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Research paper

Spray-congealed solid lipid microparticles as a new tool for the controlled release of bisphosphonates from a calcium phosphate bone cement

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ABSTRACT

The aim of this work was to develop an innovative drug delivery system potentially useful for the local delivery of Bisphosphonates to bone tissue. We propose the use of Solid Lipid Microparticles (MPs), up to now mainly used for oral and topical drug delivery, as carrier for bisphosphonates due to the favourable biocompatibility and lower toxicity of the lipids compared with many polymers. The delivery platform consisted of a biomimetic α-tricalcium phosphate-gelatin cement (CPC) enriched with alendronate loaded MPs (MPs-AL) produced by the spray congealing technology. Alendronate direct addition to cement composition is limited since Alendronate is able to sequester calcium from calcium phosphates, thus preventing the setting of the cements. At variance, this approach permitted to load a relatively high amount of the drug on the CPC and allowed the controlled release of the highly water soluble alendronate. A Design of Experiment (DoE) was employed for the screening of the effects of the formulation variables related to the presence of unloaded microparticle (MPs) on the cement most important mechanical properties. Then, MPs loaded with 10% w/w of alendronate were produced using five different carriers (Stearic Acid, Stearilic Alcohol, Cutina HR, Tristearin and Precirol ATO5). All MPs-AL exhibited a spherical shape, encapsulation efficiency higher than 90% and prevalent particle size ranging from 100 to 150 μm. Solid state characterization (DSC, HSM and X-ray powder diffraction) demonstrated that encapsulation of alendronate into MPs did not alter its crystal structure. MPs-AL addition to the cement provoked a modest lengthening of the setting times and of the hardening reaction leading to the complete transformation of α-tricalcium phosphate into calcium-deficient hydroxyapatite, without significantly affect the cement mechanical properties. Moreover, the results of in vitro AL release study performed on cements enriched with MPs-AL showed that the system allows a controlled release of the drug over time.

1. Introduction

Calcium phosphate cements (CPCs) are biocompatibile and bioactive materials, able to activate osteogenesis [1–4]. CPCs involve mixing a solid phase with a liquid phase, which provides a mouldable paste that stiffens during setting and hardening into a solid phase. As a consequence, they are ideal materials for orthopedic implants that can perfectly fit to a bone cavity. Bone healing process is promoted by CPCs resorption, which can occur via direct action of bone cells and/or through chemical dissolution/hydrolysis in the body fluids [5]. The solid phase of CPCs is constituted of one or several calcium phosphates, whereas the liquid phase is often water or a phosphate solution. Since the development of the first CPC formulation [6], a variety of different compositions has been proposed and the role of a number of parameters on the properties of the cements has been investigated [4,7–9]. Brushite, ($CaHPO_4{\cdot}2H_2O$, DCPD), and hydroxyapatite, both in the stoichiometric form ($Ca_{10}(PO_4)_6(OH)_2$, HA) or as calcium-deficient hydroxyapatite (CDHA) are the possible end members of the cementitious reaction [10].

Most apatitic CPCs relies on the hydrolysis of α-tricalcium

Abbreviations: MPs, microspheres; AL, sodium alendronate; MPs-Excipient, microspheres obtained from different excipients; Alc, stearylic alcohol; Aci, stearic acid; Cut, cutina HR; Pre, precirol ATO5; Ste, tristearin; MPs-AL, alendronate-loaded microspheres; MPs-Excipient-AL, alendronate-loaded microspheres obtained from different excipients; CPCs, calcium phosphate cements; CPCs-MPs, calcium phosphate cements enriched by microspheres; CPCs-MPs-AL, calcium phosphate cements enriched by alendronate loaded microspheres; CPCs-excipient-AL, calcium phosphate cements enriched by alendronate-loaded microspheres of a specific excipient

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phosphate (α-TCP) powder that constitutes the main component of the solid phase and yields an apatitic phase similar to the biological one as result of the hardening process in an aqueous environment at 37 °C [11]. The not exhothermic setting reaction of CPCs has several advantages, including the possibility to load biological molecules and drugs. The possibility to use CPCs as drug delivery systems has been tested through combination of the drugs with the solid or liquid phase, or through introduction in a particulate carrier added to cement formulation [13]. Antibiotics, anti-inflammatory agents, anti-osteoporotic and anticancer drugs, growth factors and proteins are the main categories of substances which have been included in the composition of CPCs [12–14]. In particular, calcium phosphate cements have been proposed to deliver bisphosphonates (BPs) to the target sites in order to overcome the problem of poor availability of these drugs, as well as to minimize the side effects provoked by their systemic administration [14,15]. BPs are potent drugs for the treatment of pathologies characterized by excessive osteoclast mediated bone resorption, such as osteoporosis, Paget's disease and bone metastases, and have also been suggested as potential anticancer agents [16,17]. BPs inclusion in CPCs was obtained through addition to the liquid phase or chemisorption on one of the component of the solid phase [18–21]. The presence of BPs was generally found to lengthen the setting time and decrease compressive strength of the cement [18,21–24].

The use of biodegradable microspheres has been proposed to load other substances, such as growth factors and antibiotics, and also to increase the porosity of the CPCs [25–30]. The most used materials for this purpose are poly(DL-lactic-co-glycolic acid) (PLGA) [26–28,30,31] and gelatin [32,33]. In particular, it was shown that encapsulation of vancomycin in PLGA microspheres provided a longer sustained release with respect to loading the drug in the cement powder [31]. It is conceivable to hypothesize that the use of microparticles (MPs) as drug carriers inside CPCs should avoid/limit the lengthening of setting times caused by substances like bisphosphonates and also provide a tailored sustained release. Herein, we investigate the possibility to use solid lipid microparticles as carrier for bisphosphonates due to the favourable biocompatibility and lower toxicity of the lipids compared with many polymers [34]. Solid lipid MPs can be produced using different technologies such as melt dispersion technique, solvent evaporation, hot and cold homogenization, spray drying and spray congealing [34]. In this work, the spray congealing technique has been selected as it does not require the use of organic or aqueous solvents and hence it is environmentally friendly and less time consuming than other methods [35]. In addition, the spray congealing technology avoids the use of surfactants and it is easily scaled-up. The aim of the work is to design and develop an efficient drug delivery system able to load relatively high amount of sodium alendronate into the calcium phosphate cements, while maintaining suitable cement mechanical properties. In particular the delivery platform consists of CPC enriched with Solid Lipid MPs. As numerous formulation parameters related to the MPs can (negatively) influence the CPCs characteristics, we firstly applied a Design of Experiment (DoE) for the evaluation of the effect of types, dimensions and amount of unloaded MPs on the CPCs most important mechanical properties. Then the study was performed using five different types of excipients to prepare MPs loaded with alendronate and added to the composition of a biomimetic gelatin-α-TCP cement. The setting and hardening properties of the cements were investigated as a function of the presence of alendronate. In addition, in vitro sodium alendronate release study were performed on AL-loaded Solid Lipid MPs and on cement enriched with AL-loaded MPs.

2. Materials and methods

2.1. Microspheres

2.1.1. Preparation of microparticles (MPs) and of alendronate-loaded microparticles (MPs-AL)

For the production of the microparticles, five different excipients were tested: Stearic Acid, Stearilic Alcohol, Cutina HR (hydrogenated castor oil) and Tristearin were purchased from Farmalabor S.R.L., Italy, while Precirol ATO5 (Glyceryl palmitostearate) was kindly supplied by Gattefossè, France. The microparticles were produced by the spray-congealing process using the wide pneumatic nozzle (WPN), which is an external-mix two-fluid atomiser already described in detail in previous work [36]. For the preparation of the MPs and MPS-AL, the excipient was heated at a temperature of 10 °C above its melting point. Alendronate sodium trihydrate (AL) (ChemOs GmbH Germany), when present, was added to the molten excipient and stirred to obtain a homogeneous suspension which was then loaded into the feeding chamber of the WPN. The batch size was 20 g. Two WPN operating parameters can be set: the pressure of the inlet air and the temperature of the device. In the preliminary studies for the production of MPs, the atomisation was carried out setting the air pressure at 3.5 bar and heating the WPN at three different temperature (50, 60 and 70 °C). Then, MPs-AL were obtained with the air pressure at 3.5 bar and the nozzle temperature at 70 °C. The atomisation of the molten fluid led to the formation of melted droplets which then solidified during the fall in the cooling chamber held at room temperature. The final MPs were collected, size separated and stored in polyethylene closed bottles at 4 ± 2 °C.

2.1.2. MPs characterization

2.1.2.1. Particle size and morphological analysis. The size distribution of MPs was evaluated by sieve analysis, using a vibrating shaker (Octagon Digital, Endecotts, London, UK) and 6 standard sieves (Scientific Instruments s.r.l., Milano, Italy) of 50, 75, 100, 150, 250 and 500 μm.

The MPs were observed by using a scanning electron microscope ESEM Quanta 200 (FEI, Cambridge, UK) at 10.0 kV accelerating voltage without any coating.

2.1.2.2. Determination of drug content. 40 mg of MPs-AL were accurately weighted and added to 10 mL of PBS pH 7.2. The suspensions were heated 10 °C above each excipient melting point to melt the carrier and gently shaken for 60 min. The solution was filtered and the drug content was assayed by a spectrophotometric method [37], that requires the use of a derivatizing agent. Briefly, a working solution of the derivatizing reagent was prepared by dissolving 12.5 mg of anhydrous ortho-phthalaldehyde (OPA, Sigma-Aldrich) in 2 mL of PBS, then 62.5 mL di 2-Mercaptoethanol (ME, Sigma-Aldrich) solution were added and the final volume of 25 mL obtained by adding PBS. This solution was freshly prepared before each experiment. For the calibration curve, proper aliquots of AL standard solution (1mg/mL in PBS) were mixed with 2 mL of derivatizing solution and 0.25 mL of NaOH 5M and the volume filled up to 25 with PBS in order to obtain a concentration of AL ranging from 5 to 100 mg/mL; the absorption was measured at 330 nm (R^2 = 0.9978).

Each formulation was analyzed in duplicate and the mean ± SD was reported. Finally, the encapsulation efficiencies (EE) were calculated as follows:

$$EE\ (\%) = (Wa/Wt) \times 100$$

where Wa is the actual drug content and Wt is the theoretical drug content.

2.1.2.3. MPs-AL dissolution studies. In vitro dissolution tests of MPs-AL were performed in PBS pH 7.2 at a temperature of 37 °C. For each sample 40 mg of MPs were poured into 10 mL of PBS under continuous stirring (50 rpm). As the AL is very soluble in water (10 mg/mL, Sigma