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Of the in vivo behavior of calcium phosphate cements and glasses as bone substitutes

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Abstract

The use of injectable self-setting calcium phosphate cements or soluble glass granules represent two different strategies for bone regeneration, each with distinct advantages and potential applications. This study compares the in vivo behavior of two calcium phosphate cements and two phosphate glasses with different composition, microstructure and solubility, using autologous bone as a control, in a rabbit model. The implanted materials were α-tricalcium phosphate cement (cement H), calcium sodium potassium phosphate cement (cement R), and two phosphate glasses in the P_2O_5–CaO–Na_2O and P_2O_5–CaO–Na_2O–TiO_2 systems. The four materials were osteoconductive, biocompatible and biodegradable. Radiological and histological studies demonstrated correct osteointegration and substitution of the implants by new bone. The reactivity of the different materials, which depends on their solubility, porosity and specific surface area, affected the resorption rate and bone formation mainly during the early stages of implantation, although this effect was weak. Thus, at 4 weeks the degradation was slightly higher in cements than in glasses, especially for cement R. However, after 12 weeks of implantation all materials showed a similar degradation degree and promoted bone neoformation equivalent to that of the control group.

Keywords: Calcium phosphates; Calcium phosphate cements; Phosphate glasses; Bone grafts; Bone regenerations

1. Introduction

Bone substitution is still an unsolved problem. Currently, the best substitutes are the bone grafts provided either by a patient (autograft) or by a donor (allograft) [1]. However, bone grafts have well-known limitations [2,3]. Due to these drawbacks, several synthetic materials for bone substitution have been developed and characterized during the last few decades. In this framework, bioceramics have raised especial interest due to their bioactivity and the possibility of tailoring their composition, tuning their degradation rate and adjusting their formulation to compositions close to that of the mineral phase of bone [4].

Different approaches have been used for the design of bone substitute materials. On the one hand, the interest for injectable self-setting materials, which have a great potential on the field or minimally invasive surgical techniques, has led to the development of calcium phosphate cements. Traditionally, the bone cements used in orthopaedics have been polymer based [5–7]. However, during the early 1980s, the idea of developing calcium phosphate-based cements was put forward by LeGeros et al. [8] and was further investigated by Brown and Chow [9]. These ceramic cements have the advantages of polymethylmethacrylate cements, i.e. they are injectable and workable materials that can adapt to different geometries and are able to set once implanted in the body. In addition, they

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are bioactive and have a chemical composition very similar to that of the mineral phase of bone. Given these features, they can be used in various medical applications as cavity fillers, and also in the improvement of fracture osteosynthesis. Several cement formulations have been developed which, upon reaction, produce different products, such as hydroxyapatite or brushite [10]. Depending on their composition, these CaP cements have different solubilities and porosities, and hypothetically this should allow the modulation of their resorption rate.

On the other hand, a different approach has led to the development of glasses with controlled solubility and a predictable dissolution rate, which can be implanted in the form of granules, alone or mixed with some fluid carrier. The development of phosphate glasses for biomedical applications was begun in the 1980s by Burnie et al. [11]. Their studies were based on the analysis of glasses with P_2O_5 as the network former and different proportions of CaO and Na_2O as modifying oxides. They obtained a wide range of glasses with different degradation rates, varying from days to months [12]. The solubility rate of these glasses is controlled by the CaO/Na_2O ratio. An increase in the CaO percentage leads to an increment in the glass stability and an increment in the amount of Na_2O increases the solubility rate of the material, as shown by Clement et al. [13]. Moreover, the incorporation of small radius and highly charged ions contributes to the improvement of the chemical stability of these glasses [14]. The solubility control represents a great advantage in comparison with CaP crystalline ceramic materials used in orthopaedic applications.

Although both calcium phosphate cements and glasses have been extensively studied, no studies have been published comparing their in vivo performance. The aim of this study is to compare the in vivo behavior, in terms of biomaterial resorption and new bone formation, of four biomaterials designed following the two approaches described above: two injectable self-setting CaP cements, with different chemical composition and porosity, and two CaP glasses in the form of granules, with different chemical composition.

The two cements compared are coded as cement H (CH), an α-tricalcium phosphate (α-TCP, $Ca_3(PO_4)_2$) cement [15–18], and cement R (CR), which consists in a mixture of monocalcium phosphate monohydrate (MCPM, $Ca(H_2PO_4)_2 \cdot H_2O$) and calcium sodium potassium phosphate (CSPP,$Ca_2NaK(PO_4)_2$). The final product of both cements is a calcium-deficient hydroxyapatite. However, the reaction product of cement R has a higher solubility than that of cement H, and is more similar to the mineral phase of bone [19–21]. Moreover, the two cements have shown different cell responses in vitro. Specifically, while cement H enhanced osteoblastic differentiation in human bone marrow osteoprogenitor cell cultures, cement R elicited a cytotoxic response [22–23].

The two phosphate glasses studied, glass 0 (G0) and glass 5 (G5), also possess different chemical compositions and solubilities. Whereas G0 belongs to the system $CaO–P_2O_5–Na_2O$, in the case of G5 titanium oxide is added with the aim of reducing the solubility of the glass. The solubility and degradation mechanisms of these glasses both in water and in simulated body fluid (SBF) have been characterized in a previous study [24]. In fact, the dissolution rate of the glass without titanium oxide (G0) in SBF was an order of magnitude higher than the glass with 5 mol.% of TiO_2 [24]. Moreover, glass G0 was found to be slightly more cytotoxic when compared with G5 in fibroblastic cell cultures [25].

An additional goal of this study was to elucidate the extent to which the in vivo solubility of a material and its cytocompatibility can be used to predict the in vivo behavior and resorption rate of the material.

2. Materials and methods

2.1. CaP cements

Two cement formulations were used in this study, cements H and R. Cement H consists of 98 wt.% α-TCP and 2 wt.% precipitated hydroxyapatite. The powder was mixed with a 2.5 wt.% Na_2HPO_4 aqueous solution, at a liquid-to-powder (L/P) ratio of 0.35 ml g^{-1} [26]. Cement R is a mixture of CSPP and MCPM in a molar ratio of 2:5, giving a Ca/P ratio of 0.86. The cement paste was made by mixing the powder mixture with distilled water at an L/P ratio of 0.55 ml g^{-1}.

The specimens for the physico-chemical characterization were prepared by injecting the cement paste in cylindrical Teflon moulds (6 mm diameter, 12 mm height). The cements were allowed to set for 15 days in Ringer's solution at 37 °C. After different time periods the compressive strength was measured in a servohydraulic testing machine (MTS Bionix 858) at a crosshead speed of 1 mm min^{-1}. The set materials were characterized by X-ray diffraction to determine the phase composition. After soaking in Ringer's solution for 15 days, the set specimens were hand-crushed using a pestle and a mortar to a fine powder. X-ray diffraction (XRD) patterns of the samples were recorded by step scanning using a microprocessor-controlled diffractometer system (Siemens D500) with an Ni-filter for the K_α of the Cu, with an integration time of 3 s at intervals of 0.058 (2y). The potential used was 40 kV and the current was 30 mA. The porosity and pore size distribution was measured by Hg-porosimetry (Micromeritics Autopore IV 9500), which allows detecting the open porosity in the range of 0.006–350 μm.

2.2. CaP glasses

The two different phosphate glasses studied, glasses G0 and G5, had molar compositions of $44.5CaO–44.5P_2O_5–11Na_2O$ and $44.5CaO–44.5P_2O_5–6Na_2O–5TiO_2$, respectively. The glasses were obtained by melting and casting, and the methodology is explained in detail elsewhere [24].

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