Periodontal Tissue Response to Coverage of Root Cavities Restored With Resin Materials: A Histomorphometric Study in Dogs

Thiago M. Martins,* Alvaro F. Bosco,* Fernando J.O. Nóbrega,* Maria J.H. Nagata,* Valdir G. Garcia,* and Stephen E. Fucini†

**Background:** The purpose of this study was to histomorphometrically evaluate the response of periodontal tissues covering Class V resin restorations in dogs.

**Methods:** After raising a mucoperiosteal flap, bony defects measuring 5 × 5 mm were created on the buccal aspect of the canines of five dogs followed by cavity preparations on the root surface measuring 3 × 3 × 1 mm. Before repositioning the flap to cover the bone defect, the cavities were restored with composite resin (CR) or resin-modified glass ionomer cement (RMGIC) or were left unrestored as control (C). The dogs were euthanized 90 days after surgery. Specimens comprising the tooth and periodontal tissues were removed, processed routinely, cut into longitudinal serial sections in the bucco-lingual direction, and stained with hematoxylin and eosin (H&E) or Masson’s trichrome. The most central sections were selected for histomorphometric analysis.

**Results:** Histomorphometric analysis revealed apical migration of epithelial tissue onto the restorative materials (RMGIC and CR). The C group presented significantly longer connective tissue attachment (P < 0.05) than the RMGIC and CR groups and significantly higher bone regeneration (P < 0.05) compared to the RMGIC group. Histologically, the cervical third (CT) of all groups had the most marked chronic inflammatory infiltrate.

**Conclusions:** Within the limits of this study, it can be concluded that the restorative materials used exhibit biocompatibility; however, both materials interfered with the development of new bone and the connective tissue attachment process. *J Periodontol 2007;78:1075-1082.*

**KEY WORDS**

Animals; dental materials; healing; regeneration.

**Extensive gingival recession associated with deep caries or cervical abrasions caused by incorrect tooth-brushing are common. In these cases, complete coverage by traditional mucogingival surgical techniques might be contraindicated because of the need for extensive root planing, which could compromise the tooth.**1,2 The combination of an adhesive restorative material and surgical coverage might be a solution.3

Dental restorative materials have been extensively studied, especially with regard to adhesion,4 polishing and final finishing,5,6 biocompatibility,7-12 and esthetics.13 Subgingival restorations may cause alterations because of direct trauma to the periodontal tissues14 or may facilitate the accumulation of subgingival plaque with consequent inflammatory alterations and/or recession of adjacent gingival tissue.15,16 Special attention should be paid to the “biologic width.”17

In 1961, Gargiulo et al.18 established the following mean dimensions in humans: histologic sulcus depth of 0.69 mm, junctional epithelium of 0.97 mm, and connective tissue attachment (CTA) of 1.07 mm.

An intense inflammatory infiltrate associated with bone resorption is usually observed when the area of CTA is violated by the presence of a restoration. This response suggests a possible attempt of
the organism to reestablish the dimensions of the biologic width.\textsuperscript{19-21} Recently, Gunay et al.\textsuperscript{22} found that placement of a restoration margin within the biologic width, <1 mm from the bone crest (BC), compromised periodontal health, resulting in higher indices of gingival bleeding and probing depth. In contrast, Dragoo\textsuperscript{23} observed that subgingival sites in patients with large root lesions restored with modified resin ionomer materials presented clinically healthy periodontal tissues well adapted to the root surface with no bleeding on probing and minimum sulcus depth. Histologic analysis revealed adhesion of fibroblasts and connective tissue to the restorations.

The response of periodontal tissues to adhesive restorative materials has been studied by a number of investigators. The roughness and subgingival position of acrylic resin restorations have been shown to be key factors in the development of gingival inflammation.\textsuperscript{24} Sites restored with resin-modified glass ionomer cement (RMGIC), compomers, and composite resin (CR) were associated with greater amounts of gingival crevicular fluid compared to unrestored sites.\textsuperscript{25} Konradson and Van Dijken\textsuperscript{26} analyzed interleukin-1 levels in gingival crevicular fluid adjacent to subgingival restorations of calcium aluminate cement, CR, and enamel and concluded that the restorations per se did not alter gingival health nor did they significantly affect interleukin-1 levels or induce gingival inflammation. Gomes et al.,\textsuperscript{27} evaluating the periodontal response to subgingival restorations of amalgam and RMGIC in dogs, observed a more intense inflammatory infiltrate at sites restored with amalgam compared to sites restored with the RMGIC. However, the control of bacterial plaque minimized the inflammatory response at most restored sites in both groups.

Therefore, soft tissue root coverage may be contraindicated for root surfaces where the cavity preparation and/or cervical abrasion exceeds a depth of 1.0 to 3.0 mm;\textsuperscript{12} thus, the choice between restoration alone or combination of a composite restoration with soft tissue root coverage is up to the clinician.\textsuperscript{3} The purpose of this study was to histomorphometrically evaluate the response of periodontal tissues covering Class V resin restorations in dogs.

**MATERIALS AND METHODS**

This research protocol was approved by São Paulo State University “Julio de Mesquita Filho” – Dental School of Araçatuba Animal Research Care Committee. All guidelines regarding the care of animal research subjects were strictly followed. Five adult male mongrel dogs, weighing 15 to 18 kg, were used in this study. The dogs were in good systemic and oral health.

Before all experimental procedures, animals were anesthetized using xylazine\textsuperscript{†} (1 mg/kg body weight, intramuscularly [IM]) and a combination of tiletamine hydrochloride with zolazepam hydrochloride\textsuperscript{§} (50 mg/kg body weight, IM).

Supra- and subgingival scaling of all teeth with Gracey curets and ultrasonic instruments, followed by coronal polishing, was done 1 week before the surgical procedure. Plaque control was maintained by daily topical application of 0.2% solution of chlorhexidine gluconate.

**Surgical and Restorative Procedures**

All surgeries were performed by the same surgeon. The treatment groups were assigned using a randomized block design. Surgical sites were locally infiltrated with 2% mepivacaine containing epinephrine (1:100,000) to reduce hemorrhage. After elevating a full-thickness flap on the buccal aspect of the upper and lower canines, an osteotomy was made at each site with a microchisel\textsuperscript{¶} to create a bone defect measuring $5 \times 5$ mm. The dimension of the defect was verified with a periodontal probe. Next, a Class V cavity preparation measuring $3 \times 3 \times 1$ mm (width $\times$ height $\times$ depth) was prepared with a high-speed cylindrical 1091 diamond bur\textsuperscript{¶¶} under irrigation with sterile saline in such a way that the apical and lateral margins of the preparation were located 1 mm from the walls of the surgically created bone defect. The coronal margin of the preparation (CMP) was made 2 mm apical to the cemento-enamel junction. Before the restorative procedure, a vertical groove was made in the enamel on the buccal aspect of the anatomic crown using a tapered fissure diamond bur to identify the center of the preparation in the mesio-distal dimension (Fig. 1). In the experimental groups, the preparations were restored with RMGIC\textsuperscript{‡} or microhybrid CR.\textsuperscript{**} The restorations were finished with fine diamond burs and polished with abrasive rubber points. Control group (C) preparations did not receive any restorative treatment. All surgical sites were thoroughly irrigated with sterile saline. The flaps were repositioned and sutured with 5.0 polyglactin 910\textsuperscript{††} in such a way that the control defect and experimental restorations were completely covered with gingival tissue. The animals were given amoxicillin (33.3 mg/kg body weight) orally twice a day for 7 days starting 2 hours before surgery.

**Post-Surgical Procedures**

All animals were given an oral analgesic (ketorolac, 5 mg/kg body weight) once a day for 3 days postoperatively. They were placed on a soft diet until euthanasia.

\textsuperscript{†} Coopazine, Coopers Brasil, São Paulo, SP, Brazil.

\textsuperscript{‡} Zoletil 50, Virbac, São Paulo, SP, Brazil.

\textsuperscript{¶} KG Sorensen, São Paulo, SP, Brazil.

\textsuperscript{¶¶} Fuji II LC, GC, Tokyo, Japan.

\textsuperscript{**} Palfique Estelite Paste, Tokuyama Dental, Tokyo, Japan.

\textsuperscript{††} Vicryl, Ethicon, Johnson & Johnson, São José dos Campos, SP, Brazil.
Plaque control was maintained throughout the experimental period by topical application of 0.2% solution of chlorhexidine gluconate spray five times weekly. Sutures were removed at 10 days postoperatively. The dogs were euthanized 90 days after the surgical procedure.

**Tissue Processing (laboratory procedures)**

Before removing block specimens made up of the tooth, gingiva, and alveolar bone, the longitudinal groove made in the crown was extended into the dentin using a carborundum disk to a depth of ~2 mm. After removal, the specimens were fixed in 10% neutral formalin. After complete decalcification in 16% EDTA, each specimen was divided longitudinally into two blocks following the groove made in the crown, and the restorative materials were removed. The specimens were processed and embedded in paraffin. Serial sections 6 μm thick were cut in a longitudinal direction (bucco-lingual) starting at the center of the root cavity. The sections were stained with either hematoxylin and eosin (H&E) or Masson’s trichrome for analysis by light microscopy.

**Histomorphometric Analysis**

The six most central sections of each specimen were selected for histologic and histometric analyses. The images of the histologic sections were captured by a digital camera connected to a light microscope with an original magnification of ×23. The digital images were saved on a computer. A composite digital image was created by combining three smaller images because it was not possible to capture the entire defect in one image at the level of magnification that was used.

The following parameters were evaluated by histomorphometric analysis using an imaging tool: 1) intensity of the connective tissue inflammatory infiltrate as proposed by Wolfson and Seltzer; 2) position of the apical limit of the junctional epithelium in relation to the apical margin of the cavity preparation (PE-AMP); 3) distance between the BC and the apical margin of the cavity preparation (BC-AMP); 4) length of the junctional and sulcular epithelia (E); 5) length of the CTA; and 6) distance between the gingival margin and the BC (GM-BC). For analysis of the intensity of the inflammatory infiltrate, the histologic specimens selected were divided into three zones corono-apically: cervical third (CT) = located coronal to the CMP; middle third (MT) = located at the level of the cavity preparation; and apical third (AT) = located apical to the apical margin of the cavity preparation.

**Statistical Analysis**

Evaluation for each tooth corresponded to an average value calculated from measurements obtained from the six most central sections of each tooth. The animal was used as the statistical unit (N = 5). Parametric tests were used to assess the variables E, CTA, and GM-BC, where the normality of the data was confirmed, and the data variances were shown

<table>
<thead>
<tr>
<th>Group</th>
<th>Zone</th>
<th>Median Score</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMGIC</td>
<td>CT</td>
<td>4</td>
<td>0.1134</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>CT</td>
<td>3a</td>
<td>0.0417</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>2.5b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>1.5b</td>
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</tr>
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<td>CT</td>
<td>4</td>
<td>0.0597</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Same letters indicate that there is no statistical difference among the groups by the Dunn test (P>0.05).

* Friedman test.

‡‡ Aphoticário–Compounding Pharmacy, Aracatuba, SP, Brazil.

§§ Image Tool UTHSCSA, Version 3.0, University of Texas Health Science Center, San Antonio, TX.
to be similar. Non-parametric tests were used to evaluate BC-AMP and the qualitative variable (PE-AMP). The following tests were used: 1) analysis of variance (ANOVA) to compare the histologic measurements (E, CTA, and GM-BC) among groups, complemented by the Tukey test; 2) the Fisher exact test to determine the relationship between the position of the epithelium in relation to the AMP (PE-AMP); 3) the Friedman test to compare the intensity of the connective tissue inflammatory infiltrate among the three areas in each group, complemented by the Dunn test; and 4) the Kruskal-Wallis test to compare the intensity of the inflammatory infiltrate in each area among groups and the BC-AMP parameter, complemented by the Dunn test. A level of $P < 0.05$ was accepted as statistically significant.

**RESULTS**

Analysis of the intensity of the connective tissue inflammatory infiltrate showed a clear predominance of a chronic infiltrate characterized by the presence of mononucleated cells (lymphocytes and plasma cells). No significant differences ($P > 0.05$) between the cervical, middle, and ATs were observed in the RMGIC or CR group. In the C group, a significant difference ($P < 0.05$) was observed between the three areas, with the CT differing from the AT but not from the MT (Table 1). Comparison of the intensity of the inflammatory infiltrate in each third among groups revealed no significant differences ($P > 0.05$).

Only in the C group was the epithelium observed coronal to the AMP. In fact, the epithelium was observed coronal to the CMP in the C group histologic analysis (Fig. 2). Migration of the epithelium onto the restorative materials was seen in both experimental groups: RMGIC and CR (Figs. 3 through 6). A significant difference ($P < 0.05$) in the position of the epithelium in relation to the AMP was observed between the experimental and C groups but not between the experimental groups.

The mean epithelium length was significantly shorter in the C group (2.24 ± 0.53 mm; Fig. 2) than...
in the RMGIC (6.16 ± 0.57 mm; Figs. 3 and 4) and CR (6.44 ± 0.23 mm; Figs. 5 and 6; P < 0.05) groups. CTA was significantly greater in the C group (2.22 ± 0.19 mm; Fig. 2) compared to the RMGIC (0.52 ± 0.14 mm; Fig. 4) and CR (0.37 ± 0.05 mm; Fig. 6; P < 0.05) groups. The C group presented a significantly shorter GM-BC distance (4.58 ± 0.20 mm; Fig. 2) than the RMGIC (6.71 ± 0.36 mm; Fig. 3) and CR (6.55 ± 0.37 mm; Fig. 5; P < 0.05) groups, which did not differ from each other (P > 0.05; Table 2). The BC in relation to the AMP (BC-AMP) was significantly more coronal in the C group (0.85 mm; Fig. 2) compared to the RMGIC group (−1.13 mm; Fig. 3; P < 0.05). The CR group (−0.36 mm) did not differ from the C or RMGIC group in this regard (Fig. 5; Table 3). One specimen showed bone formation with the BC coronal to the AMP.

**DISCUSSION**

The search for a restorative material that could be used on exposed root surfaces affected by deep caries or cervical abrasions before surgical coverage has been the subject of various studies. Although amalgam shows less accumulation of microorganisms than CR, it was not included in the present study because of its unfavorable esthetic characteristics, lower fibroblast adheriveness, and inferior bone biocompatibility compared to resin materials.

No significant differences in the intensity of the connective tissue inflammatory infiltrate were observed between the control and experimental groups in the present study (Table 1). Therefore, it seems that the restorative materials evaluated did not influence the inflammatory reaction per se in accordance with Konradsson and Van Dijken. The degree of surface roughness of restorations is directly related to the accumulation of bacteria and the presence of gingival inflammation. The favorable periodontal tissue response observed in the present study is likely a consequence of the careful finishing and polishing of the restorations prior to flap closure and the care taken with bacterial plaque control throughout the experiment.

The biocompatibility of restorative materials has been the subject of many studies. Lewis et al. examined the effects of components released from glass ionomer cements on the growth and metabolism of hamster oral epithelial cells and observed that the...
Leachable components of these materials affected the rate of progression of these cells through the cell cycle rather than cause cell death because of toxicity. In a scanning electron microscope study, Oliva et al. showed the adherence of osteoblastic cells to the surface of various glass ionomer cements, including the cement \(\text{Fuji II Lc, GC.}\) Brentegani et al. histologically evaluated the biocompatibility of a glass ionomer cement in rat dental alveoli. The authors concluded that the material tested was biologically compatible, being progressively incorporated into bone tissue during the alveolar wound healing process. However, when Lucksanasombool et al. compared the biocompatibility of glass ionomer cements and conventional bone cement (polymethyl methacrylate) in marrow cavities of rat femurs, they observed that the polymethyl methacrylate bone cement did not interfere with bone healing, whereas the glass ionomer cements (with smaller glass particles) and the RMGICs impaired bone formation.

In the present experiment, the bone defect created favored bone growth in the C group (0.85 mm), partially filling the prepared root cavity (Fig. 2). In the experimental groups, the BC remained apical to AMP (RMGIC: −1.13 mm; CR: −0.36 mm; Figs. 3 and 5). However, significant bone resorption was not observed in relation to the previously created distance (1 mm). These observations showed the biocompatibility of the restorative materials tested. A biologic distance between the restorative material and bone tissue corresponding to the space of CTA to the root cementum (RMGIC: 0.52 ± 0.14 mm; CR: 0.37 ± 0.05 mm) was maintained (Figs. 4 and 6). These findings are in accordance with other studies that have shown that bone growth and connective tissue reattachment do not usually occur on these restorative materials (Figs. 3 to 6). However, bone growth was noted on the CR in one specimen of this study, and bone growth was also noted on the glass ionomer in one specimen of a study by Gomes et al.

During laboratory processing and removal of the restorative materials, some fragments of the epithelial tissue were lost. CTA was only observed at sites where no restorative material was present. Therefore, CTA was significantly greater in the C group (2.22 ± 0.19 mm; Fig. 2) compared to the RMGIC (0.52 ± 0.14 mm; Fig. 4) and CR (0.37 ± 0.05 mm; Fig. 6) groups,

Table 2.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Group</th>
<th>Mean ± SD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>RMGIC</td>
<td>6.16 ± 0.57\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2.24 ± 0.53\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>6.44 ± 0.23\textsuperscript{a}</td>
</tr>
<tr>
<td>CTA</td>
<td>RMGIC</td>
<td>0.52 ± 0.14\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2.22 ± 0.19\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>0.37 ± 0.05\textsuperscript{a}</td>
</tr>
<tr>
<td>GM-BC</td>
<td>RMGIC</td>
<td>6.71 ± 0.36\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.58 ± 0.20\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>CR</td>
<td>6.55 ± 0.37\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Same letters indicate that there is no statistical difference among the groups by the Tukey test (\(P>0.05\)).

Table 3.

<table>
<thead>
<tr>
<th>Mean ± SD and Median of BC-AMP With Comparison Among Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>RMGIC</td>
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<tr>
<td>C</td>
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<tr>
<td>CR</td>
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Same letters indicate that there is no statistical difference among the groups by the Dunn test (\(P>0.05\)).

* Kruskal-Wallis test.
† Bone level apical to the apical limit of the cavity preparation.

\(\text{Fuji II Lc, GC.}\) \(\text{Vidrion F, S.S. White, Rio de Janeiro, RJ, Brazil.}\)
which did not differ from each other. These results do not corroborate the findings of Dragoo,\textsuperscript{23} who reported a large amount of connective tissue adhesion to glass ionomer cement restorations (4.13 mm).

In the present study, the length of the epithelium was significantly shorter ($P < 0.05$) in the C group (2.24 ± 0.53 mm; Fig. 2) compared to the RMGIC (6.16 ± 0.57 mm) and CR (6.44 ± 0.23 mm; Figs. 3 and 5; Table 2) groups, which did not differ from one another. The apical migration of the epithelium beyond the apical margins of the restorations (RMGIC and CR groups; Figs. 4 and 6) in the present study was also observed in other histologic studies that evaluated different restorative materials (i.e., amalgam and glass ionomer cement).\textsuperscript{16,20,23,27}

The CTA of the flap to the tooth is mediated by the deposition of new or additional root cementum and connective tissue derived from periodontal ligament and periosteum. Because the migration of epithelium along the root surface is not a desirable phenomenon, a pattern of an effective healing should exclude the epithelium tissue, except at the marginal area where a new sulcus and junctional epithelial attachment are formed.\textsuperscript{36} Apical proliferation seems to be greatest during the 10- to 14-day postoperative period. Further apical migration is not observable after 21 to 28 days, a period that shows cementoid deposition and CTA.\textsuperscript{36} Through case reports,\textsuperscript{3,35,37} the literature has shown that apical migration is not observable after 21 to 28 days, a period that shows cementoid deposition and CTA.\textsuperscript{36}

**CONCLUSION**

Within the limits of this study, it can be concluded that the restorative materials used exhibited biocompatibility; however, both materials interfered with the development of new bone and the CTA process.

**ACKNOWLEDGMENTS**

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