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Optimization of spray-dried hyaluronic acid microspheres to formulate drug-loaded bone substitute materials

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ABSTRACT

We present here our first results concerning the evaluation of hyaluronic acid (HA) as a candidate to formulate an organic–mineral cement with sustained release properties. Incorporating drug-loaded microspheres in mineral bone cements is an alternative strategy to improve their ability as drug delivery materials. To synthesize microspheres according to a reproducible process and control at the same time their morphology and their encapsulation efficiency is one of the main challenges of the conception of such drug-loaded bone substitute. In this context, we investigated the potentialities of two HA, differing by their molecular weight, to form microspheres by a spray-drying technique. Erythrosin B (EB) was encapsulated as a model drug and spray-drying process conditions were optimized. To perform this, the rheological behavior and viscosity of HA solutions have been related to their spray-drying ability, and then to the resulting microparticles morphological properties and size distribution. Reproducible microspheres, answering to the requirements in terms of size and encapsulation efficiency, have been obtained from both HA. However the HA exhibiting the lowest molecular weight, HA600, led to smaller microparticles, with a higher polydispersity index. Their swelling ability, related to their stability upon rehydration, also appeared reduced. In this context, HA850, with the highest molecular weight, was selected and the possibility to modulate drug release by HA850 microspheres incorporation into a mineral cement was explored. EB release kinetics from HA microspheres, HA microspheres loaded cement and reference cement were followed at 37 °C, in Tris buffer at pH 7.4, using European Pharmacopoeia flow-through cells. Results showed that HA microspheres incorporation into a mineral cement permitted to modify the cement drug release profile and led to a sustained release.

1. Introduction

Hyaluronic acid (HA) is an abundant non-sulfated glycosaminoglycan component of synovial fluid and extracellular matrices. It is a mucopolysaccharide consisting of repeating units of D-glucuronic acid and N-acetyl-D-glucosamine (Fig. 1). It is a well-known biocompatible, nonimmunogenic and biodegradable polymer, having widespread applications in drug delivery, tissue engineering and viscosupplementation [1–6]. Besides being an important structural component in cartilage, HA is also essential for bone remodeling [7]. HA is an attractive starting material for the construction of bulk gels or hydrogel particles [8] but its applications in bone tissue engineering are limited by its poor mechanical properties. For this reason, it is often associated with calcium phosphates in order to obtain reinforced and/or injectable bone cements, consisting in HA gels containing hydroxyapatite [9,10] or calcium phosphate cements CPCs containing HA [11,12].

To minimize the risk of post-operative failure, surgeons often require the possibility to co-administer therapeutic agents and/or biologically active components limiting inflammation, bacteria proliferation or promoting bone reconstruction (antibiotics, growth factors…). In this context, CPCs can be used as local drug delivery systems [13,14]. An alternative approach for drug loading is to incorporate the drug in polymeric microspheres before blending with CPC. This strategy offers two advantages: polymer microparticles could help to modulate drug delivery [15–20], in combination with cohesion and/or enhanced resorption and remodelling capability [13,14].

Synthetic polymers, mainly poly(lactic-*co*-glycolic) acid PLGA, have been tested for this purpose [15,16,20], but their acidic degradation remains problematic. Surprisingly, polysaccharides have rarely been exploited to form microparticles [18] although they are frequently added to CPCs as rheological modifiers or cohesion promoters [21–23]. HA has never been tested for this purpose.

In this context, we decided to evaluate hyaluronic acid as a candidate to formulate a loaded microspheres–mineral cement whose release properties could be tailored on demand.

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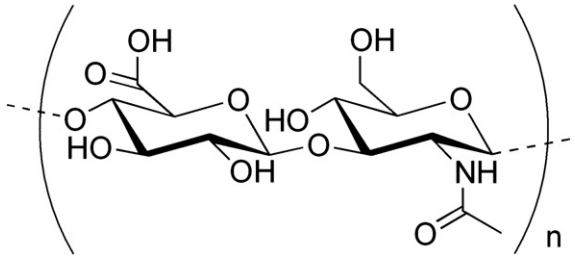

Fig. 1. Chemical structure of the monomer units of hyaluronic acid (HA).

To synthesize microspheres according to a reproducible process and control at the same time their morphology and their encapsulation efficiency is one of the main challenges of such organic–mineral cement design. Process conditions have to be optimized to fit the desired particles characteristics: (i) high encapsulation efficiency, (ii) spherical morphology, (iii) particles mean size and size distribution maintaining the injectability property of the final organic–mineral cement.

In this context, we explored the potentialities of two HA, differing by their molecular weight, to form microspheres with a spray-drying method. Spray-drying method consists of atomizing chemical solutions into droplets dispersed and dried inside a carrier gas, which induces a concentration of non-volatile species and the solidification of particles. It is extensively used in pharmaceutical industries [24,25], since the textural and release properties of the materials are highly tunable thanks to the variation of the solution components.

Erythrosin B (EB), a fluorescein derivative, was encapsulated as a model drug and spray-drying process conditions were optimized. To perform this, the rheological behavior and viscosity of HA solutions have been related to their spray drying ability, and then to the resulting microparticles morphological properties and size distribution. Then the release ability of a HA microspheres–mineral cement was evaluated and compared to the microspheres and the mineral cement release profiles.

2. Materials and methods

Two hyaluronic acid (HA) samples with high molecular weight were tested in this study. HA600 (Cristalhyal, Soliance, France, kindly provided by Pr R. Auzély-Velty from the CERMAV laboratory, Grenoble, France) and HA850 (Primalhyal, Soliance, France) exhibited an average molecular weight of 600,000 Da and 850,000 Da, respectively. Erythrosin B (Tetraiodofluorescein sodium salt, dye content 91.7%) was purchased from Alfa Aesar and Tris (Tris(hydroxymethyl)aminomethane Trizma® base BioXtra, purity ≥99.9%) from Sigma.

2.1. Preparation of HA and HA-EB solutions

Polymer solutions, with HA concentrations ranging from 1 g/L to 10 g/L, were obtained by dispersing polymer powder in deionized water under continuous stirring for 24 hours at room temperature.

2.2. Rheological characterization of HA in solution

Polymers rheological behavior, at concentrations ranging from 1 g/L to 10 g/L, was studied using a controlled stress rheometer (Haake Rheostress RS 75) equipped with a cone plate geometry (6 cm diameter; 1° angle). Measurements were obtained with imposed shear rate ranging from 0 to 2000 s^{-1}.

2.3. Production of HA microspheres: Spray-drying

Microparticles were produced using a Buchi mini Spray Dryer model 190 (Buchi, Germany). Briefly, the polymer solutions, with HA concentrations ranging from 1 g/L to 6 g/L, were fed into the instrument by a peristaltic pump and sprayed with a 0.7 mm nozzle, by means of a flow of compressed air, in the drying chamber of the apparatus. A flow of heated air aspirated by a pump induced the quick evaporation of the solvent from the drops, leading to the formation of solid microparticles. The instrumental settings are reported in Table 1. The obtained particles, after separation from the exhausted air cyclone, settled into a bottom collector and were kept in sealed tubes at ambient temperature prior use.

2.4. Microparticles characterization

Morphology (shape and surface) of the dried microspheres was observed by scanning electron microscopy (SEM) using a LEO 435VP microscope after metallization by silver coating under vacuum by SPI Sputter coating unit.

Particles size distributions were measured using a laser particle sizer Mastersizer 2000 (Malvern, UK) based on a laser light scattering technique. Each sample was measured in triplicate. The volume weighted mean diameters, (D [4,3]) and mean $D(0.5)$, were used to describe the particles size. The polydispersity or span of size distributions was evaluated by calculation of samples polydispersity index $PI = [D(0.9) - D(0.1)]/D(0.5)$

2.4.1. Microparticles recovery: Yield

Microparticles recovery efficiencies were calculated as percentage of weight of the obtained microparticles, taking as reference the total amount of polymer (and EB if added) used for their preparation.

2.4.2. Drug content of microparticles: Encapsulation efficiency

The amount of encapsulated drug per milligram of dried microspheres was determined by visible spectroscopy (Spectrophotometer HP 8451A) at 528 nm (wavelength value corresponding to the λ_{max} of EB), after complete degradation (i.e. after 72 hours) of 100 mg of microspheres into Tris buffer 0.1 M solution at pH 7.4, under stirring at 100 rpm at room temperature. Encapsulation efficiency was calculated by the ratio between this EB amount to the EB amount initially incorporated in the polymer solution.

2.5. Preparation of reference and microspheres-loaded cements

The reference mineral cement (MC) paste was prepared by mixing the appropriate amount of liquid phase (deionized water) with a powder mixture of brushite (DCPD, $CaHPO_4$, $2H_2O$) and vaterite ($CaCO_3$), as previously published [26]. Powder and liquid phase were mixed using a liquid/solid weight ratio of 0.7. In the case of reference EB-cements (MC-EB), EB was added in the solid phase. In the case of microspheres-loaded cements (MC-HA-EB), the required amount of microspheres (10% w/w of the solid phase of the cement) was added to the reference paste after 1 minute of mixing, and mixed until visually homogeneous distribution of the microparticles within the paste was obtained. The pastes were then filled into silicone moulds, placed in sealed containers and let at 37 °C in a water saturated atmosphere for 48 hours, while setting and hardening.

Table 1
Spray drying conditions. Spray-drying parameters of the microspheres manufactured (HA or EB-loaded HA microspheres) for this study using a Büchi B-290 equipment.

Spray-dryer parameters	HA600	HA600-EB	HA850	HA850-EB
Atomizing gas flow rate (L/h)	357	357	357	357
Drying gas flow rate (L/h)	30	30	30	30
Feedstock flow rate (L/h)	0.34	0.34	0.34	0.34
Inlet temperature (°C)	120	120	120	120
Outlet temperature (°C)	55	59	57	65