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Background: This study compared clinical and radiographic findings for the treatment of Class II furcation defects in human mandibular molars using anorganic bovine-derived hydroxyapatite matrix (ABM)/cell-binding peptide (P-15) or open flap debridement (OFD).

Methods: Twelve subjects showing two comparable Class II furcation defects in their mandibular molars were enrolled. The defects in each subject were assigned randomly to the test (ABM/P-15) or the control (OFD) group. Clinical measurements and standardized radiographs were taken at baseline and 6 to 7 months after surgery.

Results: There were no statistically significant differences between the test and control groups for any clinical or radiographic parameter (P > 0.05). On comparing the baseline and final measurements, the gain in horizontal clinical attachment level and reduction in gingival recession were significant only in the test group (P ≤ 0.02), whereas the gain in the vertical clinical attachment level was significant in both groups (P ≤ 0.04). In the test group, four of 12 sites showed complete closure, and five showed partial closure; in the control group, three defects showed complete closure, and four showed partial closure (P = 0.42). Subtraction radiography revealed similar gains in bone height and increases in mean bone density with both treatments (P > 0.05).

Conclusions: ABM/P-15 yielded favorable results in the treatment of Class II furcation defects over a 6-month evaluation period; however, there was no difference compared to OFD. Further studies using a larger sample size are needed to confirm the present findings. J Periodontol 2007;78:2277-2283.

KEY WORDS
Clinical trial; furcation defect; periodontal regeneration; periodontitis.

The ultimate goal of periodontal therapy is to regenerate the tissues lost as a consequence of periodontal disease. Several regenerative approaches, including bone graft materials associated or not with membranes and growth factors, have been used when attempting to regenerate the periodontium. In the treatment of Class II furcation defects, although guided tissue regeneration (GTR) is more effective than open flap debridement (OFD) in reducing open horizontal furcation depths, horizontal and vertical attachment levels, and probing depths,1 the clinical results are modest and irregular.2-11 Factors that negatively influence the results of regenerative furcation therapy include greater distance between the roof of furcation and crest of bone and between roof of furcation and base of defect, deeper horizontal defect, divergence of roots at the crest of bone, and higher presurgical horizontal clinical attachment level (h-CAL).12

Qian and Bhatnagar13 developed a synthetic bone substitute composed of a peptide component (P-15) adsorbed on an anorganic bone mineral (ABM). P-15 is a synthetic clone of a highly conserved, linear polypeptide with a 15
amino acid sequence (GTPGPQGIAGQRGVV) identical to that contained in residues 766 to 780 of the α1 chain of type I collagen, a protein uniquely involved in cell adherence, particularly in fibroblasts and osteoblasts.14,15 ABM is a natural, microporous, bovine-derived bone mineral occurring in a particulate form that consists of smooth, porous particles with a mean diameter of 300 μm. The ABM/P-15 combination consists of 200 ng P-15 and 1 g ABM.

In the treatment of intrabony defects, the ABM/P-15 combination is clinically effective and superior to ABM alone for reentry bone parameters16 and clinically superior compared to OFD and to demineralized freeze-dried bone allograft (DFDBA).17 In Class III furcation defects in dogs, there was no significant difference between the expanded polytetrafluoroethylene (ePTFE) membrane associated with ABM/P-15 and the ePTFE membrane alone when new bone, connective tissue, and epithelium were evaluated.18

ABM/P-15 is commercially available in particulate or flow formulations. The particulate form of ABM/P-15 is difficult to deliver to the surgical site, does not remain where placed, and can be deformed, displaced, or reduced in amount during flap manipulation. It does not adhere to tooth or bone and does not form a firm, cohesive, and stable mass.19 The ABM/P-15 flow formulation was developed as a new presentation that contains a biocompatible, inert carrier made of water, glycerol, and sodium carboxymethylcellulose, imparting a gel-like consistency to the material, which is intended to resolve the technical limitations without adversely affecting healing.

The aim of this study was to compare, clinically and radiographically, the results of ABM/cell-binding peptide flow and OFD in the treatment of human Class II furcation defects in mandibular molars over a 6-month period.

**MATERIALS AND METHODS**

The present study protocol was approved by the Institutional Ethics Committee of the São Leopoldo Mandic Dental Research Center, and all subjects included in the study signed an informed consent form. The subjects were selected from patients referred for regular dental treatment to the São Leopoldo Mandic Dental Research Center. Subject selection, surgeries, and follow-up were performed from January 2004 to January 2006.

This randomized, double-masked, intrasubject, controlled study was conducted in 12 non-smoking subjects (five males and seven females), aged 34 to 63 years (mean, 44.3 years), who were diagnosed with chronic periodontitis. The subjects had a total of 24 defects.

Criteria for inclusion were good systemic health, no use of medication known to interfere with periodontal healing, no contraindication for periodontal surgery, non-smoking, two Class II furcation defects in contralateral lower molars, and no restorations or caries in the area to be treated.

The study protocol entailed a screening appointment followed by oral hygiene instruction, full-mouth scaling and root planing (SRP) to establish optimal biofilm control and gingival health condition, occlusal adjustment if necessary, baseline evaluation, surgical therapy, post-surgical appointments every 2 weeks, and postoperative evaluation 6 to 7 months later.

The clinical measurements were obtained by one examiner using a University of North Carolina periodontal probe.‡ A prefabricated stent was used to reproduce probing site and angulation, and an endodontic rubber stopper was adapted to the probe and slid to the margin of the stent or to the tooth surface during the vertical and horizontal measurements, respectively. The measurements were obtained using the probe scale and checked with a caliper.7 The parameters were recorded at the center of the furcation site. The examiner was unaware of the treatment distribution.

The following clinical parameters were obtained at baseline (≥1 month after initial therapy, which was immediately before surgery) and 6 to 7 months after surgery: relative vertical clinical attachment level (v-CAL), h-CAL, relative gingival recession (GR), and bleeding on probing. Probing depth (PD) was established by calculating the difference between v-CAL and GR. The plaque index (PI)20 was used to assess brushing and flossing performance throughout the study. The furcation defects were classified using a Nabers probe: Class 0, no furcation; Class I, horizontal PD ≤3 mm; Class II, horizontal PD >3 mm; or Class III, through-and-through furcation involvement.

Both surgeries were performed at the same appointment. The surgeries consisted of intrasulcular incisions, mucoperiosteal flaps, and the removal of granulation tissue. The root surfaces were scaled and planed thoroughly with manual and rotary instruments and rinsed with sterile saline. The defects in each subject were assigned randomly to one of two treatment groups: the test group (ABM/P-15)¶ or the control group (OFD). In the test group, the furcation defect was filled completely with ABM/P-15. The flaps were displaced coronally and secured with interdental ePTFE sutures. The flap sutures were removed after 14 days. All surgical procedures were performed by a trained operator.

An analgesic (acetaminophen,§ 750 mg, four times a day) was prescribed for the first day. The subjects were instructed to abstain from brushing and flossing after 14 days. All surgical procedures were performed by a trained operator.

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¶ PCP-UNC 15, Hu-Friedy, Chicago, IL.
§ Tylenol, Cilag Farmacêutica, São Paulo, SP, Brazil.

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around the surgical area until suture removal 14 days later and to consume only soft food during the first week after surgery. They also were instructed to avoid mechanical trauma to the treatment sites. The subjects used 0.12% chlorhexidine mouthwash twice a day for 8 weeks. Subjects were seen every 2 weeks for 6 to 7 months for professional prophylaxis and reinforcement of their oral hygiene procedures.

Subtraction Radiography Analysis
Standardized, vertical, bitewing radiographs were obtained immediately before surgery (baseline) and 6 to 7 months postoperatively. The radiographs were taken using the long-cone paralleling technique. A commercial system** was modified and connected to an individual acrylic bite-block with occlusal registration in auto-polymerizing acrylic resin.7

For each site, the baseline radiograph was placed under a video camera, digitized at 512 × 480 pixels spatial resolution and 8 bits (256 gray levels) color resolution, and stored on a computer. The follow-up radiograph was aligned under the video camera and digitized. Pairs of radiographs were subtracted following correction for contrast and planar geometric discrepancies as described previously.21 The resulting subtraction image showed areas of bone loss and bone gain against a neutral gray background (Fig. 1).

Figure 1. Subtraction image. Area of bone gain in the furcation defect (arrow) against a neutral gray background. This area of bone gain was isolated, and its height and density were measured. (29 × 39 mm [300 × 300 dots per inch].)

Areas of bone loss or gain were isolated using a morphologically aided technique that also removes background noise from the image. The signed subtraction image was converted to a binary image (black and white with no gray shades) using an interactively controlled threshold. The operator adjusted the threshold until the area of bony change appeared white against a background of salt and pepper noise. An erode operation was performed on the binary image to aid in removing the isolated pixel noise. Then a dilate operation was performed to restore the areas of bony change to their original size.21

Computer software was used to measure the length of the region of bone loss or gain (in millimeters) that intersected with the tooth root and the bone density in the furcation area. The measurements of bone loss or gain were made interactively along each root surface using a mouse as the pointing device.

Statistical Analysis
The differences in the clinical parameters between baseline and after 6 to 7 months and between the test and control groups were compared using the Student t test for paired data. The PI, bleeding index, and bone changes were evaluated using the Wilcoxon test.

The difference between the groups with regard to the frequencies of furcation lesions classified as Class I or completely closed at the time of final examination was calculated using the McNemar $\chi^2$ test.

A significance level of $P = 0.05$ was used for all comparisons, and calculations were performed using a statistical software package.††

RESULTS
All subjects tolerated the surgical procedures well, experienced no postoperative complications, complied with the study protocol, and completed the 6- to 7-month follow-up.

Full-mouth PI was maintained at <20%. The differences in PI and bleeding on probing between the baseline and final evaluations were statistically significant in both groups ($P < 0.01$); however, there were no differences between the two groups.

Table 1 provides the descriptive statistics for the clinical parameters at baseline and after 6 to 7 months. The initial differences in the clinical parameters between the groups were not statistically significant ($P > 0.4$), attesting to the fact that the defects were similar in both groups. The differences between the test and control treatments were not statistically significant for any clinical parameter ($P > 0.05$).

On comparing the baseline and final measurements, h-CAL gain and GR reduction were statistically significant only in the test group ($P \leq 0.02$). The gain in

** XCP, Rinn, Elgin, IL.
†† SPSS, Chicago, IL.
v-CAL was significant in both groups ($P \leq 0.04$). The improvement in PD was not significant in either group.

Using a Nabers probe in the test group, four of 12 sites showed complete clinical closure, whereas five showed partial closure. In the control group, three defects showed complete closure, and four defects showed partial closure ($P = 0.42$).

The subtraction analysis revealed a similar gain in bone height (BH) in both groups (ABM/P-15, 0.92 ± 0.89 mm; OFD, 0.57 ± 0.60 mm; $P = 0.26$). All sites showed a gain in BH, except for a single case that presented bone loss at the test and control sites. An increase in mean bone density after both treatments was recorded (ABM/P-15, 30.72%; OFD, 22.60%). However, the difference between the two groups was not significant ($P = 0.29$).

Table 2 provides the primary outcome variables (h-CAL and BH) given for each single pair of defects at baseline and after 6 to 7 months.

**DISCUSSION**

The present study compared two treatments, ABM/P-15 and OFD, for Class II furcation defects. The evaluations were performed 6 to 7 months after surgery because most clinical changes occur within this period. On comparing the baseline and final data for the parameters measured, we found that ABM/P-15 promoted a significant gain in h-CAL and reduction in GR, whereas OFD did not. However, the differences between the ABM/P-15 and OFD treatments were not significant. Further, similar gains in BH and increases in bone density were observed after both treatments.

ABM/P-15 provided positive results in the treatment of intrabony defects. However, compared to OFD, DFDBA, and ABM, the improvements were quite modest. To the best of our knowledge, no controlled study has assessed the behavior of ABM/P-15 in the treatment of Class II furcation defects. The treatment of such defects still constitutes a significant challenge for the periodontist, and the primary goal of treatment is the complete clinical closure of the defect. In the present study, the difference between the treatments with regard to clinical closure was not statistically significant, although 75% and 59% of the furcation lesions were classified as Class I or completely closed in the test and control groups, respectively, after treatment. In addition, gain in h-CAL was significant only in the ABM/P-15 group. Thus, the horizontal improvement seemed to be clinically superior in the ABM/P-15 group. The h-CAL reported here includes horizontal and vertical components. Further, the measurement using a rigid, straight probe demands an angulation of the probe to reach the furcation concavity. Therefore, even Class I or completely closed furcations, classified using a Nabers probe, presented a high h-CAL.

Many other regenerative approaches have been tested in the treatment of furcation defects; however, regeneration is also modest and irregular. The mean reduction in horizontal furcation depth ranges from 0.2 to 4.4 mm, and complete closure ranges from 0% to 66% of cases after GTR. Following the use of enamel matrix derivative, the mean reduction in horizontal furcation depth was 2.6 ± 1.8 mm, with complete or partial closure occurring in 77.7% of defects, which was similar to the present findings.

When designing the study, a power of 80% for the main outcome variable (h-CAL) was set. The mean improvement was estimated to be 1.5 mm for the control group and 2.5 mm for the test group. The estimated sigma (SD) was 0.75 for the control group and 0.85 for the test group. Twenty-three defects were required, considering the type I error of 0.05. We used 24 defects in the present study; however, the obtained
sigma was higher than that estimated, which resulted in a power of 43%. Therefore, studies using more subjects are required to evaluate whether ABM/P-15 provides better results than OFD in the treatment of Class II furcation defects.

The ideal histologic outcome after periodontal therapy is the regeneration of the cementum, periodontal ligament, and alveolar bone, which cannot be achieved using OFD. The immediate following periodontal surgery, a cascade of cellular and molecular events initiates wound repair. Initially, a clot forms, and neutrophils and monocytes, followed by fibroblasts and endothelial cells, invade the clot forming a granulation tissue. Concomitantly, the epithelial cells alter their expression of integrins and migrate to cover the wound and root surface, jeopardizing regeneration of the cementum, periodontal ligament, and alveolar bone, resulting in a long junctional epithelium. The GTR technique avoids epithelial cell migration into the root and allows periodontal regeneration to some degree. However, the subsequent limited regeneration and frequent membrane exposure have stimulated the search for alternative regenerative therapies. ABM/P-15 also seems to avoid the apical downgrowth of epithelium and maintains the space necessary for periodontal regeneration because it promotes true periodontal regeneration. Although the clinical differences between the treatments were not statistically different, the improvement in v-CAL, h-CAL, and GR seen in the present study in the ABM/P-15 group may represent a clinical improvement in the furcation defects based on true periodontal regeneration. In contrast, in the control group, the improvement probably was based mainly on the formation of a long junctional epithelium. Because both groups received SRP at the beginning of the study, followed by periodontal maintenance, the clinical benefit of SRP and periodontal maintenance may have been so effective that a possibly superior effect of ABM/P-15 became masked. It is well known that SRP and periodontal maintenance result in significant clinical improvements after 6 months.

A significant decrease in gingival recession of 0.9 mm was found in the ABM/P-15 group, which may be related to the molecular characteristics of ABM/P-15 that enhance cell attachment, and proliferation may have provided the root coverage. The ABM/P-15 enhanced the attachment and proliferation of periodontal ligament fibroblasts and bone marrow cells to the ABM, which induces mineralizing matrix production by periodontal ligament fibroblasts.

### Table 2.

**Primary Outcome Variables for Each Pair of Defects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>ABM/P-15</th>
<th></th>
<th></th>
<th>OFD</th>
<th></th>
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<tr>
<td></td>
<td>h-CAL (mm)</td>
<td>h-CAL (mm)</td>
<td>BH Gain (mm)</td>
<td>h-CAL (mm)</td>
<td>h-CAL (mm)</td>
<td>BH Gain (mm)</td>
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<tr>
<td></td>
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<td>Final</td>
<td></td>
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<td>Final</td>
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<td>5.8</td>
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<tr>
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<td>6.0</td>
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<tr>
<td>9</td>
<td>9.4</td>
<td>5.5</td>
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<td>9.0</td>
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<tr>
<td>10</td>
<td>8.9</td>
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<tr>
<td>12</td>
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<td>0.9</td>
<td>8.4</td>
<td>6.5</td>
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</table>
and gingival fibroblasts, and induces an osteogenic phenotype in these cells.\textsuperscript{29,31} Although the gain in bone density and in BH were greater in the ABM/P-15 group, the differences between the two groups were not significant, which may be related to the short evaluation period in the present study.

We noted rare and isolated radiopaque particles (1.1 ± 1.3 spots/defect) in 50% of the defects, which was described previously. Yukna et al.\textsuperscript{16} found granular particles clinically at reentry and radiopaque particles radiographically 6 months after the treatment of infrabony defects using particulate ABM/P-15. However, they did not note the frequency of this finding. ABM/P-15 flow may present rapid resorption.\textsuperscript{19} Possibly, the lower density of particles in the ABM/P-15 flow compared to the particulate graft provided better interparticulate spacing that facilitated more rapid vascularization and resorption. In the present study, there was no significant correlation between the presence of radiopaque particles and the bone parameter findings. However, the presence of particles might have limited the probe penetration during the clinical evaluation.

CONCLUSIONS

When used in the treatment of Class II furcation defects, ABM/P-15 yielded favorable clinical and radiographic results over a 6-month evaluation period; however, there was no difference compared to OFD. Studies of longer duration using more subjects are required to evaluate the durability of the clinical effects and to confirm the present findings.

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REFERENCES


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