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In vivo degradation of low temperature calcium and magnesium phosphate ceramics in a heterotopic model

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ABSTRACT

Bone replacement using synthetic and degradable materials is desirable in various clinical conditions. Most applied commercial materials are based on hydroxyapatite, which is not chemically degradable under physiological conditions. Here we report the effect of a long-term intramuscular implantation regime on the dissolution of various low temperature calcium and magnesium phosphate ceramics in vivo. The specimens were analysed by consecutive radiographs, micro-computed tomography scans, compressive strength testing, scanning electron microscopy and X-ray diffractometry. After 15 months in vivo, the investigated materials brushite ($CaHPO_4{\cdot}2H_2O$), newberyite ($MgHPO_4{\cdot}3H_2O$), struvite ($MgNH_4PO_4{\cdot}6H_2O$) and hydroxyapatite ($Ca_9(PO_4)_5HPO_4OH$) showed significant differences regarding changes of their characteristics. Struvite presented the highest loss of mechanical performance (95%), followed by newberyite (67%) and brushite (41%). This was accompanied by both a distinct extent of cement dissolution as well as changes of the phase composition of the retrieved cement implants. While the secondary phosphate phases (brushite, newberyite, struvite) completely dissolved, re-precipitates of whitlockite and octacalcium phosphate were formed in either particulate or whisker-like morphology. Furthermore, for the first time the possibility of a macropore-free volume degradation mechanism of bioceramics was demonstrated.

1. Introduction

The demand for synthetic bone replacement materials is incessant since they offer the advantages of having a well-defined chemistry and architecture, unlimited availability without causing any donor site morbidity and the exclusion of infection transmission compared with homologous or xenogenous grafts. Because of the chemical similarity to the mineral phase of bone, favoured synthetic materials are based on calcium phosphate chemistry. These materials are either applied as sintered monoliths or granules of β-tricalcium phosphate (β-TCP) [1] or hydroxyapatite (HA) [2] as well as self-setting cements based on calcium and magnesium phosphate chemistry [3,4]. Depending on the composition and the pH of the cement paste, various matrices are obtained. At a neutral pH, stoichiometric or calcium deficient hydroxyapatite 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 $CaHPO_4{\cdot}(2H_2O)$ [5,6]. In the presence of a strong acidic environment, most calcium phosphates hydrolyse to the protonated secondary calcium phosphates monetite ($CaHPO_4$) or brushite ($CaHPO_4{\cdot}2H_2O$) [7]. Degradation of the above-mentioned low temperature bioceramics is based on different mechanisms. A passive degradation by simple chemical dissolution will only occur if the solubility product of the ceramic is several times higher than the corresponding ion concentrations in the surrounding body fluid. This is possible for secondary, protonated calcium phosphates like monetite ($CaHPO_4$) and brushite ($CaHPO_4{\cdot}2H_2O$) [8–11]. However, changes of the phase composition by precipitation processes may occur within brushite cements depending on composition, porosity and fluid exchange within the ceramic, decreasing their solubility [12]. Secondly, bioceramic degradation is also possible by osteoclastic bone remodeling [13,14]. The local acidic environment, which is 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.

Biocements could also be formed by reacting magnesium compounds (e.g. MgO or $Mg_3(PO_4)_2$) with either ammonium ions to form the biomineral struvite ($MgNH_4PO_4{\cdot}6H_2O$) [15] or with primary phosphates or phosphoric acid to form newberyite ($MgHPO_4{\cdot}3H_2O$) matrices [16]. These magnesium phosphate cements are thought to be degradable by both chemical dissolution and osteoclastic activity in the body similar to brushite cements.

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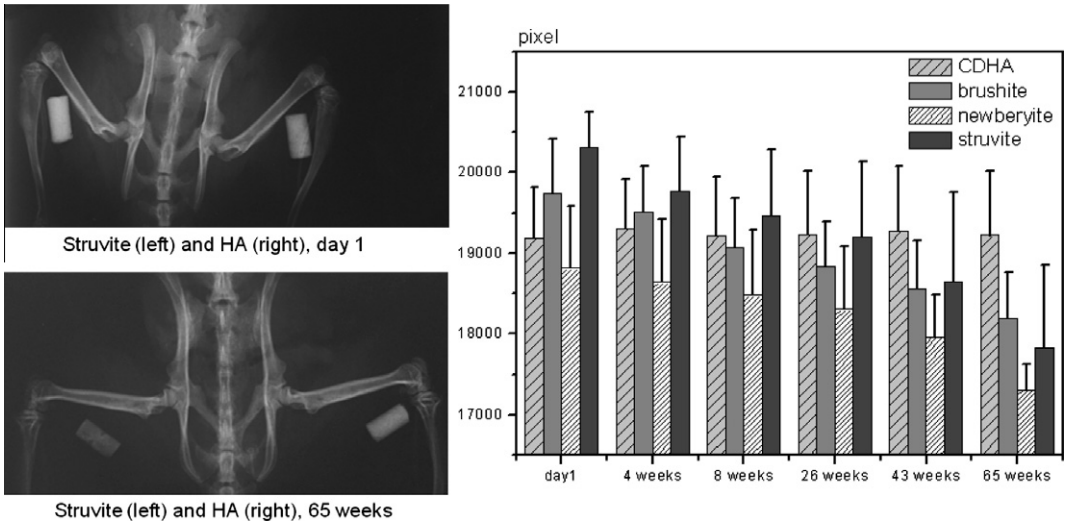

Fig. 1. Examples of X-ray micrographs for struvite (left) and calcium-deficient hydroxyapatite (right) cements immediately after implantation (day 1) and after 15 months in situ. Radiographic analysis of the implants in situ, starting on day 1 until the end of the course (65 weeks). The number of pixels reflects the implant area two-dimensionally. HA remains constant all over the course. The other ceramics present a significant ($p < 0.01$) reduction of their radiographic projection areas. The final projection areas (week 65) were found to be 87.7% (struvite), 92% (newberyite) and 92.2% (brushite) compared with the corresponding starting values (day 1). The results are displayed as mean of pixels of six specimens and the error bar represents 1 standard deviation.

A major advantage of the struvite cement is their setting at a physiological pH value combined with high early strength of the set matrix as demonstrated in previous in vitro studies [17,18]. However, little is known about the long-term in vivo behaviour of these magnesium phosphate cements.

To evaluate the chemical dissolution of various low temperature calcium and magnesium phosphate ceramics and changes of their phase composition in vivo without osteoclastic degradation, a long-term intramuscular implantation regime over 15 months was performed in this study. Additionally, the heterotopic model reflects the clinical situation in the case of reconstruction of large and complex bone defects since implant structures for such defects are extensively surrounded by soft tissues. Pre-set cylinders of 5 mm diameter and 10 mm length were implanted into the femoral extensor muscles of Sprague–Dawley rats and the implant degradation was followed by radiographic control for up to 15 months. The explanted specimens were additionally analysed by micro-computed tomography (μ-CT) analysis and X-ray diffraction with regard to changes of their density and phase composition. Remaining compressive strength was tested and the fractured surfaces were observed by scanning electron microscopy (SEM).

2. Materials and methods

2.1. Cement preparation and implant moulding

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. $Mg_{2.25}Ca_{0.75}(PO_4)_2$ was synthesized accordingly by using a powder mixture of $CaHPO_4$, $CaCO_3$, $MgHPO_4{\cdot}3H_2O$ and $Mg(OH)_2$ (Fluka-Sigma–Aldrich, Germany) in a molar ratio of 2:1:6:3 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 2 h (α-TCP) and for 1 h ($Mg_{2.25}Ca_{0.75}(PO_4)_2$).

Calcium deficient hydroxyapatite (CDHA) cement samples were obtained by mixing the α-TCP powder with a 2.5 wt.% Na_2HPO_4 (Merck, Germany) solution at a powder to liquid ratio (PLR) of 3.0 g ml^{-1}. Brushite cements were produced by mixing the β-TCP powder in an equimolar ratio with monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2{\cdot}H_2O$, Sigma–Aldrich, Germany) in a coffee grinder followed by mixing these powders with 0.05 M citric acid at a constant PLR of 3.0 g ml^{-1}. Newberyite cement was obtained similar to brushite cement by replacing β-TCP by $Mg_{2.25}Ca_{0.75}(PO_4)_2$. Struvite samples were prepared by mixing $Mg_{2.25}Ca_{0.75}(PO_4)_2$ and 3.5 M $(NH_4)_2HPO_4$ solution (Merck, Germany) at a PLR of 3.0 g ml^{-1}. The cement pastes were then transferred into cylindric silicon rubber moulds (d = 5 mm, h = 10 mm) and allowed to set for 24 h at room temperature. All samples were stored in phosphate buffered saline (PBS) (Merck, Germany) for 7 days with a daily change of the buffer to remove unreacted acidic or ionic cement reactants. Finally the specimens were sterilized by soaking in 70% ethanol for 10 min followed by air-drying.

2.2. Animal experiments

Implantation experiments were performed intramuscularly in Sprague–Dawley rats (n = 12) with a median age of 9 weeks and an average weight of 318 g. For surgery an intraperitoneal anaesthesia with a mixture of Ketavet (120 mg kg^{-1} weight) and Rompun (5 mg kg^{-1} weight) was conducted. Experiments were approved by the local ethical committee and by the veterinary authorities (No. 55.2-2531.01-80/07). Cement specimens were implanted into the left and right femoral extensor muscles of each animal, where both (1) the cement composition at the left side within an animal was different from the right side and (2) the combination of the compositions for all animals was alternating. Thus the number of specimens was n = 24 with n = 6 per cement type. Radiographic imaging of the implants was performed immediately following implantation as well as after 1, 2, 6, 10 and 15 months after sedation of the animals with isoflurane. For imaging a Faxitron 43855A X-ray system (40 kV, 40 s) with a Kodak X-OMAT MA film and an Agfa Curix 242S film processor were used. The radiograms were digitalized and analysed by determining the implant projection area (number of pixels) using Adobe Photoshop 7.0 software. All animals were sacrificed after 15 months post-implantation and the specimens including the surrounding soft tissues were explanted immediately and stored in 3.5% formaldehyde until further usage within 2 weeks.

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