Histopathological and Histochemical Evaluation of Pulpal Response to Biodentine Compared to Portland Cement in Pulpotomized Dogs’ Teeth

Moustafa Mohammed Sayed1*, Nagawa Mohammed Ali Khattab2 and Wael Hamada Ahmed3

1Dentist at Assiut University Hospital, Faculty of Dentistry, Misr University for Science and Technology, Egypt
2Professor and Head of Pediatric and Community Dentistry Department, Faculty of Dentistry, Minia University, Egypt
3Lecturer of Pediatric and Community Dentistry Department, Faculty of Dentistry, Minia University, Egypt

*Corresponding Author: Moustafa Mohammed Sayed, Dentist at Assiut University Hospital, Faculty of Dentistry, Misr University for Science and Technology, Egypt.

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Abstract

Background: Several medicaments have been used in pulpotomy procedures of primary and young permanent teeth with the objective to maintain pulp vitality and promote healing of the pulp remnants.

Aim of Study: The aim of this study was to evaluate the histological response of pulpotomized dental pulp in dogs to Biodentine and Portland cement.

Materials and Methods: The study included 72 teeth on 3 apparently healthy adult Mongrel dogs it was classified into 2 groups according to the material. Group 1: included 36 teeth capped by Biodentine, group 2: include 36 teeth capped by Portland cement.

The inflammatory response, hard tissue formation and tissue fibrosis over 1, 2, 3 months periods were recorded.

Results: Histopathological and histochemical analysis showed complete dentinal bridge formation and an absence of inflammatory pulp response. Layers of well-arranged odontoblast and odontoblast-like cells were found to form tubular dentin under the osteodentin. Statistical analysis showed no significant differences between the Biodentine and Portland cement experimental groups during the observation period. Although all specimens of both groups showed formation of dentine bridge, the dentine bridge formed by Biodentine was of better quality than Portland cement.

Conclusion: Within the limitation of the current study Biodentine was superior to Portland cement in terms of inflammatory cell response and better quality of dentine bridge.

Keywords: Biodentine; Portland Cement; Pulpotomy; Histopathology

Introduction

The preservation and protection of the dental pulp with specific emphasis on regeneration is the new treatment strategy in the fields of pediatric dentistry, endodontics and dental traumatology. The use of hydraulic calcium silicate cement apparently stimulates pulpal cell recruitment and differentiation, up-regulates transformation factors (gene expression), and promotes dentinogenesis [1].

Recently, great interest has been focused on Portland cement (PC) as an alternative to MTA, and several experimental studies have compared both materials [2]. PC differs from MTA by the absence of bismuth ions [3] and presence of potassium ions [4]. Both materials have comparable antibacterial activity [5] and almost identical properties macroscopically, microscopically and by X-ray diffraction

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analysis [6]. It has also been shown that PC and MTA have similar effects on pulp cells when used for direct pulp-capping in rat teeth [7]. In addition, both MTA and PC allowed for dentin bridge formation after pulpotomy performed on dogs. Min., et al. [8] observed that PC allowed the expression of mRNAs of a dentin-specific protein and a non-collagenous protein involved in mineralization in cultured human pulp cells.

De-Deus., et al. [9] presented a case in which substantial periapical healing occurred with the use of PC to create an apical plug in the root of an immature tooth in human. Finally, Conti., et al. [10] have recently documented the clinical success of two cases in which PC was applied as a medicament after pulpotomy of mandibular primary molars.

The main advantages of Biodentine (Septodont company) over Portland cement (CEMEX cement company) include its ease of handling, high viscosity, shorter setting time (12 minutes), and better physical properties [11], in addition to containing raw material with a known degree of purity [12]. It stimulates the deposition of hydroxyapatite on its surface when exposed to tissue fluids [13], presents color stability [14], is not genotoxic [15], and has low cytotoxicity [16], preserving gingival fibroblast viability [17]. In the few in vitro studies available so far, Biodentine presented compatibility to dental pulp cells and stimulated the formation of tertiary dentin. It also induced the differentiation of cultured pulp cells into odontoblast-like cells [18] and mineralized foci formation, similarly to MTA and calcium hydroxide [19].

Although Biodentine has already been in use as a restorative material [20] and has been indicated as a biomaterial for direct contact with exposed pulp, its biological effects should be further investigated.

Aim of Study

The aim of this study was to evaluate the response of pulpotomized dental pulp in dogs to Biodentine and Portland cement, the evaluation was carried out by histopathological and histochemical analysis.

Materials and Methods

Three apparently healthy adult Mongrel dogs aged from 2 to 3 years and their weight ranged from 12 to 20 kg were selected for the study, the mandibular and maxillary left and right first, second, third and fourth premolars and first molar (providing 72 teeth) were selected for the study.

The teeth were divided into 2 groups (split mouth technique) according to the pulp-capping material, Biodentine (n = 36 teeth) and Portland cement (n = 36 teeth). The animals had anesthesia immediately through intravenous injection of a mixture of ketamine and xylazine and maintained with 2.5% thiopental sodium intravenous till the end of procedure.

After animal were anesthetized, the working field was disinfected by 5% tincture iodine. A dry field was achieved by means of cotton rolls and gauze swabs on the facial surfaces of the teeth, class V cavities were prepared approximately 1 mm coronal to the gingival margin by inverted cone carbide. Coronal pulp was amputated with sharp curette instrument and the exposure site was rinsed with sterile saline until physiologic hemostasis was achieved controlling bleeding through the pulp chamber.

The tested materials were gently applied onto the remaining radicular pulp tissue at the amputation site, according to the manufacturers’ instructions. After initial setting of the materials, a glass-ionomer restoration (SDI Riva self-cure) was prepared on the top of Biodentine and Portland cement and left to harden.

The first dog was sacrificed after one month, the second was sacrificed after two months, and the third was sacrificed after three months. Both of mandible and maxilla of each dog were removed and sectioned into two halves at the midline.

Teeth were surgically extracted from each jaw by removing the bone around each tooth. Thereafter, they were placed in 10% neutral buffered formalin for 48 to 72 hours. The specimens were decalcified by immersion in 40 cm 10% neutral buffered formalin, 320 cm dis-
tilled water and 40 cm nitric acid and for period ranged from 10 to 20 days. Specimens were washed under running tap water thoroughly up to 24 hours. After decalcification, specimens were dehydrated in 70% ethanol alcohol and then embedded in paraffin. The paraffin embedded specimens were serially cut in a bucco-lingual plane parallel to the tooth main vertical axis through the cavity preparation and the pulp into sections of 60μm thickness.

Histopathological evaluation: all samples were stained with (H and E) and examined under light microscope to evaluate:

a) Inflammatory response using scoring system of Katoh., et al. 1993 [21].
   Score 0 = Absence of inflammation, Score 1 = Mild inflammation at pulpotomized site, Score 2 = Moderate inflammation at pulpotomized site, and Score 3 = Severe inflammation or pulp necrosis.

b) Dentine bridge using scoring system Parolia., et al. 2010 [22],
   Grade 0 = No hard tissue deposition, Grade 1 = Mild hard tissue deposition beneath the exposed area, Grade 2 = Moderate hard tissue deposition beneath the exposed area, Grade 3 = Heavy hard tissue deposition beneath the exposed area.

Histochemical evaluation: all samples were stained by masson-trichrome stain for the demonstration of collagen fibers formation, using scoring system of Onoe., et al. 1994 [23].
   Score 0 = no fibrosis, Score 1 = Mild fibrosis, Score 2 = Moderate fibrosis, Score 3 = Severe fibrosis.

Results
Histopathological evaluation

At the first evaluation period (one month after capping of pulp with tested materials) the pulp response to both material was the same, in both groups 83.3% of the specimens showed absence of inflammation response limited to pulpotomized site, while 16.7% of the specimens showed mild inflammation at pulpotomized site.

At the second evaluation period (two months after capping of pulp with tested materials) the pulp response to both material was the same, in both groups 75% of the specimens showed absence of inflammation response limited to pulpotomized site, while 25% of the specimens showed mild inflammation.

At third month of follow up period 100% of specimens capped with Biodentine showed absence of inflammation cell at the exposed pulp. In Portland cement 75% of specimens showed absence of inflammatory reaction while 25% specimens showed mild inflammation (Table 1, Figures 1,2).

<table>
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<td>2 months</td>
<td>3 months</td>
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<td>9 (75%)</td>
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Table 1: Comparison between inflammatory response of dental pulp to Biodentine and Portland cement at different periods.

For dentine bridge formation, Biodentine group showed dentine bridge formation faster and better quality than Portland cement group.

After one month of pulpotomy procedure by Biodentine 25% of the specimens showed absence of hard tissue formation and 75% showed mild of hard tissue formation. While in Portland cement 100% showed absence of hard tissue formation.

After two months of pulpotomy procedure 16.7% specimens in Biodentine specimens showed mild formation of hard tissue and 83.3% specimens showed moderate formation of hard tissue. While in Portland cement 25% specimens showed absence of tissue formation, 75% specimens showed mild formation of hard tissue formation.

After three months of capping procedure 16.7% in specimens showed mild formation of hard tissue and 83.3% showed severe formation of hard tissue. While in Portland cement 25% specimens showed mild formation of hard tissue, 75% specimens showed moderate formation of hard tissue (Table 2, Figures 3, 4).

<table>
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<td>Grade 3</td>
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Table 2: Comparison between grades of Dentine Bridge formation in response to Biodentine and Portland cement at different periods.

Figure 3: Photomicrograph of dental pulp capped with Biodentine showing formation of dentine (asterisk), (A) Mild formation of dentine matrix after one month, (B) Moderate formation of dentinal bridge after two months (C) Heavy deposition of dentinal bridge after three months. d (dentin), p (pulp), ob (odontoblast). (HE, 200× magnification).
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Figure 4: Photomicrograph of dental pulp capped with Portland cement showing formation of dentine (asterisk) (A) No hard tissue formation after one month, (B) Mild formation of dentine matrix after two months pulpotomy. (C) Moderate formation of dentinal bridge after three months, d (dentin), p (pulp), ob (odontoblast). (HE, 200× magnification).

Histochemical evaluation: After one month of pulpotomy procedure by Biodentine 25% of the specimens showed no fibrosis and 75% showed mild fibrosis. While in Portland cement group 100% specimens showed no fibrosis.

After two months of pulpotomy procedure by Biodentine 25% showed mild fibrosis, 75% showed moderate fibrosis. While Portland cement group 25% specimens showed no fibrosis, and 75% showed mild fibrosis.

After three months of pulpotomy procedure 16.66% of specimens in Biodentine group showed moderate fibrosis, and 83.3% showed severe fibrosis. While Portland cement group 25% of the specimens showed mild fibrosis, and 75% showed moderate fibrosis (Table 3, Figures 5, 6).

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*Table 3: Comparison between formation of tissue fibrosis in response to Biodentine and Portland cement at different.*

*Figure 5: Photomicrograph of dental pulp capped with Biodentine showing tissue fibrosis (A) Mild amount of tissue fibrosis after one month. (B) Moderate amount of tissue fibrosis after two months (C) Sever amount of tissue fibrosis after three months, d (dentin), p (pulp) (Masson’s trichrome, 200× magnification).*

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Discussion

Pulpotomy can be carried out with various materials based on their biocompatibility, sealing ability and antimicrobial efficacy. Latest bioactive materials such as Portland cement, Mineral Trioxide Aggregate (MTA) and Biodentine were suggested [24]. Therefore, the current study was conducted to compare the pulp response to Biodentine and Portland cement, both histopathologically and histochemically.

Dogs was the selected animal model for the current study because the mechanism of induction and synthesis of dentin in this animal are the same as in human beings, even though, the rate of reparative dentinogenesis may differ. Dogs are believed to be a suitable experimental model because their pulp tissue is comparable to that of humans. Dog dentition include four premolars and two or three molars in each quadrant which provides a good number of teeth allowing the comparison of more than one material in the same dog [25].

The current study was carried out using split mouth technique so that both medicaments could be tested in the same animal in alternate sides of the mouth. Pulpotomy procedure were performed and tested materials were applied according to assigned groups.

Histopathological evaluation was performed to report the inflammatory response, as well as dentine bridge formation using Hematoxylin, Eosin stains [26] and also observe the fibrosis degree by Mathontrichrome stain [27]. Therefore, Three dogs were sacrificed after 1, 2 and 3 months respectively to observe the histopathological changes in response to tested materials.

Results of the current study revealed that, specimens capped with Biodentine exhibited less inflammatory reaction compared to Portland cement across all follow up periods, findings goes in accordance with Shayegan., et al. [28], Tran., et al. [29], Nowicka., et al. [12] and De Rossi., et al. [30] who found that Biodentine has delayed the inflammatory response. The low inflammatory reaction in Biodentine indicated that it has excellent sealing properties and prevent microleakage and pulpal inflammation by providing a predictable secondary barrier under the surface seal [12].

Initially, at the first month, a distinct superficial necrosis occurred due to pulp tissue exposure. The necrosis induced an inflammation because necrotic tissue acts as a foreign body in a healthy environment. This necrotic tissue had been metabolized and replaced by granulation tissue due to cellular reaction of the surrounding connective tissue. The formation of granulation tissue through the proliferation of capillaries and fibroblasts initiated the repair of the pulp tissue. Thus, the inflammatory reaction is an essential part of the wound healing process [31]. If the underlying cause for the necrosis has been eliminated the inflammation will heal fast. If the reason for the necrosis persists over a longer period of time, an inflammation with severe granulation and poor healing will occur.

At the third month the teeth that capped with Biodentine showed no signs of inflammations which is supported by the evidence that Biodentine has more potent antibacterial and antifungal effect [32]. Therefore, the inflammatory reaction period and healing progress fast.

On the other hand, Fonseca., et al. [33] found that the number of inflammatory cells was significantly higher in the Biodentine group in comparison with the MTA in the initial periods; however, the morphological analysis showed clearly that the inflammatory reaction was moderate at 7 days, whilst at 15 and 30 days, a mild inflammatory reaction was observed. At 60 days, a significant reduction in the number of inflammatory cells was verified. Difference may be attributed to difference in the type of studied animal, the current study was carried out on dogs’ but Fonseca study was on rats, also the experiment was done by putting a polyethylene tube filled with Biodentine and placed into the dorsal subcutaneous of rats; not in direct contact with pulp tissue as in the current study.

While, specimens capped with Portland cement exhibited more inflammatory reaction compared to Biodentine across all follow up periods, findings goes in accordance with Bidar., et al. [34] who found that Portland cement showed inflammation rate slightly higher when compared with MTA in dogs’ teeth. However there is no any statistical significant difference in inflammation between Portland cement and MTA.

Although, there were no statistical significant difference between groups in terms of dentine bridge formation, dentine bridge formed in response to Biodentine was faster and of a better quality than Portland cement group. A finding that goes in accordance with Tran., et al. [29] and Nowicka., et al. [12] who showed that the reparative structures induced by Biodentine were homogenous and in continuity with primary dentine. This better quality of dentine bridge might be due to release of TGF-B1 in pulp cells that stimulated odontoblasts to increase their activity and activate reparative dentinogenesis [20].

In Portland cement, calcium oxide that forms calcium hydroxide when mixed with water reacts with the carbon dioxide from the pulp tissue produces calcite crystals. Then, a rich extracellular network of fibronectin in close contact with these crystals can be observed [29].

Moreover, there were no statistical significant difference between groups in terms of collagen fibrous formation. However, collagen fibers formed in response to Biodentine was faster and better quality than Portland cement group. Newly formed collagen fibrils were distinguishable near the exposure site in Biodentine group after one month of its application which increase in quality over the follow up period, while increase in quality of fibrosis was observed after 2 months of application of Portland cement. A finding that goes in accordance with Tran., et al. [29]. This better quality of Dentine Bridge might be due to the silicon ions released from Biodentine. Silicon was
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reported to promote osteoblast proliferation and gene expression by involvement in metabolism, collagen synthesis, bone mineralization, and connective tissue cross-linking [35]. This study could possibly explain the increased thickness of the dentine bridge in the Biodentine group as compared to the Portland cement group at the end of the evaluation period (3 months).

Conclusions

1. Biodentine was superior to Portland cement in terms of less intense inflammatory cell response, better quality dentin bridge formation and faster collagen formation
2. Inflammatory cell response decreased gradually over time while hard tissue deposition increased in thickness by time in both materials.

Bibliography


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