BONE REGENERATION USING DEPROTEINIZED BOVINE BONE MINERAL) IN A CRESTAL SINUS ELEVATION TECHNIQUE: A HISTOLOGICAL AND HISTOMORPHOMETRICAL PILOT STUDY

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Abstract

The aim of this study is to evaluate the percentage of new bone formation (NBF) and residual bone substitute following the use of a bovine demineralized xenograft (DBBM) in a sinus floor crestal approach elevation technique.

Nine patients with unilateral or bilateral edentulous sites with less than 2 mm residual bone height under the sinus, were included in this study. Future site development was performed using deproteinized bovine bone mineral (DBBM) as graft material. In the second stage, performed after 6 to 9 months, nine implants were placed after harvesting bone biopsies from the regenerated sites. Histological and histomorphometric analysis were then undertaken to assess the regenerated bone.

All the implants were placed successfully after 6 to 9 months following sinus floor elevation technique for future site development without any complications. The average percentage of new bone formation and residual bone substitute were of 24.37 % ± 13.12 % and 5.87 % ± 8.38 %, respectively.

Within the validity of this crestal approach in the treatment of the subantral region presenting 2 mm or less residual bone height, the DBBM has proved to be an osteoconductive biomaterial in bone regeneration procedures.

Keywords: Demineralized bovine xenograft – histology- maxillary sinus - new bone formation - sinus floor augmentation.

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RÉGÉNÉRATION OSSEUSE À L'AIDE D'UN MINÉRAL OSSEUX BOVIN DÉPROTÉINISÉ DANS UNE TECHNIQUE D'ÉLEVATION SINUSIENNE PAR VOIE CRESTALE : UNE ÉTUDE PILOTE HISTOLOGIQUE ET HISTOMORPHOMÉTRIQUE

Résumé

La réhabilitation des crêtes sévèrement atrophiées a été réalisée avec succès au cours des deux dernières décennies par diverses techniques d'augmentation du sinus avec des matériaux de substitution osseuse. Toutefois, le débat continue sur la technique et le choix du matériau de substitution à utiliser ainsi que sur le délai de la pose des implants.

L'objectif de cette étude est d'évaluer par histologie et par histomorphométrie la quantité d'os néoformé ainsi que celle du matériau résiduel après élévation sinusienne par la technique d'élévation crestale en présence d'une xénogreffe tel que l'os bovin déprotéinisé, en vue d'une restauration implantaire ultérieure. Ce travail vise aussi à comparer la néoformation osseuse et le pourcentage de matériau résiduel à trois niveaux : superficiel, moyen et profond.

Neuf patients présentant des édentations maxillaires postérieures uni- ou bilatérales avec des hauteurs osseuses résiduelles ≤2mm ont été sélectionnés pour l'étude. Ces patients ont subi une élévation sinusienne par la technique d'approche crestale de Summers modifiée avec un matériau xénogène d'origine bovine. La pose des implants a été différée à 6 mois. Les biopsies ont été faites au cours du second temps chirurgical, lors de la mise en place des implants, pour les études histologiques et histomorphométriques.

Les résultats statistiques ont montré que le pourcentage d'os nouvellement formé était plus élevé au niveau des coupes moyennes (25.38%±12.51%) mais cette différence n'était pas significative (p=0.335). Concernant le matériel résiduel, le pourcentage était plus élevé au niveau des coupes profondes (9.70%±12.59%), mais sans différence significative (p=0.086).

L'os bovin déprotéinisé semble être un matériau bioconducteur, à faible résorption, lors des élévations sinusiennes par approche crestale.

Mots-clés : os bovin déprotéinisé, élévation sinusienne, néoformation osseuse, sinus maxillaire.

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Introduction

After loss of maxillary molar teeth, a rapid resorption of bone below the maxillary sinus floor occurs. This phenomenon often results in an insufficient bone quantity to place dental implants due to the combination of a decreased residual bone height and a widened maxillary sinus [1, 2]. Conventionally, two main techniques have been described in the literature to elevate the maxillary sinus floor before or during implant placement. The amount of residual bone height often dictates the technique of choice and whether or not implants are placed simultaneously or after a healing period to allow bone formation [3, 4].

Whenever the existing bone height is insufficient and extensive bone augmentation is required, the most commonly used bone augmentation technique is the sinus floor elevation from a lateral window [3]. However, when adequate residual bone height to support simultaneous implant placement is available, a less invasive procedure is indicated. This technique was introduced by Summers in 1994 [5]. It is characterized by the use of specific root analog instruments (osteotomes) without the preparation of a lateral window with or without bone addition. In comparison to the lateral window approach, the osteotome procedure is less invasive, accompanied with less complications and operation time [6, 7]. However, it has rarely been applied in cases of inadequate bone height.

Over the years, the osteotome technique has been modified in order to elevate the maxillary sinus floor to a level that permits future implant placement [8, 9]. In this case, only the placement of bone graft material is performed by crestal approach and, after 6-9 months, when the consolidation of the graft is insured, the implants will be inserted. This approach provides a less invasive sinus lifting and bone grafting even in inadequate residual bone height cases.

The aim of the present study is to analyze histologically and histomorphometrically, bone biopsies harvested from sites with residual bone height ≤ 2 mm and who had underwent site augmentation, using demineralized bovine bone mineral as a graft material.

Materials and methods

Biomaterial

The bone-substitute material used in the present study was the bovine demineralized xenograft (DBBM particles of 0.5–1 mm diameter) (Bio-Oss; Geistlich, Switzerland). The DBBM is a deproteinised bone mineral matrix produced from bovine bone through a multi-stage purification process, with 75–80 % porosity and interconnected pores.

Patient selection

Nine systemically healthy patients, 7 men and 2 women, 40 to 65 years of age, with no contraindication for surgical implant therapy were selected for this study based on the following inclusion criteria:

- Unilateral or bilateral maxillary posterior edentulism involving the premolar-molar areas;
- Presence of 2mm and less of residual subsinus bone height as evidenced at the baseline preoperative retro-alveolar radiographic examination;
- Absence of ongoing sinus pathology;
- Absence of localized soft (e.g. fungal) or hard tissue lesions (e.g. residual granulomas or cystic lesions) in posterior sites.

The following patients were excluded from the study:

- Patients with recent extractions (less than 6 months) in the involved area;
- Patients with thin ridges as evidenced clinically or through CT scan.

Smokers (1 pack/day or less) were not excluded from study.

The patients were given detailed information about the nature and procedures of the project. A comprehensive treatment plan including endodontic, periodontal and restorative therapy was implemented pre-surgically as required. All patients received prophylaxis and oral hygiene instructions 2 to 6 weeks prior to the surgical procedure.

Surgical technique

Immediately prior to surgery, the patients were asked to rinse with chlorhexidine digluconate solution 0.12% for 1 minute. A crestal horizontal antero-posterior incision was made and minimal full-thickness flap was elevated to expose 3-4 mm of the buccal and palatal aspects of the alveolar crest. Releasing incisions at anterior or posterior ends of the horizontal incision were only realized when needed. A modified version of the Summers technique [10] for maxillary crestal sinus floor elevation was performed. An appropriately sized osteotome with a concave tip (3i, Implant Innovation Inc., Palm Beach Gardens, FL, USA) was then used under gentle malleting and/or slight hand pressure to infracture the crestal cortex. Two or more circular openings were carried out based on the mesio-distal extension of the sinus. In the presence of extremely residual bone height (2mm or less), the openings were connected to create rectangular crestal window because of the spontaneous collapse of the cortical shell located between the osteotomy sites. Particulate anorganic bovine bone matrix (Bio-Oss®, 0.25-1mm, Geistlish, Switzerland) mixed with patient's blood procured from the surgical site were carefully packed into the sinus cavity without excessive pressure using the flat part of the elevator (Nevins Kramer, Hu Friedy, Leimen, Germany) against the grafting material. Small increments of grafting particles were then progressively added to backfill the osteotomy sites and to create additional apical displacement and broader dissection of Schneiderian membrane. This last step was repeated until the necessary volume of subsinus bone was

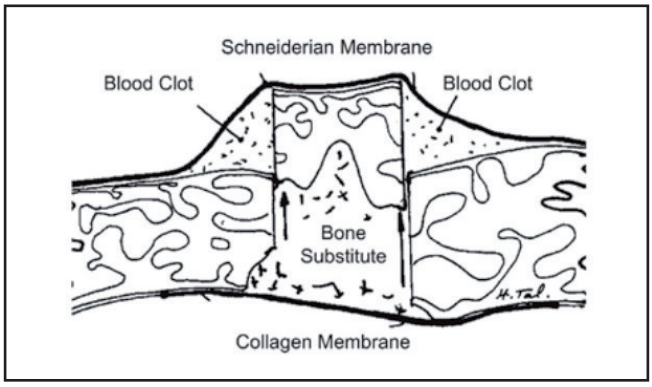

Fig. 1: Illustration of the crest following the osteotome elevation technique [10].

Fig. 2: Radiograph showing the residual bone height ≤ 2mm.

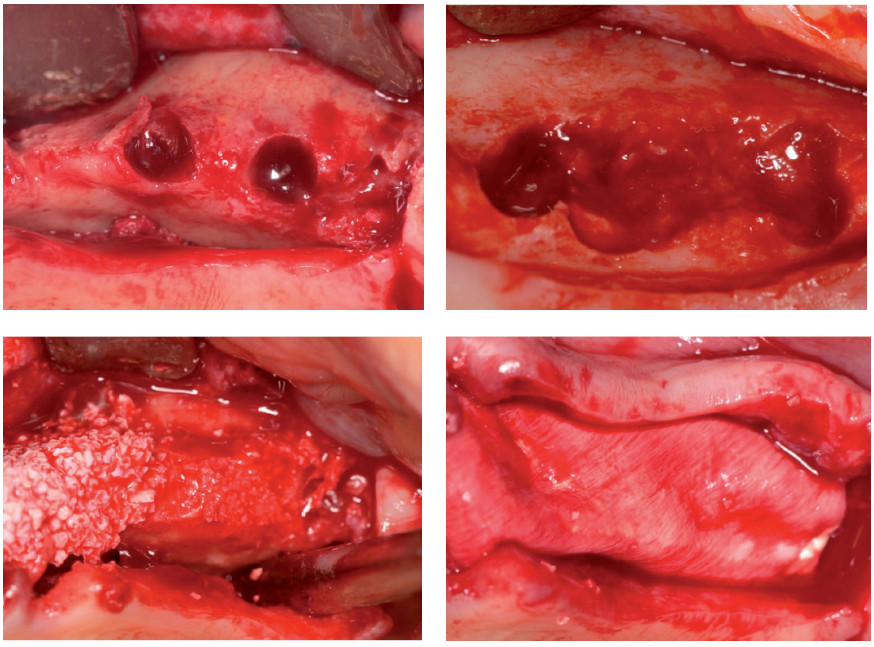

Fig. 3: Crestal views of the surgical procedure. a) Crest after two openings using the osteotomes b) Opening connected to create a rectangular crestal window. c) Placement of the grafting material. d) Coverage of the crest by a collagen membrane.

achieved. A periapical radiograph was taken at the end of graft placement to evaluate the pattern and volume of augmentation obtained in the sinus cavity. The crestal sinus access cavity was then covered with a collagen membrane (Bio-Gide®, Geistlich, Switzerland) to prevent migration of graft particules. The flaps were sutured with resorbable polyglycolic acid 5-0 sutures (Surgisorb, Vauxhall Industrial Estate, Wales, United Kingdom). A second standardized periapical radiograph was taken following flap closure applying the same exposure and conditions as abovementioned (Figs. 1- 3).

Postoperative care

When required, partial or complete removable dentures were relined with a soft reliner (Sofreliner S, Tokuyama Corp., Tokyo, Japan) and delivered to the patients immediately postoperatively. Antibiotics (amoxicillin 1g twice daily) and non-steroidal anti-inflammatory medications were prescribed for 6 and 4 days, respectively. Analgesics were recommended as needed. Sutures were removed 7 -10 days following surgery. Postsurgical visits were scheduled at one-week intervals during the first month and monthly thereafter to check the course of healing.

Six to nine months after surgery, the second-stage operation was carried out to place the implants (Fig. 4). At that stage, a bone biopsy at the regenerated area was taken with a 3.4-mm-wide trephine bur (Straumann AG, Basel, Switzerland). After three months, the implants were successfully loaded. All surgeries were performed by one experienced periodontist.

Histological procedures

The biopsies, harvested from implant placement sites were fixed in 10% formalin, dehydrated in a scale of increasing alcohol content, embedded in paraffin and, finally, sectioned along

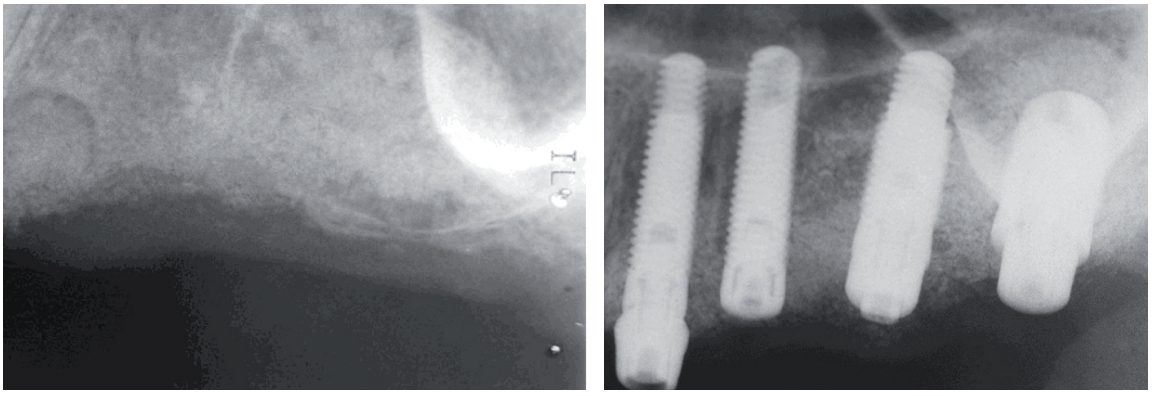

Fig. 4: Peri-apical radiographs before and after implant placement.

the major axis of the biological samples using a microtome (Cutting machine Exakt - Apparatebau Nordersted, Allemagne). Three sections per biopsy were obtained (superficial layer, X, intermediate layer, Y, deep layer, Z) then stained with Giemsa-Paragon and observed under an optical microscope (Olympus BX 60, Olympus Corporation, Tokyo, Japan). The aforementioned microscope was equipped with a digital camera (Olympus E330) making it therefore possible to photograph the samples.

Histomorphometric analysis

The images were subjected to histomorphometric analysis using dedicated software (Image J) to calculate the amount of new bone formation and residual bone graft material.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows (Chicago, IL, USA, version 24.0). The level of significance level was set at $p \leq 0.05$. The normality distribution of the continuous variable was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Kruskal-Wallis tests were used to compare the percentage of residual bone graft between the three layers (superficial, intermediate and deep).

Results

Nine crestal sinus lift procedures (2 bilateral and 7 unilateral) for future site development were performed and a total of 21 dental implants were placed. During the follow-up period, none of the patients had any complication. The residual bone height (RBH) measured on the pre-op CBCT was 2 mm and less. The mean post-operative maxillary sinus floor elevation obtained was $8<x<12$mm and more. At the time of implant placement, the average total bone height was 10 mm.

Histomorphometric results

The mean percentage occupied by new bone was 24.37% ± 13.12 % (table 1). The percentage of new bone seems to follow a gradient decrease from the residual ridge towards the top of the grafted bone; however this decrease was statistically non-significant (Fig. 5).

The mean percentage of residual bone graft was 5.87 % ± 8.38 % (Table 2). The percentage of residual bone graft increased from the residual ridge towards the top of the grafted bone but was statistically non-significant (Fig. 6).

A negative significant correlation was found between the percentage of newly formed bone and residual bone graft at the superficial (X), intermediate (Y) and deep layers (Z) ($r= 0.303$; $p=0.007$; $N=77$). When the amount of newly formed bone increased, the percentage of residual bone graft decreased significantly (Fig. 7).

Histological results

At low magnification (x2): the histological sections consisted of bone trabeculae and fragments of substitute material giving a compact structure to the biopsy. Bone remodeling with osseointegration of the graft fragments was present. Intimate contact between the newly formed bone and the granules of the substitute material (Bio-Oss®) was noticed. Connective tissue and bone marrow were developed around newly formed bone areas /graft materials (Fig. 8).

At higher magnification (x4; x10): the DBBM particles were surrounded by newly formed bone (Fig. 9). The presence of adipocytes in the medullary spaces indicates a maturation of these spaces. Lamellar bone patches and woven bone were observed. The fibers are stained blue in the extracellular matrix.

Discussion

The present study evaluated the percentage of new bone formation and residual bone substitute following a modified version of the osteotome sinus floor elevation technique for future site development. Favorable new bone formation was observed on all sections and adequate sinus floor elevation was achieved to permit implant placement in all patients. The advantages of this procedure is its reduced invasiveness, operating time, well tolerance by the patients, lower morbidity and reduced minor complications [11].

In this study, the histologic and histomorphometric analysis of bone biopsies suggest the validity of this method

		Mean ± Std. Deviation (%)	Minimum	Maximum
Percentage of newly formed bone	X	24.09 ±13.01	3.0 %	49.0 %
	Y	25.38 ± 12.51	7.0 %	57.0 %
	Z	22.76 ± 15.00	3.0 %	56.0 %
	Total	**24.37 ± 13.12**	**3.0 %**	**57.0 %**

Table 1: Percentage of newly formed bone.

		N	Mean ± Std. Deviation (%)	Minimum	Maximum
Percentage of the residual material	X	26	5.47 ± 7.57	0.0	31.3
	Y	34	4.26 ± 5.58	0.0	28.4
	Z	17	9.70 ± 12.59	0.1	44.3
	Total	**77**	**5.87 ± 8.38**	**0.0**	**44**

Table 2: Percentage of the residual material.

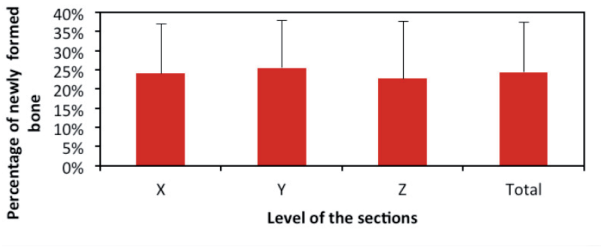

Fig. 5: Percentage of newly formed bone.

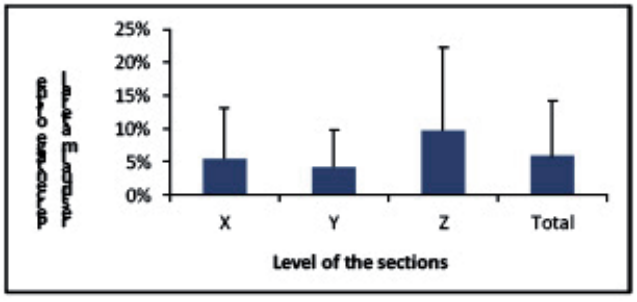

Fig. 6: Percentage of the residual material.

in regenerating a considerable amount of new bone in a highly resorbed crests (residual bone height ≤ 2 mm) rendering implant placement feasible after 6 to 9 months. This is in agreement with other studies evaluating the use of a similar approach for future site development when the residual bone height is < 5 mm [8, 12].

Similar histologic features have been reported in the literature along with comparable clinical outcomes regarding the efficacy of different types of bone replacement grafts used in the sinus compartment [13, 14].To date, no correlation has been reported between the best type of bone grafts for sinus augmentation and clinical outcomes [15]. In this study, the DBBM was chosen by the authors due to its slow resorption rate which allows a favorable space-maintenance property during the remodeling phase in order to give time for new bone formation [13, 16]. In fact, a negative significant correlation was found between the percentage of newly formed bone and residual bone graft at the superficial, intermediate and deep layers of the biopsies which demonstrates the gradual osteoclastic rehashing of the residual graft material along with a gradual new bone formation in the space-maintained compartment.

One may speculate that only a limited amount of sinus floor elevation can be obtained with the osteotome technique as compared to external sinus lift procedures. This may constitutes a drawback in the treatment of edentulous sites with a diminished residual bone height, as the outcome is considered less predictable [8, 17-20]. However, in our study the use of the modified osteotome sinus floor elevation procedure for future site development resulted in a new bone height ranging from 10 to 15 mm, outcomes that are comparable to sinus floor elevation using the lateral window. This is in agreement with other papers who used a similar approach for sinus floor elevation and reported an average total bone gain of 7.92 mm [12] and 9.93 mm [8].

The absence of membrane perforation in the present study was verified using the Valsalva maneuver and the repeated x-rays during the follow-up periods that were taken to confirm the

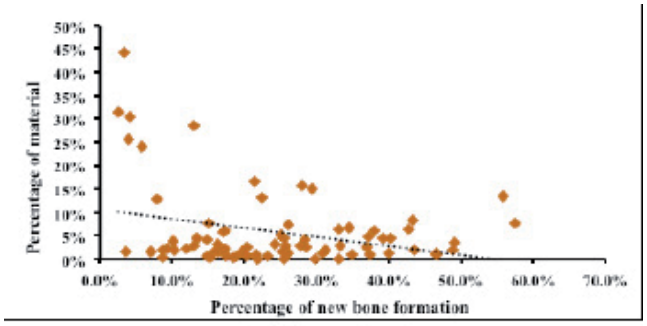

Fig. 7: Correlation between the percentage of newly formed bone and the residual material.

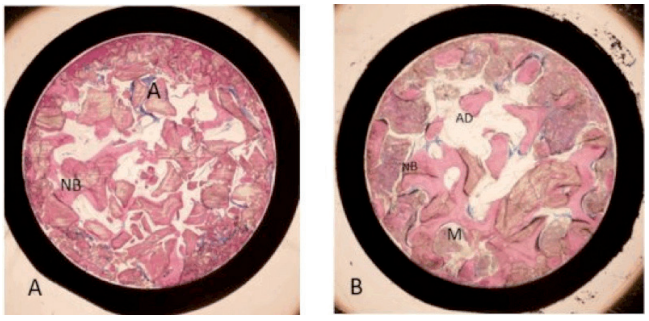

Fig. 8: Histological sections at 6 months post-operative maxillary sinus floor augmentation with DBBM (2x). A: Intimate bone (NB) to graft contact. B: Bone marrow (AD) in the center of the biopsy.

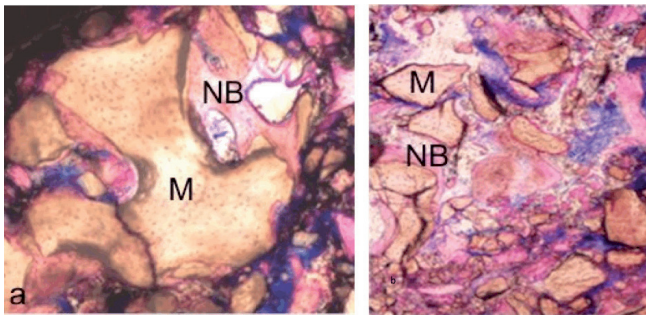

Fig. 9: DBBM particles (a) (x10) surrounded by newly formed bone (b) (x4).

absence of graft leakage. In fact, when the procedure is well conducted, the incidence of sinus membrane perforation using the transalveolar approach is generally low and is accompanied by a lower complication rate than the lateral window technique [21].

An important issue that may influence osseointegration, as well as early and long-term implant survival, is tobacco smoking [22, 23] (22,23). Tobacco smoking is a well-established risk factor that decreases local vascularization, as well as having adverse impacts on immune, inflammatory and healing responses in the periodontal and peri-implant tissues [24, 25]. (24,25)

On the other hand, tobacco smoking may negatively modify bone-to-implant contact, bone filling and bone density due to a constraining effect on the proliferation of precursor cells and vascularization of the implant site [26] (26).

The detrimental effect of smoking on implant survival in sites with sinus floor augmentation was clearly demonstrated in a systematic review performed by Chambrone et al. [27] (27). Over half of the individual studies revealed a greater implant failure rate in smokers, and the pooled estimates derived from the number of failing implants as a proportion of the total number of implants placed identified an 87% increase in the risk of loss of implants placed in grafted sinuses in smokers. Conversely, the detrimental effect of smoking was not confirmed when only prospective data were assessed.

Conclusion

Within the limitation of this study, it can be concluded that the use of the modified osteotome sinus floor elevation technique for future site development in edentulous sites with less than 2 mm residual bone height using DBBM as a graft material is a valid technique in the regeneration of bone volume in the subantral region. This procedure seems promising with low values of residual bone height and further long-term research with a larger study sample is necessary.

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