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Vertical bone augmentation with 3D-synthetic monetite blocks in the rabbit calvaria

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Abstract

Introduction: Long-term success of osteointegrated dental implants requires sufficient volume of healthy bone at the recipient sites. However, this is frequently lacking as a result of trauma, tooth loss, or infection. Onlay autografting is amongst the most predictable techniques for craniofacial vertical bone augmentation, however, complications related to donor site morbidity are common and alternatives to onlay autografts are desirable.

Aim: To develop and evaluate a new synthetic onlay block for vertical bone augmentation.

Material and methods: Sixteen synthetic monetite monolithic discs-shaped blocks were prepared using a 3D-printing technique. The blocks were computer-designed, and had a diameter of 9.0 mm, a thickness of either 4.0 mm (n = 8) or 3.0 mm (n = 8) and one 0.5-mm wide central hole that enabled their surgical fixation with osteosynthesis screws. The blocks were randomly allocated to each side of the calvaria (right or left) of eight New Zealand rabbits and fixed with screws to achieve vertical bone augmentation. Eight weeks after the surgical intervention, the animals were sacrificed and the calvaria were retrieved for histological analysis. The following parameters were analysed: the interaction between the graft and the original bone surface, the amount of bone ingrowth within the graft and the gain in bone height achieved by the procedure. Wilcoxon *t*-test was used to evaluate significant differences between the two types of monetite bone block grafts.

Results: The blocks were easy to handle and no damage or fracture was registered while being screw-fixated to the calvarial bone. As a result, the surgical procedure was easy and quick. After a healing of 8 weeks, the synthetic blocks were strongly fused to the calvarial bone surface. Upon histological observation, the monetite blocks appeared to be infiltrated by newly formed bone, without histological signs of necrosis, osteolysis or foreign body reaction. Histomorphometry revealed that bone augmentation occurred within and over the monetite block. The 4.0- and 3.0-mm high blocks were filled with newly formed bone with 35% and 41% of their respective volumes. These observations indicated that craniofacial bone augmentations of at least 4 mm could be achieved with synthetic monetite blocks.

Conclusion: Within the limits of our study, this novel material may be able to eliminate the need for autologous bone transplantation for the augmentation of large vertical bone defects.

Key words: 3D-synthetic bone graft; bone block surgery; implants; vertical bone augmentation

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Conflict of interest and source of funding statement

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Introduction

Several biomaterials and surgical techniques have been developed to facilitate implant placement in severely resorbed alveolar bone (Tonetti et al. 2008). Autografts, allografts and xenografts applied using different surgical techniques, such as guided bone regeneration (GBR) and onlay block surgery have been tested (McAllister & Haghighat 2007).

It has been shown that vertical bone augmentation with different procedures and biomaterials, is possible, however, the number of complications and failures are too high to recommend a widespread use of such procedures (Esposito et al. 2010). Vertical GBR is a highly sensitive technique, inaccessible to many operators, that often fails due to wound dehiscence (Simion et al. 1994, 2007, Tinti et al. 1996, Tinti & Parma-Benfenati 1998, Rocchietta et al. 2008, Torres et al. 2010). On the other hand, autograft onlay block surgery presents good prognosis at the recipient site, however, the requirement of bone harvesting is associated with higher costs and great morbidity at the donor site (Felice et al. 2009a, b, c). Another drawback of autograft onlay in vertical bone augmentation is their pronounced resorption, especially in sites receiving mechanical load and soft tissue tension (Araújo et al. 2002, Chiapasco et al. 2007).

For these reasons, recent research has been focused on the development and evaluation of biomaterials that could replace onlay bone autografts (Araújo et al. 2002, Felice et al. 2009a, b, c, Tamimi et al. 2009, Rothamel et al. 2009). However, at the present time there is no satisfactory synthetic alternative to onlay bone autografts for vertical augmentation of the alveolar bone, and therefore new biomaterials must be developed.

Recent studies have shown that the acidic calcium phosphates, brushite and monetite, are osteoconductive, osteinductive and resorb in vivo (Tamimi et al. 2006, 2008, 2009, 2010, Habibovic et al. 2008, Alkhraisat et al. 2010). Moreover, they can be used in vertical bone augmentation procedures, and can be 3D printed allowing precise host bone-implant specific conformation. In previous studies, we have shown that 2-mm craniofacial bone augmentation is possible with 3D-printed monetite blocks. However, despite this interesting finding, the amount of bone augmentation needed for implant placement is often more than 2 mm (Tamimi et al. 2009).

The purpose of the current study was to develop 3D-printed monetite blocks, and to assess its safety and efficacy in vertical bone augmentation procedures of 3.0 and 4.0 mm on the rabbit calvaria.

Material and Methods

3D-Monetite bone block synthesis

Onlay blocks were prepared using a previously described 3D-printing technique (Gbureck et al. 2007). Briefly, α/β-tri-calcium phosphate (α/β-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 β-TCP powder and diluted phosphoric acid (H_3PO_4) (Merck, Darmstadt, Germany) with concentration of 20% weight. The implant design was drafted using CAD software (Alibre design Xpress 10.0, Alibre, TX, USA). The samples were cylindrical tablets of 9.0-mm diameter, either 4.0- and 3.0-mm thick, with a 0.5-mm central hole for fixation with osteosynthesis screws (Figure 1A). After printing, samples were removed from the powder bed, cleaned of residual un-reacted 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) (Gbureck et al. 2007, Habibovic et al. 2008). The final phase composition of the samples was approximately 63% monetite and 37% un-reacted TCP (Gbureck et al. 2007), with a total porosity of 44% and a compressive strength of 15 MPa.

Surgical procedure

The surgical 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 in this study. The rabbits were anaesthetized, the heads were shaved and the cutaneous surface was disinfected with povidone iodine solution, prior to the operation. A ca. 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. Sixteen 3D-printed monetite blocks with heights of 3.0 (n = 8) and 4.0 mm (n = 8) were randomly allocated on each side of the calvarial (right or left) and secured with 5.0- and 6.0-mm long osteosynthesis titanium screws respectively (AO/ASIF 4.0 self-drilling screws; Synthes, Synthes GmbH&Co, Umkirch, Germany). To avoid brain damage, the screws were introduced only 2.0 mm into de native bone (Figure 1). The incision was closed with a silk 3-0 suture. Antimicrobial prophylaxis was administered for 5 days (Oxytetracycline, Terramicina®, Pfizer, Spain),

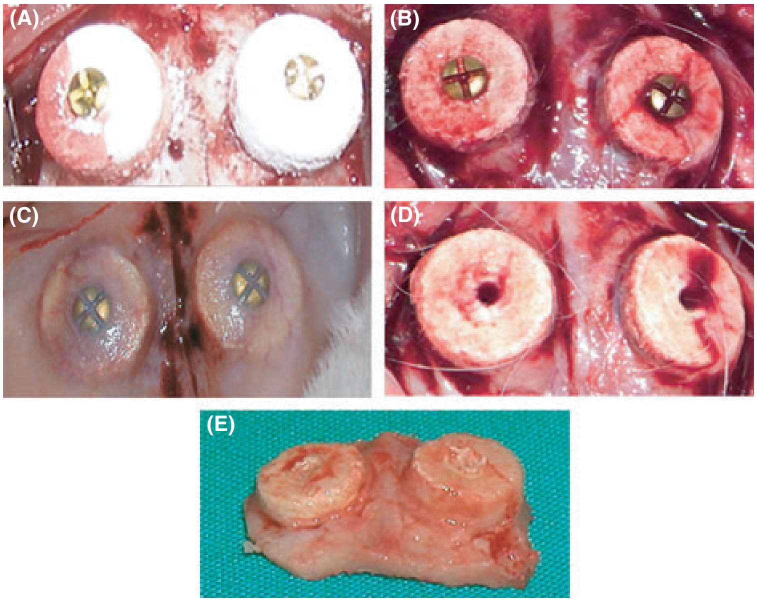

Fig. 1. (A) Surgical placement of the 3D-printed monetite blocks. (B) Photograph showing blood infiltration of the monetite block soon after surgical placement. (C) Monetite blocks upon explanation (8 weeks postoperatively). (D) Removal of the osteosynthesis screws upon surgical explanations. The blocks were integrated to the bone and did not move despite the removal of the screw. (E) Calvarial bone explants including the blocks with the two heights studied (3.0 and 4.0 mm).

and analgesia was given for pain control for 3 days (Buprenorphine Hydrochloride; Buprex®, Schering-Plough, NJ, USA). To better assess the bone remodelling process without increasing the number of animals, tetracycline injections were given at week 4 for histological labelling of the growing bone. After an implantation period of 8 weeks, the animals were sacrificed and the monolithic blocks were extracted for histological and histomorphometrical analysis on non-decalcified sections (Figure 1).

Histology and histomorphometry

Histological analyses were performed on dehydrated and resin-embedded sections. Briefly, explants were fixed in 2.5% glutaraldehyde 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 icrosystems GmbH), and the samples were stained with methylene blue/basic fuchsine, and with picrius/sirius.

The optical images of six coronal sections crossing the centre of the blocks were used to perform the histomorphometrical analysis of each implanted area. For each histological section, the area occupied by the remaining unresorbed material was identified and measured, as well as the bone growing around and within the blocks. These values were used to calculate the percentage of bone volume, and remaining material within the augmented tissues. To calculate the percentage of material resorbed, a base line histomorphometry measurement was taken from three histological sections of resin-embedded un-implanted monetite blocks. The percentage of monetite onlay resorption was calculated by subtracting the remaining graft size and area percentage from the block size and porosity of the un-implanted block.

The augmented bone area was divided into 15 smaller areas, using a 3 × 5 grit, to perform localized histomorphometrical analysis (Figure 2A). Interpolation of the localized histomorphometrical values was used to depict the average distribution of bone within the blocks and provide a statistical mapping of the histological section (Renka & Cline 1984) (Origin 7.0; Origin Lab Co., Northampton, MA, USA).

To evaluate the bone height gained with the onlay blocks, histological sections crossing through the screw hole at the centre of the blocks were evaluated. Direct vertical bone height measurements were not possible due to the variability in the anatomical convexity of the calvarial surface. Therefore, perceptual values of bone height gained relative to the distance between the original calvarial surface and the superior surface of the implant, were calculated every 2.0 mm along the mediolateral axis of the block.

Statistical analysis

The augmented bone volume, and the bone height gained were assessed for differences in the two study groups. Due to the small sample size, a non-parametric Wilcoxon test for paired samples was used to evaluate differences between the two study groups: 3.0-mm and 4.0-mm high onlay blocks. Statistical significance was set at a value of $p < 0.05$.

Results

Clinical observations

No complications were registered during the implantation of monetite bone blocks (Figure 1). Healing proceeded uneventfully for all 16 surgical sites during the 8 weeks follow-up period, and no signs of rejection were observed. Surgical re-entry revealed that the blocks' shape and size has been preserved, and no loss of the screws or blocks was observed. Upon removal of the ostesynthesis screws, the blocks appeared to be stable and fused to the native bone indicating possible osteointegration (Figure 1D and E).

Histological observations

Upon histological observation, the monetite blocks appeared to be infiltrated by newly formed bone, without histological signs of necrosis, osteolysis or foreign body reaction

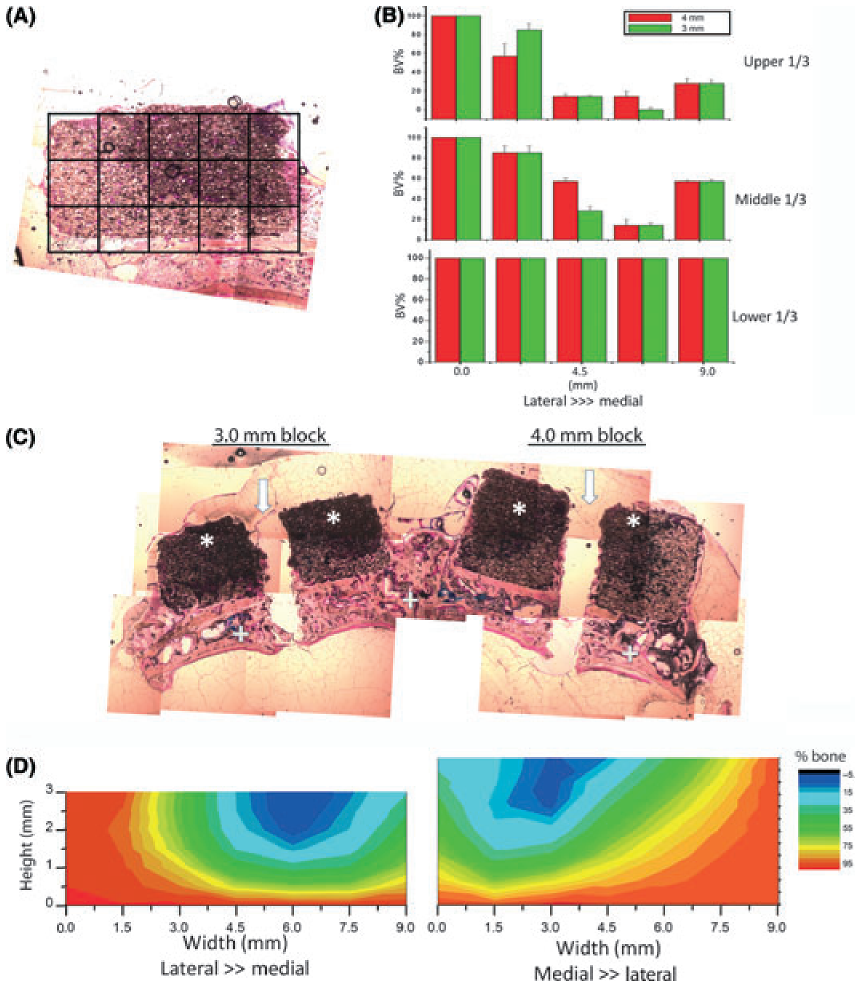

Fig. 2. (A) Bone block histological section divided into 15 smaller areas, using a 3 × 5 grit (a.u.) for histomorphometrical measurements. (B) Histomorphometrical measurements of the areas delimited by the grit shown Figure 2A. (C) Coronal cross section of the 3.0-mm and 4.0-mm blocks cut through their corresponding screw hole (white arrow) showing the remaining material (*), and the original calvaria surface (+). (D) Interpolation map created from the data presented in Figure 2B. These maps show the averaged distribution of bone growth within the blocks. Bone formation followed a similar pattern in all the samples of the two types of blocks studied. Red colour showed intense new bone formation on the lateral side and on the entire inferior surface of the blocks. Yellow and greens colours indicate moderate bone formation in the mid-central and medial part of the block and blue depicted absence of bone formation in mid-superior, and medial-superior area of the blocks.

(Figure 2–4). In all histological sections, it was possible to observe intimate contact between the remaining material and the native calvarial bone at the border between the two surfaces, indicating osteointegration of the monetite blocks. Newly formed bone was observed to be covering the lateral border of the blocks, reaching the lateral-superior surface of all the 3.0- and 4.0-mm high blocks (Figure 3 A–D). New bone formation was also observed in the inferior surface of the block at the interface with native bone, where biomaterial resorption was also apparent (Figure 3A). The screw hole in the blocks was also highly infiltrated with newly formed bone that grew alongside the osteosynthesis screw, from the calvarial surface up to halfway the length of the block. At higher magnification (Figure 3B–D), the monetite blocks appeared to be highly porous and infiltrated with newly formed bone. Direct contact between newly formed bone and the remaining monetite block was observed indicating good osteoconduction properties of this synthetic material in a vertical bone augmentation application (Figure 3D).

Histological observations suggest that the high porosity and material resorption allowed significant bone infiltration within the bone graft matrix. Monetite resorption appeared to be more pronounced on the graft-bone interface, and on the lateral margin of the implant, probably due to better perfusion in those areas (Figure 3–4).

The presence of bone within the bone block grafts was further confirmed by picro-sirius red stain which revealed the presence of collagen on the inferior and lateral-superior regions of the implants (Figure 4A–B) (Junqueira et al. 1979, Junqueira et al. 1986). Fluorescence microscopy revealed tetracycline deposition lines within the monetite blocks, and up to their superior end, indicating early bone formation (4 weeks after implantation) throughout the implants (Figure 4C–D).

Histomorphometry

Newly formed bone and remaining material could be observed within the monetite blocks of 3.0 and 4.0 mm heights (Figure 2C). The percentage of bone volume observed within the monetite blocks, and the percentage of material resorbed were higher in the 3.0 mm blocks than in the 4.0 mm one's, however, differences were not statistically significant ($p > 0.05$) (Figure 5B) (Table 1).

Interpolation map of the histomorphometrical analyses confirmed that bone growth within the blocks was heterogeneous, but it followed a consistent pattern (Figure 2B–D). Bone was abundant on the lateral, medial and inferior regions of the blocks, but it was very scarce in the central-superior surface (Figure 2B–D).

Bone height analysis

In all the 3- and 4-mm implanted blocks, new bone formation reached the top of the implant at least on the lateral side of the blocks. Accordingly, the maximum bone height gained with the 4.0 mm blocks was significantly higher than that obtained with the 3.0 mm blocks

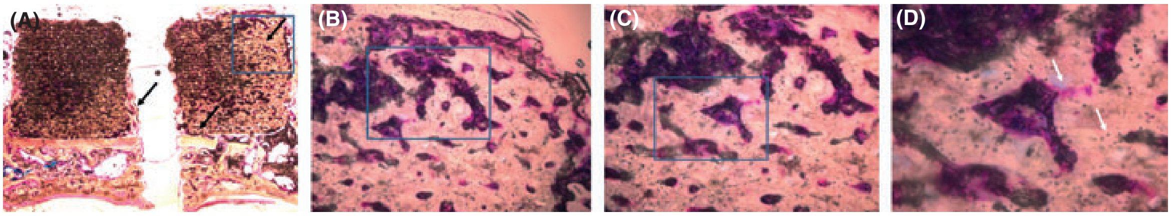

Fig. 3. Histological micrographs of the 4.0-mm high blocks at different magnifications (A–D). The sections were stained with methylene blue and basic fuchsine. At low magnifications, bone ingrowth can be observed on the lateral side of the blocks, in the surface in direct contact with native bone and in the screw hole (black arrows) (A). At higher magnifications, bone tissue (fucsia) can be identified infiltrating the blocks itself (dark brown) (B and C). At even higher magnifications, osteocytes can be observed infiltrated within the new bone formation (white arrows) (D).

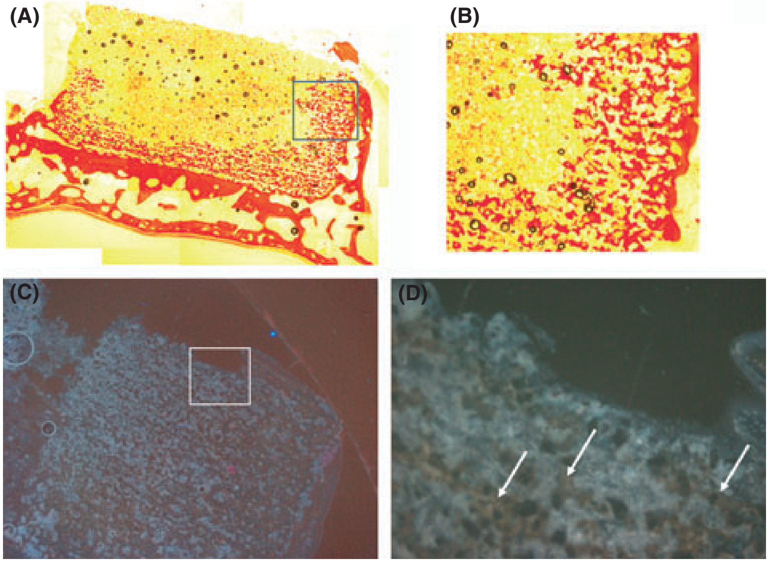

Fig. 4. Histological section of 3D-printed monolithic monetite implant (4.0-mm high), screwed on the calvarial bone of a rabbit. (A and B) Histological micrographs of sections dyed with picros/sirius stain; the red colour indicated bone/osteoid tissue; it can be observed that bone formation occurs at the top of the implant on the lateral side. (C and D) Fluorescence microscopy of histological sections showing tetracycline lines (white arrows) throughout the monetite block and on its superior surface.

($p < 0.01$). This result indicated that a 4 mm bone height augmentation could be obtained with the onlay 3D-printed monetite blocks.

Bone height levels across the blocks followed a similar pattern in both the 3.0- and the 4.0-mm high blocks, with no significant differences in the two groups($p > 0.05$) (Figure 2B and 5A). It was observed that maximum bone levels were reached on the lateral end of the blocks, whereas the lowest ones were always registered at the central region of the blocks. Relatively high levels of bone height gained were also observed on the medial end of the block. The central part of the block showed the lowest levels of bone infiltration (Figure 5A).

Discussion

In this study, the monetite blocks were easy to handle, and no damage or fracture of the blocks was registered during the surgical intervention. This indicates that the mechanical quality of the 3D-printed monetite blocks met the requirements needed for craniofacial surgeries. Moreover, by designing the blocks with a screw hole in the centre, screw fixation was easy and tension-free. As a result, the surgical procedure was straight forward and quick. Xenogenic derived bone blocks have already been reported to achieve vertical bone augmentation in the mandible. However, these materials are quite brittle and fragile. Consequently, these graft materials often break during and following the screw fixation process, resulting in a complicated surgical technique, and a hindered bone graft healing process (Simion et al. 2006, 2009 Felice et al. 2009a, b, c).

Within 8 weeks of implantation, the monetite blocks were macroscopically fully incorporated to the calvarial bone achieving a bone height augmentation of up to 4 mm.

To the best of our knowledge, this is the first study reporting 4.0 mm vertical bone augmentation with synthetic onlay blocks.

Upon histological observation, the monetite blocks appeared to be infiltrated by newly formed bone, without histological signs of necrosis, osteolysis or foreign body reaction. These results were similar to those reported in previous studies where monetite based biomaterials have already proven their excellent bone compatibility upon implantation in bone defects or on bone surfaces (Tamimi et al. 2009, 2010).

Bone formation within the blocks was heterogeneous, but it followed the same pattern in all histological sections: it was always more pronounced on the lateral end of the implants. This phenomenon can be explained by the anatomical arrangement of the calvarial blood vessels in mammals. The parietal bone is supplied by the posterior branch of the middle meningeal artery that emanates from the maxillary artery. On each parietal bone, one perfused major branch of the meningeal artery runs laterally, curving towards the sagittal suture (Slotte et al. 2005). So, it is easily comprehensible that the major evidence of newly formed bone was observed in the areas of major blood supply; in other words, in the lateral side and the surface of

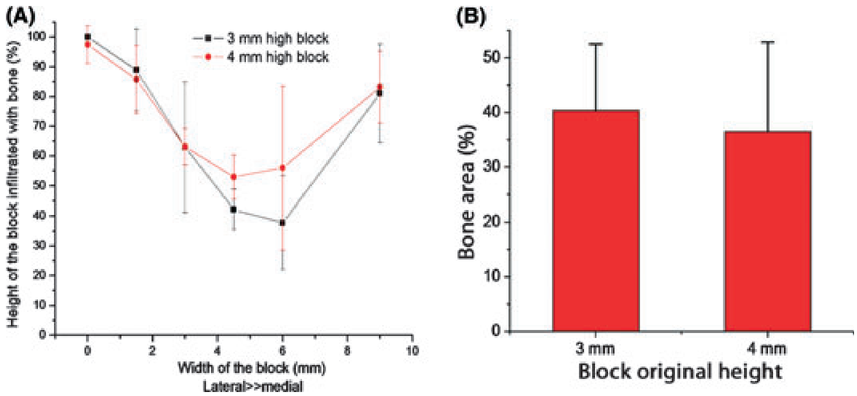

Fig. 5. (A) Relative bone height gained along the mediolateral axis of the blocks. The measurements represent the percentage of bone height gained between the original calvarial surface and the superior surface of the monetite blocks. The lateral end of the blocks achieved the maximum percentage of bone height augmentation in both blocks. The central part shows approximately 50% of bone height augmentation and medial side reached between 70% and 80% of bone height formation. (B) The percentage of bone tissue measured within the blocks' augmented area.

Table 1. Morphometrical data for each implanted block and rabbit

Rabbit	3-mm blocks			4-mm blocks		
	Bone area %	RG %	GR %	BA %	RG	GR %
1	41 SD 3	49 SD4	39 SD 5	36 SD 5	56 SD 7	32 SD 7
2	25 SD 1	69 SD1	15 SD 2	14 SD 7	83 SD 9	0 SD 9
3	34 SD 9	58 SD11	28 SD13	22 SD 8	73 SD 10	11 SD 10
4	49 SD 4	40 SD5	51 SD7	55 SD 7	33 SD 9	60 SD 9
5	48 SD 7	41 SD8	49 SD10	57 SD 6	30 SD 7	63 SD 7
6	44 SD 6	46 SD8	43 SD9	50 SD 2	39 SD 3	53 SD 3
7	33 SD 2	59 SD2	27 SD3	45 SD 4	45 SD 5	45 SD 5
8	48 SD 7	41 SD8	49 SD10	17 SD 4	79 SD 5	3 SD 6
Mean	40 SD 8	50 SD 10	38 SD13	37 SD 17	55 SD 21	33 SD26

RG, remaining graft; GR, graft resorbed; SD, standard deviation.

the block in direct contact with the native bone. These results point out the importance of graft's vascularization, as areas with better blood supply achieved more new bone formation. Moreover, a higher percentage of new bone was observed in the 3.0-mm high block than in the 4.0-mm ones. This fact was probably due to the proximity of the bone graft to the vascular supply of the native bone. Indeed, in a previous study using 2-mm high monetite block, we observed an even higher percentage of new bone formation (43%) compared with the 3-mm bone high blocks for the same implantation time (Tamimi et al. 2010).

Despite its heterogeneous distribution, the total percentage of new bone within the augmented site was 40% and 37% of the 3.0- and 4.0-mm blocks respectively. This percentage of bone is comparable to that obtained with other bone augmentation procedures by which dental implants have been successfully stabilized (Berglundh & Lindhe 1997, Artzi et al. 2000, Carmagnola et al. 2003, Tamimi et al. 2010). Accordingly, 3D-printed monetite onlay blocks might be of interest in bone augmentation procedures for dental implant placement, although future studies will have to be performed to confirm this hypothesis. Nevertheless, the heterogeneous pattern of bone growth obtained with the monetite onlay blocks may influence the hypothetical placement of implants at a later stage. So far, our studies have been focused on the use of monolithic blocks of monetite implanted for 8 weeks. However, to obtain better bone distribution and more bone volume within the blocks, longer implantation periods, and new block designs that would favour better bone distribution will have to be investigated.

The parietal bone of adult mammals has poor vascular supply and low content of bone marrow. Therefore, some authors have suggested that it resembles the atrophic mandible and therefore, it is considered to be a reliable site for testing bone augmentation procedures for oral surgery (Bays 1983, Schmitz & Hollinger 1986). Moreover the parietal bone is an intra-membranous origin type of bone just like the mandible. Accordingly, the results obtained in this study might as well be achieved in surgical interventions of the mandible, even though future studies will be needed to confirm this hypothesis (Bays 1983).

The ostesynthesis screws used in this study only required the bone thickness of the rabbit calvaria (ca. 2.0 mm), to fixate the monetite onlay blocks. The calvaria bone thickness (ca. 2.0 mm) added to the maximum bone augmentation obtained with the blocks (4.0 mm) results in a total bone height of ca. 6.0 mm. This amount of bone height is sufficient for the placement of short implants (5.0–6.0 mm) (Stellingsma et al. 2004, Felice et al. 2009a, b, c). Therefore, vertical bone augmentation achieved by monetite blocks in this animal model could be a strong indicative of the potential this new technology may have for the treatment of highly resorbed mandibles (ca. 2.0-mm bone thickness left over the mandibular canal). Although, further studies will have to be made to confirm this point.

Conclusion

Synthetic onlay blocks made of monetite, can be fixed to bone surfaces by using ostesynthesis screws, and achieve vertical bone augmentations as high as 4.0 mm.

Within the limits of this study, this novel material may eliminate the need for autologous bone transplantation for the augmentation of large vertical bone defects.

References

Alkhraisat, M. H., Rueda, C. R., Blanco, L. J., Tamimi, F. M., Torres, J., Gbureck, U. & Lopez-cabarcos, E. (2010) Effect of silica gel on the cohesion, properties and biological performance of brushite cement. *Acta Biomaterialia* **6**, 257–265.

Araújo, M. G., Sonohara, M., Hayacibara, R., Cardaropoli, G. & Lindhe, J. (2002) Lateral ridge augmentation by the use of grafts comprised of autologous bone or a biomaterial. An experiment in the dog. *Journal of Clinical Periodontology* **29**, 1122–1131.

Artzi, Z., Tal, H. & Dayan, D. (2000) Porous bovine bone mineral in healing of human extraction sockets. Part I. Histomorphometric evaluations at 9 months. *Journal of Periodontology* **71**, 1015–1023.

Bays, R. A. (1983) The influence of systemic bone disease on bone resorption following mandibular augmentation. *Oral Surgery Oral Medicine Oral Pathology* **55**, 223–231.

Berglundh, T. & Lindhe, J. (1997) Healing around implants placed in bone defects treated with Bio-Oss. *Clinical Oral Implants Research* **8**, 117–124.

Carmagnola, D., Adriaens, P. & Berglundh, T. (2003) Healing of human extraction sockets filled with Bio-Oss®. *Clinical Oral Implants Research* **14**, 137–143.

Chiapasco, M., Zaniboni, M. & Rimondini, L. (2007) Autogenous onlay bone grafts *versus* alveolar distraction osteogenesis for the correction of vertically deficient edentulous ridges: a 2–4-year prospective study on humans. *Clinical Oral Implants Research* **18**, 432–440.

Esposito, M., Grusovin, M. G., Felice, P., Karatzopoulos, G., Worthington, H. V. & Coulthard, P. (2010) The efficacy of horizontal and vertical bone augmentation procedures for dental implants – a Cochrane systematic review. *European Journal of Oral Implantology* **2**, 167–184.

Felice, P., Cannizzaro, G., Checchi, V., Marchetti, C., Pellegrino, G., Censi, P. & Esposito, M. (2009a) Vertical bone augmentation *versus* 7-mm-long implants in posterior atrophic mandibles. Results of a randomised controlled clinical trial of up to 4 months after loading. *European Journal of Oral Implantology* **2**, 7–20.

Felice, P., Checci, V., Pistilli, R., Scarano, A., Pellegrino, G. & Esposito, M. (2009c) Bone augmentation *versus* 5-mm dental implants in posterior atrophic jaws. Four-month post-loading results from a randomised controlled clinical trial. *European Journal of Oral Implantology* **2**, 267–281.

Felice, P., Marchetti, C., Iezzi, G., Piattelli, A., Worthington, H., Pellegrino, G. & Esposito, M. (2009b) Vertical ridge augmentation of the atrophic posterior mandible with interpositional block grafts: bone from the iliac crest vs. bovine anorganic bone. Clinical and histological results up to one year after loading from a randomized-controlled clinical trial. *Clinical Oral Implants Research* **20**, 1386–1393.

Gbureck, U., Hölzel, T., Klammert, U., Würzler, K., Müller, F. A. & Barralet, J. E. (2007) Resorbable dicalcium phosphate bone substitutes prepared by 3D powder printing. *Advanced Functional Materials* **17**, 3940–3945.

Habibovic, P., Gbureck, U., Doillon, C. J., Bassett, D. C., van Blitterswijk, C. A. & Barralet, J. E. (2008) Osteoconduction and osteoinduction of low-temperature 3D printed bioceramic implants. *Biomaterials* **29**, 944–953.

Junqueira, L. C., Assis Figuereido, M. T., Torioni, H. & Montes, G. S. (1986) Differential histologic diagnosis of osteoid. A study on human osteosarcoma collagen by the histochemical picrosirius-polarization method. *The Journal of Pathology* **148**, 189–196.

Junqueira, L. C., Bignolas, G. & Brentani, R. R. (1979) Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *The Histochemical Journal* **11**, 447–455.

McAllister, B. S. & Haghighat, K. (2007) Bone augmentation techniques. *Journal of Periodontology* **78**, 377–396.

Renka, R. J. & Cline, A. K. (1984) A triangle-based C^1interpolation method. Rocky mountain. *Journal of Mathematics* **14**, 223–237.

Rocchietta, I., Fontana, F. & Simion, M. (2008) Clinical outcomes of vertical bone augmentation to enable dental implant placement: a systematic review. *Journal of Clinical Periodontology* **35**, 203–215.

Rothamel, D., Schwarz, F., Herten, M., Ferrari, D., Mischkowski, R. A., Sager, M. & Becker, J. (2009). Vertical ridge augmentation using xenogenous bone blocks: a histomorphometric study in dogs.; *International Journal Oral Maxillofacial Implants* **24**: 243–250.

Schmitz, J. P. & Hollinger, J. O. (1986) The critical size defect as an experimental model forcraniomandibulofacial nonunions. *Clinical Orthopaedics and Related Research* **205**, 299–308.

Simion, M., Fontana, F., Rasperini, G. & Maiorana, C. (2007) Vertical ridge augmentation by expanded-polytetrafluoroethylene membrane and a combination of intraoral autogenous bone graft and deproteinized anorganic bovine bone (Bio Oss). *Clin Oral Implants Res* **18**, 620–629.

Simion, M., Nevins, M., Rocchietta, I., Fontana, F., Maschera, E., Schupbach, P. & Kim, D. M. (2009) Vertical ridge augmentation using an equine block infused with recombinant human platelet-derived growth factor-BB: a histologic study in a canine model. *International Journal Periodontics and Restorative Dentistry* **29**, 245–255.

Simion, M., Rocchietta, I., Kim, D., Nevins, M. & Fiorellini, J. (2006) Vertical ridge augmentation by means of deproteinized bovine bone block and recombinant human platelet-derived growth factor-BB: a histologic study in a dog model. *International Journal Periodontics and Restorative Dentistry* **26**, 415–423.

Simion, M., Trisi, P. & Piattelli, A. (1994) Vertical ridge augmentation using a membrane technique associated with osseointegrated implants. *International Journal Periodontics and Restorative Dentistry* **14**, 496–511.

Slotte, C., Lundgren, D. & Sennerby, L. (2005) Bone morphology and vascularization of untreated and guided bone augmentation-treated rabbit calvaria: evaluation of an augmentation model. *Clinical Oral Implants Research* **16**, 228–235.

Stellingsma, C., Vissink, A., Meijer, H. J., Kuiper, C. & Raghoebar, G. M. (2004) Implantology and the severely resorbed edentulous mandible. *Critical Reviews in Oral Biolology and Medicine* **8**, 240–248.

Tamimi, F., Torres, J., Bassett, D., Barralet, J. & Cabarcos, E. L. (2010) Resorption of monetite granules in alveolar bone defects in human patients. *Biomaterials* **31**, 2762–2769.

Tamimi, F., Torres, J., Gbureck, U., Lopez-Cabarcos, E., Bassett, D. C., Alkhraisat, M. H. & Barralet, J. E. (2009) Craniofacial vertical bone augmentation: a comparison between 3D printed monolithic monetite blocks and autologous onlay grafts in the rabbit. *Biomaterials* **30**, 6318–6326.

Tamimi, F., Torres, J., Kathan, C., Baca, R., Clemente, C., Blanco, L. & López-Cabarcos, E. (2008) Bone regeneration in rabbit calvaria with novel monetite granules. *Journal of Biomedical Material Research. Part A* **87**, 980–985.

Tamimi, F., Torres, J., Tresguerres, I., Clemente, C., López-Cabarcos, E. & Jerez Blanco, L. (2006) Bone augmentation in rabbit calvaria: comparative study between Bio-oss® and a novel β-TCP/DCPD granulate. *Journal of Clinical Periodontology* **33**, 922–928.

Tinti, C. & Parma-Benfenati, S. (1998) Vertical ridge augmentation: surgical protocol and retrospective evaluation of 48 consecutively inserted implants. *International Journal of Periodontics and Restorative Dentistry* **18**, 434–443.

Tinti, C., Parma-Benfenati, S. & Polizzi, G. (1996) Vertical ridge augmentation: what is the limit? *International Journal of Periodontics and Restorative Dentistry* **16**, 220–229.

Tonetti, M. S. & Hämmerle, C. H.; European Workshop on Periodontology Group C (2008). Advances in bone augmentation to enable dental implant placement: Consensus Report of the Sixth European Workshop on Periodontology. *Journal of Clinical Periodontology* **35**:168–172.

Torres, J., Tamimi, F., Alkhraisat, M. H., Manchón, A., Linares, R., Prados-Frutos, J. C., Hernández, G. & López Cabarcos, E. (2010) Platelet-rich plasma may prevent titanium-mesh exposure in alveolar ridge augmentation with anorganic bovine bone. *Journal of Clinical Periodontology* **37**, 943–951.

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Clinical Relevance

Scientific rationale for the study: Onlay autografting is amongst the most predictable techniques for craniofacial vertical bone augmentation. However, complications related to donor site surgery are common and alternatives to onlay autografts are desirable.

Principal findings: Craniofacial bone augmentations of at least 4 mm could be achieved with 3D-printed synthetic monetite blocks used as onlay bone grafts.

Practical implications: 3D-synthetic monetite blocks may be a suitable biomaterial for bone augmentation purposes.