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ORIGINAL ARTICLE

Comparative Study of Alloplastic and Xenogeneic Biomaterials Used for in Dentistry

Jairo Marcos Gross[1] · Carlos Nelson Elias[2] · Jorge José de Carvalho[1] · Andrea Gross[1] · André Antônio Pelegrine[1] · Bruno Salles Sotto Maior[1] · Ronaldo Sergio de Biasi[2] · Igor da Silva Brum[1]

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Abstract
Biomaterials are routinely used in dentistry for tissue engineering. The purpose of the present work is to compare the performance of a new alloplastic biomaterial (Blue-Bone®), xenogeneic biomaterial (Bio-Oss®), and a mixture of both biomaterials with 50% of autogenous bone. 32 Wistar rats underwent a surgical procedure in which a circular disc of bone was removed from the calvaria with a trephine drill 10 mm in diameter to create a critical bone defect, which was filled with the biomaterials under study. After 40 days, the animals were euthanized and the calvaria was removed for processing and analysis. Histomorphometric determination of vital mineralized tissue (VMT), no-vital mineralized tissue (NVMT), and on-mineralized tissue (NVMT) was performed. The results showed that, while Bio-Oss® had the best performance when used in conjunction with autogenous bone, the addition of autogenous bone did not significantly improve Blue-Bone® performance.

Keywords Alloplastic biomaterials · Xenogeneic biomaterials · Bone regeneration

Introduction

Critical bone reconstruction represents the greatest challenge for successful dental implants. Autogenous bone grafting is considered the gold standard for this type of bone reconstruction since synthetic biomaterials do not have potential cells that contribute to neoformation [1–3]. Different types of synthetic and natural biomaterials are available in the market, and it is essential to evaluate their effectiveness and potential for bone formation. The use of animals for this type of experiment is perfectly justifiable since the most appropriate analysis for assessing the quality of the formed tissue called histomorphometry demands the removal of large bone fragments [4, 5].

The use of bone grafts before dental implant insertion has been a frequent practice to obtain adequate prosthetic rehabilitation. The improvement of the technique and the knowledge of its clinical evolution requires a review of the concepts of bone grafting to obtain the best behavior of the material and a minimum of complications.

The use of xenografts (biomaterials obtained from other species) involves the risk of disease transmission. The use of allografts (synthetic biomaterials) increases the probability of rejection due to the lack of osteoinductive characteristics [6]. Many authors suggest the use of alloplastic biomaterials (synthetic materials that contain some of the essential chemical components of natural bone (e.g., calcium and phosphate) [7].

Histology tests of the ossification process at different stages after surgery are important to outline treatment alternatives and propose changes in the development of other materials, enabling a more reliable advance with safer results [8–11].

The purpose of the present study was to evaluate the performance of two biomaterials (Blue-Bone® and Bio-Oss®) in the bone reconstruction of critical defects. Blue-Bone® (Regener Biomateriais Co, Curitiba, Brazil) is an alloplastic biomaterial made with a mixture of nanometric hydroxyapatite (80%) and ß-TCP (20%). Bio-Oss® (Geistlich Pharma AG, Wolhusen, Switzerland) is a deproteinized bovine bone material.

✉ Carlos Nelson Elias
elias@ime.eb.br

[1] Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[2] Instituto Militar de Engenharia, Rio de Janeiro, RJ, Brazil

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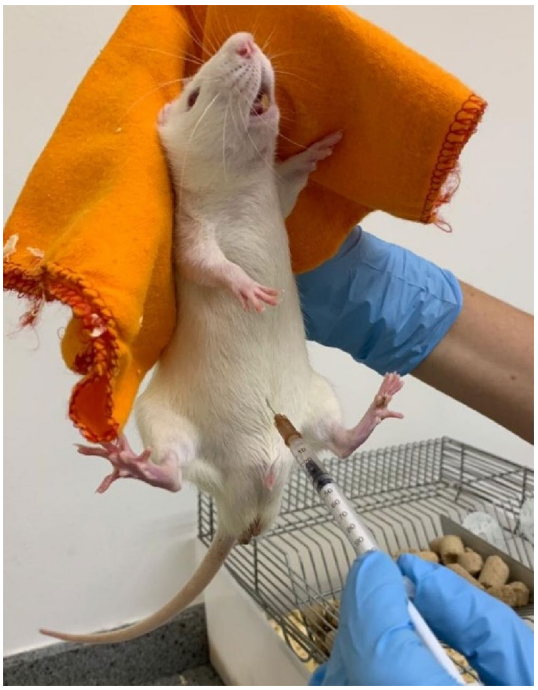

Fig. 1 Intramuscular anesthesia Ketamine 40–80 IU (mg/kg) and Xylazine 5–10 IU (mg/kg)

Methodology

The number of samples was calculated using the G*Power 3.1.5 free software, adopting the analysis of variance model. For a medium effect size of 0.50, as proposed by Cohen (1988), with a significance level of 5% and a power of 80%, the calculations indicated the need for 8 animals for each group. The present study used 32 Wistar rats.

Thirty-two bone defects were created in the calvaria of 32 rats (Fig. 1), which were divided into 4 groups, according to the type of filling biomaterial:

- Group 1: the defect was filled with pure Bio-Oss® hydrated in saline solution (N=8).
- Group 2: the defect was filled with Bio-Oss® mixed with 50% of autogenous bone taken from the defect (N=8).
- Group 3: the defect was filled with pure Blue-Bone® hydrated in saline solution (N=8).
- Group 4: the defect was filled with Blue-Bone® mixed with 50% of autogenous bone taken from the defect (N=8).

After anesthesia, a trichotomy was performed in the region of the calvaria, and antisepsis was performed with 10% polyvinylpyrrolidone-iodine (PVPI) with 1% active iodine. Figure 2 shows the trichotomy in the region of the calvaria.

After trichotomy and antisepsis, the specimens were placed on a special bed (Fig. 3) for the procedure. A 2 cm straight incision was made in the calvaria and flaps were folded back (Fig. 4). Critical bone defects were created with a trephine drill 10 mm in diameter (Fig. 5) rotating at 750 rpm, coupled in a 20:1 reducing angle to an electric motor, and under abundant cooling with sterile saline solution.

After preparing the critical bone defect, the hole was filled with biomaterial. The weight of the biomaterial inserted into the defect was measured on a precision scale (0.01 g accuracy). Equal weights of biomaterial were inserted. After filling the cavity with the biomaterial, a suture was performed to keep the graft in place, preserving the periosteum (Fig. 6).

After filling and covering, the skin was sutured using simple stitches with 4.0 Shalon mononylon thread (Fig. 6).

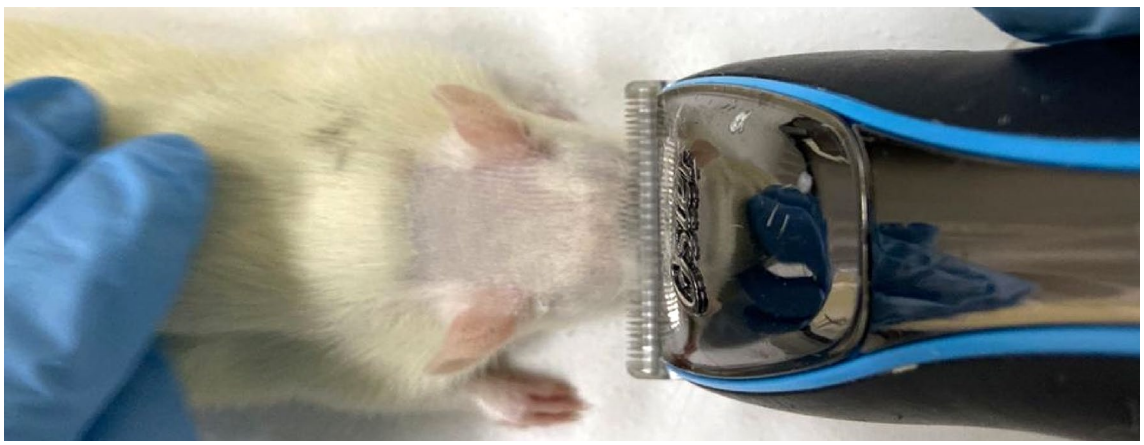

Fig. 2 Trichotomy in the region of the calvaria

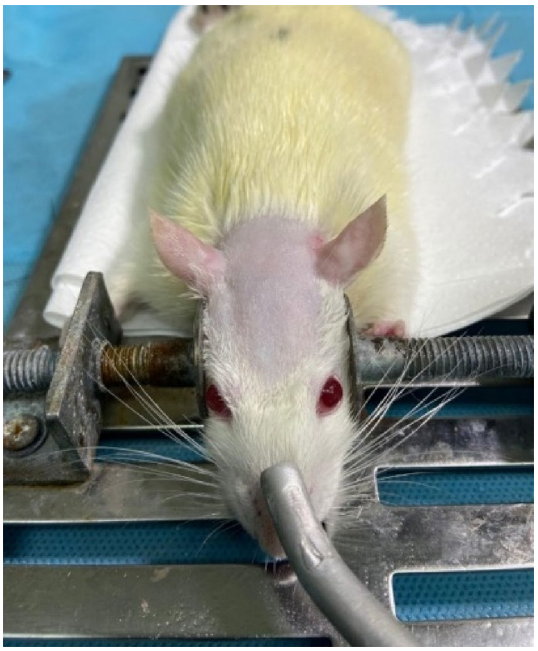

Fig. 3 Specimen accommodated in a special bed

After surgery, the animals were placed in individual cages and received a single dose of Benzetacil 600,000 IU.

The animals were sacrificed on the 40th postoperative day. This time is equivalent to eight months of human life. Euthanasia was performed by administering a lethal intravenous dose of ketamine (2 mL) and xylazine (1 mL). The calvarias were removed using diamond discs and immediately taken to the laboratory for processing (Fig. 7).

Preparation and analysis of the calvaria samples followed standard procedures, as described below:

(a) The samples were decalcified in 20% formic acid (Merck© Darmstadt–Germany) for a period of six days;
(b) the samples were washed in running water for 24 h;
(c) the samples went through the process of dehydration and clearing in a Leica® histological tissue processor for 12 h following the following sequence with one-hour immersion in each product: 70% alcohol, 80% alcohol, 90% alcohol, 95% alcohol, absolute alcohol I, absolute alcohol II, absolute alcohol III, alcohol/xylene, xylene I, xylene II, paraffin I and paraffin II (Fig. 8).
(d) the material was packed in a special container and embedded in Synth® histological paraffin in embedding equipment (Fig. 9).
(e) microtomy was performed in a Leica® RM2245 microtome (Fig. 10) with cuts 4 µm thick, distended in glass slides and kept in an oven at 60 °C for 1 h.

The data collected in the analysis of vital mineralized tissue (VMT), no-vital (NVT), and non-mineralized tissue (NVMT) tissues were evaluated for compliance with the assumptions of normality (Shapiro–Wilk tests) and homogeneity of variance (Levene tests).

Extremely discrepant data were identified using the boxplot method in one sample that received the Blue-Bone® biomaterial mixed with autogenous bone. After removing these samples from the analysis, normality and homogeneity of variance were met. Next, a two-way analysis of variance was applied to compare the performance of the biomaterials.

Statistical analyses were conducted using the SPSS 23 program (SPSS Inc., Chicago, IL, USA), adopting a significance level of 5%, setting the rejection level at 5% ($p < 0.05$).

Results

For each blade, 4 cuts were obtained represented by the Roman numerals I, II, III, and IV. The two best cuts were chosen, an average of 8 pictures were taken. Figures 11 and 12 show representative slides from different groups of samples.

The pictures were analyzed using the software Image J for Windows® for histomorphometry analysis. This program makes it possible to select the area of interest for each type of tissue in the picture. The results are shown in Tables 1 and 2, with the area in square micrometers.

Table 2 does not show the data for the rats that died during the experiment, or, as stated before, were extremely discrepant.

A two-way analysis of the experimental data (Table 3 and Fig. 13) showed that there was no statistically significant difference between pure Bio-Oss® and pure Blue-Bone® samples ($p = 0.056$). Pure for Bio-Oss® and Blue-Bone® samples had a lower percentage of MVT than mixed samples.

Discussion

The results of the present study show that there is a statistically significant difference percentage in the quality of the tissues formed when cavities are filled with xenograft or pure allograft. The percentage of non-mineralized tissue (NVMT) inside the defect filled with Blue-Bone® biomaterial is significantly higher than inside the defect filled with Bio-Oss® (Fig. 13). The mixed of pure biomaterial with bone did not present a significant difference in the percentage of non-mineralized tissue. This result may be linked to the preparation of defects with diameters (10 mm) greater than the critical size used in several studies. Most studies use defects of 3 to 5 mm in diameter

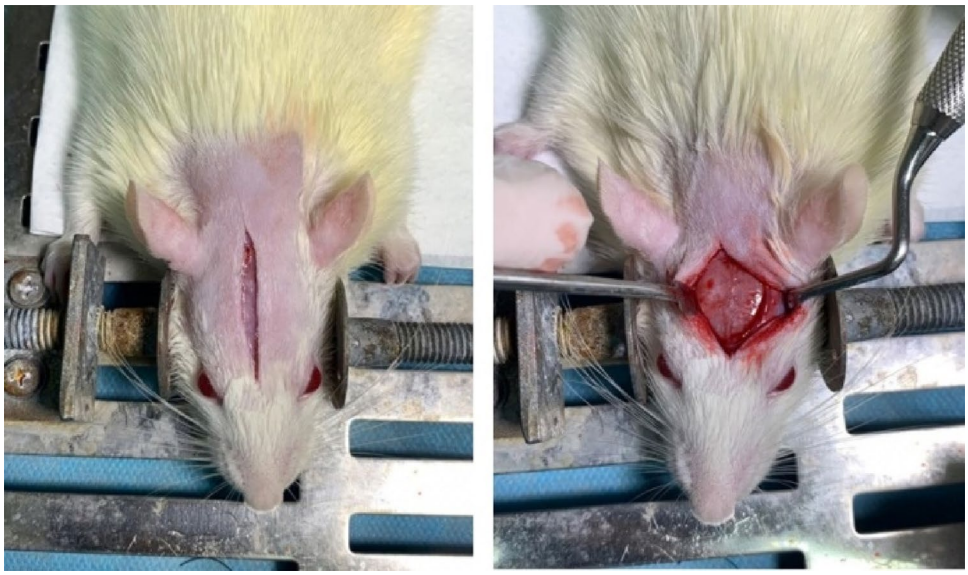

Fig. 4 Straight incision 2 cm long and the flaps folded back

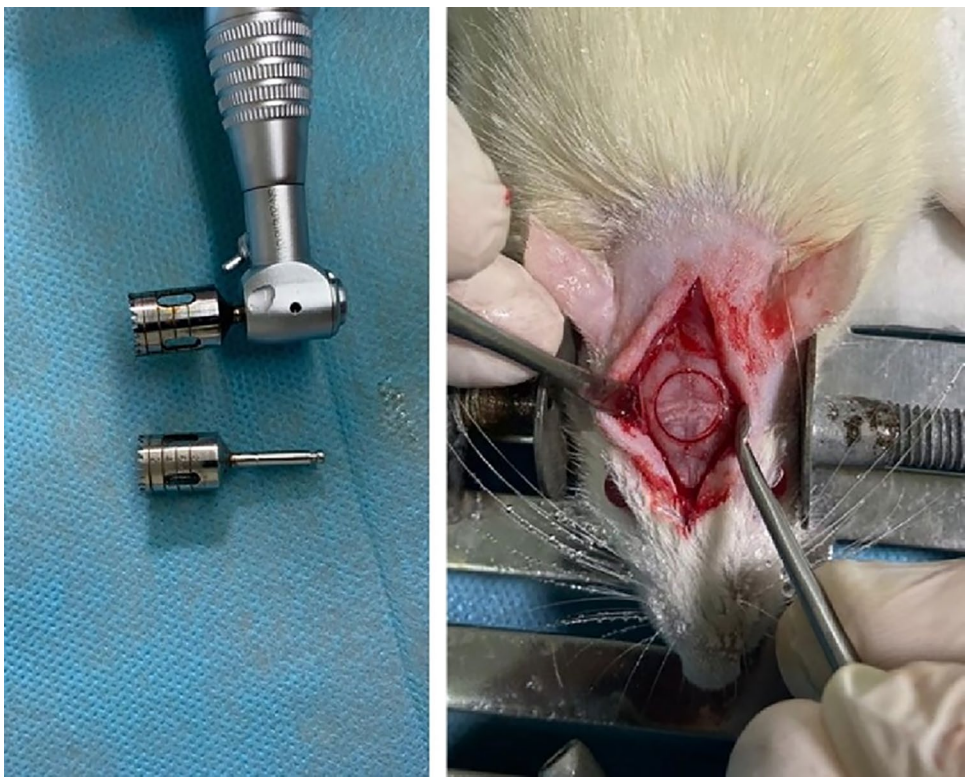

Fig. 5 Critical bone defect created with a 10 mm diameter trephine drill

[12–14]. As the defect size increases, the displacement of the graft from its position increases.

The biocompatibilities of the products were determined by the absence of areas with acute and persistent inflammatory events 40 days after surgery. In the macroscopic analysis, it was observed that all the lesions caused in the bone were similar. The lesions of Bio-Oss® and Blue-Bone® did not show the presence of exudate in the bone pockets and the remaining material was aggregated without signs of an active infectious process. This result corroborates data obtained by Zecha et al., (Tables 1 and 2).

Kotake et al. investigated the influence of the F1 protein extracted from the rubber tree *Hevea brasiliensis*, which has important properties for tissue repair and is associated

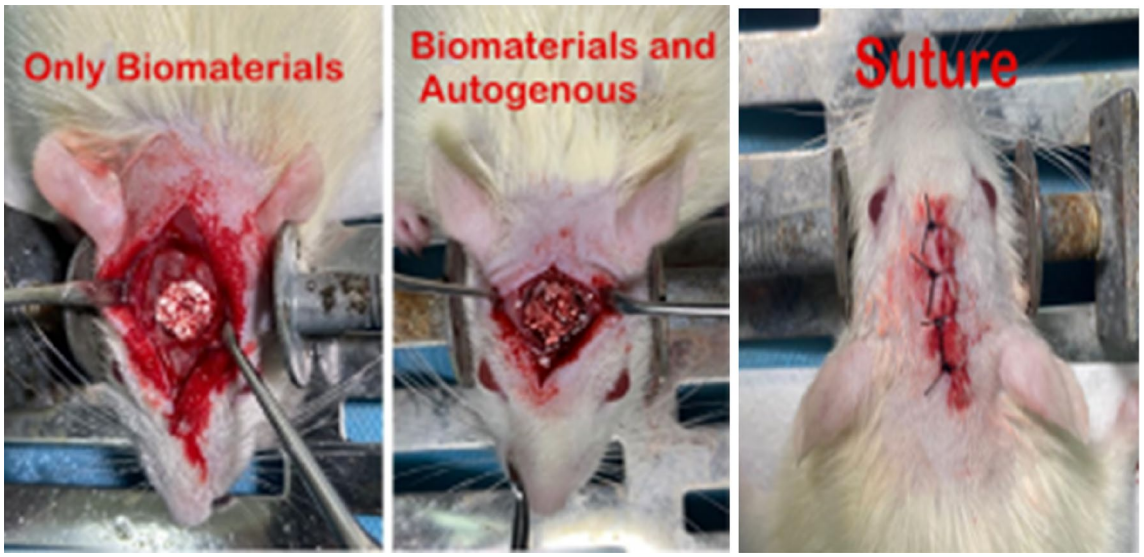

Fig. 6 Procedure of one of the graft surgeries with Blue-Bone®

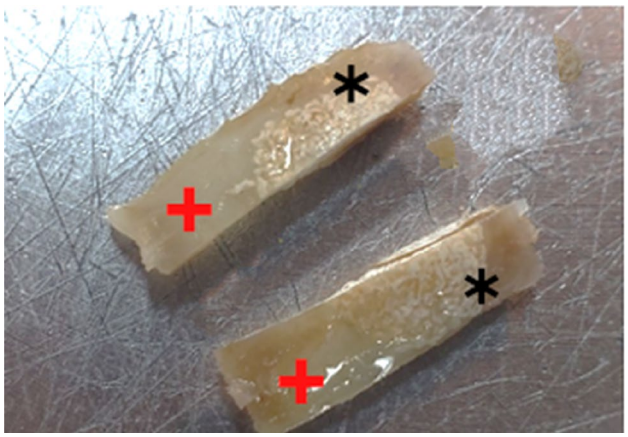

Fig. 7 Specimen divided in half ready for slide preparation. + native bone, * biomaterial

Fig. 8 Leica Diaphanizer

with neoangiogenesis, cell adhesion and extracellular matrix formation [15]. The main objective of their studies was to investigate the association of the F1 protein to different bone grafts in the repair of critical bone defects in the calvaria of Wistar rats. A total of 112 Wistar rats were divided into autograft, allograft and xenograft used pure and/or associated with F1 protein, with a waiting period of 4 and 6 weeks. Stereological results for autografts and xenografts associated or not with F1 protein had greater bone neoformation ($p < 0.05$), leading to the conclusion that bone graft associated or not with F1 increases angiogenesis and osteogenesis. This alternative is more viable compared to the present work

Fig. 9 Paraffin embedding

Fig. 10 Leica® RM2245 microtome

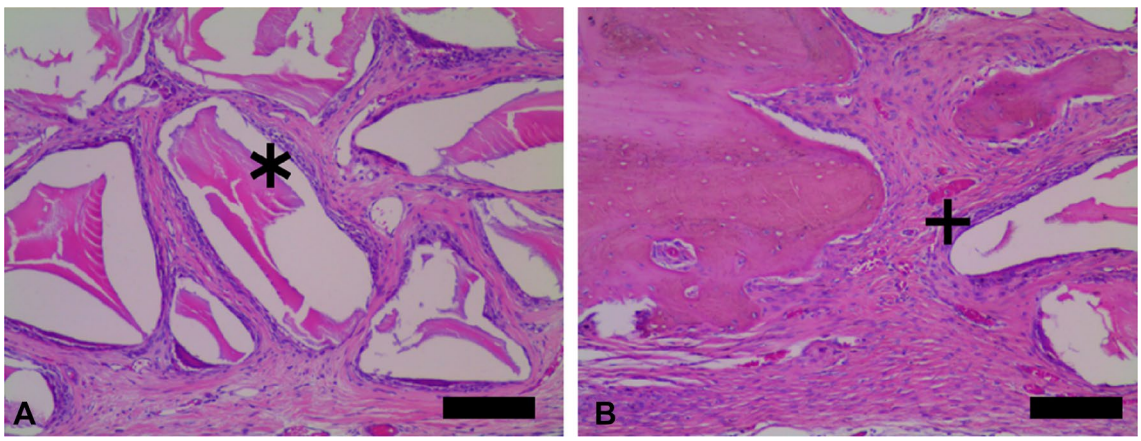

Fig. 11 **a** Pure Bio-Oss® biomaterial; the biomaterial and NVMT can be seen (*). **b** Bio-Oss® mixed with autogenous bone; one can see the biomaterial, NVMT (+) containing fibrous and connective tissue and blood cells (NVMT)

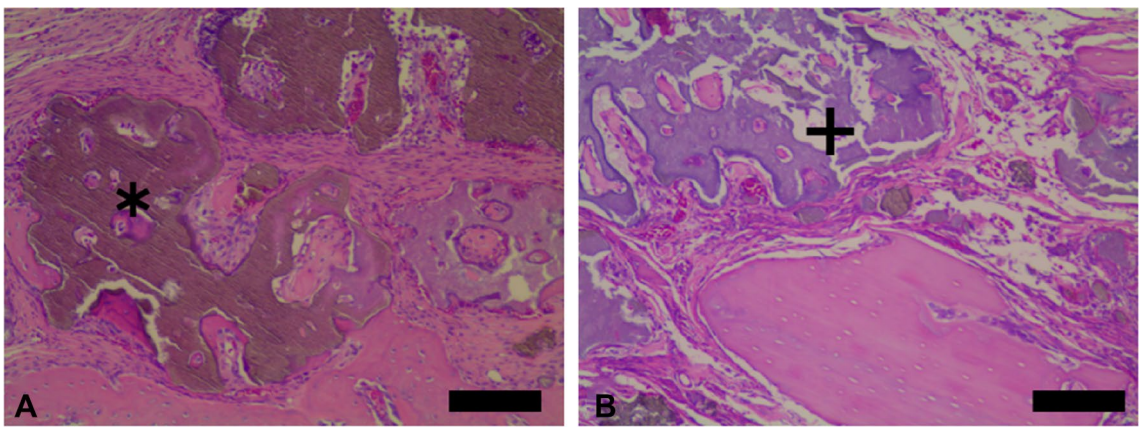

Fig. 12 **a** Pure Blue-Bone® showing the biomaterial and NVMT can be observed. **b** Blue-Bone® mixed with autogenous bone; the biomaterial and NVMT can be observed

Table 1 Data on tissue quality for pure Bio-Oss® samples and samples of Bio-Oss® mixed with 50% of autogenous bone

Sample		Pure Bio-Oss		Sample	Bio-Oss + 50% bone	
		Mean	SD		Mean	SD
1	NMT	63.45	1.51	9	77.62	0.12
2	NMT	64.56	2.98	10	85.27	4.70
3	NMT	56.3	5.27	11	65.42	3.69
4	NMT	45.54	2.36	12	53.31	16.25
5	NMT	67.63	1.26	13	79.77	1.16
6	NMT	57.31	2.18	14	53.42	4.86
7	NMT	43.36	4.10	15	58.92	5.27
8	NMT	44.27	2.70	16	66.60	10.85
Mean		55.30			67.54	
SD		9.77			12.23	
1	NVMT	35.84	2.83	9	12.18	2.55
2	NVMT	34.93	4.56	10	12.53	1.59
3	NVMT	40.55	2.08	11	16.25	3.72
4	NVMT	47.91	2.33	12	25.19	12.27
5	NVMT	31.61	0.68	13	23.59	0.30
6	NVMT	39.95	0.02	14	32.55	3.74
7	NVMT	51.87	4.11	15	18.35	1.74
8	NVMT	50.45	3.12	16	25.22	2.64
Mean		41.64			20.73	
SD		7.61			7.11	
1	VMT	0.71	0.95	9	10.2	2.43
2	VMT	0.51	0.15	10	2.2	3.11
3	VMT	3.15	0.71	11	18.33	3.72
4	VMT	6.55	4.45	12	21.49	3.98
5	VMT	0.76	1.07	13	16.64	1.47
6	VMT	2.74	1.50	14	14.03	8.60
7	VMT	4.77	4.12	15	22.72	7.00
8	VMT	5.28	1.41	16	8.18	8.21
Mean		3.06			14.22	
SD		2.31			7.02	

because there would be no need to remove autogenous bone to achieve better vascularization compared to the Bio Oss xenograft.

The incorporation of autogenous bone when handling the biomaterial is a common practice. This option is frequently used to promote better cellularity and vascularity. The result showed that the pure Bio-Oss® xenograft has a better performance than the association of Bio-Oss® with autogenous bone [16]. This result may be attributed to the fact that Bio-Oss® macrogeometry has a non-porous structure. This lack of porosity means that the granules are sequestered and regeneration takes place between the granules. The development of nanomaterials such as Blue-Bone® make it possible to promote the formation of a more cellular and vascularized bone matrix, due to its framework providing ideal conditions for the bone remodeling process to occur more effectively [4, 5, 17].

The addition of autogenous bone requires a second surgery which generates discomfort for the patient [18–20]. Blue-Bone® proved that there is no need to add autogenous bone to promote a vascularized bone matrix (Fig. 13). This finding is of great relevance because it demonstrates that it is possible to regenerate large areas with bone defects using only a biomaterial, bringing more predictability and comfort for the surgeon and the patient [21].

Table 2 Data on tissue quality for pure Blue-Bone® samples and samples of Blue-Bone ® mixed with 50% of autogenous bone

Sample		Pure Blu-Bone		Sample	Blue-Bone + 50% Bone	
		Mean	SD		Mean	SD
17	NMT	81.84	6.75	25	72.04	37.48
18	NMT	81.85	5.10	26	73.54	0.30
19	NMT	87.16	1.09	27	78.14	0.29
20	NMT	85.02	11.10	28	79.04	4.96
21	NMT	82.14	3.73	29	75.83	0.25
22	NMT	81.01	2.56	30	74.47	1.07
23	NMT	–	–	31	78.77	3.49
24	NMT	89.42	0.56	32	–	–
Mean		84.06			75.98	
SD		3.22			2.76	
17	NVMT	16.49	5.01	25	22.89	38.12
18	NVMT	16.16	3.00	26	20.4	5.93
19	NVMT	11.94	0.24	27	13.99	0.65
20	NVMT	14.69	3.37	28	16.49	0.50
21	NVMT	17.61	2.44	29	17.21	0.05
22	NVMT	18.44	2.44	30	16.94	0.05
23	NVMT	–	–	31	9.96	1.42
24	NVMT	9.69	0.56	32	–	–
Mean		15.00			16.84	
SD		3.16			4.18	
17	VMT	1.67	1.74	25	5.07	0.64
18	VMT	1.99	2.10	26	6.06	6.23
19	VMT	0.9	0.85	27	7.87	0.36
20	VMT	0.29	0.09	28	4.47	4.14
21	VMT	0.25	0.35	29	6.96	0.76
22	VMT	0.55	0.13	30	8.59	1.02
23	VMT	–	–	31	11.27	2.06
24	VMT	0.89		32	–	–
Mean		0.93			7.18	
SD		0.67			2.32	

Table 3 Percentage (mean and standard deviation) of tissue quality from pure biomaterials and samples of biomaterials mixed with 50% of autogenous bone

	Bio-Oss		Blue-Bone	
	Pure	Mixed	Pure	Mixed
NMT	55.30% (9.77%)	67.54% (12.23%)	84.06% (3.22%)	75.98% (2.76%)
NVMT	41.64% (7.61%)	20.73% (7.11%)	15.0% (3.16%)	16.84 (4.18%)
VMT	3.06% (2.31%)	14.22% (7.02%)	0.93% (0.67%)	7.18% (2.32%)

Conclusion

Based on the results obtained in this work, it can be concluded that:

(a) The Blue-Bone® biomaterial does not need to be mixed with an autogenous bone to promote a more cellular and vascularized bone matrix.
(b) Filling of cavities with Bio-Oss® presents better results when mixed with an autogenous bone when compared to the pure material.

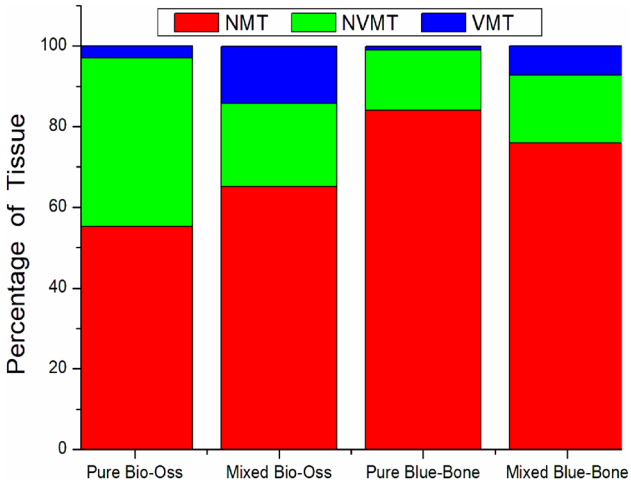

Fig. 13 Percentage of vital and no-vital mineralized tissues and non-mineralized for pure and mixed samples

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Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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