Annals of Anatomy 200 (2015) 126–133

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

Research article

Monetite granules versus particulate autologous bone in bone regeneration

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ARTICLE INFO

Article history:
Received 29 November 2014
Received in revised form 5 March 2015
Accepted 18 March 2015

Keywords:
Monetite
Autologous bone graft
Bone regeneration
Rabbit critical size defects
Histomorphometric study

ABSTRACT

Objective: The aim of this study was to test bone tissue response to monetite granules in comparison with intramembranous autologous bone graft in a rabbit calvaria critical size defect model.
Materials and methods: Novel monetite granules were synthesized by thermal conversion of set brushite cement. Eight female New Zealand rabbits were used for this study. Two identical 10 mm diameter bicortical cranial defects were created in each animal. One of the defects was grafted with monetite granules while the contralateral was grafted with granules of intramembranous autologous bone as control. Animals were sacrificed 8 weeks after surgery and biopsies were taken for histological and histomorphometrical evaluation under light microscopy. Wilcoxon test was used for statistical analysis.
Results: The bone defects treated with either autologous bone or monetite granules were able to heal within the study period. Upon histological observation the defects treated with autologous bone granules resembled the adjacent intact calvaria, whereas the defects treated with monetite showed a high infiltration of new bone and only 13.4 ± 8.4% of remaining granules. The percentage of bone volume in the defects of the control group (71 ± 9%) was 16% higher than in the study group (55 ± 10%) ($p < 0.05$). The percentage of augmented mineralized tissue volume in the study group (68 ± 18%) was not significantly different from the control group ($p > 0.05$).
Conclusion: The amount of augmented mineralized tissue in the bone defects obtained with monetite granules was not significantly different from that obtained with autologous bone. This study confirms the potential of monetite based biomaterials as an alternative to autologous bone graft.

1. Introduction

Bone regeneration in oral and maxillofacial surgery has become an essential therapeutic tool used to increase bone quality and quantity in areas where insufficient bone volume prevents the correct stabilization of dental implants (McAllister and Haghighat, 2007). Bone regeneration can be achieved by applying a biomaterial that would enhance bone formation in specific surgical sites. These biomaterials should combine osteogenic, osteoinductive and osteoconductive properties (Albrektsson and Johansson, 2001). Another desirable characteristic of bone substitutes would be their ability to be remodeled and replaced by newly formed bone. Ideally, the biomaterial resorption rate should be similar to the bone resorption/growth rate. Therefore, the biomaterials used in bone regeneration should be resorbed *in vivo* and replaced by autologous bone in order to increase the bone implant contact (Bohner, 2000; Bohner and Gbureck, 2005). Autologous bone remains the only biomaterial that fulfills all the desirable mentioned properties; and it is therefore considered the gold standard material for bone regeneration. However, its' harvesting provokes high costs and post-operative morbidity; therefore research is still required to develop alternative biomaterials to overcome the current limitations of autografts (Albert et al., 2006).

Calcium phosphates have been extensively investigated as biomaterials for bone regeneration (Bohner and Gbureck, 2005). Most calcium phosphate research has been focused on the use of hydroxyapatites due to their close resemblance to the mineral component of bone. However, despite the excellent osteoconductive properties of hydroxyapatites, their poor *in vivo* resorption limits their clinical performance (Bohner, 2000; Bohner and Gbureck, 2005;

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http://dx.doi.org/10.1016/j.aanat.2015.03.008
0940-9602/

Hallman et al., 2001). On the other hand, more soluble calcium phosphates such as dicalcium phosphate anhydrate (DCPA), also known as monetite, have recently engendered great interest due to their rapid *in vivo* resorbability which is much faster than hydroxyapatite (Gbureck et al., 2007; Tamimi et al., 2008). New evidence has pointed out the benefits of using such materials in bone regeneration (Tamimi et al., 2008, 2009, 2010).

Monetite bioceramics prepared by hydrothermal conversion of brushite can be formed as granules, blocks and 3D-printed scaffolds (Gbureck et al., 2007; Tamimi et al., 2009, 2010, 2012). Monetite granules have been shown to be osteoconductive in critical sized cranial defects in rabbits (Tamimi et al., 2008), while studies in goats have confirmed that 3D-printed monetite blocks could also be osteoinductive (Habibovic et al., 2008). Moreover, these materials are bioresorbable, and their long-term *in vivo* performance has been shown to be superior to other calcium phosphates such as bovine hydroxyapatite and brushite (Tamimi et al., 2012, 2006; Gbureck et al., 2007). The resorption mechanism of thermally synthesized monetite is mainly due to cellular activity and by passive dissolution (Grossardt et al., 2010). Even though early literature on monetite powder indicated that they could transform to hydroxyapatite *in vivo* (Tamimi et al., 2012), it seems that monetite biomaterials prepared by thermal dehydration of brushite cements behave differently. Reports on this new type of material have failed to show conversion to hydroxyapatite for implantation periods of up to 8 weeks in rabbits (Tamimi et al., 2014).

For these reasons, we believe that using monetite granules as a resorbable bone graft material could be of special interest as an alternative to autologous bone grafts. To the best of our knowledge, no studies have been found in the literature comparing the effects of monetite granules versus autologous bone particles in bone regeneration procedures. The aim of this study was to compare the bone regeneration behavior of monetite granules with the gold standard, autologous bone. In order to achieve this we prepared monetite and autologous bone graft granules with similar particle sizes (0.5–2.0 mm) and compared their performance in a critical size defect model in rabbit calvaria.

2. Materials and methods

2.1. Bone graft synthesis

Calcium carbonate (CC), dicalcium phosphate dihydrate (DCPD), monocalcium phosphate anhydrate (MCPA), sodium pyrophosphate, and phosphoric acid were purchased from Sigma-Aldrich with the highest purity and used without further purification. Beta-tricalcium phosphate (β-TCP) was synthesized by mixing equimolar amounts of CC and DCPD at 900 °C for 14 h. Monetite granules were synthesized by thermal conversion of brushite cement granules. The brushite cement powder was prepared by mixing 1.428 g of β-TCP (57 wt.%,), 0.8 g of MCPA, and 0.012 g of sodium pyrophosphate as a retardant. (Mariño et al., 2007; Tamimi et al., 2008). The cement was set into blocks by mixing the brushite powder with 1 M phosphoric acid solution in a powder-to-liquid ratio of 2.5 g/ml. Phosphoric acid was used to achieve a high yield of β-TCP conversion to brushite. After setting of the cement, brushite blocks were obtained. These blocks were then used to prepare granules by milling them with a mortar and a pestle. The granules were separated with sieves of 2.0 mm and 0.5 mm pore diameter and washed with distilled water. The brushite granules were placed in heat sealable paper bags (500 mg in each bag) and autoclaved at sterilizing conditions (121 °C, 100% humidity and 15 psi, for 20 min) in order to transform brushite into monetite. The autoclave treatment also provided the biomaterial sterilization before surgical implantation.

2.2. Characterization of the material

A calibrated photograph was taken of a portion of the granules, and the distribution of the particles was calculated using ImageJ software (Wayne Rasband; National Institute of Health, Bethesda, Maryland) and Origin 8.0 software (Origin Lab Co., Northampton, MA). The crystal composition of the granules was investigated using X-ray diffraction (XRD). The XRD studies were performed with a Philips Xpert PW3050 diffractometer (H&M Analytical Services Inc., Allentown NJ, USA). The diffractograms were recorded covering an angular interval 2θ between 4° and 80°, using a step size of 0.038° with time per step of 3 s. The mineral composition of the preset brushite cement and the monetite granules were checked with the reference patterns ICSD 016132 for brushite, ICSD 31046 for monetite, and ICSD 06191 for β-TCP, using the Xpert plus software. Bulk porosity was calculated from density measurements with a helium pycnometer (AccuPyc 1330, Micromeretics; Bedfordshire, UK), and relative surface area was measured through BET nitrogen adsorption (ASAP 2020 Micromeretics; Bedfordshire, UK). Bioceramic microstructure was observed using scanning electron microscopy (Hitachi S-3000N-VP-SEM; Tokyo, Japan), at an accelerating voltage of 20 kV, and micro-CT (SkyScan1172; SkyScan; Kontich, Belgium) set at a resolution of 6.0 μm and 0.5 mm Al filter. Accordingly, the pores detectable by micro-CT were 6.0 μm or larger, and were considered as macropores. The high proportion of macropores was probably due to high water content in the cement, and due to autoclave hydrolysis of brushite that shrinks the brushite crystals into smaller monetite crystals. Elemental composition of the bioceramics was assessed with energy dispersive X-ray (EDX) analysis using an Oxford detector and INCA software (Oxford Instruments, Abingdon, UK). For compressive strength mechanical testing, cylindrical samples (12.0 mm × 6.0 mm ∅) of the bioceramic were incubated in phosphate buffered saline (PBS) at 37 °C for 24 h; then the samples were analyzed with a universal testing machine (Instron® 5569, Instron Corp., Canton, MA) set at a cross-head speed of 0.1 mm/min. All the physical–chemical and mechanical characterization analyses of the bioceramics were performed in triplicate. The compressive strength of the ceramic was measured in monolithic cylindrical specimens because granules had an irregular shape; monetite monoliths were then crushed into granules. We acknowledge that the preparation of the granules with this method might have affected their mechanical properties by initiating cracks within the material. However, since the tested biomaterial (granules) in this study is not meant for load bearing sites, this possible detriment in the mechanical properties of the granules might not be of relevance to the biological performance of the material.

2.3. Surgical procedure

Before beginning the "*in vivo*" animal study, the study protocol was approved by the ethical committee for animal experiments of the University Rey Juan Carlos (URJC). Experiments were conducted in accordance with the guidelines laid down by the European Union Council Directive of 24 November 1986 (86/609/EEC), and all the necessary measurements were taken to minimize pain and discomfort to the animals. Eight healthy 6-month-old female New Zealand rabbits weighing between 3.9 and 4.4 kg were used. The animals were accommodated in the official stable for animal assays of the URJC at 22–24 °C with 55–70% humidity, light cycles of 12 h, and air renewal 15 times per hour. The rabbits were fed with a Panlab™ (Barcelona, Spain) diet while drinking was permitted *ad libitum*.

The surgical procedure is shown in Fig. 1. All rabbits were anesthetized with an intramuscular dose of 0.75 mg/kg ketamine