



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

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 in each side of the calvaria (right or left) of 8 New Zealand rabbits and fixed with the screws in order 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 analyzed: 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 for evaluated significantly differences between the two types of monetite bone blocks 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 in 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: vertical bone augmentation, implants, 3D synthetic bone graft, bone block surgery

Clinical Relevance

Scientific rationale:

Onlay autografting is amongst the most predictable techniques for cranio facial 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.

Introduction

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

It has been shown that vertical bone augmentation bone with different procedures and biomaterials, is possible, however, the number of complications and failures is still too high to recommend a widespread use of such procedures (Esposito 2008). Vertical GBR is a highly sensitive technique, inaccessible for many operators, that often fails due to wound dehiscence (Simion et al. 1994, Tinti et al. 1996, Tinti & Benfenati 1998, Simion et al. 2007, Rochietta 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 2009 a,b,c). Another drawback of autograft onlays in vertical bone augmentation is their pronounced resorption, especially in sites receiving mechanical loads and soft tissue tensions (Araujo et al. 2002, Chiapasco & Zaniboni 2011).

For these reasons, recent research has been focused on the development and evaluation of biomaterials that could replace onlay bone autografts (Felice et al. 2009, Tamimi et al. 2009, Rothamel et al. 2009, Araujo et al. 2002). 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, osteoinductive and resorb *in vivo* (Tamimi et al. 2006,2007,2009,2010, Alkhraisat et al. 2010 Habibovic et al. 2008). 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 wt%. The implant design was drafted using CAD software (Alibre design Xpress 10.0). The samples were cylindrical tablets 9.0 mm in 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 from residual unreacted TCP powder and stored in 20% H_3PO_4 for 3 x 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% unreacted 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 for 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 ~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 3.0mm (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). In order 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), and analgesia was given for pain control for 3 days (Buprenorphine Hydrochloride; Buprex®; Schering-Plough, NJ). In order 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 24h and infiltrated with resin for another 24h before embedding in polymerisation 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/ basic fuchsine, and with picric/sirius.

The optical images of 6 coronal sections crossing the centre of the blocks were used to perform the histomorphometric 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. In order to calculate the percentage of material resorbed, a base line histomorphometry measurement was taken from 3 histological sections of resin embedded un-implanted monentite blocks. The percentage of monentite 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 3x5 grit, in order to performed localized histomorphometrical analysis (Figure 2A). Interpolation of the localized histomorphometric values was used to depict the average distribution of bone within the blocks and provide a statistical mapping of the histological section (Renka et al. 1984) (Origin 7.0; Origin Lab Co.; Northampton; MA).

In order 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 eight 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 osteosynthesis 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 (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 mm 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 3 A). The screw hole in the blocks was also highly infiltrated with newly formed bone that grew along side the osteosynthesis screw from the calvarial surface up to halfway the length of the block. At higher magnification (Figure 3 B-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 3 D).

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 4 A-B) (Junqueira 1979,1986,). 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 4 C-D).

Histomorphometry

Newly formed bone and remaining material could be observed within the monetite blocks of 3.0 mm 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).

Table 1. Morphometric data for each implanted block and rabbit

Rabbit	3mm 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

Interpolation map of the histomorphometric 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,C,D).

Bone height analysis

In all of the 3mm and 4mm 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 ($p<0.01$). This result indicated that a 4mm 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 mm 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, while 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 the bone graft healing process (Simion et al. 2006, Felice et al. 2009).

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; Tamimi et al. 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 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 the block in direct contact with the native bone. These results point out the importance of graft's vascularisation, 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 to 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 et al. 1997, Carmagnola et al. 2003, Artzi & Dayan 2000, Tamimi 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 in order to

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3 confirm this hypothesis. Nevertheless, the heterogeneous pattern of bone growth
4 obtained with the monetite onlay blocks may influence the hypothetical placement of
5 implants at a later stage. So far our studies have been focused on the use of monolithic
6 blocks of monetite implanted for 8 weeks. However, in order to obtain better bone
7 distribution and more bone volume within the blocks, longer implantation periods, and
8 new block designs that would favour better bone distribution will have to be
9 investigated.
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14 The parietal bone of adult mammals has poor vascular supply and low content of bone
15 marrow. Therefore some authors have suggested that it resembles the atrophic mandible
16 and therefore, it is considered to be a reliable site for testing bone augmentation
17 procedures for oral surgery (Schmitz & Hollinger 1986, Bays 1983). Moreover the
18 parietal bone is an intra-membranous origin type of bone just like the mandible.
19 Accordingly the results obtained in this study might as well be achieved in surgical
20 interventions of the mandible, even though future studies will be needed in order to
21 confirm this hypothesis (Bays 1983).
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26 The ostesynthesis screws used in this study only required the bone thickness of the
27 rabbit calvaria (~2.0 mm), in order to fixate the monetite onlay blocks. The sum of the
28 calvaria bone thickness (~2.0 mm) with the maximum bone augmentation obtained with
29 the blocks (4.0 mm) sum ~6.0mm. This amount of bone height is sufficient for the
30 placement of short implants (5.0-6.0mm) (Felice 2009, Stellingsma 2004). Therefore
31 vertical bone augmentation achieved by monetite blocks in this animal model could be a
32 strong indicative of the potential this new technology may have for the treatment of
33 highly resorbed mandibles (~2.0 mm bone thickness left over the mandibular canal).
34 Although further studies will have to be made in order to confirm this point.
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38 39 **Conclusion**

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41 Synthetic onlay blocks made of monetite, can be fixed to bone surfaces by using
42 ostesynthesis screws, and achieve vertical bone augmentations as high as 4.0mm.
43 Within the limits of this study, this novel material may eliminate the need for
44 autologous bone transplantation for the augmentation of large vertical bone defects
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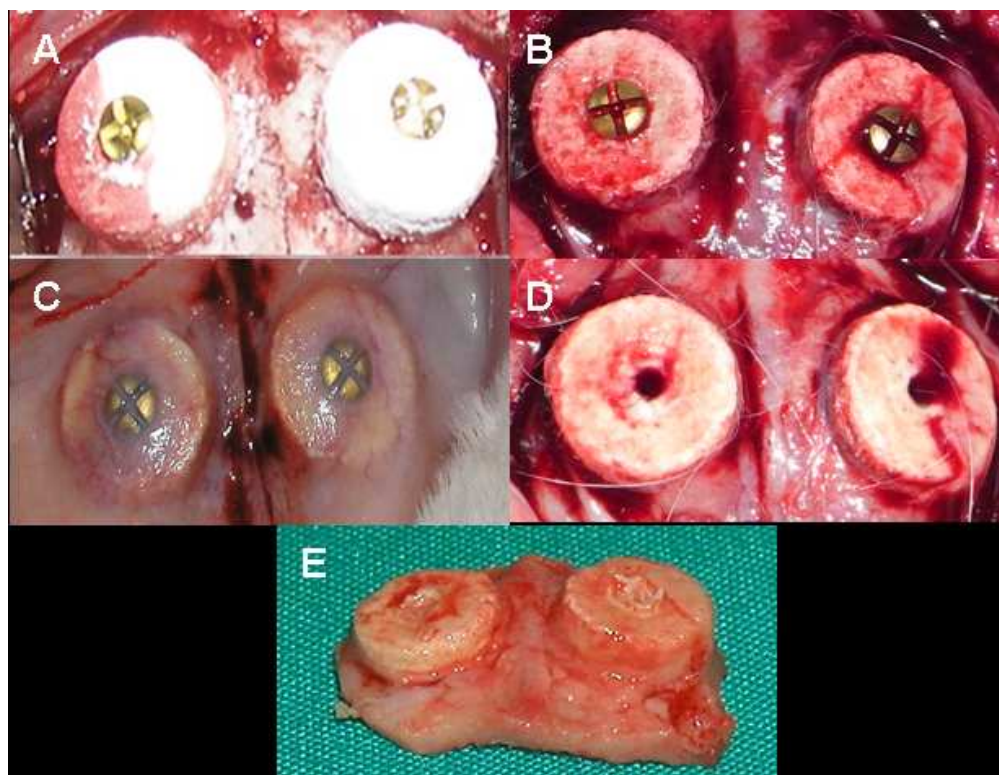


Figure 1. A: Surgical placement of the 3D printed monetite blocks. B: Photograph showing blood infiltration of the monetite block soon after surgical placement. C: Monetite blocks upon explanation (8 weeks postoperatively). D: Removal of the osteosynthesis screws upon surgical explanations. The blocks were integrated to the bone and did not move despite the removal of the screw. E: Calvarial bone explants including the blocks with the 2 heights studied (3.0 mm and 4.0 mm). 104x80mm (150 x 150 DPI)

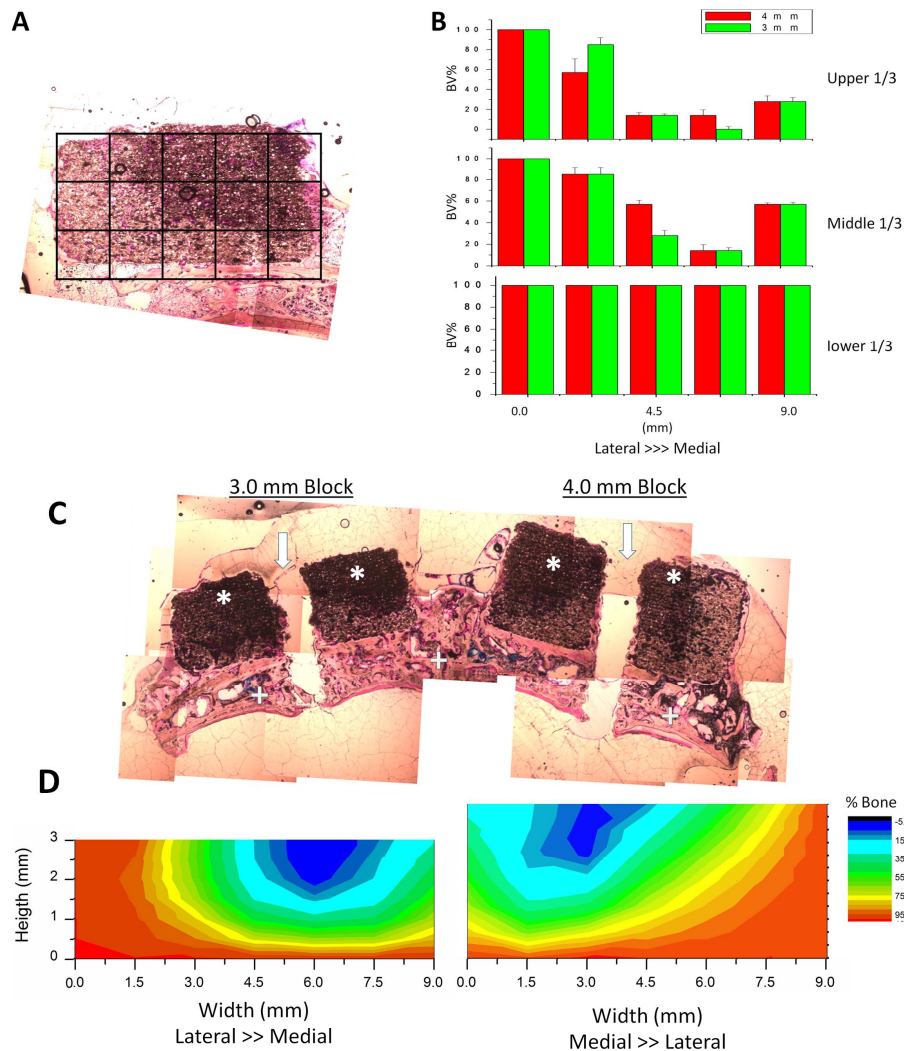


Figure 2. A: Bone block histological section divided into 15 smaller areas, using a 3x5 grit (a.u.) for histomorphometric measurements. B: Histomorphometric measurements of the areas delimited by the grit shown figure 2A. C: Coronal cross section of the 3.0mm and 4.0 mm blocks cut through their corresponding 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 indicated 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.

760x1013mm (77 x 77 DPI)

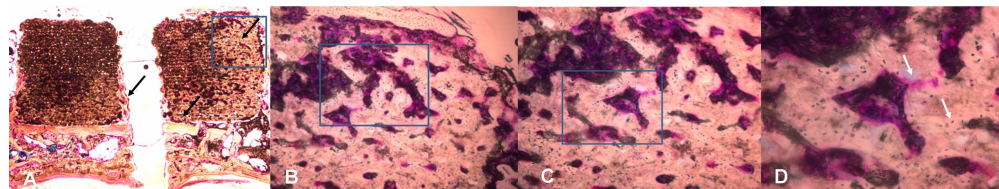


Figure 3. Histological micrographs of the 4.0 mm high blocks at different magnifications (A-D). The sections were stained with methylene blue and basic fuchsin. At low magnifications, bone ingrowth can be observed on the lateral side of the blocks, in the surface in direct contact with native bone and in the screw hole (black arrows) (A). At higher magnifications, bone tissue (fuchsin) 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).
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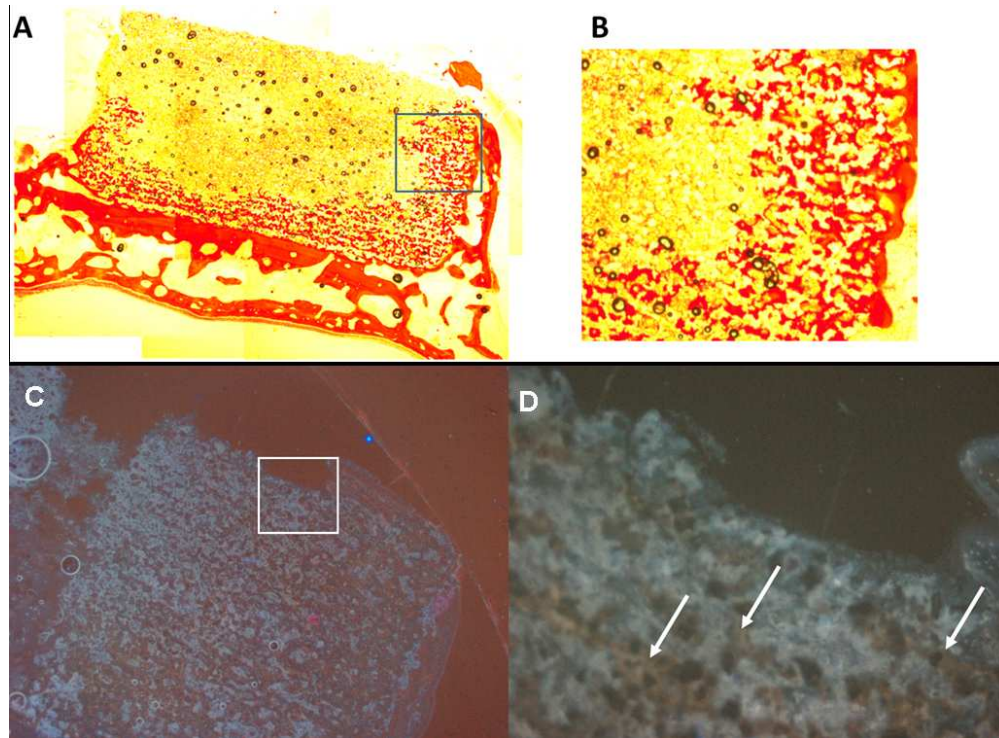


Figure 4. Histological section of 3D printed monolithic monetite implant (4.0 mm high), screwed on the calvarial bone of a rabbit. (A and B) Histological micrographs of sections dyed with picrosirius stain; the red color indicated bone/osteoid tissue; it can be observed that bone formation occurs at 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.

174x128mm (150 x 150 DPI)

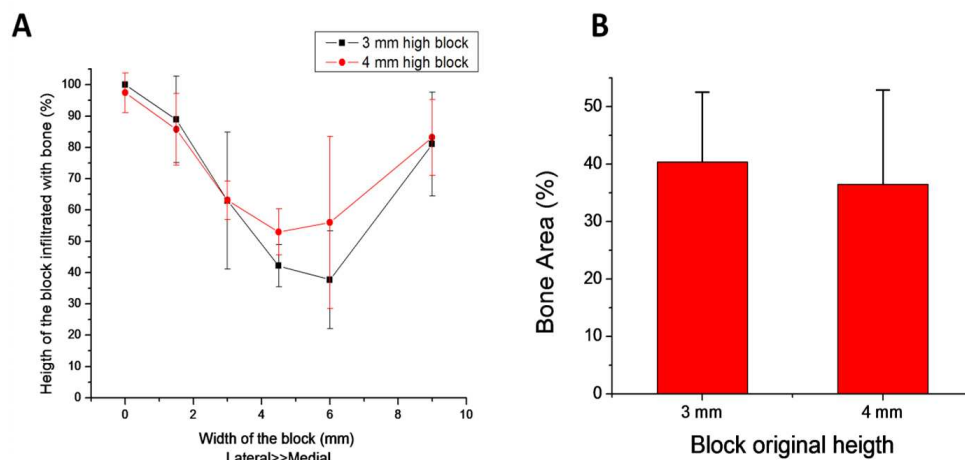


Figure 5. A: Relative bone height gained along the mediolateral axis of the blocks. The measurements represent the percentage of bone height gained between the original calvarial surface and the superior surface of the monetite blocks. The lateral end of the blocks achieved the maximum percentage of bone height augmentation in both blocks. The central part shows approximately 50% of bone height augmentation and medial side reached between 70-80% of bone height formation. B: the percentage of bone tissue measured within the blocks' augmented area. 265x117mm (137 x 150 DPI)