J Clin Periodontol 2005; 32: 193–199 doi: 10.1111/j.1600-051X.2005.00656.x

Journal of Clinical Periodontology

The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material

Zvi Artzi[1], Avital Kozlovsky[1], Carlos E. Nemcovsky[1] and Miron Weinreb[2]

Departments of [1]Periodontology and [2]Oral Biology, The Maurice and Gabriela Goldschleger School of Dental Medicine, Tel Aviv University, Tel Aviv, Israel

Artzi Z, Kozlovsky A, Nemcovsky CE, Weinreb M: The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material. J Clin Periodontol 2005; 32: 193–199. doi: 10.1111/j.1600-051X.2005.00656.x. © Blackwell Munksgaard, 2005.

Abstract

Objectives: Bone replacement substitutes are almost unavoidable in augmentation procedures such as sinus grafting. The objective of the present study was to evaluate the osteoconductive capability of two different scaffold fillers in inducing newly formed bone in this procedure.

Material and Methods: Sinus floor augmentation and implant placement were carried out bilaterally in 12 patients. Bovine bone mineral (BBM) was grafted on one side and β-tricalcium phosphate (β-TCP) on the contralateral side. Both were mixed (1:1 ratio) with autogenous cortical bone chips harvested from the mandible by a scraper. Hard tissue specimen cores were retrieved from the augmented sites (at the previous window area) at 12 months. Decalcified sections were stained with haematoxylin–eosin and the fraction area of new bone and filler particles was measured. In addition to the effect of the filler on new bone formation, the latter was tested to determine whether it correlated with the tissue depth and residual amount of the grafted material.

Results: Bone area fraction increased significantly from peripheral to deeper areas at both grafted sites in all cores: from 26.0% to 37.7% at the β-TCP sites and from 33.5% to 53.7% at the BBM-grafted sites. At each depth the amount of new bone in BBM sites was significantly greater than that in TCP sites. However, the average area fraction of grafted material particles was similar in both fillers and all depth levels (β-TCP = 27.9–23.2% and BBM = 29.2–22.6%, NS). A significant negative correlation was found between bone area fraction and particle area fraction at the middle ($p = 0.009$) and deep ($p = 0.014$) depths in the β-TCP sites, but not at the BBM sites.

Conclusion: At 12 months post-augmentation, the two examined bone fillers, β-TCP and BBM, promoted new bone formation in sinus grafting but the amount of newly formed bone was significantly greater in BBM-grafted sites. However, both exhibited similar residual grafted material area fraction at this healing period. This could imply that BBM possesses better osteoconductive properties.

Key words: bone grafted material; bone regeneration; bovine bone; follow-up studies; sinus augmentation; β-tricalcium phosphate

Accepted for publication 11 May 2004

Sinus floor augmentation is a highly successful and predictable procedure to increase bone volume for implant placement in the partially to severely atrophic maxilla (Jensen et al. 1998, Misch 1999, ten-Bruggenkate 1999, Olson et al. 2000). Autogenous bone graft, harvested in multiple forms (particles, strips, or blocks), meets many criteria and is an ideal graft. It harbours osteoinductive and osteoconductive properties, because it contains growth

factors and has a scaffold effect for osteogenic transfer. Its immunogenetic competence and rapid healing, uncomparable with any non-autologous source, make autologous source the gold standard for bone re-constructive surgery (Burchardt 1983).

The key to the success of any bone graft is primarily determined by the degree of vascularization. However, rapid re-vascularization and complete resorption of autogenous bone (Davis et al. 1984) may not ultimately serve its long-term goals, especially in certain augmentation cases, e.g., sinus grafting or vertical ridge regeneration procedures. These would require a long-lasting active osteoconductive guiding scaffold to support osseointegrated implants in function. The advantages of a combined autologous and non-autologous source, which would serve as an inductive vehicle, and also act as a slow resorbable osteoconductive vehicle, would probably be the graft of choice (Laurencin & Lu 1999, Lynch 1999, Temenoff et al. 1999, Yildirim et al. 2000, Hallman et al. 2001a, b, 2002a, b).

Bovine bone mineral (BBM) (Bio-Oss®, Geistlich Biomaterials, Wolhusen, Switzerland) is an excellent biocompatible and osteoconductive material (Spector 1994, Jensen et al. 1996, Berglundh & Lindhe 1997, Boyne 1997, Hämmerle et al. 1997, Skoglund et al. 1997, Artzi & Nemcovsky 1998), and has proved to be an appropriate scaffold in ridge deficiencies, peri-implant destruction, and sinus augmentation procedures (Smiler et al. 1992, Wetzel et al. 1995, Dies et al. 1996, Hürzeler et al. 1997, Valentini & Abensur 1997, Piattelli et al. 1999, Artzi et al. 2000, 2001a, b, 2002, Hallman et al. 2001b, 2002a). Extensive morphometric data also show that it is highly predictable (Haas et al. 1998, Valentini et al. 1998, Hanisch et al. 1999; Terheyden et al. 1999; Yildirim et al. 2000, Artzi et al. 2001b, 2002, Hallman et al. 2001a, 2002a, b). However, the rate and mechanism of its resorption are still unclear (Berglundh & Lindhe 1997, Skoglund et al. 1997, Schlegel & Donath 1998, Taylor et al. 2002, Artzi et al. 2003a, b, Sartori et al. 2003).

Beta tricalcium phosphate (β-TCP) (Cerasorb®, Curasan, Kleinostheim, Germany), a ceramic alloplast, is another popular graft material, which has shown promising results (Breitbart et al. 1995, Gao et al. 1997, Buser et al. 1998, Ohsawa et al. 2000, Szabo et al. 2001). Unlike BBM, this material had shown extensive resorption within 12–84 months (Yamada et al. 1997, Wiltfang et al. 2002, Artzi et al. 2004). This raises the question as to what is the impact of the material resorption rate on the amount of newly formed bone established in the augmented sites.

Therefore, the aim of this study was to evaluate morphometrically the amount of newly formed bone around two different bone grafts, a synthetic (β-TCP) and a natural (BBM) one, and to determine whether there was a correlation between new bone formation and the residual amount of the grafted material particles at 12 months' healing period.

Materials and Methods

The study comprised of 12 bilateral sinus floor augmentation procedures, using a one- or two-stage technique. In the one-stage approach, implants (TiUnite® Replace Select Straight, 3.5–4.3 mm, Nobel Biocare, Goteburg, Sweden or MTX® Tapered Screwvent or Spline, 3.7–5.0 mm, Centerpulse Dental Inc, Carlesbad, CA, USA) were placed in conjunction with the augmentation procedure, and in the two-stage approach, implant placement occurred at 6 months. However, specimens were retrieved from both procedures at 12 months post-augmentation upon implant cover screw exposure.

The study consisted of 12 patients (seven women, five men), ranging in age from 42 to 64 years (average 50.1 years). Patients disclosed no systemic disorders and no requirement of any routine medication. Each surgical step was explained and patients signed a consent form. The Ethics Committee of the University approved the study.

A panoramic radiograph and a computerized tomography (CT) scan of the maxilla were taken for each patient. Antral spaces were evaluated at 3 mm serial sections. Residual bone height, as measured on the serial sections of the CT scan, ranged between 1 and 7 mm (average 3.8 mm). In five sites (two were bilateral in two patients), where the residual alveolus was 1–2 mm in height, the two-stage technique was used, and implants ($n = 15$) were placed after 6 months. In 19 sites, where a one-stage procedure was used, residual bone height was at least 3 mm. Implants ($n = 62$) were simultaneously placed with the augmentation material.

In each patient, one side was randomly grafted with a combination (1:1 ratio) of BBM and autogenous bone chips and the contra-lateral side, at 2-week intervals, with a combination of β-TCP and autogenous bone chips (1:1 ratio).

Pre-medication followed the protocol suggested by Misch (1999). That is, Dexamethasone (Rekah Pharmaceuticals, Holon, Israel) 9 mg before surgery, 6 mg after 24 h, and 3 mg after 48 h, as an anti-inflammatory drug. Systemic antibiotics were also administered 1 h before surgery, Amoxicillin (Moxypen®, Teva Pharmaceuticals, Petach Tikvah, Israel) 1 g and 500 mg (QID) for 1 week. As an analgesic agent, Ibuprufen (Etopan, Taro, Haifa Bay, Israel) 200 mg, two tablets initial dose, and one tablet as needed later, was also prescribed. The sequential surgical steps are well known and follow the pioneering technique of Boyne and James (1980) and Tatum (1986). The technique has been previously described in detail (Nevins & Fiorellini 1998, Jensen 1999, Misch 1999, Artzi et al. 2001b, 2003c, Hallman et al. 2001a).

Autogenous bone chips were collected by cortical scraping of the lateral posterior area of the mandible near the external oblique line using a special manual scraper device (MxGrafter®, Maxilon Laboratories, Inc., Hollis, NH, USA). At the maxillary antrum, the fractured lateral bony wall was pushed gently inward and upward, along with careful reflection of the Schneiderian membrane. Cortical bone chips were then harvested. After local anaesthesia of the long buccal nerve, a blunt incision was made on the lateral posterior part of the mandible extending from the buccal masticatory mucosa to the ascending ramus with a Bard-Parker No. 15 c blade (Martin GmbH & Co. KG., Tuttlingen, Germany). A full periosteal thickness flap exposed the lateral retromolar mandibular bone area. By using the safe manual bone scraper, numerous bone chips were harvested from the cortical plate. These were mixed at a 1:1 ratio with either BBM (250–1000 µm) or β-TCP (500–1000 µm), and blood coagulum was added for moisture.

In the immediate implant placement approach, i.e., one-stage, grafted material was applied in increments before

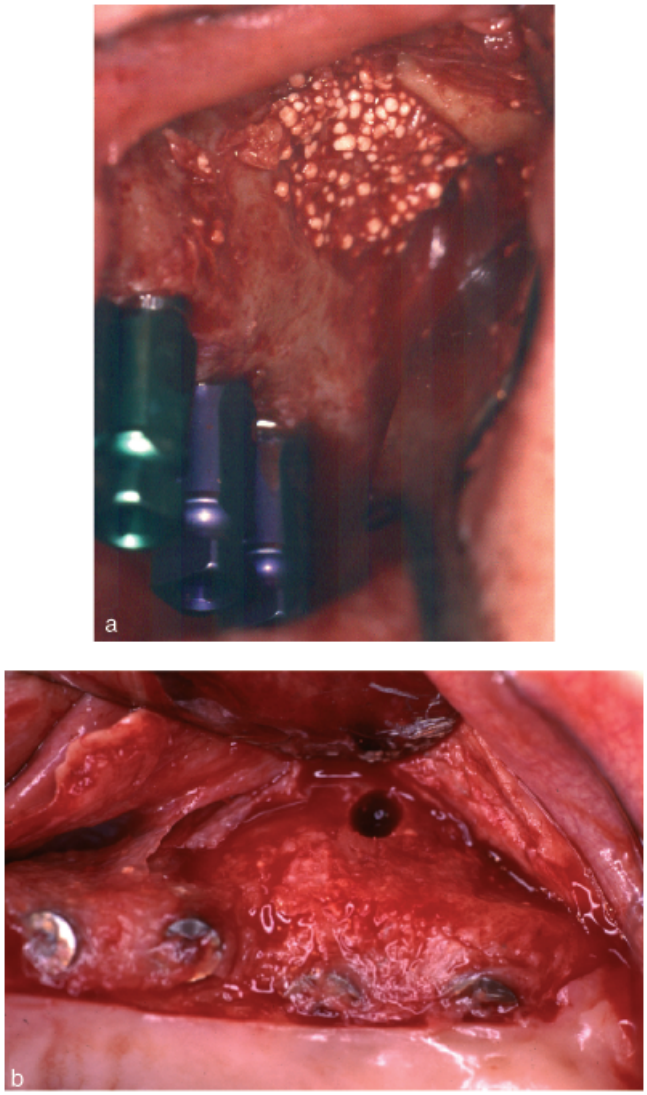

Fig. 1. (a) A mix of β-TCP and autogenous bone chips augments the sinus floor and occludes the lateral window orifice. (b) Specimen core taken with trephine at the previous location of the window area, avoiding contact with implants.

and after implant placement. The site was filled with the grafted particles and the obturated window boundaries were measured to the crestal bone level (Fig. 1a). Also, in this technique, the location of the implant body was considered for accurate orientation of the future specimen harvesting phase (Fig. 1b). Prior to soft tissue closure, the entire obturated lateral window was covered by an absorbable double-layer collagen membrane (Bio-Gide®, Geistlich Biomaterials, Wolhusen, Switzerland).

At the five sites (three grafted with β-TCP and two with BBM) of the two-stage approach, implants were placed 6 months post-augmentation. In both techniques, the cover screw was exposed 12 months post-augmentation. A periapical radiograph showed osseous housing around implants and their exact location before harvesting a sample of the augmented tissue sample. At that time, cylindrical bone samples were harvested by a 2 mm internal diameter trephine bur exactly from the previous location of the lateral fractured window area. Specimen retrieval depth varied from 6 to 8 mm. The current periapical radiograph and previous measurement during the augmentation surgical phase ensured avoidance of hazards and over-approximation of the trephine bur during cautious drilling.

Tissue samples were trimmed to a standard length (6 mm) and marked with black ink to identify the peripheral *versus* deep ends. Specimens were fixed in 10% neutral buffered formalin for 1 week and then decalcified with 5% formic acid for 2 weeks. The decalcified cylindrical specimens were embedded in paraffin and transversely cut into serial sections, 5 µm in width, using a microtome (Leica RM 2245, Leica Microsystems, Nussloch GmbH, Germany). Each core was cut uniformly from the peripheral to deeper region. Morphometric analysis was performed on a pair of sections (25 µm apart) from each of the peripheral, middle, and deepest regions of each specimen core, stained with haematoxylin–eosin. Data from each pair were averaged and used as such, while a mean of the three examined areas of each sample was also calculated.

In each section, the area fraction of bone and grafted material particles was measured using an adaptation of the point-counting procedure (Chalkey 1943, Bellhouse 1981, Dayan et al. 1992, Artzi et al. 2000). Briefly, each section was examined in a projection microscope (Visopan, Reichart, Leica AG, Vienna, Austria) at $\times$ 20 magnification. A 64-square (1.5 cm $\times$ 1.5 cm) graticule was superimposed on the screen. Point counting was performed on bone and grafted particles (BBM or β-TCP). Whenever the graticule-square centre (marked by a ''+'') hit one of the two components, the specified component scored one point. The sum of the points overlying each component (Pi) was calculated. Area fraction percentage of each component in each section was determined as $Pi/\Sigma i$, where Σi represents the total number of points superimposed on each section.

The two measured parameters (mean bone area and particle area of the two sections representing each depth) were statistically analysed (SPSS Inc, Chicago, IL, USA). One- and two-way analysis of variance (ANOVA) tested whether the parameters were influenced by tissue depth and type of filler. Paired t-tests evaluated the difference in both parameters between the two fillers (i.e., two sides of the same patient) at each depth. A correlation analysis (two-tailed Pearson Correlation Coefficients) tested the association between bone and particle area fractions of each material at each depth.

Results

All ($n = 77$) implants except one showed solid stability and no crestal bone resorption. With the two-stage technique, all surgical sites ($n = 15$)

healed uneventfully and all implants were shown to be osseointegrated, clinically. However, with the one-stage technique ($n = 62$), one implant (in a β-TCP site) that did not establish initial stability at the time of surgical placement could not integrate and was removed after 1 month. This implant was replaced after 4 months subsequent to complete healing of the site.

Histomorphometrically, bone area fraction in all grafted sites increased from peripheral to deeper zones of the specimen cores (Table 1). Thus, bone area fraction increased from 26% to 37.7% (average 32%; SD = 8.4) at the β-TCP-grafted sites (Figs 2a, b) and from 35.9% to 53.2% (average 45.6%, SD = 10.9) at the BBM-grafted sites (Figs 3a, b). This depth increase was statistically significant for both fillers ($p < 0.005$). At each depth, bone area in BBM sites was significantly greater than that in β-TCP sites ($p < 0.02$, $p < 0.001$ and $p < 0.002$ at peripheral, middle and deep zones, respectively). Two-way ANOVA, which showed that bone area was significantly influenced by depth and filler, corroborated these conclusions.

However, particle area fraction was affected by depth but not by the type of filler. It decreased from 27.9% in peripheral zones to 23.2% in deep zones of β-TCP sites and from 29.2% to 22.6% in BBM sites (Table 2). There was no difference between particle area fractions of the two fillers at any depth.

When correlation coefficients between bone area and particle area at each depth were examined, a significant negative correlation was found in β-TCP sites ($R = 0.72$ $p = 0.009$ and $R = 0.68$ $p = 0.014$ in deep and middle zones, respectively) but not in BBM sites (Table 3).

Table 1. Bone area fraction (%) in the different grafted sites (mean ± SD)

Depth	β-TCP	BBM	Paired *t*-test
Peripheral	26.0 ± 4.7	35.9 ± 9.4	$p = 0.017$
Middle	32.1 ± 5.5	47.8 ± 8.4	$p < 0.001$
Deep	37.7 ± 9.9	53.2 ± 6.9	$p = 0.002$
Average	32.0 ± 8.4	45.6 ± 10.9	

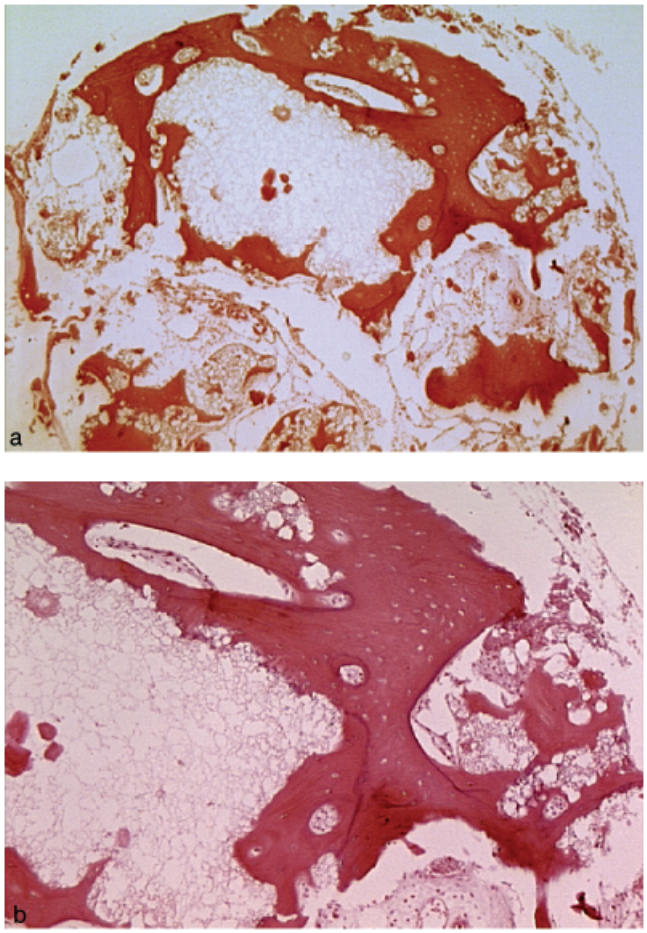

Fig. 2. (a) Representative section from a β-TCP specimen core, in which material particles (P), newly formed bone (B), and intervening connective tissue (C) are clearly visible (haematoxylin–eosin staining × 60 original magnification). (b) Higher magnification of (a). Vital newly formed bone with osteocytes in the lacunae resides in intimate contact with the grafted material (P) (haematoxylin–eosin staining × 120 original magnification).

Discussion

The two non-autologous bone grafts used in this study, whether a natural derivative (BBM) or a synthetic substitute (β-TCP), proved to be biocompatible and osteoconductive and promoted bone formation. Eventually, both materials enabled regeneration of newly formed bone to accommodate implants for fixed prosthetic reconstruction in the posterior atrophic maxilla.

In this procedure an absorbable GTR membrane was applied to enhance osteopromotion (Ohnishi et al. 2000). A recent comparative study in monkeys (Schou et al. 2003) has shown the advantage of BBM particles in experimental peri-implant sites treated in accordance with GTR principles. The advantage of GTR membrane over lattice at the wound site is well documented (Dahlin et al. 1988, 1989, 1990, Tarnow et al. 2000, Wikesjo et al. 2003), and would facilitate bone formation and absorption of the bone graft material.

Although different resorption rates of TCP and BBM have been reported (Berglundh & Lindhe 1997, Skoglund et al. 1997, Yamada et al. 1997, Schlegel & Donath 1998, Taylor et al. 2002, Wiltfang et al. 2002, Artzi et al. 2003a, b, 2004, Sartori et al. 2003), both showed a similar area fraction of the grafted particles at 12 months in this study. Nevertheless, complete resorption of β-TCP has been reported after a healing period of 24 months (Artzi et al. 2004). Irrespective of particle area fraction resemblance, bone area fraction at the BBM sites was significantly

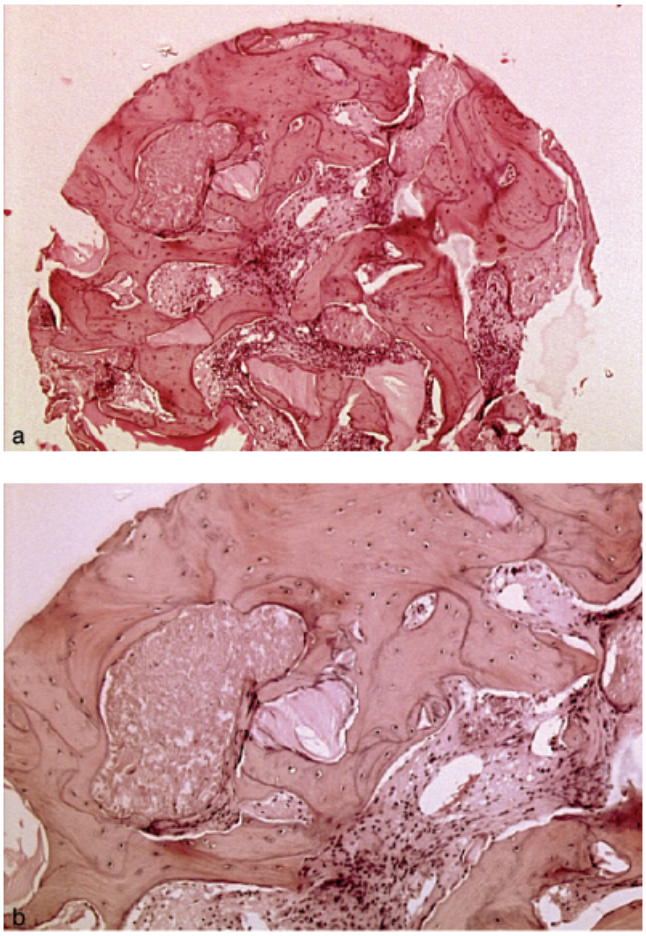

Fig. 3. (a) Representative section from a BBM specimen core. Substantial newly formed bone (B) surrounds the grafted material (P), while connective tissue is mainly found in the periphery (haematoxylin–eosin staining × 60 original magnification). (b) Higher magnification of (a). Newly formed bone with numerous osteocytes completely surrounds the grafted material (P) (haematoxylin–eosin staining × 120 original magnification).

Table 2. Particles area fraction (%) in the different grafted sites (mean ± SD)

Depth	β-TCP	BBM	Paired *t*-test
Peripheral	27.9 ± 4.4	29.2 ± 6.7	NS
Middle	24.6 ± 6.5	26.0 ± 7.8	NS
Deep	23.2 ± 4.9	22.6 ± 6.9	NS
Average	25.3 ± 5.6	26.0 ± 7.5	

NS, statistically not significant, β-TCP, β-tricalcium phosphate; BBM, bovine bone mineral.

Table 3. Correlation coefficients (*R*) between bone and particle area fractions

Depth	β-TCP	BBM
Peripheral	0.47	0.25
Middle	0.72*	0.46
Deep	0.68**	0.15

β-TCP, β-tricalcium phosphate; BBM, bovine bone mineral. *$p = 0.009$; **$p = 0.014$.

greater than at the β-TCP-grafted sites. This could indicate the degree of osteoconductivity of each material. In the same environment, BBM proved to promote a significantly greater amount of new bone formation over β-TCP after 12 months. Despite the slow resorption rate of BBM, it did not inhibit the continual formation of newly formed bone, but progressively enhanced it (Artzi et al. 2003a, b). It can be assumed that the macro- and micro-porous configurations of BBM particles (Spector 1999, Rosen et al. 2002) result in better osteoconductive properties and the continual presence of the material established hard augmented tissue combined by the new bone and the grafted material as a new dense ''cancellous network''. In a recent study (Xu et al. 2004), BBM, as a grafted material in maxillary sinus in rabbits, contributed to the stability of the augmentation site by inhibiting bone resorption. Therefore, it is not surprising that the presence of residual grafted material at the BBM sites is not commensurate with the amount of newly formed bone, as shown by the Pearson correlation test, while at the β-TCP sites, the grafted material negatively correlated to the amount of new bone at the deeper (middle and deep) augmentation zones. This correlation was not found at the peripheral zone, probably because of the temporarily impaired vascularization and selective barrier function of the double layer collagen membrane at the early healing period that was determinant. The negative correlation between particle and bone area fractions at the β-TCP actually showed that the amount of newly formed bone depended on the resorbable capability of this scaffold. β-TCP, which is also a porous material, but a ceramic synthetic one, acts differently, both chemically and functionally, thus establishing a different bone re-modelling process over time.

The increasing/decreasing pattern along the depth of the augmented site has been shown in sinus grafting procedures (Artzi et al. 2001b, 2002). An undisturbed vascular supply to the grafting site is of utmost importance (Solar et al. 1999). It appears that the amount of new regenerated tissue is not only time dependable (Valentini et al. 2000, Sartori et al. 2003) but also dependent on augmentation location and depth. The recipient osseous site and walls and the proximity of the Schneiderian membrane, as vascular nourishment sources, probably influence the amount of tissue generation.

Conclusion

Both materials tested in this study (β-TCP and BBM) are suitable for promoting bone formation. However, at 12 months, the greater amount of newly formed bone in BBM-grafted sites compared with β-TCP sites, in light of the similar residual material area fraction, suggests that BBM possesses better osteoconductive properties.

Acknowledgements

The authors wish to thank Mrs Hana Vered for histological preparation, and Ms Rita Lazar for editorial assistance.

References

Artzi, Z., Givol, N., Rohrer, M. D., Nemcovsky, C. E., Prasad, H. S. & Tal, H. (2003a) Qualitative and quantitative expression of bovine bone mineral in experimental bone defects. Part 1: description of a dog model and histological observations. *Journal of Periodontology* **74**, 1143–1152.

Artzi, Z., Givol, N., Rohrer, M. D., Nemcovsky, C. E., Prasad, H. S. & Tal, H. (2003b) Qualitative and quantitative expression of bovine bone mineral in experimental bone defects. Part 2. Morphometric analysis. *Journal of Periodontology* **74**, 1159–1160.

Artzi, Z. & Nemcovsky, C. E. (1998) The application of deproteinized bovine bone mineral for ridge preservation prior to implantation. Clinical and histological observations in a case report. *Journal of Periodontology* **69**, 1062–1067.

Artzi, Z., Nemcovsky, C. E. & Dayan, D. (2002) Bovine-HA spongiosa blocks and immediate implant placement in sinus augmentation procedures. Histopathological and histomorphometric observations on different histological stainings in 10 consecutive patients. *Clinical Oral Implants Research* **13**, 420–427.

Artzi, Z., Nemcovsky, C. E. & Dayan, D. (2003c) Nonceramic hydroxyapatite bone derivative in sinus augmentation procedures: clinical and histomorphometric observations in 10 consecutive cases. *International Journal of Periodontics and Restorative Dentistry* **23**, 381–389.

Artzi, Z., Nemcovsky, C. E. & Tal, H. (2001a) Efficacy of porous bovine bone mineral in various types of osseous deficiencies. Clinical observations and literature review. *International Journal of Periodontics and Restorative Dentistry* **21**, 395–405.

Artzi, Z., Nemcovsky, C. E., Tal, H. & Dayan, D. (2001b) Histopathological morphometric evaluation of 2 different hydroxyapatite-bone derivatives in sinus augmentation procedures: a comparative study in humans. *Journal of Periodontology* **72**, 911–920.

Artzi, Z., Tal, H. & Dayan, D. (2000) Porous bovine bone mineral in healing of human extraction sockets Part 1 Histomorphometric evaluation at 9 months. *Journal of Periodontology* **71**, 1015–1023.

Artzi, Z., Weinreb, M., Givol, N., Rohrer, M. D., Nemcovsky, C. E., Prasad, H. S. & Tal, H. (2004) Biomaterial resorbability and healing site morphology of inorganic bovine bone and beta tricalcium phosphate in the canine. A 24-month longitudinal histologic study and morphometric analysis. *International Journal of Oral and Maxillofacial Implants* **19**, 357–368.

Bellhouse, D. R. (1981) Area estimation by point counting techniques. *Biometrics* **37**, 303–312.

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

Boyne, P. J. (1997) Bone grafts: materials. In: Evensen, L. (ed). *Osseous Reconstruction of the Maxilla and the Mandible*, pp. 13–21. Carol Stream, IL: Quintessence Publishing Co.

Boyne, P. J. & James, R. A. (1980) Grafting of the maxillary sinus floor with autogenous marrow and bone. *Journal of Oral Surgery* **38**, 613–616.

Breitbart, A. S., Staffenberg, D. A., Thorne, C. H., Glat, P. M., Cunningham, N. S., Reddi, A. H., Ricci, J. & Steiner, G. (1995) Tricalcium phosphate and osteogenin: a bioactive onlay bone graft substitute. *Plastic and Reconstructive Surgery* **96**, 699–708.

Burchardt, H. (1983) The biology of bone graft repair. *Clinical Orthopaedics and Related Research* **174**, 28–42.

Buser, D., Hoffmann, B., Bernard, J. P., Lussi, A., Mettler, D. & Schenk, R. K. (1998) Evaluation of filling materials in membrane-protected bone defects. A comparative histomorphometric study in the mandible of miniature pigs. *Clinical Oral Implants Research* **9**, 137–150.

Chalkey, H. W. (1943) Method for quantitative morphologic analysis of tissues. *Journal of the National Cancer Institute* **4**, 47–53.

Dahlin, C., Gottlow, J., Linde, A. & Nyman, S. (1990) Healing of maxillary and mandibular bone defects using a membrane technique. An experimental study in monkeys. *Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery* **24**, 13–19.

Dahlin, C., Linde, A., Gottlow, J. & Nyman, S. (1988) Healing of bone defects by guided tissue regeneration. *Plastic and Reconstructive Surgery* **81**, 672–676.

Dahlin, C., Sennerby, L., Lekholm, U., Linde, A. & Nyman, S. (1989) Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits. *International Journal of Oral and Maxillofacial Implants* **4**, 19–25.

Davis, W. H., Martinoff, J. T. & Kaminishi, R. M. (1984) Long-term follow up of transoral rib grafts for mandibular atrophy. *Journal of Oral and Maxillofacial Surgery* **42**, 606–609.

Dayan, D., Bodner, L. & Horowitz, I. (1992) Effect of salivary hypofunction on the healing of extraction wounds: a histomorphometric study in rats. *Journal of Oral and Maxillofacial Surgery* **50**, 354–358.

Dies, F., Etienne, D., Bou Abboud, N. & Ouhayoun, J. P. (1996) Bone regeneration in extraction sites after immediate placement of an e-PTFE membrane with or without a biomaterial. Report on 12 consecutive cases. *Clinical Oral Implants Research* **7**, 277–285.

Gao, T. J., Lindholm, T. S., Kommonen, B., Ragni, P., Paronzini, A. & Lindholm, T. C. (1997) Stabilization of an inserted tricalcium phosphate spacer enhances the healing of a segmental tibial defect in sheep. *Archives of Orthopaedic and Trauma Surgery* **116**, 290–294.

Haas, R., Donath, K., Fodinger, M. & Watzek, G. (1998) Bovine hydroxyapatite for maxillary sinus grafting: comparative histomorphometric findings in sheep. *Clinical Oral Implants Research* **9**, 107–116.

Hallman, M., Cederlund, A., Lindskog, S., Lundgren, S. & Sennerby, L. (2001a) A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. Results after 6 to 8 months of healing. *Clinical Oral Implants Research* **12**, 135–143.

Hallman, M., Hedin, M., Sennerby, L. & Lundgren, S. (2002a) A prospective 1-year clinical and radiographic study of implants placed after maxillary sinus floor augmentation with bovine hydroxyapatite and autogenous bone. *Journal of Oral and Maxillofacial Surgery* **60**, 277–284.

Hallman, M., Lundgren, S. & Sennerby, L. (2001b) Histologic analysis of clinical biopsies taken 6 months and 3 years after maxillary sinus floor augmentation with 80% bovine hydroxyapatite and 20% autogenous bone mixed with fibrin glue. *Clinical Implant Dentistry and Related Research* **3**, 87–96.

Hallman, M., Sennerby, L. & Lundgren, S. (2002b) A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. *International Journal of Oral and Maxillofacial Implants* **17**, 635–643.

Hämmerle, C. H. F., Olah, A. J., Schmid, J., Fluckiger, L., Gogolewski, S., Winkler, J. R. & Lang, N. P. (1997) The biological effect of deproteinized bovine bone on bone neoformation on the rabbit skull. *Clinical Oral Implants Research* **8**, 198–207.

Hanisch, O., Lozada, J. L., Holmes, R. E., Calhoun, C. J., Kan, J. Y. & Spiekermann, H. (1999) Maxillary sinus augmentation prior to placement of endosseous implants: a histomorphometric analysis. *International Journal of Oral and Maxillofacial Implants* **14**, 329–336.

Hürzeler, M. B., Quiñones, C. R., Kirsch, A., Gloker, C., Schupbach, P., Strub, J. R. & Caffesse, R. G. (1997) Maxillary sinus augmentation using different grafting materials and dental implants in monkeys. Part I. Evaluation of anorganic bovine-derived bone matrix. *Clinical Oral Implants Research* **8**, 476–486.

Jensen, O. T. (1999) Treatment planning for sinus grafts. In: Jensen, O. T. (ed). *The Sinus Bone Graft*, pp. 49–68. Carol Stream, IL: Quintessence Publishing Co, Inc.

Jensen, S. S., Aaboe, M., Pinholt, E. M., Hjørting-Hansen, E., Melsen, F. & Ruyter, I. E. (1996) Tissue reaction and material characteristics of four bone substitutes.

International Journal of Oral and Maxillofacial Implants **11**, 55–66.

Jensen, O. T., Shulman, L. B., Block, M. S. & Iacono, V. J. (1998) Report of the sinus consensus conference of 1996. *International Journal of Oral and Maxillofacial Implants* **13** (Suppl), 11–45.

Laurencin, C. T. & Lu, H. H. (1999) Polymer–ceramic composites or bone–tissue engineering. In: Davies, J. E. (ed). *Bone Engineering*, pp. 462–472. Toronto, Ontario: EM Squared Inc.

Lynch, S. E. (1999) A clinical example of tissue engineering. In: Lynch, S. E., Genco, R. J. & Marx, R. E. (eds). *Tissue Engineering*, pp. XI–XVIII. Carol Stream, IL: Quintessence Publishing Co, Inc.

Misch, C. E. (1999) The maxillary sinus lift and sinus graft surgery. In: Misch, C. E. (ed). *Implant Dentistry*, pp. 469–496. St Louis: Mosby Publishing, Inc.

Nevins, M. & Fiorellini, J. P. (1998) The maxillary sinus floor augmentation procedure to support implant prostheses. In: Nevins, M. & Mellonig, J. T. (eds). *Implant Therapy*, pp. 171–196. Carol Stream, IL: Quintessence Publishing Co, Inc.

Ohnishi, H., Fujii, N., Futami, T., Taguchi, N., Kusakari, H. & Maeda, T. (2000) A histochemical investigation of the bone formation process by guided bone regeneration in rat jaws. Effect of PTFE membrane application periods on newly formed bone. *Journal of Periodontology* **71**, 341–352.

Ohsawa, K., Neo, M., Matsuoka, H., Akiyama, H., Ito, H., Kohno, H. & Nakamura, T. (2000) The expression of bone matrix protein mRNAs around beta-TCP particles implanted into bone. *Journal of Biomedical Materials Research* **52**, 460–466.

Olson, J. W., Dent, C. D., Morris, W. F. & Ochi, S. (2000) Long term assessment (5 to 71 months) of endosseous dental implants placed in the augmented maxillary sinus. *Annals of Periodontology* **5**, 152–156.

Piattelli, M., Favero, G. A., Scarano, A., Orsini, G. & Piattelli, A. (1999) Bone reactions to anorganic bovine bone (Bio-Oss) used in sinus procedures: a histologic long-term report of 20 cases in humans. *International Journal of Oral and Maxillofacial Implants* **14**, 835–840.

Rosen, V. B., Hobbs, L. W. & Spector, M. (2002) The ultrastructure of anorganic bovine bone and selected synthetic hydroxyapatites used as bone graft substitute materials. *Biomaterials* **23**, 921–928.

Sartori, S., Silvestri, M., Forni, F., Icaro Cornaglia, A., Tesei, P. & Cattaneo, V. (2003) Ten-year follow-up in a maxillary sinus augmentation using anorganic bovine bone (Bio-Oss). A case report with histomorphometric evaluation. *Clinical Oral Implants Research* **14**, 369–372.

Schlegel, A. K. & Donath, K. (1998) Bio-Oss – a resorbable bone substitute? *Journal of Long-Term Effects of Medical Implants* **8**, 201–209.

Schou, S., Holmstrup, P., Jorgensen, T., Skovgaard, L. T., Stoltze, K., Hjørting-Hansen, E. & Wenzel, A. (2003) Anorganic porous bovine-derived bone mineral (Bio-Oss) and ePTFE membrane in the treatment of peri-implantitis in cynomolgus monkeys. *Clinical Oral Implants Research* **14**, 535–547.

Skoglund, A., Hising, P. & Young, C. (1997) A clinical and histologic examination in humans of the osseous response to implanted natural bone mineral. *International Journal of Oral and Maxillofacial Implants* **12**, 194–199.

Smiler, D. G., Johnson, P. W., Lozada, J. L., Misch, C., Rosenlicht, J. L., Tatum, O. H. Jr & Wagner, J. R. (1992) Sinus lift grafts and endosseous implants: treatment of the atrophic posterior maxilla. *Dental Clinics of North America* **36**, 151–186.

Solar, P., Geyerhofer, U., Traxler, H., Windisch, A., Ulm, C. & Watzek, G. (1999) Blood supply to the maxillary sinus relevant to sinus floor elevation procedures. *Clin Oral Implants Res* **10**, 34–44.

Spector, M. (1994) Anorganic bovine bone and ceramic analogs of bone mineral as implants to facilitate bone regeneration. *Clinics in Plastic Surgery* **21**, 437–444.

Spector, M. (1999) Basic principles of tissue engineering. In: Lynch, S. E., Genco, R. J. & Marx, R. E. (eds). *Tissue Engineering*, pp. 3–16. Carol Stream, IL: Quintessence Publishing Co.

Szabo, G., Suba, Z., Hrabak, K., Barabas, J. & Nemeth, Z. (2001) Autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevations (2- and 3-dimensional computed tomographic, histologic, and histomorphometric observations): preliminary results. *International Journal of Oral and Maxillofacial Implants* **16**, 681–692.

Tarnow, D. P., Wallace, S. S., Froum, S. J., Rohrer, M. D. & Cho, S. C. (2000) Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: part 3 of an ongoing prospective study. *International Journal of Periodontics and Restorative Dentistry* **20**, 117–25.

Tatum, H. Jr. (1986) Maxillary and sinus implant reconstructions. *Dental Clinics of North America* **30**, 207–229.

Taylor, J. C., Cuff, S. E., Leger, J. P., Morra, A. & Anderson, G. I. (2002) In vitro osteoclast resorption of bone substitute biomaterials used for implant site augmentation: a pilot study. *International Journal of Oral and Maxillofacial Implants* **17**, 321–330.

Temenoff, J. S., Lu, L. & Mikos, A. G. (1999) Bone-tissue engineering using synthetic biodegradable polymer scaffolds. In: Davies, J. E. (ed). *Bone Engineering*, pp. 454–461. Toronto, Ontario: EM Squared Inc.

ten-Bruggenkate, C. M. (1999) Sinus floor elevation and its predictability. In: Lang, N. P., Karring, T. & Lindhe, J. (eds). *Proceedings of the 3rd European Workshop on Periodontology: Implant Dentistry*, pp. 535–543. Berlin: Quintessence Publishing Co.

Terheyden, H., Jepsen, S., Moller, B., Tucker, M. M. & Rueger, D. C. (1999) Sinus floor augmentation with simultaneous placement of dental implants using a combination of deproteinized bone xenografts and recombinant human osteogenic protein-1. A histometric study in miniature pigs. *Clinical Oral Implants Research* **10**, 510–521.

Valentini, P. & Abensur, D. (1997) Maxillary sinus floor elevation for implant placement with demineralized freeze-dried bone and bovine bone (Bio-Oss): a clinical study of 20 patients. *International Journal of Periodontics and Restorative Dentistry* **17**, 233–241.

Valentini, P., Abensur, D., Densari, D., Graziani, J. N. & Hämmerle, C. H. F. (1998) Histological evaluation of Bio-Oss® in a 2-stage sinus floor elevation and implantation procedure. *Clinical Oral Implants Research* **9**, 59–64.

Valentini, P., Abensur, D., Wenz, B., Peetz, M. & Schenk, R. (2000) Sinus grafting with porous bone mineral (Bio-Oss) for implant placement: a 5-year study on 15 patients. *International Journal of Periodontics Restorative Dentistry* **20**, 245–53.

Wetzel, A. C., Stich, A. & Caffesse, R. G. (1995) Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. *Clinical Oral Implants Research* **6**, 155–163.

Wikesjo, U. M., Lim, W. H., Thomson, R. C. & Hardwick, W. R. (2003) Periodontal repair in dogs: gingival tissue occlusion, a critical requirement for GTR? *Journal of Clinical Periodontology* **30**, 655–64.

Wiltfang, J., Merten, H. A., Schlegel, K. A., Schultze-Mosgau, S., Kloss, F. R., Rupprecht, S. & Kessler, P. (2002) Degradation characteristics of alpha and beta tricalcium-phosphate (TCP) in minipigs. *Journal of Biomedical Materials Research* **63**, 115–121.

Xu, H., Shimizu, Y., Asai, S. & Ooya, K. (2004) Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation. *Clinical Oral Implants Research* **15**, 126–133.

Yamada, S., Heymann, D., Bouler, J. M. & Daculsi, G. (1997) Osteoclastic resorption of calcium phosphate ceramics with different hydroxyapatite/beta-tricalcium phosphate ratios. *Biomaterials* **18**, 1037–1041.

Yildirim, M., Spiekermann, H., Biesterfeld, S. & Edelhoff, D. (2000) Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood: a histologic and histomorphometric study in humans. *Clinical Oral Implants Research* **11**, 214–229.

Address:
Dr Zvi Artzi
Department of Periodontology
School of Dental Medicine
Tel Aviv University
Ramat Aviv
Tel Aviv 61390
Israel
E-mail: zviartzi@post.tau.ac.il