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Self-hardening and thermoresponsive alpha tricalcium phosphate/pluronic pastes

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ABSTRACT

Although calcium phosphate cements (CPCs) are used for bone regeneration in a wide range of clinical applications, various physicochemical phenomena are known to hinder their potential use in minimally invasive surgery or in highly vascularized surgical sites, mainly because of their lack of injectability or their low washout resistance. The present work shows that the combination of CPCs with an inverse-thermoresponsive hydrogel is a good strategy for finely tuning the cohesive and rheological properties of CPCs to achieve clinical acceptable injectability to prevent phase separation during implantation and cohesion to avoid washout of the paste. The thermoresponsive CPC developed combines alpha-tricalcium phosphate with an aqueous solution of pluronic F127, which exhibits an inverse thermoresponsive behaviour, with a gelling transformation at around body temperature. These novel CPCs exhibited temperature-dependent properties. Addition of the polymer enhanced the injectability of the paste, even at a low liquid-to-powder ratio, and allowed the rheological properties of the cement to be tuned, with the injection force decreasing with the temperature of the paste. Moreover, the cohesion of the paste was also temperature-dependent and increased as the temperature of the host medium increased due to gelling induced in the paste. The thermoresponsive cement exhibited excellent cohesion and clinically acceptable setting times at 37 °C, irrespective of the initial temperature of the paste. The addition of pluronic F127 slightly delayed the setting reaction in the early stages but did not hinder the full transformation to calcium-deficient hydroxyapatite. Moreover, the frozen storage of premixed thermoresponsive cement pastes was explored, the main physicochemical properties of the cements being maintained upon thawing, even after 18 months of frozen storage. This avoids the need to mix the cement in the operating theatre and allows its use off-the-shelf. The reverse thermoresponsive cements studied herein open up new perspectives in the surgical field, where the sequential gelling/hardening of these novel cements could allow for a better and safer clinical application.

Statement of Significance

Calcium phosphate cements are attractive bone substitutes due to their similarity to the bone mineral phase. Although they can be injectable, cohesion and stability of the paste are crucial in terms of performance and safety. A common strategy is the combination with hydrogels. However, this often results in a decrease of viscosity with increasing temperature, which can lead to extravasation and particle leakage from the bone defect. The preferred evolution would be the opposite: a low viscosity would enhance mixing and injection, and an instantaneous increase of viscosity after injection would ensure washout resistance to the blood flow. Here we develop for the first time a calcium phosphate cement exhibiting reverse thermoresponsive properties using a poloxamer featuring inverse thermal gelling.

1. Introduction

Minimally invasive surgical (MIS) techniques reduce the risk, damage and pain caused to the patient, as well as healthcare costs, compared to traditional surgical procedures [1,2]. In the field of orthopaedic surgery, some interventions such as vertebroplasty,

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kyphoplasty or the treatment of osteonecrosis of the hip, are performed via MIS [3]. In order to perform these procedures safely, however, the properties of the material used should be adapted to each step of the process in order to overcome key problems found during material injection [4,5].

Self-setting calcium phosphate slurries, also referred to as calcium phosphate cements (CPCs), are well known bone-filler materials with more than 30 years of use in orthopaedics [6]. Unlike acrylic bone cements based on polymethyl methacrylate (PMMA), the exothermic of the CPC hardening reaction is very low, and the end product of the cementitious reaction is a calcium phosphate that is very similar to the mineral phase of bone. Although CPCs are considered to be injectable materials, balancing their characteristics to achieve injectable pastes with adequate cohesion, setting time and sufficient mechanical strength for clinical application remains a challenge in the field of injectable bone substitutes [7–9]. Specifically, the cohesion and stability of the paste is a crucial issue in terms of material performance and safety. Indeed, serious health risks were detected some years ago in what is currently known as the Norian case [10,11]. In this catastrophic experience, a commercial CPC formulation was used off-label in vertebroplasty, with particle leakage into the blood stream after injection eventually being held responsible for causing blood clotting and subsequent embolism, which resulted in the death of several patients. Therefore, one of the most important clinical requirements for CPCs is immediate and complete cohesion after mixing, even in highly vascularised bone defects.

Several strategies have been explored to enhance the injectability of CPCs [12,13], either by reducing the permeability of the cement powder [14], modifying the particle surface properties [9,15] or increasing the viscosity of the liquid cement phase [16]. However, even though a high viscosity of the liquid cement phase can be beneficial to the cohesive and anti-washout properties, as well as for avoiding leakage and migration of the material after implantation, it is detrimental to handling of the material by the surgeon [17,18]. Furthermore, most hydrogels used as liquid phase thickeners exhibit a decrease of viscosity with increasing temperature, as is the case for gelatine or agarose, which is not suitable for clinical applications since the temperature of the surgical site is higher than that of the operating theatre. This makes the cement more likely to undergo extravasation from the bone defect once injected. The preferred evolution would rather be the opposite: whereas a low viscosity would be convenient for mixing and injection without phase separation, an instantaneous increase of viscosity once the material fills the bone defect would be beneficial [3], since this would ensure stability and washout resistance to the blood flow and/or to other surrounding fluids.

Hence, the use of polymeric additives featuring an inverse thermal gelling, like pluronics also referred to as poloxamers, is of particular interest for controlling the properties of injectable biomaterials [19]. To the best of our knowledge there are no publications focusing on the combination of reverse thermoresponsive hydrogels with self-hardening calcium phosphate cements, apart from a study using pluronic as surfactant in the synthesis of calcium phosphate foams loaded with simvastatin [20] where the concentration used was below the critical micelle concentration, and therefore did not present a thermoresponsive behaviour. Other studies used pluronic as a carrier of non-reactive millimetre-sized calcium phosphate granules [21]. Pluronics are triblock (A-B-A) amphiphilic copolymers comprising two external blocks of polyoxoethylene (PEO) and a central block of polyoxopropylene (PPO) [22]. These copolymers present an inverse thermal gelling above the critical micelle concentration driven by the formation of entangled micelles due to the difference in lower critical solution temperature (LCST) between PEO and PPO [22]. This induces self-assembly of the macromers above the LCST of the PPO blocks. A further increase in temperature leads to the formation of a gel [23]. The gelling temperature and the strength of the gel depend on the concentration (above the critical micelle concentration) and the lengths of the PEO/PPO blocks. A prominent member of this family of polymers known as pluronic F127 (or Poloxamer 407) has a gelling temperature close to physiological temperature at relatively low concentration (18–30 wt%). Some studies have reported that 20 wt% water solutions of pluronic F127 in a phosphate buffer exhibited a gelling temperature of about 27 °C, whereas 25 wt% and 30 wt% solutions exhibited this gelling at much lower temperatures of 19 °C and 10 °C, respectively [24,25]. These findings highlight the high dependence of gelling temperature on the concentration of the aqueous pluronic solution. Other pluronics, *i.e.* poloxamer 188, require a high concentration (≈50 wt%) in order to gel at physiological temperature, which can be accompanied by considerable swelling [22]. The effect of ions on the gelling of pluronic F127 20 wt% hydrogels has also been studied. Su et al. reported that the presence of salts such as sodium phosphate promoted the formation of micelles at lower temperature than normally observed without ions [26,27].

Pluronic solutions exhibit a complex rheological behaviour, being liquid below the sol-gel transition temperature and experiencing a drastic change in shear moduli/viscosity around the sol-gel transition temperature. Nonetheless, the elastic shear modulus increases linearly after the onset of this transition and subsequently increases with a lower slope, thus resulting in an "S"-shaped viscosity versus temperature curve [25]. This curve is shifted towards lower temperature when electrolytes, *i.e.* phosphate buffer, are incorporated into the solution, producing lower gelling temperatures for the same pluronic concentration [25]. The excellent biocompatibility of pluronic F127 hydrogels [28] have made them particularly attractive for a number of biomedical applications, especially wound dressings, local drug delivery systems, temporary embolization of blood vessels and cell encapsulation [29]. Furthermore pluronic F127 is among the few hydrogels that are able to maintain their properties after gamma irradiation sterilization at 25 kGy, without producing any detectable by-products and maintaining their biocompatibility [30]. The objective of this work is to explore the potential of combining pluronic F127 with self-setting calcium phosphate pastes, with the aim of conferring a reverse thermoresponsive behaviour on them.

2. Materials and methods

2.1. Solid phase preparation

Alpha-tricalcium phosphate (α-TCP, α-$Ca_3(PO_4)_2$) and beta-tricalcium phosphate (β-TCP, β-$Ca_3(PO_4)_2$) were obtained by solid-state reaction from a 2:1 molar mixture of calcium carbonate ($CaCO_3$, Sigma Aldrich) and anhydrous dicalcium phosphate ($CaHPO_4$, Sigma Aldrich) at 1400 and 1100 °C respectively. Air quenching was performed for α-TCP after heat treatment to prevent the formation of β-TCP during cooling. Powders of these two phosphates were obtained by dry milling (Pulverisette 6, Fritsch Gmbh) for 40 min at 450 rpm with 10 agate balls (30 mm diameter), followed by 60 min at 500 rpm (with the same balls) and finally 60 min at 500 rpm with 100 agate balls (10 mm diameter). Powders were used as milled, *i.e.* without sieving step. The phase composition of the obtained powders was assessed by X-ray diffraction (XRD; D8 Advance, Bruker). The particle size distribution and the specific surface area were determined by laser diffraction (LS 13 320, Beckman Coulter) and nitrogen adsorption (ASAP 2020, Micromeritics) respectively, as described in Montufar et al. [9], obtaining the following values: α-TCP (d_{25} = 1.1 μm, d_{50} = 2.1 μm and d_{75} = 4.2 μm and SSA = 2.4 m^2/g) and β-TCP (d_{25} = 0.8 μm, d_{50} = 2.0 μm and d_{75} = 4.6 μm and SSA = 3.4 m^2/g).