Furcation Perforation: Periradicular Tissue Response to Biodentine as a Repair Material by Histopathologic and Indirect Immunofluorescence Analyses

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Abstract

Introduction: The purpose of this study was to evaluate the *in vivo* response of periradicular tissues after sealing of furcation perforations with Biodentine, mineral trioxide aggregate (MTA), and gutta-percha by means of histopathologic and indirect immunofluorescence analyses. **Methods:** Thirty teeth of 3 dogs were divided into 3 groups: Biodentine (n = 14 teeth), MTA (negative control, n = 10 teeth), and gutta-percha (positive control, n = 6 teeth). After endodontic treatment, perforations were made on the center of the pulp chamber floor and filled with the materials. After 120 days, the animals were killed, and blocks containing the teeth and periradicular tissues were processed histotechnically for histopathologic semiquantitative (new mineralized tissue formation and bone resorption at the perforation site) and quantitative (thickness and area of newly formed mineralized tissue and number of inflammatory cells) analyses and RUNX2 immunofluorescence assay. Data were analyzed by χ^2, Fisher exact test, Mann-Whitney test, one-way analysis of variance, Kruskal-Wallis test, and Dunn posttest (α = 0.05). **Results:** MTA and Biodentine induced the formation of significantly more new mineralized tissue ($P < .0001$) than gutta-percha, which did not induce the formation of mineralized tissue in any case. Complete sealing of the perforations was more frequent with MTA, which formed mineralized tissue with greater thickness and area. Biodentine and MTA exhibited no bone resorption in the furcation region, fewer inflammatory cells, and greater RUNX2 immunostaining intensity than gutta-percha. **Conclusions:** Although MTA presented higher frequency of complete sealing and greater thickness and area of newly formed mineralized tissue, Biodentine also had good histopathologic results and can be considered as an adequate furcation perforation repair material. *(J Endod 2017;43:1137–1142)*

Key Words

Biodentine, furcation perforations, histopathological analysis, MTA, RUNX2

Significance

In vivo study of periradicular tissue response to new furcation perforation repair materials such as Biodentine is very important so that it can be used clinically in a secure and efficient manner.

Furcation perforations are iatrogenic or pathologic communications between the root canal space and the periradicular tissues caused by caries lesions or resorption (1). The trauma of a perforation at the furcation region can induce an inflammatory response, causing destruction of the periodontal ligament and resorption of bone and dental tissues. These events can quickly produce a pathway of communication with the gingival sulcus and lead to irreversible loss of periodontal attachment in the region (2).

Although a variety of materials have been suggested for the repair of furcation perforations, mineral trioxide aggregate (MTA) remains the gold standard and material of choice (3, 4). *In vivo* studies (5, 6) have demonstrated favorable periradicular tissue response to the use of MTA in furcation perforations and induction of the formation of mineralized tissue. In addition, its good biological behavior has been demonstrated in case reports that indicate clinical and radiographic success after use of this material for sealing furcation perforations (7, 8).

A negative point of MTA is the potential marginal gingiva and coronal tooth discoloration because of the presence of iron oxide in its composition, although white MTA has attenuated this problem without affecting the material's characteristics (9). There is

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also evidence that bismuth oxide, used as radiopacifier, affects its physicochemical properties (10).

Tricalcium silicate–based sealers have been developed in an attempt to have materials that combine antimicrobial activity and good sealing ability without the presence of heavy metals, which cause tooth crown staining and might have other harmful effects to the health. Recently launched in the European market, Biodentine (Septodont, St-Maur-des-Fosses, France) contains tricalcium silicate powder, dicalcium silicate, calcium carbonate, and zirconium oxide as radiopacifier. Its water-based liquid component contains calcium chloride as a setting accelerator and a water-soluble polymer (polycarboxylate), which provides good flowability (11). In the same way as MTA, Biodentine has tissue compatibility, bioactivity (12, 13), and resistance to compression (14). Although both are tricalcium silicate–based materials, Biodentine does not contain aluminate, which shortens its setting time and avoids potential risks to the health (11). Biodentine maintains the bone-biomaterial interface (15), has good capacity to seal (16), is not genotoxic (17), has low cytotoxicity (18), and promotes less coronal tooth discoloration than MTA (19).

With regard to the biological aspects, Biodentine has been shown to induce the differentiation of dental pulp cells into odontoblast-like cells and stimulate biomineralization (20). It also has been shown to stimulate the formation of mineralized tissue morphologically similar to osteodentin, which expressed odontoblast markers, and increased the secretion of transforming growth factor beta 1 from pulp cells in a similar manner to MTA and calcium hydroxide (21). In a previous study, our research group found that Biodentine had tissue compatibility and induced the formation of mineralized tissue bridges in pulpotomized dog's teeth with similar morphology and integrity but thicker than those formed with use of MTA (22). A recent study showed that in contact with dental pulp cells, both MTA and Biodentine were capable of inducing the expression of mineralization markers such as osteopontin, alkaline phosphatase, and runt-related transcription factor 2 (RUNX2) *in vitro* and *in vivo* (23).

Although sealing of furcation perforations is one of the indications of Biodentine, its biological effects when used for this purpose have not been investigated *in vivo*. The objective of this *in vivo* study was to evaluate the response of periradicular tissues of dogs' teeth after sealing of furcation perforations with Biodentine in comparison with MTA (negative control) and gutta-percha (positive control).

Materials and Methods

After approval of the research project, all animal procedures were conducted in accordance with the ethical guidelines and regulations of the institutional Animal Research Ethics Committee (process number 2014.1.76.58.4).

Operative Procedures

The permanent second and third maxillary premolars and second, third, and fourth mandibular premolars of 3 beagle dogs (age, 12 months; mean weight, 15 kg) were selected for the study, providing 30 teeth, which were distributed into 3 groups according to the furcation repair material: Biodentine (n = 14 teeth), ProRoot White MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK; negative control, n = 10 teeth), and gutta-percha bar (Odahcan Maillefer, Dentsply, Teresópolis, RJ, Brazil; positive control, n = 6 teeth). To have all variables evaluated in the same animal and in different dental quadrants, each hemiarch received one material chosen at random by using a change-over system.

For the operative procedures, the animals were preanesthetized and then received inhalation anesthesia with isoflurane. Standardized periapical radiographs were taken by using custom-made film-holding devices to confirm complete root formation and absence of pathologies. After pumice prophylaxis, rubber dam isolation, disinfection of the operative field with 2% chlorhexidine gluconate, coronal access, and pulpectomy, the working length was determined 2 mm short of the radiographic apex with an electronic apex locator (Root ZXII; J Morita Corp, Kyoto, Japan). Root canal shaping was performed with ProTaper Universal rotary instruments (Dentsply Tulsa Dental Specialties,) activated by an electric engine (X-Smart; Dentsply Tulsa Dental Specialties) under irrigation with 3.6 mL 1% NaOCl at each change of instrument. The canals were filled by lateral condensation of gutta-percha cones and AH Plus sealer (Dentsply De Trey, Konstanz, Germany).

After cleaning of the pulp chamber with saline, a perforation was made in the central region of the pulpal chamber floor with #1012 round diamond bur (KG Sorensen, São Paulo, SP, Brazil). In all groups, the diameter of the perforation was standardized as being the diameter of this bur. A new bur was used for every 3 perforations. Hemostasis was achieved with abundant sterile saline irrigation and gentle pressure with sterile cotton pellets, and the perforations were filled with the tested materials. Biodentine or MTA was prepared according to the manufacturers' instructions and gently placed into the perforations with a curette. After setting of the materials, the coronal access cavity was sealed with silver amalgam (Sybraloy; Kerr Corporation, Orange, CA). After 120 days, the animals were killed.

Histotechnical Processing

The maxillas and mandibles were removed, dissected, and sectioned to obtain individual blocks containing the teeth and adjacent periradicular tissues. The pieces were fixed in 10% buffered formalin for 48 hours, demineralized in an EDTA-based solution, and embedded in paraffin, and sagittal 5-μm-thick serial sections were obtained along the tooth extension. An experienced examiner blinded to the groups performed all analyses.

Histopathologic Analysis

For the semiquantitative analysis of the furcation perforations and adjacent periradicular tissues, the sections were stained with hematoxylin-eosin (HE) and examined by using an Axio Imager.M1 microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) operating in the conventional light mode. Scores were attributed to the following parameters: newly formed mineralized tissue: 0, absent; 1, partial; 2, complete (seal of the perforation); and bone resorption at the furcation region: 0, absent; 1, present. All sections from the evaluated teeth (around 20 sections) were examined by a blinded experienced pathologist. After that, 1 representative section (including the region of interest) from each tooth was selected and used for measurements.

The quantitative analysis of the thickness of the mineralized tissue formed in the furcation perforation was performed in the same HE-stained sections by using Axiovision Rel. 4.8 digital image processing software (Carl Zeiss MicroImaging GmbH), by using an Axio Imager M1 (Carl Zeiss) and a camera coupled to a computer system. In each section, the mineralized tissue thickness was measured in the cervical-occlusal direction by the average (in μm) of 3 linear measurements, 1 in the center of the furcation perforation and the other 2 at equidistant points from this first measurement. The distance from these 2 equidistant points was selected according to the amount of mineralized tissue formed, pointed on the tissue borders. The total area (in μm^2) of mineralized tissue formed was also measured (Fig. 1).

In addition, the number of inflammatory cells present in the central region of the furcation perforation, adjacent to the repair material

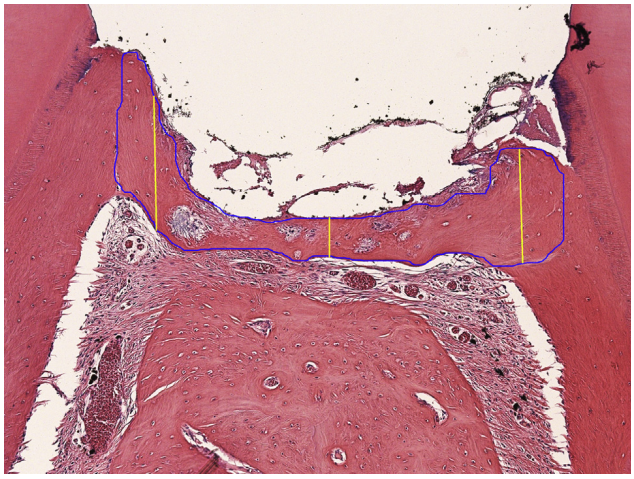

Figure 1. Representative photomicrograph of HE-stained section to illustrate the methodology used to measure the thickness (*yellow line*) and total area of mineralized tissue formed (*blue line*).

or to newly formed mineralized tissue, was counted within an area of approximately 1 mm^2.

Indirect Immunofluorescence: RUNX2 Labeling

Indirect immunofluorescence assay was performed to determine the expression of RUNX2 as a transcription factor for the synthesis of mineralization proteins and as an indicator of cell differentiation with mineralization phenotype.

The histologic slides were processed histotechnically according to Daltoé et al (23). The regions of interest were photographed by fluorescence microscopy at ×40 magnification by using Alexa Fluor (Carl Zeiss) and 40,6-diamidino-2-phenylindole (DAPI) filters. Next, the images were analyzed by using the "analyze particles" tool of Image J software (National Institutes of Health, Bethesda, MD), and the number of positively stained cells was determined by adjusting the threshold and calibrating the measurements by using a measuring scale. The percentage (frequency) of positively stained cells was evaluated in the periodontal ligament space adjacent to the mineralized tissue formed at the furcation perforation site and in the central region of this tissue within a 1 mm^2 area. In the cases without formation of mineralized tissue, the analysis was performed in the corresponding region.

Statistical Analysis

The score data of newly formed mineralized tissue were transformed into percentage (%) and analyzed by the χ^2 test, and bone resorption data (dichotomous data) were analyzed by the Fisher exact test. The quantitative results were analyzed by the D'Agostino & Pearson test for normality of the data. Mineralized tissue thickness and area data were analyzed by the Mann-Whitney test, and inflammatory cell count data were analyzed by one-way analysis of variance. Quantitative data of RUNX2 fluorescence intensity were analyzed by Kruskal-Wallis test and Dunn posttest. All tests were performed by using the Graph Pad Prism 6.0 statistical software (Graph Pad Software Inc, San Diego, CA), and the significance level was set at 5% for all analyses.

Results

Histopathologic Analysis

Regarding the semiquantitative analysis (scores) of newly formed mineralized tissue, gutta-percha (positive control) was significantly different ($P < .0001$) from Biodentine and MTA, which also differed from each other ($P < .0001$). From the 3 materials, MTA presented the highest frequency of complete sealing of furcation perforations. Expressing these results in percentage, Biodentine showed 7.1% absent mineralized tissue, 57.1% mineralized tissue partially sealing the perforation, and 35.8% mineralized tissue completely sealing the perforation. MTA showed 11.1%, 11.1%, and 77.8%, respectively, and gutta-percha showed 100% absence of new mineralized tissue formation. In summary, Biodentine and MTA induced repair by the formation of mineralized tissue sealing totally or partially the furcation perforation in almost all cases (92.9% and 88.9%, respectively), whereas no formation of mineralized tissue was observed in the positive control group (gutta-percha).

Comparing the furcation repair materials with respect to the thickness of the mineralized tissue formed in the furcation perforation, MTA induced the formation of a significantly thicker tissue ($P = .0148$) than Biodentine. Likewise, the area of newly formed mineralized tissue was significantly greater ($P = .0089$) in the MTA group. These results are presented in Figure 2.

Regarding the semiquantitative analysis (scores) of bone resorption in the furcation perforation region, gutta-percha (positive control) was significantly different ($P < .0001$) from Biodentine and MTA, which were similar to each other ($P = 1.00$). Expressing these results in percentage, Biodentine and MTA showed no bone resorption in 100% of the cases, whereas in the positive control group bone resorption was present in 100% of the cases.

Inflammatory cell count revealed significant difference only between Biodentine and the positive control group ($P = .0396$), which showed a larger number of inflammatory cells. MTA had intermediate values and did not differ significantly from the other groups (Fig. 3). Figure 4 shows representative histopathologic results of the groups treated with Biodentine (*A–C*), MTA (*D–F*), and gutta-percha (*G–I*).

Indirect Immunofluorescence: RUNX2 Labeling

RUNX2 immunofluorescence intensity revealed significant difference only between Biodentine and gutta-percha (positive control) ($P = .0036$), with stronger intensity for Biodentine. MTA showed mild intensity, but with statistically insignificant difference from the other groups ($P > .05$) (Fig. 5). Figure 6 shows representative photomicrographs of each group.

Discussion

The choice of the material used to seal pathologic or accidental furcation perforations has a decisive role in the repair of these lesions (4). The formation of mineralized tissue promoting wound healing is the ideal response and is an important indicator of treatment success (6).

The results of the histopathologic analysis showed that MTA (negative control) presented higher frequency of complete sealing of furcation perforation and greater thickness and area of newly formed mineralized tissue, which are in agreement with previous studies (5–8). The mechanism of action of MTA that could justify these findings is well-established in the literature. MTA is a bioactive cement with tissue compatibility and antimicrobial action, which is able to create an ideal microenvironment for repair (3). Because of its cementogenic and osteogenic properties, it can stimulate periodontal ligament cell growth, adhesion of osteoblasts, and bone regeneration (24). In contact with tissues, MTA stimulates immune system cells to release lymphokines that induce cementum repair and regeneration and activate bone markers required for biomineralization and healing of periapical bone defects (25). Despite all its benefits, MTA also has some

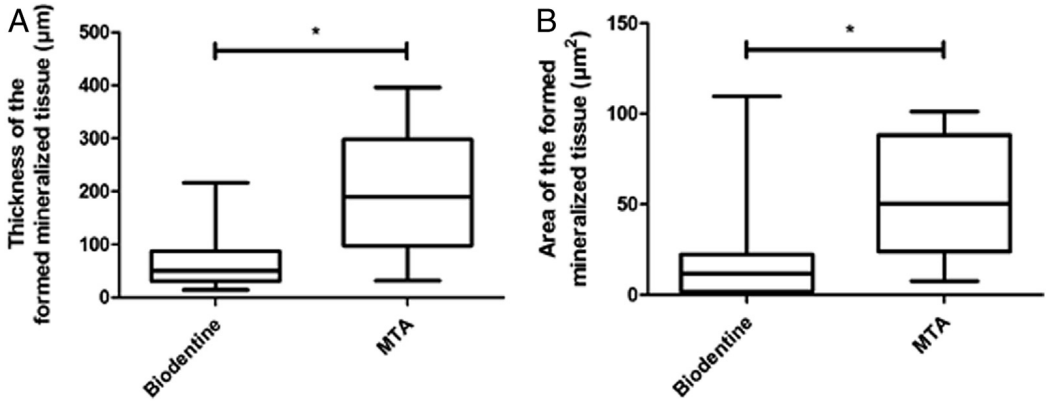

Figure 2. Box plots presenting minimum value, first quartile, median, third quartile, and maximum value of the thickness (*A*) and area (*B*) of the newly formed mineralized tissue in the furcation perforation region, when comparing Biodentine and MTA. *Statistically significant difference.

disadvantages. The main drawbacks include the potential for coronal tooth discoloration, presence of some toxic elements, long setting time, and difficult handling and difficult removal after setting (26, 27).

Although in the present study the thickness and area of newly formed mineralized tissue were significantly greater and the frequency of completely sealed perforations was significantly higher after use of MTA, Biodentine induced repair by the formation of mineralized tissue sealing totally or partially the furcation perforations in almost all cases (92.9%), which shows good biological performance of this material. Moreover, both materials showed 100% absence of bone resorption in the furcation perforation region. To the best of our knowledge this is the first *in vivo* study to evaluate the response of periradicular tissues to Biodentine used for sealing of furcation perforations, which precludes a direct comparison with the results of other investigations.

Some of Biodentine's properties and its mechanism of action could substantiate our findings. Because of their similar composition and bioactivity (12), Biodentine has been considered a suitable substitute for MTA and has been shown to induce greater deposition of hydroxyapatite when exposed to tissue fluids (11). Studies have also

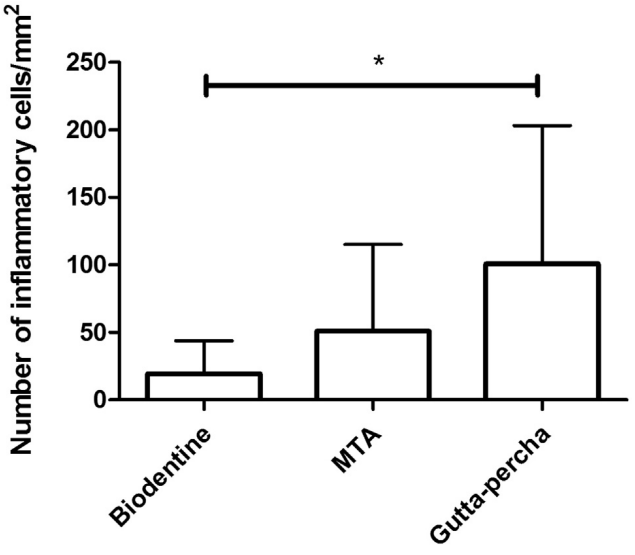

Figure 3. Mean and standard deviation of number of inflammatory cells per mm^2 in furcation perforations sealed with Biodentine, MTA, and gutta-percha. *Statistically significant difference.

demonstrated excellent physical and chemical properties, such as mechanical strength (28), high pH and calcium ion release (29), excellent sealing performance (16), antimicrobial activity, and low solubility (30) after use of Biodentine. From the biological point of view, the absence of genotoxicity (17), low cytotoxicity (18), high capacity to maintain dental pulp cell viability in three-dimensional culture (31), and mineralization potential (23) make Biodentine an ideal material for clinical use. Until now, there is conflicting evidence in the literature according to tooth discoloration caused by Biodentine. Although Beatty and Svec (32) showed that Biodentine caused a perceptible color change in bovine teeth in comparison with other cements, Marconyak et al (19) demonstrated that this material showed significantly less discoloration compared with white ProRoot MTA, MTA Angelus, and ProRoot MTA.

Mori et al (33) reported that Biodentine stimulates fibroblast activation and collagen fiber formation with insignificant inflammatory reaction in rat connective tissue. In contact with Biodentine, fibroblasts adhered to the material's surface (18), and undifferentiated mesenchymal cells exhibited significantly high levels of osteogenic genes with increased alkaline phosphatase expression, demonstrating the osteoblastogenic capacity of this material (34). These findings could explain, in part, the high capacity of Biodentine to induce furcation perforation repair, with predominance of mild/absent inflammatory infiltrate in the region.

In the present study, the groups sealed with MTA and Biodentine presented fewer inflammatory cells than the positive control (gutta-percha), although this difference was significant only for Biodentine. A recent *in vivo* study in rat subcutaneous tissue showed that the number of inflammatory cells and interleukin-6–immunolabeled cells was significantly higher after 7 and 15 days of exposure to Biodentine compared with MTA. However, after 60 days, there was a significant regression of the inflammatory reaction, and both materials exhibited capsules with numerous fibroblasts and collagen fiber bundles (13), which corroborate our findings. Those results could explain the few cases with inflammatory cell infiltrate after sealing of the perforations with MTA or Biodentine. In addition, it is well-established that inflammatory cells participate in mineralized tissue repair and act in the activation of other cells that are essential for bone formation, repair, and remodeling (35).

RUNX2 is a mineralization marker and an essential transcription factor for osteoblast differentiation. The occurrence of furcation perforation repair was also confirmed by the immunofluorescence assay, which revealed increased RUNX2 expression in the periodontal

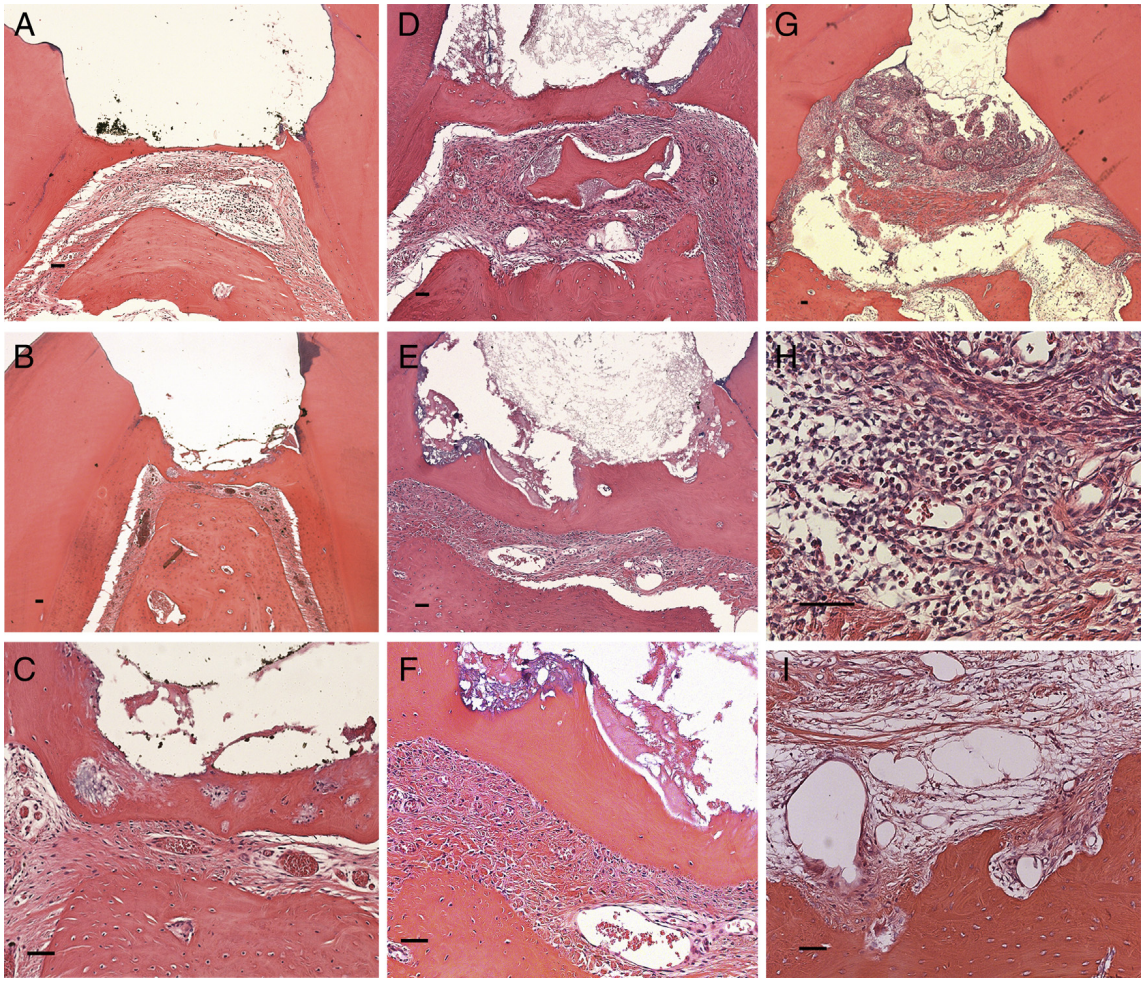

Figure 4. Photomicrographs representative of HE-stained sections of groups treated with Biodentine (*A–C*), MTA (*D–F*), and gutta-percha (*G–I*). (*A*) Newly formed mineralized tissue sealing completely the furcation perforation. (*B*) Newly formed mineralized tissue at higher magnification and repair of this region. (*C*) Absence of inflammatory cells or bone tissue resorption. (*D*) Mineralized tissue sealing completely the furcation perforation, with greater thickness and area. (*E*) Newly formed mineralized tissue at higher magnification, with greater thickness and area and repair of this region. (*F*) Few inflammatory cells and several osteoblasts covering the bone surface, indicating ongoing repair. (*G*) Absence of newly formed mineralized tissue formation at site of perforation. (*H*) Intense inflammatory infiltrate. (*I*) Bone resorption lacunae with osteoclasts on their surface. Scale bar = 50 μm.

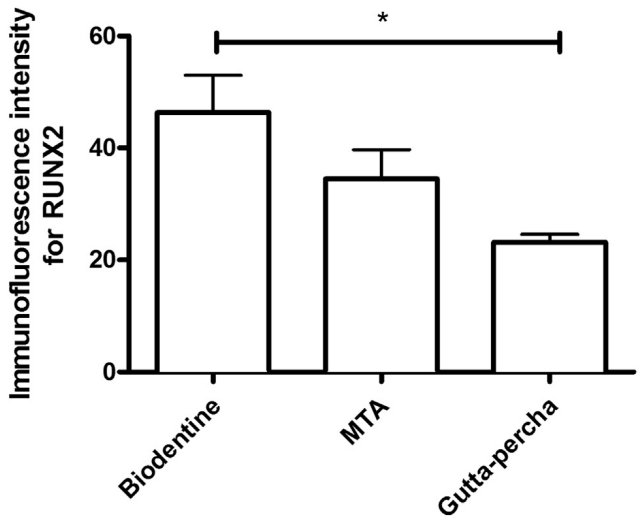

Figure 5. RUNX2 immunofluorescence intensity (mean and standard deviation) in groups treated with Biodentine, MTA, and gutta-percha. *Statistically significant difference.

ligament and in the new mineralized tissue formed adjacent to Biodentine and MTA, although statistical significance was found only in the comparison between Biodentine and the control group. A recent cell culture study (31) showed RUNX2 (marker of mineralization) expression by differentiated pulp cells in both MTA and Biodentine groups at all evaluation periods. It is known that during bone development, RUNX2 induces osteoblastic differentiation, upregulates the expression of several genes of bone matrix proteins, and increases the number of immature osteoblasts (36). Therefore, although the exact nature of mineralized tissue formed in response to Biodentine is not known, RUNX2 expression in the present study confirms the biomineralization role and the repair induction capacity of this material when used to seal furcation perforations.

In contact with periradicular tissues in this study, Biodentine induced the repair of furcation perforations because of the creation of a biomineralizing microenvironment with mild inflammatory response. It may be speculated that if a longer evaluation period were used, this material could present frequency and thickness of completely formed mineralized tissue comparable with MTA.

Under the conditions of this *in vivo* study, it may be concluded that Biodentine and MTA provided good histopathologic results and can be

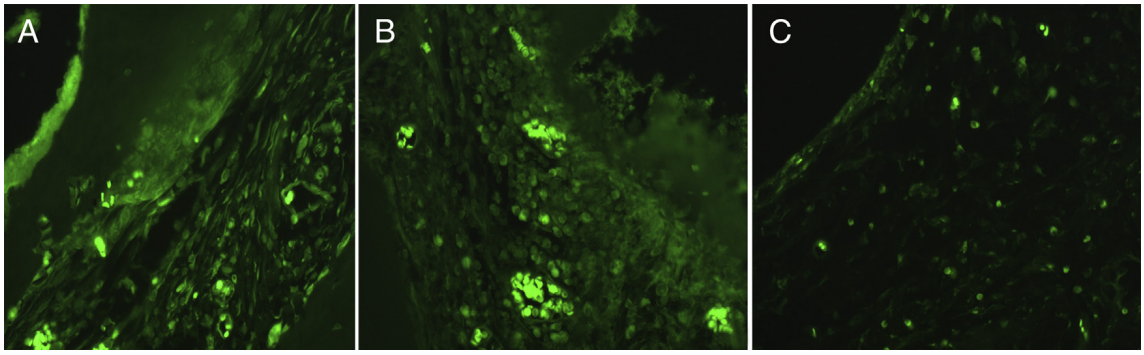

Figure 6. Representative photomicrographs of indirect immunofluorescence RUNX2 labeling showing strong intensity in group treated with Biodentine (*A*), mild staining in group treated with MTA (*B*), and weak intensity in group treated with gutta-percha (positive control) (*C*) (original magnification, × 40).

considered as adequate furcation perforation repair materials. However, MTA induced the formation of mineralized tissue with greater thickness and area and presented higher frequency of complete sealing of the furcation perforations. RUNX2 appears to be an important osteogenic transcription factor for the biomineralizing potential of both materials.

Acknowledgments

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The authors deny any conflicts of interest related to this study.

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