Comparison of Pulpal Responses to Pulpotomy and Pulp Capping with Biodentine and Mineral Trioxide Aggregate in Dogs

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Abstract

Introduction: This study evaluated the pulpal and periapical responses of dogs' teeth after pulpotomy and pulp capping with a new tricalcium silicate–based cement (Biodentine) when compared with mineral trioxide aggregate (MTA) by radiographic, histopathologic, and histomicrobiological analyses. **Methods:** Sixty roots (30 teeth) of dogs were divided into 2 groups, Biodentine (n = 36 roots) and ProRoot MTA (control, n = 24 roots). Animals were killed after 120 days, and the teeth were subjected to histotechnical processing (hematoxylin-eosin and Brown and Brenn staining). Qualitative and quantitative histopathologic data were analyzed by Fisher exact and Mann-Whitney tests (α = 0.05). **Results:** Radiographically, mineralized tissue bridge formation was observed in more specimens treated with Biodentine (96.8%) than with MTA (72.2%) (P = .02). Integrity of the lamina dura and absence of periapical bone rarefaction and root resorption (external and internal) were observed in all specimens. Histopathologic and histomicrobiological analyses revealed mineralized tissue bridge formation, pulpal vitality, odontoblast layer integrity, preserved periodontal ligament, and absence of bone or root resorption and microorganisms in both groups. Although the bridges formed at the amputation site had similar morphology, they were significantly thicker in the Biodentine group (P < .0001). Comparison between the radiographic and histopathologic results showed that radiographic visualization of more bridges in the Biodentine group was related to bridge thickness because radiographic diagnosis was flawed for bridges with thickness less than 0.5 mm. Fluorescence microscopy improved the visualization of bridge structure. **Conclusions:** Biodentine presented tissue compatibility and allowed for mineralized tissue bridge formation after pulpotomy in all specimens with similar morphology and integrity to those formed with use of MTA. Periapical radiographs failed in detecting mineralized tissue bridges with thickness less than 0.5 mm. *(J Endod 2014;40:1362–1369)*

Key Words

Biodentine, mineral trioxide aggregate, pulpotomy

Since the mid-1990s, mineral trioxide aggregate (MTA) has been recognized as the reference material for conservative pulp vitality treatments, with high success rates (90%–100%) in clinical, radiographic, and histopathologic studies (1, 2). However, MTA has a long setting time (2.75 hours), need of hydration during setting (3), and difficult handling characteristics (4) and may contain heavy metals (5). Therefore, researchers continue searching for materials with improved physical properties (6), adequate sealability, and antimicrobial action similar to that of calcium hydroxide (7).

A tricalcium silicate–based cement has recently been launched for use as a dentin substitute in restorative procedures. Biodentine (Septodont, St-Maur-des-Fossés, France) is composed of highly purified tricalcium silicate, dicalcium silicate, calcium oxide and carbonate, and zirconium oxide (radiopacifier); a calcium chloride liquid agent to reduce the setting time; and a water-soluble polymer to provide adequate flow capacity (8). The main advantages of Biodentine over MTA include its ease of handling, high viscosity, shorter setting time (12 minutes), and better physical properties (9), in addition to containing raw material with a known degree of purity (10). This material stimulates the deposition of hydroxyapatite on its surface when exposed to tissue fluids (11), presents color stability (12), is not genotoxic (13), and has low cytotoxicity (14), preserving gingival fibroblast viability (15). In the few *in vitro* studies available so far, Biodentine presented compatibility to dental pulp cells and stimulated the formation of tertiary dentin (3, 16, 17). It also induced the differentiation of cultured pulp cells into odontoblast-like cells (16) and mineralized foci formation, similarly to MTA and calcium hydroxide (17).

Although one of the indications for use of Biodentine is pulpal therapy, its effect after pulpotomy in permanent teeth has not yet been investigated. To the best of our knowledge, 2 studies have evaluated this material in conservative pulpal therapies. In one study, it was used as a direct pulp-capping agent in human third molars (9); in the other, it was used in pulpotomies in porcine primary teeth (18). Although Biodentine has already been in use as a restorative material (19) and has been indicated as a biomaterial for direct contact with exposed pulp, its biological effects should be

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further investigated. The aim of this study was to evaluate pulpal and periapical response of dogs' teeth after pulpotomy and pulp capping with Biodentine and MTA by radiographic, histopathologic, and histomicrobiological analyses.

Materials and Methods

All animal procedures conformed to the ethical guidelines and regulations of the institutional Animal Research Ethics Committee (process number 2002.1.121.53.0). The present study was based on the protocol recommended by the ISO standard 7405:2008 (20).

The second and third maxillary premolars and the second, third, and fourth mandibular permanent premolars of 3 beagle dogs aged 12 months and weighing 15 kg on average were selected for the study, providing 30 teeth (60 roots).

The roots were distributed into 2 groups according to the pulp-capping material, Biodentine (n = 36 roots) and ProRoot White MTA (Dentsply Tulsa Dental Specialties, Tulsa, OK; control group, n = 24 roots). Materials' composition is given in Table 1.

Standardized periapical radiographs were taken by using custom-made film-holding devices. The animals were preanesthetized 15 minutes before the operative procedures and received inhalation anesthesia with isoflurane. Prophylaxis and root scaling were performed, all teeth were isolated with a rubber dam, and the operative field was disinfected with 2% chlorhexidine gluconate. After coronal access, coronal pulp was removed with sharp curettes up to the canal entrance level, and hemostasis was achieved with abundant sterile saline irrigation of the pulp chamber. The tested materials were gently applied onto the remaining radicular pulp tissue at the amputation site, according to the manufacturers' instructions. All variables were evaluated in the same animal in different dental quadrants. After initial setting of the materials, a glass ionomer (Vidrion; S. S. White, Rio de Janeiro, RJ, Brazil) base was prepared on the top of MTA or Biodentine and left to harden, and then silver amalgam (Velvalloy; S. S. White) was condensed on top of the glass ionomer to restore the coronal access cavity.

After 120 days, new periapical radiographs were taken, and the animals were killed. The preoperative and postoperative radiographs were evaluated by an experienced examiner blinded to the groups, who attributed scores to each of the following radiographic parameters: absence (0) or presence (1) of mineralized tissue bridge and radiolucent areas suggestive of periapical lesion or root resorptions (internal and external). The lamina dura was evaluated as preserved (0) or altered (1).

The maxillas and mandibles were dissected and sectioned to obtain individual roots, which were fixed in 10% buffered formalin for 48 hours, demineralized in EDTA, and embedded in paraffin. Sagittal 5-μm-thick serial sections were stained with hematoxylin–eosin (HE) for histopathologic analysis and with the modified Brown and Brenn method (21) for histomicrobiological analysis.

TABLE 1. Composition of the Materials Used

Material	Composition according to manufacturer
Biodentine	Powder: tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, iron oxide, zirconium oxide Liquid: calcium chloride, hydrosoluble polymer
ProRoot White MTA	Powder: Portland cement, bismuth oxide, calcium sulfate dihydrate or gypsum (5%), tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite Liquid: water

Qualitative and quantitative histopathologic analyses were performed on the HE-stained specimens by using an Axio Imager.M1 microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) operating in the conventional light and fluorescence modes (22). An experienced examiner blinded to the groups conducted all analyses.

Under conventional light, the qualitative histopathologic analysis consisted of describing the characteristics of the remaining radicular pulp tissue, mineralized tissue bridge, and apical and periapical tissues. The quantitative analysis consisted of the attribution of scores to histopathologic parameters by using a scoring system modified from Nowicka et al (9) and Leonardo et al (23) (Table 2).

Under fluorescent light, a quantitative analysis was performed in the same HE-stained sections by using the microscope at ×10 magnification operating in fluorescence mode by using Alexa Fluor 488 (Carl Zeiss) filter with G365 excitation, FT395 reflectors, and LP420 emission to provide an additional evaluation of pulp response after pulpotomy. In each specimen, the thickness of the mineralized tissue bridge was measured by using the Axiovision Rel. 4.8 digital image processing software with the Axiocam microscope (Carl Zeiss) and a camera coupled to a computer system. The mineralized tissue bridge thickness was measured in the cervical-occlusal direction by the average (in mm) of 3 linear measurements, 1 made in the central region of the pulp amputation site and the other 2 at equidistant points from this first measurement.

The radiographic and histopathologic results were analyzed by Fisher exact test at 95% confidence interval by using the SPSS statistical program (SPSS, Inc, Chicago, IL). Mineralized tissue thickness data were analyzed by the Mann-Whitney test by using the GraphPad Prism 4.0 statistical software (Graph Pad Software, Inc, San Diego, CA). The significance level was set at $\alpha = 0.05$.

Results

Of the 36 roots treated with Biodentine, 31 roots were examined radiographically, and 30 roots were examined histopathologically. Of the 24 roots treated with MTA, 18 roots were examined radiographically, and 18 were subjected to histopathologic analysis. The other roots were excluded from the study because of restoration fracture, dislodgement, or failures during the histotechnical processing.

Radiographic Findings

In the roots capped with Biodentine, mineralized tissue bridge formation was observed in 30 of 31 specimens (96.8%). In the roots capped with MTA, mineralized tissue formation occurred in 13 of 18 specimens (72.2%). In both groups, in 100% of the specimens the lamina dura was preserved, and there was no image suggestive of periapical bone rarefaction or internal/external root resorption. Formation of mineralized tissue was significantly more frequent (P = .02) after use of Biodentine compared with MTA. Preservation of the integrity of the lamina dura and periapical region and absence of internal or external root resorption processes were observed after use of Biodentine and MTA without statistically significant difference between the materials ($P > .05$).

Histopathologic Findings

Conventional Light Microscopy. On the basis of the histopathologic results, capping with Biodentine and MTA produced a very similar and regular pulpal and periapical response pattern (Table 2 and Figs. 1–4). Formation of a mineralized tissue bridge obliterating