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ORIGINAL ARTICLE

Maxillary Bone Microstructure After Lateral Sinus Flour Augmentation with Deproteinized Bovine Bone Material in Severe Alveolar Bone Atrophy: Comparative Micro-CT Study

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Abstract
Background The most common material used for lateral sinus floor augmentation (LSFA) is xenogenic deproteinized bovine bone (DBB). However, in cases with significantly decreased residual bone height (RBH) (2–3 mm or less) where the osteogenic potential and vasculature of the recipient area are compromised the efficacy of the DBB is debatable. The aim of the study was to investigate the structural properties of the subantral bone regenerate by micro-CT in patients treated for significant alveolar ridge atrophy (RBH less than 3 mm) with LSFA and DBB grafts.
Materials and Methods Twenty patients (10 men and 10 women) were included in the study and divided into two groups: (1) the main group–10 patients with edentulous posterior maxilla and RBH less than 3 mm, where LSFA was performed to create the appropriate bone volume for installation of the implants; (2) the control group–10 patients with RBH more than 10 mm and no necessity for bone augmentation procedures. In all patients, the bone samples were taken during the insertion of the implants in the area of interest. All samples were analyzed by micro-CT method. 3-D morphometric parameters were evaluated and compared in both groups.
Results There were no significant differences in parameters that reflected 3-D morphometric structure and density of the edentulous alveolar bone with sufficient bone volume and augmented bone in patients with RBH less than 3 mm ($p > 0.05$). The slight differences were observed in trabecular architecture: In operated patients, the lower porosity, bone volume-to-surface ratio, anisotropy rate and increased trabecular pattern factor as a result of continuous functional remodeling process were observed.
Conclusion Maxillary sinus floor elevation with DBB alone is a strategy, providing an appropriate 3-D architecture of the newly formed bone tissue and adequate primary stability of the dental implants. The biological properties and long-term remodeling of the augmented bone in patients of this category requires the further investigation.

Keywords Lateral sinus floor augmentation · Deproteinized bovine bone · Microcomputed tomography

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Introduction

Atrophy of the alveolar bone after tooth loss is often associated with increasing pneumatization of the maxillary sinus. This causes limitations for implant supporting prosthetic restoration in the posterior maxilla region. The sufficient bone volume required for installation of dental implants in such cases can be created by bone augmentation procedures including the lateral sinus floor augmentation (LSFA). Since LSFA was developed by Tatum (1977) and Boyne and James (1980), it became the routine surgical

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procedure with high predictability and success rate [1]. Currently, it is the method of choice for severely atrophied alveolar ridges, with nearly 90% long-term implant survival rates.

Previously, most of the authors strongly recommended the autogenous bone as grafting material for LSFA due to its excellent osteoconductive and osteoinductive properties. However, the autogenous bone grafts are associated with excessive donor site morbiity, unpredictable resorption and bone volume loss. Therefore, xenogenic materials such as deproteinized bovine bone (DBB) were proposed as an alternative grafting material for LSFA [2]. DBB has good osteoconductivity and high volumetric stability. The clinical research demonstrated that DBB in general may be as effective as autogenous bone for sinus floor augmentation. However according to San Lie [3], DBB requires a special consideration in cases with significantly decreased residual bone height (RBH) (2–3 mm or less) where the osteogenic potential and vasculature of the recipient area are compromised and the volume of the bone graft for remodeling is significant. In such cases, some authors recommend to prolong healing period before implant placement (up to 8–10 months) or combine DBB with autologous bone and/or growth factors for increased osteoinductivity. They reported that the main drawbacks of the material are the increased healing period and compromised bone quality in comparison with autologous bone. It is known, that to provide a good primary stability of the dental implants and their long-term function, the quality of the subantral bone regenerate is not less important as its volume.

In terms 4–12 months recommended for implant installation, the regenerated bone is presented by the mixture of the residual no-vital particles of the bone graft, connective tissue and the newly formed bone. The structural and the mechanical properties of augmented bone may be significantly different from the alveolar bone with normal architectonics and depend on the material used. Their careful analysis is necessary to determine the indications for different bone grafting materials an prognosis of implant stability in the early postoperative period. However, the hystomorphometric studies currently used for the investigation of the bone graft turnover had strong limitations in 3-D analysis of the regenerate microstructure [4, 5].

Recently, the use of microcomputed tomography (micro-CT) offers the advantage of precise analysis of the bone microstructure and remodeling [6], based on 3-D CT scans with high resolution (a voxel size between 0.3 and 100 μm) [7, 8]. Micro-CT can be used for visualization and structural evaluation of the newly formed bone by calculating the number of indices, reflecting the bone microarchitecture strongly associated with mechanical or/and biological properties of the bone [9].

Bone grafting materials/subantral regenerates with intricate interior structures can be scrutinized using micro-CT, and any spatial location of the architecture can be digitally isolated out using specific software. To evaluate the quality of the regenerate, it is possible to measure the bone volume and surface area, thus allowing the calculation of porosity as well as the newly formed bone mineral density. All these parameters are important for primary stability of the dental implants and their long-term functional capacity [6, 10–12].

The aim of the present study was to investigate the structural properties of the subantral bone regenerate by micro-CT in patients treated for significant alveolar ridge atrophy (RBH less than 3 mm) with LSFA and DBB grafts.

Materials and Methods

The present study focuses on microstructural characteristics of the bone tissue in edentulous posterior maxilla of patients, who required implant-supported prosthetic constructions. Twenty patients (10 men and 10 women) were included in the study divided into two groups: (1) the main group – 10 patients who needed LSFA to create the appropriate bone volume for installation of the implant with RBH less than 3 mm; (2) the control group – 10 patients with RBH more than 10 mm and no necessity for bone augmentation procedures.

The inclusion criteria for both groups were (1) patients with edentulous posterior maxilla that required implant installation for further prosthetic rehabilitation with non removable dentures; (2) tooth extraction in the area of interest more than 12 months ago; and (3) comprehensive clinical and radiological documentation of the case.

The exclusion criteria were: age under 16 years, decompensated or sub-compensated concomitant somatic pathologies, mental illnesses, alcoholism or drug addiction, active radiation or chemotherapy, non-compliance with medical recommendations and lack of interaction with a physician, existing pathology of the paranasal sinuses, aggressive forms of periodontal disease, systemic bone disorders including osteoporosis, residual alveolar bone width less than 6 mm, perforation of the Shneiderian membrane during the LSFA procedure in the main group and patient's refusal to participate in the study. Patients were recruited at Stomatological Medical Center of the Bogomolets National Medical University (Kyiv, Ukraine). Informed consent was obtained from all individual participants included in the study. The study was approved by the Ethics Committee of the Bogomolets National

Medical University (Protocol № 139/26.11.2020) the micro-CT investigations were performed at the Department of Medical Physics and Informatics, University of Pecs, Hungary.

Surgical Procedures

All patients prior to surgical interventions underwent the con-beam CT scan to measure the residual alveolar bone volume and create the plan of rehabilitation with implant supported dentures. In the main group in all cases, the standard LSFA procedure was performed under the local anesthesia: After the elevation of the mucoperiosteal flap, the lateral wall of the maxillary sinus was removed with round diamond bur size ø5 mm (NTI, ISO 9001:2008), the Shneiderian membrane was carefully elevated, and the space, created under the membrane, was filled with cancellous DBB (TutoboneTM, Germany, ø1–2 mm) as a single grafting material. Antibiotics and nonsteroid analgetics were prescribed according to the protocol described by Esposito M. [13] After 6 months of the bone healing period, the implants were placed at the augmented area according to the standard two-stage protocol. The diameter of the implants in all cases was more than 4 mm. Before implant placement the samples of the bone tissue were taken for further micro-CT analysis with a standard trephine burs (Medesy, Italy. 3.0 mm outer diameter and 2.0 mm inner diameter). Thus the specimen contained both the residual alveolar bone and bone regenerate in the grafted area. The specimens were fixed in 4% neutral-buffered paraformaldehyde and sent for the micro-CT structural analysis. The further preparation of the hole for implant placement was performed by the standard drills according to the protocol, recommended by the implant manufacturer. In the control group, the bone samples were taken from alveolar crest at the same manner followed by the placement of the two-stage osteointegrated implants. The torque was measured during the implant installation in both groups, and the complications were recorded within a 6-month follow-up.

Micro-computed Tomography

All bone samples were subsequently measured and analyzed. The images were taken with a SkyScan 1176 micro-CT device manufactured by Bruker. The voltage was 80 kV, the current was 310 uA, and the exposure time was 700 ms long during every scan with a 0.5-mm aluminum filter in use and with 0.7-degree increments in a 180-degree rotation. One scan took approximately 36 min.

Bone phantoms and a water sample were recorded with the same settings. The side of one pixel corresponded to 8.74 µm. The raw images were reconstructed with NRecon (v.: 1.7.4.2) software. The position of the bone samples was standardized on the recordings and then a circular ROI with a diameter of 2 mm was selected which was recorded as long as there was a contiguous bone sample. The selected area was studied using CTAn (v.: 1.20.8.0 +) software. Before the evaluation, the software was calibrated to measure bone density using the previously prepared bone phantom and water sample images. The threshold was set individually for each sample so that the software recognizes the bony formulas as accurately as possible for each (Figs. 1, 2). 3D reconstructions were generated from these datasets and colored to show the different bone mass values using the CT vox software developed by Bruker Inc (Fig. 3) [14].

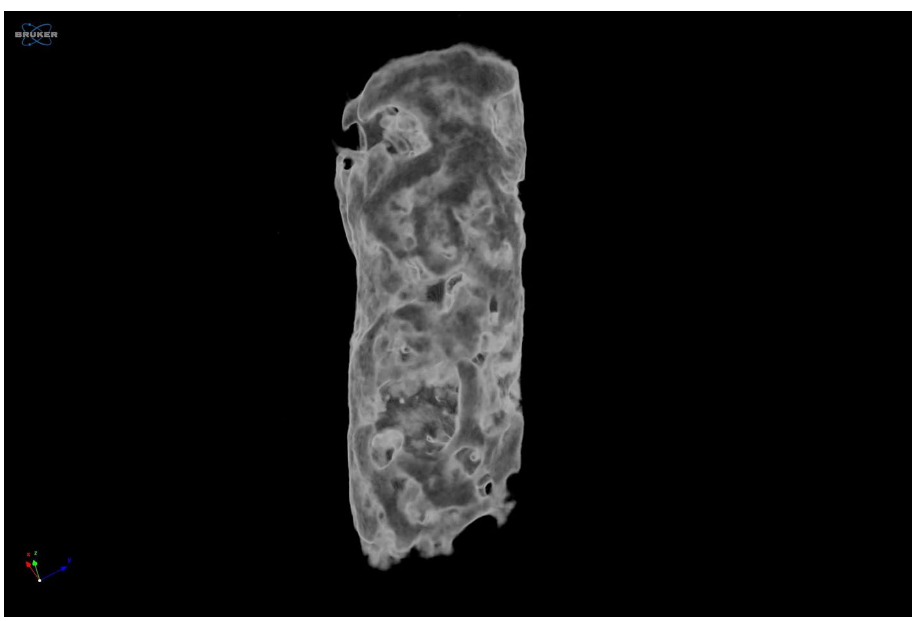

Fig. 1 Demonstrative 3-D reconstruction pictures of bone sample after LSFA surgery

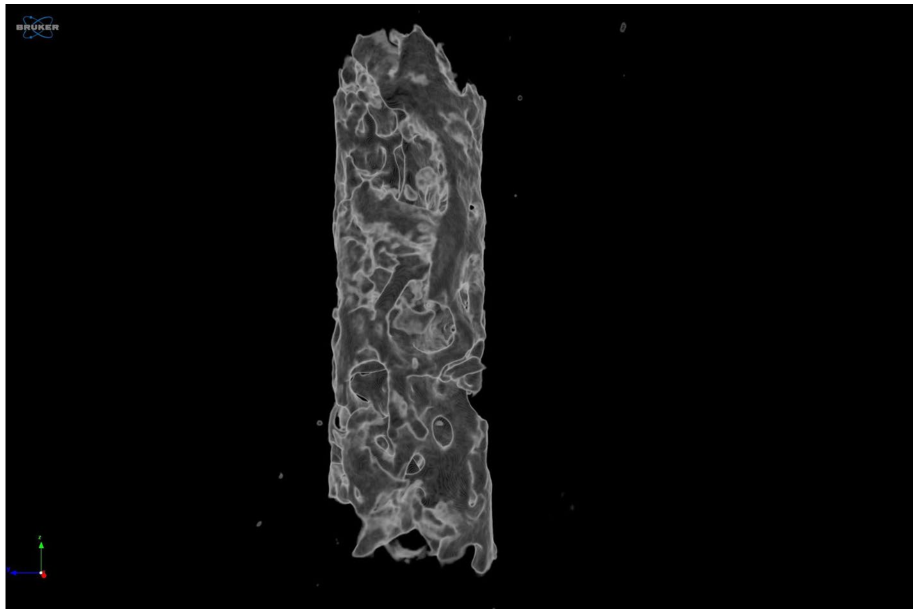

Fig. 2 Demonstrative 3-D reconstruction pictures of the normal alveolar bone sample

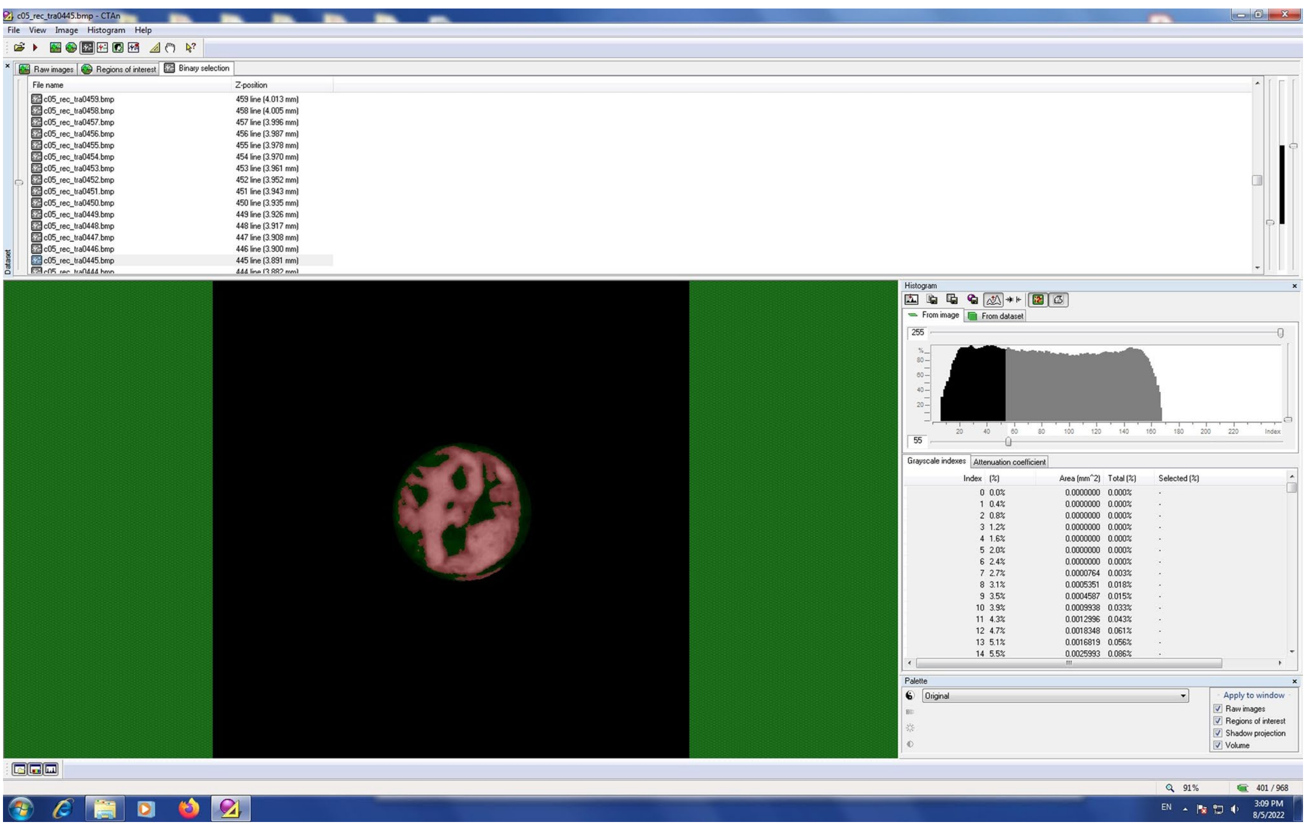

Fig. 3 The bone mass values based on micro-CT in ROI

Statistical Analyses

For quantitative variables obtained in micro-CT analysis Shapiro–Wilk test was used to check the distribution. Means and standard deviations (SD) for normally distributed data or medians and interquartile range for no normally distributed data were estimated and reported. For two groups, comparison t-tests (normal distribution) or Mann–Whitney test (not normal distribution) were used. The associations between variables were studied by

Spearman correlation coefficient. Two-tailed tests were used with critical level of significance set at $p < 0.05$. The data were analyzed with statistical software EZR v. 1.55 (graphical user interface for *R* statistical software version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) [15].

Results

The age of the patients included to the study ranged from 25 to 65 years (the mean age in the main group consisted 48,8 ± 15,5 and in the control group—48,4 ± 13,4 years). RBH in the main group ranged from 1 to 3 mm (the mean value 2,5 ± 0,65 mm). In the control group, it ranged from 10 to 19 mm (with a mean value of 12.6 ± 2.41 mm). All LSFA procedures in the main group were performed without perforations of the Schneiderian membrane. No clinical signs of infection development and/or the graft failure were observed during the period before implant placement. The bone density outside the implant, measured by conventional cone beam CT was 668,16 ± 137,08 in research and 514.56 ± 115.51 in control groups corresponded to the 3-d bone type by Misch C.E. (1990). In both groups, implants were installed with a good primary stability (maximal torque on installation was 35–50 Ncm). No signs of periimplantitis or implant failure were observed at 1-year follow-up period.

Two bone samples (one from each group) were excluded from the further micro-CT analysis due to a damaged morphological structure and integrity, resulted from failure of the bone harvest procedure.

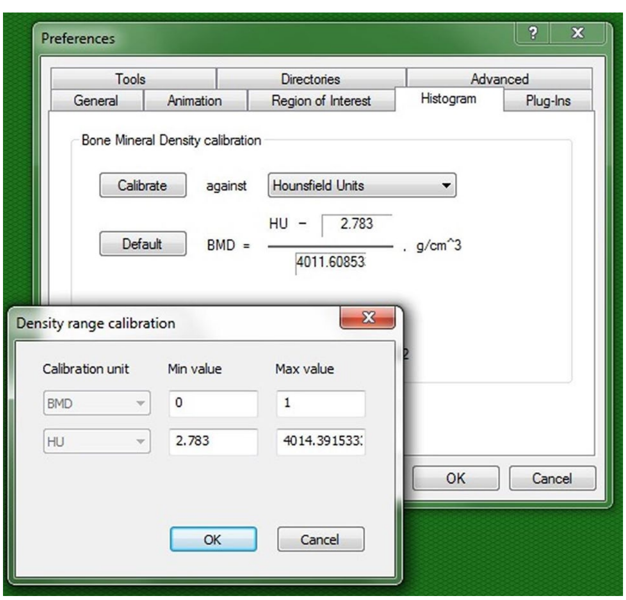

Fig. 4 Bone mineral density calibration method

The quantitation of bone morphometric parameters in the volume of interest (Fig. 4) analyzed by micro-CT is shown in Table 1. No significant differences in 3-D morphometric parameters or all bone structures in ROI were observed in the biopsies of the augmented bone (the main group) and intact alveolar bone (control). There was a trend toward higher total porosity rate and bone volume-to-surface ratio in the control group. At the same time, the trabecular number and thickness was higher at the main group. The structure model indices demonstrated the predominance of the plate-like trabecular architecture in both groups, but lower trabecular pattern factor in the control group signified better connected trabecular lattices. The degree of the anisotropy in the control group was also higher.

Discussion

The LSFA is a predictable and widely used procedure. It provides the possibility to create the sufficient bone volume for the placement of dental implants with adequate primary stability. The numerous clinical studies demonstrated that LSFA is an effective option even in cases with low RBH (less than 2–3 mm). However, the choice of appropriate bone grafting material is still widely discussed in the literature. The autologous bone grafts with the best osteoinductive and osteoconductive properties during a long time were considered as the "golden standard" for LSFA procedure. The main drawbacks and limitations of the technique were associated with donor's additional morbidity and unpredictable volume loss during the bone remodeling process. Many authors recommended to use the xenogenic deproteinized bone grafts as an alternative to the authologous bone. This approach gained a significant popularity due to good osteoconductivity and volumetric stability of the xenogenic materials. The big number of clinical and experimental studies with the use of morphological, radiological (including CT and micro-CT) and biomechanical methods proved that the xenogenic bone grafts (mainly DBB) provides the quality of the newly formed bone equivalent to the autologous bone grafts.

Ji-Hyun Lee et al. [16], based on the histomorphometric, microradiographic, and clinical outcomes of the LSFA procedures with DBB used as the single grafting material, demonstrated that xenogenic bone does not require prolonged healing time before implant placement in comparison with autologous bone grafts.

The meta-analyses found no significant differences in clinical and morphological outcomes of DBB, autologous bone or mixture of the DBB and autologous bone. All these data prove in favor of DBB as a predictable and minimally invasive modality for LSFA with controlled risk and high efficacy [17, 18].

Table 1 Statistical analysis of bone samples based on micro CT

Parameter	3D integrated analysis of all objects in VOI*		Statistical significance of the differences
	Control group (9 samples)	Main group (9 samples)	*P*
Total bone volume, mm^3	7.15 ± 3.72	6.63 ± 2.8	0.74
Bone surface, mm^2	93.7 ± 33.8	96.2 ± 24.2	0.857
Percent bone volume, %	39.2 ± 15.1	41.6 ± 16	0.754
Bone surface/volume ratio, mm^{-1}	16.1 (11.9–18.5)	13.7(10.9–21.3)	> 0.999
Bone surface density	5.29 ± 0.91	6.11 ± 1.44	0.171
Trabecular thickness	0.227 (0.196–0.328)	0.275 (0.179–0.347)	> 0.999
Trabecular separation	0.35 ± 0.08	0.33 ± 0.09	0.579
Trabecular number	1.38 ± 0.27	1.62 ± 0.55	0.269
Trabecular pattern factor	2.5 ± 2.05	4.09 ± 5.93	0.458
Structure model index	0.84 ± 0.67	1.02 ± 1.41	0.736
Degree of anisotropy	1.8 ± 0.43	1.57 ± 0.53	0.328
Degree_of_anisotropy_вскобках	0.42 ± 0.12	0.31 ± 0.19	0.154
Number of closed pores	40 (13–60)	33 (28–67)	0.587
Closed porosity (percent)	0.036 (0.007–0.08)	0.042 (0.03–0.07)	0.863
Volume of open pore space	10.2 (7.5–12.1)	8.2 (6.9–10.9)	0.436
Open porosity (percent)	60.8 ± 15.2	58.4 ± 16	0.753
Total volume of pore space	10.2 (7.5–12.1)	8.174 (7.0–10.9)	0.489
Total porosity (percent)	60.8 ± 15.1	58.4 ± 16	0.754

Data are presented as mean ± SD or median (interquartile range)

*volume of interest

However, there is still a strong prejudice concerning the use of the xenogenic bone as a single grafting material in cases with low RBH. In such cases, the residual bone could not provide the adequate primary stability for the dental implant and has the compromised regenerative potential. Thus the bone remodeling and new bone formation is less active under such conditions. Many authors recommend the use of the bone grafts with high osteoinductivity (autologous bone, combination of bone grafts with PRP or rBMP) and prolonged healing time before the implant placement [19, 20].

The present study presented a 3-D morphometric analysis of DBB as a single grafting material in cases with low RBH using high resolution micro-CT.

We take the edentulous alveolar bone of the posterior maxilla with sufficient volume and quality as a control. The presence of such bone with organospecific architectonics is usually considered as an optimal situation for installation of the dental implants.

The design of the study was based on 3-D analysis of the bone samples taken directly from the area of implant placement, so the parameters obtained had a direct influence on the outcomes of the dental implantation. Obviously in the main group where the standard augmentation procedures were performed, the samples included both residual alveolar bone and bone regenerate. The 6-month term after LSFA procedure was chosen as an optimal healing period for bone augmentation procedures according to the recommendations of interventions for replacing missing teeth: augmentation procedures of the maxillary sinus [12]. The micro-CT method was used for analysis of the bone samples at preclinical and clinical evaluation of the bone plastic materials and scaffolds for tissue engineering [21], their architecture, degradation and remodelling, as well as the healing capability of bone regenerative materials and new bone formation within the scaffold or mineral matrix. It is the gold standard for assessing BMD and bone microstructural features with high resolution and accurately [22].

The results of the present study revealed that there were no significant differences in all morphometric parameters between two groups. This indicates that the DBB used as a single bone grafting materials provides the bone with structural characteristics that match the natural alveolar bone of the posterior maxilla even in cases with significant decrease in RBH.

This support the clinical suggestions of [23, 24]. In contrast, a controlled biopsy study reported better results of the autologous bone application for edentulous maxillary augmentation [25]. Meanwhile, it does not reject negative factors such as additional trauma and the repneumatization effect.

Minor differences between two groups demonstrated a tendency to higher total porosity, degree of the anisotropy and bone volume-to-surface ratio in the control group, while the trabecular number and thickness, as well as the trabecular pattern factor were higher in the main group. These differences indicate of better organized trabecular structure resulting from extended period of functional remodeling and adaptation to the exciting functional loads in the control group. As it was shown in the previous studies such factors as trabecular thickness, connectivity, anisotropy and special complexity of the trabecular mesh as well as the bone mineral density are important determinants of bone strength and stiffness important for functional capacity of the bone under specific loading conditions [26]. However in the present study due to the small number of cases the abovementioned differences were not statistically significant.

The present study has some limitations to be considered. Compared to histomorphometric studies, micro-CT has no possibilities to analyze the cell proliferation and differentiation, microcirculation and vascularization inside the bone, rate of mineral apposition and bone remodeling. So biological features of the augmented bone as well as the long-term patterns of its remodeling require the further investigation. The number of cases was relatively small (9 cases in each group) with marked individual variability for some parameters; the dimensions of granules and type of the DBB may significantly influence the final structural outcome, so it also can be the subject for the further investigations.

Conclusion

Within the limitations of the study, there were no significant differences in 3-D morphometric structure and density of the edentulous alveolar bone with sufficient bone volume (control group) and augmented bone in patients where LSFA procedure with DBB alone was performed at RBH less than 3 mm (main group).

The slight differences in trabecular architecture revealed in the present study demonstrated the higher porosity, bone volume-to-surface ratio, anisotropy rate and decreased trabecular pattern factor in the control group as a result of continuous functional remodeling process.

Therefore, maxillary sinus floor elevation with DBB alone is a strategy, providing an appropriate 3-D architecture of the newly formed bone tissue and adequate primary stability of the dental implants.

The biological properties and long-term remodeling of the augmented bone in patients of this category requires the further investigation.

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Author contributions AK, OS contributed to conception and design, analysis and interpretation of data, drafting the article. OS, TK contributed to data collection, analysis and interpretation of data. AK, ZH critically revised the article and supervised the study.

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Declarations

Conflict of interest The authors declare no conflict of interest. The founders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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