Calcium phosphate bone cements for local vancomycin delivery

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ABSTRACT

Among calcium phosphate biomaterials, calcium phosphate bone cements (CPCs) have attracted increased attention because of their ability of self-setting in vivo and injectability, opening the new opportunities for minimally invasive surgical procedures. However, any surgical procedure carries potential inflammation and bone infection risks, which could be prevented combining CPC with anti-inflammatory drugs, thus overcoming the disadvantages of systemic antibiotic therapy and controlling the initial burst and total release of active ingredient. Within the current study α-tricalcium phosphate based CPCs were prepared and it was found that decreasing the solid to liquid phase ratio from 1.89 g/ml to 1.23 g/ml, initial burst release of vancomycin within the first 24 h increased from $40.0 \pm 2.1\%$ up to $57.8 \pm 1.2\%$ and intrinsic properties of CPC were changed. CPC modification with vancomycin loaded poly(lactic acid) (PLA) microcapsules decreased the initial burst release of drug down to $7.7 \pm 0.6\%$, while only $30.4 \pm 1.3\%$ of drug was transferred into the dissolution medium within 43 days, compared to pure vancomycin loaded CPC, where 100% drug release was observed already after 12 days. During the current research a new approach was found in order to increase the drug bioavailability. Modification of CPC with novel PLA/vancomycin microcapsules loaded and coated with nanosized hydroxyapatite resulted in $85.3 \pm 3.1\%$ of vancomycin release within 43 days.

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1. Introduction

In the last decades musculoskeletal diseases and disorders are becoming a great problem all over the world and every year the number of patients suffering from these diseases dramatically increases [1,2]. Although the development of medications and techniques directed to the treatment of these diseases, even in the initial stages, is in great progress, still there are some unclear issues regarding the balance between maximal drug efficiency and minimal side effects [3,4].

The most prospective materials for the bone tissue replacement and regeneration are calcium phosphates (CaP) due to their biocompatibility, osteoconductivity and similarity to the natural bone mineral phase [5,6]. Among CaP biomaterials, CaP bone cements have attracted extended attention because of their ability of self-setting in vivo, moldability and injectability, opening the new opportunities for minimally invasive surgical procedures [2,7,8]. However, any surgical procedure carries potential inflammation and bone infection risks, traditionally prevented through prolonged (2–6 weeks) systemic antibiotic therapy [9–11]. The main disadvantages of systemic therapy are that only a small fraction of any given dose actually reaches the surgical site, thus the use of local or site specific antibiotic delivery system could be a solution to achieve high

drug levels at an infection site and to minimize systemic side effects [12,13].

If compared to the drug delivery systems based on bioceramic materials, where drugs can be added using different strategies, like sorption, physical/mechanical aspects or chemical linking, drugs in the CPC can be easily dispersed throughout the entire cement matrix [4, 14]. However, it should be considered that CPC setting reaction, porosity and mechanical properties can be considerably affected by the antibiotic introduction within the calcium phosphate bone cement [15,16]. Three basic strategies can be used for the drug incorporation into calcium phosphate bone cements, like mixing the drug with cement solid phase or dissolving the active substance in the liquid phase, impregnation of CPC with drug solution or modifying the CPC with microencapsulated drug forms. The main advantages of the third strategy over the others, is based on the possibility to obtain long term drug delivery systems, at the same time decreasing the initial burst release, characteristic for the calcium phosphate bone cements [17].

In spite of all the advantages, calcium phosphate bone cements possess poor degradability, limiting the bone regeneration rate and low mechanical properties, limiting their use in load-bearing applications [18–20]. To overcome the limitations of poor degradability, CPCs can be modified with polymers, which upon the degradation could release the acidic monomers, enhancing CPC degradation [21–23]. Among the various polymer systems, poly(lactic acid) (PLA) and its copolymers with glycolic acid have been used as macroporosity inducers (in the form of microspheres, microcapsules or microfibers) [24–26], not only because of their degradation product acidic nature,

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but also due to their biocompatibility, tailorable properties and approval from the Food and Drug Administration to be used in clinics [27,28].

In order to overcome the disadvantages of systemic therapy, the poor degradability of the CPC and to ensure prolonged (2–6 weeks) antibiotic delivery to the targeted site, in the current research two strategies of vancomycin incorporation into the CPC matrix were compared. Local drug delivery systems were prepared either dispersing the drug throughout the CPC matrix, estimating the solid to liquid phase ratio changes onto the drug release kinetics and cement properties, or modifying the CPC with microencapsulated vancomycin forms, evaluating the effect of nanosized hydroxyapatite addition during the microencapsulation process onto the drug release profile and cement properties.

2. Materials and methods

2.1. Materials

Poly(lactic acid) (PLA) (Biomer L9000) with molecular weight of 200–300 kDa and polyvinyl alcohol (PVA) with molecular weight of 25 kDa (98 mol% hydrolyzed) were purchased from Polysciences (Warrington, FL). Vancomycin hydrochloride (from streptomyces orientalis), dichloromethane ($\geq$99.8%), orthophosphoric acid ($\geq$85%), calcium oxide ($\geq$97%) and isopropyl alcohol ($\geq$99.7) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium dihydrogen phosphate and sodium hydrogen phosphate were purchased from Acros Organics (Geel, Belgium).

2.2. Synthesis of nanosized hydroxyapatite (HAp)

Calcium phosphate powders where synthesized using wet precipitation reaction between calcium hydroxide and orthophosphoric acid as described previously [29]. The concentrations of $Ca(OH)_2$ suspension and H_3PO_4 solution used for the synthesis process were 0.45 M and 2.00 M respectively. The obtained precipitates were vacuum filtered and the final concentration of HAp suspension was 0.2 g/ml.

2.3. Preparation of vancomycin hydrochloride loaded PLA microcapsules

Vancomycin hydrochloride loaded PLA microcapsules were prepared using the slightly modified double emulsification technique described previously [30]. Briefly, PLA (1 g) was dissolved in 10 ml of dichloromethane. PVA (4 g) was dissolved in 100 ml of water. Vancomycin hydrochloride (1 g) was added to the polymer solution in dichloromethane. S/O (solid phase/organic phase) primary suspension was properly homogenized for 30 s at 7000 rpm and added to 100 ml of 4% aqueous PVA solution. S/O/W (solid phase/organic phase/water phase) double emulsion was homogenized for 30 s at 7000 rpm. After emulsification, the organic solvent was extracted in 2 l of water for 30 min. Then the microcapsules formed were separated by centrifugation for 5 min at 3000 rpm and dried at 40 °C for 24 h.

2.4. Preparation of vancomycin hydrochloride/PLA/HAp microcapsules

PLA (1 g) was dissolved in 10 ml of dichloromethane. PVA (4 g) was dissolved in 100 ml of water. Vancomycin hydrochloride (1 g) was dissolved in 1 g of HAp suspension ($c_{HAp} = 0.2$ g/ml). An aqueous suspension of VANK and HAp was added to the PLA solution in dichloromethane. $S/W_1/O$ (solid phase/primary water phase/organic phase) primary suspension was properly homogenized for 30 s at 7000 rpm and added to 100 ml of 4% aqueous PVA solution. $S/W_1/O/W_2$ double emulsion was homogenized for 30 s at 7000 rpm. After emulsification, the organic solvent was extracted in 2 L of water for 30 min. Then the microcapsules formed were separated by centrifugation for 5 min at 3000 rpm and dried at 40 °C for 24 h.

2.5. Characterization of microcapsules

Microanalysis (apparatus — Vario MACRO CHNS, Hanau, Germany) was used to determine the nitrogen content in samples and the total drug load (DL) in microparticles was calculated according to Eq. (1):

$$DL(\%) = \frac{N_{el}}{N_{tot}} \cdot 100, \tag{1}$$

where N_{el} is the nitrogen content found using microanalysis and N_{tot} is the calculated nitrogen content in vancomycin hydrochloride.

The average microcapsule size and particle size distribution were determined using a laser particle size analyzer (ANALYSETTE 22, measuring range from 0.01–1000 μm, laser wavelength 650 nm). Each sample was measured in triplicate.

The surface morphology and inner structure of microcapsules were examined using scanning electron microscopy (SEM, Tescan Mira \LMU, Czech Republic) at an acceleration voltage of 3–7 kV. Each sample was sputter coated with gold prior to imaging.

2.6. Preparation of calcium phosphate bone cements

CPC solid phase was α-tricalcium phosphate powder (α-TCP), prepared by heating a mixture of $CaCO_3$ and $CaHPO_4$ (molar ratio 1:2) at 1300 °C for 4 h with subsequent quenching in air. The obtained α-TCP was milled in a planetary ball mill (Fritsch, Pulverisette 5, Germany) for 1 h at 320 rpm in isopropyl alcohol. Cement liquid phase was a mixture of 0.5 M Na_2HPO_4 and 0.5 M NaH_2PO_4 solutions (volume ratio 20:1). The CPC samples with solid to liquid phase ratios of 1.89 g/ml, 1.75 g/ml and 1.23 g/ml were used in this study. In order to prepare calcium phosphate bone cement composites, all solid additives were properly mixed prior to the addition of liquid phase. The solid and liquid phases of the CPC were intensively mixed for 30 s and then placed into the teflon molds (7 mm in diameter and 16 mm in height). For CPC compositions and their detailed preparation see Table 1.

2.7. Characterization of calcium phosphate bone cements

The phase composition of prepared powders (cement solid phase and HAp) was analyzed using X-ray powder diffractometry (XRD, PANalytical X'Pert PRO, Westborough, MA). XRD patterns were recorded using Ni-filter and Cu Kα radiation at 40 kV and 30 mA, 2θ range of 5–60°. HAp suspension was dried at 100 °C for 24 h, followed by heat treatment in air atmosphere at 1100 °C for 1 h, before the XRD analysis.

The specific surface area of α-TCP and as-synthesized HAp powder was determined using the BET method (ISO 9277:2010, Quadrasorb SI-KR/MP, Quantachrome Instruments, Boynton Beach, FL) measuring the amount of physically adsorbed N_2 gas (purity 99.99%) at −196.15 °C. Before analysis all samples were degassed for 24 h at 100 °C. For the calculations the Brunauer–Emmett–Teller model has been applied. The value of specific surface area found was used to calculate the average α-TCP and as-synthesized HAp particle size, assuming that particles are spherical and the theoretical density of α-TCP = 2.86 g/cm^3 and for HAp = 3.14 g/cm^3 [6]. Calculations were done according to Eq. (2):

$$D = \frac{6}{S \cdot \rho}, \tag{2}$$

where D is the average particle diameter, S is the specific surface area and ρ is the theoretical density.

Samples for mechanical tests were prepared by placing the CPC paste into cylindrical teflon molds (7.0 mm diameter × 16 mm height). After 24 h samples were removed from the teflon molds and the compressive strength was measured at a loading rate of 0.5 mm min^{-1}