Oral rehabilitation by means of implants often requires alveolar bone volume augmentation. When the width of the alveolar crest is not enough for implant placement or does not allow the placement of the implant in an ideal position, a surgical technique for bone volume augmentation might be required. A recent systematic review with meta-analysis reported the dimensional changes after lateral bone augmentation. Various techniques were evaluated, and among these, the most frequently used was the autologous bone block. It was reported that this technique produced a mean width gain of 4.25 mm, as evaluated at the surgical reentry. In another review with meta-analysis, the lateral gain as evaluated at the surgical session using autogenous bone blocks was 3.7 mm. After 6 months, < 1 mm of width was lost, so the insertion of implants was possible and a success rate of 98.9% to 100% was achieved.

Grafting procedures using autogenous bone blocks require the inclusion of a donor site with increased morbidity and pain for the patients. Hence, the use of blocks of allograft, xenograft, or alloplastic grafts represents an interesting alternative for lateral alveolar ridge augmentation.

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**Purpose:** To evaluate the impact on healing of bioactivation with argon plasma of a xenogeneic graft with adequate fixation but poor adaptation to the native host bone. **Materials and Methods:** Xenogeneic grafts were either treated with argon plasma or left untreated and randomly secured with a titanium screw to both lateral aspects of the mandible angle of rabbits. A discrepancy was obtained between the xenograft and the mandible due to the convexity of the recipient site. Collagen membranes were placed on the grafts. Thirty animals were included and euthanized in groups of 10 after 2, 6, and 10 weeks, respectively. Histomorphometric evaluations were performed on ground sections. Newly formed bone was the primary outcome, while the distance between the peak of new bone inside the graft and the upper surface of the graft, the xenograft percentages, and the area of the xenograft were considered as secondary variables. The Wilcoxon test was applied for statistical analyses. **Results:** After 2 weeks of healing, gaps of ~0.5 mm were observed at the interface between the graft and the recipient sites, and new bone was mainly located in the interface and within the inferior regions of the grafts. New bone increased over time in all regions, including those in the upper zones of the graft, reaching proportions of 20.3% ± 6.5% and 19.3% ± 7.4% (P = .484) after 10 weeks in the plasma and control groups, respectively. The mean distance between the peak of new bone inside the graft and the upper surface of the graft decreased between 2 and 10 weeks of healing from 1.4 mm to 0.4 mm and from 1.7 mm to 0.3 mm at the plasma and control sites, respectively. **Conclusion:** The xenogeneic grafts of both groups were incorporated into the recipient sites by newly formed bone that presented a growth close to the upper surface of the graft. The bioactivation with argon plasma did not improve healing. **Keywords:** argon plasma, biomaterials, bone graft, histology, surgical procedure
The use of corticocancellous fresh-frozen allograft bone blocks had been described for augmenting atrophic posterior mandibles in humans. Fresh-frozen allografts presented approximately 20% of newly formed bone after 6 months. However, a substantial volumetric contraction of the blocks (~40%) was described for those allografts during a period comprised between surgery and the first year of functional loading.

Recently, a randomized, controlled, split-mouth, prospective clinical trial compared autografts and xenograft in blocks for anterior maxillary horizontal bone augmentation. Although lower insertion torques were obtained during implant placement, xenograft bone blocks were demonstrated to be an appropriate alternative to autogenous bone, with suitable volumetric stability over time. Nevertheless, experimental studies reported contradictory results using xenograft blocks. Some experiments registered minimal amounts of new bone formation within xenogeneic bone, while others reported optimal results.

Key factors for good incorporation of a block graft are rigid fixation and optimal adaptation between the graft and recipient site, reducing the gap at the interface, which is obtained by trimming the graft and recontouring the recipient site. Moreover, the addition of particles of autogenous bone or xenograft to fill the gaps between the graft and recipient site was suggested, aiming to improve the incorporation and reduce the resorption of the graft. The healing in the interface between an autogenic bone block and the recipient site was described in an experiment in rabbits. Despite the presence of gaps of 0.5 to 0.6 mm between these two units, the blocks were found to be incorporated to the recipient sites after 40 days of healing.

A treatment of titanium surfaces based on the activation of the external electronic mantle of the surface material by plasma of argon was introduced for abutment cleaning. This biophysical alteration of the surface finally affects the wettability of the material and then increases the cell/material adhesion.

This approach was initially adopted in the field of implant dentistry to bioactivate abutments and implants in vitro, in vivo, and human studies and then was translated to the graft material and bone regeneration. The treatment with plasma of argon improves hydrophilicity and free energy of the surface without altering surface morphology and chemical composition. It has been shown to enhance bone apposition on both implants and particulate graft material. In an experiment in dogs, implants were either treated with plasma of argon or left untreated. A higher bone-to-implant contact was observed at the treated compared with the untreated surfaces. In an experiment on sinus elevation in rabbits, a deproteinized bovine bone mineral was used as filler. A higher rate of bone formation was observed at the sites filled with the activated biomaterial compared with the control sites, even though the difference was statistically significant only in the most central regions of the sinus.

Even though a lack of intimate adaptation between the bone graft and recipient site yielded to a good incorporation of the graft, a similar situation using a xenograft block might be detrimental for proper healing. In this case, the bioactivation of a xenogeneic block with argon plasma might improve healing.

Hence, the aim of the present study was to evaluate the impact on healing of bioactivation with argon plasma of a xenogeneic graft with adequate fixation but poor adaptation to the native host bone.

**MATERIALS AND METHODS**

**Ethical Statement**

The ethical approval for the present study was given by the Ethical Committee at the School of Dentistry, of Ribeirão Preto, University of Sao Paulo (USP), protocol number 2018.1.9.58.9 on March 21, 2018, for 20 animals, increased to a total of 30 animals, with the addendum being approved on May 23, 2019, aiming to add a further period of healing. The study was carried out based on the ARRIVE guidelines and the animal welfare regulations were respected.

**Study Design**

The present split-mouth design study included a bilateral augmentation at the lateral aspect of the mandible in rabbits. The experiment was performed with xenogeneic block grafts of similar dimensions. One block was bioactivated with argon plasma (plasma sites), while the other was left untreated (control sites). Thirty rabbits, provided by Chacara Uniao, were included and divided into three groups of 10 animals, euthanized after 2, 6, and 10 weeks, respectively.

**Xenogeneic Block Graft**

Customized blocks of collagenated cancellous bone of equine derivation were preformed in cylinders of 7 mm in diameter and 3 mm in height (Tecnoss). The xenografts were provided with through holes, one central of 1.2 mm in diameter for fixation, and four adjunctive holes of 0.8 mm in diameter, aiming to improve the propagation of newly formed bone.

**Bioactivation with Argon Plasma**

The xenogeneic blocks were placed in a small cap (Fig 1a) and then in an argon plasma reactor (10 W, 1 bar for 12 minutes, Plasma R, Diener), and the protocol for the bioactivation was carried out.
Experimental Procedures

For sedation and anesthesia induction, acepromazine 1.0 mg/kg (Acepran, Vetnil) was injected subcutaneously, and xylazine 3.0 mg/kg (Dopaser, Hertape Calier) and ketamine hydrochloride 50 mg/kg (Ketamin Agener, União Quimica Farmacêutica Nacional S/A) were injected intramuscularly. Anesthesia was injected locally. An extraoral submandibular incision of ~3 cm was performed bilaterally in the lateral aspect of the angle of the mandible. The skin, muscles, and periosteum were incised, and the flaps were elevated, exposing the cortical bone of the experimental region. A standardized stent was used to perform nine perforations in the cortical layer (Fig 1b), and the block graft, 7 mm in diameter and 3 mm in height, was tightened onto the lateral aspect of the mandible using a fixation screw in titanium (Fig 1c), 10 mm long and 1.5 mm in diameter (Graft screw standard head, Neodent). No decorticalization of the convex shape of the recipient site was done to flatten its surface to have a better adaptation to the graft. Consequently, a gap occurred in the interface between the recipient convex site and the graft, especially in the peripheral regions. No biomaterial particles were used to fill the gap. A collagen membrane (Bio-Gide, Geistlich Biomaterials) was placed to cover the grafts, bilaterally (Fig 1d). Two layers of sutures were applied, profound resorbable for muscles and periosteum and superficial for the skin.

Euthanasia

The animals were sedated and euthanized with a lethal dose of sodium thiopental (1.0 g, 2 mL, Thiopentax, Cristália Produtos Químicos Farmacêuticos).

Experimental Animals

Thirty New Zealand rabbits, ~5 months old and a mean weight of 3.6 kg, were included in the experiment.

Housing and Husbandry

The animals were housed at the investigative center of the Dentistry School, USP, Ribeirão Preto (Brazil). Each animal was hosted in a separate cage in rooms with controlled light and temperatures. Professionals took care of the wounds, provided the planned drugs, and controlled the biologic functions during the experimental period. Full access to water and food was allowed. Antibiotics and anti-inflammatory/analgesic drugs were administrated (details are reported elsewhere).33

Fig 1 (a) The block was placed in a small cap and then in an argon plasma reactor. (b) The lateral aspect of the angle of the mandible was exposed, and the cortical layer was perforated with burs. (c) The xenogeneic block was secured with a fixation screw. (d) A collagen membrane was placed on the graft.
Sample Size
No similar experiments with xenogeneic block grafts exposed to argon plasma were performed before. Hence, for sample evaluation, a difference of 10% of new bone formed within the grafts between the two groups was judged to be of clinical relevance. A sample of 10 animals in each group was estimated, applying 10% as standard deviation, 0.8 as power, and alpha set at .05.

Randomization and Allocation Concealment
The randomization was performed at the website www.randomization.com, adopting blocks of 10 units by an author (D.B.) not involved in the surgical procedures. The masking of the information about treatment allocation sites was carried out by means of coded sealed opaque envelopes. At each surgery, the blocks were prepared, with one assistant inserting the screw, and one of the blocks was placed in the plasma reactor for the treatment. After the first exposure of the lateral aspect of the mandible and the recipient site preparation, the coded envelope was opened by an author not involved in the surgery (S.P.X.), and the allocation treatment was disclosed. The surgeon (E.R.S.) was blinded until this moment.

Histologic Preparation
After the euthanasia, specimens containing the blocks were harvested and maintained in 10% buffered formalin. The specimens were reduced in dimensions, dehydrated, and included in resin. The fixation screw was identified, and a mesiodistal cut was performed following the screw. Cutting and grinding equipment (Exakt, Apparatebau) was used for histologic preparation. Two ground sections of each specimen were obtained and stained with either Stevenel's blue and alizarin red or toluidine blue.

High-resolution photomicrographs (100× magnification) were scanned with a Digital Sight DS-2Mv (Nikon Corporation) coupled with a microscope (Eclipse Ci, Nikon Corporation) equipped with an EK14 motorized stage (Nikon Corporation).

Histologic Analyses
The histologic analyses were performed by an expert blinded examiner (K.A.A.A.). Prior to the measurements, a calibration with another expert (D.B.) was performed, and a K > 0.90 interobserver reliability was achieved.

The following histometric measurements were performed (Fig 2): (1) the height of the interface between the xenogeneic graft and the native host bone, measured in four regions, two bilaterally to the fixation screw (internal interface) and two in the peripheral edges of the graft (external interface); (2) the distance (new bone [NB] to the upper surface of the graft [SG]) between the peak of NB inside the graft and SG; and (3) the area occupied by the resorbed block xenograft.

Histomorphometric Analyses
The histomorphometric evaluations were performed by an expert blinded examiner (K.A.A.A.). Prior to the measurements, a calibration with another expert (D.B.) was performed, and a K > 0.90 interobserver reliability was achieved.

The histomorphometric measurements were accomplished using the NIS-Elements D 5.11 software (Laboratory Imaging, Nikon Corporation). Four regions were identified, both sides of the fixation screw: inferior/internal, inferior/external, superior/internal, and superior/external. One grid containing 16 × 10 squares of 75 microns (1,200 × 750 microns; 0.9 mm²) in dimensions was superposed to the image in each region. The following tissues were assessed: new bone, xenograft, soft tissues (marrow spaces, dense and loose tissues, connective tissue), vessels, inflammatory infiltrate, and osteoclastic zones.

Experimental Outcomes
The primary outcome was the percentage of new bone within the augmented region. The NB-SG height, the xenograft percentages, and the area of the xenograft were considered as secondary variables.

Separated evaluations were performed in four regions: inferior/internal, inferior/external, superior/internal, and superior/external. Moreover, the data were pooled together for the regions located in the following half parts of the graft (compartments), each side of the fixation screw: inferior (inferior/internal and inferior/external), superior (superior/internal and superior/external), internal (inferior/internal and superior/internal), and external (inferior/external and superior/external) compartments. Mean values were obtained for each region and compartment for the data assessed in both sides lateral to the fixation screw.

Statistical Methods
The differences in means between the plasma and the control sites were analyzed in the full area, for all tissues, and in the various regions and compartments, for new bone and xenograft. Moreover, differences in means were also analyzed between the inferior and superior compartments, and the internal and external compartments. The Wilcoxon test was applied using the IBM SPSS Statistics software (IBM). The statistical significance was set at .05.
RESULTS

Two grafts of the plasma sites presented a strong inflammatory infiltrate within membranes, graft, and the peripheral zone. In one case, the fixation screw lost the retention for an intense resorptive process that occurred at the cortical layer. At these sites, no new bone formation from the cortical layer or inside the grafts was observed, so the histologic data were not included in the analysis. Consequently, the sample for the 10-week group was reduced to n = 8, while n = 10 was maintained for both the 2- and 6-week groups.

Discrepancies of the adaptation of the xenogeneic block onto the host site were evidenced. The internal interface distances were 0.32 ± 0.16 mm in the plasma sites and 0.36 ± 0.20 mm in the control sites. The corresponding distance at the external interface was 0.55 ± 0.16 mm and 0.60 ± 0.23 mm. No statistically significant differences were found between the plasma and control sites, while the differences between the internal and external interfaces were statistically significant for both sites. These outcomes provide the evidence of the convexity of the native host bone and the presence of a gap between the graft and recipient site.

A similar pattern of healing was found for both groups of treatments in all periods evaluated.

Two Weeks of Healing

After 2 weeks, woven bone was found forming from the cortical layer and from the bur perforations performed in the cortical bone during the surgery (Figs 3a and 3b). The new bone grew inside the graft-host interface, sprouting fingerlike ridges from the cortical layer toward the graft, gaining connections with its trabecula and with the fixation screw surface (Fig 4a). Woven bone also formed at the periphery of the block, propagating from the parent cortical host bone within the interface and toward the graft surface (Fig 4b). In this period of healing (Table 1, Fig 5a), new bone within the graft and interface was 7.8% ± 3.8% in the plasma sites, and 6.7% ± 3.6% in the control sites (P = .359). The highest fraction was found in the inferior/external quadrant of the plasma group (14.4%; Table 2). The inferior compartment (Table 3; Fig 5a) presented statistically significantly higher percentages of new bone in both the plasma and the control sites (~12% to 14%) compared with the superior compartment (~1% to 2%). The internal and external compartments presented similar fractions of new bone for both the plasma and the control sites (~6% to 8%). None of the differences between the plasma and control sites were found to be statistically significant. The NB-SG height was 1.4 ± 0.6 mm and 1.7 ± 0.5 mm at the plasma and control sites, respectively (P = .114; Table 4).

Six Weeks of Healing

After 6 weeks of healing, the consolidation process of the graft to the native host bone was observed (Figs 6a and 6b). Higher amounts of new bone were found inside the graft compared with the previous
period, especially located in the inferior compartment (Fig 7a). Bone ingrowth also increased in the superior compartment due to bone apposition onto the fixation screw and the trabecula surfaces (Fig 7b). New bone increased to 15.2% ± 4.4% and 14.9% ± 5.2% at the plasma and control sites, respectively (Table 1; Fig 5b). Statistically significantly higher amounts of new bone were found in the inferior (~20% to 22%) compared with the superior compartments (8% to 9%), as well as in the internal (~17%) compared with the external (~13%) compartments, in both the plasma and the control sites (Table 3). None of the differences between the plasma and control sites were statistically significant. The distance NB-SG was reduced to 1.0 ± 0.5 mm and 0.9 ± 0.7 mm at the plasma and control sites, respectively ($P = .646$; Table 4).

**Ten Weeks of Healing**

After 10 weeks of healing, new bone further grew deeper into the graft in both the plasma and control sites (Figs 8a and 8b). In some cases, the new bone reached the most coronal regions of the xenograft block (Fig 9a). The healing was still in progress in several areas, as substantiated by the presence of osteoid.
Parts of the trabecula were substituted by new bone, and marrow structures were occupying intertrabecular spaces, reaching fractions of 36% to 38% (Table 1; Fig 5c). At this stage of healing, the proportions of new bone were similar in all compartments and increased to 20.3% ± 6.5% and 19.3% ± 7.4% in the plasma and control sites, respectively (P = .484).

The highest amounts of new bone were found in the inferior (~22%) compartment, and the lowest in the superior (16% to 19%) compartment, with the difference between them being statistically significant only for the plasma group (P = .799; Table 3). The distance NB-SG further decreased to 0.4 ± 0.4 mm and 0.3 ± 0.2 mm at the plasma and control sites, respectively (P = .484).

Between 2 and 6 weeks, the resorptive processes reduced the proportions of xenograft from 37.0% ± 8.1% to 25.0% ± 8.4% in the plasma sites, and from 34.3% ± 4.9% to 25.6% ± 5.4% in the control sites (Table 4). Minor changes were seen between 6 and 10 weeks of healing. The area of the graft decreased between 2 and 10 weeks from 17.2 ± 1.6 mm² to 12.5 ± 2.7 mm² in the plasma sites, and from 17.9 ± 1.6 mm² to 11.7 ± 2.9 mm² in the control sites.

### Table 2: New Bone and Xenograft Percentages in the Various Regions of the Graft Analyzed in the Plasma and Control Sites After 2, 6, and 10 Weeks of Healing

<table>
<thead>
<tr>
<th></th>
<th>Inferior/Internal</th>
<th>Inferior/External</th>
<th>Superior/Internal</th>
<th>Superior/External</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 wk plasma</strong></td>
<td>13.2 ± 6.0 (5.0; 21.4)</td>
<td>14.4 ± 6.9 (10.1; 18.7)</td>
<td>1.4 ± 1.8 (0.3; 2.5)</td>
<td>2.2 ± 2.5 (0.6; 3.8)</td>
</tr>
<tr>
<td><strong>2 wk control</strong></td>
<td>13.1 ± 5.5 (9.7; 16.5)</td>
<td>11.3 ± 6.5 (7.2; 15.3)</td>
<td>1.4 ± 2.3 (0.0; 2.8)</td>
<td>1.0 ± 1.7 (0.0; 2.1)</td>
</tr>
<tr>
<td>P value (n = 10)</td>
<td>.878</td>
<td>.203</td>
<td>.478</td>
<td>.107</td>
</tr>
<tr>
<td><strong>6 wk plasma</strong></td>
<td>22.4 ± 5.6 (19.0; 25.9)</td>
<td>21.7 ± 5.6 (18.2; 25.2)</td>
<td>11.5 ± 8.6 (6.2; 16.9)</td>
<td>5.2 ± 7.2 (0.7; 9.7)</td>
</tr>
<tr>
<td><strong>6 wk control</strong></td>
<td>21.8 ± 4.3 (19.1; 24.4)</td>
<td>19.3 ± 7.7 (14.5; 24.0)</td>
<td>11.3 ± 6.8 (7.1; 15.5)</td>
<td>7.4 ± 5.4 (4.0; 10.7)</td>
</tr>
<tr>
<td>P value (n = 10)</td>
<td>.445</td>
<td>.445</td>
<td>.959</td>
<td>.343</td>
</tr>
<tr>
<td><strong>10 wk plasma</strong></td>
<td>22.6 ± 8.0 (17.0; 28.1)</td>
<td>21.1 ± 6.8 (16.4; 25.8)</td>
<td>19.6 ± 9.7 (12.8; 26.3)</td>
<td>18.1 ± 5.0 (14.7; 21.6)</td>
</tr>
<tr>
<td><strong>10 wk control</strong></td>
<td>21.5 ± 8.8 (15.4; 27.6)</td>
<td>22.7 ± 8.0 (17.1; 28.2)</td>
<td>24.0 ± 6.8 (19.3; 28.8)</td>
<td>15.8 ± 10.4 (8.6; 23.1)</td>
</tr>
<tr>
<td>P value (n = 8)</td>
<td>.779</td>
<td>.624</td>
<td>.735</td>
<td>.401</td>
</tr>
</tbody>
</table>

None of the difference between plasma and control sites was statistically significant. Mean values ± SD; 95% confidence interval (lower; upper) in percentages.

### Table 3: Pooled Data of New Bone and Xenograft Percentages in the Inferior, Superior, Internal, and External Compartments of the Graft in the Plasma and the Control Sites: Analyses Performed After 2, 6, and 10 Weeks of Healing

<table>
<thead>
<tr>
<th></th>
<th>Inferior</th>
<th>Superior</th>
<th>Internal</th>
<th>External</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2 wk plasma</strong></td>
<td>13.8 ± 6.1* (10.0; 17.6)</td>
<td>1.8 ± 1.8* (0.6; 2.9)</td>
<td>7.3 ± 3.7 (5.0; 9.6)</td>
<td>8.3 ± 4.2 (5.7; 10.9)</td>
</tr>
<tr>
<td><strong>2 wk control</strong></td>
<td>12.2 ± 5.6* (8.7; 15.5)</td>
<td>1.2 ± 1.9* (0.0; 2.4)</td>
<td>7.3 ± 3.7 (5.0; 9.6)</td>
<td>6.1 ± 3.9 (3.7; 6.6)</td>
</tr>
<tr>
<td>P value (n = 10)</td>
<td>.314</td>
<td>.233</td>
<td>1.000</td>
<td>.285</td>
</tr>
<tr>
<td><strong>6 wk plasma</strong></td>
<td>22.1 ± 4.0* (19.6; 24.5)</td>
<td>8.4 ± 7.5* (3.8; 13.0)</td>
<td>17.0 ± 4.6* (14.2; 19.8)</td>
<td>13.5 ± 5.2* (10.2; 16.7)</td>
</tr>
<tr>
<td><strong>6 wk control</strong></td>
<td>20.5 ± 5.2* (17.3; 23.7)</td>
<td>9.3 ± 5.8* (5.8; 12.9)</td>
<td>16.5 ± 4.8* (13.5; 19.5)</td>
<td>13.3 ± 5.9* (9.7; 17.0)</td>
</tr>
<tr>
<td>P value (n = 10)</td>
<td>.386</td>
<td>.721</td>
<td>.646</td>
<td>.919</td>
</tr>
<tr>
<td><strong>10 wk plasma</strong></td>
<td>21.8 ± 6.8* (17.1; 26.5)</td>
<td>18.9 ± 6.9* (14.1; 23.6)</td>
<td>21.1 ± 7.9 (15.6; 26.5)</td>
<td>19.6 ± 5.5 (15.8; 23.4)</td>
</tr>
<tr>
<td><strong>10 wk control</strong></td>
<td>22.1 ± 7.4 (17.0; 27.2)</td>
<td>16.4 ± 9.6 (9.8; 23.1)</td>
<td>19.3 ± 8.1 (13.7; 24.9)</td>
<td>19.3 ± 7.2 (14.3; 24.2)</td>
</tr>
<tr>
<td>P value (n = 8)</td>
<td>.889</td>
<td>.575</td>
<td>.674</td>
<td>.624</td>
</tr>
</tbody>
</table>

The differences between plasma and control sites were not statistically significant. *P < .05 between inferior and superior regions and between internal and external regions. Mean values ± SD and 95% confidence interval (lower; upper) in percentages.
The aim of the present study was to evaluate the impact on healing of bioactivation with argon plasma of a xenogeneic graft with adequate fixation but poor adaptation to the native host bone. The grafts were found consolidated to the recipient sites due to the new bone ingrowth within the graft. Similar patterns of healing were observed in both the plasma and control sites, and no statistically significant differences were found in new bone formation in any of the regions examined. This, in turn, means that the bioactivation with argon plasma did not improve the healing.

The selection of the periods of healing was based on the results from a previous study in which autogenous bone blocks were used in a similar model applying a similar protocol. In that experiment, a complete incorporation of the block was obtained after 40 days. In the present experiment, besides the inclusion of intermediate periods of healing, a longer final period of evaluation was applied (10 weeks), assuming that the xenograft incorporation might require a longer time compared with autogenous bone blocks.

A good adaptation of the grafts to the recipient sites has been suggested to improve healing. In the present study, after 2 weeks of healing, the distance between the block and the native host bone was approximately 0.3 to 0.4 mm in the internal zone and 0.5 to 0.6 mm in the external zone. This showed that the adaptation of the block to the recipient site presented deficiencies, resulting in a gap in the interface. Due to the convexity of the host bone, the discrepancy was higher in the periphery of the blocks compared with the central region. Despite these discrepancies,

### Table 4 Areas of the Residual Graft (in mm²) and Height and Width (in mm) of the Regenerated Zone, Including Graft and Bone Exceeding the Perimeter of the Graft

<table>
<thead>
<tr>
<th>Area</th>
<th>NB-SG</th>
<th>Internal interface</th>
<th>External interface</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 wk plasma</td>
<td>17.2 ± 1.6 (16.2; 18.2)</td>
<td>1.4 ± 0.6 (1.0; 1.7)</td>
<td>0.32 ± 0.16 (0.22; 0.42)</td>
</tr>
<tr>
<td>2 wk control</td>
<td>17.9 ± 1.6 (16.9; 18.8)</td>
<td>1.7 ± 0.5 (1.4; 2.0)</td>
<td>0.36 ± 0.20 (0.24; 0.48)</td>
</tr>
<tr>
<td>6 wk plasma</td>
<td>15.3 ± 3.8 (12.9; 17.7)</td>
<td>1.0 ± 0.5 (0.7; 1.2)</td>
<td>NA</td>
</tr>
<tr>
<td>6 wk control</td>
<td>16.7 ± 1.6 (15.7; 17.7)</td>
<td>0.9 ± 0.7 (0.5; 1.3)</td>
<td>NA</td>
</tr>
<tr>
<td>10 wk plasma</td>
<td>12.6 ± 2.7 (10.7; 14.5)</td>
<td>0.4 ± 0.4 (0.1; 0.6)</td>
<td>NA</td>
</tr>
<tr>
<td>10 wk control</td>
<td>11.5 ± 2.8 (9.6; 13.4)</td>
<td>0.3 ± 0.2 (0.1; 0.5)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*P < .05 value for the difference between plasma and control sites; #P < .05 value for the difference between external and internal interfaces.
Mean values ± SD.

### DISCUSSION

The aim of the present study was to evaluate the impact on healing of bioactivation with argon plasma of a xenogeneic graft with adequate fixation but poor adaptation to the native host bone. The grafts were found consolidated to the recipient sites due to the new bone ingrowth within the graft. Similar patterns of healing were observed in both the plasma and control sites, and no statistically significant differences were found in new bone formation in any of the regions examined. This, in turn, means that the bioactivation with argon plasma did not improve the healing.

The selection of the periods of healing was based on the results from a previous study in which autogenous bone blocks were used in a similar model applying a similar protocol. In that experiment, a complete incorporation of the block was obtained after 40 days. In the present experiment, besides the inclusion of intermediate periods of healing, a longer final period of evaluation was applied (10 weeks), assuming that the xenograft incorporation might require a longer time compared with autogenous bone blocks.

A good adaptation of the grafts to the recipient sites has been suggested to improve healing. In the present study, after 2 weeks of healing, the distance between the block and the native host bone was approximately 0.3 to 0.4 mm in the internal zone and 0.5 to 0.6 mm in the external zone. This showed that the adaptation of the block to the recipient site presented deficiencies, resulting in a gap in the interface. Due to the convexity of the host bone, the discrepancy was higher in the periphery of the blocks compared with the central region. Despite these discrepancies,
the blocks were over time incorporated by new bone that originated from the host bone, filled the gap in the interface zone, and propagated toward and inside the xenogeneic block. These results are in agreement with another similar experimental study in rabbits in which, however, autogenous bone blocks were used. Also, in that study, discrepancies were found between the graft and the convex host bone, and no biomaterial in particles was used to fill the gap in the interface. Nevertheless, after 40 days of healing, the blocks were consolidated to the host bone, and the interface was filled with new bone.

The bioactivation with argon plasma did not provide statistically significantly higher bone formation compared with the control sites. These results are in agreement with the data reported from another experimental
study in which, however, granules of deproteinized bovine bone mineral were used for sinus elevation in rabbits. However, in that study, for the central regions of the elevated area, ie, those not directly in contact with the sinus bone walls, the treatment in plasma of argon yielded better outcomes in terms of bone formation.

At the same time, the outcomes of the present study are in line with another experimental study on critical defects on the calvaria of rabbits that failed to demonstrate any difference in bone regeneration between activated graft and control sites.

Controversial outcomes, however, might also be due to plasma conditions that are not perfectly set: in fact, several in vitro studies demonstrated the positive effect of this technology in enhancing bone cell adaptation and protein deposition. The clinical time to perform the surgery might lead to wasting the bioactivation effect, although this was not in correlation with enhanced microbiologic contamination.

The present study showed that new bone grew over time from the recipient cortical layer toward the top of the xenogeneic graft. After 2 weeks of healing, both in the plasma and in the control sites, the proportions of new bone were higher at the base of the graft compared with the superior compartments. However, after 10 weeks of healing, due to the osteoconductivity of the biomaterial, as well as of the titanium fixation screw, new bone also increased in the superior compartment. The distance between the peak of new bone inside the graft and the upper surface of the graft (NB-SG) decreased over time, reaching the upper surface of the graft in some specimens. The xenogeneic block was partly resorbed over time, mainly at the base and on the lateral aspects, both in the plasma (~26%) and in the control (~36%) sites.

Bone growth within a graft had also been described in an experimental study in dogs. In that experiment, defects were prepared in the lateral aspect of the mandible at the time of tooth extractions. After 8 weeks of healing, synthetic biomaterial in blocks containing different concentrations of HA/βTCP 15:85% or 60:40%, respectively, were placed in the defects for lateral alveolar crest augmentation. Collagen membranes were used to protect the grafts. The horizontal gain in bone formation from the parent bone was studied after 4, 8, and 16 weeks, and the greater horizontal extension of new bone was obtained at the HA/β-TCP 60:40% graft, while the 15:85% type presented very limited bone formation. This, in turn, means that the characteristics of the biomaterial affect bone ingrowth inside the graft. This agrees with the outcomes reported by other experimental studies. In an experiment in dogs, buccal defects were created at the time of tooth extraction, and after 3 months, blocks of autogenous bone or made of deproteinized bovine bone mineral were fixed into the defects for lateral augmentation and covered with collagen membranes. After 3 months, implants were placed in the interface between the blocks and the parent bone. Three months after, biopsy specimens were collected for histologic analysis. It was shown that the autogenous grafts were completely incorporated into the parent bone, and the implants presented...
optimal osseointegration in both the parent bone and the grafted site. Conversely, the deproteinized bovine bone mineral grafts were not incorporated into the parent bone, from which they were separated by a layer of connective tissue, and only rarely presented truly little amounts of new bone at the interface with the parent alveolar crest. Moreover, while the implants were integrated into the native alveolar bone in the lingual aspect, at the buccal grafted sites, almost no new bone was formed.

In the present study, the cortical bone was perforated before the fixation of the graft, and new bone was found forming from these osteotomies, spreading outside the cortical layer toward the graft. This is in agreement with other similar studies in rabbits in which perforations of the cortical layer of the recipient beds were performed.13,15,16,22 This surgical protocol had been shown to improve the incorporation of the grafts into the recipient beds.40,41

A limitation of the present study was the use of a rabbit model, so the translation of the outcomes to humans should be made with caution, due to the different rate of healing.62 However, it is obvious that attention should be paid in the choice of the biomaterial used for horizontal bone augmentation. Another limitation is that the amounts on new bone found within the graft do not guarantee the success of an implant placed subsequently to or simultaneously with the grafting procedure. Even though new bone was found around the titanium fixation screw, experimental studies incorporating protocols with standard implants should be performed. Moreover, different biomaterials that did not present optimal results in previous experiments might be investigated, applying argon plasma bioactivation. The presence of several areas with osteoid tissue in the present study suggests investigating longer periods to allow the formation of higher amounts of new bone. The presence of a large inflammatory infiltrate in two plasma sites compared with the very low percentages detected in the other sites should not be directly related to the argon plasma treatment. However, these two occurrences might draw attention to the protocol procedures after the bioactivation due to the increased cell adhesion properties acquired after the treatment that might also attract contaminants.

CONCLUSIONS

The xenogeneic grafts of both groups were incorporated into the recipient sites by newly formed bone that presented a growth close to the upper surface of the graft. The bioactivation with argon plasma did not improve the healing.

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