Vertical Bone Augmentation Using Different Osteoconductive Scaffolds Combined with Barrier Domes in the Rat Calvarium

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ABSTRACT

Purpose: To compare the regenerative potential for vertical bone augmentation of various osteoconductive scaffolds when used in conjunction with barrier domes.

Materials and Methods: Following exposure and perforation of the calvarium, a gold occlusive dome was filled with the tested scaffold and anchored by fixation screws. Flaps were repositioned and secured. The four treatment groups, three to five rats each, were as follows: Bio-Oss collagen (BOC), β-tricalcium phosphate (TCP), collagen sponge (COL), and empty domes (C). Rats were sacrificed 8 weeks later, and specimens were prepared for histological and histomorphometric analysis. Vertical bone height and total tissue height were measured.

Results: The newly regenerated bone appeared mature, highly vascularized, and with no signs of inflammation. Vertical bone height in the TCP group (mean 2.04 ± 0.2 mm) was greater than all other groups (0.76 ± 0.02, 1.52 ± 0.18, and 1.77 ± 0.61 mm for the BOC, C, and COL, respectively) but significantly only for the BOC group (p = .0145). Total tissue height was significantly higher (p < .0001) in both BOC and TCP groups (4.48 ± 0.23 and 5.5 ± 0.24 mm, respectively) compared with COL (3.22 ± 0.11 mm) and C (2.39 ± 0.3 mm) groups.

Conclusion: TCP in conjunction with barrier dome resulted in greater vertical bone augmentation in the calvarium of rats.

KEY WORDS: guided bone regeneration (GBR), osteoconductive scaffolds, vertical bone augmentation

INTRODUCTION

Severe alveolar bone loss may be associated with trauma, malignancy, and periodontal or peri-implant diseases.1 Restoring the lost bone is essential for the rehabilitation of the patient’s function, phonetics, and aesthetics. Nowadays, the commonly used methods that are employed to gain substantial vertical bone augmentation are distraction osteogenesis and bone blocks (autologic/allogenic or xenogenic). These techniques are surgically complicated, not always predictable, and are associated with significant morbidity.2,3 Guided tissue regeneration (GBR) technique is also used for such defects, as a stand-alone or in combination with other materials. The biological principle of GBR includes space maintenance, cell exclusion, and clot stabilization.4,5 However, preclinical studies that attempted to grow bone extracortically using GBR techniques demonstrated only modest success.6

Both the calvarium and jaw bones are developmentally formed through the pathway of intramembranous bone formation,7 thus, the calvarium bone may serve as a legitimate experimental model to test extracortical vertical bone formation in small animals.8

Various osteoconductive biomaterials have been evaluated for bone regeneration purposes. These biomaterials provide volume for new bone formation and act as scaffolds for ingrowth of osteoblasts. Some of the more commonly used materials include the following:
Bovine derived xenograft (BDX) – this material is widely used in maxillofacial bone augmentation procedures. It contains deproteinized bovine-derived natural bone mineral with physical and chemical characteristics that resemble other animals and human bone. The porosity of the scaffold increases surface area, thus allowing vessels and bone forming cells to migrate along the scaffold’s framework and generate new bone.9–11 The biocompatibility and osteoconductivity of BDX were previously proven.12–14 Recently, a composite BDX plus collagen sponge (COL) was introduced. The structure of this material might further allow adherence and growth of cells into this scaffold.15

β-tricalcium phosphate (β-TCP) is a synthetic scaffold used clinically for the reconstruction of intrabony defects. These materials provide a mineral matrix phase similar to that found in bone tissue. Following application, βTCP is resorbed by osteoclastic activity and replaced by newly formed bone. Additionally, βTCP as a synthetic material does not pose the risk of transmitting pathogenic agents (as is the case with allografts and xenographs). It is also being resorbed more rapidly when compared with a xenograft.16

COLs usually used in order to stabilize blood clot in extraction socket immediately postop. However, recent publications have supported the potential use of COL as a carrier platform for cells or growth factors.17,18

Previous studies have suggested that these materials alone or modified with growth factors are sufficient for bone regeneration; however, these results are derived from studies of intrabony defect bone regeneration19,20 and might not be sufficient for extracortical vertical bone regeneration.

Thus, the aim of this study is to test and compare the potential of three different scaffolds to improve extracortical vertical bone formation in conjunction with barrier domes in a rat calvaria model.

MATERIALS AND METHODS

The study protocol was initially approved by the committee for the supervision of animal experiments at the faculty of Medicine, Technion (I.I.T.). Sixteen male Lewis rats (13 w, 300 g) were allocated to one of the following groups (three to five rats each):

1) Bio-Oss collagen (BOC) – BDX plus collagen scaffold (Bio-Oss Collagen®, Geistlich Biomaterials, Wolhusen, Switzerland), n = 5;
2) TCP – β-TCP scaffold (Ossaplast®, Ossacur Medical Products, Oberstenfeld, Germany), n = 5;
3) COL – haptide-coated collagen sponge (Hapto Biotech Ltd., Jerusalem, Israel), n = 3;
4) Empty dome (C) – empty capsule, n = 3.

Rats were anesthetized by an intramuscular injection of ketamin (Ketaset, Fort Dodge, IO, USA) 10 mg/100-g body weight and xylasin (Eurovet, Cuijk, Holland) 0.5 mg/100-g body weight. About 0.3 mg/kg body-weight antibiotics (cephalexin, Norbrook Laboratories, Newry, Ireland) and analgesic (boprenorphine, Vetamark, Petah Tikva, Israel) were injected subcutaneous preoperatively and 3 days postoperatively.

A U-shaped incision served to raise a full thickness flap and exposed the parietal bone. Next, small perforations of the cortical bone were performed under cooling conditions with saline using a dental handpiece and a diamond bur. Rigid gold domes (10 mm in diameter and 7 mm in height) were filled with one of the tested scaffolds as follows: 0.2-g β-TCP particles or 4 ¥ 4 ¥ 5 mm block of BOC, 4 ¥ 4 ¥ 5 mm of COL, or left empty (in the C group). The domes were secured to the calvaria using fixation screws via its anchoring rings (Figure 1). The flaps were repositioned and sutured with minimal tension, using resorbable sutures. Immediately postop and during the whole experiment, each rat was kept in a separate cage and were fed rat chow and water ad libitum.

Two months later, rats were sacrificed and the capsules were removed. The part of the calvarium surrounding the regenerated area was sawed out and fixed in 10% neutral buffered formalin for 2 days.
Histological Preparations and Histomorphometric Analysis

The fixed samples were decalcified in Calci-Clear Rapid (National Diagnostic, Atlanta, GA, USA) for 2 to 3 days and processed for paraffin embedding. Five-micrometer sections were stained with hematoxylin and eosin (H&E) for the assessment of bone morphology. Scaffold degradation and inflammatory infiltration were graded as high, medium, or low. For histomorphometric measurements, four H&E stained slides from each specimen were captured by a microscope mounted Charge-Coupled Devices (CCD) camera (Olympus DP70, Olympus, Tokyo, Japan) with a calibration scale.

Two parameters were measured using image J software (National Institutes of Health, Bethesda, MD, USA):

1) vertical bone height – maximal osseous height measured from the bottom of the calvaria to the crest of the newly formed bone;
2) overall tissue height – the vertical dimension of tissue measured from the base of the calvaria to the top of the newly formed tissue. This tissue included bone, residual scaffold, and connective tissue.

Statistical Analysis

A StatPlus®5.7.8 statistical package (AnalystSoft, Vancouver, BC, Canada) was used. Descriptive statistics including mean and standard error was initially tabulated. Multiple comparison analysis was done using analysis of variance with Bonferroni correction. A significant value was set at $p < .05$.

RESULTS

All rats survived the surgical procedures and healing was uneventful. Although the surgical sites had initially healed per-primum, a focal necrosis of the skin overlying the capsule was noticed approximately 1 month after the surgery. This caused spontaneous exposure of the capsules in five rats (two rats from TCP group and one rat from each of the other groups). As this was not associated with signs of inflammation around these sites, a remedial action was therefore not needed. Moreover, spontaneous exposure of the capsules did not affect vertical bone augmentation.

Clinical macroscopic view following removal of the capsule showed that in the control group most of the space under the capsule remained empty with only minimal irregular new tissue that was regenerated (Figure 2A). In the TCP group, hard tissue filled most of the space under the capsule. This augmented tissue appeared nonhomogenous and was composed of particulate residual scaffold material surrounded by new host tissue (see Figure 2B). Similar clinical view was also noticeable in the BOC group. On the contrary, in the

Figure 2 Clinical view. A, Control – a wide platform (2) of new bone extends vertically form the calvaria (1), from which an additional spike (3) of regenerate bone extends vertically. B, TCP – new augmented hard tissue (a), continuous with the original calvaria (c). Nonhomogenous appearance of the newly formed tissue composed of particulate residual scaffold (black arrow) surrounded by new host tissue. C and D, Collagen sponge-residual scaffold still occupied the space under the capsule (black arrow in C). D, Minimal new tissue formed vertically and covered by soft yellowish tissue.
COL group, most of the COL was still present inside the capsule (see Figure 2C). Minimal new tissue was formed vertically and was covered by soft yellowish tissue (see Figure 2D).

Descriptive Histology and Histomorphometric Measurements

In all the rats the bone under the capsule appeared mature. Reversion lines were observed suggesting a bone remodeling process. The newly formed bone was continuous with the native calvaria (Figure 3A).

Mean vertical bone height in the BOC group was 0.76 \pm 0.02 mm, which was similar to the original calvaria thickness that ranged from 0.71 to 0.83 mm (Figure 4). In the COL and C groups, a modest 1- to 1.5-mm vertical bone gain was observed (resulting in a total bone height of 1.52 \pm 0.18 and 1.77 \pm 0.61 mm, respectively); however, these differences were not statistically significant. The TCP group showed the greatest vertical bone gain. Mean bone height in this group (2.04 \pm 0.2 mm) was significantly greater than the BOC group \((p = 0.0145)\).

The overall height of augmented tissue (i.e., bone, residual graft, and connective tissue) in the C group ranged from 2.0 to 3.2 mm (mean 2.39 \pm 0.3 mm). Bone was covered by thin layer of connective tissue (\(-0.5\) mm) with slight inflammation (polymorphonuclear [PMN] and mononuclear cells). In the COL group, the mean tissue height was 3.22 \pm 0.11 mm. In this group, newly formed bone was covered by a thick fibrous tissue

Figure 3 Histology (hematoxylin and eosin stain). A, Control (scale bar 2 mm) – a wide platform of new bone extends vertically (2), on top of the calvarium (1), from which an additional spike (3) of new bone extends vertically. Blue arrows point at the lateral borders of the regenerated bone. B, Collagen sponge (scale bar 2 mm) – bone is covered by a thick fibrous tissue of residual collagen scaffold. C and E, Bio-Oss collagen – residual scaffold particles are surrounded by highly infiltrated connective tissue in C (scale bar 2 mm). Osteoclasts (OCs) can be observed adjacent to a Bio-Oss particle in higher magnification in E. D and F, tricalcium phosphate (TCP) – bone is continues with the calvaria and covered by dense connective tissue surrounding residual scaffold particles in D (scale 200 mm). New bone (NB) was formed by osteoblasts (OBs) in close proximity to TCP particles shown in F (higher magnification).
and residual collagen scaffold; the tissue was vastly infiltrated with erythrocytes and inflammatory cells (see Figure 3B). In the BOC and TCP groups, the vertical heights of the newly augmented tissues were comparable (4.48 ± 0.23 and 5.5 ± 0.24 mm, respectively), which were significantly greater than those of the COL and C groups (p < .0001). In the BOC group, this newly regenerate tissue was mostly composed of residual scaffold that was surrounded by connective tissue (see Figure 3, C and D). Particles of the residual scaffold were encapsulated by loose connective tissue that was infiltrated with PMN and mononuclear cells (see Figure 3C). Osteoclasts were observed adjacent to the BOC particles (see Figure 3E). In the TCP group, islands of dense connective tissue surrounded the residual scaffolds with numerous areas of newly formed lamellar bone aligned by osteoblasts and osteoid (see Figure 3, D and F).

**DISCUSSION**

The TCP group yielded the best results in both parameters: overall tissue height (5.5 ± 0.24 mm) and vertical bone growth (2.04 ± 0.2 mm). The osteoconductivity of TCP was previously reported. Rojbani and colleagues tested the osteoconductivity of β-TCP by its implantation into critical size defects in rats’ calvaria.16 The results showed higher bone formation in the β-TCP group compared with empty controls that were filled with connective tissue. In another clinical trial, Martinez and colleagues implanted pure β-TCP for bone augmentation in the maxillary sinus.21 Histomorphometric analysis obtained 8 months later revealed 35% new bone volume. Furthermore, in an in vitro study that compared human mesenchymal stem cell (MSC) growth and function (cell adherence, cellular activity, and osteogenic gene expression) on six different scaffolds, the authors concluded that of all synthetic scaffolds, β-TCPs have shown the best growth behavior for MSC.22

The response observed in the BOC group was somewhat disappointing: while the overall tissue height was sizable (4.48 ± 0.23 mm) and almost comparable to the TCP group, net bone gain was almost zero. Most of the available literature supports the osteoconductive potential of Bio-Oss alone when transplanted in extraction socket or maxillary sinus.12,21,23,24 However, only few studies used BOC, with conflicting results. Araújo and Lindhe grafted BOC in dogs’ extraction sockets.15 Histological analysis 6 months later revealed that the placement of BOC in the fresh extraction socket did not enhance new bone formation. Another study by the same group followed the dynamic of healing following BOC grafting into dogs’ extraction socket.25 Histological specimens were evaluated after 1 to 4 weeks. Similar to our results, massive inflammatory reaction was found. In the first 2 weeks of healing, polymorphonuclear cells and osteoclasts were observed near the surface of the foreign particles. However, in contrast to our findings, in the later stages of healing the osteoclasts disappeared from the Bio-Oss granules and were followed by osteoblasts that laid down bone mineral in the provisional matrix. According to these later results, it is possible that
better results might be achieved with longer healing period.

The empty domes have yielded some new bone (vertical bone height of 1.77 ± 0.61 mm). This might be primarily attributed not only to the GBR technique but also to the cortical perforation that was performed in all these sites. Lundgren and colleagues explored vertical bone augmentation by fixing 3-mm height domes to calvaria of three rabbits. Three months later, histological analysis revealed complete bone fill of all the domes. Kostopoulos and Karring demonstrated that the structural integrity of the barrier material and the sufficient adaptability of its borders to the underlying bone are prerequisites for predictable extracortical bone formation; both of these GBR principals were kept in our study. Moreover, we performed bone perforation to allow progenitor cell migration from the bone marrow to the GBR-treated site and to facilitate angiogenesis. The contribution of decortication prior to a GBR procedure for vertical bone augmentation is controversial. In preclinical trials, Min and colleagues and Majzoub and colleagues compared the effect of cortical perforations on bone formation under rigid domes that were fixed to rabbits’ calvaria. In both studies, intramarrow penetration accelerated bone formation. On the contrary, Lundgren and colleagues in the same experimental model reported that decortication of the calvarial bone in the rabbit does not result in more bone formation compared with no removal of the cortical bone plate. In the current study, the contribution of cortical perforations per se to bone formation could not be evaluated as we preformed cortical perforations in all of the cases.

The maximal vertical bone height obtained in this study was approximately 3 mm (mean 2 mm or less), thus highlighting the limitations of GBR even in conjunction with osteoconductive materials. The addition of growth factors or cells to scaffolds might improve bone formation. Ben-David and colleagues in a study of critical size defects in mice calvaria demonstrated improved bone formation by addition of bone-marrow-derived MSC to hydrogel scaffold compared with scaffold alone. Seebach and colleagues in critical size defect in rats’ tibiae demonstrated complete bridging of the defect by the addition of stem cells to TCP scaffold compared with partial bone fill using scaffold alone. The major limit of this study is the relatively low number of rats in each group. However, as the histological and histomorphometric analyses showed minimal intragroup variations (as seen in Figure 4) despite the low number of specimens, differences between groups are clear.

It is very likely that in the present experimental model, the limiting factor for vertical bone formation is the amount of osteoprogenitor cells that migrate from the periosteum and perforated calvaria to populate the newly formed augmented tissue. Therefore, in order to further improve vertical bone formation, osteoprogenitor cell transplantation mixed with β-TCP under the rigid dome is currently intensively studied.

CONCLUSIONS

The combination approach using osteoconductive scaffold with dome-shaped rigid barriers resulted in moderate vertical bone regeneration with minimal complications. TCP in conjunction with barrier dome resulted in greater vertical bone augmentation in the calvarium of rats.

REFERENCES

8. Lundgren D, Lundgren AK, Sennerby L, Nyman S. Augmentation of intramembranous bone beyond the skeletal


