

Histomorphometric comparison between Bio-oss and Sintlife dental in post-extraction bone regeneration: a clinical experimental study

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Abstract

This clinical experimental study aims to compare the histomorphometric outcomes of two biomaterials—Bio-Oss, a bovine bone substitute, and Sintlife Dental, a magnesium-enriched hydroxyapatite—in post-extraction alveolar ridge regeneration. Twenty patients requiring tooth extraction were randomly assigned to either the Test group (Sintlife Dental) or the Control group (Bio-Oss). Biopsies were collected approximately six months after surgery using quantitative histology to evaluate new bone formation, residual graft material, and intertrabecular spaces. Results indicated that both biomaterials supported bone regeneration; however, Bio-Oss showed a higher percentage of newly formed bone and more residual material, aligning with its reputation as the gold standard in bone grafting. Sintlife Dental exhibited a greater tendency toward resorption and fibrous tissue formation. Patient-specific factors such as anatomical conditions and residual bone volume influenced regenerative outcomes. These findings support the effective use of both materials in clinical practice, with Bio-Oss demonstrating superior performance in this sample. Ongoing research with larger cohorts aims to deepen the understanding of biomaterial-bone interactions.

Keywords: Bone regeneration, Histomorphometric analysis, Post-extraction grafting, Bio-Oss, Sintlife Dental

Introduction

Bone ridge resorption following tooth loss often precludes the possibility of pursuing implant-prosthetic rehabilitation. To achieve this critical goal, filling the post-extraction socket with a space-maintaining material, per the previously described regenerative techniques, seeks to increase bone volume and quality. Adequate bone volume enables high-level aesthetic outcomes while preserving the overlying soft tissues from collapse. In this experimental study, sites selected for tooth extraction were chosen to ensure minimally traumatic techniques that preserve the integrity of the socket as much as possible. Through randomization, the sites were divided into two groups. The sockets in the Test group were filled with magnesium-enriched hydroxyapatite (SINTLIFE DENTAL), while the Control group sockets were filled with bovine bone substitute (BIO-OSS) (1-4). Clinical measurements and histological samples were taken immediately



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after suturing and again at 4 months post-op to assess and compare the two healing processes' dimensional changes and histomorphometric variations.

This study assessed the osteoconductive properties of two biomaterials used in post-extraction sites. Histomorphometry, or quantitative histology, was selected because it enables the measurement of key bone parameters, including the bone remodeling index. It is commonly used to diagnose skeletal metabolic diseases. As a quantitative method, histomorphometry is also highly useful for "measuring" bone response to implanted biomaterials in surgical settings.

Various biomaterials have been used over the years with high implant success rates in treated areas; however, few studies have measured the quantity and quality of regenerated bone, both of which are essential for implant osseointegration (5-8). Many studies have highlighted autologous bone as the "gold standard" because of its unique osteoinductive and osteoconductive properties. Still, other osteosubstitute materials like allografts, xenografts, and alloplastic implants have also produced satisfactory outcomes.

Materials and Methods

Patient Selection Criteria

The study involved 20 patients—13 females and 7 males—aged between 28 and 67. They could provide informed consent and required the extraction of a single tooth due to root fractures, periodontal issues (within inclusion limits), failed endodontic treatments, destructive caries, or orthodontic reasons.

Exclusion criteria included:

- Lack of consent
- Poor oral hygiene with no potential for improvement
- Poor patient compliance
- Acute or chronic systemic conditions (e.g., uncontrolled diabetes, bleeding disorders, immunodeficiency, ischemic heart disease, hypertension)
- Active infection at the extraction site
- Untreated aggressive periodontitis
- Lack of adjacent teeth at the treatment site
- Vertical bone loss ≥ 4 mm and/or horizontal bone loss ≥ 2 mm (radiographically assessed)
- Inability to attend follow-ups
- Smoking > 10 cigarettes/day
- Pathological changes at the extraction site (cysts, tumors, osteomyelitis)
- Alcohol or drug dependence
- Psychiatric illness
- Previous radiation therapy to the implant site
- Pregnancy

Patients were recruited from September 2013 to March 2014 from a single dental practice and by one operator (3-10). The principles of the Helsinki Declaration regarding clinical research on humans were followed. The protocol was reviewed and approved by the University of L'Aquila Ethics Committee. All patients provided informed consent and agreed not to undergo prosthetic rehabilitation at the treated site during the 6-month observation period.

Surgical Procedure

All patients were clinically evaluated, with data collected on age, sex, smoking habits, indications for extraction (clinical and radiographic), tooth location, and presence or absence of adjacent teeth. (14-17). After signing consent, all patients received at least one session of professional oral hygiene to optimize healing conditions.

A surgical guide was created on the study model, referencing teeth anterior and posterior to the site, to help with clinical measurements. The computer randomly assigned extraction sites to either the Test or Control groups, with sealed, coded envelopes prepared by an independent operator.

All patients received prophylactic antibiotics (amoxicillin 2g or clindamycin 600mg for penicillin-allergic patients) 1 hour before extraction. They continued post-operatively with amoxicillin 1g or clindamycin 300mg twice daily for 4 days. Chlorhexidine 0.2% mouth rinses were used for 1 minute pre-op and twice daily for 3 weeks post-op. Local anesthesia was achieved with lidocaine/adrenaline 1:50,000.

All extractions were performed by an experienced surgeon using a standardized, minimally traumatic technique to preserve alveolar walls: A circular incision was made around the tooth, avoiding flap elevation. A periosteal elevator was used to sever periodontal ligament fibers without leveraging. A luxator was then gently used to mobilize the root (18-23). If mobility was sufficient, the tooth was removed with the luxator; otherwise, appropriate forceps were used. The socket was then cleaned with a surgical curette to remove residual ligament and promote bleeding for clot formation.

After extraction, the randomized envelope was opened to assign the site to either the Test group (magnesium-enriched hydroxyapatite, SINT LIFE, no membrane) or the Control group (bovine bone substitute BIO-OSS with suturing to stabilize the blood clot) (Figs. 1-2-3).

Harvesting

For each patient examined, a bone sample was collected using a full-thickness flap and trephine burs (hollow, serrated burs with internal cooling, mounted on a hand-piece) from the area where the grafting biomaterial was placed, approximately 6 months after the surgery (Fig.4).

Fixation

Samples were immersed in fixative for 9 days to stabilize tissue components (21-25). The goal was to prevent post-mortem degeneration and preserve in vivo tissue morphology, structure, and reactivity. Formalin 4% was used as the fixative.

Embedding

Samples were embedded in methacrylate, which enables thin sectioning. The process involved dehydration through graded alcohols and acetone, followed by immersion in uncatalyzed and hypocalyzed methacrylate solutions before final embedding with a catalyst to harden the resin.

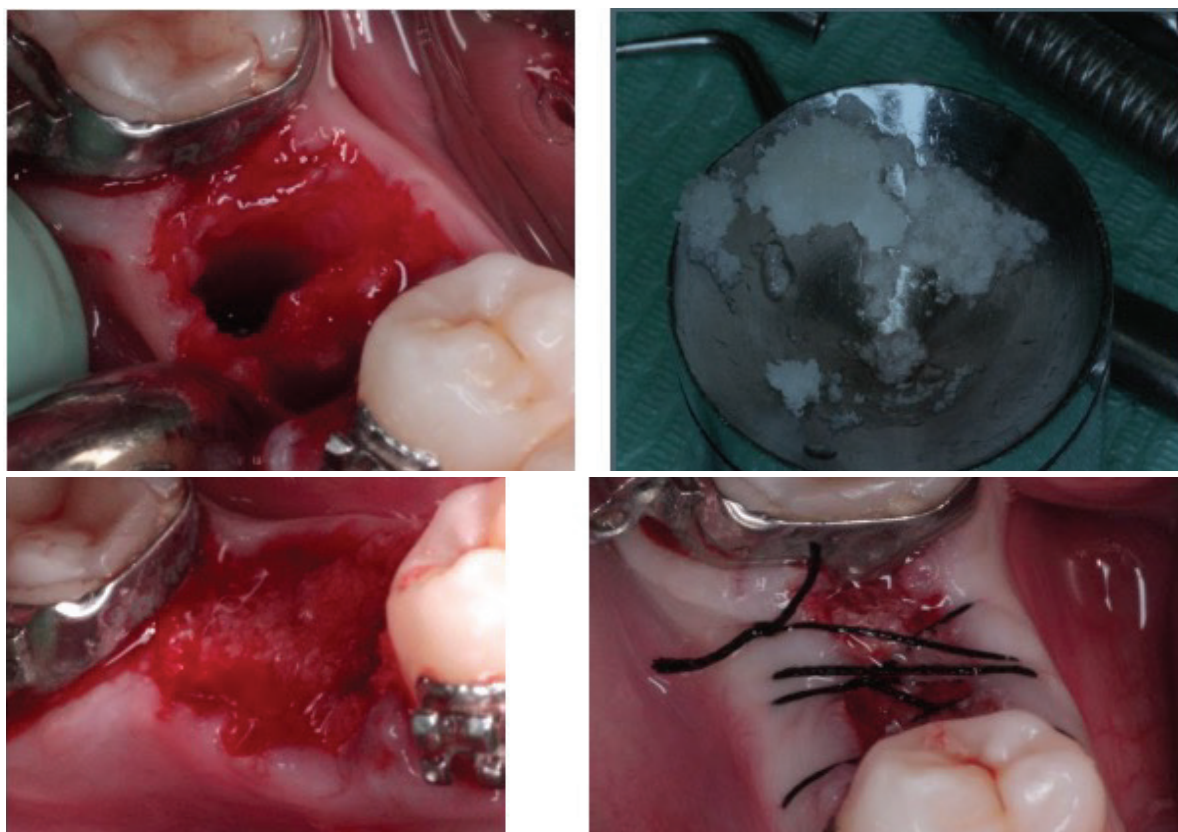


Figure 1. Socket filled with grafting material.



Figure 2. Sintlife.



Figure 3. Bio oss.

- **DEHYDRATION AND NON-CATALYZED SOLUTION**

The sample is first washed with PBS (Dulbecco's phosphate-buffered saline), followed by graded ethanol (EtOH) dehydration, increasing to absolute ethanol concentration. The operating protocol includes incubation in 50% acetone for 15 minutes, 95% acetone for 30 minutes, and 100% acetone for 30 minutes. The sample is treated with a “Non-catalyzed Solution” (containing methacrylate and ethylene glycol monobutyl ether in a 10:1 ratio) for 7 days.

- **HYPOCATALYZED SOLUTION**

The next step involves prolonged treatment in “Hypocatalyzed Solution” (composed of the non-catalyzed solution and benzoyl peroxide in a ratio of 4.5 ml: 1 mg) for 7 days.

- **METHACRYLATE EMBEDDING**

This final step produces a methacrylate resin block containing the bone sample, allowing it to be sectioned. Preparation of the methacrylate involves adding a catalyst (solution B) to the hypocatalyzed solution to achieve polymerization. The components are mixed in a 4 ml: 1 ml ratio.

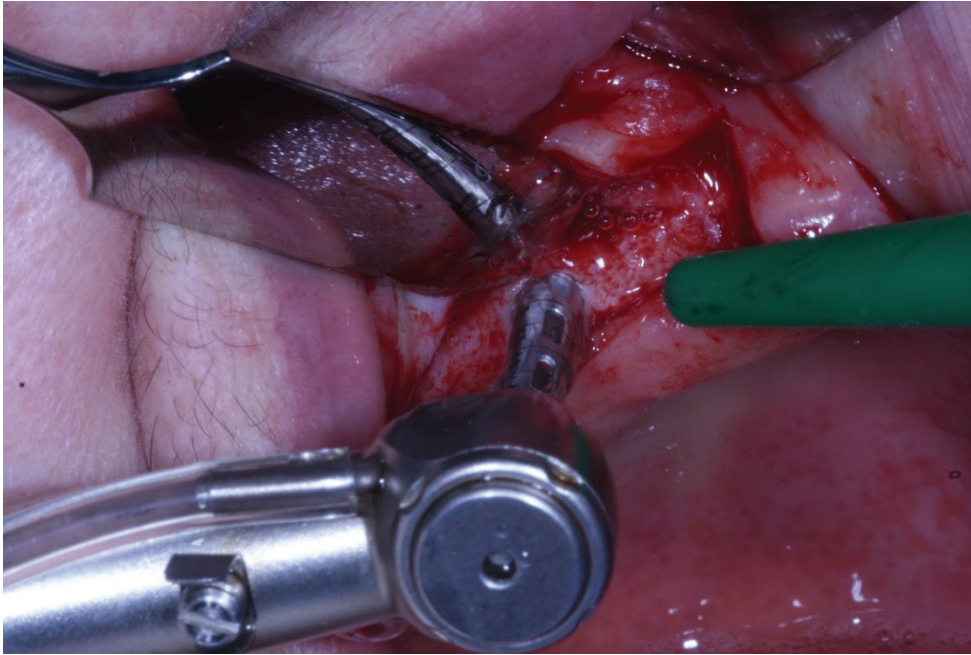


Figure 4. Harvesting of a bone sample with a trephine bur.

Cutting, coloring, and preparation of slides

The piece thus obtained was subsequently cut longitudinally using a microtome (Fig. 5).



Figure 5. Microtome

Histological sections, approximately 5 microns (μm) thick, were prepared for staining and analysis. The sections, placed on microscope slides, were immersed in xylene, the embedding solvent, to facilitate rehydration and subsequent staining. Once stained according to the investigation requirements, the section was sealed with Canada balsam and a coverslip.

Multiple sections were prepared from each sample; some were stained with methylene blue/azure II to analyze structural parameters, while the remaining

sections were stained with TRAcP to analyze osteoclastic cellular parameters.

Histomorphometric Analysis

The histomorphometric analysis involved the entire sectioned sample, and the following were measured:

- number of osteoclasts/bone surface (number/ mm^2),
- osteoclast surface/bone surface (percentage),
- osteoblast surface/bone surface (percentage),
- bone volume/total volume (percentage).

Histomorphometric measurements were conducted using an interactive image analysis software (IAS 2000, Delta Sistemi, Rome, Italy) (24-25). The histomorphometric indices' nomenclature, symbols, and measurement units followed the guidelines of the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research.

Cells were fixed in 3% paraformaldehyde in 0.1 M cacodylate buffer for 15 minutes, then rinsed with the same buffer. TRAcP activity was detected histochemically using the Sigma-Aldrich kit #386, following the manufacturer's instructions.

Statistics

Data are presented as mean $\pm$ SEM. Statistical analysis was conducted using the one-way ANOVA (Analysis of Variance) test and the Student's t-test. A p-value < 0.05 was considered statistically significant.

Histological Images

The following images (Fig. 6) display the histological analyses of some of our patients who took part in the study:

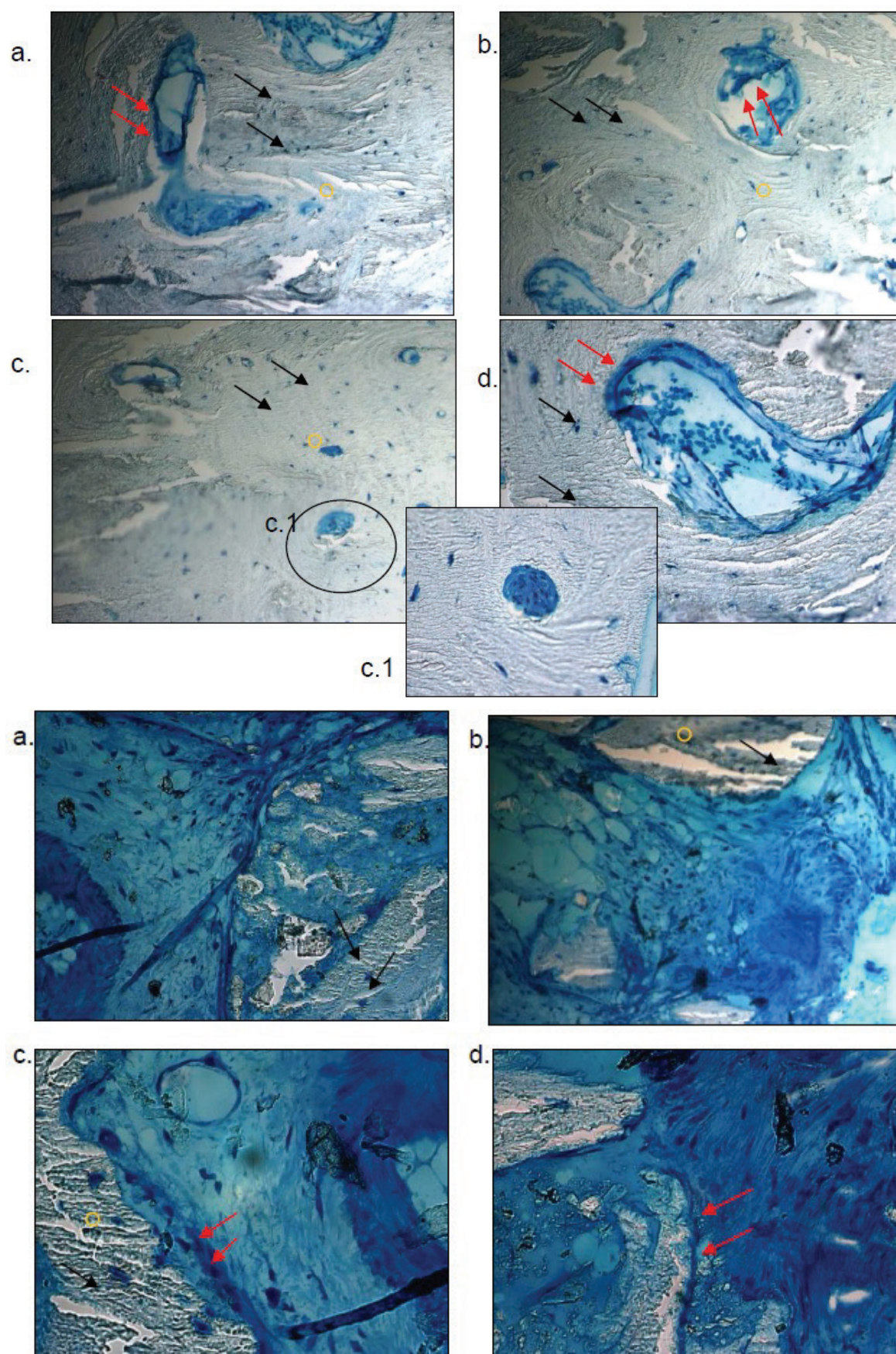


Figure 6. Histological analysis of the study sample Red arrows = osteoblasts / Black arrows = osteocytes

Table 1. Results of the study

Parameter	Sint Life	Bio-Oss
New bone	37% $\pm$ 2.3%	39% $\pm$ 1.6%
Intertrabecular spaces	23% $\pm$ 1.6%	34% $\pm$ 1.6%
Residual material	37% $\pm$ 3.1%	31% $\pm$ 1.4%

Results

The results show that the xenografts (Bio-Oss) achieve better bone regeneration compared to Sint Life, with a higher amount of newly formed bone (as indicated by the parameter BV/TV) and a lower amount of fibrous bone, which has unfavorable characteristics for implant biomechanics (24-26). This was evident from the histomorphometric examination and the histological sections analyzed under an optical microscope.

The percentage of residual material, measured through histomorphometry, was also higher for the xenografts than the alloplastic implants—a significant factor that enables their replacement with new bone tissue (osteoconduction).

For the two groups treated with different biomaterials, the following parameters were examined:

- BV/TV, representing the amount of newly formed bone,
- Percentage of intertrabecular spaces,
- Percentage of residual material.

The results obtained were as follows (Table 1):

Bio-Oss:

Sections from some samples revealed osteoblastic activity, with bone deposition directly on the surface of the particles. Most of these particles were surrounded by newly formed, mature, and compact bone tissue without bone lacunae along the interface (28-29).

The bone was consistently found in close contact with the particles. Additionally, no inflammatory infiltrate was observed.

Histomorphometry showed that the newly formed bone accounted for 39% $\pm$ 1.6%, the intertrabecular spaces comprised 34% $\pm$ 1.6%, and the residual material constituted 31% $\pm$ 1.4%.

Sint-life dental

The newly formed bone tissue exhibited lamellar features and was not immediately visible at the bone-graft interface. Instead, small capillaries, fibroblasts, and macrophages were seen farther from the interface. However, inside most particles, tissue with the typical staining of new bone matrix was apparent, while many bone lacunae were found on their surface (25-29).

No necrosis, inflammatory infiltrate, or foreign body reaction was observed. Newly formed bone accounted for 37% $\pm$ 2.3%, intertrabecular spaces for 23% $\pm$ 1.6%, and residual particles for 37% $\pm$ 3.2%.

Conclusions

All examined samples showed newly formed bone; thus, it can be concluded that all biomaterials considered in this study promoted bone neoformation. However, bone neoformation alone is insufficient to determine one biomaterial's usefulness over another. Therefore, this study also evaluated the amount of mineralized bone, the extent of intertrabecular spaces, and the residual biomaterial remaining.

Based on the analyzed parameters, differences were observed among the various biomaterials, especially regarding the amount of residual biomaterial (22). Sint Life Dental seems more easily resorbable than Bio-Oss, which exhibits a higher residual percentage after the same time (consistent with international literature identifying Bio-Oss as the "gold standard" among bone graft biomaterials).

Regarding the type of replacement—either with bone or fibrous tissue—Sint Life Dental produced the highest percentage of fibrous tissue formation, probably due to its shorter persistence at the graft site, followed closely by Bio-Oss.

Nevertheless, based on the statistics, significant differences were observed between samples regarding the type of regenerated tissue, even when the same material was used.

These variations are linked to individual patient factors that play an equally important role, including:

- The general health status of the patient,
- Anatomical conditions of the alveolar bone,
- Amount of residual bone.

Indeed, radiological studies conducted before and after bone graft surgery show that the anatomical shape and residual bone thickness are crucial factors in the procedure's success.

Regarding anatomy, specific anatomical features promote graft integration because increased vascularization leads to bone formation.

Residual bone volume refers to the thickness of the marrow, which is a key factor because it supports the graft's vascularization, allowing blood cells to invade and facilitating the graft's resorption and replacement with new tissue.

Based on these findings, our comparative study shows that all biomaterials produced valid results, with individual traits that may make one more suitable (28-31).

Bio-Oss is the material with the best results from relatively few treated patients.

The current research study is ongoing and aims to evaluate all possible interactions between bone graft biomaterials and bone more thoroughly. The goal is to increase the sample size and potentially consider more parameters.

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