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Craniofacial vertical bone augmentation: A comparison between 3D printed monolithic monetite blocks and autologous onlay grafts in the rabbit

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ABSTRACT

Onlay autografting is amongst the most predictable techniques for craniofacial vertical bone augmentation, however, complications related to donor site surgery are common and synthetic alternatives to onlay autografts are desirable. Recent studies have shown that the acidic calcium phosphates, brushite and monetite, are osteoconductive, osteoinductive and resorb faster *in vivo* than hydroxyapatite. Moreover, they can be 3D printed allowing precise host bone–implant conformation. The objectives of this study were to confirm that craniofacial screw fixation of 3D printed monetite blocks was possible and to compare the resulting vertical bone augmentation with autograft. 3D printed monolithic monetite onlay implants were fixed with osteosynthesis screws on the calvarial bone surface of New Zealand rabbits. After 8 weeks, integration between the implant and the calvarial bone surface was observed in all cases. Histomorphometry revealed that 42% of the monetite was resorbed and that the new bone formed within the implant occupied 43% of its volume, sufficient for immediate dental implant placement. Bone tissue within the autologous onlay occupied 60% of the volume. We observed that patterns of regeneration within the implants differed throughout the material and propose that this was due to the anatomy and blood supply pattern in the region. Rapid prototyped monetite being resorbable osteoconductive and osteoinductive would appear to be a promising biomaterial for many bone regeneration strategies.

1. Introduction

Advances in biomaterials and surgical techniques have contributed to an increase in the application of dental implants for the restoration of partially and totally edentulous patients. An important factor to predict the long-term success of osseointegrated implants is a sufficient volume of healthy bone at recipient sites [1]. However, this is frequently lacking as a result of trauma, tooth loss or infection such as advanced periodontitis [1].

Vertical alveolar bone loss in partially edentulous patients renders prosthetic rehabilitation difficult and presents a major challenge for dental implant placement due to anatomical restrictions and surgical difficulties. The nasal cavity, maxillary sinus and the mandibular inferior alveolar nerve limit the bone height available for implant placement. In addition a large empty space between the maxillary and mandibular ridge complicates the final treatment outcome.

Clinical and histological data support the use of vertical ridge augmentation techniques to enable dental implant placement. The main approaches considered in clinical practice include guided bone regeneration (GBR) [1–10], distraction osteogenesis [11–20] and onlay bone grafts [21–26]. Table 1 summarizes the results of some of the most relevant articles in the literature regarding vertical bone augmentation. It is apparent that although distraction osteogenesis can produce significantly greater bone height than GBR and onlay bone grafting, there is a higher rate of complication associated with this technique [27]. GBR appears to generate a similar amount of new bone to onlay bone grafting but carries a higher rate of complication.

The principles of GBR were applied in the early 1990s to atrophic jaws [2]. Severe vertical defects were treated by means of titanium reinforced non-resorbable barrier membranes in conjunction with titanium dental implants. Vertical ridge augmentation can be achieved successfully using GBR. However, success appears to be

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Table 1
Summary of clinical studies reporting the average bone height gained and complication rate of vertical bone augmentation by GBR, distraction osteogenesis and onlay autografts.

Surgical technique											
GBR				Distraction osteogenesis				Onlay bone graft			
Pts/graft no	ABH (mm)	Complcn (%)	Refs	Pts/site	ABH (mm)	Complcn (%)	Refs	Pts	ABH (mm)	Complcn (%)	Refs
5/6	4.0	16.7	[2]	10/13	7.5	23	[11]	25	4.22	4	[24]
6/6	4.95	16.7	[3]	7/10	7.0	30	[12]	9	2.2	0	[25]
1/1	2.5	0	[4]	14/14	10.3	14.3	[13]	8	4.6	25	[23]
2/2	ID	0	[5]	28/28	6.5	50	[14]	56	ID	7.1	[26]
18/22	Na	13.6	[6]	10/10	ID (6–8)	10	[15]				
20/22	5.02	18	[7]	37/37	9.9	21.6	[16]				
6/6	3.14	ID	[8]	10/10	ID	20	[17]				
1/1	ID	0	[9]	10/10	7.3	30	[18]				
7/10	3.15	10	[10]	7/7	ID (10–15)	28.6	[19]				
				37/45	8.2	75.7	[20]				
				10/10	6–12	20	[21]				
				10/10	5.3	70	[22]				
				11/11	6.1	27.3	[22]				
				9/9	5.3	33.3	[23]				
Pool ABH ± SD	4.3 ± 0.5	13.3 ± 15.5			8.0 ± 1.5	34.1 ± 24.6			3.9 ± 0.9	7.1 ± 8.8	

ABH: average bone height obtained; Pts: patients; Complcn: complications; Ref: references; SD: standard deviation. ID: insufficient data.

highly technique-sensitive and therefore application to a wide community of operators and clinical settings remains unclear [1–9]. Another major limitation of this technique appears to be the ability to regenerate bone only along the axis of the applied force [1,10–22].

Bone block onlay grafts were also introduced in the early 1990s to increase the vertical height of the maxilla and mandible [28]. This technique involves extracting a block of autologous bone from a donor site such as the iliac crest or the mandibular *ramus*, and fixing the block with osteosynthesis screws onto the recipient site. Onlay bone grafting appears to have acceptable results and minor complications at the recipient site; however, complications are often noted at the donor site. At this moment there is no satisfactory synthetic alternative to onlay autologous bone grafts for maxillofacial bone augmentation.

Synthetic calcium phosphates are excellent biomaterials for bone regeneration. However, the most commonly used calcium phosphates such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) have limited *in vivo* resorption and remodeling capacity, and are therefore unsuitable as onlay bone graft substitutes for vertical bone augmentation [29,30]. Recent studies have demonstrated the potential of dicalcium phosphate compounds with higher solubilities at physiological pH, in vertical bone augmentation procedures [31,32]. For instance, dicalcium phosphate dihydrate (brushite) application in the form of cements or granules has shown good potential for maxillofacial bone augmentation [31]. However, *in vivo*, brushite has a tendency to reprecipitate as insoluble HA slowing its replacement by bone. A closely related compound, dicalcium phosphate anhydrous ($CaHPO_4$) commonly known as monetite is slightly less soluble and appears not to transform to HA. It has recently been found to be osteoconductive and resorbable *in vivo* [33–35], which is of great interest for maxillofacial bone augmentation.

We have recently developed a 3D-powder printing technique enabling computer designed monetite blocks to be made easily for specific bone regeneration applications [33]. In this study we sought to answer two questions. Firstly would screw fixation of a printed bioceramic enable satisfactory osteointegration, and secondly, how would bone tissue behave following abutment with a monetite block.

2. Materials and methods

Onlay blocks were prepared using a previously described 3D printing technique [33]. Briefly, tricalcium phosphate (TCP) was synthesized by heating a mixture of dicalcium phosphate anhydrous ($CaHPO_4$, monetite) (Merck, Darmstadt, Germany) and calcium carbonate ($CaCO_3$, calcite) (Merck, Darmstadt, Germany) in a 2:1 molar ratio to 1400 °C for 7 h followed by quenching to room temperature. The sintered cake was crushed with a pestle and mortar and passed through a 160 μm sieve. Subsequent milling of TCP was performed in a planetary ball mill (PM400, Retsch, Germany) for 10 min. Printing of cement samples was performed with a 3D-powder printing system (Z-Corporation, USA) using the TCP powder and diluted phosphoric acid (H_3PO_4) (Merck, Darmstadt, Germany) with concentration of 20 wt%. The implant design was drafted using CAD software (Alibre design Xpress 10.0). The samples were cylindrical tablets 9.0 mm in diameter, 2.0 mm thick, with a 0.5 mm central hole for fixation with osteosynthesis screws (Fig. 1). After printing, samples were removed from the powder bed, cleaned from residual unreacted TCP powder and stored in 20% H_3PO_4 for 3 × 60 s to increase the degree of reaction to DCPD. The blocks were then dehydrated into monetite (dicalcium phosphate anhydrous) and simultaneously sterilized by autoclaving (121 °C; humidity 100%; 30 min) (see Fig. 1A) [33,35]. The final phase composition of the samples was approximately 63% monetite and 37% unreacted TCP [33] with a total porosity of 44% and a compressive strength of 15 MPa.

Prior to implantation, X-ray diffraction (XRD) patterns of the materials were recorded using monochromatic CuKα radiation (D5005, Siemens, Karlsruhe, Germany). Data were collected from $2\theta = 20°–40°$ with a step size of 0.02° and a normalized count time of 1 s/step. The phase composition was checked by means of The International Centre for Diffraction Data reference patterns for α-TCP (PDF Ref. 09-0348), β-TCP (PDF Ref. 09-0169), monetite (PDF Ref. 09-0080) and brushite (PDF Ref. 09-0077). Post implantation, XRD patterns were recorded on poly-methylmethacrylate (PMMA) embedded implants using the same method.

The implantation protocol was approved by the ethical committee for animal experiments of the Rey Juan Carlos University of Madrid. Experiments were conducted in accordance with the guidelines described by the European Communities Council Directive of 24 November 1986 (86/609/EEC), and adequate measures were taken to minimize pain and discomfort to the animals. Eight New Zealand rabbits (3.5–4.0 kg) were used for this study. The rabbits were anaesthetized, the head was shaved and the cutaneous surface was disinfected with povidone iodine solution prior to the operation. A ~5 cm long full depth incision was made on the *linea media* of the *calvaria* and the periosteum was separated from the bone surface with a periosteal elevator. A trephine burr was then used to cut two bilateral circular full thickness autograft cores (10 mm diameter) in the posterior part of the exposed cranium. The circular autologous onlay bone grafts (9.0 mm in diameter) and the monetite blocks were secured with osteosynthesis titanium screws (AO/ASIF 4.0 self-drilling screws; Synthes, Synthes GmbH&Co, Umkirch, Germany) side by side on the anterior part of the exposed cranium (Fig. 1).

The incision was closed with a silk 3-0 suture and the animals were sacrificed after 8 weeks. Histological examinations were performed on dehydrated and resin embedded sections. Briefly, explants were fixed in 2.5% gluteraldhyde solutions and dehydrated in ascending concentrations of ethanol. The samples were then pre-infiltrated for 24 h and infiltrated with resin for another 24 h before embedding in polymerization resin at −20 °C for 14 days (Technovit, Leica Microsystems GmbH Wetzlar; Germany). Following embedding, histological sections were taken using a micro saw (Leica Microsystems GmbH, Wetzlar; Germany), and the samples were stained with methylene blue (MB) and basic fuchsine (BF). Un-implanted monetite blocks were also resin embedded to be analyzed as well by optical and electronical microscopy.