The vertical course of bone regeneration in maxillary sinus floor augmentations: A histomorphometric analysis of human biopsies

Florian Beck\textsuperscript{1,*} | Karoline Maria Reich\textsuperscript{2,3,*} | Stefan Lettner\textsuperscript{2,3} | Patrick Heimel\textsuperscript{2,3} | Stefan Tangl\textsuperscript{2,3} | Heinz Redl\textsuperscript{3,4} | Christian Ulm\textsuperscript{1}

\textsuperscript{1} Division of Oral Surgery, University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria
\textsuperscript{2} Karl Donath Laboratory for Hard Tissue and Biomaterial Research, Division of Oral Surgery, University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria
\textsuperscript{3} Austrian Cluster for Tissue Regeneration, Vienna, Austria
\textsuperscript{4} Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Vienna, Austria

Correspondence
Dr. Florian Beck, Division of Oral Surgery, University Clinic of Dentistry, Medical University of Vienna, Sensengasse 2a, 1090 Vienna, Austria.
Email: florian.beck@meduniwien.ac.at

\textsuperscript{*} Florian Beck and Karoline Maria Reich contributed equally to the authorship of this work and are considered joint first authors.

Abstract

\textbf{Background:} Maxillary sinus floor augmentation (MSFA) is a well-established and predictable augmentation method in severely resorbed maxillae. However, data on the vertical course of bone graft consolidation within the maxillary sinus are rare. The aim of the present study was to quantify the vertical distribution of new bone formation (nBF) in MSFA and to characterize the vertical gradient of bone graft consolidation.

\textbf{Methods:} Eighty-five human sinus biopsies were harvested 6 ± 1 months after MSFA. Histological thin-ground sections were prepared and histomorphometrically analyzed. The volume of newly formed bone (nBV/TV) was measured in serial zones of 100 \( \mu \)m proceeding from the bottom of the sinus floor (SF) up to the apical top of the biopsy. The gradient of nBV/TV within the augmentation area was determined by the vertical distribution of nBV/TV along these zones.

\textbf{Results:} In the premolar region, nBV/TV slightly declined from 20.4\% in the zone adjacent to the SF to 17.7\% at a distance of 8 mm. The gradient was steeper in the molar region: nBV/TV decreased from 18.7\% to 12.8\%. This decline was even more distinct when the volume fraction and the height of the residual bone of the SF were low.

\textbf{Conclusions:} nBF follows a gradient from native bone of the SF towards the apical part of the augmentation area. The distance to primordial bone thus plays a critical role for bone regeneration in MSFA, particularly in the molar region.

\textbf{KEYWORDS}
bone regeneration, bone substitutes, histological analysis, maxillary sinus floor elevation

\section{1 \ INTRODUCTION}

The maxillary sinus floor augmentation (MSFA) is an effective and predictable therapy to increase bone supply in the edentulous maxillary region.\textsuperscript{1,2} Besides a successful surgical procedure, the decisive factor for augmentation success is the consolidation of the grafted material into bone.
Table 1  Number of biopsies with deproteinized bovine bone mineral and adjuncts

| Bone graft materials | n | %  |
|----------------------|---|----|
| DBBM                 | 7 | 8.2|
| DBBM + aB            | 44| 51.8|
| DBBM + aB + PC       | 14| 16.4|
| DBBM + aB + PC       | 5 | 5.9|
| Total                | 85| 100|

aB, autologous bone; aBcells, autologous bone cells; DBBM, deproteinized bovine bone mineral; MSC, mesenchymal stem cells; PC, platelet concentrates.

Controversy exists in the literature regarding the tissue from which bone regeneration originates in MSFA. Both, the residual bone of the sinus floor (SF) and the Schneiderian membrane (SM) are discussed in this context:3‒6 Palma et al.7 performed MSFA in capuchin primates and found de novo bone formation in contact with SM, indicating an osteogenic potential of SM. This observation is in accordance with Srouji et al.8 and Rong et al.9 who studied the role of the SM in an ectopic tissue transplant model in nude mice and in a sinus lifting model in canines. Both reported that the SM appears to have osteogenic and osteoinductive properties and thus might contribute to bone formation in MSFA.

Jungner et al.4 by contrast could not detect any bone formation originating from the SM in capuchin primates. In fact, the authors found new bone formation (nBF) sprouting from the bottom of the SF, extending into the elevated area surrounding the implant. This is in line with Busenlechner et al.10 and Fuerst et al.11 who reported that in mini-pigs, graft consolidation after MSFA largely depends on the osteoinductive potential of the surrounding bone. A graft consolidation gradient was identified showing more new bone in zones adjacent to the SF and less new bone in the more distant zones.

At present, clinical recommendations for MSFA mainly address the choice of surgical approach, single- versus two-stage procedure based on minimum requirements for the width and the height of the residual alveolar ridge.12,13 However, recommendations on the lifting height of the SM and subsequently the augmentation height itself are rare and usually only consider the possible installation of an implant length of 12 to 13 mm.13‒16 The question whether a larger augmentation height actually results in a larger graft consolidation height or whether excessive bone grafting might even be detrimental to successful graft consolidation remains open.

Based on this background, the aim of the present study was to quantify the vertical distribution of nBF in human sinus biopsies 6 months after MSFA by means of modern histomorphometry. We hypothesized that nBF in the augmented area follows a gradient from the native bone of the SF towards the apical part of the augmented area and the elevated SM. Unlike previous studies10,11,17,18 that investigated nBF in a few pre-defined discrete zones, we herewith introduced a new method that allows describing the vertical distribution and extension of new bone continuously over the whole length of the biopsy.

2 MATERIALS AND METHODS

Seven Medical Universities (Departments of Oral Surgery, Department of Prosthodontics and Departments of Oral and Maxillofacial Surgery) from Austria (n = 2) and Germany (n = 5) provided biopsies of MSFA for a multicenter study of Reich et al.19 The present study investigates the histologic material under a new scientific perspective which has not been studied to date. This study was approved by the human subjects ethics board of Austria and Germany [Austria: 102/2004, 22/2007, 18-053ex06/07; Germany: 837.274.04(4432)] and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

2.1 Eligibility criteria for recruitment

Patients destined for a two-stage approach of MSFA with a residual alveolar bone height (oldB.Ht) of the posterior maxilla of <5 mm, requiring at least one dental implant (premolar or molar region) and aged >18 years were enrolled. Exclusion criteria were recent tooth extraction at the site of implant installation (within the last 3 to 18 months), periodontal disease, pathological conditions of the maxillary sinus, metabolic or degenerative diseases of the bone (e.g., osteoporosis, diabetes mellitus, hyperparathyroidism), long-term medication with corticosteroids or NSAIDs, smoking (>5 cigarettes/day), and alcoholism. All patients provided written informed consent.

2.2 MSFA and sample processing

MSFA procedures were performed following a lateral approach20,21 under local or general anaesthesia. After window preparation and careful elevation of SM, the grafting material was placed into the created void space. Patients with obvious signs of inflammation, large SM perforations or other complications were excluded from this study.

After a healing period of 6 ± 1 months, sinus biopsies from the premolar and/or the molar region were
Histomorphometric measurement of the vertical distribution of nBV/TV over the length of the augmentation area (illustrated by a histological thin-ground section and a SEM image and their corresponding color-coded classification images). A) stained histological thin-ground section, B) color-coded classification image of A; C) SEM image, D) color-coded classification image of C; blue: bone of the sinus floor, red: newly formed bone, yellow: bone substitute particles, white: marrow area/soft tissue; green: border between the SF and the augmentation area). In parallel to the green line, contour lines were set at an interval of 100 μm to measure nBV/TV within each of the resulting serial zones. The gradient over the full length of the augmentation height was calculated for all biopsies harvested using a trephine burr before implant placement. Implants were inserted along the long axis of the drill hole.

2.3 Eligibility of MSFA biopsy samples

Biopsies originating from previous clinical studies have already been used for a multicenter study published by Reich et al. analyzing the impact of the maxillary region and patients’ age and sex on bone regeneration after MSFA.

This current study re-analyzed parts of the original samples based on the following criteria:

Biopsies were included if:

1. both the native bone of the SF and the augmented area were present
2. deproteinized bovine bone mineral (DBBM; BioOss, Geistlich Pharma, Wolhusen, Switzerland) was used as bone substitute alone or in combination with adjuncts: autologous bone (aB) harvested intraorally, culture-expanded aB cells (aB cells) isolated from the anterior iliac crest, aB with platelet concentrate, and aB with mesenchymal stem cells aspirated from the tibia (Table 1, see Supplementary Material S1 in online Journal of Periodontology).

2.4 Histology

Biopsy specimens (n = 85) were fixed in phosphate-buffered formalin, dehydrated in ascending grades of alcohol, and embedded in a light-curing resin (Technovit 7200 VLC + BPO; Kulzer, Wehrheim, Germany). Undecalculated thin-ground sections were produced (EXAKT Apparatebau, Norderstedt, Germany) along the long axis of the biopsies as described by Donath. Forty-three (50.6%) of the sections were stained using Levai–Laczko dye and digitized with a camera mounted on a microscope (Nikon DXM 1200/Microphot-FXA, Tokyo, Japan). Multiple single images per specimen were merged to obtain high resolu-
tion overview images (2.212 μm per pixel) (Lucia G 4.71, LIM., Praha, Czech Republic).

SEM images were generated of the remaining 42 sections (49.4%) using back-scattered electrons at 15/20 kV (JSM-6310, Jeol, Tokyo, Japan) with a resolution of 2.695 μm per pixel. The comparability of the results the SEM and the histological image sources, was previously checked using an intraclass correlation coefficient within 10 biopsy specimens (0.92 or >0.92)\textsuperscript{19}.

2.5  Histomorphometric analysis

Digital images were semi-automatically segmented and classified into different tissue types using Definiens Developer XD (Definiens, Munich, Germany): pre-existing bone of the SF as well as newly formed bone, bone substitute material, and soft tissue/marrow area within the augmentation area. If areas were inaccurately classified, correction was performed manually under visual control.

2.5.1  Gradient of new bone volume fraction (nBV/TV) in serial zones

The “old” bone of the SF was separated from the actual augmentation area with a manually drawn line (Adobe Photoshop, Adobe, San Jose, CA). In parallel to this borderline, contour lines at an interval of 100 μm were set over the whole length of the augmentation area. nBV/TV was measured within each of these created serial 100-μm zones, beginning from the zone adjacent to the SF up to the apical top of the augmentation area (Fig. 1). The gradient was calculated based on these values.

2.6  Statistical analysis

2.6.1  Generalized mixed model predicting the vertical distribution of nBV/TV

Bone volume per tissue volume was modeled as a generalized mixed model with Gaussian error term and log link,\textsuperscript{27} adding biopsy ID, patient ID, augmentation material, and centre ID as nested random effects. To correct for potential confounding of the effect of various biomaterials, the augmentation materials were incorporated into the mixed effects multiple regression model as a single fixed factor as described by Katz.\textsuperscript{28} This allows to take into account possible influences of the confounding factors on the correlation of the interesting variables.\textsuperscript{29} The distance of the respective zone to the SF was included as a main covariate of interest. Region (premolar/molar), sex, bone volume fraction and bone height of the pre-existing bone of the SF, referred to as oldBV/TV and oldB.Ht, were included as potential confounders. Further, interaction terms with the distance of the respective zone to the SF were considered to allow for different slopes for all the above.

This model predicts the volume of newly formed bone for a given distance to the SF as illustrated by a marginal model plot (Figs. 2 through 4). The prediction line in this plot represents a fictional “median patient” that is generated from the data of the sample. To determine if the region has an influence on the vertical distribution of nBV/TV over the length of the biopsy, premolar, and molar biopsies were analyzed separately.

Normality and homoscedasticity was checked graphically using residual plots.

2.6.2  Influence of oldB.Ht and oldBV/TV of the pre-existing residual bone of the sinus floor on the prediction of nBV/TV

To analyze to what extent the status of the residual bone of the SF had an influence on the prediction of the course of nBV/TV, we show the results of the above model for the three quartiles of the parameters oldB.Ht and oldBV/TV. This allows a model prediction for a fictional patient with “low oldBV/TV” (Q1, 26.6%), “medium oldBV/TV” (Q2, 38.4%), and “high oldBV/TV” (Q3, 48.8%) and “low oldB.Ht” (Q1, 0.7 mm), “medium oldB.Ht” (Q2, 1.2 mm), and “high oldB.Ht” (Q3, 1.6 mm), respectively. Tests on the significance of changes in these predictions were calculated. Regression lines including a 95% confidence interval are shown in a marginal model plot for typical (mode, median, or quantile) values of the confounders. All computations were done using R (version 3.5.1),\textsuperscript{30} and graphics were created using ggplot2.\textsuperscript{31}

3  RESULTS

3.1  Sample characteristics

The final study sample consisted of 85 biopsies augmented with DBBM alone or in combination with adjuncts (Table 1), providing a total of 3,925 measurements. The potential confounding effect of different adjuncts was considered in the generalized mixed model and corrected in the statistical evaluation using multivariable regressions.\textsuperscript{28,29}
3.2 nBV/TV in the premolar and the molar region

The vertical distribution of nBV/TV over the length of the augmentation area is depicted in Figure 2, demonstrating a mild negative gradient of nBV/TV along the augmentation area in the premolar region. The mean nBV/TV slightly declined from 20.4% in the first 100-μm zone adjacent to SF to 17.7% at a distance of 8 mm. In the molar region, the gradient was more pronounced within the same distance: nBV/TV decreased from 18.7% to 12.8%.

The distance of a zone to the SF was highly associated with nBV/TV within the respective zone (P ≤ 0.001).

3.3 Influence of oldBV/TV and oldB.Ht on the prediction of nBV/TV

To analyze the impact of the status of the residual bone of the SF on the prediction of nBV/TV, regression lines were calculated at low (Q1, 26.6%/0.7 mm), median (Q2, 38.4%/1.2 mm), and high (Q3, 48.8%/1.6 mm) values of oldBV/TV (%) and oldB.Ht (mm), respectively (Figs. 3 and 4):

oldBV/TV (median: 42.0%) significantly influenced the prediction of nBV/TV in both regions (P < 0.001). In the premolar region, the regression of nBV/TV for high oldBV/TV showed an exponential decline with increasing distance to the SF which most probably is a distortion due to the low number of long biopsies in this quartile. As indicated by the confidence band (Fig. 3, light blue) variation of new bone values within the respective zones is high, specifically in the premolar region.
FIGURE 3 Different regression lines of the gradient of nBV/TV considering the volume fraction of the pre-existing residual bone of the sinus floor (oldBV/TV). The lines present slightly different predictions of nBV/TV, referring to low (Q1, 26.6%), median (Q2, 38.4%), and high (Q3, 48.8%) values of oldBV/TV (%). PM, premolar region; M, molar region.

FIGURE 4 Different regression lines of the gradient of nBV/TV considering the height of the pre-existing residual alveolar bone (oldB.Ht). The lines present slightly different predictions of nBV/TV, referring to low (Q1, 0.7 mm), median (Q2, 1.2 mm) and high (Q3, 1.6 mm) values of oldB.Ht (mm). PM, premolar region; M, molar region.

FIGURE 5 Distribution of augmentation heights (Histogram overlaid with kernel density estimate). The majority of biopsies had an augmentation height of 3 to 4 mm, followed by 2 to 3 mm and 7 to 8 mm. Biopsies with an augmentation height of >8 mm were less common.

In the molar region, the decline of nBV/TV was significantly more distinct when oldBV/TV was low. While for high oldBV/TV, nBV/TV is limited to be 16.0% at a 8 mm distance, it is only 10.7% for low oldBV/TV.

oldB.Ht (median: 1.4 mm) also had a significant effect on the prediction of nBV/TV ($P < 0.001$). In the premolar region, predictions followed the same trend as observed for oldBV/TV. By contrast, in the molar region, nBV/TV inclined from 15.2% to 19.4% when oldB.Ht was high, which again may be distorted by the low number of biopsies and indicated by the wide confidence band (Fig. 4, light green). The decline of nBV/TV was significantly more distinct when oldB.Ht was low/median, namely decreasing from 19.8% to 11.5% and 18.7% to 12.8%, respectively.

This implies that a low quantity of the pre-existing residual bone of the SF is expected to result in less nBF in the apical parts of the augmentation area in the molar region. Medium and high oldBV/TV and oldB.Ht on the other side alleviate this negative gradient observed for low oldBV/TV and low oldB.Ht.

4 | DISCUSSION

The elevation height of SM is generally determined by the oral surgeon based on the height of the residual bone of the SF, the desired implant length, the choice of bone graft and on preferences and experience. Clinical
recommendations on the elevation height of SM are rare and hardly have scientific basis. Whether a higher MSFA actually results in a larger graft consolidation remains open to date.

Thus, the aim of the present study was to quantify the vertical distribution of nBF in human sinus biopsies 6 months after MSFA. Assuming that the regeneration potential of bone is (predominantly) vested in the residual bone of the SF, we hypothesized that the graft consolidation follows a gradient from native bone of the SF towards the apical part of the augmentation.

In fact, our results demonstrated that nBF declined with increasing distance to the residual bone of the SF which suggests the critical role of primordial bone for bone regeneration in MSFA.

The observed negative gradient is in line with others who reported a decrease of nBF in the more apical parts of the augmented area in animal and clinical studies. Other studies could not detect a gradient, but a rather uniform distribution of new bone.

The reason for these conflicting findings might be attributed to different (animal) models, evaluation methods (qualitative description of histological images, definition of analyzed zones) and healing periods. The latter must be regarded as a very important factor, since nBF and graft consolidation are dynamic processes that proceed and change the bone volume over time.

Interestingly, the predicted decline of nBF/TV was steeper in the molar than in the premolar region after 6 months. While nBF/TV adjacent to the SF was relatively similar in the premolar (20.4%) and the molar region (18.7%), the vertical distribution was very different: nBF/TV only slightly declined to 17.7% at a distance of 8 mm from the SF in the premolar region but dropped to 12.8% in the molar region. This suggests that nBF under-achieves in the molar region even when the augmentation height is ≥ 8 mm. In other words, a large augmentation height in the molar region might not contribute to nBF in the more apical areas 6 months after MSFA.

While some studies found new bone sprouting also from the SM, our results could not provide indications that SM plays a major role in this context. Our model was not designed to measure the influence of the SM since biopsy cores seldom extend exactly to the SM. However, the histological analysis of the biopsies revealed that in the most apical part of many biopsies, graft particles close to the SM were frequently not integrated into new bone but tended to be surrounded by or encapsulated in poorly vascularized, fibrous connective tissue. This might be interpreted as an indirect hint that the osteogenic/osteointuctive role of the SM is at least only minor compared with that of SF.

Fibrous tissue in the apical region of the augmentation area was also described by others using various bone grafts for MSFA: Similarly to our histological observation, these fibrous areas were rich in fibroblasts and poor in blood vessels resembling scar tissue. Both, the sinus mucosa and the trap door/window of the lateral approach in MSFA were considered as a source of soft tissue invasion. Based on the histological observations that fibrous tissue formation predominantly occurs in the apical portion of the augmentation area, it seems reasonable to assume that nBF from the SF and soft tissue ingrowth from the apical side (SM or lateral window) stand in competition with each other. From this point of view, an excessive augmentation height not necessarily results in a larger graft consolidation height but conceivably might be considered as a space holder critical to counteract fibrous tissue invasion from the SM or the lateral bone window.

The second focus of the present study was laid on the role of the status of the SF on the vertical gradient of newly formed bone within the augmentation area. As demonstrated in a previous study, the height and the volume fraction of the SF had a significant impact on the overall volume of newly formed bone within the total augmentation area. The worse the status of the residual alveolar bone, the less total nBF/TV was present in the augmentation area. As shown in this current study, oldB.Ht and oldBV/TV also had a significant impact on the vertical course of nBF/TV over the length of the augmentation: In the molar region, the gradient of nBF/TV was notably steeper when the status of the residual bone was poor: nBF/TV is limited to 10.7% at a distance of 8 mm from the SF when oldBV/TV is low. If, however, the bone volume fraction of the SF is high, nBF amounts to 16%. Unfortunately, long biopsies (augmentation height >8 mm) from the premolar region with high and dense residual bone are relatively rare which might have distorted the predictions for these subgroups (Figs. 3 and 4).

Given that the osteogenic potential in MSFA originates from the residual bone, it is plausible that a better overall state results in an augmented, accelerated healing capacity also in the apical parts of the augmentation. In practical terms, a timely augmentation as long as residual bone is not subjected to atrophy seems particularly reasonable in the molar region. Avila-Ortiz et al. and Price et al. could not demonstrate a relationship between the dimensions of
the residual crest and graft consolidation. However, they used circular histological cross-sections of human biopsies whereas our study used longitudinal sections through the long axis of biopsies, which allowed the continuous measurement of nBF over the whole length of the augmentation. To obtain a more holistic picture of the influencing factors of nBF also the dimensions of the maxillary sinus should be considered. As reported by Klijn et al., the width of the residual alveolar crest is significantly lower in the premolar than in the molar region. The same is true for the width of the maxillary sinus, the distance between the medial/palatal and the lateral/buccal sinus wall is shorter and therefore narrower in the premolar region than in the molar region. Avila et al. determined the impact of the buccal and palatal sinus walls on the amount of nBF 6 months after MSFA. The larger the buccopalatal distance, that is, the wider the sinus (as expected in the molar region), the less new bone was formed. By contrast, Pignaton et al. could neither detect an influence of the sinus width (narrow/average/wide) nor the residual bone height (<2 mm and >2 mm) on the outcome of MSFA after 8 months. The situation in the augmented sinus is comparable with a three-wall defect as in periodontal bony defects or experimental monocortical drillhole defects in the animal calvaria. Unfortunately, we could only assess the influence of one wall, that is, the SF. The impact of the spatial relationship of the sinus walls and nBV/TV remains issue of future studies.

The same applies for the spatial relationship of nBV/TV and SM. Particularly in long biopsies, apical graft particles were often not integrated and tended to be encapsulated by fibrous tissue only. These rather loose graft particles often fragmented when the biopsy cores were harvested. In some cases it could not be entirely avoided that the most apical non-consolidated graft particles became lost during biopsy processing. However, completely fragmented biopsies were not included in the study to avoid bias. The low number of long biopsies is indeed a limitation of this study. In future studies, emphasis should be directed on the cautious extraction of biopsies and on imaging methods (such as computed tomography or MRI) before biopsy extraction. Computed tomography scans would provide valuable information about the exact position and extension of the biopsy, the sinus dimensions, and the vicinity of the biopsy to the sinus walls – another osteogenic source for bone regeneration within the maxillary sinus.

In this context, it needs to be mentioned that the height of the residual bone (“oldB.Ht”) in the present study was calculated by dividing the area of the pre-existing bone region by the diameter (width) of the biopsy. This method was applied to obtain a robust, consistent measurement also of the few “geometric outliers” in which pre-existing bone was slightly angled or oblique due to natural anatomic variations of the SF.

The most significant limitation of this study is the heterogeneity of DBBM used as a biomaterial alone or in combination with different adjuncts. Most of the biopsy samples (59 of 85) were a combination of DBBM and aB/cells. In fact, statistical methods were used to compensate for the influence of DBBM and its combinations. Further studies using a more homogeneous sample are needed to verify the observed gradient.

5 | CONCLUSIONS

The results of the present study demonstrate that nBF declines with increasing distance to the residual bone of the SF. This gradient was more pronounced in the molar than in the premolar region. A poor overall condition of the residual bone of the SF is expected to result in less nBF in the apical parts of the augmentation area, particularly in the molar region. After a healing period of 6 months post-MSFA, graft particles in the apical portion of the augmentation areas tend to be loose and encapsulated by fibrous tissue. This most apical part of the MSFA (>8 mm distance to SF) appears to make only a minor contribution to the treatment outcome since nBF at this distance was relatively low. This might have clinical implications on the planning of the lifting height of SM in MSFA and thus on the choice of the implant length thereby providing the basis for the establishment of MSFA height recommendations in the future.

ACKNOWLEDGMENTS

The authors thank Tina Rybacek, Doris Moser, Georg Watzek, Gabor Fuert, Michael Payer. We thank the Helios-Klinikum Erfurt, the University of Aachen, the Medical University of Mainz/Rhein, the University of Schleswig Holstein and the Charité in Germany for providing histological biopsies. No external funding was obtained for performing this study. The authors report no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

Dr. Beck contributed to concept/design, data collection, and drafting article and approval of the article. Dr. Reich contributed to concept/design, data collection, and drafting and approval of the article. Lettner contributed to concept/design, data collection, and approval of the article. Heimel contributed to concept/design, data collection, and drafting and approval of the article. Dr. Tangl contributed to data collection and drafting and approval of the article.
REFERENCES

1. Esposito M, Grusovin MG, Rees J, et al. Effectiveness of sinus lift procedures for dental implant rehabilitation: a Cochrane systematic review. *Eur J Oral Implantol*. 2010;3(1):7-26.

2. Raghoebar GM, Oncelin P, Boven GC, Vissink A, Meijer HJA. Long-term effectiveness of maxillary sinus floor augmentation: a systematic review and meta-analysis. *J Clin Periodontol*. 2019;46(S21):307-318.

3. Gruber R, Kandler B, Fuerst G, Fischer MB, Watzek G. Porcine sinus mucosa holds cells that respond to bone morphogenetic protein (BMP)-6 and BMP-7 with increased osteogenic differentiation in vitro. *Clin Oral Implants Res*. 2004;15(5):575-580.

4. Jungner M, Cricchio G, Salata LA, et al. On the early mechanisms of bone formation after maxillary sinus membrane elevation: an experimental histological and immunohistochemical study. *Clin Implant Dent Relat Res*. 2015;17(6):1092-1102.

5. Price AM, Nunn M, Oppenheim FG, Van Dyke TE. *De novo* bone formation after the sinus lift procedure. *J Periodontol*. 2011;82(9):1245-1255.

6. Scala A, Botticelli D, Faida RS, Garcia Rangel I, Américo de Oliveira J, Lang NP. Lack of influence of the Schneiderian membrane in forming new bone apical to implants simultaneously installed with sinus floor elevation: an experimental study in monkeys. *Clin Oral Implants Res*. 2012;23(2):175-181.

7. Palma VC, Magro-Filho O, Oliveira D, et al. Bone reformation and implant integration following maxillary sinus membrane elevation: an experimental study in primates. *Clin Implant Dent Relat Res*. 2006;8(1):11-24.

8. Srouji S, Ben-David D, Lotan R, Riminucci M, Livne E, Bianco P. The innate osteogenic potential of the maxillary sinus (Schneiderian) membrane: an ectopic tissue transplant model simulating sinus lifting. *Int J Oral Maxillofac Surg*. 2010;39(8):793-801.

9. Rong Q, Li X, Chen SL, Zhu SX, Huang DY. Effect of the Schneiderian membrane on the formation of bone after lifting the floor of the maxillary sinus: an experimental study in dogs. *Br J Oral Maxillofac Surg*. 2015;53(7):607-612.

10. Busenlechner D, Huber CD, Vasak C, Dobsak A, Gruber R, Watzek G. Sinus augmentation analysis revised: the gradient of graft consolidation. *Clin Oral Implants Res*. 2009;20(10):1078-1083.

11. Fuerst G, Tangl S, Gruber R, Gahlteiner A, Sanromán F, Watzek G. Bone formation following sinus grafting with autogenous bone-derived cells and bovine bone mineral in minipigs: preliminary findings. *Clin Oral Implants Res*. 2004;15(6):733-740.

12. Al-Dajani M. Recent trends in sinus lift surgery and their clinical implications: recent trends in sinus lift surgery. *Clin Implant Dent Relat Res*. 2016;18(1):204-212.

13. Van Den Bergh JPA, Ten Bruggenkate CM, Disch FJM, Tuinzing DB. Anatomical aspects of sinus floor elevations. *Clin Oral Implants Res*. 2000;11(3):256-265.

14. ten Bruggenkate CM, van den Bergh JPA. Maxillary sinus floor elevation: a valuable pre-prosthetic procedure. *Periodontol 2000*. 1998;7(1):176-182.

15. Nkenke E, Schlegel A, Schultze-Mosgau S, Neukam FW, Wiltfang J. The endoscopically controlled osteotome sinus floor elevation: a preliminary prospective study. *Int J Oral Maxillofac Implants*. 2002;17(4):557-566.

16. Raghoebar GM, Vissink A, Reintsema H, Batenburg RHK. Bone grafting of the floor of the maxillary sinus for the placement of endosseous implants. *Br J Oral Maxillofac Surg*. 1997;35(2):119-125.

17. Alayan J, Vaquette C, Saifzadeh S, Hutmacher D, Ivanovski S. A histomorphometric assessment of collagen-stabilized anorganic bovine bone mineral in maxillary sinus augmentation – a randomized controlled trial in sheep. *Clin Oral Implants Res*. 2016;27(6):734-743.

18. Kolerman R, Nissan J, Rahamov N, Malvo-Guirado JL, Green NT, Tal H. Sinus augmentation analysis of the gradient of graft consolidation: a split-mouth histomorphometric study. *Clin Oral Investig*. 2019;23(8):3397-3406.

19. Reich KM, Huber CD, Heimel P, Ulm C, Redl H, Tangl S. A quantification of regenerated bone tissue in human sinus biopsies: influences of anatomical region, age and sex. *Clin Oral Implants Res*. 2016;27(5):583-590.

20. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg*. 1980;38(8):613-616.

21. Tatum H. Maxillary and sinus implant reconstructions. *Dent Clin North Am*. 1986;30(2):207-229.

22. Fuerst G, Strbac GD, Vasak C, et al. Are culture-expanded autogenous bone cells a clinically reliable option for sinus grafting?. *Clin Oral Implants Res*. 2009;20(2):135-139.

23. Payer M, Lobherger B, Strunk D, Reich KM, Acham S, Jakse N. Effects of directly autotransplanted tibial bone marrow aspirates on bone regeneration and osseointegration of dental implants. *Clin Oral Implants Res*. 2014;25(4):468-474.

24. Wagner W, Wiltfang J, Pilstner H, et al. Bone formation with a biphasic calcium phosphate combined with fibrin sealant in maxillary sinus floor elevation for delayed dental implant. *Clin Oral Implants Res*. 2012;23(9):1112-1117.

25. Donath K. Die Trenn-Dünnschliff-Technik zur Herstellung histologischer Präparate von nicht schneidbaren Geweben und Materialien. *Präp*. 1988;34:197-206.

26. Leva J, Laczko G. A simple differential staining method for semi-thin sections of ossifying cartilage and bone tissues embedded in epoxy resin. *Mikroskopie*. 1975;31(1-2):1-4.

27. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67(1).

28. Katz MH. *Multivariable Analysis: A Practical Guide for Clinicians and Public Health Researchers*. 3rd ed. New York: Cambridge University Press; 2011.

29. McMann AC, Mohamed MA, Courneya J, et al. Regression modelling and other methods to control confounding. *Occup Environ Med*. 2005;62(7):500-506.

30. R Core Team. *R: A language and environment for statistical computing*. (Version 3.6.1) R Foundation for Statistical Computing, Vienna, Austria. 2019; Available from: http://www.R-project.org/
32. Alayan J, Vaquette C, Farah C, Ivanovski S. A histomorphometric assessment of collagen-stabilized anorganic bovine bone mineral in maxillary sinus augmentation – a prospective clinical trial. *Clin Oral Implants Res.* 2016;27(7):850-858.

33. Avila G, Wang H-L, Galindo-Moreno P, et al. The influence of the bucco-palatal distance on sinus augmentation outcomes. *J Periodontol.* 2010;81(7):1041-1050.

34. Stavropoulos A, Becker J, Capsius B, Açil Y, Wagner W, Terheyden H. Histological evaluation of maxillary sinus floor augmentation with recombinant human growth and differentiation factor-5-coated β-tricalcium phosphate: results of a multicenter randomized clinical trial. *J Clin Periodontol.* 2011;38(10):966-974.

35. Danesh-Sani SA, Engebretson SP, Janal MN. Histomorphometric results of different grafting materials and effect of healing time on bone maturation after sinus floor augmentation: a systematic review and meta-analysis. *J Periodontal Res.* 2017;52(3):301-312.

36. Hanisch O, Lozada JL, Holmes RE, Calhoun CJ, Kan JY, Speikermann H. Maxillary sinus augmentation prior to placement of endosseous implants: a histomorphometric analysis. *Int J Oral Maxillofac Implants.* 1999;14(3):329-336.

37. Wang F, Zhou W, Monje A, Huang W, Wang Y, Wu Y. Influence of healing period upon bone turn over on maxillary sinus floor augmentation grafted solely with deproteinized bovine bone mineral: a prospective human histological and clinical trial: bone turn over on sinus augmentation with DBBM. *Clin Implant Dent Relat Res.* 2017;19(2):341-350.

38. Lundgren S, Anderson S, Gualini F, Sennerby L. Bone reformation with sinus membrane elevation: a new surgical technique for maxillary sinus floor augmentation. *Clin Implant Dent Relat Res.* 2004;6(3):165-173.

39. Xu H, Shimizu Y, Ooya K. Histomorphometric study of the stability of newly formed bone after elevation of the floor of the maxillary sinus. *Br J Oral Maxillofac Surg.* 2005;43(6):493-499.

40. Danesh-Sani SA, Wallace SS, Movahed A, et al. Maxillary sinus grafting with biphasic bone ceramic or autogenous bone: clinical, histologic, and histomorphometric results from a randomized controlled clinical trial. *Implant Dent.* 2016;25(5):588-593.

41. Hallman M, Cederlund A, Lindskog S, Lundgren S, Sennerby L. A clinical histologic study of bovine hydroxyapatite in combination with autogenous bone and fibrin glue for maxillary sinus floor augmentation. *Clin Oral Implants Res.* 2001;12(2):135-143.

42. Jensen OT, Sennerby L. Histologic analysis of clinically retrieved titanium microimplants placed in conjunction with maxillary sinus floor augmentation. *Int J Oral Maxillofac Implants.* 1998;13(4):513-521.

43. Choi K-S, Kan JYK, Boyne PJ, Goodacre CJ, Lozada JL, Rungharasaeng K. The effects of resorbable membrane on human maxillary sinus graft: a pilot study. *Int J Oral Maxillofac Implants.* 2009;24(1):73-80.

44. Avila-Ortiz G, Neiva R, Galindo-Moreno P, Rudek I, Benavides E, Wang H-L. Analysis of the influence of residual alveolar bone height on sinus augmentation outcomes. *Clin Implant Dent Relat Res.* 2012;23(9):1082-1088.

45. Klijn RJ, van den Beucken JJJP, Bronkhorst EM, Berge SJ, Meijer GI, Jansen JA. Predictive value of ridge dimensions on autologous bone graft resorption in staged maxillary sinus augmentation surgery using Cone-Beam CT. *Clin Oral Implants Res.* 2012;23(4):409-415.

46. Chan H-L, Suarez F, Monje A, Benavides E, Wang H-L. Evaluation of maxillary sinus width on cone-beam computed tomography for sinus augmentation and new sinus classification based on sinus width. *Clin Oral Implants Res.* 2014;25(6):647-652.

47. Teng M, Cheng Q, Liao J, Zhang X, Mo A, Liang X. Sinus width analysis and new classification with clinical implications for augmentation. *Clin Implant Dent Relat Res.* 2016;18(1):89-96.

48. Pignaton TB, Wenzel A, de Almeida Ferreira CE, et al. Influence of residual bone height and sinus width on the outcome of maxillary sinus bone augmentation using anorganic bovine bone. *Clin Oral Implants Res.* 2019;30(4):315-323.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

---

**How to cite this article**: Beck F, Reich KM, Lettnner S, et al. The vertical course of bone regeneration in maxillary sinus floor augmentations: A histomorphometric analysis of human biopsies. *J Periodontol.* 2021;92:263–272. https://doi.org/10.1002/JPER.19-0656