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Effect of processing conditions of dicalcium phosphate cements on graft resorption and bone formation

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ABSTRACT

Dicalcium phosphate cements (brushite and monetite) are resorbable biomaterials with osteoconductive potential for bone repair and regeneration that have yet to gain widespread commercial use. Brushite can be converted to monetite by heat treatments additionally resulting in various changes in the physicochemical properties. However, since conversion is most commonly performed using autoclave sterilisation (wet heating), it is uncertain whether the properties observed for monetite as a result of heating brushite under dry conditions affect resorption and bone formation favourably. This study was designed to produce monetite grafts of differing physical form by autoclaving and dry heating (under vacuum) to be compared with brushite biomaterials in an orthotopic pre-clinical implantation model in rabbit for 12 weeks. It was observed that monetite grafts had higher porosity and specific surface area than their brushite precursors. The autoclaved monetite grafts had compressive strength reduced by 50% when compared with their brushite precursors. However, the dry heat converted monetite grafts had compressive strength comparable with brushite. Results from *in vivo* experiments revealed that both types of monetite graft materials resorbed faster than brushite and more bone formation was achieved. There was no significant difference in the amount of bone formed between the two types of monetite grafts. The implanted brushite grafts underwent phase transformation to form hydroxyapatite, which ultimately limited bioresorption. However, this was not observed in both types of monetite grafts. In summary, both autoclaving and dry heating the preset brushite cement grafts resulted in monetite biomaterials which were more resorbable with potential to be investigated and optimized for orthopaedic and maxillofacial bone repair and regeneration applications.

Statement of Significance

We present in this original research article a comparison between dicalcium phosphate cement based grafts (brushite and 2 types of monetite grafts prepared by wet and dry thermal processing) with regards to resorption and bone formation *in vivo* after orthotopic implantation in rabbit condylar femural region. To the best of our knowledge this is the first *in vivo* study that reports a comparison resorption and bone formation using brushite and two types of monetite biomaterials. Also, we have included in the manuscript a summary of all the *in vivo* studies performed on brushite and monetite biomaterials to date. This includes cement composition, physical properties (porosity and surface area), implantation and histomorphometrical details such as animal species, site of implantation, observation period, percentage bone tissue formation and residual graft material. In addition, we calculated the percentage resorption of graft materials based upon various implantation sites and included that into the discussion section. The results of this original research provides greater understanding of the resorption processes of dicalcium phosphate based grafts, allowing preparation of bone substitute materials with more predictable resorption profiles in future.

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1. Introduction

Bone substitutes are frequently used in dental and orthopaedic surgery [1,2] and currently, autografts are considered to be the best option due to having high biological acceptability after implantation [3,4]. However, disadvantages such as limited availability, donor site morbidity and increased procedural cost [4–7] give rise to reservations over the use of autografts and have lead towards research to find more suitable alternatives. Calcium phosphate biomaterials have similar composition to bone and are of interest as bone substitutes [8]. The mineral named brushite (dicalcium phosphate dihydrate, DCPD), has the ability to support new bone tissue formation but with varying amounts of woven bone and fibrovascular tissue [9–11]. Ideally, resorption of graft materials should be concurrent with new bone formation, in order to obtain a stabilized repair and eventually a fully healed bone defect [12]. The rate of dissolution and resorption is thought to be dependant on the chemical composition and physical characteristics of the calcium phosphate bioceramics [13,14]. Although initially after implantation brushite cement biomaterials do resorb, they tend to react with the surrounding environment and convert to insoluble hydroxyapatite (HA) [15,16], whereupon resorption slows down. This phase conversion affects the rate of resorption negatively and ultimately limits their clinical usefulness for certain applications [17,18].

Monetite (dicalcium phosphate anhydrous, DCPA) can be prepared by either modifying the precipitation conditions of brushite to promote setting as monetite [16], or by dehydration of preset brushite cements [15,19]. The following equation represents conversion of DCPD to DCPA:

$$CaHPO_4 \cdot 2H_2O \rightarrow CaHPO_4 + 2H_2O \quad (1)$$

The dehydration of brushite can be carried out under wet or dry conditions which alter physical properties of the produced monetite biomaterials. Heating in absence of moisture is known to cause shrinkage of the material [15]. However, by maintaining adequate humidity, pressure and temperature (by autoclaving which is also used for sterilisation) this can be prevented [20]. Monetite prepared by autoclaving has inferior mechanical properties in comparison to their brushite precursor bioceramics [17] but show the ability to regenerate bone in animal and human bone defects and to also stimulate vertical bone augmentation [19,21–23]. These monetite materials also demonstrate higher volumes of bone regeneration achieved than with HA based graft substitutes [19,24]. Monetite grafts prepared by autoclaving resorb at a faster rate than brushite [17,25] and do not convert to HA [19,24] *in vivo*.

Pre-clinical reports on monetite usually refer to autoclaved materials and there has been no direct comparison in literature between autoclaved and dry heat converted monetite with regards to resorption in an orthotopic implantation model. A recent study has compared two types of monetite grafts prepared by autoclaving and dry heat conversion and implanted in a calvarial rabbit model has shown that the autoclaved monetite resorbs more with greater bone augmented within the graft area [26]. This current study was designed to compare graft resorption and bone response *in vivo* between brushite and the two types of monetite prepared by autoclaving and dry heat (under vacuum) conversion. The study was tested for the hypotheses, that both monetite bioceramics would resorb to a greater extent *in vivo* compared to brushite, and that there would be a discernable difference between the two monetites.

2. Method and materials

2.1. Synthesis

Brushite cement grafts were prepared with a mixture of β-TCP (Merck) and commercially available monocalcium phosphate hydrate (MCPM) (ABCR, GmbH & Co.KG) using a ratio of 1.2:1 respectively. The cements were produced at P/L mixing ratio of 3. The powders were hand ground with a pestle and mortar and cement pastes prepared by mixing the powder with appropriate amount of distilled water on a glass slab for 20 s. Once all of the powder was combined with the liquid, the cement paste was kneaded for a further 30 s. The manipulated cement slurry was cast into a polytetrafluoroethylene (PTFE) split mould forming hardened cement cylinders (∼4 mm height and 3 mm diameter). The cylinders were allowed to set for 24 h at 37 °C ± 1 °C in a vacuum desiccator to form brushite. At the end of the incubation period, the samples were removed from the moulds and weighed until constant mass was reached. Three different batches of fifteen cylinders each were produced to obtain a total of forty-five cylinders.

Monetite cement grafts ($n = 60$ in total) were synthesized by conversion of the preset brushite cement cylinders utilizing two different methods: dry heat and wet heat conversion. For dry heat conversion, brushite cylinders ($n = 30$) were heated at 250 °C for 30 min under vacuum (80 mTorr). Wet heat transformation was performed with brushite cylinders ($n = 30$) being autoclaved at sterilising conditions (121 °C, 100% humidity and 15 psi, for 20 min).

2.2. Characterisation of the biomaterial grafts

The phase purity of the brushite and monetite grafts was confirmed using X-ray diffraction (XRD). Data was collected (Bruker Discover D8 diffractometer) with Ni filtered CuKα radiation ($\lambda = 1.54$ Å) with a two dimensional VANTEC area detector at 40 kV and 40 mA. A step size of 0.02° was used to measure from 10 to 40° 2θ over 3 frames with a count time of 300 s per frame. The phase composition was checked by means of the International Centre for Diffraction Data reference patterns for brushite (PDF Ref. 09-0077) and monetite (PDF Ref. 09-0080). The XRD patterns were analyzed using DIFFRAC plus EVA software 14.0 (AXS, Bruker, Germany). The microstructural morphology of the prepared grafts was examined with a scanning electron microscope (SEM) (Hitachi S-4700 FE-SEM; Tokyo, Japan) operating at an accelerating voltage of 20 kV using a back-scattered electron (BSE) detector. Elemental composition (Ca/P ratio) of the bioceramics was assessed by employing energy dispersive X-ray (EDX) analysis using Oxford detector with a SEM (Hitachi S-4700 FE-SEM; Tokyo, Japan) and INCA software (Oxford Instruments, Abingdon, UK). The true density of the grafts was determined using a helium pycnometer (Accupyc 1330, Micromeritics). The volume of each sample was measured 10 times following 10 purges of the measurement chamber with helium. The relative porosity (bulk porosity) of grafts was calculated from apparent and true density measurements. The pore size distribution of the prepared brushite and monetite grafts was measured by using mercury intrusion porosimetry (9420, Micromeritics, Bedfordshire, UK). The specific surface area (SSA) of grafts was determined by using the Brunauer–Emmett–Teller (BET) method with helium adsorption–desorption (Tristar3000, Micromeritics). The compressive strength of the grafts was also measured before implantation. The geometrical measurements were made in triplicate and the samples were mounted on the testing machine (5544, Instron) so that the long axes of the graft cylinders were perpendicular to the lower anvil. A compressive force was then applied to the upper surface of the cylinders at a constant crosshead displacement rate of 1 mm/min until failure occurred. The applied load was measured using a 100 N load cell (5544, Instron). Mean compressive strength was determined from the average of 10 measurements.

2.3. In vivo implantation

An animal study was performed in order to evaluate *in vivo* differences between the three types of prepared biomaterial grafts