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Do Ca^{2+}-adsorbing ceramics reduce the release of calcium ions from gypsum-based biomaterials?

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ABSTRACT

Bone implantable materials based on calcium sulfate dihydrate dissolve quickly in tissue liquids and release calcium ions at very high levels. This phenomenon induces temporary toxicity for osteoblasts, may cause local inflammation and delay the healing process. Reduction in the calcium ion release rate by gypsum could be therefore beneficial for the healing of gypsum-filled bone defects. The aim of this study concerned the potential use of calcium phosphate ceramics of various porosities for the reduction of high Ca^{2+} ion release from gypsum-based materials. Highly porous ceramics failed to reduce the level of Ca^{2+} ions released to the medium in a continuous flow system. However, it succeeded to shorten the period of high calcium level. It was not the phase composition but the high porosity of ceramics that was found crucial for both the shortening of the Ca^{2+} release-related toxicity period and intensification of apatite deposition on the composite. Nonporous ceramics was completely ineffective for this purpose and did not show any ability to absorb calcium ions at a significant level. Moreover, according to our observations, complex studies imitating in vivo systems, rather than standard tests, are essential for the proper evaluation of implantable biomaterials.

1. Introduction

Bone-implantable materials comprise compounds and composites of different chemical formulas and chemical and biological properties. Some of them are based on calcium sulfate hemihydrate (Plaster of Paris; $CaSO_4 \cdot ½H_2O$; CSH), a material which transforms into calcium sulfate dihydrate (CSD) after hydration. It is widely recognized as bioactive and safe and is commercially available as a component of CSH-based bone-repair products, e.g., Stimulan® (Biocomposites Ltd., UK) and Osteoset™ (Wright Medical Technology, Inc., USA). Despite many advantages, the relatively rapid rate of in vivo gypsum resorption may disturb the rate of appropriate, gradual replacement of the biomaterial by the newly formed bone. Furthermore, due to the rapid resorption of gypsum and gypsum-based composites, the resulting calcium-rich fluid is relatively toxic for human osteoblasts [1,2]. The presence of OsteoSet® pellets (calcium sulfate bone void filler) caused a decrease in osteogenic cell proliferation [3]. In vitro study on porcine osteoblasts showed that Ca^{2+} concentrations (7–10 mM) which are approximately 3–4.5 times greater than optimum Ca^{2+} concentration (2.2 mM) in control DMEM medium strongly inhibited cell proliferation and alkaline phosphatase (ALP) activity [4]. It was found for mesenchymal stem cells (MSCs), that Ca^{+2} concentrations higher than optimum significantly inhibited cell differentiation [5]. Physiological changes in extracellular calcium concentrations may also affect osteoblast function [6]. It is therefore not surprising that the elevated Ca^{+2} concentrations in the fluids incubated with gypsum-based implants, highly exceeding those optimal for osteoblast cells, dramatically reduce their metabolism and viability [2].

The problem of calcium sulfate-associated toxicity is rarely commented. Gypsum-based biomaterials are claimed generally safe and free of adverse reactions [7]. However, as summarized by Apaydin & Torabineyad [8], investigations into the use of calcium sulfate as a promoter of bone healing after surgery have produced mixed results. Moreover, both older [9] and more recent [10,11] reports indicate the problem of gypsum-related post-implantation inflammation. In one of these reports, severe inflammatory reaction developed in 20% cases of Osteoset pellet implantation, due to the Ca^{2+} accumulation in the fluid surrounding the implant [10]. In some cases, calcium accumulation in tissue liquids can be so high that the Ca^{2+} level in blood serum can increase significantly (as reported for a dog in vivo model) [12]. Gypsum-incited inflammatory response remains for up to 2 months [9–11]. Preparation of gypsum-based materials which release calcium ions at a reduced level could therefore be beneficial for the survival and proliferation of osteoblast cells. This would reduce the risk of inflammation and accelerate the healing of gypsum-filled bone defects.

It has already been shown that the application of calcium-chelating polysaccharides (alginates or rhizobial exopolysaccharides)

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as a supplement to gypsum-based composite does not provide a sufficient, long-term efficacy in the removal of the excess of gypsum-released Ca^{2+} ions [13]. However, another concept may be tested for this purpose: the use of calcium ion-adsorbing ceramics. This type of ceramics is represented by unsintered hydroxyapatite (HAp). It was demonstrated that some composite materials based on unsintered hydroxyapatite showed an unexpected cytotoxic effect (reduced cell viability). This phenomenon appeared due to a massive Ca^{2+} and Mg^{2+} ion uptake from the medium, reaching critically low values [14]. Probably, the ion uptake was caused by the highly specific surface area of unsintered ceramics. Another study confirmed the effect of high porosity of ceramics on an increased Ca^{2+} and Mg^{2+} uptake from the growth medium and decreased viability of osteoblast cells [15]. Highly porous ceramics is generally not used for biomedical purposes due to its excessive brittleness and a cytotoxic effect exerted on osteoblasts. However, in combination with calcium sulfate dihydrate, it could form a composite material of acceptable rate of the Ca^{2+} ion release and appropriate mechanical parameters.

Another profit may result from this strategy: in vivo resorption of the CSD-HAp composite is expected to be reduced in comparison with pure gypsum. The resorption rate of bone fillers based on pure CSD is claimed to be too fast to ensure its appropriate, gradual replacement by a newly formed bone tissue. The addition of HAp, the most stable and the least soluble form of calcium phosphate, could delay the rate of the composite dissolution and in vivo replacement by bone tissue. Moreover, such a biphasic construct could generate in vivo porosity due to the quick resorption of gypsum phase in aqueous solutions [16,17]. The question of pore size impact on biomaterials osteoconduction has been studied for several decades. Various sources define various pore sizes as minimum (50–100 μm) and optimal (300 μm) for osteoconduction [18,19]. Chang et al. postulated that not only pore size but also pore geometry affected in vivo osteoconduction and induced different histological changes [20]. Therefore, the formation of large pores during the gradual dissolution of HAp-gypsum composites in aqueous solution is a promising strategy to improve the osteoconductivity of bone void fillers.

Gypsum–HAp composites have already been tested as potential bone repair materials [21]. However, to our knowledge, the influence of calcium-absorbing ceramics on the reduction of Ca^{2+} release from gypsum has never been studied. In this report, we prepared calcium phosphate granules from bulk material sintered at various temperatures, as sintering temperature alters microporosity, specific surface area and the crystal size of ceramics [22]. Such obtained ceramics were used for the preparation of ceramic–gypsum composite materials. We focused on the influence of ceramics sintered at different temperatures on the removal of gypsum-released calcium ions. Based on these results, a possible impact of the porous ceramics on the reduction of gypsum-related inflammation risk and increase in bone healing effect was discussed.

2. Experimental procedures

2.1. Materials

Calcium sulfate hemihydrate (CSH) and the DMEM/F12 medium were obtained from Sigma. Ca^{2+}, HPO_4^{2-} and Mg^{2+} ion concentration was estimated spectrophotometrically using commercial kits (Calcium CPC, Magnesium and Phosphorus; Biomaxima, Poland), following the manufacturer's protocol. The components for granules and SBF preparation were provided by Sigma (USA) and POCH (Gliwice, Poland).

2.2. Granule preparation

Ceramic powder used for granule preparation was prepared using a wet chemical precipitation method described elsewhere [23,24], with some modifications. Calcium hydroxide and orthophosphoric acid were used as the starting materials. The Ca/P molar ratio was 1.67. Briefly, 1.67 mol H_3PO_4 was added dropwise (using peristaltic pump at a flow rate of 7 ml/min) to well dispersed 1 mol $Ca(OH)_2$ in aqueous suspension under constant stirring at 28 ± 3 °C. pH of the suspension was stabilized at 11.0 using 2 M NaOH solution. The resulting precipitate was aged for 96 h at room temperature. Subsequently, the precipitate was washed several times with distilled water until neutral pH was obtained. Then, the precipitate was decanted, dried out at 90 °C overnight, crumbled into smaller pieces and divided into four portions. Three portions were calcined at 400 °C, 800 °C and 1150 °C, respectively, for 2 h; the last portion was left without calcination. Finally, granules were sieved and fractions 0.2–0.5 mm were selected for experiments.

2.3. Granule characterization

2.3.1. Pore size distribution determination

An Autopore IV 9500 (Micrometrics) Inc., USA mercury porosimeter was used to determine the pore size distribution according to the standard ISO 15901-1:2005 [25]. Before intruding mercury in step-wise pressure increments in the range between 0.036 and 413 MPa, ceramic granules with open pore structures were oven-dried at 105 °C and degassed in vacuum under the pressure of 6.67 Pa at the temperature of 20 °C. PSD (pore size distribution) as f(r) was determined based on the Washburn equation (1921):

$$P = \frac{2\gamma_{Hg}\cos\theta}{r}$$

where: P is the external pressure (Pa) applied in the vacuum chamber, γ_{Hg} is the surface tension of mercury (485 J m^{-2}), θ is the contact angle of mercury (130°), and r is the pore radius of pore aperture (m) for a cylindrical pore. This approach allows the determination of pore radii ranging from 0.0015 to 47 μm. Average pore radius ($2V/A$) was obtained by assuming that all pores are right cylinders, thus when the volume ($V = \pi r^2 L$) is divided by the pore area ($A = 2\pi rL$), the average pore radius (r) equals $2V/A$.

2.3.2. XRD and FTIR analysis

X-ray diffraction data were acquired using a copper Kα radiation and scintillation detector on a Bragg–Brentano focusing diffractometer type HZG-4 (Zeiss). The X-ray source was a conventional sealed 1500 W X-ray tube operated at 36 kV and 25 mA. Diffraction data were collected by step counting in the range $2\Theta = 30 \div 38°$ at 0.01° intervals for 2 s per data point.

Prior to the FTIR measurement, the ceramic samples were ground in a mortar and dried at 105 °C for 20 h to remove traces of adsorbed water. Dried powders were compressed into KBR tablets of IR grade. The FTIR transmission spectra were obtained using an IR spectrometer (Vertex 70, Bruker, USA), 64 scans and 1 cm^{-1} resolution.

2.3.3. Evaluation of ionic reactivity

Evaluation of ionic reactivity was performed based on a previously described procedure [2]. Briefly, granules sintered at various temperatures (90 °C, 400 °C, 800 °C and 1150 °C) were placed on a 24-well plate (200 mg per well) and sterilized using the ethylene dioxide method (1 h at 55 °C with a subsequent degassation for 20 h). Subsequently, 2 ml of sterile DMEM/F12 medium was added to each well. The plate was incubated for 72 h at 37 °C. During the experiment, the medium was collected every 24 h and replaced with a fresh medium. The removed medium was evaluated for Ca^{2+}, Mg^{2+} and HPO_4^{2-} ion concentration. The experiment was performed in five repetitions and the results were expressed as mean values ± SD (standard deviations).