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Cross-linked and hydrophobized hyaluronic acid-based controlled drug release systems

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ABSTRACT

This work demonstrates the preparation, structural characterization, and the kinetics of the drug release of hyaluronic acid (HyA)-based colloidal drug delivery systems which contain hydrophobic ketoprofen (KP) as model molecule. Because of the highly hydrophilic character of HyA the cross-linked derivatives at different cross-linking ratio have been synthesized. The hydrophobized variants of HyA have also been produced by modifying the polymer chains with cetyltrimethylammonium bromide (CTAB) at various HyA/CTAB ratios. Due to modifications the coherent structure of HyA changes into an incoherent colloidal system that were verified by rheological investigations. Nearly 70% of the encapsulated KP dissolve from the totally cross-linked HyA carrier but the release rate of KP is about 20% (after 8 h) from the CTAB-modified colloidal system at HyA monomer/CTAB 1:0.8 mass ratio. It has been verified that the modified HyA may be a potential candidate for controlled drug release of hydrophobic KP molecules.

1. Introduction

All areas encapsulating nano- or microparticle-based drug delivery systems have become some of the most fascinating research areas in modern pharmaceutical development. Several biodegradable macromolecules such as polyesters, proteins, polysaccharides, polyelectrolytes, lipids *etc*. (Benko et al., 2015; Csapó et al., 2016; Danhier et al., 2012; Kumari, Yadav, & Yadav, 2010; Padilla De Jesus, Ihre, Gagne, Frenchet, & Szoka, 2002; Palumbo, Pitarresi, Mandracchia, Tripodo, & Giammona, 2006; Pasqui, De Cagna, & Barbucci, 2012), or inorganic materials (layer double hydroxides (LDH), clays, mesoporous silica *etc*. (Deák, Csapó, Juhász, Dékány, & Janovák, 2018; Varga, Benko, Sebok, Bohus et al., 2014) are used as drug carriers in order to achieve a targeted drug delivery system and also a controlled drug release process. Generally, albumin proteins, biocompatible polymers, liposomes, or solid lipid NPs have been utilized for encapsulation of non-steroidal anti-inflammatory drugs (NSAID) such as ibuprofen, meloxicam *etc*. (Benko et al., 2015; Csapó et al., 2016; Varga, Benko, Sebok, Dékány, 2014). Ketoprofen (KP) also belongs to NSAID and is widely used to treat postoperative pain, including patients after a gastric resection.

Hyaluronic acid (HyA) is a well-known linear polysaccharide of alternating units of β-1,4-*D*-glucuronic acid and β-1,3-*N*-acetyl-D-glucosamine. (Berkó et al., 2013; Bodnár et al., 2009; Hashad, Ishak, Geneidi, & Mansour, 2017; Lee et al., 2015; Maroda et al., 2011; Wang et al., 2017) Because of the biocompatible, biodegradable, non-toxic, non-immunogenic and non-inflammatory features this biomaterial is an ideal candidate for several medical and pharmaceutical applications. At physiological conditions (pH, ionic strength) the HyA molecules have a negative charge (hyaluronate form) which results in an extremely high hydrophilic property. Thanks to the hydrophilic character of HyA it is present in several biological fluids, the highest amount can be found in the extracellular matrix of the soft connective tissues. The main disadvantage of this hydrophilic character is that HyA molecules, without chemical structural modification, cannot be simply used as a carrier. In most cases chemical preparation routes have been selected in order to synthesize the hydrophobized derivatives of HyA (*e.g.* biocompatible polymer (HyA/polylactic acid (Mayol et al., 2014)) or different alkyl- and aryl-functionalized derivatives (HyA/decylamine(DA)) (Lee et al., 2015; Vafaei et al., 2016). Some cases the HyA has been used as surface functionalizing agent for preparation of *e.g.* core-shell type NPs (HyA/Human serum albumin-covered chitosan NPs) via electrostatic adsorption of the negatively charged HyA onto the surface of core NPs which has a positive surface charge (Hashad et al., 2017). Besides these derivatives the cross-linked variants of HyA can also be used to

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encapsulate different drugs (Berkó et al., 2013; Bodnár et al., 2009; Maroda et al., 2011). Various techniques have been developed for the production of cross-linked HyA, but one of the commonly used method is the carbodiimde technique. (Bodnár et al., 2009; Maroda et al., 2011) During this procedure the covalent cross-linking of the carboxyl functional groups of HyA molecules is carried out with a diamine in an aqueous medium at room temperature. The main advantage of this technique is that stable colloidal particles can be formed in water without the use of any surfactant or other organic solvent. Another possibility is the chemical modification of the HyA molecules by linking aliphatic or aromatic functional groups to the previously mentioned carboxyl moiety which gives the HyA macromolecules hydrophobicity. (Choi et al., 2009) Moreover, the less-known neutralization of HyA via the formation of electrostatic interactions using positively charged amines containing long aliphatic chains like cetyltrimethylammonium bromide (CTAB) also results in HyA particles having hydrophobic nature. (Kargerová and Pekař, 2014; Oueslati et al., 2014; Sauerová et al., 2015) In previous work by other research groups, the KP was encapsulated in different polymers (*e.g.* poly (D, L-lactic acid) (PDLLA) or Eudragit) or alginate and gelatin-based carriers but the HyA has not been used before for the direct encapsulation of KP molecules. (Arida & Al-Tabakha, 2007; Del Gaudio, Russo, Rosaria Lauro, Colombo, & Aquino, 2009; Vučen et al., 2013)

In this work hydrophobic KP, as the model drug molecule, has been used to develop and characterize different types of modified HyA-based systems for controlled drug release. The cross-linking of HyA has been carried out in aqueous media at different ratios of cross-linking (50; 75 and 100%). Moreover, the hydrophobized derivatives of HyA have also been prepared by using CTAB at three different HyA monomer/CTAB mass ratios (1:0.2; 1:0.5; 1:0.8). Besides structural characterizations the drug release process has also been investigated and the experimental results of different colloidal systems were interpreted. One of the main motivation of this work was to introduce that the extreme hydrophilic HyA after structural modifications is applicable for encapsulation of highly hydrophobic KP small molecules which results in the formation of an effective HyA-based nanosized colloidal systems and a controlled KP release is also feasible.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium salt (HyA, 200–500 kDa) was obtained from Gedeon Richter Plc. Ketoprofen (KP; $C_{16}H_{14}O_3$; ≥98%) and CTAB ($CH_3(CH_2)_{15}N(Br)(CH_3)_3$; 95%), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \times 12H_2O$; 98.5%) and sodium phosphate monobasic monohydrate ($NaH_2PO_4 \times H_2O$; ≥99%) were purchased from Sigma-Aldrich. Sodium chloride (NaCl; a.r.), from Molar Chemicals, was used to prepare isotonic (150 mM) NaCl solution. For the cross-linking reaction 2,2′(ethylenedioxy)bis(ethylamine) (EDEA; $NH_2CH_2CH_2OCH_2CH_2OCH_2CH_2NH_2$; 98%) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimid methiodide (EDC methiodide; $C_2H_5N{=}C{=}N(CH_2)_3N(CH_3)_3I$) were obtained from Sigma-Aldrich. Highly purified water was obtained by deionization and filtration with a Millipore purification apparatus (18.2 MΩ·cm at 25 °C). All solvents and reagents used were of analytical grade and no further purification were made.

2.2. Experimental procedures of the HyA modifications

Cross-linked (cl) HyA derivatives were prepared according to the previously published procedure reported by Bodnár et al. (2009) The synthesis was performed at room temperature. Namely, 200 mg HyA was dissolved in water to produce 1 mg/mL solution then the pH was adjusted to pH = 5.5. The stoichiometric ratio of cross-linking was 50%, 75% and 100% resulting in cl-HyA/50%, cl-HyA/75% and cl-HyA/100% samples. Accordingly, 1.88 mL, 2.82 mL and 3.76 mL EDEA solution (1 v/v%, pH = 5.5) was added to the HyA solution and mixed for 30 min. Then 80 mg, 120 mg and 160 mg EDC methiodide was dissolved in water and added to the mixture drop by drop, respectively. After an overnight stirring the product was purified by dialysis for 7 days against distilled water and the aqueous solution of the final product was freeze-dried. For CTAB modification different calculated amount of surfactant was added to the aqueous solution of HyA to change the hydrophobicity. The mixture was stirred for 30 min before further use.

2.3. Preparation of KP-containing systems

Because of the low solubility of KP in pure Milli-Q water (c_{max} = 0.051 mg/mL) all drug containing samples were prepared in phosphate buffer solution (PBS) at pH = 7.4 at 25 °C using constant ionic strength (0.9% NaCl) which highly increased in the KP solubility (c_{KP} = 20 mg/mL). In all cases constant KP (c_{KP} = 20 mg/mL) and constant HyA concentrations (100.0–100.0 mg lyophilized HyA and cl-HyA/mL) were used. The aqueous KP solutions were added to the different individual cl-HyA and HyA/CTAB samples which resulted in the formation of a gel-like structure after 24 h. After KP loading the samples were diluted to 0.1% and centrifuged (8000 rpm, 10 min). The supernatant contained only 4.5–5.0% remained KP molecules determined by the previously registered spectrophotometric calibration curve. According to this determination method the loading efficiency is *ca.* 95.5–95.0%.

2.4. Methods for structural characterizations

High-resolution transmission electron microscopy (HRTEM) images were recorded on a Tecnai G2 instrument at 200 kV accelerating voltage and they were analyzed using ImageJ software. The particle size and zeta potential values were determined by dynamic light scatteing (DLS) with a Zetasizer Nano ZS ZEN 4003 apparatus (Malvern Ins., UK) equipped with a He-Ne laser (λ = 633 nm). The measurements were performed at 25 ± 0.1 °C, with an angle detection of 173° in a clear disposable zeta cell. In order to determine the maximum amount of CTAB to be added prior to precipitation 0.02 M of CTAB was added stepwise (20–20 μL/step) to 0.2 mg/mL concentration of HyA in PBS and the zeta potential values were registered with DLS. Turbidity measurements were performed by a Precision Bench Turbidity Meter LP2000 (Hanna Ins.), while the conductivity was measured by a Radelkis OK- 114 conductometer equipped with an electrode with sheet plates. The Fourier transform infrared (FT-IR) spectroscopy studies were performed by using Jasco FT/IR-4700 spectrometer with ATR PRO ONE Single-reflection accessory (ABL&JASCO, Hungary). Spectra were registered at 4 cm^{-1} optical resolution by averaging 256 interferograms.

2.5. Isothermal titration calorimetry (ITC) studies

Thermometric titration experiments were performed at 298.15 K with a computer-controlled VP-ITC power-compensation microcalorimeter (MicroCal) in order to determine the degree of the charge compensation of the CTAB in presence of HyA. Deionized water or HyA solution (1.4 mL) in the sample cell was titrated under constant stirring with 300 μL of CTAB solution in aliquots of 10 μL in periodic time intervals of 5 min. The enthalpograms (calorimeter power signal vs time) were evaluated by means of Origin Microcal 7.1. software. ITC curves were successfully fitted by using the sigmoidal Boltzmann equation. The modified version of Boltzmann equation has been used to improve the precision of the determination of the critical micellization concentration (*cmc)* (Juhász, Tabajdi, Dékány, Csapó, 2017; Juhász, Csapó, Vécsei, Dékány, 2017; Király and Dekány, 2001).