Pyrophosphate modification prevents long term stability of brushite cement

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Statement of Purpose: As brushite ($CaHPO_4.2H_2O$) is sparingly soluble in physiological conditions it has the potential to be more completely resorbed *in-vivo* than hydroxyapatite based bone replacement materials, which can remain in place for decades. The metastable nature of brushite, however, means that in physiological conditions it hydrolyses slowly to form hydroxyapatite causing long-term implant stability [1]. The addition of a magnesium salt to stabilize the brushite component has been shown to be successful *in vitro* [2] but not *in vivo* [3]. Our group has recently demonstrated that by forming brushite cement containing an amorphous calcium pyrophosphate component, it is possible to stabilize brushite cement for in excess of 90 days *in-vitro* allowing extensive cement degradation to occur [4]. This stabilization was thought to be as a result of the propensity of pyrophosphate ions for preventing hydroxyapatite formation. To determine whether this phenomenon can operate *in vivo*, a pyrophosphate modified brushite cement was implanted into an ovine model. The sites of implantation were histologically examined after periods of up to 1 year and the cement was examined to determine whether brushite hydrolysis had occurred using Rietveld refinement of X-ray diffraction data.

Methods: Sample Fabrication - Two different cement formulations were examined in this study. The non-modified brushite cement material (control) was formed by incrementally mixing β-tricalcium phosphate (Plasma-Biotal, Derbyshire, UK) with a 2M orthophosphoric acid solution containing trisodium citrate at a concentration of 50 mM (Sigma-Aldrich, Gillingham, UK) at a powder to liquid ratio (P:L) of 1.75 g/mL. The pyrophosphate modified brushite cement was formed from the incremental combination of β-TCP with 540 mg of polyphosphoric acid (105, Rhodia, West Midlands, UK) and 720 mg double distilled water (P:L=2.25 g/mL). Following the combination of the powder and liquid components, the cement pastes were cast into a PTFE mould to form specimens of diameter 6.4 mm and height 12 mm. The cement samples were left to set for 1 h at 37˚C before removal from the mould. Prior to implantation samples were washed in distilled water and air dried, sealed into sterilisation bags and sterilised by gamma irradiation (26 kGy). **Implantation –** A total of 18 sheep were used in this experiment. The sheep were put under general anaesthesia and then defects were made in the medial side of the proximal tibia in each hind leg. Implants were push fitted into the defects (diameter 6.4 mm, depth 12 mm) according to a randomisation schedule and the soft tissue closed. Fluorescent bone markers were administered intravenously at various stages during the study. **Histological analysis –** 3, 6 and 12 months after implantation the sheep were sacrificed and the bone surrounding the implants harvested. Each sample was bisected transversely. One half was decalcified and processed for wax embedding and the other resin embedded and analysed for the presence of fluorescent bone

marker staining and new bone growth (using alizarin red). The cements harvested from the bone at each time-point were analysed by X-ray diffractions to determine composition.

Results/Discussion: Histological sections through the harvested implant site 3 and 12 months post implantation are shown in Figure 1. After three months there was little difference between the pyrophoposphate modified and non-pyrophosphate modified materials (Fig. 1A and B), with the defect in each case containing a dense core of cement. By 12 months post implantation, however, the contrast between cements was marked. In the case of the pyrophosphate modified brushite cement, extensive resorption had taken place and the cement had been replaced by new bone (indicated by the green pseudo colouring), however, the brushite control material remained *in situ* as a dense core of cement with comparatively little bone formation. In order to examine the factors that may have influenced the degradation rates of each cement, the phase composition of the cement cores were determined using Rietveld refinement phase analysis. The non-modified brushite cement consisted of β-TCP and poorly crystalline hydroxyapatite, suggesting that all of the brushite had undergone hydrolysis. Whereas the pyrophosphate modified cement still consisted entirely of brushite and β-TCP.
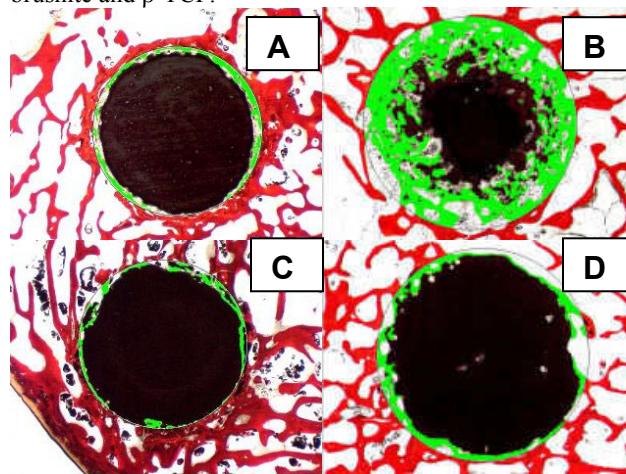

Figure 1. Alizarin red stained sections through the pyrophosphate modified brushite cement 3 (A) and 12 months (B) post implantation and the control brushite cement 3 (C) and 12 months (D) post implantation.

Conclusions: In this study, it has been demonstrated that the modification of brushite cement with an amorphous calcium pyrophosphate compounds prevents the formation of hydroxyapatite by brushite hydrolysis and thereby facilitates more complete degradation *in vivo* when compared with a non-modified brushite cement control group.

References:
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