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Ready-to-use injectable calcium phosphate bone cement paste as drug carrier

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ABSTRACT

Current developments in calcium phosphate cement (CPC) technology concern the use of ready-to-use injectable cement pastes by dispersing the cement powder in a water-miscible solvent, such that, after injection into the physiological environment, setting of cements occurs by diffusion of water into the cement paste. It has also been demonstrated recently that the combination of a water-immiscible carrier liquid combined with suitable surfactants facilitates a discontinuous liquid exchange in CPC, enabling the cement setting reaction to take place. This paper reports on the use of these novel cement paste formulations as a controlled release system of antibiotics (gentamicin, vancomycin). Cement pastes were applied either as a one-component material, in which the solid drugs were physically dispersed, or as a two-component system, where the drugs were dissolved in an aqueous phase that was homogeneously mixed with the cement paste using a static mixing device during injection. Drug release profiles of both antibiotics from pre-mixed one- and two-component cements were characterized by an initial burst release of ~7–28%, followed by a typical square root of time release kinetic for vancomycin. Gentamicin release rates also decreased during the first days of the release study, but after ~1 week, the release rates were more or less constant over a period of several weeks. This anomalous release kinetic was attributed to participation of the sulfate counter ion in the cement setting reaction altering the drug solubility. The drug-loaded cement pastes showed high antimicrobial potency against *Staphylococcus aureus* in an agar diffusion test regime, while other cement properties such as mechanical performance or phase composition after setting were only marginally affected.

1. Introduction

Infection in bone (e.g., osteomyelitis) is one of the largest problems in orthopedic surgery, since it often results in a loss of bone tissue and the removal of implants in a second operation [1,2]. Owing to the limited accessibility of infected bone tissue to systemically administered drugs, a localized delivery of antibiotics is a common treatment of postoperative infections, e.g., using poly(-methyl methacrylate) (PMMA) beads as carriers for the drugs [3] or mixing self-setting PMMA cement with antibiotics [4]. However, PMMA-based materials are not resorbable [5] and require surgical removal, after which they may be replaced by either new material to prolong the antibiotic therapy or a permanent natural or synthetic bone graft. A significant step forward would be the use of degradable bone grafts impregnated with antibiotics, e.g., using sintered calcium phosphate phases [6] or self-setting calcium phosphate cements (CPC) [7–11]. In contrast to PMMA, this type of cement consists of a porous ceramic matrix, which is formed by a continuous dissolution–precipitation reaction after adding an aqueous phase to the cement powder. Depending on the pH value of the cement paste, two types of CPC can be distinguished: while at neutral and basic pH nanocrystalline hydroxyapatite is formed, a strong acidic pH by the addition of primary phosphates or phosphoric acid results in the formation of protonated secondary calcium phosphates such as brushite or monetite [12,13]. Since the set cement matrix is microporous, CPC have captured increasing attention for the controlled release of water-soluble drugs, such as antibiotics or bone growth factors [14].

CPC are commonly applied as powder/liquid formulations in which the cement powder is mixed during surgery with the aqueous cement liquid to produce the cement paste [15–17]. The paste is either modeled into an open defect by means of a spatula or it is (after transfer into a syringe) injected using minimally invasive operation techniques. The latter procedure exhibits intrinsic handling problems, since cement setting starts immediately after mixing the cement powder and liquid, resulting in a continuous change in the material properties and leaving only a small timeframe for cement application by the surgeon. Current developments in CPC technology concern the use of ready-to-use

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injectable cement pastes by dispersing the cement powder in a water-miscible solvent (e.g., glycerine [18,19], PEG [20]) such that, after injection into the physiological environment, setting of cements occurs by diffusion of water into the cement paste [21–23]. A new approach consists in the combination of a water-immiscible carrier liquid combined with suitable surfactants, which facilitates a discontinuous liquid exchange in CPC, enabling the cement setting reaction to take place [24,25].

The present study reports these novel cement paste formulations for use as a controlled-release system of antibiotics (gentamicin, vancomycin). Cement pastes were applied either as one-component material, in which the solid drugs were physically dispersed, or as a two-component system, where the drugs were dissolved in an aqueous phase, which was homogeneously mixed with the cement paste using a static mixing device during injection. Drug release kinetics were studied over a period of up to 56 days, and the influence of the admixed antibiotics on the material properties of the cement such as phase composition, mechanical performance or pore size distribution was determined.

2. Materials and methods

The cement powder composition used in this study was similar to the formulation of Biocement D, originally developed by Driessens and co-workers [26], and contained 60 wt.% α-tricalcium phosphate (α-$Ca_3(PO_4)_2$), 26 wt.% dicalcium phosphate anhydrous ($CaHPO_4$), 10 wt.% calcium carbonate ($CaCO_3$) and 4 wt.% precipitated hydroxyapatite. Tricalcium phosphate (TCP) powders were produced by sintering mixtures of $CaHPO_4$and $CaCO_3$in a 2:1 M ratio at temperatures of 1300 °C following quenching in air. The powder components were mixed in an agate ball mill (Pulverisette 5, Fritsch, Germany) with 30 g agate balls (Fritsch) at 200 rpm for 45 min. Mixing of the CPC powder and 4% Na_2HPO_4-solution with a powder to liquid ratio of 2.5 g ml^{-1} resulted in a water-based cement paste, which was used as reference material in the study. Ready-to-use cement pastes were obtained according to a previous study by Heinemann et al. [25]. Briefly, the CPC powder was mixed with 2.5% finely ground K_2HPO_4 in an oil-based suspension (synthetic short chain triglyceride Miglyol 812 with 8–12 °C saturated fatty acids) at an oil to powder ratio of 0.16 g ml^{-1}. The oil phase contained two surface-active agents, 14.7% (w/w) castor oil ethoxylate 35 (Cremophor ELP, BASF, Germany) and 4.9% (w/w) hexadecyl-phosphate (Cetyl-phosphate, Amphisol A, Brenntag AG, Germany). CPC powder and oil phase were mixed in a stainless steel mixer (Stephan Mischer, Stephan Machinery GmbH, Germany) until homogeneity. In accordance with standardized tests the cement paste was proved to be cyto compatible in vitro (DIN ISO 10993-5) and showed no sensitization and intracutaneous reactivity in animal studies (DIN ISO 10993-10) or systemic toxicity (DIN ISO 10993-11) [25]. The biocompatibility was demonstrated in an animal study (DIN ISO 10993-6) and showed reactions similar to a commercial CPC over a period of 90 days (data not shown) [25]. A study with human mesenchymal stem cells cultured on the used cement paste showed the ability of the cell to proliferate and differentiate into osteoblasts [27]. The cement pastes were applied as either one-component material or as a two-component system in which a second aqueous phase was homogeneously mixed with the cement paste at a 1:4 volume ratio using a static mixing device (Medmix, Switzerland) during injection (Fig. 1). Cement modification with gentamicin (molar mass, 463 g mol^{-1}; Fluka, Steinheim, Germany) or vancomycin (molar mass, 1486 g mol^{-1}; actavis, Munich, Germany) was performed (1) by mixing 1.24 or 2.48 wt.% solid antibiotic with the one-component cement paste during manufacture or (2) by dissolving 2.5, 5.0 or 10.0 wt.% antibiotic in the aqueous phase of the two-component cement system. Antibiotic-loaded reference cements were prepared by mixing the cement powder with a solution containing 10 wt.% gentamicin and 4 wt.% Na_2HPO_4 at a powder to liquid ratio of 2.5 g ml^{-1} or by adding 100 mg dry vancomycin to 2.5 g cement powder following mixing with 1 ml Na_2HPO_4 solution.

2.1. Release study

Cubic samples (6 × 6 × 12 mm) were fabricated in silicon molds and immersed in 3 ml of PBS buffer (composition: 8.0 g NaCl, 1.1 g Na_2HPO_4, 0.2 g KCl, 0.2 g KH_2PO_4) after 10 min setting. The release study was performed at 37 °C in an incubator with an orbital platform shaker (incubator 1000, Unimax 1010, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), at a constant shaking rate of 60 rpm. The buffer was changed after each measurement. The vancomycin content of the eluate was determined directly by UV–vis spectroscopy at 237 nm. One milliliter of gentamicin containing eluates was mixed with 1 ml isopropanol and 1 ml of a solution containing 100 mg *o*-phthaldialdehyde, 1 ml methanol, 0.2 ml β-mercapthoethanol and 2 g sodium tetraborate in 100 ml water and measured after 45 min reaction time with UV–vis at

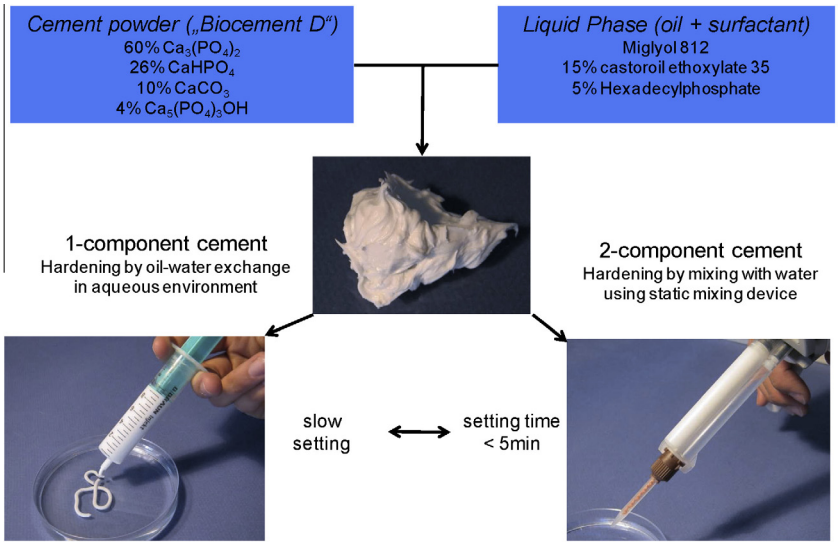

Fig. 1. Preparation regime of ready-to-use CPC pastes and their application in one- and two-component drug delivery devices.