



# Efficacy of Mineral Trioxide Aggregate and Photobiomodulation on Pulp Capping of Dogs' Teeth

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## Abstract

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**AIM:** The present study assessed the effect of mineral trioxide aggregate (MTA) and photobiomodulation (PBM) on pulp capping of exposed pulp of dogs' teeth.

**METHODS:** Forty-eight teeth in three mongrel dogs were randomly divided into two major study groups; Group I where MTA was used as a pulp capping agent and Group II in which both MTA+PBM were used. The groups were equally subdivided according to the observation period following completion of pulp capping into Subgroup (A) 1 week, Subgroup (B) 2, and Subgroup (C) 16 weeks. The teeth were examined for histological inflammatory response as well as dentine bridge formation.

**RESULTS:** With regard to inflammatory response at 1 week significantly, less intense inflammation was observed in MTA+PBM (Group II) compared to the MTA (Group I) for the same time period with no significant difference for between Group I and Group II for other time intervals. As for dentin bridge formation, PBM+MTA groups showed statistically significant thicker dentine bridge formation at 16 weeks than MTA alone group for the same time period with no significant difference for between Group I and Group II for other time intervals.

**CONCLUSIONS:** Under the conditions of this study, PBM appeared to be a beneficial adjunct in dental pulp capping procedures in which MTA was the pulp capping material.

## Introduction

The dental pulp has crucial defensive, nutritive, formative, and sensory functions. Following pulp exposure, direct pulp capping procedures enable the pulp tissue to form a reactionary hard tissue barrier at the exposure site. Various pulp capping materials have been developed and used in dental pulp capping procedures with new materials continually being developed [1].

Ca(OH)<sub>2</sub> had been the chosen material for direct pulp capping due to its ability induce hard tissue bridge [2] as well as IRS antibacterial potential [3]. The response of the dental pulp when initially capped by Ca(OH)<sub>2</sub> until it forms hard tissue has been described in the literature [4], [5].

Mineral trioxide aggregate (MTA) was more recently introduced as an alternative dental pulp capping material which consists of a powder of tricalcium oxide, silicon oxide, and bismuth oxide. Other particles that are hydrophilic in nature as tricalcium silicate and tricalcium aluminate are also present [6], [7].

Photobiomodulation (PBM) has been reported to suppress inflammation and biostimulate tissues to improve tissue repair mechanisms [8]. In a recent systematic review conducted by Sleep *et al.* (2021), it was concluded that PBM had a positive effect on dental pulp regenerative ability [9]. Diode laser of low power and a wavelength ranging from 810 to 903 nm showed high stimulation of healing of dental pulp tissue [10], [11].

More than 1 study assessed the outcomes of using PBM in conjunction with direct pulp capping [12], [13] and its effect on regeneration potential of dental pulp complex [14]. PBM elevates the secretion of certain proteins that are of high value in the dentin bridge formation cycle in addition to another set of proteins that signal the initiation of dentinogenesis [15], [16], [17].

This study was conducted to determine the histologic effects of MTA with and without PBM on exposed pulps of dogs' teeth at observation periods 1 week, 8 weeks, and 16 weeks following direct pulp capping procedures.

## Materials and Methods

### Ethical approval

The National Research Centre (Giza, Egypt) provided ethical approval for this research with acceptance code 13/144.

### Animal model

Three male mongrel dogs with an estimated weight of 65 pounds and 18 months old were quarantined for 10 days before initiating the pulp capping procedures study. Direct pulp capping was done on 16 teeth per dog and a total of 48 teeth were capped.

In this study, all teeth chosen were healthy without dental decay or periodontal disease.

All dogs were observed in Kasr El Aini Animal Research Unit (Cairo, Egypt). The animals were monitored every day before any treatment started.

### Anesthesia

According to Dominguez *et al.* (2003) [18], general anesthesia was achieved by an intramuscular injection of 1–2.2 mg/kg of Rompun and 20 mg/kg of ketamine. Local anesthesia intraorally was achieved in lower arch by a nerve block using 1.8 ml of 2% lidocaine containing 1:100,000 adrenalin. In the upper arch infiltration, anesthesia of 1.8 ml of 2% lidocaine containing 1:100,000 adrenalin was given [18].

### Pulp capping

In each of the four quadrants of the oral cavity, two incisors as well as two premolars were treated. One of the incisors was treated with MTA alone and the other with MTA+PBM. Furthermore, one of the premolars was treated with MTA alone and the other with MTA+PBM.

Under copious water irrigation, class V cavity preparations were formed on facial teeth aspects, one mm coronal to the gingival margin using size #2 high-speed round diamond till pulpal shadow had occurred. Then, the pulpal exposure was created manually with a sterile sharp probe at the center of each prepared cavity. Saline was used to wash the exposure site until bleeding stopped [18].

In all teeth, MTA (Angelus, Londrina, Brazil) was prepared following the manufacturer's guidelines and used to cover the exposure. In all groups, the cavity was finally restored with glass ionomer (Fuji II LC, GC Industrial Co., Tokyo, Japan).

### Laser irradiation

A therapeutic laser apparatus "soft-laser-SL-202" manufactured by scientific-and-production allegation (Petrolaser, Stachek, 47, Saint-Petersburg, Russia) of a power of 50 mW and 870 nm wavelength was used as an infrared PBM laser source.

Infrared laser was used to irradiate half of the treated teeth on each dog. The laser irradiation was done in a direct manner immediately after final restoration with glass ionomer. This was followed by consecutive sessions of laser application at 48 h and finally at 96 h with 48 h gaps between irradiation sessions [19]. Only teeth on the right side of each arch were laser irradiated while their contralateral was not irradiated [19]. The full irradiation interval through the study was 120 s/tooth.

The laser beam was directed to the buccal and palatal/lingual surfaces of each tooth for 20 s per tooth surface.

Contact mode was the method of laser application [19] with a 2 mm spot diameter through a continuous wave.

### Study groups

Teeth were grouped as shown in Table 1:

**Table 1: Grouping of all samples used in the present study**

Group period	Group I (n = 24) (MTA)	Group II (n = 24) (MTA+PBM)
1 week (A)	IA (n = 8)	IIA (n = 8)
8 week (B)	IB (n = 8)	IIB (n = 8)
16 week (C)	IC (n = 8)	IIC (n = 8)

### Animal sacrifice and tissue processing

With accordance to Dominguez *et al.* (2003) [18], the dogs were sacrificed after completing their previously decided dates at 1 week, 8 weeks, and 16 weeks. Twenty ml of 20% potassium chloride solution were injected into the external jugular vein. When the dogs' active pulse stopped, the carotid artery was perfused with 1 L of saline solution followed by 1 L of 10% buffered formalin. Sections of the mandible and maxilla were isolated. All sections were put in solution 10% formalin to achieve tissue fixation [18].

Specimens were placed in 0.5 M EDTA at 4°C for 4–8 months to achieve demineralization. The block specimens were immersed in paraffin wax and sectioned serially at 5.0–7.0 using a microtome. Fifteen randomly chosen sections were used from each specimen. Three of those sections were stained with hematoxylin and eosin (H and E) [18].

### Histological assessment

Slides were examined by an oral pathology specialist. Asgary *et al.*'s (2008) [20] scoring system was modified and used to assign a grade to specimens as outlined in Table 2.

**Table 2: Scoring method used to assess the inflammatory response and dentine bridge thickness in this study modified from Asgary et al.' (2008) [20]**

Grading				
Feature	Category	I	II	III
Pulp inflammation	Type	Chronic and acute inflammation	Chronic inflammation	No inflammation
	Intensity	Severe >60 inflammatory cells	Mild (0–30) to moderate (0–60) inflammatory cells	No inflammation
	Extension	Entire coronal pulp	Localized under exposure area	No inflammation
Hard tissue formation	Continuity formation	No hard tissue	Incomplete dentine bridge	Complete dentine bridge
	Thickness	<100 µm	Between 100 µm and 250 µm	More than 250 µm

**Statistical analysis**

For comparing categorical descriptive data of the scoring system used in the study, Chi-square test was performed. For quantitative analysis of inflammatory intensity and dentine bridge thickness numerical results, Mann–Whitney U-test and Wilcoxon signed-rank test were used. Statistically significant results were set at  $p < 0.05$ .

**Results**

The outcomes of histological evaluation are explained according to the inflammatory reaction and hard tissue formation as follows:

**Inflammatory response Figures 1-3, Tables 3-5**

With regard to *inflammatory type*, there was no statistically significant difference between Group I (MTA Only) and Group II (MTA+PBM) subgroups (Table 3).

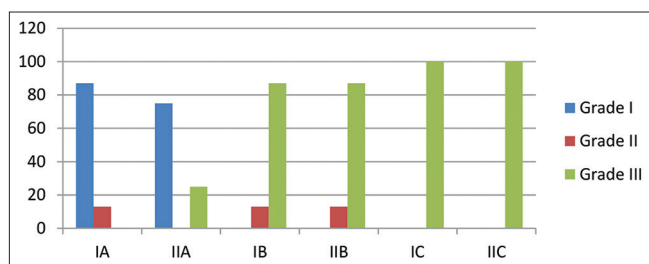


Figure 1: Bar chart illustrating the frequency of inflammatory type in all groups at different observation times

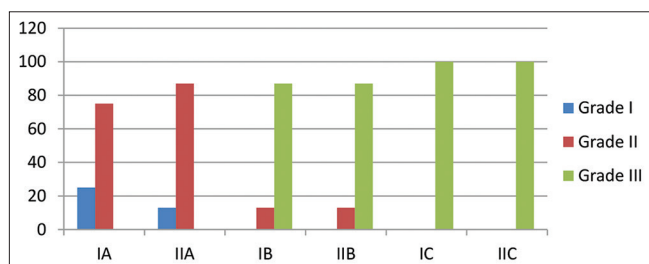


Figure 2: Bar chart illustrating the frequency of inflammatory intensity in all groups at different observation times

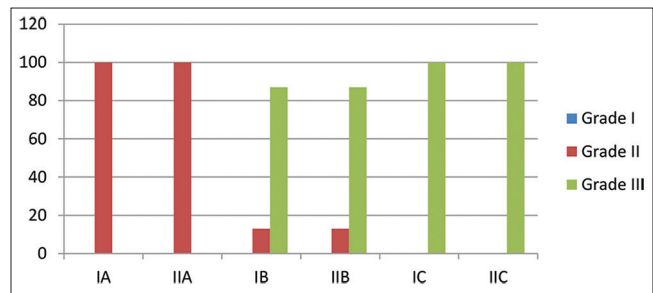


Figure 3: Bar chart illustrating the frequency of inflammatory extension in all groups at different observation times

For *inflammatory intensity* grading analysis according to Asgary et al. was performed, there was no statistically significant difference between Group I (MTA) and Group II (MTA+PBM) subgroups (Table 3).

**Table 3: Inflammatory results of all groups according to Asgary et al. scoring system (modified)**

Inflammation	Grading	IA (%)	IIA (%)	IB (%)	IIB (%)	IC (%)	IIC (%)
Type	I	7 (87)	6 (75)	0 (0)	0 (0)	0 (0)	0 (0)
	II	1 (13)	0 (0)	1 (13)	1 (13)	0 (0)	0 (0)
	III	0 (0)	2 (25)	7 (87)	7 (87)	8 (100)	8 (100)
	p-value	0.214		1.000		1.000	
Intensity	I	2 (25)	0 (13)	0 (0)	0 (0)	0 (0)	0 (0)
	II	6 (75)	6 (75)	1 (13)	1 (13)	0 (0)	0 (0)
	III	0 (0)	2 (25)	7 (87)	7 (87)	8 (100)	8 (100)
	p-value	0.135		1.000		1.000	
Extension	I	0 (0)	0 (13)	0 (0)	0 (0)	0 (0)	0 (0)
	II	8 (100)	6 (75)	1 (13)	1 (13)	0 (0)	0 (0)
	III	0 (0)	2 (25)	7 (87)	7 (87)	8 (100)	8 (100)
	p-value	0.318		1.000		1.000	

However, when quantitative analysis of inflammatory intensity was performed – Table 5, it showed that at 1 week, Group II (MTA+PBM) showed a statistically significant less inflammatory intensity than Group I (MTA) ( $p = 0.001$ ). Quantitatively, there was no statistically significant difference between Group I (MTA) and Group II (MTA+PBM) at 8 and 16 weeks.

**Table 4: Dentine bridge results of all groups according to Asgary et al. scoring system (modified)**

Dentine bridge	Grading	IA (%)	IIA (%)	IB (%)	IIB (%)	IC (%)	IIC (%)
Continuity	I	8 (100)	7 (87)	0 (0)	0 (0)	0 (0)	0 (0)
	II	0 (0)	1 (13)	5 (63)	5 (63)	0 (0)	0 (0)
	III	0 (0)	0 (0)	3 (37)	3 (37)	8 (100)	8 (100)
	p-value	0.586		1.000		1.000	
Thickness	I	8 (100)	8 (100)	0 (0)	0 (0)	0 (0)	0 (0)
	II	0 (0)	0 (0)	5 (63)	2 (25)	2 (25)	0 (0)
	III	0 (0)	0 (0)	3 (37)	6 (75)	6 (75)	8 (100)
	p-value	1.000		0.318		0.318	

Coming to the inflammatory extension, all specimens with signs of inflammation extension showed a localized inflammatory reaction (Grade II) with no statistically significant difference between Group I (MTA) and Group II (MTA+PBM) (Table 3).

**Table 5: Demonstrating quantitative statistical analysis of mean inflammatory cell intensity**

Groups	Subgroup A	Subgroup B	Subgroup C
Group I			
Range	67–32	19–0	0
Mean ± SD	48 ± 12.329	2.38 ± 6.718	0 ± 0
Group II			
Range	34–0	4–0	0
Mean ± SD	17.13 ± 11.862	0.5 ± 1.414	0 ± 0
p-value	0.001*	0.927	1.000

$p \leq 0.05$  is significant.

**Dentine bridge formation Figures 4 and 5, Tables 4 and 6**

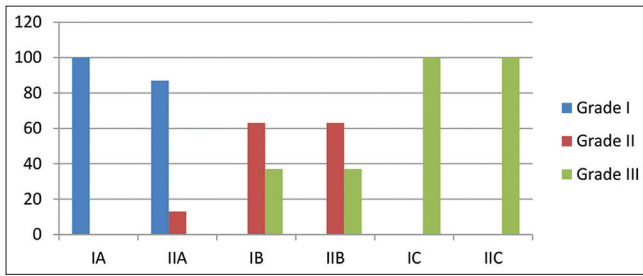


Figure 4: Bar chart illustrating dentine bridge continuity in all groups at different observation times

Regarding *Dentine Bridge Continuity* and *Dentine Bridge thickness* at all three observation times, there was no statistically significant difference between Group I and Group II, as shown in Table 4 and Figures 6-8.

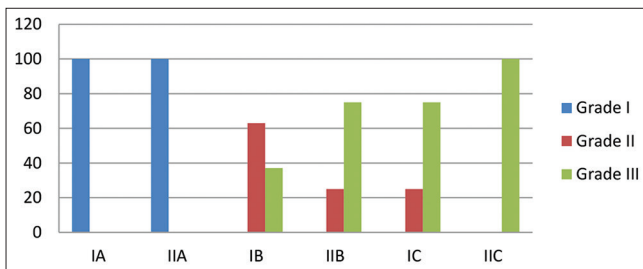


Figure 5: Bar chart illustrating dentine bridge thickness of all groups at different observation times

However, when quantitative analysis of *dentine bridge thickness* represented in Table 6 was performed, at 16 weeks, Group II (MTA+PBM) showed a statistically significant thicker dentine bridge formation than Group I (MTA) ( $p = 0.001$ ). There was no statistically significant difference between other Group I and Group II at other observation periods (Figures 6-8).

**Table 6: Demonstrating quantitative statistical analysis of mean dentine bridge thickness**

Groups	Subgroup A	Subgroup B	Subgroup C
Group I			
Range	0.000–0.000	403.5–153.8	488.75–124.000
Mean ± SD	0.000 ± 0.000	284.250 ± 101.552	345.625 ± 146.265
Group II			
Range	0.000–0.000	437.8–182.3	731.75–497.5
Mean ± SD	0.000 ± 0.000	331.656 ± 94.976	623.375 ± 81.501
p-value	1.000	0.462	0.001*

**Discussion**

One of the main goals of direct pulp capping procedures is to allow odontoblast-like cells to form a dentine bridge between the capping material and the pulp tissue. Many direct pulp capping materials and techniques have been studied in literature to try to assess advantages and disadvantage of each [21], [22].

The success of direct pulp capping procedures depends on many factors [23], [24] including infection

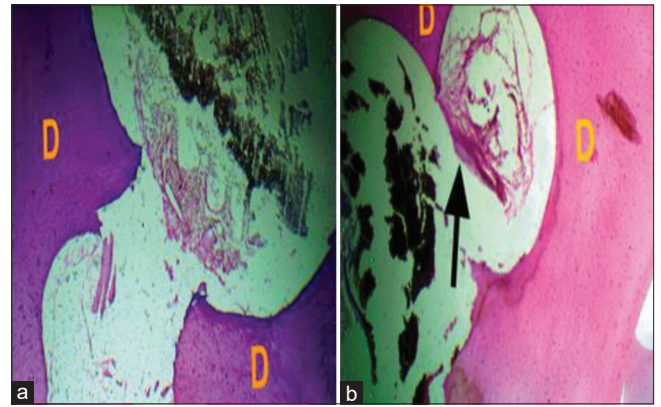


Figure 6: ( $\times 40$ ) Photomicrograph of 1 week/MTA group (a) with no dentine bridge formation. (b) (MTA+PBM) group shows beginning of formation of hard tissue deposits (arrow) but no dentine bridge can still be seen

control, absence of inflammation, and the pulp capping material itself that is used [25], [26].

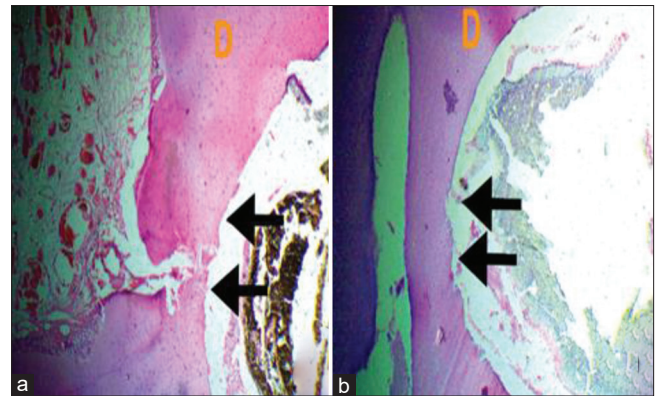


Figure 7: ( $\times 40$ ) Photomicrograph of 8 weeks/MTA group (a) with almost complete dentine bridge formation (arrow). (b) (MTA+PBM) group shows a complete dentine bridge formation (arrow)

Although many investigators chose only one evaluation time usually 8 weeks [27] [28], [29] and up to 150 days [18], [20] before the animal models were sacrificed, the authors of the present study chose three different time periods of 1 week, 8 weeks, and 16 weeks. This allowed the examination of inflammatory reactions at an early stage of reparative process (1 week) and a follow-up the reparative process to 8 and 16 weeks.

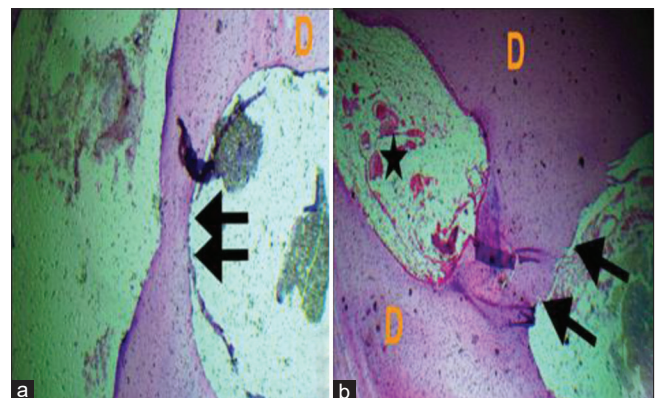


Figure 8: ( $\times 40$ ) Photomicrograph of 16 weeks/MTA group (a) with a complete dentine bridge formation (arrow). (b) MTA+PBM group shows a thicker complete dentine bridge formation (arrow)

The effect of PBM with pulp capping procedures has been studied by 2016 Bidar *et al.* [30] who found that despite laser irradiation being effective before pulp capping, the material type that was used in direct pulp capping was of more importance in the success of vital pulp therapy. The authors of the mentioned study stated that one of the limitations in their study was that the laser application was only performed 1 time before placing the pulp capping material on the exposure site [30]. This limitation was avoided in our study in which laser was applied in consecutive sessions after the placement of the capping material immediately and 2 and 4 days.

Previous to our research and to the best of our knowledge, only Toomarian *et al.* [19] investigated the stimulatory laser irradiation on dental hard tissue in consecutive sessions. They directed the laser toward the middle root area of the lower molar teeth of one side of the oral cavity at consecutive intervals of 48 h to examine its effect on root development of rat molars. They concluded that 808 nm PBM accelerates the development of the molar roots of rats with lower inflammatory reactions.

De Santana *et al.* [14] found that 780 nm PBM increased density of primary odontoblasts when applied to the roots of extruded rat incisors when applied through the palatal mucosa every 48 h. Neiburger [31] showed a faster healing of gingival ulceration following intermittent PBM.

Glass ionomer above the capping material which is proved to have a good sealing ability and shear bond strength [32] which helps to protect the pulp capping material until healing occurs.

The significantly less intense inflammatory reaction in Group II (MTA+PBM) than Group I (MTA) can be supported by a research conducted by Bidar *et al.* [30] who evaluated the effect of PBM on pulpal response of dogs' teeth after pulp capping. They found that the lowest degree of pulpal inflammation was present in PBM when used in conjunction with pulp capping material.

The continuity of a dentine bridge could be considered as a sign of success of direct pulp capping procedures [33]. In the present study, none of the subgroups showed signs of necrosis. Previously, in Arafa *et al.*'s [34] study, none of the teeth capped showed any signs of clinical failure.

Thicker and more uniform and thicker dentin bridge formation, less inflammatory response, and less necrosis of pulpal tissues were observed in the previous studies [25], [35]. This may be explained by a previous study [36] which showed that MTA liberated more growth factors. Furthermore, MTA induced more cell proliferation and a higher alkaline phosphatase (ALP) activity [37]. The release of growth factors and pulp cell differentiation are important conditions for the formation of dental hard tissue [38].

At 1 week, there was an earlier hard tissue formation, and at 8 weeks, there was an earlier thickening of dentine bridge in the lased groups. Nawam *et al.* [39] who investigated the effects of diode PBM on dentinogenesis and angiogenesis of the pulp tissue observed that both lower [1 J/cm<sup>2</sup>] and higher [3 J/cm<sup>2</sup>] energy density showed a statistically significant upregulation of odontogenic genes.

Moreover, Paschalidou *et al.* [40] found that low-level laser irradiation at different energy fluencies increased viability, migration, odontogenic differentiation, and mineralization of stem cells from human exfoliated deciduous teeth.

In an attempt to understand more the effects of PBM on dentinogenesis, Ohbayashi *et al.* [41] found that low-level laser at 803 nm near-infrared increased ALP activity and specific molecular expression of dental pulp cells. ALP is a known marker for early differentiation of odontoblasts. This enzyme plays a crucial function in calcified tissue formation, most likely by regulating phosphate transport.

Like the histological results obtained within our present study, calcified nodules were formed after PBM was performed at wavelengths of 830 nm and a power output of 600 mW at maximum [41].

In another study by Ferreira *et al.* [15], it was found that diode laser with a 670 nm wavelength and energy density of 4 J/cm<sup>2</sup> increased the expression of proteins such as collagen, fibronectin, and tenascin. These proteins are able to perform a crucial function in calcified tissue formation as well as the differentiation of fibroblasts and odontoblasts during dentinogenesis [42], [43].

Collagen which forms approximately 90% of the dentin organic composition is made by the odontoblasts before being secreted into pre-dentin as a network of collagen fibers which later act as a scaffold for mineral crystals [16]. Fibronectin and tenascin may also play a role in dentin mineralization and in the ability of fibroblast differentiation to form mineralized tissue [15], [17].

The effects of 870 nm PBM on dentin barrier accelerated formation could be due to any of the mentioned mechanisms or a combination of any of them.

## Conclusions

Under the conditions of this study, PBM is a beneficial adjunct in dental pulp capping procedures in which MTA is used as a pulp capping material.

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