Injuries due to accidental trauma, traumatic tooth extractions, advanced periodontal disease, and periodontal surgery are among the most common causes of alveolar bone loss. For cases of bone deficiency such as these, bone regeneration is a necessity.

Bone substitutes act as filling materials, reducing the risk of soft tissue collapse into the defect and providing a framework that allows colonization by cells involved in bone formation, encouraging neoformation in the medium or long term. Such substitutes should also favor the proliferation of new vessels and maintain their biologic support so as to be gradually replaced by new bone. One of the most widely used bone substitutes is deproteinized bovine bone, which is similar to the inorganic portion of human autogenous bone and has similar chemical and physical properties. Studies have demonstrated the superior biocompatibility and osteoconductivity of bovine bone compared to other bone substitutes. Despite being widely used and acting as an excellent osteoconductor, bovine bone does not exhibit osteogenic or osteoinductive properties. As a result, bovine bone has a longer healing time than autogenous bone. Therefore, techniques and materials that can improve the properties of bovine bone are interesting alternatives to bone healing.

Photobiomodulation therapy (PBMT) also has an effect on bone healing by increasing osteogenesis, ATP synthesis, fibroblastic proliferation, collagen synthesis, and bone cell activity. The positive effects of PBMT on bone regeneration have been previously suggested in previous studies, systematic reviews, and meta-analyses. This technique can be considered as an option to assist bone substitutes in the regenerative process and accelerate rehabilitation treatment. PBMT is a noninvasive therapeutic modality with biologic properties that has been extensively studied, with favorable results in bone regeneration in animal and human models. However, few studies so far have defined an adequate and consistent protocol for the use of lasers in bone regeneration with bone grafts or bone substitutes, making it necessary to determine protocols that are effective for treating each type of defect. Therefore, this study aimed to evaluate different protocols as adjuncts in the healing of bone defects grafted with inorganic bovine bone.

**Evaluation of Different Photobiomodulation Therapy Protocols as Adjuncts in the Healing of Bone Defects Grafted with Inorganic Bovine Bone**

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**Purpose:** This study evaluated the effect of two photobiomodulation therapy protocols on bone regeneration in critical-size bone defects grafted with inorganic bovine bone. **Materials and Methods:** A critical-size defect was created in 30 adult male rat calvaria, which were divided equally and randomly into three experimental groups (n = 10): (1) DBBM (deproteinized bovine bone mineral); (2) DBBM + PBMT 4 J (4 J; photobiomodulation therapy; GaAlAs, 730 nm, 100 mW, 140 J/cm²); and (3) DBBM + PBMT 6 J (6 J; GaAlAs, 730 nm, 100 mW, 210 J/cm²). Animals were euthanized after 30 days. The neoformed bone area (NBA), linear bone extension (LBE), and area of the remaining particles (ARP) were evaluated. The data were subjected to nonparametric Kolmogorov-Smirnov test and ANOVA, followed by Tukey post hoc test to identify differences between the groups (P < .05). **Results:** The 6 J group showed the highest average NBA (48.57% ± 28.22%) and demonstrated a statistically significant difference in NBA and LBE. A higher mean ARP was found in the DBBM group (38.73 ± 6.95) than in the groups irradiated by photobiomodulation therapy, with statistically significant differences (P < .05). **Conclusion:** The 6 J protocol showed the best results, promoting greater bone formation with greater resorption of residual particles. Int J Oral Maxillofac Implants 2022;37:1244–1249. doi: 10.11607/jomi.9613

**Keywords:** biomaterials, bone regeneration, bone substitutes, lasers
PBMT application protocols for bone regeneration. The null hypothesis was that different PBMT protocols do not influence the bone healing process.

MATERIALS AND METHODS

Experimental Model

This study was approved by the Ethics Committee on Teaching and Research in Animals of the Bauru Dental School, University of São Paulo (Proc N° 038/2013). A total of 30 adult male rats (Wistar, rattus norvegicus, albino) weighing 250 to 300 g were used. The animals were obtained from the Central Animal Hospital of the Bauru Dental School, University of São Paulo. The treatment protocol for the animals was performed in accordance with previous studies. Sample calculation considering a previous study by de Almeida et al20 resulted in n = 7 per group, with an effect size f = 0.95, α error probability = 0.05, power (1-β error probability) = 0.95, number of groups = 3, and a total sample size = 21 through an analysis in GPower 3.1.9.7. For this study, a total of 30 rats were randomly sorted into three experimental groups (n = 10): (1) DBBM (deproteinized bovine bone mineral; Bio-Oss 0.25–1 mm, Geistlich Pharma); (2) DBBM + PBMT 4 J (4 J; GaAlAs, 730 nm, 100 mW; Thera Lase DMC); (3) DBBM + PBMT 6 J (6 J; GaAlAs, 730 nm, 100 mW, 210 J/cm²). After the critical-size defect was determined, the treatment selected for each sample was randomly computer-generated using a number table by another team member. Thus, both the operator (L.A.E.) and the person responsible for the analyses (M.C.S.) were unaware of the treatment to be performed.20

Surgical Procedure

While ensuring preservation of the dura mater, a critical defect 5 mm in diameter was created using a trephine attached to a low-rotation handpiece (JIGC Indústria e Comércio de Materiais Dentários SA). With the aid of the surgical guide, "L" markings were made 2 mm anterior and 2 mm posterior to the margins of the surgical defect using an FG 700 carbide-conical bur (Microdont Microusinagem de Precisão) under continuous irrigation with sterile saline before they were subsequently filled with amalgam. These markings were made to identify the most central part of the original surgical defect during laboratory processing and to locate the original bone margins during histometric analysis.20-22 In the DBBM group, the surgical defect was filled with DBBM (0.02 g), whereas in the 4 J (140 J/cm²) and 6 J (210 J/cm²) groups, the defects were filled with the bone graft and photobiomodulation therapy was performed. All surgical procedures to create the critical-size defect (Fig 1) have been described in detail in previous studies by this research group.20-22

PBMT Application Protocols

PBMT was applied to four points on the bone surface and at the central point of the critical-size defect. This method was proposed by de Almeida et al.20 A TheraLase DMC laser with a continuous emission mode (GaAlAs), wavelength of 730 nm, power of 100 mW, and fluency of 140 J/cm² (4 J and 40 seconds per point in a single application) was used in the 4 J group, while a fluency of 210 J/cm² (6 J and 60 seconds per point in a single application) was used in the 6 J group. Thus, over the course of the study, a total of 20 J was delivered to the PBMT 4 J group and 30 J was delivered to the PBMT 6 J group. The animals were euthanized 30 days after the operation with a combination of 5 mg/mL ketamine hydrochloride and xylazine. The area of the original surgical defect and the surrounding tissues were removed in blocks. After the blocks were processed, they were embedded in paraffin (Histosec Pastilles, Merck). Starting from the center of the original surgical defect, 6-µm-thick longitudinal serial slices were obtained. Histologic sections were stained using hematoxylin and eosin (HE) and Masson’s trichrome (TM) techniques for analysis by light microscopy.

Histomorphometric Analysis

Four histologic sections representing the central area of the original surgical defect were selected for histologic and histometric analyses.20 In each image, the area to be analyzed was delimitated corresponding to the total area (TA) in mm², representing 100% of the area to be evaluated. Within this area, the neoformed bone area (NBA) and the areas of remaining particles of the implanted material (APR) were delimited, measured in mm², and calculated as percentages of TA.20 Measurements of the linear extension of the created defect were performed with the boundaries of the extremities delimited for the measurement of TA. From this, measurements of the linear extension of the new bone (LBE) were also calculated (in mm) and evaluated as a percentage of the total extension of the original bone (LBE).
surgical defect. Histomorphometric analysis was performed using ImageLab 2000 software (Bio-Rad Laboratories). All analyses were performed by an examiner who was previously calibrated and blinded to the study (M.C.S.).

Statistical Analysis

Kolmogorov-Smirnov test and ANOVA were used to test the normality of the data, followed by Tukey post hoc test to identify the differences between the groups. A significance level of .05 was used.

RESULTS

Qualitative Histologic Analysis

During laboratory processing, one animal from the 4 J group was lost. In all groups, no significant inflammatory infiltrate was observed during the 30-day postoperative period. The amount of new bone that formed varied between the different treatment groups (Fig 2).

In the DBBM group, the defect was almost entirely filled with bone graft particles. The fibrous connective tissue was well-organized around the BO particles (HE = 4×). New bone was found extending toward the center of the critical-size defect (Fig 2a).

In the 4 J group, the connective tissue was well-organized around the DBBM particles that filled a large part of the defect. New bone was found extending toward the center, but there was no complete closure of the defect in any of the specimens (Fig 2b).

In the 6 J group, closure of the defect in bone extension was observed in three specimens, and the thickness of the original calvaria was restored in all specimens (Fig 2c). The connective tissue was observed to be well-organized and vascularized, involving DBBM particles that occupied part of the original defect (Fig 3a). In some specimens, bone formation was observed inside and between DBBM particles (Fig 3b).

Histometric and Statistical Analysis

Tables 1, 2, and 3 describe the NBA, LBE, and APR, respectively, with descriptive statistics of means, standard deviations, and comparisons between groups.

The 6 J group had the highest mean NBA (48.57 ± 28.22), while the DBBM group had the lowest (11.36 ± 7.89). The 6 J group showed a statistically significant difference in relation to the other groups (Table 1 and Fig 4).

The 6 J group had a significantly higher LBE (73.17 ± 24.87) in relation to the other groups (Table 2 and Fig 5).

The highest RPA was found in the DBBM group (38.73 ± 6.95), with a statistically significant difference
to the other groups. Between the 4 J and 6 J groups, there was no statistically significant difference (Table 3 and Fig 6).

**DISCUSSION**

Of the different PBMT protocols associated with DBBM, the 6 J group provided a larger area of NBA and LBE.

There is no consensus on the best way to use PBMT in bone regeneration,\(^{13}\) with a variety of protocols\(^{11,15,23}\) and the use of several experimental models with different results reported in the literature.\(^{15,24,25}\)

In the present study, better quantitative results for bone neoformation were observed in the groups that received laser application, regardless of the dose. The effect of PBMT on bone regeneration can be multifactorial, and includes its ability to act on the differentiation of undifferentiated mesenchymal cells in osteoblasts\(^{26}\) by increasing the production of bone matrix,\(^{27}\) as was frequently observed in specimens irradiated with 6 J in the present study. PBMT initially increases vasculization,\(^{28}\) collagen production,\(^{14}\) and the release of growth factors in the surgical area.\(^{29}\)

Many of these events may be related to the amount of energy received by the system, and thus, the bio-modulation of bone healing.

Among the various parameters required for the application of PBMT, fluency seems to be the most important.\(^{16,30}\)

In the present study, better results were obtained when PBMT was used with a fluency of 210 J/cm\(^2\) compared to the 140 J/cm\(^2\) protocol, corroborating the results of previous studies\(^{20–22}\) that demonstrated new bone formation when using PBMT with 210 J/cm\(^2\) fluency during the operation.

Based on the results of the 4 J and 6 J groups, a single application of PBMT in the intraoperative period proved to be effective for bone healing in regard to the formation of highly organized and cellularized fibrous connective tissue, as well as bone neoformation around graft material particles.

In addition, the direct application of PBMT on surgical defects may have provided greater bone formation, corroborating the findings of Marques et al.\(^{25}\) Although some studies have reported that several sessions are necessary to achieve PBMT effects on bone healing,\(^{19}\) research on new protocols to improve PBMT results with

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**Table 1** Mean, Standard Deviation, Q25, Median, and Q75 of the Newly Formed Bone Area Values and Comparison Between Groups (NBA%)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBBM(^b)</td>
<td>10</td>
<td>11.36</td>
<td>7.89</td>
<td>6.26</td>
<td>9.49</td>
<td>14.11</td>
</tr>
<tr>
<td>DBBM + PBMT (4 J)(^b)</td>
<td>9</td>
<td>23.53</td>
<td>5.31</td>
<td>19.29</td>
<td>21.42</td>
<td>28.16</td>
</tr>
<tr>
<td>DBBM + PBMT (6 J)(^A)</td>
<td>10</td>
<td>48.57</td>
<td>28.22</td>
<td>29.08</td>
<td>42.22</td>
<td>76.31</td>
</tr>
</tbody>
</table>

Different uppercase and lowercase letters (A,b) indicate significant difference (\(P < .05\)); matching lowercase letters (b,b) indicate that there was no significant difference (\(P > .05\)); DBBM vs. DBBM + PBMT 6 J: \(P = .000\); DBBM vs. DBBM + PBMT 4 J: \(P = .301\); DBBM + PBMT 4 J vs. DBBM + PBMT 6 J: \(P = .001\).

**Table 2** Mean, Standard Deviation, Q25, Median, and Q75 of the Linear Extension Values of Newly Formed Bone and Comparison Between Groups (LBE%)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBBM(^b)</td>
<td>10</td>
<td>27.38</td>
<td>15.54</td>
<td>17.10</td>
<td>23.60</td>
<td>36.38</td>
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<tr>
<td>DBBM + PBMT (4 J)(^b)</td>
<td>9</td>
<td>29.39</td>
<td>9.95</td>
<td>22.22</td>
<td>26.37</td>
<td>38.51</td>
</tr>
<tr>
<td>DBBM + PBMT (6 J)(^A)</td>
<td>10</td>
<td>73.17</td>
<td>24.87</td>
<td>50.98</td>
<td>79.41</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Different uppercase and lowercase letters indicate significant difference (A,B, \(P < .05\)), equal lowercase letters (b,b) indicate that there was no significant difference (\(P > .05\)); DBBM vs. DBBM + PBMT 6 J: \(P = .000\); DBBM vs. DBBM + PBMT 4 J: \(P = .968\); DBBM + PBMT 4 J vs. DBBM + PBMT 6 J: \(P = .000\).

**Table 3** Mean, Standard Deviation, Q25, Median, and Q75 of the Remaining Particle Area Values and Comparison Between Groups (RPA%)

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Q25</th>
<th>Median</th>
<th>Q75</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBBM(^A)</td>
<td>10</td>
<td>38.73</td>
<td>6.95</td>
<td>34.62</td>
<td>37.71</td>
<td>42.97</td>
</tr>
<tr>
<td>DBBM + PBMT (4 J)(^b)</td>
<td>9</td>
<td>24.64</td>
<td>5.61</td>
<td>19.08</td>
<td>26.06</td>
<td>29.29</td>
</tr>
<tr>
<td>DBBM + PBMT (6 J)(^b)</td>
<td>10</td>
<td>16.74</td>
<td>15.25</td>
<td>0.00</td>
<td>22.42</td>
<td>28.38</td>
</tr>
</tbody>
</table>

Different uppercase and lowercase letters (A, b) indicate significant difference (\(P < .05\)); matching lowercase letters (b,b) indicate that there was no significant difference (\(P > .05\)); DBBM vs. DBBM + PBMT 6 J: \(P = .000\); DBBM vs. DBBM + PBMT 4 J: \(P = .017\); DBBM + PBMT 4 J vs. DBBM + PBMT 6 J: \(P = .238\).
fewer sessions provides interesting alternatives, since photobiomodulation therapy acts in the early stages of bone healing. A recent study on alveolar bone regeneration in rabbits demonstrated no statistically significant difference between single and multiple PBMT applications, which suggests that, in agreement with the present study, a single application is effective. It is difficult to consider any protocol to be adequate due to the variety of dosimetry and different types of treatments (eg, single or multiple sessions and/or applications). Therefore, protocols using PBMT with fewer applications should be investigated. The association of PBMT with DBBM provided better results compared with biomaterial alone. The use of lasers has improved the performance of biomaterials in the formation of new bone, whether associated with bone morphogenetic proteins, alloplastic materials, or xenogens such as deproteinized bovine bone. However, the literature is not conclusive regarding the effects of lasers on bone substitutes, probably due to the heterogeneity of the studies, which had different protocols and experimental models. Furthermore, to date, no studies comparing different protocols for the application of PBMT on the most common bone substitutes in clinical practice have been found in the literature. The application of PBMT (6 J) to a bone defect filled with DBBM demonstrated a better result in the area and extension of new bone than of PBMT (4 J). In histologic analysis, total closure of the defect was observed in three specimens in the 6 J group. There are no studies in the literature that used the same laser parameters and the same animal model for comparison. The fact that PBMT increases angiogenesis may favor tissue mineralization, as it is directly related to the growth and differentiation of osteoblasts. The shape and interconnected pore system of DBBM particles seem to have a size and structure that favors the growth of blood vessels. Although the DBBM group had lower results than the groups with the additional PBMT, it is possible to observe maintenance of the original defect thickness in the histologic analysis. The granules of the bovine bone graft undergo slow resorption, and instead of being reabsorbed, they remain surrounded by newly formed bone, thereby offering a framework for bone formation, maintaining tissue position, and preventing the resorption of the defect, while additionally having osteoconductive properties with a physical structure similar to that of autogenous bone that allows space for the formation of blood vessels. The hydrophilic capacity favors the deposition of osteogenic proteins, which are released by osteogenic cells in the vicinity of the particles, leading to bone formation on the particle surface. Fewer particles were observed in the groups treated with DBBM associated with different PBMT protocols compared to DBBM alone. There was a smaller number of particles accompanied by greater histologic bone remodeling in the 6 J group, corroborating the findings of Cunha et al. However, these results differ from those found by Bosco et al, which demonstrated that the PBMT was not able to accelerate the resorption of residual particles. This difference in results can be explained by the different protocols and trademarks of the xenogenous graft between in the study in question and the present study. The reduction in the number of residual particles found in the defect can be explained by the actions of the laser in stimulating osteoclastic activity. Several PBMT protocols are currently used, with results that seem to be dependent on the different parameters, making it difficult to compare results and to reach a consensus on the performance of PBMT due to the varying experimental models, dosages, and healing times. These factors must be considered in future research, following better standardization.
CONCLUSIONS

The 6 J group protocol promotes greater bone formation with greater resorption of residual particles, suggesting that it is an advantageous option for the treatment of bone defects. Thus, the null hypothesis was rejected.

ACKNOWLEDGMENTS

This study was supported by São Paulo Research Foundation (FAPESP #2010/10538-0) and Finance Code 001. The authors state that there is no conflict of interest.

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