Sinus Floor Elevation Using the Lateral Approach and Window Repositioning and a Xenogeneic Bone Substitute as a Grafting Material: A Histologic, Histomorphometric, and Radiographic Analysis

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Purpose: Sinus floor elevation using the lateral approach and bone window repositioning and a xenogeneic bone substitute (Cerabone) has been well documented clinically. The purpose of this histologic and histomorphometric study was to determine the fate of the window, its contributing role in the healing process, and the osseoconductivity and resorption potential of the high-temperature sintered bovine bone used, as well as to correlate the histomorphometric results with sinus depth and lateral wall thickness as determined on cone beam computed tomography (CBCT).

Materials and Methods: Thirty biopsy specimens were harvested from the lateral side of the maxilla of patients operated on for sinus floor elevation and implant placement at two postoperative periods: early, group 1 (mean: 5.73 ± 0.44 months); and late, group 2 (mean: 8.68 ± 1.76 months). Sinus depth and lateral wall thickness were determined on CBCT and correlated to graft maturation. Results: The repositioned bone window was microscopically detectable in both study groups and looked well integrated. Bone was found growing out of the repositioned window toward the center of the graft, most often forming a trabecular network independently from the bone matrix, which is in favor of osteogenic potential of the window. Also, newly built bone was found directly attached to the surfaces of the window, indicating bone growth via osseoconducton. Repositioned window sides showed signs of low-grade inflammation. Active osteoclasts were only found to be associated with the newly built bone matrix, hinting at an active bone remodeling process. No signs of biodegradation or remodeling of the window were detected using the tartrate-resistant acid phosphatase (TRAP) technique. The histomorphometric analysis of the tissue distribution showed similar values of newly formed bone in group 1 (22.77% ± 5.89%) and in group 2 (26.15% ± 11.18%) and connective tissue values in both study groups (42.29% ± 8.98% for group 1 vs 46.03% ± 5.84% for group 2). No significant differences were found between group 1 (34.94% ± 7.10%) and group 2 (27.82% ± 11.97%) for xenogeneic bone substitute values. Statistically significant differences were only found between connective tissue values and newly built bone values (P < .01 and P < .001, respectively). Furthermore, a significant difference was found between connective tissue values and that of bone substitute up to 12 months (P < .01). No significant correlation was found between sinus depth and lateral window thickness and histomorphometric results. Conclusion: The repositioned window technique appears to be a good osteoconductive barrier for bone formation. Its osteogenic potential needs to be confirmed immunochemically. High-temperature sintered bovine bone proved to be an effective slowly resorbing osseoconductive material. Int J Oral Maxillofac Implants 2018;33:1089–1096. doi: 10.11607/jomi.6226

Keywords: bone window repositioning, Cerabone, histomorphometry, sinus floor elevation, xenogeneic bone substitute

Sinus floor elevation is a safe, effective, and highly predictable procedure for the management of the deficient posterior maxilla.1,2 Its purpose is to create bone of sufficient quality and quantity to allow implant placement and resist long-term functional loading. Many factors, some established and others controversial, intervene in the healing process and influence the outcome, mainly in terms of regenerated bone quality and healing time: sinus volume, height and depth,3 quality of the residual subcrestal bone,4–6 coverage of the lateral osteotomy site,7–9 grafting material,10 and technical performance of the surgery.11 Reduction of healing time and improvement of the regenerated bone quality remain the main focuses of current endeavors.

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Sinus floor elevation using the lateral approach and window repositioning is a well-documented technique. It was described by Lundgren et al in 2004 to create a closed compartment in a nongrafted sinus elevation and documented in animal studies, and it proved to be an effective and successful surgical procedure clinically. The repositioned window seems to be a good alternative to membrane placement over the osteotomy site with minimal clinical complications. Several grafting materials, autograft, allograft, alloplastic, or xenografts, were extensively used over the past decades in sinus grafting and were well documented clinically and histomorphometrically. Various physical and chemical treatments to purify xenografts from immunogenic components may influence the graft healing potential. Residual organic remnants may induce immune reactions and negatively influence the healing events. The xenograft used in the present study (Cerabone) is a cancellous bovine bone that has been heat treated at >1,250°C to eliminate all organic components. It has been shown that high-temperature treatment may have an effect on the ultrastructure of the raw material and possibly on its regenerative potential.

Histologic studies to determine the fate of the repositioned window and its role in the healing process are scant. In an immunochemical study in rabbits comparing new bone formation in sinuses grafted with anorganic bovine bone (ABB) and covered with a collagen membrane to nongrafted sites with bone window repositioning, Sohn et al found that the repositioned window may accelerate new bone formation.

The purpose of this histologic and histomorphometric study was to determine the fate of the repositioned window and its role in the healing process, namely, serving as a source of osteogenic cells, to correlate graft maturation with lateral wall thickness and sinus depth, and to determine the effectiveness of the xenograft used (Cerabone) as an osteoconductive material.

MATERIALS AND METHODS

Surgical Technique
A total of 109 sinus elevations using the lateral approach and window repositioning were performed in 102 consecutively treated patients with severe bone loss in the posterior maxilla. The surgical technique and clinical outcomes were already reported by Tawil et al in a separate paper. Briefly, the surgical technique consisted of exposing the lateral wall of the maxilla through a full-thickness mucoperiosteal flap. An approximately 12 × 7-mm bone window was cut and lifted outward. The sinus mucosa was then elevated medially and superiorly to allow for bone grafting. The sinus was grafted with xenogeneic bone (0.5- to 1-mm grain size, Cerabone, Botiss biomaterials) soaked in the patient’s venous blood. Implants were placed where residual ridge bone quality allowed good primary stability after insertion. The graft was then packed around and over the implants and the window repositioned on the osteotomy site. In cases where residual bone quality did not allow for implant placement, the site was closed, and 6 months were awaited for graft maturation. The implants were then placed. They were uncovered after 5 to 6 months. In total, 30 biopsy specimens were harvested from 30 randomly selected patients who gave their consent for this histologic and histomorphometric study. Biopsy specimens were analyzed at two postoperative periods: at 20 to 24 weeks (group 1, n = 15) and 32 to 60 weeks (group 2, n = 15). A 6- to 8-mm-deep bone core was harvested from the lateral side of the maxilla where the window was repositioned, at approximately 10-mm distance from the crestal bone using a trephine bur with a 2-mm inner diameter. The postoperative time was dictated by the patient availability for treatment within a specific time frame or according to the surgical procedure applied: immediate or delayed approach. The number of postoperative healing days was calculated for each biopsy specimen, and two healing periods were therefore determined, allowing the comparison of early (5.73 ± 0.44 months) to late (8.68 ± 1.76 months) graft maturation. Six of the 30 biopsy specimens were harvested from sites with a tear in the sinus mucosa during elevation. Also, 5 of the 30 analyzed sites came from patients with delayed implant placement.

Preoperative cone beam computed tomography (CBCT) was done on all patients, and sinus depth was measured buccopalatally at a 10-mm distance from the ridge crest. Also, the thickness of the lateral maxillary wall was determined on CBCT. In all cases, histomorphometric results were matched with sinus depth and wall thickness to evaluate the influence of these parameters on graft maturation.

Histologic Preparation
Directly after harvesting, biopsy specimens were fixed in 4% formalin for 24 hours and transferred afterward to phosphate-buffered saline (PBS) until their further histologic workup. They were dehydrated in a series of increasing alcohol concentrations and xylol and embedded in methyl methacrylate (Technovit 9100, Heraeus Kulzer). Sections with a thickness of 3 to 5 µm were done with a rotation microtome (Leica). Finally, the slides were stained by hematoxylin-eosin (h&e), toluoidine blue, and histochemically for the detection of tartrate-resistant acid phosphatase (TRAP).
Histologic analysis was done with special attention to the fate of the repositioned bone window, its integration in the adjacent native bone, and its contribution to the healing process. Additionally, the interactions between xenogeneic bone substitute and surrounding tissues were analyzed with regard to bone substitute osseoconductive potential and tissue inflammatory response. Two independent investigators (F.W. and M.B.) conducted the evaluation by means of a light microscope (Leica DMRB, Leica Microsystems) using the following parameters: integration pattern of the biomaterial granules, fibrosis, hemorrhage, necrosis, vascularization, presence of neutrophils, lymphocytes, plasma cells, macrophages, multinucleated giant cells (MNGCs), and TRAP+ osteoclast-like cells. Differences between the two study groups were noted. Images were recorded by means of a digital camera (AxioCam 105 color, Carl Zeiss) and a computer system running Zen software (version 2.3, blue edition, Carl Zeiss).

**Histomorphometric Analysis**

The histologic slides stained with toluidine blue and TRAP were initially digitized using a light microscope (Axioskope 40, Carl Zeiss) connected with a scanning table (EK 14 mot, Märzhäuser), a digital camera (AxioCam MRC 5, Carl Zeiss), and a computer running Zeiss AxioCam software (AxioVs40, version 4.8.2.0, Carl Zeiss) at 5× magnification.

The area tool of the NIS Elements software (Basic Research, version 4.51, Nikon) was used to measure the total implant area and the respective fractions of the newly built bone tissue, the remaining bone substitute, and connective tissue within the total scans. Different fraction values were related to the total implantation area. Histomorphometric analysis of the numbers of TRAP+ and TRAP− MNGCs was performed. The total scans, ie, digitized TRAP slides, were used to count the number of MNGCs and their subforms. The MNGCs were counted manually using the counting tool of the NIS Elements software, and the number of each cell fraction was then calculated by relating to the total implantation area of the respective slide (cell number/mm²).

**Statistics**

The quantitative data were statistically analyzed by analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc assessment to compare groups using the GraphPad Prism 7.02 software (GraphPad Software). Differences were considered significant when P values were less than .05 (*P < .05), and highly significant when P values were less than .01 (**P < .01) or less than .001 (**P < .001). Finally, the data were presented as the mean ± standard deviation. Pearson correlation coefficient was used to assess the relationship between continuous variables in the radiographic data to graft maturation analysis. The level of significance was set at P < .05.

**RESULTS**

**Histologic Observations**

Twenty-seven of the 30 harvested biopsy specimens were deemed suitable for histologic analysis. Three biopsy specimens were discarded because of lack of measurable material related to difficulties in retrieving the biopsy specimen in one piece over its full length.

Similar tissue reactions and equivalent integration of both the repositioned window and the xenogeneic bone substitute were observed in both study groups 1 and 2. Histologic images from the study groups including cases up to 12 months are shown in Figs 1 and 2.

Histologic analysis showed that the repositioned bone window was microscopically detectable in both study groups (Figs 1a and 2a). It looked well integrated and seemed to have survived the grafting procedure. Bone was also found growing out of the repositioned window toward the center of the graft (Fig 1a), most often forming a trabecular network independently from the bone matrix, which is in favor of osteogenic potential of the window.

Newly built bone tissue was found directly attached to the surfaces of the repositioned window, indicating bone growth via osseoconduction (Fig 1b). Further analysis showed in more detail that the repositioned window became integrated with the newly built bone matrix (Fig 1b). In both study groups, uncalcified bone matrix was found, indicating that the process of bone maturation was still in progress (Fig 1b). The repositioned window sides showed signs of low-grade inflammation, ie, increased vascularization and inflammatory cells such as macrophages and lymphocytes beside moderate numbers of fibroblasts (Fig 1b). No evidence of soft tissue invasion could be observed on the repositioned window side in any of the examined biopsy specimens. Active osteoclasts were only found associated with the newly built bone matrix, hinting at an active process of bone remodeling (Fig 1c). No signs of biodegradation or remodeling of the window were detected, as no MNGCs or TRAP+ osteoclasts were associated with this matrix (Fig 1c).

Histologic analysis additionally revealed that the xenogeneic bone substitute (Cerabone) used for sinus augmentation was detectable in both study groups (Fig 2a). Newly built bone was found covering most of the surface areas of the material granules, indicating its good osseoconductive potential (Fig 2b). At the surface areas covered by connective tissue, mononucleated cells of the macrophage lineage were mainly observed beside moderate numbers of MNGCs (Fig 2c).
Both the mononucleated and the multinucleated cells at the material-tissue interfaces expressed TRAP molecules, which may be an indicator of an ongoing degradation process of the bone substitute (Fig 2d). No difference in healing could be observed between sites with an initial sinus mucosa tear and sites with no tear.

**Histomorphometric Results**

The histomorphometric analysis of the tissue distribution showed similar values of newly formed bone in group 1 (34.94% ± 7.10%) and in group 2 (27.82% ± 11.97%) (Fig 3a). Similar values of connective tissue were found in both study groups (42.29% ± 8.98% for group 1 vs 46.03% ± 5.84% for group 2). Statistically significant differences were only found between connective tissue values compared with newly built bone values (**P < .01 and ***P < .001), respectively (Fig 3a). Furthermore, a significant difference was found between connective tissue values and that of the bone substitute up to 12 months (**P < .01).

A high number of TRAP-positive and TRAP-negative MNGCs/mm² were found at both time periods.
TRAP-positive MNGCs were 8.55 ± 5.72 in group 1 and 4.34 ± 1.69 in group 2. TRAP-negative MNGCs were 17.62 ± 5.27 in group 1 and 9.17 ± 5.27 in group 2 (Fig 3b). Furthermore, the analysis detected significant differences between the total numbers of MNGCs (group 1: 25.92 ± 9.89 MNGCs/mm²; group 2: 17.67 ± 8.34 MNGCs/mm²) and TRAP-positive numbers of MNGCs at both study time points (*P < .05) (Fig 3b).

**Sinus Depth and Wall Thickness as Related to Graft Maturation**

The mean lateral wall thickness varied between 1 and 2.3 mm with a mean value of 1.419 mm (SD = 0.420). No significant correlation was found between wall thickness and the percentage of new bone formation (P = .733). Likewise, sinus depth varied in the current series between 11.5 and 18.2 mm with a mean value of 14.981 (SD = 1.459), with 75% of the values ranging between 14 and 16 mm. No significant correlation was found between sinus depth and percentage of new bone formation (P = .528).

**DISCUSSION**

The histologic results of the present study seem to indicate an interesting contribution of the repositioned bone window to new bone formation. Windows looked well incorporated in the adjacent native bone with no significant signs of remodeling and a bone matrix deposition in favor of an osseoconductive process (Fig 1b). Greater bone density close to the repositioned window and continuity of the window with the newly formed bone within the graft were observed. Bone was seen growing out from the window side, indicating a potential osteogenic role in the healing process.

In biopsy specimens harvested vertically from intended implant sites, Cordaro et al observed a greater percentage of newly formed bone closer to the residual crest compared with that in the remaining part of the biopsy specimen and attributed it to cellular and vascular ingrowth from the adjacent residual maxillary bone. Conversely, in biopsy specimens harvested from the lateral maxilla in the case of a membrane-protected osteotomy site following window removal, bone gradient formation was observed inward with greater bone formation in the inner part of the graft (53.2%) compared with the zone closer to the previous lateral border of the maxilla (35.9%), although such a gradient could not be identified in other studies. In a classical description of bone formation following external sinus elevation, bone was shown to grow centripetally mainly from the floor and the lateral borders of the maxilla toward the center of the graft, with the last part to mineralize being the external cortical plate. In the present study, it is interesting to note that a greater density of bone was observed close to the repositioned lateral window with new bone forming in continuity with it, which may indicate a potential enhancing role of the window in the regenerative process through the release of osteopromoting biomolecules like in autogenous bone grafting, or the creation of a secluded space like in guided bone regeneration (GBR). However, inductive factors originating from the window need to be confirmed in an appropriate experimental model. So far, only one rabbit study of maxillary sinus augmentation with and without bone grafting using the repositioned window technique on.
the nongrafted side evaluated the augmented sinuses by immunohistochemical analysis of proliferating cell nuclear antigen (PCNA), collagen type I, and osteocalcin content. Newly formed bone was shown expanding from the repositioned window toward the center of the sinus cavity, with the window acting as a starting point to induce new bone formation. Bone increased in density in the sinus cavity as healing progressed, and positive expression of osteocalcin and PCNA-positive cells along the side of the repositioned window were observed, which is in favor of the osteogenic potential of the window. In the present study, newly formed bone was a mean 22.77% ± 5.89% in group 1 and 26.15% ± 11.18% in group 2, with no statistical difference between the two groups, which closely compares with values obtained from studies using ABB as a sole grafting material, where percentage of new bone formation ranged between 12.4% and 34.2%.34–37 The values obtained seem to indicate a modest enhancement of the repositioned window technique to the overall new bone formation. However, it is interesting to note that the ratio of mineralized tissues to bone marrow in the present study compares favorably to values found in native maxillary bone, where morphometric analyses showed a mean 51.2% ± 8.1% bone marrow, while mineralized tissues represented a mean 45.7% ± 7.9% of the total bone mass.38 However, it is important to note that these values may vary with age, sex, and biopsy site.

Histomorphometric analysis was performed on biopsy specimens harvested from the lateral wall of the maxilla at approximately 10 mm from the alveolar ridge. Sinus depth was evaluated on CBCT at 10 mm from the ridge crest and was a mean 14.98 mm, with 75% of the values ranging between 14 and 16 mm. The homogeneity of the values explains the absence of correlation between depth and percentage of new bone formation. The difference in the outcome with the Avila et al study3 is related to differences in the protocol, where biopsy specimens were harvested vertically and the measurements correlated with sinus depth at 8, 10, and 12 mm.

New bone formation seems to depend on material particle size and significantly increases with large particles (26.7%) compared with small particles (18.8%).39 It may also depend on the surgical technique, whether or not a membrane was used to cover the lateral osteotomy site. Tawil et al7 observed a significant increase in vital bone formation in membrane-protected sites (26.6%) compared with nonprotected sites (11.9%), although these results were not confirmed in a recent comparative clinical trial by Barone et al,9 where no significant differences in vital bone could be detected between membrane-protected sites (30.7% ± 15.5%) and nonmembrane sites (28.1% ± 19.4%), although a positive effect of membrane placement on graft reabsorption rate and reduction of connective tissue formation was observed.

New bone formation may vary with sinus depth: the deeper the sinus, the lower the percentage of new bone. It may also vary with the volume of the grafted area,40 the biopsy technique, its location and depth,4 and the dimension of the lateral window:11 the larger the window, the lower the percentage of new bone and the higher the percentage of residual graft. It is interesting to note that the size of the window would not be a critical issue anymore in the case of the repositioned window technique.

New bone formation may vary with postoperative healing time. In biopsy specimens harvested at 4 to 5 months, 11.8% ± 9.2% of new bone was found compared with 21.4% ± 8.6% at 7 to 9 months, with no significant differences in nonmineralized tissues and percentage of residual graft.34 In the present study, no significant difference was found between the early 5– to 6-month group and the 8- to 12-month group, which may indicate a potential favorable effect of the repositioned window on graft maturation. However, this may need to be confirmed with a larger biopsy sample.

It has often been claimed that one of the requested properties of bone substitute biomaterials is their ability to resorb and be replaced by new bone at a speed that allows proper and timely bone regeneration. Xenogenic material resorbs very slowly and can still be identified several years after grafting.41–43 Yet, it is recognized as very effective in sinus grafting, maintaining graft volume with excellent clinical outcomes and high implant survival rates.1,44,45 Several bone substitutes with different microarchitecture and macroarchitecture, pore size, interconnecting channels, resorption rate, collagen content, corticocancellous composition, deproteinization, and sintering temperature and compressive strength were developed and evaluated clinically and histomorphometrically: porous phyrogenic hydroxyapatite,56,47 microporous biphasic calcium phosphate,48 calcium carbonate coral derived material,49 corticocancellous mixture of porcine origin,50 and ABB.8,51–53 They all have proper microporosities and macroporosities that allow vascular invasion, bone apposition, and ingrowth of new bone,54 and mechanical characteristics close to the tissue to be regenerated.55,56 They are biologically stable and maintain with minor differences graft volume in time with no adverse reaction. Their resorption rate varies based on their composition. ABB resorption depends on several factors, namely, its cortical or cancellous nature, its particle size, and chemical deproteinization and sintering. It seems to increase as crystallinity decreases. A high sintering temperature tends to lower biomaterial degradation because of the increase in crystallinity.57
Cerabone is subjected to temperatures up to 1,250 degrees for total deproteinization and sintering. In the present study, resorption of the biomaterial proved to be ongoing during the early and later phases of healing. The high temperature for deproteinization did not seem to affect its osteoconductive or resorption potential. However, longer-term studies are still needed to determine its exact resorption rate.

Cells involved in the resorption process are MNGCs that can be identified using the TRAP technique. ABB has been found in close contact with giant cells 3 years postgrafting without existing signs of resorption.58 Osteoclastic cells were thought to be unlikely to be related to deproteinized bovine bone resorption. Araújo et al59 observed that multinucleated cells disappear from the healing scene after 3 months of healing as they undergo apoptosis. Yet, Tadjoein et al60 observed TRAP+ multinucleated cells in contact with ABB, suggesting gradual resorption by osteoclastic activity. Likewise, Galindo-Moreno et al61 described TRAP+ multinucleated cells on ABB particles, and within them, promotion of central resorption and the presence of different multinucleated cells and TRAP+ osteoclasts after 6 months of healing, and assumed that different stages of differentiation of these resorptive cells may coexist in relation to specific cellular events. TRAP+ cells may express their resorptive capacity exclusively on graft particles and not on vital bone, indicating selective resorption of xenogenic material. In the present study, TRAP+ osteoclasts were seen in contact with Cerabone in Howship lacunae, indicating a natural resorptive process at both evaluation periods.

Also, mononucleated cells of the macrophage lineage were mainly observed in connective tissue areas beside moderate numbers of MNGCs. They both expressed the lytic enzyme TRAP at the material-tissue interfaces, which may be an indicator of an ongoing remodeling process. Although lower values of the remaining bone substitute at the later study time point together with the significantly higher values of connective tissue may indicate the slow biodegradation of the xenogenic bone substitute in the present study, the decreasing number of MNGCs, especially of its TRAP-positive subforms, rather point to a reduction of the cell-mediated resorptive process. Bone substitute as a “constant tissue fraction” should be “added” to the fraction of bone tissue and must be seen as a “bone-like long-term implant”.

CONCLUSIONS

The histologic and histomorphometric results clearly indicate osseoconductive and osteogenic potential of the repositioned window with an interesting, yet limited, contribution to the overall graft maturation. High-temperature sintered bovine bone proved to be an effective slowly resorbing osseoconductive material. However, its precise resorption rate needs to be confirmed in long-term studies.

ACKNOWLEDGMENTS

The authors would like to thank Mrs Zheni Zaka and Mrs Claudia Beutler for their excellent technical assistance. Furthermore, the authors would like to thank Mrs Britta Bartelt for postprocessing of the images and figures and Botiss Biomaterial for supporting the histologic study. The authors declare no conflict of interest with Botiss Biomaterial. The authors reported no conflicts of interest related to this study.

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