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Chemical functionalization of hyaluronic acid for drug delivery applications

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ABSTRACT

Functionalized hyaluronic acid (HA) derivatives were obtained by ring opening mechanism of maleic anhydride (MA). FTIR and H^1 NMR spectroscopy were used to confirm the chemical linkage of MA on the hyaluronic acid chains. Thermal analysis (TG-DTG and DSC) and GPC data for the new products revealed the formation of new functional groups, without significant changes in molecular weight and thermal stability. New gels based on hyaluronic acid modified derivatives were obtained by acrylic acid copolymerization in the presence of a redox initiation system. The resulted circular and interconnected pores of the gels were visualized by SEM. The release profiles of an ophthalmic model drug, pilocarpine from tested gels were studied in simulated media. Evaluation of the cytotoxicity and cell proliferation properties indicates the potential of the new systems to be used in contact with biological media in drug delivery applications.

1. Introduction

Hyaluronic acid (HA) is a linear polysaccharide formed by repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine linked by β (1, 4) and β (1, 3) glycoside bonds (Fig. 1). In physiological conditions, HA appears in the form of sodium salt (HAs), negatively charged and highly hydrophilic.

The architecture of this natural biopolymer exhibits excellent physicochemical properties such as biocompatibility, non-toxicity, non-immunogenic, non-inflammatory and totally biodegradable features [1]. Also, HA is intensively studied for its unique features, such as high water-binding capacity and interesting viscoelastic behavior [2,3].

HA derivatives are currently used in a various number of biomedical applications: arthritis treatment [4], ophthalmic surgery [5], tissue engineering [6], wound healing [7] and drug delivery [8,9].

The potential in drug delivery is correlated with the number of receptors in the body with specific affinity for HA that can be targeted, e.g. RHAMM (receptor for hyaluronic-mediated motility) and CD44 [10,11] and the degradation process of HA. During enzymatic degradation of HA-drug systems CD44 receptors favors the internalization of HA by cells that enable intracellular delivery of drugs. It was already demonstrated that HA is naturally degraded in the body by an enzymatic complex mechanism involving hyaluronidase enzymes and HA cell internalization is facilitated by CD44 cell surface receptors [12,13] or by reactive oxygen species (ROS) [14]. In this context, HA became an extensively explored carrier system for long-acting delivery and depot system for drugs. An important requirement of the HA-drug carrier system is represented by the prolonged residence time of the drugs [15] determined by the molecular weight. The biological properties are dependent on its molecular weight [16], high molecular weight of HA exhibits anti-angiogenic and anti-inflammatory properties, whereas low molecular weight fragments (<100 kDa) have the opposite biological activity [17].

To get a proper HA based drug delivery system with optimal control release [18,19] many chemical modifications modulated by the available functional groups (carboxyl, hydroxyl) from the backbone chain were investigated. Creuzetet al. [20] used an adipic dihydrazide-modified HA intermediate obtained in the presence of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide for the preparation of new water-soluble alkylated derivatives of HA using alkyl aldehydes in water/ethanol mixtures in the presence of sodium cyanoborohydride. Amphiphilic water soluble derivatives of HA were obtained also using alkyl bromides in DMSO [21]. Alkyl silyl diazomethanes were used for the preparation of methyl ester HA derivatives in methanol/diethyl ether mixtures using the protonated form of HA [22]. Carboxyl group has a great impact also in the recognition sites for HA receptors and hyaluronidase, therefore the chemical modification of this moiety would change its biological behaviors in the body [4].

An alternative to maintain carboxylic group integrity [23] could be represented by the chemical modification involving the hydroxyl

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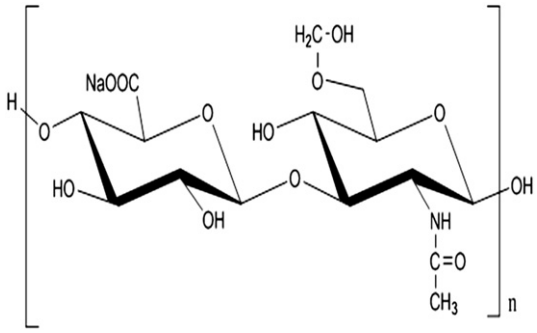

Fig. 1. Chemical structure of HAs.

moiety. HA's hydroxyl group was modified by Kawaguchi et al. [24] using acylchlorides in N, N dimethylformamide in the presence of pyridine in order to obtain new polysaccharide surfactants. Partially hydrophobic and water soluble alkyl ether HA derivatives were synthesized in a water/DMSO mixture by chemical reaction of alkyloxymethyloxiranes [25]. Aryl-or alkyl-vinyl sulfones were used in order to produce aryl/alkyl-vinyl sulfone HA derivatives in water/acetone mixtures in the presence of sodium hydroxide [26], with possible applications in the cosmetics and biomedical industries. Of great interest was also poly(lactic acid) mono-acyl chloride for the hydroxyl group modification, a reaction in DMSO using the cetyltrimethyl ammonium salt of HA. The product was intended to be used in the field of biodegradable plastic materials for the preparation of sanitary and surgical articles, in pharmaceutical and cosmetic fields [27]. However, these methods were conducted to compounds with low molecular weight which can be forward involved in inflammatory process at cellular level, low residence time of the drugs immune-stimulatory and angiogenic effects [28]. Lu et al. [29] examined the corneal endothelial cell and retinal pigment epithelial cell responses to 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) or glutaraldehyde (GTA) cross-linked hyaluronic acid materials. The results concluded that HA crosslinked hydrogels can be successfully applied as delivery vehicles for corneal endothelial cell therapy. In another study, Lai et al. [30] optimized the solvent composition-mediated carbodiimide cross-linking of HA materials by the determination of structural stability, enzymatic degradability and cytocompatibility. The cell viability on human retinal pigment epithelial cell line and pro-inflammatory cytokine expression strongly depended on the solvent concentration and the cross-linking degree.

Functionalized HA derivatives can be used as drug delivery systems in various forms (hydrogels, gels, nanoparticles) for their physicochemical and biological improved properties. Viscous solutions and gels based on bioadhesive polymers are common formulations administrated in the cul-de-sac. Gels permit longer residence time in the precorneal area, an increased contact time of the drug with the tissue and enhanced bioavailability, therefore facilitating transcorneal penetration.

Acrylic acid monomers are frequently used in the ophthalmic drug delivery formulations due to their ability to modulate new properties, control the release profile (prolonged residence time) and improve biological interactions (bioadhesion, cellular internalization) [31].

The present study offered an alternative method in obtaining HA high molecular weight derivatives as precursor for the improvement of drug efficiency, solubility and absorption of hydrophobic active principles. Thus, it presents the synthesis of new HA derivatives by reaction of the pending hydroxyl groups with maleic anhydride, through a ring opening mechanism in organic media, and physicochemical characterization of the obtained functionalized polymer. Furthermore, HA-based derivatives were used for the formulation of new gels by acrylic acid copolymerization in the presence of a redox system, followed by pilocarpine loading/release profile in an ophthalmic simulated media.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium salt (HAs) (from Streptococcus equi; solubility in H_2O of 5 mg/mL), acrylic acid (AA), ammonium persulfate (APS), N,N,N′,N′-tetramethylethylenediamine (TEMED) and pilocarpine hydrochloride were purchased from Sigma Aldrich; maleic anhydride (MA) (≥99.0% (NT) purity) was obtained from Fluka. Amberlite™ IR 120 and acetone, chloroform, and dimethylsulfoxide (DMSO) were acquired from Fisher. Dialysis tubing membrane (Visking 12–14,000 Da) was procured from Medicell International Ltd. DMEM (Dulbecco's Modified Eagle Medium, with 4500 mg/mL glucose, 110 mg/L sodium pyruvate and 0.584 mg/L L-glutamine); BFS (bovine fetal serum, heat inactivated, non-USA origin, sterile-filtered, suitable for cell culture); P/S/N (penicillin/streptomycin/neomycin solution with 5000 units penicillin, 5 mg streptomycin and 10 mg neomycin/mL, sterile-filtered, suitable for cell culture); PBS solution (phosphate buffered saline solution, sterilized, suitable for cell culture); and MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide).

APS was purified by recrystallization from a water/methanol mixture (1/2 v/v).

2.2. HA derivatives preparation

Chemical modification of HA was done in two steps:

A. The exchange of HAs into its acidic form (HAa) by passing it through an ionic exchange column (H^+ Amberlite™ IR 120) for solubility in organic solvents (due to the hydrolysis process of MA in water) (Scheme 1A).
   Briefly, the Amberlite™ IR 120 exchange column was washed with 2 N HCl solution for 30 min and with distilled water until neutral pH. An aqueous solution of HAs (0.1%) was passed through the column, drop-wise. The obtained product was freeze-dried and used in the next step (batch HAa).
B. HA derivatives preparation by reaction of the hydroxyl groups with maleic anhydride, through a ring opening mechanism in organic solvent (Scheme 1B).
   MA is one of the most reactive anhydride which confers the possibility to obtain non-toxic compounds with vinylidene and carboxyl group in the structure by a ring opening mechanism [32,33].

Various concentrations of MA (corresponding to a HAa/MA molar ratio of 1/2, 1/3 and 1/10 denoting batches HA2, HA3, HA10) were added to 0.1% HAa organic solution (DMSO) at 60 °C. The reaction mixture was stirred under nitrogen atmosphere for 24 h. The final product was precipitated with chloroform and separated by centrifugation (3000 rpm, 5 min), washed with acetone, followed by dialysis in water until the conductivity of the washing water registered a constant value, and finally dried and stored for further analysis.

2.3. Gel preparation

The synthesis of gels (HAgel) based on HA derivatives (batch HA10) and AA involves a simple co-polymerization reaction in the presence of an initiator redox system (APS/TEMED) in aqueous solution under temperature. The gel formation mechanism is presented in Scheme 1C. Briefly, an aqueous solution of HA10 (0.1%) was mixed with the monomer solution (1/20 w/w ratio) under stirring at room temperature. Then, an aqueous APS solution (5%) followed by the TEMED was added to the mixture, in the percentage of 1% of AA concentration. The reaction blend was homogenized 10 min and moved to the oven (50 °C) for 24 h. The obtained gels were purified by repeated washing with bidistilled water, followed by freeze-drying. The gels were stored for further use.