

## VOLUMETRIC EVALUATION OF BONE GRAFT IN GUIDED BONE REGENERATION USING P4HB MESH AND TITANIUM MESH. COMPARATIVE STUDY

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### ABSTRACT

**Aim of the study** The aim of the present study was to compare the stability of bone graft volume in guided bone regeneration procedures in patients with alveolar process atrophy who were treated using titanium mesh and resorbable polymeric mesh made of poly-4-hydroxybutyrate (P4HB). **Materials and methods** A randomized clinical study was conducted on 70 patients, divided into two groups. The study group consisted of 35 patients treated with guided bone regeneration surgery using resorbable P4HB mesh, while the control group comprised 35 patients with similar pathologies who underwent guided bone regeneration using a titanium membrane. A CT scan was performed 2-3 weeks after the initial procedure to calculate the added/augmented graft volume (VGA-added/initial) using Slider 3D software. The second surgical stage was performed at least 8 months later, which included a CT scan to measure the volume of regenerated bone tissue (VON) using the same software. Data were analyzed using the R Studio software (Version 2024.04.1+748). **Results** The study found that the volume of the augmented graft did not undergo significant changes during the regeneration period in either group, with volumetric values showing a close correlation throughout the same period. **Conclusions** The polymeric mesh made of P4HB provides three-dimensional stability to the bone graft during healing, comparable to that of titanium mesh.

**Keywords** titanium mesh; Poly-4-hydroxybutyrate (P4HB) mesh; guided bone regeneration

### INTRODUCTION

Alveolar ridge resorption and restoration are major concerns in dental practice, prompting further research in this area. To meet the need for adequate peri-implant bone tissue, various restoration methods have been developed. These include using bone blocks from intraoral and extraoral donor sites in the same patient, autologous bone lamellae as described in the Khoury method, directed bone expansion, directed bone elongation osteogenesis, and guided bone regeneration (GBR). According to D. Buser, approximately 50% of placed dental implants require a surgical bone restoration procedure

to ensure sufficient bone volume for long-term stability [1, p. 17].

Currently, guided bone regeneration is an excellent option for restoring lost bone volume when dental implants are needed [1]. According to Retzepi M. (2010), using a membrane for tissue separation is essential for guided bone regeneration to succeed, whether bone grafts or other materials are used. Membranes or meshes enable proper separation of soft tissues from the graft and provide mechanical support to maintain the three-dimensional structure of regeneration [2]. For effective performance, the meshes used in guided bone regeneration must meet several requirements, including

biocompatibility, non-toxicity, non-allergenicity, and the ability to support adjacent soft tissues [3, 4]. Currently, the most common separation meshes are made of PTFE (polytetrafluoroethylene) and titanium, which have certain disadvantages, including the need for additional surgical procedures to remove them. These procedures can hinder wound healing and complicate patient recovery. Furthermore, titanium meshes are rigid and can sometimes cause dehiscence when exposed in the oral cavity, negatively impacting treatment success [5,6,7,8]. To address the limitations of PTFE and titanium meshes, we have developed a new technique for guided bone regeneration of pre-implant alveolar ridges, which involves using a resorbable polymeric surgical mesh with a barrier and retention function made of poly-4-hydroxybutyrate (P4HB).

### AIM OF THE STUDY

Comparative assessment of bone graft volume stability in guided bone regeneration procedures in patients with alveolar process atrophy, who were treated using titanium mesh and resorbable polymeric mesh made of poly-4-hydroxybutyrate (P4HB).

### MATERIALS AND METHODS

We conducted a randomized clinical study involving 70 patients with alveolar process bone defects who were referred for implant-prosthetic rehabilitation and required guided bone regeneration surgery to repair the bone defect. The study was carried out from 2019 to 2024. The research group was divided into two: a study group and a control group, each consisting of 35 patients.

The study group included 35 patients with atrophy of the alveolar processes of the jaws who underwent guided bone regeneration surgery using the resorbable P4HB mesh. A grafting material composed of a mixture of

mineralized bovine bone and autologous bone in a ratio of at least 4:1 was employed. To improve shaping and facilitate adaptation during surgery, this mixture was secured with autologous platelet concentrate. The resorbable P4HB mesh, which functions to maintain and stabilize the augmentation, was fixed to the bone bed with 4-6 titanium pins placed both on the oral and buccal sides.

The control group also included 35 patients with the same conditions. In these patients, guided bone regeneration surgery was performed using a technically perforated titanium membrane. The membrane fixation method and grafting material were the same as those used in the study group.

### *Surgical intervention protocol*

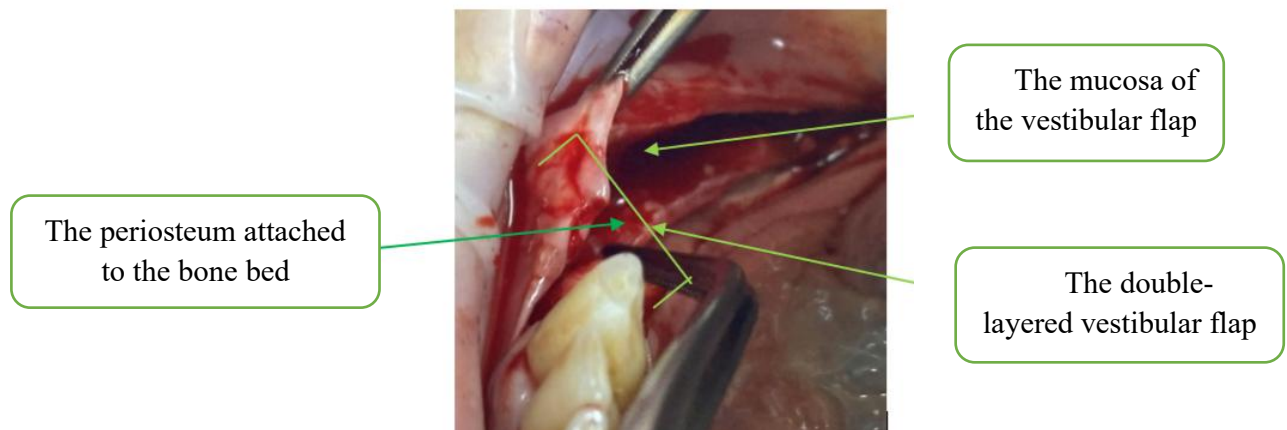
The patient's preparation for surgery included preoperative antimicrobial treatment with azithromycin 500 mg, taken as two tablets orally. As oral antiseptic solutions, Polividone iodine 10% and chlorhexidine (CHX) 0.05% solution were used. The perioral skin was first disinfected with a 70% alcohol solution, then with a 10% Povidone-iodine solution. Afterward, the patient was covered with a sterile drape. Anesthesia for all patients in the study was administered using 4% articaine with epinephrine 1:100,000. The primary anesthesia method was the peripheral truncular approach, complemented by loco-regional infiltration through plexal infiltration to reduce bleeding without deforming the structure of the future flap.

### *Flap design*

The creation of the flap to cover the guided bone regeneration elements was the same for all patients and was performed according to the technique and recommendations of Nada and the authors in 2020, who describe a double-layered

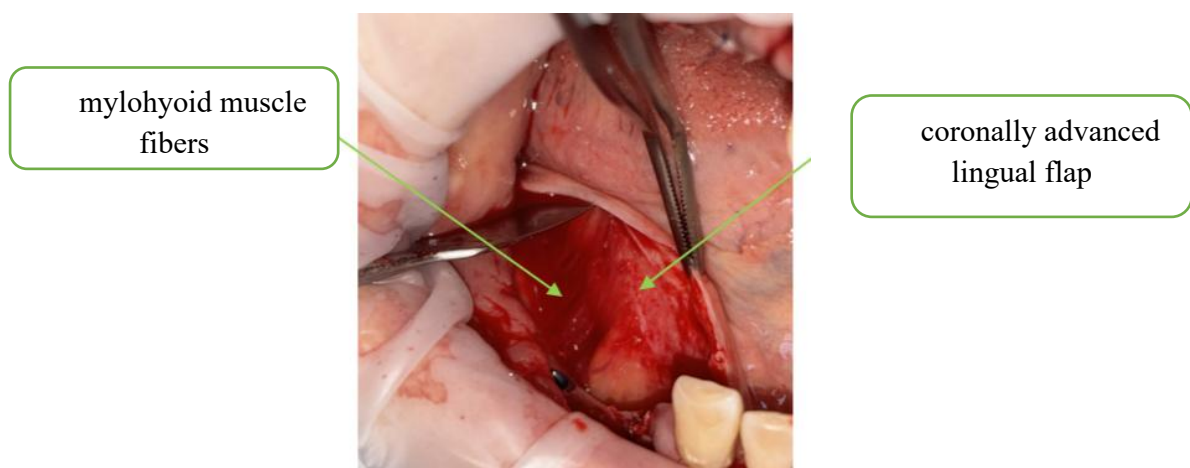
vestibular flap, consisting of one layer of periosteum and another of gingivomucosa, avoiding vertical incisions. Figure 1 shows a terminal breach on the right in the mandible

with the creation of a double-layered vestibular flap composed of the periosteum attached to the bone bed and the gingivomucosa lifted from it.



**Figure 1. The creation of a double-layered vestibular flap**

In the case of the lingual flap, it was performed by lifting the lingual gingivomucosa from the alveolar ridge, along with lifting and exposing the mylohyoid muscle fibers. These fibers were detached from the mucosa using a blunt instrument until enough coronal advancement was achieved. In figure 2, we can see the creation of a coronally advanced lingual flap with atraumatic detachment of the mylohyoid muscle fibers.



**Figure 2. Coronally advanced lingual flap**

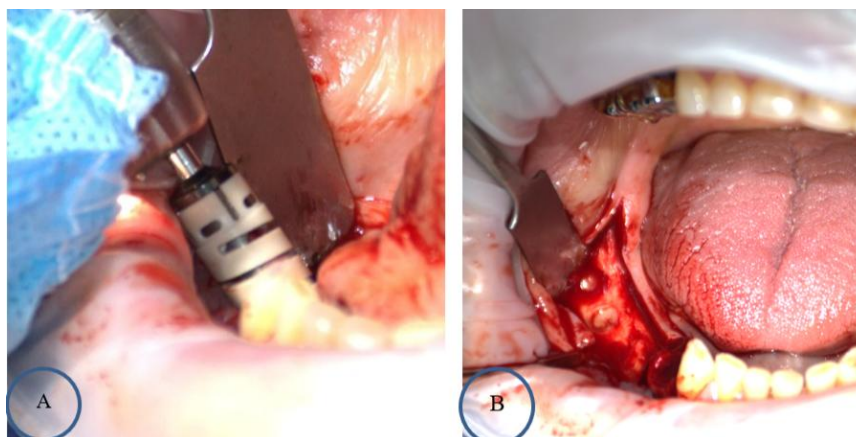
#### ***The preparation of the bone bed***

The preparation of the bone bed involved the next stage of the procedure, which was to ready it for augmentation. After deperiosteation and flap creation, the bone surface was carefully smoothed using a bone scraper. With a physiodispenser equipped

with a bur: No. 1 or a pilot bur, the bone collector bur "Triple Blade Bone Collect," or the disposable scraper "SafesScraper Rtwist," several holes or openings were drilled into the cortical surface in the areas of the bone defect. The depth of these holes depended on

the cortical thickness and the presence of punctate bleeding. The aim was to reach the bone marrow, enabling the migration of pluripotent cells and the collection of the patient's bone marrow-derived cells. Figure 3

illustrates the surgical process of creating communications (figure 3B) with the bone marrow using the "Triple Blade Bone Collect" bone cutter (figure 3A).



**Figure 3. Preparation of the augmentation site and collection of bone chips using the Triple Blade Bone Collector.**

#### *The preparation of the bone graft*

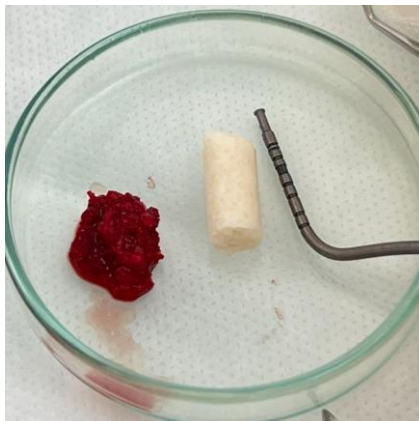
The preparation of the bone graft for the augmentation procedure involves creating a mixture of mineralized bovine bone (OBM) and autologous bone sawdust, with a ratio of at least 4:1, a protocol used throughout the entire research group. The autologous bone sawdust was obtained by drilling holes into the bone marrow or creating neoalveoli during implant insertion in the same session. When dental implants were not placed in the same session and the autograft was insufficient, bone was collected from the

cortex of the augmentation site using the "Triple Blade Bone Collect" device, a reusable bur with three blades and a shock-absorbing housing designed to collect bone sawdust (figure 4 A). Alternatively, the disposable "SafesScraper Rtwist" instrument (figure 4 B) was used; it consists of a handle, a bone sawdust collector, and a semicircular blade that, when moved more aggressively along the bone cortex surface, collects bone tissue in the form of sawdust. This instrument was employed mainly in cases where the augmentation site had a thin cortex.



**Figure 4. A. Triple Blade Bone Collect B. Disposable Bone Harvesters "SafesScraper Rtwist"**

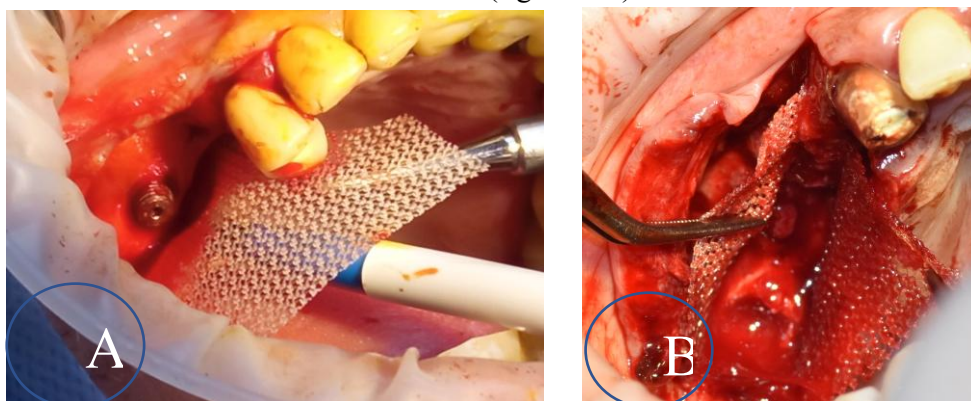
For easier shaping and handling, the grafting mixture was stabilized with an autologous blood platelet concentrate, resulting in a mixture composed of autologous bone, mineralized bovine bone, and autologous blood platelets. This mixture had a gelatinous and semi-solid consistency that was easily molded during augmentation (figure 5).



**Figure 5. Mixture composed of autologous bone, mineralized bovine bone, and autologous blood platelets**

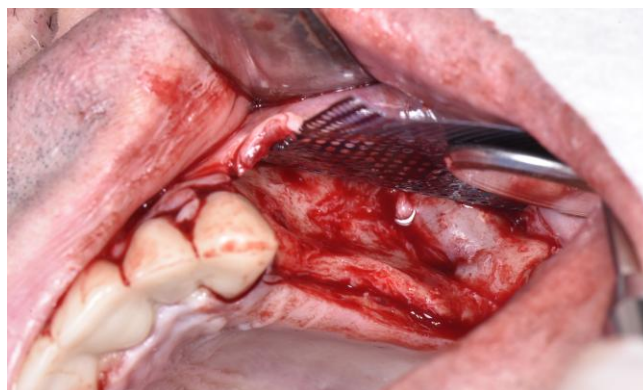
#### ***Mesh fixation***

In the case of the study group where the resorbable mesh made of poly-4-hydroxybutyrate (P4HB) was used as a separation and maintenance device, it was prepared differently depending on the degree of atrophy of the alveolar processes. The mesh was prepared as follows: in grade IV and V atrophies, when there is both vertical and horizontal resorption, the dental implants were inserted immediately and partially into the native bone. The resorbable mesh was fixed on each edge of the defect, oral and buccal, with 4-6 titanium pins (figure 6 A). In grade VI atrophies, where the defect also includes basal bone atrophy, the same procedure was followed, except that the dental implants were not inserted in the same session (figure 6 B).



**Figure 6. Fixation of mesh from lingual and buccal PH4B, image A – atrophy grade V, image B – atrophy grade VI**

In the control group, where a titanium membrane was used as a maintenance and stabilization device, the regeneration site was prepared similarly to the study group. Regardless of the type of atrophy, the titanium membrane was shaped and adapted with surgical scissors during the operation after measurements taken on the operating field with a graduated probe. Its primary fixation depended on the case and the operator's convenience, and it was also fixed with 4-6 titanium pins on the oral or vestibular side. (figure 7)



**Figure 7. Adapting the titanium device and fixing it with titanium pins**

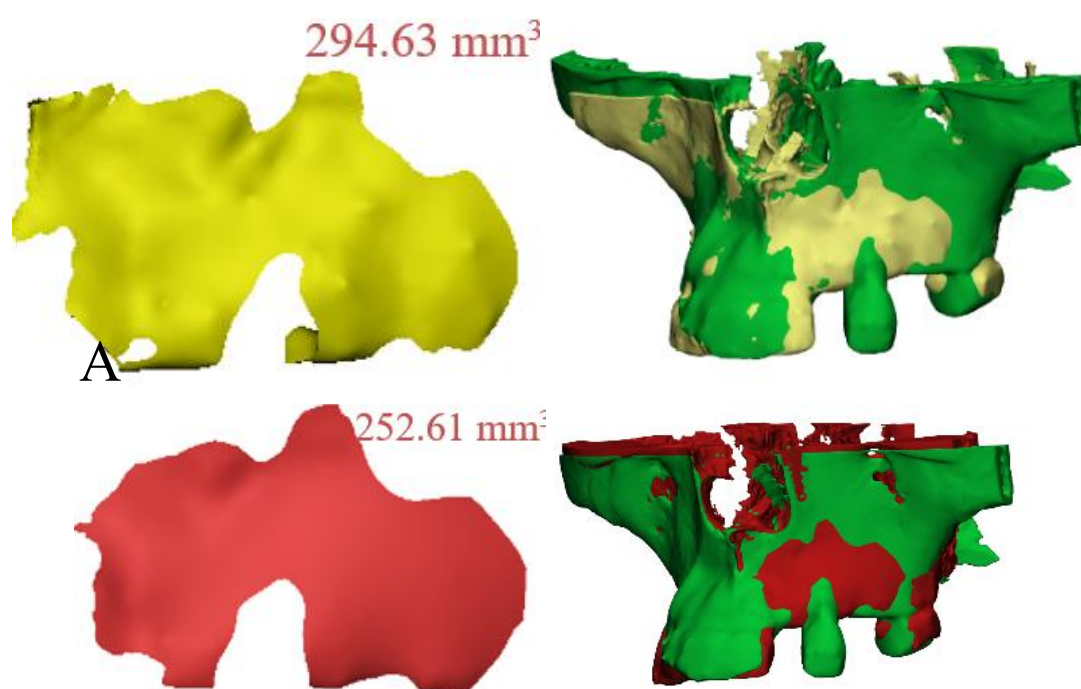
### ***The gingivomucosal suture***

The gingivomucosal suture and primary wound closure were performed in three layers, as recommended by Nada et al. [9]. Therefore, to reduce tension on the vestibular flap, the respective periosteum was first sutured to the oral mucoperiosteal flap using mattress sutures, making this the first step in decreasing tension. The second suture level also used mattress sutures without tension in the thread; however, this level was tensioned until the gingival edges of the flaps were in contact. The third suture level involved sutures for final wound closure, consisting of either interrupted or continuous sutures.

Home treatment included continued antibiotic therapy with Azithromycin 500 mg once daily for at least 5 days and up to 7 days, depending on the extent of the procedure. For pain management, patients took NSAID tablets, specifically Naproxen Na 500 mg. Oral rinses with 0.05% Chlorhexidine (CHX) solution were performed three times daily until the last suture was removed.

Approximately 2-3 weeks later, depending on the healing of the gingivomucosa and the patient's condition, a computed tomography scan was performed. During this scan, the added or augmented graft volume (VGA-added/initial) was calculated using Slider 3D software. (figure 8A).

The second surgical stage was performed at least 8 months after the first and included both clinical and paraclinical evaluations. The clinical stage involved inspecting and assessing the soft tissue integrity in the regeneration area, while the paraclinical stage involved conducting a radiological examination, specifically a computed tomography scan, where the condition of the regenerated bone tissue was visualized and measurements necessary for the research were taken, particularly the volume of regenerated bone tissue (VON), using the same software (figure 8B).



**Figure 8** Graphical representation of the calculation of the added graft volume (VGA) - image A and the volume of new bone obtained (VON) - image B

The collected data were processed using R Studio (Version 2024.04.1+748), enabling effective statistical analysis. For numerical variables, the following descriptive statistics were calculated: minimum, maximum, mean with standard deviation, median with interquartile range (IQR). The comparison of numerical variables between the control group and the study group was conducted using non-parametric tests for independent and dependent samples (Mann-Whitney, Wilcoxon tests), depending on the relationship between the groups. Visualization included box plots, jitter plots, and violin plots combined. Correlation analysis between numerical variables was performed using Spearman's  $\rho$  test, and the results were adjusted for multiple comparisons using the Holm method. This analysis was presented using a heatmap (CI 95%,  $p = 0.05$ ).

## RESULTS

In the study, 140 computed tomography scans from 70 patients who underwent bone reconstruction surgery were analyzed. Using Slider 3D software, the volumetric values of the added or initially augmented graft (VGA) and the volumetric values of the newly obtained or regenerated bone tissue (VON) were calculated. The data were collected, statistically analyzed, and visually represented.

### *General characteristics of the variable augmented graft volume $\text{cm}^3$ (VGA) per study*

Table I shows the overall data for the graft volume added at the growth site, measured immediately after the first CBCT scan using the free software program Slicer 3D. This measurement in 70 patients revealed a mean of  $2.0 \text{ cm}^3$  (AI 0.6), a median of  $2.0 \text{ cm}^3$  (AI 0.9), a minimum of  $0.9 \text{ cm}^3$ , and a maximum of  $3.2 \text{ cm}^3$  (95% CI 1.8, 2.1).

**Table I. The representation of VGA variables**

| Variables            | N = 70 <sup>1</sup>                | 95% CI <sup>2</sup> |
|----------------------|------------------------------------|---------------------|
| VGA, cm <sup>3</sup> | 2.0 (0.6)<br>2.0 (0.9)<br>0.9; 3.2 | 1.8, 2.1            |

**General characteristics of the variable volume of newly regenerated bone per study cm<sup>3</sup> (VON)**

This characteristic was measured after the healing period using CBCT and the same software, resulting in a mean volume of newly regenerated tissue of 1.9 cm<sup>3</sup> (AI 0.6), a median of 1.8 cm<sup>3</sup> (AI 0.8), a minimum of 0.9, and a maximum of 3.1 (95% CI 1.7, 1.7,2.0) (Table II). Based on the data in this table, we can see that the value of this characteristic decreased insignificantly.

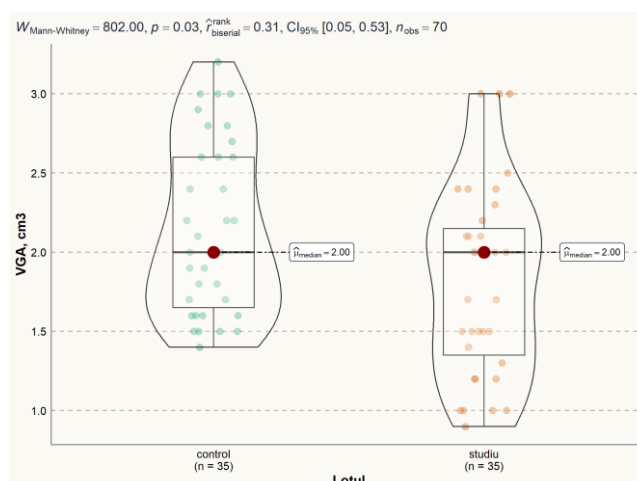
**Table II. The representation of VON variables**

| Variables            | N = 70 <sup>1</sup>             | 95% CI <sup>2</sup> |
|----------------------|---------------------------------|---------------------|
| VON, cm <sup>3</sup> | 1.9(0.6)<br>1.8(0.8)<br>0.9 3.1 | 1.7, 2.0            |

**The comparison of the added/augmented graft volume in cm<sup>3</sup> (VGA)**

Between the two research groups, a Mann-Whitney test (figure 9) revealed a significant statistical difference (Mann-Whitney = 802, p=0.031). The research group had a median of 2.0 with AI=1, while the study group also had a median of 2.0 but with AI=0.8. The clinical effect size, estimated using the biserial rank test, was 0.31 with a 95% confidence interval (CI) of 0.05 to 0.53, indicating a moderate effect. However, the wide CI (ranging from 5% to 53%) suggests difficulty in determining the effect size across

the entire population, as it encompasses all possible variations. Additionally, after applying the Hochberg correction for multiple comparisons, the p-value adjusted to 0.3, which is not statistically significant. From a clinical perspective, according to Istvan Urban, the key factor is not the augmented graft volume but the graft content. He notes that vascularization and biocompatibility are more important than resorption time, especially for regenerations larger than 4 mm. In such cases, the ratio of mineralized bovine bone (xenograft) to the patient's own bone (autograft) should be at least 1:1. [10]

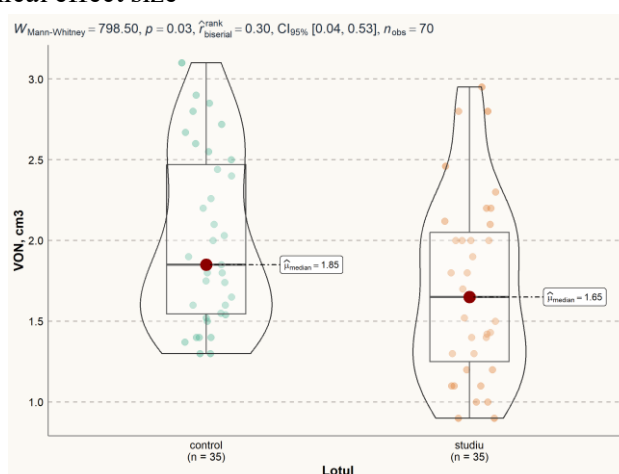


**Figure 9. Boxplot/violin plot, completed with (dots) jitter plot for the variable Initial Graft Volume Added/Augmented (VGA) expressed in cm<sup>3</sup>. Mann-Whitney test**

### Comparative evaluation of the variable of the volume of newly obtained/regenerated bone tissue cm<sup>3</sup> (VON)

The research groups were analyzed similarly using the Mann-Whitney test (figure 10). This parameter was calculated with the same software at least 8 months apart, before the second surgical intervention. In evaluating this parameter, we also found statistical significance with a p-value of 0.03 and a Mann-Whitney test score of 798. The control group had a median of 1.9 with an AI of 0.9, while the study group had a median of 1.65 with an AI of 0.8. The clinical effect size

from the biserial rank test was 0.30, which is considered moderate, with a 95% confidence interval (CI) of 0.05 to 0.53. However, as with the (VGA) parameter, the 95% CI is very wide, and this parameter cannot cover several possible levels. Additionally, after adjusting for multiple comparisons using the Hochberg method, the p-value increased to 0.3, indicating no statistical significance. From this graph, we see that the (VON) parameter values in the study group showed a more noticeable decrease compared to the control group. Clinically, this did not interfere with the second surgical stage.



**Figure 10. Boxplot/violin plot, completed with (dots) jitter plot for the variable New Bone Volume (VON) expressed in cm<sup>3</sup>. Mann-Whitney test**

### Analysis of the correlation between VGA and VON

The analysis was conducted throughout the research study. Data calculation and interpretation were performed using the Spearman test, supplemented by corrections for multiple testing with the Holm method, where association values of the VGA and VON parameters of 0.96 were obtained in 70 cases, with the maximum being 1. (figure 11).

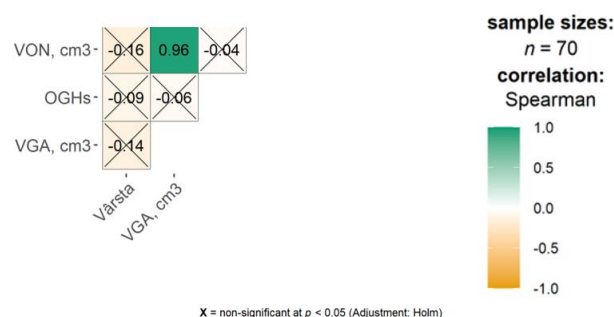


Figure 11. Analysis of correlations between variables in research. Spearman test

### DISCUSSIONS

The first membranes with barrier function, used in bone growth in the jaw, were made of polytetrafluoroethylene (PTFE). Due to their low mechanical strength, titanium strips were later added to their structure to provide rigidity and three-dimensional stability, preventing collapse (Buser, D 1993) [11]. It was subsequently demonstrated that these membranes have several disadvantages, particularly in relation to the additional surgical steps required for their removal, which significantly impact the wound regeneration process [12]. It can be stated that P4HB biopolymer resorbable meshes meet several essential criteria for successful guided bone regeneration, including biocompatibility, appropriate biodegradation, good mechanical and physical properties, sufficient strength to prevent collapse, and a practical barrier function [13, 14]. Additionally, our research demonstrates that they can be easily shaped and adapted to the bone growth zone, and they have an increased affinity for soft tissues, promoting the migration of proinflammatory cells such as polymorphonuclear cells and fibroblasts,

followed by mesh resorption and the formation of a sturdy collagen-fibrous structure. The property of complete resorption allows avoiding the additional trauma of removal and facilitates a less invasive GBR procedure. Notably, the mesh's resorption time depends on the individual's aseptic inflammatory reaction and ranges between 6-9 months, which is the optimal period for bone formation and complete maturation.

### Scientific news

For the first time, a new technique for guided bone regeneration in the jaw has been developed using a resorbable synthetic biopolymer that degrades over a period similar to the osteoformation period.

### CONCLUSIONS

Following the comparative evaluation of the two research techniques, we obtained significant results, especially in the correlation values and volumetric stability of the bone graft. Patients who underwent guided bone regeneration using either the resorbable polymer mesh or the titanium mesh showed that the volume of the

regenerated graft remained relatively unchanged compared to the initial measurements. Using the Mann-Whitney test, we found a median decrease of 0.15 cm<sup>3</sup> in the volume of the regenerated graft over time in the control group (initially 2.0 cm<sup>3</sup>, decreasing to 1.85 cm<sup>3</sup> at follow-up). According to the box-plot diagram, this decrease primarily affected high values (initial maximum 3.3; maximum 3.1 at follow-up), while low values remained stable during the healing process. In the study group of patients treated with the P4HB resorbable polymer mesh, we observed a median decrease of 0.35 cm<sup>3</sup>. This change was consistent across the entire group, with maximum and minimum values showing no significant alterations during the healing process. From these results, we conclude that the polymer mesh tends to cause a more noticeable reduction in the volume of regenerated bone tissue, likely due to its less rigid mechanical properties compared to those of titanium mesh. Nonetheless,

clinically, this reduction was insignificant and did not interfere with the next surgical stage. This parameter of bone graft volume also showed statistically and clinically significant results in the Spearman test. The correlation analysis between VGA and VON yielded a value of 0.96, indicating a strong relationship between the volume of newly regenerated bone tissue and the graft volume, regardless of whether titanium or resorbable polymer mesh was used.

The comparative study of the two separation devices regarding the volumetric stability of the regenerating bone tissue shows no significant changes in this parameter over time, with a strong correlation between VGA and VON. Therefore, we can conclude that the polymeric mesh made of poly-4-hydroxybutyrate (P4HB) provides adequate three-dimensional stability of the space for guided bone regeneration in alveolar process atrophy, similar to the titanium mesh.

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