electrophoretic mobilities to electrokinetic potential values. The accuracy of the measurements was ± 5 mV. The samples were identical to those in the DLS experiments.