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Alveolar ridge augmentation with the Bone Into Bone technique: a histological and histomorphometric analysis

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ABSTRACT

Aim Barriers made of cortical bone of heterologous origin are now used as a possible substitute of non-resorbable membranes and bone blocks for the regeneration of bone defects. This report analyzes the efficacy of these barriers, when they are inserted in a surgical slot, vestibular to the defect, using the new Bone into Bone (BiB) technique.

Methods A group of 20 patients were treated with the BiB technique, and 32 implants were placed. Bone samples were collected with trephine burs 8 months after surgery and submitted to histological and histomorphometric analysis. The linear horizontal and vertical changes between pre-operative and post-operative radiographs were measured on the CT Scan.

Results A mean width gain of 2.36 ± 0. 0.69 mm and a mean height gain of 3.13 ± 0.90 mm was recorded. The biomaterial was almost completely resorbed, and it accounted for 7.39% ± 7.70 of the samples.

Conclusion Comparing the current results with those of other investigations, the BiB technique is an efficient alternative to other regenerative approaches, as it provides several advantages, both in terms of reduced morbidity and ease of the procedure. More reports are needed to evaluate the stability of the regenerated bone and to directly compare this technique to other, more commonly used, regenerative procedures.

INTRODUCTION

The presence of a bone defect in an edentulous area requires the restoration of the bone structures for the subsequent placement of a dental implant. Both guided bone regeneration (GBR) and bone blocks grafts are usually considered predictable procedures; in GBR (1) the surgeon applies a membrane over a bone defect filled with osteoconductive material, and the regenerative abilities of the bone clot can be employed to fill the defect with newly formed bone, that has been proven capable of bearing the load of an implant-supported restoration (2). The first membranes adopted for this procedure were non resorbable, titanium-reinforced, polytetrafluoroethylene membranes, which can be modeled following the surgeon's needs and the anatomy of the defect. While these membranes have shown a high regenerative capacity (3), they are also accompanied by a high risk of complications (4), which are also extremely difficult to treat, and can jeopardize the procedure (5,6). Other solutions have been adopted for this procedure, resorbable membranes are one alternative as they are easier to adapt to the defect and are resorbable, so there is no need for a second surgery to remove the membrane.

The introduction of barriers made of cortical bone of heterologous origin (7,8), which physicochemical properties have been appropriately studied and compared to several others membranes (9), could substitute the application of non-resorbable membranes and autologous bone block as a means to create and maintain a space for particulate bone graft placement. These resorbable laminas can be stabilized on the surgical site via fixation screws (10), or various adhesives (11): in order to insert fixation screws the flap has to be extended apically to the defect, and the application of an adhesive involves a second, human-derived biomaterial, with an increase in costs and complexity of the procedure.

The present study describes the Bone into Bone (BiB) technique, that involves the application of a cortical lamina (Lamina, Roen, Turin, Italy) inserted into a surgically created slot on the vestibular aspect of the defect, without adopting other means of fixation. The aim of this investigation is to assess the efficacy of this

procedure at an 8 month follow-up, with a histological and histomorphometric analysis, accompanied by a radiographical evaluation of the ridge dimension.

MATERIALS AND METHODS

All patients were informed of the various steps included in the protocol and provided full informed consent. The Declaration of Helsinki guidelines, as revised in 2013, were followed in the present investigation. Study subjects were recruited between September 2018 and January 2019; the study was performed between February 2019 and September 2020.

Inclusion/exclusion criteria

Inclusion criteria were as follows.

1. At least 18 years old.
2. Good oral hygiene.
3. Bone augmentation procedure required, in order to achieve adequate horizontal and/or vertical dimension of the edentulous ridge to place one or more implants in a prosthetically-guided position.

Exclusion criteria were as follows.

1. Systemic diseases that could impair normal healing processes (such as diabetes).
2. Smoking habits (>10 cigarettes/day).
3. Bruxism.
4. Non treated periodontal diseases.

Pre-treatment

Before the surgical procedure, all patients underwent a preliminary preparatory phase that included mechanical debridement (with ultrasonic and, if needed, manual instruments) so that no pocket with a PPD ≥5mm was present. Data on the subjects' age, gender, and dental status were obtained and their dental history was updated immediately before the surgery.

Surgical procedure

All patients underwent an antibiotic prophylaxis 1 hour before surgery with 2 g Amoxicillin + Clavulanic Acid in tablets (Augmentin 875 mg/125 mg, GSK, UK) and a 1-minute mouthrinse with 0.20% chlorhexidine digluconate, immediately before surgery. After administration of local anesthetic, a crestal incision in the edentulous area, continuing with an intrasulcular incision on the closest adjacent teeth on both sides of the defect, was made and a vestibular full-thickness flap and a lingual full-thickness flap were raised. Using a 6 mm bur, mounted on a surgical handpiece, a 2 mm slot was made on the vestibular aspect of the defect (Fig. 1), to insert and stabilize the lamina, and in order to promote blood clot formation, some perforations were made on the cortical bone of the edentulous area with a round bur on a surgical handpiece. A soft lamina, which has a semi-rigid consistency and should not be hydrated before its application, was then modeled with sterile scissors and inserted in the surgically prepared slot (Fig. 2), creating a space where the osteoconductive bone material could be inserted. The space between the lamina and the lingual wall of the defect was filled with a 1:1 mixture of porcine collagenated heterologous bone and autologous bone particles, retrieved from the surgical area, apically to the bone defect, with a bone scraper. The grafted area was then covered with a resorbable collagen membrane, stabilized without any conventional means of fixation, and the flap was closed with a single line of interrupted sutures that were removed 7 days after surgery.

After 8 months, a full-thickness flap was elevated, and implants were placed following a submerged healing protocol. Hard tissue biopsies were taken using a 4-mm-diameter trephine bur, limiting the procedure to the augmented bone. A unique identification number was assigned to each biopsy specimen.

Subjects were prescribed post-operative antibiotics for 6 days (Amoxicillin 0.75 g + Clavulanic acid 0.25 g 2/die) and anti-inflammatory therapy (Ibuprofen 400 mg 3/die days 1-2, 2/die, 400 mg/2 die days 3-4).

After a healing time of three months, implants were uncovered, and healing abutments were placed. After two weeks, an impression was taken using alginate impression material and stock trays to manufacture a custom impression tray. The final impression was taken

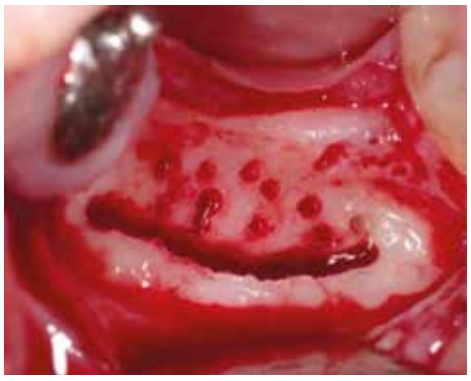

FIG. 1 The slot created for the insertion of the lamina. The cortical perforation, needed to induce bleeding, can be seen.

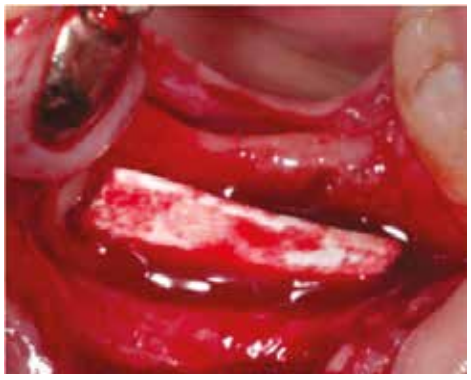

FIG. 2 The lamina inserted and stabilized in the apical slot.

using the open tray technique and polyether impression material.
Veneered zirconia restorations were fabricated by the technician and cemented on stock-abutments, previously screwed to the implant fixture with a torque control ratchet set at 25 N/cm, with a glass ionomer cement; carefully removing any excess cement with an explorer.

Histological and histomorphometric analyses

Bone biopsies were fixed in 10% phosphate-buffered formalin, followed by decalcification in a hydrochloric acid/formic acid solution (4/5%). After decalcification, the samples were dehydrated in a series of alcohol baths and then embedded in paraffin. 5-µm-thick histological sections were then prepared and stained with hematoxylin/eosin.
The slides were then subjected to digital scanning at various magnifications. Histomorphometric analyses were conducted using the ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland) to evaluate the presence and characteristics of the newly formed bone, of the remaining grafted material, and the integration of the grafted material with the surrounding tissues. On each slide the percentages of bone, residual biomaterial, and soft tissue (either bone marrow or unmineralized connective tissue) were measured.

Radiographic analysis

A post-operative CT scan was taken 8 months after the regenerative procedure and before the second surgery; sagittal cuts of the pre-operative and post-operative CT scan were superimposed, using the adjacent teeth as reference. On the preoperative radiograph [T0], presurgical crestal width (W0) and presurgical crestal height (H0) were measured linearly near the planned implant insertion site. Keeping the same palatal and apical reference points, the postsurgical (T1) crestal width (W1) and postsurgical crestal height (H1) were measured linearly on the postoperative radiograph (12). Horizontal bone gain (WG) and (HG) vertical bone gain were then measured as the difference between W1 and W0, and H1 and H0. When more than one implant was planned, only the greatest horizontal and vertical bone gain were obtained and used for the analyses.
All linear measurements were taken with coDiagnostiX (coDiagnostiX, Dental Wings, Chemnitz, Germany) software, rounded to the nearest 0.1 mm. Both the pre-operative and post-operative CT scan were taken with the CBCTNewtom 5G XL (Cefla s.c., Verona, Italy).

Statistical analysis

The primary outcome was to radiographically observe the stability of the regenerated bone at a follow-up of 8 months. The secondary outcomes were to observe the histological aspect of these tissues, and to analyse their composition.
Continuous variables were presented as mean±standard deviation, while qualitative variables were described as absolute and relative frequencies.
In the statistical analysis, the bone defect, as defined by W0, H0, W1 and H1, was considered as the statistical unit. A comparison of the differences in width and height between T0 and T1 was conducted. Given their non-normal distribution, the paired samples Wilcoxon signed rank test was used, and the significance threshold was set at $p<0.05$. Statistical analysis was performed with R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

Mean age	60.18 years
Gender	
Male	9
Female	11
Treated Site	
Mandible	11
Maxilla	9
Misch Classification	
C	7
Ch	5
Cw	8

TABLE 1 Sample characteristics.

RESULTS

Baseline characteristics of subjects, and classification of bone defects according to Misch et al. (13) are shown in Table 1.
In the present study, 20 patients, 9 men and 11 women, aged between 52 and 76 years, were treated with the Bone into Bone technique, and 32 implants were placed by a single experienced surgeon (ML).
The histological images were evaluated; in only a few slides remaining granules could be discerned, and they were largely resorbed. No signs of infection were present, and the biomaterial was perfectly integrated (Fig. 3). The lamina was not observable neither clinically nor on the histological images. The bone morphology showed a mature, mineralized bone, with a lamellar structure with different thicknesses. Also bridges of newly formed bone could be seen, surrounded by non-mineralized, soft tissue, mostly bone marrow (Fig. 4). Similarly to other regenerative procedures, a higher percentage of new bone could be observed near the apical portion of the defect than near its coronal extension. Mineralized mature bone composed 30.28% ± 5.32 of the samples, while the percentage of remaining, not yet resorbed biomaterial was 7.39% ± 7.70. The remaining part of the samples was occupied by soft tissues (either bone marrow

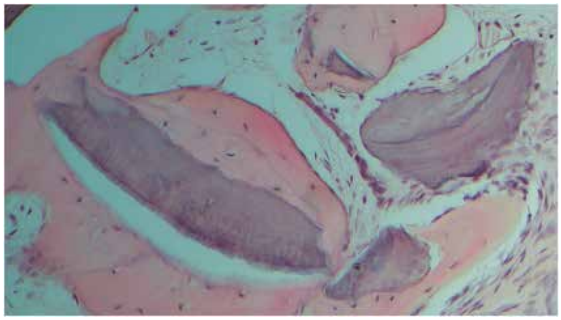

FIG. 3 The bone samples obtained 8 months after surgery. A particle of unresorbed biomaterial integrated into new, mature bone.

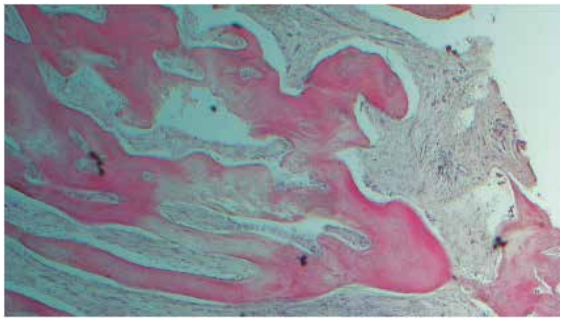

FIG. 4 The bone samples obtained 8 months after surgery. Bridges of bone surrounded by soft tissue.

or unmineralized connective tissue), which accounted for 62.61% ± 11.13 of the tissues.
The mean width of the ridge ranged from a pre-operative value of 4.89 mm to a post-operative value, measured 8 months after surgery, of 7.25 mm with a mean WG of 2.36 ± 0.69 mm while the mean height ranged from a pre-operative value of 8.01 mm to a post-operative value of 11.15 mm with a mean HG of 3.13 ± 0.90 mm; these differences were deemed to be statistically significant ($p<0.05$) (Table 2). All patients healed uneventfully, and no complications were recorded.

DISCUSSION

Modern Dental implants have high success rates in terms of both function and esthetics (14–16); edentulous areas are, however, often characterized by a severe bone atrophy due to the continued bone resorption that occurs after tooth loss (17). Although many studies have confirmed the reliability of short and ultrashort implants in the atrophic maxilla (18), and in the atrophic mandible (19), a three-dimensional reconstruction is often needed to obtain a restoration that is satisfactory in terms of esthetics and function (20,21). This is especially true in the posterior mandible, because of the inferior alveolar nerve (22), where most long-term edentulous patients do not have adequate bone volume so that this area typically requires bone augmentation.
In cases of three dimensional ridge defect, a non-resorbable membrane, with a supporting titanium framework, has usually been advocated as necessary (23); the adoption of these membranes is usually associated with a significant risk of membrane exposure, which can lead to contamination and infection of the biomaterials and could potentially harm the whole regenerative process, even if appropriately treated (5). Additionally the membrane has to be removed with a second surgery, notably increasing the impact on the patient.
As an alternative to these procedures, resorbable cortical barriers have been introduced. Their efficacy has been proven, both in the regeneration of vertical and horizontal defects by several studies (24,25), and in sinus floor augmentation (26). Festa et al. (27) have also adopted this membrane in socket preservation, and its application, combined with a cortico-cancellous porcine bone xenograft, can reduce hard tissue resorption after tooth loss.
Our current results are in line with these reports, as in the enrolled subjects a significant regeneration of the ridge in both dimensions was observed, and at the re-entry surgery the lamina appeared fully integrated with the surrounding soft tissues and native bone, similarly to what other reports have stated (28).
This paper is also, to the best of our knowledge, the first one to provide histological and histomorphometric analysis of the efficacy of lamina in human subjects: a previous histologic evaluation conducted in the canine model (29), where an extraction socket was filled with collagen-modified porcine bone graft material and with the cortical membrane positioned on the vestibular side of the defect to restore the buccal contour, showed that new bone had formed and that the lamina was slowly being resorbed and integrated in newly formed bone.

Variables (mm)	Mean (N=20)	Standard deviation	Standard error	95% Confidence interval
HG	3.13	0.90	0.15	2.81 3.45
WG	2.36	0.69	0.12	2.11 2.61

HG= Height Gain (postoperative crestal height- preoperative crestal height); WG= Width Gain (postoperative crestal width-preoperative crestal width)

TABLE 2 Radiographically recorded changes of the regenerated sites.

Our results are quite similar, apart from the differences between the two models; the lamina, even if only stabilized thanks to the vestibular slot, managed to provide the needed stability to the graft that allowed for the regeneration of the defects; the lamina was completely resorbed after 8 months and could not be clinically observed at the second surgery. The application of the heterologous lamina provides several advantages when compared to other regeneration techniques; the procedure is much easier than other regenerative approaches, particularly the ones that require a donor site and the retrieval of a bone block, making this procedure accessible for a broader number of operators.
In previous reports, the lamina was stabilized with fixation screws; in order to insert these screws, the flap has to be extended apically to expose sound bone where to fixate the lamina (7). As in the surgical technique used, the lamina is stabilized thanks to a surgically created slot, and less particulate bone material is inserted, so there is no need for such extension and passivation of the flap; also, the bleeding of the slot immediately reaches the lamina, and therefore the bone substitute that is positioned underneath, thus improving the vascularization of the graft.
Therefore, the current technique notably improves the morbidity associated with other regenerative techniques and with techniques that adopt fixation screws to stabilize the lamina, as it also eliminates the need of a re-intervention, and it notably reduces the risk of dehiscence and membrane exposure that is associated with non-resorbable membranes; they do carry some risk of exposure, but we recommend to only remove the lamina if there is a clear infection of the graft, because its consistency allows to achieve a complete second intention healing of the wound.
This report has however several limitations: given the small dimension of our sample, the absence of a control group, and the short follow-up, it is possible that the real capabilities of this technique are quite different from what was encountered. Another limitation is related to our 2D volumetric measurement method as we investigated changes in width and height providing linear values; we therefore advocate for more studies where these dimensions are volumetrically measured and compared.

CONCLUSION

The present report shows that stabilizing the heterologous lamina only with a surgically created slot vestibular to the defect gives good clinical and histological results; further studies should be planned to evaluate the limitations of this technique when applied to severely deficient maxillary of mandibular ridges, and to compare this technique to other procedures commonly adopted in the treatment of bone defects.

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The authors report no conflicts of interest related to this study.

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