CLINICAL ORAL IMPLANTS RESEARCH

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Role of mechanical compression on bone regeneration around a particulate bone graft material: an experimental study in rabbit calvaria

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Abstract

Purpose: This experimental study was designed to analyze the effect of different compressive forces on the bone regeneration around a particulate bone graft material.

Material and methods: Eighty 6-mm-diameter defects were created in the calvaria of 20 New Zealand rabbits (4 defects per rabbit calvaria). All the defects were filled with particles of synthetic bone. Two standardized compressive forces were then applied, 4.1 g to half the defects (Test A) and 8.2 g to the other half (Test B), all for 1 min. The graft sites were allowed to heal for 6 weeks, after which the rabbits were euthanized. The calvarium vault of each animal was extracted, radiographed, and prepared for histomorphometric analysis. The percentage of defect fill, bone density, new bone formation, and residual bone graft material were recorded, and the results were subjected to statistical analysis.

Results: Histological evaluation found that defect closure among the Test A (lower compression) group ranged from 38.34 (95% lower CI) to 55.8 (95% upper CI) (mean 47 $\pm$ 8.5%), while among the Test B group (higher compression), it ranged from 81.26 (95% lower CI) to 95.32 (mean 88 $\pm$ 7.3%). Significantly more closure was achieved for the Test B group ($P < 0.05$). Histomorphometric comparison of the two groups found significantly more new bone formation, higher bone density, and a higher percentage of defect fill in the defects subjected to the higher compression level ($P < 0.05$).

Conclusions: Increasing the compressive force applied to bone graft particulate used to fill small defects created in rabbit calvaria appears to be beneficial.

Different grafting materials have been used in oral and maxillofacial surgery, each with various properties promoting osteogenesis, osteoconduction, and/or osteoinduction (Artzi et al. 2001; Giannoudis et al. 2005; Allegrini et al. 2008; Cardaropoli et al. 2012). In combination with these materials, different surgical methods enable reconstruction of the narrow alveolar ridge and deficiencies in ridge height and width for successful implant placement (Aghaloo & Moy 2007). Alveolar socket preservation techniques and lateral and vertical augmentations are surgical procedures with long-term success if specific requirements are met (Mardas et al. 2010; Block et al. 2012; Vignoletti et al. 2012; Avila-Ortiz et al. 2014).

The success or failure of augmentation procedures depends on revascularization and replacement of the grafted bone with vital, load-bearing bone. If the defect is contained, new bone formation is usually possible due to good vascularization in the surgical site from the surrounding bony walls. Otherwise, graft resorption and replacement by new bone become questionable. The etiology of graft failure has been discussed in the literature (Machtei 2001; Chiapasco et al. 2006; Haemmerle et al. 2008).

Different recommendations have been provided for the use and handling of particulate bone grafts; some authors recommend packing particulated graft material in socket preservation techniques (Artzi & Nemcowsky 1998; Brugnami et al. 1999; Froum et al. 2002; Kotsakis et al. 2014), and others suggest the use of titanium pins (tags), fixation screws, or tenting screws to immobilize both resorbable and non-resorbable membranes (with or without titanium reinforcement) for lateral and/or vertical augmentations (Buser et al. 1996; Fugazzotto 1998; Simion et al. 1998; Urban et al. 2009).

Also to improve the handling and enable filling of defects in a single step, manufacturers have developed particulated bone substitutes with collagen or bioadhesive gels (putty forms) (Kotsakis et al. 2014).

In the other hand, some clinicians have speculated that packing/overcompression of the bone substitute particles should be avoided to allow for in-growth of vascular elements, blood flow, and revascularization of the graft (Krauser & Schetritt 2012) and prevent problems during healing, including graft rejection.

However, scientific evidence has been lacking about the effects of impaction and condensation of particulated grafting material within defects or the need for compression of the grafting material underneath a membrane in cases of guided bone regeneration (GBR).

Therefore, the purpose of this study was to evaluate the effects of two different standardized compressive forces on a particulate bone graft through the evaluation of the new bone formation, bone density, graft resorption rate, and connective tissue in defects created in rabbit calvaria.

Material and methods

Graft material

Defects were filled with Osteon™ Sinus (Dentium®, Englewood Cliffs, NJ, USA), 100% synthetic bone graft material (HA 70%/β-TCP 30%). The particle size of this material is 1–2 mm, with interconnected pores of 300–500 μm.

Animal model and implantation procedure

The study protocol was approved by the Ethics Committee of Murcia University, Spain (13-5-2013), which followed the guidelines established by the Council Directive of the European Union (53/2013; February 1, 2013) for animal care and experimentation. Twenty New Zealand rabbits that were all 30–35 weeks old and weighing from 3900 to 4500 g were used in the study. The animals were fed a daily diet of pellets, and they were watered *ad libitum* throughout the study period.

General anesthesia included ketamine plus chlorbutol (5–8 mg/kg intravenously), 0.5–1 mg/kg acepromazine maleate as a coadjuvant, and 0.05 mg/kg atropine. Amoxicillin (0.1 ml/kg) was administered intramuscularly at the end of surgery. A 4-cm linear (anterior–posterior) incision was made in the middle portion of each animal's head to expose the cranial vault. Complete flaps were raised, and parieto-occipital and parieto-frontal bone sutures were located. Using a 6-mm-diameter trephine bur (Neodent V, Curitiba, Brazil), 4 identical circular defects were created in each animal's calvarium. To distinguish between the defects receiving less compression (test group A) and more compression (test group B), titanium tags (1 mm length) were inserted close to the left anterior defect in each rabbit calvaria. This facilitated sample orientation for the histological, radiographic, and thermal analyses.

After creation of the test defects, 0.12 gr of the particulate graft material was carefully packed inside each defect and compressed by two different standardized compressive forces: 4.1 or 8.2 gr. The standardized compressive forces were obtained using sterile packages of stainless steel coins. All coins were of the same diameter, but one package weighed 4.1 g, while the second package contained twice as many coins and thus weighed 8.2 g.

The lighter coin package was placed over two of the defects in each animal, and the heavier coin package was placed over the other two defects. Each package was left in position for 1 min at each compression site—a total of 2 min of compression with the heavy package and 2 min of compression with the lighter coin package per animal. No additional pressure was applied to the packages. If any graft material was inadvertently removed as a result of clinging to the compression package, an attempt was made to refill the defect using new graft material.

Then, a new recompression was performed for one additional minute.

A 20 × 30 mm and 300-μm-thick piece of porcine type I collagen membrane (Dentium®) was placed over each set of 4 defects to cover them completely. Periosteal closure was achieved with resorbable vicryl 4-0 suture (Ethicon, Norderstedt, Germany); monofilament nylon 4-0 suture (Ethicon) was used for the superficial sutures including the skin. Figure 1 illustrates the procedure used on each animal.

After 6 weeks, the animals were euthanized by means of an intracardiac overdose of thiopental, after being sedated following the same anesthetic procedure that was used for the first surgery. The soft tissues were then dissected, and the calvarium vault of each rabbit was extracted.

Radiographic and thermal imaging procedures

To evaluate the bone density (d) of each graft site and compare the results of the two compression groups, a digital radiograph was taken perpendicular to each of the 20 extracted calvaria bone sections. All radiographs were taken using the Kodak RVG 6100 Digital Radiography System at 32 KV and 40 mA and with automatic light metering. The images were processed with the ImageJ software developed by the National Institute of Health (Bethesda, MD), a 3D plug-in with a thermal LUT with a grid size of 128 × 128, smoothing of 6.0, and perspective of 0.2 at a scale of 1 : 1. A color thermal gradient was used to analyze the changes in density inside a 6-mm-diameter zone of interest.

A scale with values from 0 to 150 was used to score the density according to Calvo-Guirado et al. 2012; the value range from 0 to 20 (d5) was assigned to the air, 20 to 50 (d4) assigned to the water, 50 to 80 (d3) assigned to soft tissues, 80 to 110 (d2) assigned to lower density bone, and 110 to 150 (d1) assigned to higher density bone. Along with the graft density (d1, d2, d3, d4, and d5), defect closure (as determined by the software and expressed as a percentage) was recorded.

Histological procedure

After being radiographed, the samples were dehydrated in an ascending series of ethanol concentrations (60–100%). Then, they were embedded in 2-hydroxy-ethyl-methacrylate, photopolymerized for 6 h with ultraviolet light, for 2 h with white light, and for 6 h with blue light to create ready-to-cut sample blocks. A saw microtome (Exakta, Norderstedt, Germany) was used to cut a 200-μm-thick coronal section from each sample. Thereafter, the sections were ground to a total thickness of 50–80 μm using a grinder (Exakta) to achieve better histological visualization without risking the loss of the samples. Surface staining was performed with the Levai Laczko method.

Histomorphometric analysis

For the histomorphometric evaluation, the areas of bone and graft particles were measured in relation to the total sample area. This was accomplished using the ImageJ software developed by the National Institute of Health (NIH). New bone (NB), residual graft material (RGM), and connective tissue (CT) were calculated and expressed as mean percentages.

The descriptive evaluation and morphometric measurement were performed under a Nikon Eclipse 80i microscope (Teknooptik AB, Huddinge, Sweden) equipped with the EasyImage 2000 system (Teknooptik AB) and 1.0× to 4.0× lenses. Two regions of interest

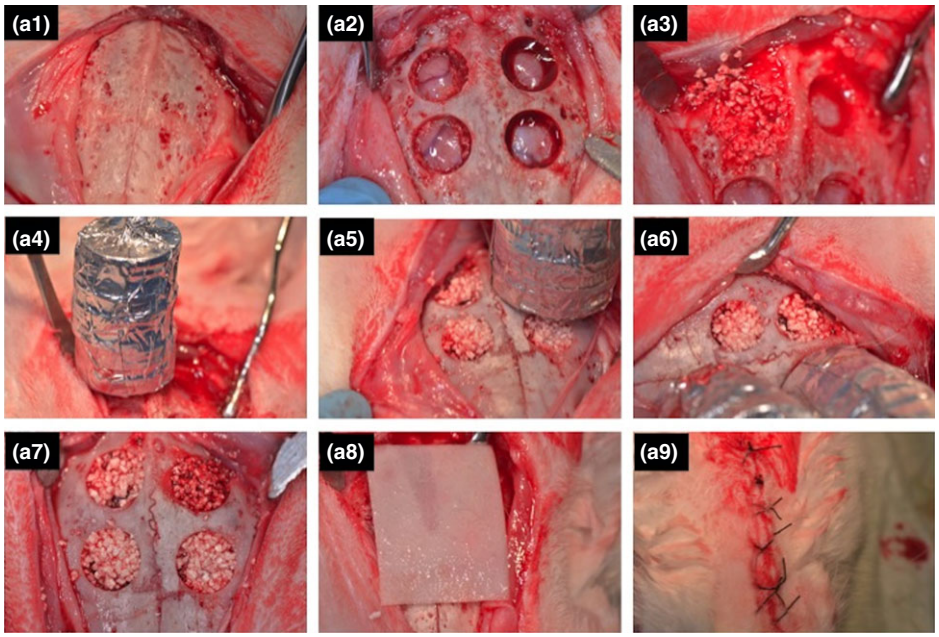

Fig. 1. a1 to a9: For each of the experimental animals, the following steps were followed: (a1) the calvarium was exposed; (a2) 4- to 6-mm defects and six identifier defects were created; (a3) the four large defects were filled with the bone graft particulate; (a4) the sterile 4.1 g package was placed on two of the defects successively and left in place for 1 min; (a5) the sterile 8.2 g package was placed for 1 min on each of the remaining two graft sites; (a6) result after compression; (a7) the four defects after grafting, compression, and restoration of any graft material dislodged by the removal of the compression weights; (a8) porcine type I collagen membrane was positioned to cover all the defects; and (a9) tissues after being sutured.

in each 6-mm-diameter defect were evaluated (Fig. 2):

ROI_1: The 2-mm-wide section at the center of the defect.

ROI_2: The 2-mm-wide sections on each side of the ROI_1 section, limited by the borders of the defect.

Statistical analysis

The statistical software SAS V9.3 (SAS Institute, Inc. Cary, NC, USA) was used to perform the statistical analysis. Mixed model regressions and the nonparametric Brunner–Langer method were applied for statistical analysis. The defect closure evaluated by thermal radiography and NB, RGM, and CT evaluated by histomorphometry were carried out for comparisons of the effects of both compressive forces. Dunnet–Hsu's correction was used for the comparisons. The level of significance was set as $P < 0.05$.

Results

Radiographic thermal analysis

Radiographic thermal imaging analysis (Fig. 3) showed changes in the radiopacity of the defects compressed with 8.2 gr compared to the defects compressed with 4.1 gr ($P < 0.05$). The analysis showed a significantly greater radiopacity in the group with higher compression (Table 1).

Defect closure

The histological evaluation (Fig. 4) found that defect closure among the Test A (lower compression) group ranged from 38.34 (95% lower CI) to 55.8 (95% upper CI) (mean 47 ± 8.5%), while among the Test B group (higher compression), it ranged from 81.26 (95% lower CI) to 95.32 (mean 88 ± 7.3%). Significantly more closure was achieved for the Test B group ($P < 0.05$).

New bone formation

Both groups showed good new bone formation, especially in the outer areas (the margins of the defect), due to the revascularization and osteogenesis from the borders (Fig. 5). However, this new bone formation was greater in the group subjected to higher compression.

Residual graft and connective tissue

Histomorphometric comparison of the two test groups found significant ($P < 0.05$) differences. Table 2 presents the findings regarding new bone (NB) formation, residual graft material (RGM), and connective tissue (CT) for both regions of interest (ROI_1 and ROI_2) in both groups.

Discussion

The present study aimed to answer a simple question: Does compressing particulate allograft bone within non-critical-sized defects in rabbit calvaria effect on bone regeneration?

For that purpose, we used two standardized compressive forces and a single material covered by a membrane for grating defects at calvaria of rabbits.

The radiographic thermal analysis demonstrated higher radiopacity and density at the areas of particulated bone grafts with higher compression. The increased bone density may be explained by the reduction in the spaces between particles and by the possible collapse of some particles under compression. Preliminary works performed in the field of orthopedics found that the impaction of particulate bone grafts have shown positive effects in the bone formation and particle stabilization (Bolder et al. 2003). Excellent clinical outcomes have been shown using this method in revision knee surgery, with mechanical stability of the particle achieved in irregularly sized, large, and shaped defects (Lotke et al. 2006).

Different bone graft materials have different properties, and those properties can affect bone turnover during healing. The shape and porosity of graft particles have an impact on subsequent blood vessel formation and revascularization. The particle shape and size; methods used to harvest and process the material; and its composition, crystallinity, and porosity also influence resistance to compressive and tensile forces (Burchardt 1983). Furthermore, the size of the defect and use of stable cell-occlusive membranes to maintain the space for tissue regeneration are of great significance.

In the present study, the initial compression of the graft particles at the defect margins appears to have stimulated the bone (trigger zone) and promoted bone regeneration. In a similar way, dental implants placed with high stability in undersized osteotomies compress the bone at the bone–implant interface and promote osteogenesis. However, knowledge about the compression threshold is inexact, and thus, osteoclastic activity and bone resorption may result.

Earlier research demonstrated that bone crystals are piezoelectric, and when they are subjected to mechanical stresses, they produce an electric current. These electrical field effects regulate bone changes in important ways (Bassett 1965). The initial signal is a deforming force that activates a large number

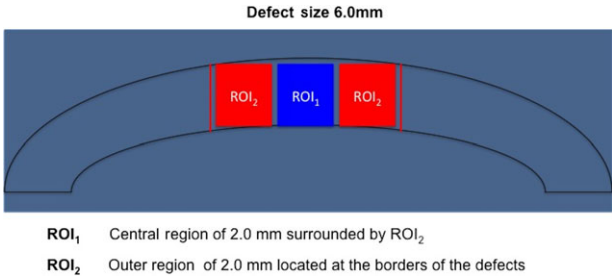

Fig. 2. Two regions of interest were identified in each of the bone slices obtained from the harvested calvaria. The 2-mm-wide center of the grafted defect was labeled ROI_1, and the 2 2-mm-wide sections on each side of the center were called ROI_2 for the purposes of the histomorphometric analysis.

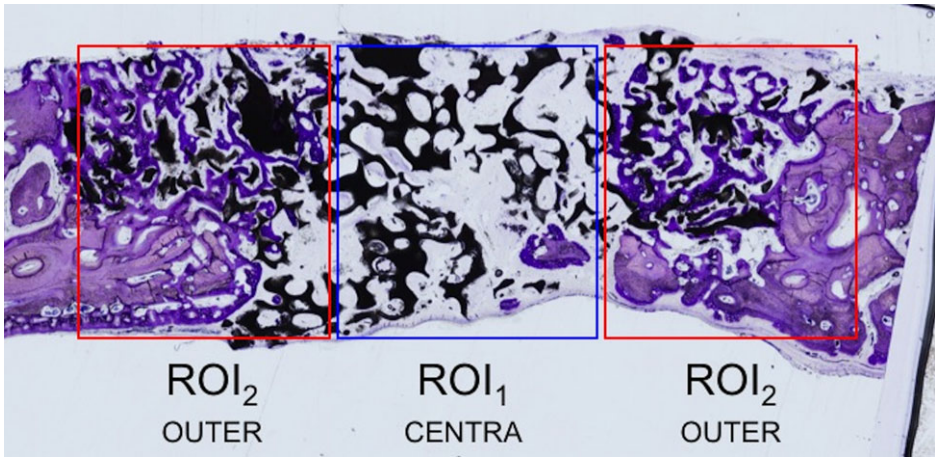

Fig. 3. New bone formation around the bone graft particles in the margins of the defect (RO_2) and less in the middle of the bone defect (RO_1) clearly visible in this specimen.

Table 1. **Mean and standard deviations, median values with upper and lower interquartile, and *P*-values for defect closure measured by thermal radiography in both study groups. Percentages of the defect closure at the sites with low and high compression according to the radiographic thermal imaging analysis showed a significantly greater radiopacity in percentages in the test. Brunner and Langer nonparametric mixed model test. Significance was set as $P < 0.05$**

Group	Descriptive statistics	Defect closure
Test A	Mean ± SD	47 ± 8.5%
4.1 grs	Median (Q1–Q3)	47.3 (39.51–56.6)
n = 40		
Test B	Mean ± SD	88 ± 7.3%*
8.2 grs	Median (Q1–Q3)	86.8 (81.4–94.2)
n = 40		
P-value	0.034	

The asterisk is indicating the value with significant higher defect closure.

of piezoelectric transducers, generating potential proportional to the applied force. Previous studies focusing on the effects of bone compression have shown that it is associated with negative electric potential and leads to osteoblastic activity and new bone formation (Bassett 1968). In contrast, flexion is associated with bone resorption. However, these effects have not been studied extensively, and the electrodynamic potential for osteogenesis has not been clearly explained. One possible explanation is that as collagen fibers are stretched on one side of the bone and compressed at the opposite side, electric charges are created within the bone crystals. This may lead to compression within the intercanalicular system of the osteocytes, with further stimuli and electric charges of the molecules and ions in the blood plasma bathing the osteocytes.

The cellular, subcellular, and biomolecular responses to different low-frequency electromagnetic fields are especially important. Electrical stimulation is known to initiate signaling pathways and may enhance osteogenic differentiation of stem and/or progenitor cells. Mechanical and electrical stimuli have been known for some time to affect the properties and regenerative capacity of skeletal tissues (Lavine & Grodzinsky 1987; Sun et al. 2007). The concept of coupling endogenous and exogenous electrical activity has been suggested as a possible tool for promoting bone fracture healing and differentiation of osteoprogenitor cells (Lavine & Grodzinsky 1987; Spadaro 1997; Wang et al. 1998; Brighton et al. 2001; Whitehead et al. 2007).

The biological response of cultured cells and tissues has been studied in *in vitro* and *in vivo* settings using molecular biology and histomorphological analyses or animal models (rats, rabbits, canines, sheep, and horses) and radiographic, histomorphological, and biomechanical analyses. Extracorporeal shock waves caused microtrauma or microfracture and induced neovascularization through hematoma formation, which would increase osteoblast or fibroblast activity (Wang et al. 2001). Unfortunately, biological studies at the cellular level cannot provide reliable information on the therapeutic effect of biophysical stimulation on tissue response at the system level. Similar investigations are expected to produce valuable information concerning the distractibility of vessels and nerves in the same operation. It has been proposed that the type of stress applied to immature or undifferentiated tissue can dictate its regeneration fate (Carter et al. 1988). Intermittent compressive forces or shear stresses in fractured bones or tensile stresses lead to endochondral or intramembranous ossification, respectively, and constant compressive forces inhibit endochondral ossification but promote cartilage formation. In contrast, high shear stresses are associated with connective tissue formation (encapsulation).

Based on the one-dimensional interfragmentary strain theory of Perren (1979), the tissue responses to the local environment. The gap distance in fragmented bones and micromovements in the gap are important for new bone formation. Specifically, when gap motion is smaller than the gap size, a new bone formation can be established. Micromovements are eliminated if compression is present. In addition, the gap distance will be reduced.

Other studies have shown that electricity may induce osteogenesis and new opportunities for bone growth and repair (Brighton et al. 1977). Woven and lamellar bone formation may be stimulated under loading conditions (McKenzie & Silva 2011).

However, there is no firm evidence that compression stimulates or promotes fracture healing (Perren et al. 1969). Mechanical studies on the strength of healing fractures in cortical bone treated with various fusion methods with and without compression have compared the strength of bones from different animals (Laurin et al. 1963; Anderson 1965; Lettin 1965; Wolff et al. 1981). Studies

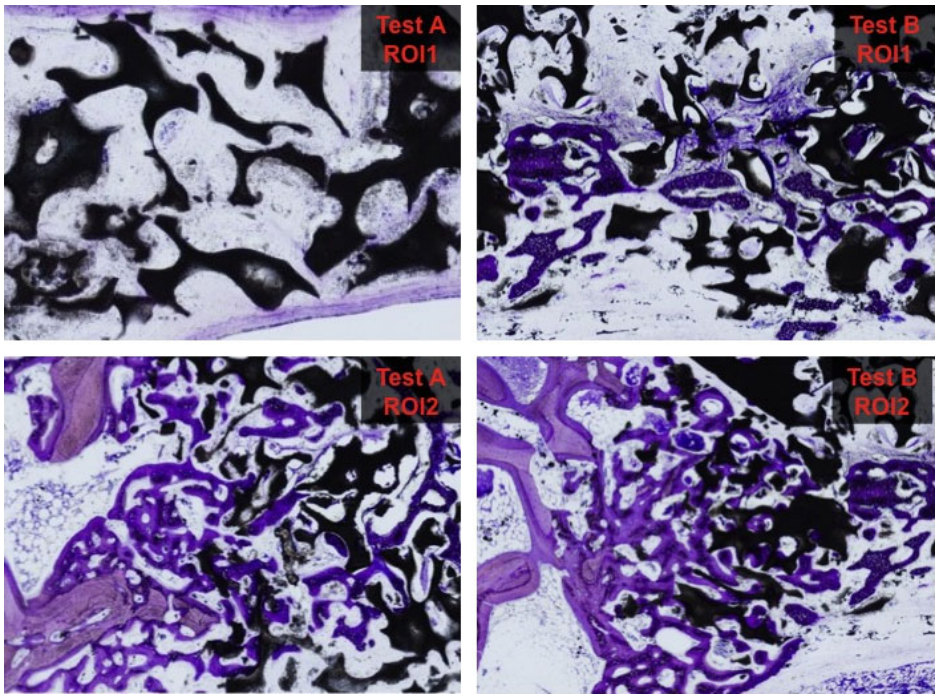

Fig. 4. Histological evaluation of the outer and central areas of the defects with the grafting substitutes under low (test A) and high (test B) compression. Upper row shows the outer areas (RO_2) and lower row represents the central areas (RO_1), showing that new bone is formed in the outer and central areas of the defects only under higher compression.

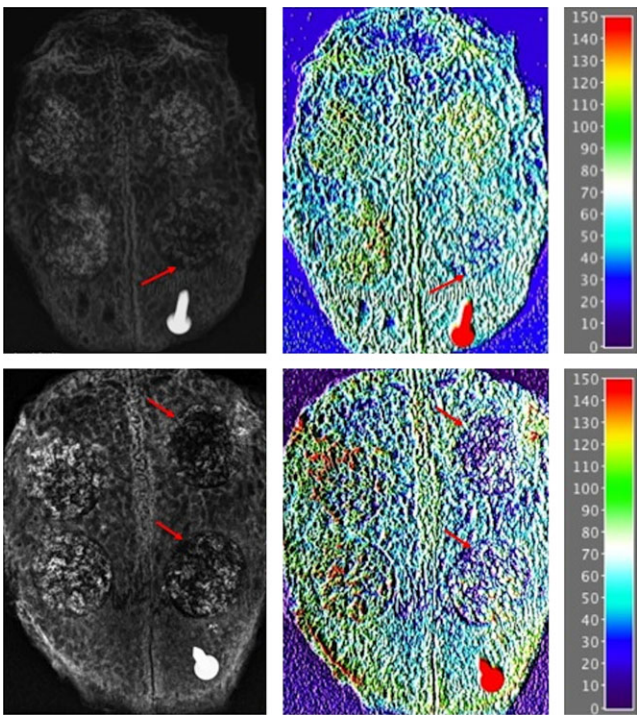

Fig. 5. Defect closure and radiographic analysis. The image composition is showing (at the left side) red arrows marking the control defects (4.1 grs) with less radiopacity compared to the test defects (8.2 grs). The thermal analysis (right photos) demonstrated better defect closure at the defects of the test group (no red arrows present).

comparing the effects of compression in the tibia of the same animals (rabbits) after osteotomy to create a fracture line showed better results for the compression group (a smaller gap) only in the early stages of healing (Holmstrom et al. 1986).

Studies involving tibial defects have elucidated the role of compressive and tensile forces in defect closure under loading conditions (Oda et al. 1996). Specifically, round bony defects in rabbit tibiae were created, and tensile or compressive forces were applied. The subsequent healing was compared with that in control defects in which no tension or compression was used. The compressive and tensile forces under loading conditions led to more new bone formation within 2–4 weeks.

When the effects of bone graft compression have been evaluated in different implant-loading protocols, immediate function has been associated with new bone formation and increased bone density at the bone–implant interface (Romanos et al. 2002), compared to unloaded conditions (Piattelli et al. 1997; Romanos et al. 2003).

The preliminary results presented here underscore the importance of packing well the graft particles used in lateral and vertical augmentation and using membranes to compress them. Previous studies by Greenstein et al. (2009) showed that decortication of the alveolar ridge in lateral augmentations did not increase the final amount of bone regenerated. However, compressing the bone particles within such decorticated areas may improve the graft consolidation and bone regeneration.

Clinical studies of sinus augmentations also support this theory. The use of plastic syringes to better condense graft particles has been recommended by various clinicians to improve the final clinical outcome in sinus-lift procedures (Wallace 2006).

Previous orthopedic studies also have shown no negative effects in acetabular revision surgery of using the bone impaction grafting technique (Arts et al. 2005). Short-term results indicate that impaction grafting is an effective method of dealing with loss of bone stock at revision hip surgery (Blom et al. 2009). Compaction of the bone graft using smaller sized chips next to the host bone and larger size ones on the component side may stabilize the acetabular component and improve the biological response of the grafted aggregates (Akiyama et al. 2011).

One problem associated with the impaction procedure is that a large number of bone particles may be removed each time the impactors are withdrawn (van Haaren et al. 2005). A possible explanation may be that the particles are less sticky than bone graft. The stickiness that binds particles together, known as cohesion in soil mechanics (Smith 1995), seems to be important in orthopedic reconstructive surgery.

Table 2. **Mean and standard deviations, median values with upper and lower interquartile, and *P*-values for NB (new bone), RGM (residual graft material), and CT (connective tissue). Findings for both regions of interest showed significantly more NB for the test group. Brunner and Langer nonparametric mixed model test. Significance was set as $P < 0.05$**

Group	Descriptive statistics	New bone formation (NB)		Residual graft material (RGM)		Connective tissue (CT)	
		ROI 1	ROI 2	ROI 1	ROI 2	ROI 1	ROI 2
Test A 4.1 grs $n = 40$	Mean ± SD	20 ± 3.1%	39 ± 5.3%	26 ± 2.6%	22 ± 1.4%	47 ± 2.3%	29 ± 3.6%
	Median (Q1–Q3)	21.24 (18.4–27.1)	40.21 (36.7–48.3)	27.3 (24.8–35.5)	23.2 (17.6–25.1)	46.4 (41.7–54.1)	46.4 (41.7–54.1)
Test B 8.2 grs $n = 40$	Mean ± SD	54 ± 3.2%	83 ± 6.4%	28 ± 3.5%	32 ± 2.3%	26 ± 3.8%	25 ± 2.2%
	Median (Q1–Q3)	53.7 (49.1–59.2)	84.2 (78.6–89.2)	28.6 (23.8–34.5)	33.58 (30.2–38.8)	25.9 (22.7–33.4)	24.3 (20.12–29.4)
P-value		>0.05	0.029	>0.05	0.036	0.039	>0.05

Bone grafts should be maintained under maximum compression to optimize fusion (Hitchon et al. 2000). Many authors have indicated the importance of implant-mediated compression on strut grafts for healing (Reidy et al. 2004; Francke et al. 2010). It is important to focus on the graft–bone interface to differentiate which fixation technique can best achieve the desired level of compression.

In general, the maintenance of implant-mediated graft compression is of major importance to ensure healing. Previous clinical experience with cervical anterior autograft fusions has endorsed this philosophy and suggested the need for further investigation (Bolger et al. 2006; Pizanis et al. 2013).

The present work has some drawbacks; first, there is a lack of control group (without compression), and this can be explained because our study design was focused only in the bone regeneration with two experimental forces; therefore, including a control group (empty) only will reduce the number of data available for each group. In addition when packing the particulate material, a certain degree of compression may be exerted, and therefore, for the control of the variables, only those compressive forces exerted by the calibrated coins were used.

The strength of this work lies in the calvaria model used (in which the regenerative potential of the material is tested in extraordinary difficult conditions because the area is poor in trabecular bone) and in the use of a membrane for stabilization (excluding other sources of bone formation as the periosteum). The standardization of the compressive forces was useful in controlling the repeatability, and we provided a new method model for the evaluation of the new bone formation through different forces.

Finally, it must be noted that grain size distribution has a major impact on the mechanical stability of bone grafts. Optimizing grain size distribution results in tighter packing of small particles within larger particles. This can increase the primary stability of allografts used in load-bearing applications and prevent implant subsidence. Some have recommended that bone banks should provide allografts in different grain sizes to enable mixing them to improve graft stability and prosthesis fixation for each specific surgical application. In a similar way, fine particulate bone powder grafts provide more viable and active osteocytes to accelerate bone defect healing than larger bone grafts (Wang et al. 2012).

Further research is necessary to understand which threshold of compressive forces is more beneficial for the bone regeneration and how specific materials must be compressed in bone defects.

Conclusion

The results of this preliminary study indicate that increasing the compressive force applied to bone graft particulate used to fill small defects created in rabbit calvaria results in increased amount of new bone formation. There is a need in maxillofacial reconstructive surgery for more studies evaluating the effects of impaction and compression of different bone grafting substitutes to obtain optimal revascularization and bone regeneration.

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