Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2022.43.006897

Microscopic and Tomographic Analyses of Alveoli Repaired by Three Alveolar Preservation Techniques: A Randomized Clinical Study

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ARTICLE INFO

Received: April 13, 2022

Published: April 22, 2022

Citation: Fernando Fusari Bento de Lima, Amílcar Sabino Damazo, Bruno Salles Sotto Maior, Carlos Eduardo Francischone. Microscopic and Tomographic Analyses of Alveoli Repaired by Three Alveolar Preservation Techniques: A Randomized Clinical Study. Biomed J Sci & Tech Res 43(3)-2022. BJSTR. MS.ID.006897.

Keywords: Bone Substitutes; Bone Regeneration; Guided Tissue Regeneration; Clinical Research

ABSTRACT

Objective: This study aimed to compare the effectiveness of three post-extraction techniques for alveolar preservation.

Material and Methods: Twenty-three fresh adult alveoli were randomly allocated to three experimental groups: Group one, gingival flap + blood clot; Group two, polypropylene barrier + blood clot; Group three, bovine bone mineral matrix + gingival flap. Tomographic scans were performed within 1 day after tooth extraction and at 6 months post-extraction. At implant installation, a bone biopsy of the treated alveolus was obtained, prepared, and examined under hematoxylin-eosin, picrosirius, and Goldner stains.

Results: Statistically significant differences between Group two and Group three ($p=0.0338$) were found regarding the remodeling of the cervical region and the whole volume of the treated alveolar ridge ($p=0.0217$). Histologically, statistically significant differences between the polypropylene barrier technique and the gingival flap techniques were observed in terms of collagen ($p<0.0001$) and calcified tissue distribution ($p<0.0001$).

Conclusions: Our findings suggest that the bovine bone mineral matrix + gingival flap strategy provides greater dimensional stability on tomography, whereas the polypropylene barrier + blood clot provides better quality and quantity of mature bone on microscopy.

Introduction

The alveolar ridge is a tooth-dependent tissue that develops in conjunction with tooth eruption and undergoes resorption and atrophy after dental extraction (Barone, et al. [1]). These morphologic changes have been widely studied (Araújo, et al. [2-10]) and the decrease in height and thickness of the alveolar ridge can hinder the three-dimensional positioning of implants, especially in the anterior region of the maxilla, where bone volume is important for aesthetic and biological reasons (Crespi, et al. [6]). Minimizing alveolar ridge atrophy is critical for ensuring adequate installation of the prosthesis on the implant (Buser, et al. [11]). However, careful extraction and immediate installation of the implant does not interrupt or inhibit the remodeling of the alveolar ridge after

dental extraction (Araújo, et al. [12,13]). Therefore, it is necessary to strive to preserve this structure as much as possible (Artzi, et al. [3,7,13-17]). Several filling techniques and materials have been proposed to help preserve the alveolar structure, including blood clot (Karaca, et al. [7]), bone or bone substitutes of various origins (Aimetti, et al. [14,17-23]) with or without membranes or barriers (Artzi, et al. [14,15,17,19,22-24]). In this study, we aimed to determine the comparative effectiveness of three techniques for preserving alveolar bone structure after dental extraction by microscopic evaluation of the newly formed bone tissue, osteoid matrix, organized collagen, and remnant graft material, as well as tomographic measurements of the width, height, area, and volume of the remaining alveolar bone after socket healing.

Materials and Methods

Patients

All procedures were approved by the local ethics committee for experiments involving humans (CAAE: 48634415.8.0000.5374 available for check at: http://plataformabrasil.saude.gov.br/login.jsf) and were conducted in accordance with the Declaration of Helsinki principles. The paper was prepared in accordance with the CONSORT 2010 Statement. All patients included in the study provided written informed consent to participate. Patients were recruited at Department of Basic Health Sciences, Faculty of Medicine, Federal University of Mato Grosso, from September 2015 through August 2016. We included adult's patient with at least one upper incisor, or premolar tooth indicated for extraction after clinical and radiographic examination. Patients who fulfilled any of the following conditions before or during the study were excluded:

i. Absence of bone reference for tomographic measurements

ii. Diabetes, osteoporosis, or osteopenia

iii. Use of bisphosphonates

iv. Smoking

v. Radiation therapy or chemotherapy

vi. Medical contraindication for dental surgeries

vii. Periodontal disease or periapical lesions in the treated or adjacent teeth

viii. Pregnancy and

ix. Bone wall defects

Randomization and Group Allocation

The sample randomization process was performed using Microsoft Excel for Mac 2011 software, version 14.5.8. Initially, a spreadsheet was elaborated where in row one, the cells that formed the column headings of the spreadsheet were named: randomized order (A one), order (B one), patient Identification (C one), randomized number (Done), and copy (E one). Column A, in the first stage of the preparation of the spreadsheet, was left without data insertion, since this was later used for the rank and division of the sample between the groups. Column B has a sequential numbering of integers with values in the range of one to 23, arranged in ascending order. Column C identified the dental element to be randomized. In all rows in column D, the formula =RANDBETWEEN (1; 23)+B2/30 (using row two of the worksheet as a reference, the rest the formula was copied to subsequent rows). The values returned in column D were copied and pasted into column E. Based on the values of column E, which represented the result of randomization, patients were ranked in column A through the function: = RANK(E2;$E$2:$E$24;1) to assign a serial number to each patient listed.

Experimental Design

This was a single-center study, with all procedures performed at Federal University of Mato Grosso (Brazil) by a master surgeon. All patients underwent extraction followed by alveolar preservation according to group allocation:

(i) Group one (control), filling the extraction socket with blood clot and closing with a gingival flap from the hard palate (Jung R, et al. [25]);

(ii) Group two, filling the socket with blood clot and closing with a polypropylene barrier (Bone Heal™; INP, Brazil);

(iii) Group three, filling the socket with acellular bovine mineral matrix (Lumina Porous; Critéria Biomateriais, Brazil) and closing with a gingival flap from the hard palate (Jung R, et al. [25]).

Volumetric computed tomography scans of the operated region were obtained at up to 24 hours and at 6 months postoperatively. After the second tomographic examination, an osseointegrated implant was installed at the treated site and a bone biopsy was collected for histomorphometric analysis.

Surgical Procedures

All surgical procedures were performed, under local anesthesia (mepivacaine hydrochloride with corbadrine), as well as under aseptic and antiseptic conditions. Postoperatively, the patients were prescribed analgesic, anti-inflammatory, and antiseptic mouthwashes. Flapless extraction was performed, in a minimally traumatic manner. Afterwards, the integrity of the alveolus was verified clinically. For teeth allocated to groups one or three, a gingival flap compatible with the recipient area and composed of epithelium and connective tissue was harvested from the hard palate using a circular scalpel; the gingival flap was accommodated

onto the recipient bed and sutured to the gingival margins using nylon suture. For teeth allocated to group two, guided bone regeneration was induced by performing an incision into the interdental papillae, followed by tunneling of the vestibular and palatine flaps. The polypropylene barrier was lodged in the surgical bed according to the manufacturer's recommendations, and the soft tissue was sutured with nylon suture. The barrier and the suture were removed after 7 days (for all patients). For teeth allocated to group three, the alveolus was filled with acellular bovine mineral matrix, hydrated in saline solution, lodged in the fresh alveolus, and sutured as described above. At 6 months postoperatively, the patients received osseointegrated implants at the treated site. A bone biopsy was obtained from the surgical bed using a trephine drill (SIN, Brazil), with an internal diameter of 2.0mm. The bone fragment was carefully removed from the trephine and placed in buffered paraformaldehyde for fixation and histological processing. The wound was sutured with nylon thread.

Tomographic Analysis

Tomographic images were analyzed using Imaging Studio version 3.3 for Windows (Anne Solutions, São Paulo, Brazil). Parasagittal sections with a thickness of 1mm were obtained at 1mm intervals. Three non-adjacent sections corresponding to the mesial, middle, and distal regions of each treated site were selected. Four types of measurements were made for each section: three measurements of thickness (cervical, middle, and apical) and one measurement of height (perpendicular to cervical thickness) (Figure 1). In the three-dimensional analysis, the roots of adjacent teeth were considered the mesial and distal references. The superior axial plane crossing the apex of the roots of the two adjacent teeth was considered the superior reference, and the cervical alveolar remnant and the vestibular and palatal cortical bones were considered references for the inferior, vestibular, and palatal references, respectively. In the first tomographic examination, the area and volume of the fresh alveolus were included in the area and total volume of the region studied, whereas only the remaining bone tissue was included in the second tomography evaluation. Tomographic measurements were performed using the "Three-dimensional polygonal editing" tool available in the software. For that, the area of interest was demarcated in each axial slice (0.1mm thickness), and from the overlap of all these demarcated areas, a three-dimensional solid was constructed, which had its total area and volume mathematically calculated by the software.

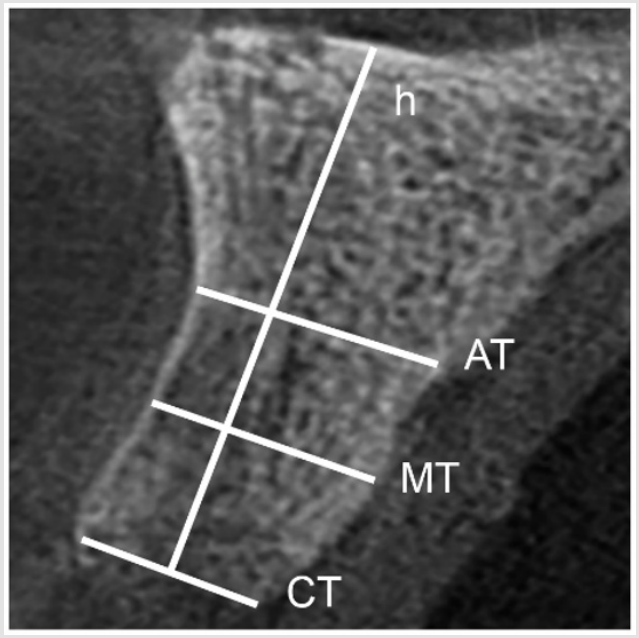

Figure 1: Diagram illustrating the reference standards used for liner measurements of the alveolar ridge on computed tomography. CT, thickness in the cervical region; MT, thickness in the middle region; AT, thickness in the apical region; h, height.

Histological Analysis

The bone biopsy samples were fixed in 4% paraformaldehyde with 0.2M phosphate buffer for 24 h at 4°C. The samples were then washed in running water, decalcified with EDTA (Sangeetha [26]), and processed in a carousel tissue processor (SLEE MTP MAINZ; Carl Zeiss Meditec AG, Germany) according to the following protocol: washing in running water, dehydration in increasing ethanol concentration solutions, clarification in xylol, and embedding in paraffin overnight. For analysis, each sample was cut into 7µm sections using a HIRAX M60 microtome (Carl Zeiss), and the sections were mounted on histological slides. After deparaffinization and rehydration, some sections were stained with hematoxylin-eosin for histomorphometric analysis. Other sections underwent Goldner trichrome staining using the Von Kossa Histokit (EasyPath, Brazil), according to the manufacturer's recommendations. The remaining sections of each specimen underwent picrosirius staining (Junqueira, et al. [27]). Tissue analyses were performed on an AxioScope A one microscope (Carl Zeiss). The software AxioVision (Carl Zeiss) was used for the quantification of tissue area. The parameters used for this evaluation were the determination of the area of the vital bone tissue, organized collagen, calcified tissue, and remnants of grafted material. These parameters were calculated as a percentage of the total area of the histological section.

Statistical Analysis

Data were analyzed using GraphPad Prism 6.01 for Windows (GraphPad Software Inc., La Jolla, CA). Two-way analysis of variance and Tukey tests were employed to evaluate the statistical significance of differences between groups.

Results

Of 56 teeth considered for this study, 23 were selected (male, 11; female, 12; age, 41.91±11.69 years) as shown in Table 1. The teeth were randomly allocated to three experimental groups according to the alveolar preservation technique employed. Four teeth were lost to follow-up (Figure 2). After the randomization process, teeth ranked from one to nine were allocated to group one, whereas those ranked from 10 to 16 were allocated to group two, and those ranked from 17 to 23 were allocated to group three. The sample size used in this study was based on other previously published articles used as reference in this study (Artzi, et al. [7,10,14,28]). The percent change in alveolar ridge dimensions on computed tomography (immediately post-extraction vs. 6 months post-extraction) did not differ among groups when considering individual slices. However, when averaging across the mesial, middle, and distal slices, groups two and three differed significantly in terms of the percent change in cervical thickness and bone volume (Table 2). On histomorphometric analysis, group two differed significantly from groups one and three in terms of the percent areas of organized collagen and calcified bone (Table 3). In all groups, hematoxylin-eosin staining revealed newly formed bone tissue with an organization representative of bone maturity, with osteocytes, and without inflammatory cells (Figure 3).

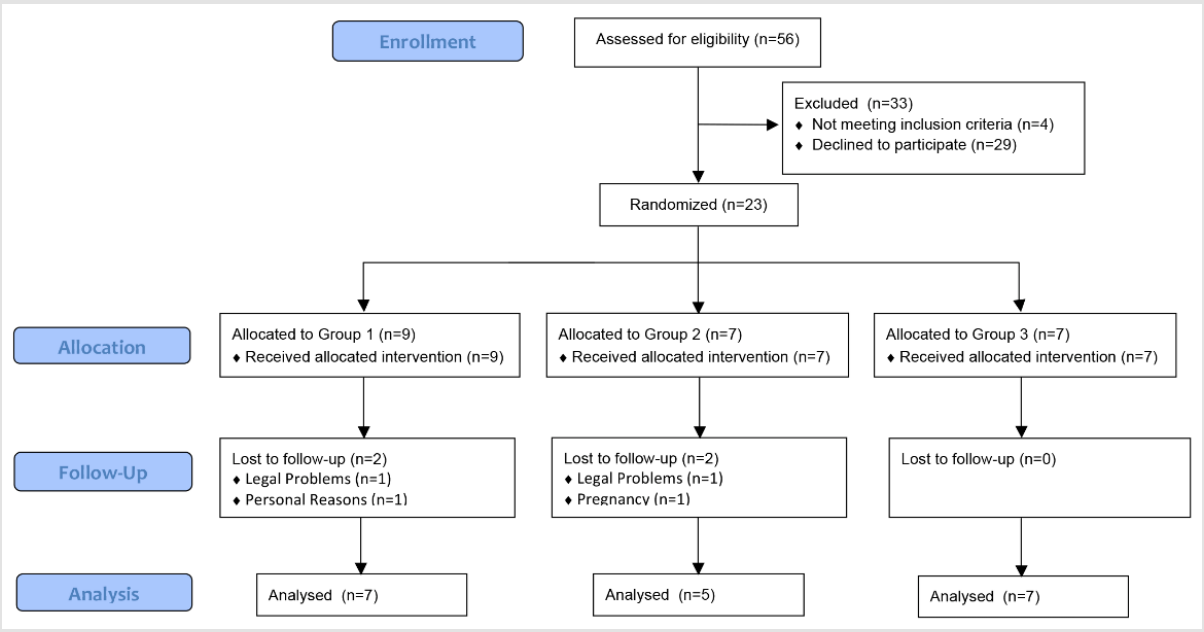

Figure 2: CONSORT diagram, prepared in accordance with the CONSORT 2020 statement. Teeth were stratified according to the alveolar preservation technique: group one, gingival flap + blood clot; group two, polypropylene barrier + blood clot; group three, bovine mineral matrix + gingival flap.

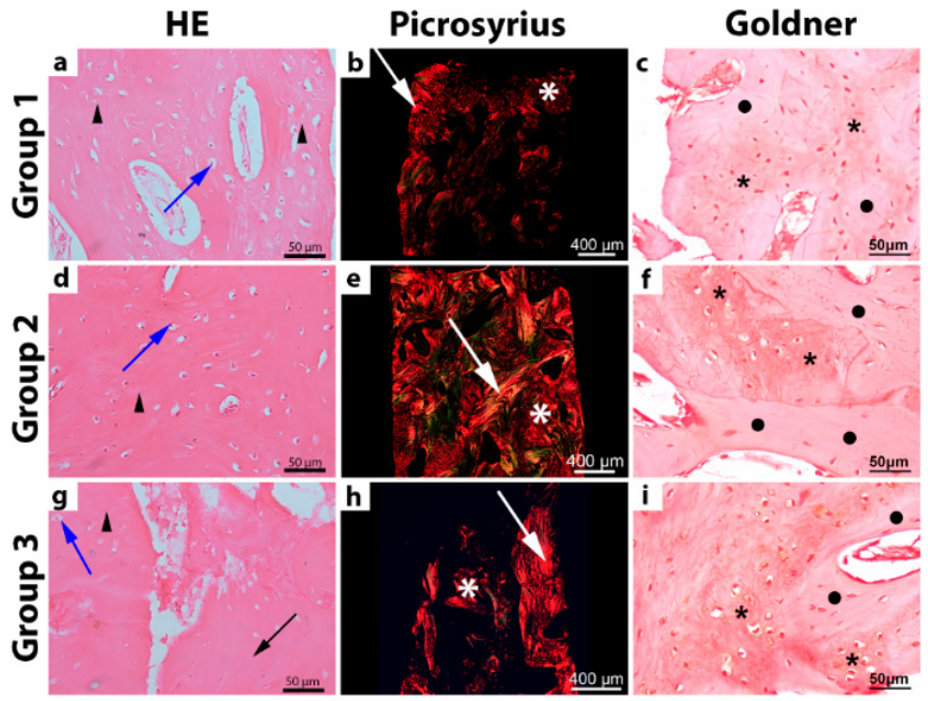

Figure 3: Microscopic appearance of alveolar bone biopsies at 6 months after extraction. Samples were stratifying according to the alveolar preservation technique: group one, gingival flap + blood clot; group two, polypropylene barrier + blood clot; group three, bovine bone mineral matrix + gingival flap. Representative brightfield photomicrographs, under hematoxylin and eosin staining are shown for groups one (A) two (D), and three (G). Newly formed bone tissue (triangles), remnant graft material (black arrow), and osteocytes (blue arrow) are labeled. Representative photomicrographs obtained, under picrosirius sating polarized, light reveal the distribution of collagen fibers in areas of bone (white arrows) and osteoid matrix (white asterisk) in teeth from groups one (B), TWO (E), And three (H). Representative brightfield photomicrographs, under Golder trichome staining, are shown for newly formed bone belonging to group one (C), two (E), and three (I). Areas of calcified bone tissue (black asterisk) and osteoid matrix (circles) are labeled.

Table 1: Baseline demographic and clinical characteristics for each patient.

Patient	Genre	Age	Tooth Trated	Reason for Extraction
1*	Male	37	21	Root Fracture
2	Male	54	21	Caries
3	Female	44	12	Caries
4	Female	25	24	Caries
5	Male	20	21	Coronary Root Fracture
6*	Female	48	13	Caries
7	Female	44	25	Caries
8	Female	41	11	Caries
9	Male	59	12	Caries
10*	Female	30	14	Caries
11	Female	41	21	Caries
12	Male	43	11	Caries
13	Female	37	11	Caries
14*	Male	37	11	Root Fracture
15	Female	34	14	Caries
16	Female	49	15	Caries

17	Male	34	24	Caries
18	Female	54	14	Coronary Root Fracture
19	Male	30	22	Caries
20	Male	64	12	Caries
21	Female	43	14	Root Fracture
22	Male	32	11	Caries
23	Male	64	21	Caries

Note: *Lost of follow up.

Table 2: Morphometric percent change in alveolar ridge dimensions on computed tomography (immediately post-extraction vs. 6 months post-extraction), in a linear two-dimensional analyses for each studied region (mesial, middle, distal and height) and averaged over the same regions; and and three-dimensional full analyses for area and volume.

Region	Slice	Group one	Group two	Group three	*p value* (ANOVA)
Cervical	Mesial	-34.28±17.70	-33.70±17.98	-16.24±1 2.24	0.0939
	Middle	-34.95±18.11	-39.61±13.67	-20.58±17.71	0.1481
	Distal	-20.22±34.32	-37.35±13.35	-21.79±22.40	0.4944
	Average	-29.81±24.37	-36.89±14.25	-19.53±17.19	0.0338*
Middle	Mesial	-16.97±17.40	-0.99±14.82	-7.50±11.40	0.1978
	Middle	-22.87±13.88	-23.39±17.05	-12.96±11.75	0.3399
	Distal	-14.18±10.11	-22.45±12.47	-11.45±5.348	0.1560
	Average	-18.01±13.90	-15.61±17.46	-10.64±9.722	0.2140
Apical	Mesial	-7.37±24.01	12.79±36.88	5.40±15.10	0.3933
	Middle	-4.36±7.423	-16.19±23.14	-4.93±21.32	0.4849
	Distal	-2.40±12.51	-11.13±21.18	-4.90±6.838	0.5582
	Average	-4.71±15.52	-4.84±29.00	-1.47±15.61	0.8348
Height	Mesial	-0.39±6.174	-5,57±5.280	-7,96±10.36	0.2157
	Middle	-2.47±12.74	-6.66±2.733	-6.85±15.81	0.7731
	Distal	-2.96±6.737	-4.85±6.339	-7.60±9.720	0.5560
	Average	-1.94±8.662	-5.69±4.709	-7.47±11.65	0.1478
Total	Area	-19.10±16.42	-25.77±13.54	-16.29±10.98	0.5124
	Volume	-23.08±12.89	-30.34±12.79	-11.08±6.120	0.0217*

Note: Positive and negative values, respectively, indicate an increase and decrease in bone tissue. Teeth were stratified according to the alveolar preservation technique: group one, gingival flap + blood clot; group two, polypropylene barrier + blood clot; group three, bovine bone mineral matrix + gingival flap. *Statistically significant difference; Tukey test indicates differences between groups two vs. three.

Table 3: Percentage area of new bone, organized collagen, and calcified bone at 6 months after extraction.

Staining	Group one	Group two	Group three	*p-value* (ANOVA)
Hematoxylin and eosin (Newly formed bone)	56.62±9.519	70.78±5.561	58.05±12.01	0.0530
Picrosirius (Organized collagen)	22.46±10.05	78.96±15.54	33.82±19.21	<0.0001**
Goldner (Calcified bone)	51.03±7.332	91.56±5.590	58.82±7.831	<0.0001**

Note: Teeth were stratified according to the alveolar preservation technique: group one, gingival flap + blood clot; group two, polypropylene barrier + blood clot; group three, bovine bone mineral matrix + gingival flap. **Statistically significant difference; Tukey test indicates differences between groups one vs. two, and two vs. three.

Samples from group two displayed some areas of lamellar bone formation. Samples from group three revealed remnant graft material in contact with newly formed bone tissue which presented an average of 9.61% of the total slice area, 6 months after the first surgery. Connective tissue and blood vessels were observed in all groups. Picrosirius staining revealed a predominance of immature collagen fibers alternating with small areas of mature collagen fibers and permeated by areas of connective tissue in group one, whereas vast regions of organized collagen (sometimes with radial and concentric organization) interlaced with scattered areas of connective tissue were observed in group two; in group three, picrosirius staining revealed organized and dense collagen interspersed with disorganized collagen which, in some regions of concentric collagen bundles, reflected immature bone (Figure 3). In all three groups, Goldner trichrome staining revealed regions of newly formed bone tissue, calcified or as osteoid matrix, with calcification foci throughout the section (Figure 3).

Discussion

This study had a similar design and sample size as employed by previous studies focused on two-stage dental implant surgery (Aimetti, et al. [17,18,20,28]). Moreover, we employed a similar evaluation strategy, including microscopic analysis with hematoxylin-eosin staining for evaluating bone vitality and cellular components (Artzi, et al, [1,6,10,14,20,29,30]), picrosirius staining for evaluating collagen fibers (Vivan, et al. [31]), and Goldner trichrome staining for distinguishing the osteoid matrix from mature bone (Vivan, et al. [31]). However, while previous studies measured only alveolar ridge height and thickness (Araújo, et al. [5,23,29,32,33]), this study also measured alveolar ridge area and volume. Such three-dimensional measurements provide a more perspective on bone remodeling after dental extraction. Reports on socket healing duration are contradictory and vary widely, from 3 months (Aimetti, et al. [18,29,30]) to 4 months (Araujo-pires, et al. [6,34-36]), 6 months (Lekovic, et al. [37,38]), 7 months (Barone, et al. [1,36]), 8 months (Mardas [39]), and even 15 years (Carmagnola, et al. [36]). The discrepancies are likely directly related to the technique and filling material used for preserving the alveoli.

As dimensional stability is higher for palatal bone than for vestibular bone (Botticelli, et al. [4]), palatal bone can be used as a stable reference for evaluating dimensional changes in the alveoli after dental extraction. Using the alveolar apex as the apical reference (Jung, et al. [33]) is controversial. Therefore, it was used an adjacent bone structure for apical reference. The presence of biomaterial in the alveolus is believed to delay socket healing (Heberer, et al. [30]), which is supported by the present results obtained for group three. Specifically, the rate of new bone formation and the percent area of organized collagen were lower in group three than in group two, as new bone formation is preceded by resorption of the grafted biomaterial. However, from a clinical perspective, 58.05% vital bone bioavailability (i.e., average percentage area of new bone in group three) is perfectly adequate for implant installation and osseointegration. On microscopic analysis, we found that the rate of new bone formation depends on the graft material. In group three patients, the rate of new bone formation was higher than that reported for freeze-dried bone allografts (Froum, et al. [20]), Bio-Oss Collagen™ (Heberer, et al. [30]), xenogenous corticocancellous bone grafts (Barone, et al. [1]), calcium sulphate (Crespi, et al. [29]), magnesium-enriched hydroxyapatite (Crespi, et al. [29]), pig bone (Crespi, et al. [6]), and autogenous bone marrow (Pelegrine, et al. [40]), but similar to the rate reported for bioactive glass (Froum, et al. [20]).

Previous reports indicate that the extraction socket heals without graft material (i.e., simple closure by flap advancement), but the rate of new bone formation ranges from 17% to 53.1% (Froum, et al. [20]), which is comparable to the value we found for group one (56.62%; gingival flap + blood clot) but much lower than the value for group two (70.78%; polypropylene barrier + blood clot). The discrepancy might be related to differences in techniques including flap elevation and interposition of an impermeable barrier between the bone plate and the periosteum, which may interfere with bone healing. Histological analysis of the connective tissue in areas not filled by bone tissue revealed results similar to those obtained using bioactive glass (Froum, et al. [20]), better than those obtained with Bio-Oss™ (Carmagnola, et al. [36]), and worse than those obtained with blood clot filling (Froum, et al. [20]). However, such observations should be interpreted in consideration of the potential effect of biopsy manipulation and processing. Furthermore, the presence of remnant graft material in biopsies is related to the speed of material resorption and subsequent replacement by bone tissue. Thus, a large volume of remnant graft material indicates lower availability of vital bone tissue.

The amount of remnant graft material in group three was comparable to that reported for Bio-Oss Collagen™ (Heberer, et al. [30]) and bioactive glass (Froum, et al. [20]), but lower than that reported for pig bone (Barone, et al. [1]), Bio-Oss™ (Carmagnola, et al. [36]), and hydroxyapatite (Crespi, et al. [6]), suggesting that bovine bone mineral matrix has a relatively high resorption rate, which contraindicates its use when aiming to maintain the alveolar ridge for longer but supports its use when aiming to maximize the percentage of vital bone tissue at 6 months postoperatively. Furthermore, in group three, the remnant graft material was in contact with new bone in the absence of inflammation, confirming that this graft material is highly biocompatible (Froum, et al, [20,39]). Guided bone regeneration displayed the best microscopic results, proving that, although the inclusion of biomaterial in the

alveolus affects healing, it can be biocompatible. With regard to the percentage of mature bone, bovine bone mineral matrix was similar to Bio-Oss Collagen™ (Schulz, et al. [38]). However, use of a polypropylene barrier (group two) was associated with higher bone calcification and thus bone maturity. Taken together, our microscopic observations suggest that alveolar treatment with graft to preserve its structure affect bone healing. Since the mature bone, osteoid matrix, and grafted material are difficult to assess precisely on tomographic images, caution is recommended when analyzing exclusively tomographic examination results of alveoli treated for structure preservation; several aspects should be considered in such cases, including tomographic findings, nature of the filling material, and time elapsed since extraction.

The mean change in linear dimensions of the dental alveoli was reported to range between 1.7% and 77.5%, depending on the grafting material (Jung, et al. [33]). In our study, the mean dimensional changes ranged between 5.4% tissue gain and 39.61% tissue reduction. A mean cervical remodeling of 18.1% was reported for Bio-Oss™ used together with a gingival flap (Jung, et al. [33]), which is similar to the value obtained in our study for group three (19.53% reduction) but smaller than the value noted for group two (36.89% reduction). The discrepancy likely stems from the choice of grafting material, since group three also showed lower rates of remodeling than those noted for group one and for beta-tricalcium phosphate (Jung, et al. [33]). Our finding that morphometric remodeling was the highest in group two suggests that installing an impermeable barrier may decrease the blood supply to the bony crest. Therefore, such a strategy would be more suitable for regions with thicker vestibular and palatal/lingual cortical bone. While the use of bovine bone mineral matrix (group three) provided superior results over those of Bio-Oss Collagen™ (Araújo, et al. [34]), some discrepancy might be attributable to the choice of anatomical reference used in the measurements. Importantly, our results confirm that alveolar height reduction also occurs at grafted sites (Araújo, et al. [34]). Previous reports estimate a 2.7% reduction in the availability of bone tissue (Araújo, et al. [34]), but methodological differences likely account for most of the discrepancies (e.g., use of two-dimensional vs. three-dimensional data, one side vs. entire alveolar ridge). In fact, group three exhibited the least pronounced decrease in alveolar volume, which was significantly smaller than the decrease noted in group two. Further studies based on three-dimensional parameters are required to confirm these findings.

Our observations in alveoli preserved using gingival flaps suggest that primary closure of the alveolus after tooth extraction can also help maintain alveolar ridge height and that the flapless surgery to maintain bone tissue vascularization helps preserve the alveolar process. On the contrary, minor losses of the grafted material in the early postoperative period should be considered. Tomographic and clinical observations agree on the changes in the grafted bone upon healing (Barone, et al. [1]), as measurements conducted at the center of the ridge crest (clinical) are equivalent to those conducted in the cervical region of the middle slice (tomography). Dimensional alterations of the alveolar ridge cannot be completely prevented by alveolar preservation (Horváth, et al. [41]), which is in agreement with the results of the present study; however, guided bone regeneration without biomaterial filling appears to have no advantage. The central portion of the alveolus is clinically interesting because increased bone resorption occurs at this site (Barone, et al. [1,5]), thus carrying increased risk of alveolar ridge micro-fractures during tooth extraction maneuvers. Therefore, we emphasize the importance of performing the extraction carefully to minimize trauma to the tissue and maintain the remaining bone structure in the medium or long term. The optimal clinical protocol for alveolar preservation after dental extraction has yet to be established. We recommend the surgeon's decision be based on the following factors: thorough knowledge of the material to be grafted, its origin, and its potential interaction with the host tissue; cortical bone thickness of the alveolus after tooth extraction; the need to elevate the mucoperiosteal flap; the opportunity to minimize tissue trauma during tooth extraction; and the timing and technique of alveolar closure.

Conclusion

Within the context of a clinical randomized, not blinded study, we conclude that the strategy bovine bone mineral matrix + gingival flap is superior to gingival flap + blood clot or polypropylene barrier + blood clot, providing higher dimensional stability of the alveolar ridge, though guided bone regeneration provided better quality and quantity of the newly formed bone tissue. Further randomized clinical studies and comparative studies using different techniques in different clinical situations are needed to establish a consensus.

Authors Contribution Statements

- Lima, F.F.B.; Sotto-Maior, B.S.; Francischone, C.E. Contributed to conception and design.
- Lima, F.F.B.; Damazo, A.S. Contributes to acquisition, analysis and interpretation of the data.
- Lima, F.F.B. Drafted the Manuscript.
- Lima, F.F.B; Damazo, A.S.; Sotto-Maior, B.S.; Francischone, C.E. Gave final approval and agrees to be accountable for all aspects of work ensuring integrity and accuracy.
- Sotto-Maior, B.S.; Francischone, C.E. critically revised the manuscript.

Acknowledgment

This study did not receive any funding from the public or private sector. The authors report no competing interests.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2022.43.006897

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