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Injectability, microstructure and release properties of sodium fusidate-loaded apatitic cement as a local drug-delivery system

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ABSTRACT

The introduction of an antibiotic, sodium fusidate (SF), into the liquid phase of calcium carbonate–calcium phosphate ($CaCO_3$–CaP) bone cement was evaluated, considering the effect of the liquid to powder ratio (L/P) on the composition and microstructure of the set cement and the injectability of the paste. In all cases, we obtained set cements composed mainly of biomimetic carbonated apatite analogous to bone mineral. With this study, we evidenced a synergistic effect of the L/P ratio and SF presence on the injectability (i.e., the filter-pressing phenomenon was suppressed) and the setting time of the SF-loaded cement paste compared to reference cement (without SF). In addition, the in vitro study of SF release, according to the European Pharmacopoeia recommendations, showed that, regardless of the L/P ratio, the cement allowed a sustained release of the antibiotic over 1 month in sodium chloride isotonic solution at 37 °C and pH 7.4; this release is discussed considering the microstructure characteristics of SF-loaded cements (i.e., porosity, pore-size distribution) before and after the release test. Finally, modelling antibiotic release kinetics with several models indicated that the SF release was controlled by a diffusion mechanism.

1. Introduction

Post-operative infection is one of the major causes of increased morbidity and the most serious and costly complications associated with orthopaedic procedures. Besides, the infected areas are often difficult to reach and treat. The development of minimally invasive surgical techniques that are able to control and reduce this post-operative risk and pain has promoted the use of injectable biomaterials, especially for orthopaedic applications [1–2]. Despite the reduction in post-surgery risks associated with the use of minimally invasive surgery techniques, new biomaterials have been developed for therapeutic purposes. These materials are capable of releasing a drug over a long period that could prevent or treat some postoperative complications. Calcium phosphate cements (CPCs) have great potential as carriers for the controlled release of drugs in bone tissue, owing to their composition that is close to bone mineral, excellent bioactivity and possible use as injectable and degradable grafting materials. They are set at low temperatures (ambient or physiological temperatures), which enables the association of CPCs with biologically active molecules, for example during paste preparation, without denaturation of the molecule or loss of its therapeutic activity. Several studies have been conducted on the association of antibiotics, such as gentamicin, cephalexin, doxycycline or vancomycin with CPCs in order to prevent implant-associated infection or to treat specific infections [3–10].

Usually, surgeons implement a prophylactic antibiotherapy by means of oral administration in order to prevent or reduce infectious pathologies after a surgery. The mostly used antibiotics in orthopaedic or traumatology are cefazolin, vancomycin, gentamycin and ciprofloxacin, but other antibiotics with good bone-tissue penetration (>30%), such as rifampicine, fosfomycine or fusidic acid, are also proposed [11–14]. In the present study, we chose a water-soluble derivative of fusidic acid, sodium fusidate (SF), which showed broad spectrum bactericidal and fungicidal properties in addition to very good bone penetration [14].

The performance of CPCs in association with an antibiotic to design drug-delivery systems is related to specific parameters, that is, the cement microstructure (i.e., porosity, tortuosity and specific surface area) and its resorbability, as well as the solubility of the drug and its interactions with the mineral matrix. An overview of the combination of pharmaceutical and biological substances with CPCs for delivery applications, in addition to the effect of the principle-loading method on the physicochemical properties of the cements, has been reported by Ginebra et al. [10].

The objective of this work is to study the physicochemical properties of biomimetic apatite cement loaded with an antibiotic, sodium fusidate, which is a soluble salt of fusidic acid. We aim to evaluate the characteristics and properties of the paste (i.e., setting time and injectability) and the

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set cement (i.e., mechanical and drug-release properties) in a view to propose a sustained drug-release biomimetic bone substitute material.

2. Materials and methods

2.1. Preparation and characterisation of the cements

The reactive powders (solid phase) were comprised of vaterite calcium carbonate ($CaCO_3$) and dicalcium phosphate dihydrate (DCPD; $CaHPO_4 \cdot 2H_2O$), which were prepared by precipitation at ambient temperature, as previously described [15]. The cements investigated in this study were obtained by mixing the reactive powders (DCPD and vaterite in a 1:1 weight ratio) with an appropriate amount of liquid phase. The reference cement (CR) was produced using pure water only as the liquid phase, whereas we used a 9 wt.% SF aqueous solution to prepare the antibiotic-loaded cements (CSF). The liquid-to-powder ratios (L/P) used to prepare the cement pastes were 0.7 and 0.8 mL/g, leading to reference cements named CR7 and CR8, and to antibiotic-loaded cements referred to as CSF7 and CSF8, respectively. L/P = 0.7 corresponds to the minimum amount of liquid phase (water or aqueous solution of SF) to be added to the powder to form a malleable paste after mixing. We also chose to work with a higher L/P ratio (0.8) which corresponds to a compromise between improvement of the injectability of the paste while adding minimum amount of water to keep the cement mechanical properties as high as possible.

After mixing the solid with the liquid phase, the obtained paste was placed in a sealed container saturated with H_2O at 37 °C for setting and hardening for 2 days.

The set and dried cements were characterised by X-ray diffraction (INEL CPS 120 diffractometer) using a Co anticathode (wavelength = 1.78897 Å), transmission Fourier transform infrared spectroscopy with KBr pellets (Nicolet 5700 spectrometer, ThermoElectron) and scanning electron microscopy (SEM LEO 435 VP). Porosity measurements (between 5 nm and 360 μm) were performed using mercury intrusion porosimetry (AutoPore III, Micromeritics).

2.2. Setting time determination

The setting time of the cement paste was determined at 37 °C. The initial and final setting times were estimated according to a protocol adapted from the ASTM C266-03 and ISO 9917-1:2007 standards, using a Gillmore needle apparatus (HM-310, Gilson Inc.). Briefly, the system comprised two stainless steel needles: one is 2.13 mm diameter and 113.4 g weight used to determine the initial setting time; the second is 1.06 mm diameter and 453.6 g weight applied to estimate the final set. When the needles did not lead to a trace on the upper surface of the cement paste, the values of setting time were determined (expressed in minutes from when the mixing began). All measurements were performed in triplicate.

2.3. Mechanical properties of the hardened cements

A cement paste, prepared as described above, was introduced into cylindrical moulds (10 mm in diameter and 20 mm in height), which were then placed in sealed containers saturated with water at 37 °C for setting and hardening. After 2 days, the hardened cement was withdrawn from the mould and left to dry for 1 week at 37 °C. Five cements were prepared for each of the tested cement formulations. The compressive strength of the cylindrical cement blocks was evaluated using a Hounsfield Series S apparatus.

2.4. Injectability of the cement paste

The injectability of the reference and antibiotic-loaded cement pastes was measured at room temperature using a TAXT2 texture analyser (Stable Micro Systems) equipped with a specific syringe system, including a 2.5 mL syringe (inner diameter of the syringe body was 9 mm and the opening/exit diameter was 2 mm) without a needle. The protocol consists of measuring the force, expressed as a load to be applied on the piston (piston surface = 64 mm^2), to extrude a volume of paste corresponding to the displacement of 15 mm of the syringe piston at a constant rate (2 $mm \cdot s^{-1}$).

Briefly, the syringe was filled with cement paste and the injectability measurement was performed 5 min after paste preparation (t = 0 corresponds to the beginning of powder –liquid mixing). This period corresponds to the time needed to prepare the paste, to introduce it into the syringe and to place the syringe system on the texture analyser before starting the injectability measurements. This period could also correspond to the time a surgeon would need to prepare the cement paste and to introduce it into a device for implantation using a minimally invasive technique.

2.5. Antibiotic release study

The study of the antibiotic release from the cement was performed in vitro, according to the European Pharmacopoeia recommendations, using a Dissolutest (Pharmatest®) equipped with a stirring system (rotating paddle rate = 100 rpm) adapted to a series of 12 bowls (1 L), which allows homogenisation of the solution composition throughout the test.

The cement samples were prepared as described above for the mechanical properties determination of hardened cements, except for the cylindrical mould dimensions, which were smaller so as to consume less of the cement paste (17.5 mm diameter × 10 mm height). Then, in order to study the drug-release kinetics, the cement blocks were immersed in 1 L of 0.9% (*w*/*w*) NaCl solution at physiological pH (pH = 7.4) and 37 °C under sink conditions for 5 weeks. The surface of each cement block in contact with the release solution was equal and limited to the top of the cylindrical block (S = 2.40 cm^2).

Some (10 mL) of the solution was removed from each bowl every day and replaced with the same volume of fresh 0.9% (*w*/*w*) NaCl solution at pH = 7.4 in order to maintain a constant solution/cement ratio throughout the experiment duration (i.e., 1 month). The pH value of the aliquot removed each day was not recorded but the pH value of the NaCl solution after 5 weeks of release test was around 8.0; this slight increase could be attributed to the release of carbonate species from the cement in the medium. The sampled solution was immediately filtered using a Millipore filter (pore diameter of 0.2 μm) to eliminate any fine particles that could be released from the cement block and impair the titration of the release solution by UV spectrophotometry. The concentration of the antibiotic in the sampled solution was then determined by UV spectrophotometry (UV-1800 SHIMADZU) at 235 nm. The absence of interference (no absorbance) was checked for the reference cement using the same electrolyte solution.

3. Results

3.1. Cement composition and microstructure

The X-ray diffraction patterns of the cement prepared in the absence (CR7) or presence of sodium fusidate (CSF7) are presented in Fig. 1. The XRD pattern of the reference cement (CR7) showed that it was composed of carbonate apatite and residual vaterite, revealing the formation of apatite through the reaction of part of the vaterite with all of the DCPD powder. The results also indicated that adding SF to the cement paste did not significantly affect the end product, as the XRD patterns of both cements are similar. FTIR spectrum of drug containing cement (CSF) compared to the spectrum of the reference cement (CR) confirmed that addition of drug into cement did not change significantly the composition of cement final composition despite the presence of the drug (Fig. 2). However FTIR spectroscopy allowed to identify on CSF spectrum bands of very low intensity, which are characteristic of