



## EVALUATION OF PULP–DENTINE COMPLEX RESPONSE TO DIFFERENT DIRECT PULP–CAPPING AGENTS (AN EXPERIMENTAL STUDY)

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### ABSTRACT

**Objectives:** The purpose of this study was to evaluate the pulpal response of dogs' teeth after direct pulp capping using Biodentine (BD) and compared it with Mineral Trioxide Aggregate (MTA). **Materials and Methods:** Following the split-mouth design, forty intact teeth in two healthy Mongrel dogs were randomly assigned to two experimental groups; group I: BD and group II: MTA. Standardized Class V cavities were prepared on the vestibular surface of each tooth where the pulp exposure was performed with a dental explorer. The pulp-exposed teeth were immediately capped with one of the tested materials. The prepared cavities were then finally restored with glass-ionomer cement. After termination of the observation periods (one week and three months), the animals were euthanized. Then, teeth were extracted for histopathological evaluations. Data collected and statistically analyzed by using Fisher's exact test. The significance level was set at  $P \leq 0.05$ .

**Results:** Histopathological analysis showed complete dentin bridge formation and an absence of inflammatory pulp response. Statistical analysis showed no significant differences between the BD and MTA experimental groups during the observation periods. However, a significantly higher thickness of the dentin bridge was found in the group of teeth treated with BD at three months.

**Conclusion:** BD may be considered an interesting alternative to MTA. Both materials produced favorable pulpal responses that were similar in nature

**KEYWORDS:** Direct pulp capping; Dogs; Biodentine; MTA

### INTRODUCTION

Preservation of pulpal vitality has been a prime concern in operative dentistry<sup>(1)</sup>. Direct pulp capping (DPC) is defined as the treatment of an exposed vital pulp by sealing the pulpal wound with a biomaterial placed directly on mechanical or traumatic exposure to facilitate the formation of reparative dentin and maintenance of vital pulp<sup>(2)</sup>. If successful, this procedure precludes the need for more invasive, more extensive and more expensive treatment<sup>(3)</sup>.

Numerous materials have been used throughout the years for pulp capping<sup>(4)</sup>. Calcium hydroxide has been the gold standard for pulp capping<sup>(5)</sup>. However, it exhibits some obvious disadvantages including pulpal inflammation and necrosis; the presence of tunnel defects in the dentin bridge, which fails to provide a hermetic seal to the underlying pulp against infection because of microleakage; high solubility in oral fluids; lack of adhesion; and degradation over time<sup>(6,7)</sup>.

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An alternative gold standard, mineral trioxide aggregate (MTA), is available as a DPC material (8). It is suggested to be superior to calcium hydroxide due to its more uniform and thicker dentin bridge formation, less inflammatory response and less necrosis of pulpal tissue (9,10). However, in spite of its well-demonstrated biocompatibility and excellent sealing ability(11), MTA has some drawbacks related to its long setting time (2h 45min) and difficult handling properties (12).

In order to overcome some of these limitations other bioactive tricalcium silicate cements have been recently introduced in the dental market. One such material is Biodentine (BD) (13). BD is an improved calcium silicate material with good mechanical properties as well as excellent biocompatibility and bioactive behavior (14,15). It has physico-mechanical properties superior to those of MTA and similar to those of dentin; it also has easier handling and shorter setting time (12 min) than MTA (16). On the biological level, BD is capable of inducing the apposition of reparative dentin, by induction of cell differentiation (17).

Therefore, it seems worthwhile to evaluate the pulpal response in mechanically exposed cavities of Mongrel dogs' teeth after direct pulp capping using BD and compared it with MTA.

**MATERIALS AND METHODS**

Tested materials used in this study are listed in (Table 1).

**TABLE (1)** Tested materials used in this study

Materials	Composition	Manufacturers
Biodentine™	<u>Powder:</u> Tricalcium silicate, dicalcium silicate, calcium carbonate and zirconium dioxide. <u>Liquid:</u> Calcium chloride and hydrosoluble polymer.	Septodont, Saint-Maur-des-fossés Cedex, France.

Materials	Composition	Manufacturers
ProRoot® White MTA	<u>Powder:</u> Tricalcium silicate, dicalcium silicate, tricalcium aluminate, gypsum and bismuth oxide. <u>Liquid:</u> Distilled water.	Dentsply Tulsa Dental, Johnson City, TN, USA.

**Ethical committee approval:**

This study was carried out in accordance with the International Guiding Principles for Biomedical Research involving Animals based on the protocol recommended by the ISO standard 7405:2008. Ethical approval of this study was gained from the Medical Research Ethics Committee, Al-Azhar University, Cairo, Egypt.

**Study design:**

A total number of 40 dogs' teeth; the third incisor, canine, two-rooted premolars and first molar dogs' teeth in each quadrant of the dogs' jaws, were equally divided into two main groups according to the type of capping material used (n=20); group I: BD and group II: MTA. Each group was then further sub-divided into two subgroups (n=10) according to the observation periods; one week and three months.

**Animal model selection:**

The inclusion criteria included the following: Age range 12–24 months, weighing range 15–20 kg and intact permanent dentition. The exclusion criteria included the following: Newly born and diseased dogs.

**Sample size calculation:**

The power analysis used dentin bridge formation as the primary outcome based upon the results of a recent study (18). Using alpha (α) level of 0.05 (5%) and Beta (β) level of 0.20 (10%) i.e. power = 80%; the minimum estimated sample size was a total of 32 teeth (8 teeth per subgroup).

### General anesthesia and pulp exposure procedures:

General anesthesia was induced by administration of a combination of Ketamine HCl (Eimc, Egypt): 5mg/kg body weight and Xylazine HCl (ADWIA, Egypt): 1mg/kg body weight given intravenously. Level of anesthesia was maintained by incremental doses of 2.5% solution of Thiopental Sodium (EPICO, Egypt): 25mg/kg body weight given I.V.

After general anesthesia, the operating field was disinfected with antiseptic solution (2% Chlorhexidine Gluconate). The teeth were isolated with a rubber dam to avoid bacterial contamination from the oral environment. A modified metal band with a central window was used to standardize the prepared cavities to be 3 mm mesio-distally and 2 mm occluso-gingivally. The preparations were cut 0.5-1 mm above the free gingiva, parallel to cement-enamel junction<sup>(19)</sup>.

Aided with magnifying glasses (Univet, magnification 5×, Italy), Class V cavities were prepared on the vestibular surfaces of teeth by using a sterile #330 high speed bur (Meisinger, Germany) under copious amounts of air-water spray coolant. Pulpal floors were further deepened until the unexposed pulp was seen shining through the dentin as a pink spot. Pin-point pulp exposure at the center of the preparation was induced with a sterile dental explorer (Hu-Friedy, USA)<sup>(20)</sup>. Bleeding was controlled by the placement of a cotton pellet moistened with sterile normal saline to achieve hemostasis.

### Pulp capping materials application:

Capping materials were applied for each group according to manufacturer's instructions as follow: For BD group, only 5 drops of BD liquid were added to the capsule containing the BD powder. The plastic capsules were placed in an amalgamator (TPC, USA) for 30 sec. For MTA group, the MTA powder was mixed with the MTA liquid on the glass slab in a 3:1 P/L ratio by weight for one min to obtain wet sand consistency.

All the cavities were then subsequently restored with GC Fuji IX<sub>CP</sub> (GC Corporation, Tokyo, Japan) to provide the suitable conditions for pulpal repair.

### Histological evaluation:

Dogs were scarified either after one week and three months by injecting an overdose of Thiopental Sodium (EPICO, Egypt) through the cephalic vein. Then, the teeth were immediately removed surgically from jaws and the apical parts of its roots were exposed to allow proper fixation of pulp tissues in 10% neutral formalin solution (Gomhorya Company, Egypt) for one week and every 48 h the solution was changed.

After fixation period, the teeth were decalcified in 20% Formic acid/25% Sodium Citrate (Gomhorya Company, Egypt) for two months. Then, the specimens were cleaved in Xylene and embedded in paraffin wax. The paraffin-embedded specimens were then serially sectioned in facio-lingual plane to an average thickness of 5 μm using microtome (Leica Biosystems Inc. USA).

The sections were stained with Hematoxylin and Eosin (HE) for histopathological evaluation, examined by light microscope (Zeiss, Germany) and scored according to the criteria<sup>(21)</sup> presented in (Table 2).

**TABLE (2)** Criteria and scores for histopathological evaluation

<b>a. Dentin bridge formation:</b>	
1	= Complete dentin bridge formation.
2	= Partial dentin bridge formation.
3	= Initial dentin bridge formation.
4	= No dentin bridge formation.
<b>b. Dentin bridge thickness:</b>	
1	= >0.25 mm.
2	= 0.1–0.25 mm.
3	= <0.1 mm.
4	= Absent bridge.
<b>c. Inflammatory response:</b>	
1	= Absent or very few inflammatory cells.
2	= Mild (< 10 inflammatory cells).
3	= Moderate (10–25 inflammatory cells).
4	= Severe (> 25 inflammatory cells).

**Statistical analysis:**

Qualitative data was presented as frequencies and percentages. Fisher’s exact test was used for comparisons between groups, as well as to compare between follow-up times. Statistical analysis was performed with IBM SPSS (Statistical Packages for the Social Sciences, Inc., NY, USA) V20 for Windows. The significance level was set at  $P \leq 0.05$ .

**RESULTS**

The summary of histological results presented in (Tables 3,4 and 5). Histopathological results showed that both capping materials were well tolerated by the pulp tissue (Figure 1).

At one-week, there was no statistically significant difference between BD and MTA groups in terms of dentin bridge formation, dentin bridge thickness and inflammatory cell response. Limited areas of pulp tissue necrosis and degenerated odontoblastic layer were only seen adjacent to both capping materials (Figure 1-A and 1-B). No or mild inflammatory cell infiltration associated with relatively dilated blood vessels were observed next to the capping materials.

After three-months, there was no statistically significant difference between BD and MTA groups in terms of dentin bridge formation and inflammatory cell response. As regard to dentin bridge thickness, there was a statistically significant difference between the two groups. BD group showed higher prevalence of dentin bridge thickness  $>0.25$  mm. A reparative dentin bridges were observed directly at the injury site in all of the pulps capped with BD or MTA (Figure 1-C and 1-D). The layers of well-arranged odontoblast-like cells were seen under the complete calcified dentinal bridge. There was no evidence of inflammation, abscess, or necrosis. Dilated engorged blood vessels were rarely encountered. Pulp tissue proper showed dense fibrous tissue formation.

**TABLE (3)** Comparison between dentin bridge formation in the two groups and the changes within each group

Time	Dentin bridge formation	BD		MTA		P-value (Between groups)
		n	%	n	%	
1-week	Complete	0	0	0	0	1.000
	Partial	9	90	8	80	
	Initial	1	10	2	20	
	No dentin bridge	0	0	0	0	
3-months	Complete	10	100	10	100	NC**
	Partial	0	0	0	0	
	Initial	0	0	0	0	
	No dentin bridge	0	0	0	0	
<b>P-value (Within group)</b>		$<0.001^*$		$<0.001^*$		

\*: Significant at  $P \leq 0.05$ , NC\*\*: Not Computed because the variable is constant

**TABLE (4)** Comparison between dentin bridge thickness in the two groups and the changes within each group

Time	Dentin bridge thickness	BD		MTA		P-value (Between groups)
		n	%	n	%	
1-week	$>0.25$ mm	0	0	0	0	NC**
	0.1 – 0.25 mm	10	100	10	100	
	$<0.1$ mm	0	0	0	0	
	Absent bridge	0	0	0	0	
3-months	$>0.25$ mm	10	100	6	60	0.042*
	0.1 – 0.25 mm	0	0	4	40	
	$<0.1$ mm	0	0	0	0	
	Absent bridge	0	0	0	0	
<b>P-value (Within group)</b>		$<0.001^*$		0.011*		

\*: Significant at  $P \leq 0.05$ , NC\*\*: Not Computed because the variable is constant

**TABLE (5)** Comparison between pulpal inflammation in the two groups and the changes within each group

Time	Pulpal inflammation	BD		MTA		P-value (Between groups)
		n	%	n	%	
1-week	Absent	8	80	7	70	1.000
	Mild	2	20	3	30	
	Moderate	0	0	0	0	
	Severe	0	0	0	0	
3-months	Absent	10	100	10	100	NC**
	Mild	0	0	0	0	
	Moderate	0	0	0	0	
	Severe	0	0	0	0	
<b>P-value (Within group)</b>		0.474		0.211		

\*. Significant at  $P \leq 0.05$ , NC\*\*: Not Computed because the variable is constant

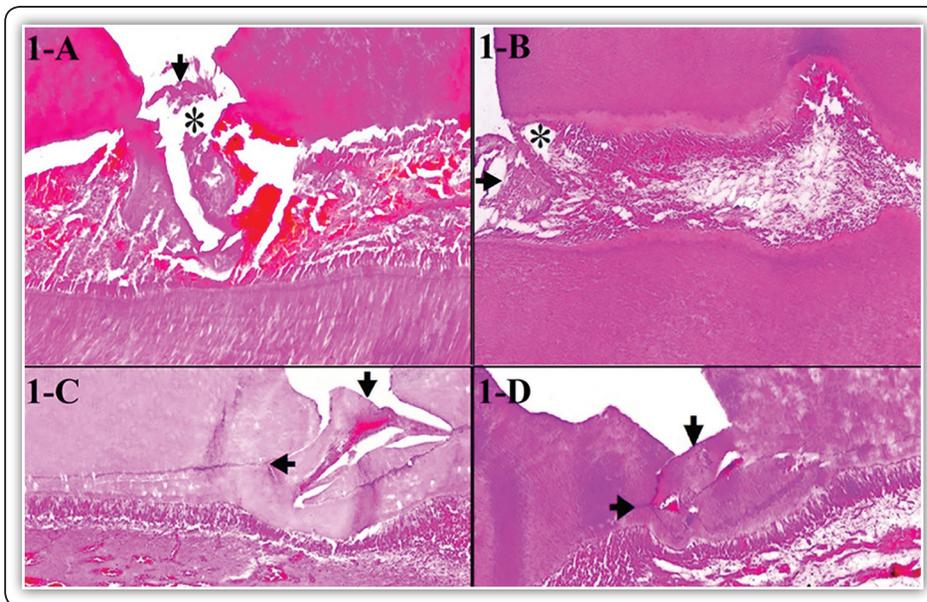


FIG (1) Photomicrograph of pulp response at follow-up periods at magnification (X40). After one-week, HE-stained sections showed limited pulp tissue necrosis (asterisks) below the initial mineralization (arrows) for BD (1A) and MTA (1B). After three-months, HE-stained sections showed complete dentin bridge formation (vertical arrows), separated from surrounding dentin with demarcation line (horizontal arrows), and normal subjacent pulpal histology (asterisks) for BD (1C) and MTA (1D).

## DISCUSSION

The true “gold standard” of pulp status is histological analysis. Unfortunately, the true state of pulp health or pathology cannot be determined by clinical signs, symptoms or radiologic appearance. However, numerous studies including histological analysis have demonstrated a chronically inflamed pulp, but the patients reported no symptoms, the

investigators discerned no signs, and no apical/radicular pathology was noted on radiographs<sup>(3)</sup>.

Pulp exposure was performed by mechanical perforation of the cavity floor with a sharp probe to avoid extensive pulp damage caused by exposure during cutting with the bur and also to create a pulp exposure of uniform size<sup>(22)</sup>.

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.

The histopathological results obtained with BD and MTA groups for one week showed limited pulp tissue necrosis and mild pulp inflammation followed by subjacent initial mineralization.

Similar to the results observed by other investigators <sup>(18,23,24)</sup> who found that BD and MTA has delayed the pulp inflammatory response and induced an early form of reparative dentin synthesis. This can be explained by the fact that BD and MTA have excellent sealing ability, which prevents microleakage and pulpal inflammation and thus provides a predictable barrier <sup>(18,23,25,26)</sup>.

On the other hand, Fonseca and others <sup>(27)</sup> found that the number of inflammatory cells was significantly higher in BD compared with MTA at 7 days, whilst 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 placing a polyethylene tube filled with BD into the dorsal subcutaneous of rats; not in direct contact with pulp tissue as in the current study.

After three months, the histopathological results of this study revealed that pulps that treated with BD and MTA were essentially free of inflammation led to the formation of a complete dentin bridge with a normal pulp tissue and intact odontoblastic-like cells under the pulp exposure site.

The findings of the current study are in accordance with findings of other investigators <sup>(23,24,28)</sup> who showed that the reparative structures induced by BD and MTA were homogenous and in continuity with primary dentin. Favorable therapeutic effects of BD were explained by a significant increase of transforming growth factor beta-1 (TGF- $\beta$ 1)

release from human dental pulp cells after DPC on entire human teeth cultures models as shown with MTA. TGF- $\beta$ 1 secretion stimulated odontoblasts, increased their secretory activity and mineralized foci appeared just beneath the material in the pulp wound area after two days <sup>(29)</sup>.

The present observations show a significantly higher thickness of dentinal bridges after pulp capping with BD at three months in comparison with MTA. These results are in agreement with those of other investigators <sup>(18,30,31)</sup> who have confirmed that the only difference in the pulpal response to MTA and BD was related to the dentin bridge thickness, which was greater with use of BD.

These results may be explained by on the basis of calcium chloride present in the liquid of BD provided by the manufacturer. Although both materials produce the same chemical compounds, it may be speculated that this reaction is more efficient in BD, which has a shorter setting time <sup>(32)</sup>. Moreover, BD permits a higher release of bioactive ions (calcium and hydroxyl ions) than MTA during the initial setting, reducing ion release over time and consequently producing more favorable conditions for pulp repair <sup>(33)</sup>.

The histopathological results obtained with BD group were overall similar to that observed with MTA group. These results are in wide agreement with those of other investigators who have confirmed that pulp capping with BD and MTA materials produced a very similar pulp response pattern <sup>(18,23,24,28,31,34)</sup>.

The similarity in the results may be due to the fact that the similarity of their chemical composition because both have tricalcium silicate. Hydration of tricalcium silicate in BD produces a calcium silicate hydrate gel and calcium hydroxide <sup>(18,28)</sup>. In MTA, calcium oxide reacts with water and tissue fluids and also produces calcium hydroxide <sup>(35)</sup>. Therefore, it is believed that the mechanism of action of BD is similar to that of MTA.

The present study was conducted under controlled experimental conditions to avoid the interference by confounding factors. The teeth had healthy pulp tissue means that differences encountered in the pulpal response can be attributed to the capping material used<sup>(36)</sup>. Therefore, Future research should explore how pulps challenged by caries might react to certain pulp agents over an extended treatment period.

## CONCLUSIONS

BD may be considered an interesting alternative to MTA. Both materials produced favorable pulpal responses that were similar in nature.

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