Biopolymeric microcapsules for the spatiotemporally-controlled delivery of growth factors for the treatment of heart failure

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INTRODUCTION AND OBJECTIVE

Microencapsulation by covalent crosslinking of biosourced polymers is a very versatile method with interesting potentialities for the improvement of drug delivery. Varying the preparation parameters (stirring speed, reaction pH…) allows to finely tune the properties of the microparticles (size distribution, mechanical resistance…). Furthermore, the microcapsule constitutive polymers are more or less likely to interact with the encapsulated drugs by hydrophobic or electrostatic interactions. The choice of these polymers then influences the release kinetics of the encapsulated substance.

Therapeutic angiogenesis is a promising approach for the treatment of cardiovascular diseases, including myocardial infarction (MI) and chronic heart failure. The main problems of current proangiogenic approaches include the limited duration of the therapy achieved with bolus delivery of naked proteins, the high costs associated with protein therapy because of the need for large doses, the transient effects of treatment likely due to the generation of unstable blood vessels, and important safety issues related to the possibility of inadvertent stimulation of angiogenesis in distant, dormant micrometastases. Angiogenic growth factors being positively charged at physiological pH, their delivery through microparticles made of crosslinked anionic polysaccharides could improve the therapy by controlling the delivery, both spatially and temporally. In a previous study, we developed albumin-alginate crosslinked microcapsules as injectable delivery systems for spatiotemporally controlled release of a growth factor combination. The evaluation of functional consequences of targeted intramyocardial growth factor delivery in chronic heart failure showed that this therapeutic angiogenic approach ameliorates cardiac function following myocardial infarction (Banquet 2011a, 2011b).

In the present study, we explored the influence of microcapsule composition, size, and loading, on growth factor release kinetics, in order to optimize the design of the growth factor delivery system.

MATERIALS AND METHODS

Preparation of the microparticles

Albumin-alginate microcapsules were prepared using a modified version of the previously described interfacial cross-linking method (Lévy 1991). Briefly, 4% human serum albumin and 2% propylene glycol alginate were dissolved in a phosphate buffer pH 7.4. This aqueous phase was emulsified in cyclohexane containing 2% sorbitan trioleate, at a stirring speed of 2000 rpm. Then, a 2.5% solution of terephthaloyl chloride in a chloroform-cyclohexane mixture was added to the emulsion and the cross-linking reaction was allowed to develop for 30 min. The microcapsules were separated from the organic phase by centrifugation, and washed. Finally, the microcapsules were freeze-dried in a Freezone 6 (LabConco). The whole procedure was conducted in aseptic conditions. Variations were made to this standard procedure. The alginate ester was replaced by other acidic polysaccharides, like acacia gum or chondroitin sulfate. Finally, for some batches, the protein was omitted in the initial aqueous phase.

The influence of the crosslinking method on the growth factor release kinetics was assessed by preparing albumin-alginate microcapsules using the transacylation method (Callewaert 2009). Briefly, 6 mL of an aqueous phase consisting of 2% PGA and 20% HSA in water was emulsified in 40 mL of an organic phase at a stirring speed of 3000 rpm. After 5 min stirring, 2 mL of a 2% NaOH solution in 95% (v/v) ethanol was added and agitation was continued for 15 min to allow the transacylation reaction to occur. Then, 2 mL of an 8.5% (v/v) acetic acid solution in ethanol was added for the neutralization of the emulsion. After 15 more min, agitation was stopped and the microspheres were washed and finally lyophilized. Variations were made to this standard procedure. The stirring speed was raised in order to prepare smaller particles.

Morphological studies of the microparticles

Diameter measurements were performed using laser diffraction (Particle Sizer LS200, Beckman-Coulter). After staining with methylene blue, the microparticles were observed with a light microscope (Olympus, BH-2) equipped with interferential phase contrast. SEM observations (JSM-5400LV, JEOL) were made after alcohol dehydration of microcapsule suspension followed by Au/Pd coating.

In vitro growth factor release

Briefly, lyophilized microcapsules were loaded with growth factors by imbibition (Hurteaux 2005), with 0.5 to 1.5 µg growth factor per 1 mg microcapsules. Four growth factors were tested: VEGF-A, FGF-2, HGF, and PDGF-BB. Growth factor release after

incubation in extracellular fluid mimetic release buffer was quantified by ELISA. Data are presented as mean amount (nanograms) of growth factor released per day per 1 mg microcapsules (n=3).

RESULTS AND DISCUSSION

Morphological study of the microparticles

The standard procedure led to spherical particles with a mean diameter of 98 µm. With the same preparation procedure, it was also possible to prepare microcapsules with an acacia gum-albumin crosslinked membrane (figure 1A), or a chondroitin-albumin crosslinked membrane.

To prepare microcapsules omitting the serumalbumin, it was necessary to raise the reaction pH in order to favour the acylation of the hydroxyl groups of the polysaccharides. Propylene glycol alginate, acacia gum, carrageenan or chondroitin led to resistant microcapsules when the initial aqueous solution was prepared in 1M NaOH (figure 1B).

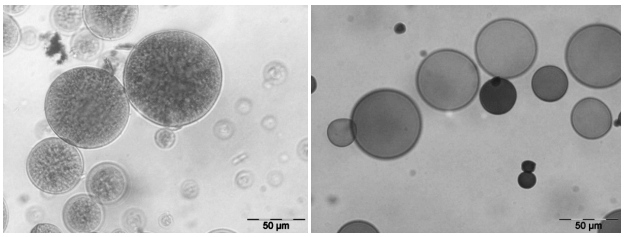

Figure 1: A-acacia gum-albumin crosslinked microcapsules; B-crosslinked chondroitin microcapsules, after staining with methylene blue. The scale bar represents 50 µm.

PGA-HSA microparticles were also prepared using the transacylation method. Three batches were prepared, and the adjustment of the stirring speed led to particles with a mean diameter of 90 µm, 40 µm or 15 µm.

In vitro growth factor release

When we compare the release kinetics of different growth factors from the standard batch, for the same initial payload, the differences are very marked (figure 2).

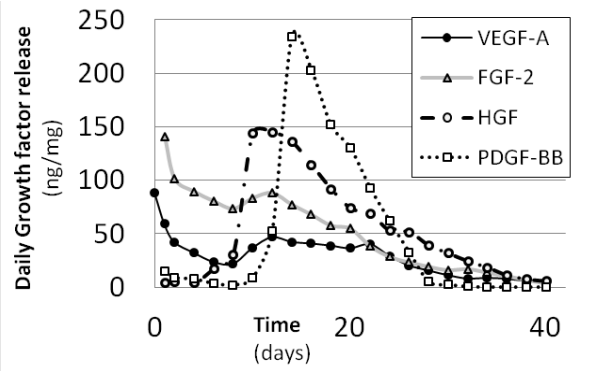

Figure 2: release kinetics from standard batch, initial payload: 1 µg/mg microcapsules.

The initial payload influenced the release kinetics of the studied growth factors from the standard batch.
For a given growth factor, the release kinetics was shown to depend on the constitution of the particle, and on the crosslinking method. For example, for the same initial VEGF payload, the released quantities from albumin-alginate microparticles crosslinked by acyl dichloride was much lower than from albumin-alginate particles prepared using the transacylation reaction.

The physicochemical properties of each studied growth factor are different. Even if they are all positively charged at neutral pH, their interactions with the microparticles may vary as a function of their isoelectric point and of their molecular weight. Another important factor is the constitution of the microparticle, with or without a co-crosslinked protein, more or less crosslinked, leading to a different number of binding sites for the growth factors.

CONCLUSIONS

Microcapsules prepared by crosslinking of anionic polysaccharides, associated with serum albumin or not, can be used to control the release of angiogenic growth factors. The release kinetics is easily modulated by the choice of the microcapsule size and payload, and of the constitutive polymers. The ideal microparticle will be chosen for each growth factor on the basis of the desired *in vivo* kinetics.

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