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Control of in vivo mineral bone cement degradation

Britta Kanter [a], Martha Geffers [b], Anita Ignatius [a], Uwe Gbureck [b,*]

[a] Centre for Musculoskeletal Research, Institute for Orthopaedic Research and Biomechanics, University of Ulm, Helmholtzstrasse 14, D-89081 Ulm, Germany
[b] Department for Functional Materials in Medicine and Dentistry, University of Würzburg, Pleicherwall 2, D-97070 Würzburg, Germany

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ABSTRACT

The current study aimed to prevent the formation of hydroxyapatite reprecipitates in brushite-forming biocements by minimizing the availability of free Ca^{2+} ions in the cement matrix. This was achieved by both maximizing the degree of cement setting to avoid unreacted, calcium-rich cement raw materials which can deliver Ca^{2+} directly to the cement matrix after dissolution, and by a reduction in porosity to reduce Ca^{2+} diffusion into the set cement matrix. In addition, a biocement based on the formation of the magnesium phosphate mineral struvite ($MgNH_4PO_4{\cdot}6H_2O$) was tested, which should prevent the formation of low-solubility hydroxyapatite reprecipitates due to the high magnesium content. Different porosity levels were fabricated by altering the powder-to-liquid ratio at which the cements were mixed and the materials were implanted into mechanically unloaded femoral defects in sheep for up to 10 months. While the higher-porosity brushite cement quantitatively transformed into crystalline octacalcium phosphate after 10 months, slowing down cement resorption, a lower-porosity brushite cement modification was found to be chemically stable with the absence of reprecipitate formation and minor cement resorption from the implant surface. In contrast, struvite-forming cements were much more degradable due to the absence of mineral reprecipitates and a nearly quantitative cement degradation was found after 10 months of implantation.

1. Introduction

Bone replacement using synthetic and degradable materials is desirable in various clinical conditions. Most applied commercial materials are based on hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) ceramics, which are poorly degradable under physiological conditions, while secondary phosphates such as brushite ($CaHPO_4{\cdot}2H_2O$), monetite ($CaHPO_4$) or struvite ($MgNH_4PO_4{\cdot}6H_2O$) have a higher solubility and hence a better degradation regime [1–4]. While HA can be formed by both sintering or aqueous precipitation reactions, the protonated phosphates can be synthesized only at low temperatures from aqueous solutions containing calcium (magnesium) and phosphate ions at a suitable pH and stoichiometry. If slowly soluble raw materials are combined with an aqueous phase at high powder-to-liquid ratios (PLRs), these mixtures undergo a cement setting reaction and harden within several minutes while precipitating the setting product [5]. Depending on the composition and the pH of the cement paste, various matrices are obtained. At a neutral pH, stoichiometric or calcium-deficient HA (CDHA) is formed, e.g. by hydrolysis of α-$Ca_3(PO_4)_2$ or by the reaction of tetracalcium phosphate ($Ca_4(PO_4)_2O$) with $CaHPO_4(\cdot 2H_2O)$ [6,7]. In the presence of a strong acidic environment, most calcium phosphates hydrolyse to the protonated secondary calcium phosphates monetite or brushite [8]. If magnesium compounds are used as cement raw materials (e.g., MgO or $Mg_3(PO_4)_2$) the resulting cements react in the presence of ammonium ions to form the biomineral struvite [9]. The resorption of the above-mentioned bioceramics is based on different mechanisms. A passive degradation by simple chemical dissolution will occur if the solubility of the ceramic is several times higher than the corresponding ion concentrations in the surrounding body fluid, which is the case for monetite, brushite or struvite ceramics [10–12]. An active bioceramic degradation is also possible by osteoclastic bone remodeling [13–15], in which the local acidic environment produced by these cells leads to an increased solubility of the ceramic material. This kind of degradation is limited by the osteoclastic activity at the interface between the ceramic and the surrounding bone.

The degradation profile of ceramic bone cements has been investigated in various in vivo studies, and the observed rate of degradation and the formation of new bone were found to vary over a broad range [10,16–21]. A severe problem of degradable biocements based on brushite is that phase changes can occur during long-term implantation. Various studies have detected the

* Corresponding author.
E-mail address: uwe.gbureck@fmz.uni-wuerzburg.de (U. Gbureck).

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formation of apatitic reprecipitates within brushite cements slowing down the resorption rate [22,23]. A concept to solve this problem was introduced by Bohner and Matter [24] by adding magnesium salts to the cement such that the released magnesium ions inhibit apatite crystal growth. Although this worked in an in vitro study [25], a corresponding in vivo experiment still showed the formation of ~40% HA over 6 months of implantation [26]. The authors concluded that the magnesium concentration within the cement was obviously too low to prevent reprecipitation of an apatitic phase.

The current study aimed to prevent the formation of HA reprecipitates by a combined chemical/physical approach. Since the Ca/P ratio of HA reprecipitates (Ca/P = 1.5–1.67) is much higher compared to brushite (Ca/P = 1.0), this process requires additional calcium ions to create an ionic electrolyte composition with a suitable stoichiometry. These calcium ions may have been stemmed from two different sources in previous works: (i) unreacted cement raw materials such as β-tricalcium phosphate (β-TCP; ~45 wt.% present in Ref. [26]) can deliver Ca^{2+} directly in the cement matrix after dissolution; and (ii) Ca^{2+} may also diffuse into the porous cement from the surrounding physiological electrolyte. To minimize both effects, the brushite cement composition in the current study was altered in such a way that (i) the degree of conversion to brushite was maximized by using an equimolar mixture of β-TCP and monocalcium phosphate anhydrous (MCPA, $Ca(H_2PO_4)_2$); and (ii) a reduction in porosity was achieved by decreasing the PLR to reduce Ca^{2+} diffusion into the set cement matrix. The cement pastes were intraoperatively formed and implanted into mechanically unloaded femoral defects of sheep. In addition, a biocement based on the formation of the magnesium phosphate struvite was tested, since this type of cement contains a high proportion of magnesium ions and should therefore also prevent the formation of low-solubility HA reprecipitates. Implants were retrieved after 4, 7 and 10 months and analysed by microcomputed tomography (μ-CT) analysis and X-ray diffraction (XRD) with regard to changes of their density and phase composition. Remaining mechanical strength was tested using an indentation test and the fractured cement surfaces were observed by scanning electron microscopy (SEM).

2. Materials and methods

2.1. Cement preparation

α/β-TCP was prepared by sintering $CaHPO_4$ (Mallinckrodt-Baker, Germany) and $CaCO_3$ (Merck, Germany) in a molar ratio of 2:1 for 5 h at 1100 °C (β-TCP) or 1400 °C (α-TCP) following quenching to room temperature. $Mg_3(PO_4)_2$ (farringtonite) was synthesized accordingly by using a 2:1 powder mixture of $MgHPO_4{\cdot}3H_2O$ and $Mg(OH)_2$ (Fluka–Sigma–Aldrich, Germany) which was sintered at 1100 °C for 6 h. The sintered cakes were crushed and passed through a 125 μm pore size-sieve followed by ball milling at 200 rpm for 10 min (β-TCP), for 4 h (α-TCP) and for 2 h ($Mg_3(PO_4)_2$).

Brushite cements were produced by mixing 10.0 g β-TCP powder in an equimolar ratio with monocalcium phosphate anhydrous (7.55 g, $Ca(H_2PO_4)_2$, Sigma–Aldrich, Germany) in an electric coffee grinder (2 × 15 s) followed by mixing these powders with 0.05 M citric acid at a PLR of 2.0 or 3.0 g ml^{-1}. After stirring for 30–45 s in a glass beaker the slurry was transferred into a 5 ml syringe. The cement was injected without any needle into the defects when it became pasty, which was for the 2 g ml^{-1} PLR after ~3 min and for the 3 g ml^{-1} PLR after 2 min. Struvite-forming cements were obtained by mixing 10.0 g $Mg_3(PO_4)_2$ with a solution containing 3.0 M $(NH_4)_2HPO_4$ and 0.5 M $NH_4H_2PO_4$ at a PLR of 2.0 and 3.0 g ml^{-1}. For the 2.0 g ml^{-1} PLR 5 ml liquid were added and stirred in a beaker for 45 s. The slurry became pasty very quickly and was injected into the defects directly after transferring it into a 5 ml syringe. For the 3.0 g ml^{-1} PLR the cement was mixed with 3.33 ml liquid on a glass plate for 45 s and the paste was directly filled into the defects with a spatula. CDHA cement samples were obtained by mixing the α-TCP powder with a 0.25 M sodium phosphate solution containing 0.083 M NaH_2PO_4 and 0.167 M Na_2HPO_4 (both Merck, Germany) at a PLR of 3.3 g ml^{-1}. The HA cement was also mixed on a glass plate and inserted with a spatula. All cement powders were sterilized prior to use for the implantation experiments by γ-irradiation with a dose of 25 kGy, while the cement liquids were autoclaved at 121 °C for 2 h.

2.2. Animal experiments

The animal study was performed according to the national regulations for the care and use of laboratory animals and was approved by the local Ethical Committee (Regierungspräsidium Tübingen, Germany, No. 1070). 49 adult female merino sheep (age: 4–7 years, weight: 94 ± 8 kg) were divided into seven groups. They received bilaterally different cement formulations and were killed at three different time points according to the scheme depicted in Table 1. Each animal of the brushite and struvite groups received two different PLRs, each in one extremity. The control group, where the defects were filled with HA or left empty, was kept for 10 months only, because we did not expect any resorption of the HA cement [18] and wanted to confirm that the defects are critical size models.

Under general halothane anesthesia and premedication with thiobarbital (Trapanals, BYK Gulden, the Netherlands) a hole 10 mm in diameter and 15 mm deep was drilled in the medial femoral condyle. The defects were cleaned of bone debris, flushed with sterile saline and dried with gauze before filling with the freshly mixed cements. After the cements were set, ~3 min, the wounds were closed in layers. The animals received 4 mg kg^{-1} carprofen (Rimadyl®Pfizer) s.c. for analgesia and 10 mg kg^{-1} amoxicillintrihydrate (Veyxyl®LA20%,Veyx) s.c. as antibiotic treatment for 4 days. After 3, 7 and 10 months the animals were killed and the femora were collected and processed for histological and biomechanical investigations. Briefly, the femoral condyles were cut into blocks of 2 cm × 2 cm × 2 cm, in which the cement cylinders were located in the center of the cuboid. For biomechanical testing a plane-parallel slice with a height of 5 mm was cut from each cuboid perpendicular to the axis of the implant and was tested immediately. The remaining bone was stored in 4% buffered formaldehyde and scanned by μ-CT. After fixation for 5 days in 4% buffered formaldehyde, the bone samples were dehydrated in an ascending series of ethanol and afterwards embedded in methylmethacrylate (Merck KGaA, Darmstadt, Germany). Using the sawing and grinding technique described by Donath and Breuner [27], histological thin slices of 80–120 μm were obtained and subsequently stained with toluidine blue and basic fuchsine. μ-CT analysis of the explants was performed with a Skyscan1172 (Bruker MICROCT, Kontich, Belgium) using 100 kV, 100 μA and a resolution of 29.7 μm and with the corresponding Skyscan™CT-analyzer software. The region of interest was a cylinder perpendicular to the axis of the implant (16 mm diameter × 1 mm height).

2.3. Setting time, porosity and mechanical performance in vitro/in vivo

The initial setting times of the cements were measured in a humidity chamber at 37 °C and >90% humidity using the Gilmore needle test with a needle of 113.98 g and 2.117 mm diameter according to the relevant ASTM standard [28]. The development of the pH value of the cement pastes during the setting process was measured using a cut-in pH electrode (Mettler-Toledo, Germany).