

# Histological evaluation of the synergistic effect of chitosan and mineral trioxide aggregate on mechanically exposed dental pulp following pulp capping in dogs' teeth

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## Abstract

**Introduction:** This work studied the synergistic effect of chitosan and mineral trioxide aggregate (MTA) on mechanically exposed dental pulp following pulp capping in dogs' teeth.

**Materials and Methods:** Class V cavities were prepared in 60 teeth of 3 mongrel dogs. These cavities were prepared approximately 1 mm coronal to the gingival margin by using a round carbide bur #2 with water cooling. These teeth were divided according to the pulp capping material into 2 groups (30 teeth each); Group I: MTA and Group II: a combination of MTA and chitosan compound. The cavities were restored by self-curing glass ionomer cement, followed by varnish application to provide the suitable conditions for pulpal repair. Both groups were further subdivided according to the evaluation period into 3 subgroups (10 teeth each); subgroup A: 7 days, subgroup B: 21 days, and subgroup C: 60 days. Histological evaluation of dentin bridge formation was performed after pulp capping in all subgroups. Data were statistically analyzed by ANOVA, Tukey's *post hoc*, Kruskal–Wallis, and Mann–Whitney U-tests. The significance level was set at  $P \leq 0.05$ .

**Results:** No statistically significant difference was found between both groups at all evaluation times ( $P > 0.05$ ). The combination of MTA and chitosan did not improve the quality of dentin bridge produced by the MTA alone.

**Conclusion:** Mixing of chitosan and MTA as a direct pulp capping material has no synergistic odontogenic effect in dog's teeth.

**Keywords:** Capping material, dental pulp, dentin bridge, dog's teeth, odontogenesis

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**Submission:** 21-11-20 **Revision:** 07-01-21 **Acceptance:** 21-01-21 **Web Publication:** 08-01-22

## INTRODUCTION

The dentin-pulp complex supports the defense mechanisms and the reparative reactions occurring as a response to

different factors such as chemical agents, trauma, various infections, or exposure. Direct pulp capping is recommended in such cases to keep pulp health and function.<sup>[1]</sup>

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**How to cite this article:** Emara RA, Abu-Seida AM, El Ashry SH. Histological evaluation of the synergistic effect of chitosan and mineral trioxide aggregate on mechanically exposed dental pulp following pulp capping in dogs' teeth. Saudi Endod J 2022;12:25-30.

### Access this article online

Quick Response Code:



Website:

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DOI:

10.4103/sej.sej\_294\_20

Many pulp capping agents have been traditionally applied to enhance healing in the exposed dental pulp. One of the most commonly used pulp capping agents is mineral trioxide aggregate (MTA). First, MTA was discovered in a gray color (GMTA) by Mahmoud Torabinejad in 1996. It is composed mainly of calcium and bismuth oxides and silica. Then, white MTA (WMTA) was introduced to the market to overcome the discoloration produced by GMTA.<sup>[2,3]</sup> The MTA produces excellent hard-tissue formation and less pulp inflammation. Unfortunately, poor handling properties, high cost, and delayed setting time are the main disadvantages of MTA.<sup>[2-4]</sup> Therefore, numerous studies have been conducted to produce an ideal pulp capping agent or at least improve the traditional ones.<sup>[5-13]</sup>

Although the MTA produces normal pulp, continuous odontoblastic layer, and complete dentin bridge formation after 2–3 months of capping in dogs' teeth,<sup>[13-15]</sup> several *in vitro* investigations have been used additives in order to improve the quality of the dentin bridge formation after pulp capping using MTA, thus improving its clinical outcomes.<sup>[10-13]</sup> However, these studies have not investigated completely the physicochemical characteristics of MTA. Therefore, the clinical application of the MTA is still unclear to some extent and the addition of some materials to the MTA may adversely affect its biocompatibility and practical uses. Calcium chloride, phosphate-containing fluid, and chlorhexidine were added to the MTA for improving its clinical outcome.<sup>[16-19]</sup>

Another technique for improving the clinical outcome of MTA as a pulp capping material is using osteogenic supplements to MTA. Most of these supplements produce various degrees of success such as dexamethasone, Vitamin D, chitosan, dentin adhesives, tri-calcium phosphates, calcitonin, enamel matrix derivatives, or simvastatin.<sup>[5,16-20]</sup>

Chitosan is a linear polysaccharide. It is produced by the deacetylation of chitin. Chitin presents in crustaceans, mainly crabs and shrimp. In dentistry, it could be used for pulp capping because it has antibacterial and anti-inflammatory actions, enhances tissue healing and bone regeneration, and chemically associates with inorganic salts such as calcium phosphate and calcium silicate.<sup>[21]</sup> The hypothesis of this study was that the addition of chitosan to MTA may improve the efficacy of the MTA as pulp capping material.

Therefore, this study investigated the synergistic odontogenic effect of chitosan and mineral trioxide aggregate on mechanically exposed dental pulp through

histological assessment of the produced dentin bridge following pulp capping in dog's teeth.

## MATERIALS AND METHODS

### Animal model

Ethical approval was obtained from the Research Ethics Committee at Faculty of Dentistry, Ain Shams University, Egypt (No: 16-06-2013-Endo). During this study, all institutional and international guidelines were followed up according to the Guide for the Care and Use of Laboratory Animals 8<sup>th</sup> edition.

Three healthy mongrel dogs were used in this study. The animals had intact dentitions and their age and weight were 2–3 years and 15–20 kg, respectively.

Before the beginning of the experimental procedures, each dog was housed in a separate kennel and observed for 2 weeks before the operative procedures to exclude any diseased dog.

### Classification of samples

In each dog, twenty teeth have been used including; three premolars (2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup>), a canine, and an incisor in each quadrant. In accordance with the capped material used, these teeth ( $n = 60$ ) were randomly divided into group I that served as a control group and had 30 teeth capped with MTA and Group II that served as an experimental group and had 30 teeth capped with a combination of MTA and chitosan compound. Both groups were represented in each dog. According to the evaluation period, both groups were further subdivided into 3 subgroups (10 teeth each) including; subgroup A: 7 days, subgroup B: 21 days, and subgroup C: 60 days. The sample size was estimated according to 80% power analysis at 95% confidence interval based on the pilot study using G power version 3.1.9.7. (Windows XP, USA).

### Operative procedure

Each dog was premedicated with Atropine sulfate (Atropine sulfate<sup>®</sup>, ADWIA CO., Egypt), 0.1 mg/kg given subcutaneously then Xylazine HCl (Xylaject<sup>®</sup>, ADWIA, CO., Egypt), 1 mg/kg given intravenously. Induction of general anesthesia was achieved by Ketamine HCl (Keiran<sup>®</sup>, EIMIC, CO., Egypt), 5 mg/kg given intravenously, then maintained by thiopental sodium (Thiopental sodium<sup>®</sup>, EIPICO, Egypt), 25 mg/kg 2.5% solution given intravenously as dose to effect.

The teeth surfaces have been disinfected by povidone-iodine solution and isolated by sterile cotton rolls. Pulp exposure was performed in 5 teeth in each quadrant. Standard class

V cavities were induced in the selected teeth approximately 1 mm coronal to the gingival margin by a round carbide bur #2 with water cooling to avoid heat generation. A modified metal band with a central window was used to standardize the prepared cavities to be 3 mm  $\pm$  0.5 mesiodistally and 2 mm  $\pm$  0.5 occlusogingivally and 3 mm  $\pm$  1 mm in depth.<sup>[15]</sup>

The pulpal floor was deepened in each cavity until the appearance of pulpal shadow. The pulp was then exposed mechanically by a sharp sterile probe. Hemostasis was performed by a cotton piece soaked with 3% sodium hypochlorite solution. Pulp capping of the exposed teeth was done according to each group. The pulp capping materials were mixed as follows:

In Group I (control group), the teeth were capped by the MTA only (Dentsply Tulsa Dental, Oklahoma, USA). The powder and liquid were added together according to the manufacturer's guidelines. This mix was then transferred to the exposed pulpal floor by an amalgam carrier and slightly packed by a suitable size condenser.

In Group II (experimental group), the teeth were capped by a combination of MTA and chitosan AI (Debeiky Pharmaceutical, Cairo, Egypt). The MTA was prepared with a modified form of its original liquid containing 10% wt chitosan.<sup>[19]</sup> The MTA powder (1 g) and the chitosan solution were mixed in 3:1 powder to liquid ratio. Once all powder particles were well incorporated into the chitosan solution using a sterile spatula, the mix was then transferred to the exposed pulpal floor by an amalgam carrier and slightly packed by a suitable size condenser.

After the end of the pulp capping procedures, all the cavities were restored by self-curing glass ionomer cement (Promedica, Germany), followed by varnish application to provide the suitable conditions for pulpal repair. Only one operator performed the experimental work in all dogs. All dogs received Carprofen tablets (Rimadyl®, Zoetis, USA), 4 mg/kg given once orally (By wrapping the tablet into a small piece of meat) as a pain killer during the experimental periods.

### Histopathological evaluation

According to the subgroup, one dog was sacrificed by intravenous injection of overdose of Thiopental sodium (Thiopental sodium®, EIPICO, Egypt) after 7 days, 21 days, and 60 days of pulp capping. Then, each tooth was extracted with its surrounding bone.

All sacrificed animals were safely discarded by burning them through the disposal unit of dead animals at the Faculty of Veterinary Medicine, Cairo University.

All teeth were placed in 10% neutral buffered formalin for 72 h. After fixation in formalin, the specimens were decalcified in formic acid 5% solution that was renewed periodically during the decalcification period (100 days). Perforations of the specimens were carried out by a needle for allowing penetration of the formic acid.

The decalcified specimens were prepared as usual for histopathology. The samples were serially sectioned through the capping area and pulp tissue (5 microns thickness). These sections were stained with H and E stain to evaluate the dentin bridge formation. The dentin bridge was graded according to a previous scoring system.<sup>[7]</sup> In this system, the scores 0, 1, and 2 represent no, partial, and complete dentin bridge formation, respectively.

The thickness of the dentin bridge was also measured by using Leica quein image analysis system (Leica Microsystems, Wetzlar, Germany). Photomicrographs for the stained sections were captured using a digital camera connected with a light microscope (Leica Microsystems, Wetzlar, Germany) for analysis and fixed at a magnification of ( $\times 20$ ) during capturing. These photomicrographs were analyzed by the computer through the line measurement button. The average dentin bridge thickness was measured by drawing lines perpendicular to the bridge using the Leica software analysis.

### Statistical analysis

Data were presented as mean, standard deviation, and frequency. Exploration of data for normality was performed using Kolmogorov–Smirnov and Shapiro–Wilk tests. Dentin bridge thickness (mm) showed a parametric distribution, so one-way ANOVA was applied for evaluation of the effect of both tested materials, followed by Tukey's *post hoc* test for pairwise comparison when ANOVA was significant. The significance level was set at  $P \leq 0.05$ . Statistical analysis was carried out with IBM SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 23 for Windows.

## RESULTS

### Dentin bridge score

Results of dentin bridge for both groups in all subgroups are listed in Table 1.

#### After 7 days

The dentin bridge score for all samples of both groups was 0.

*After 21 days*

Only 2 samples in Group I (MTA) exhibited a partial dentin bridge formation (score 1), while 2 samples of Group II (MTA + Chitosan) had a complete dentin bridge formation (score 2).

*After 60 days*

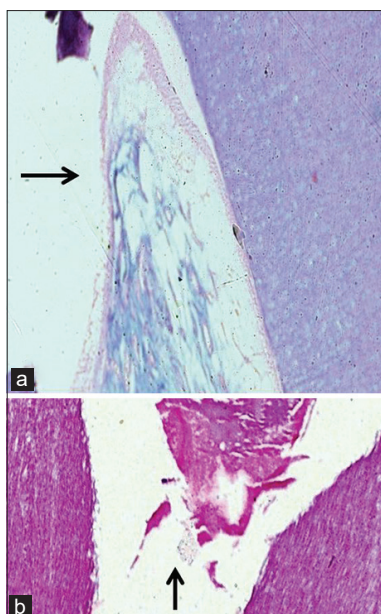
Only 2 samples of Group I (MTA) had a complete dentin bridge (score 2), while 4 samples of Group II (MTA + Chitosan) exhibited a partial dentin bridge formation (score 1).

No statistically significant difference was noticed between both groups at the different evaluation periods ( $P < 0.05$ ). Furthermore, there was no statistically significant difference between all subgroups within each group ( $P < 0.05$ ) as shown in Table 1.

**Dentin bridge thickness**

The results for both groups at different observation times are collected in Table 2. Representative samples for the newly formed hard tissue in the samples of all subgroups of both groups are shown in [Figures 1-3].

No statistically significant differences were observed between both groups at all evaluation periods ( $P < 0.05$ ). No statistically significant differences were also recorded between subgroups B (21 days) and subgroup C (60 days) in each group ( $P < 0.05$ ).



**Figure 1:** (a) A photomicrograph of the mineral trioxide aggregate group after 1 week (H and E,  $\times 20$ ) showing the absence of dentine bridge formation at the exposure site (arrow). (b) A photomicrograph of the mineral trioxide aggregate and chitosan group after 1 week (H and E,  $\times 10$ ) showing no dentin bridge formation at the exposure site

**DISCUSSION**

The goal of pulp capping, as a treatment of the pulp exposure, is to enhance the dentinogenesis. There are several factors influencing the healing after pulp exposure such as pulp vitality, bacterial infection, size of injury, and effect of therapy.<sup>[1,2]</sup> This work investigated the influence of the addition of chitosan to the MTA on the dentin bridge formation following pulp capping of the mechanically exposed pulps in dogs' teeth. The hypothesis of this study was rejected because the combination of MTA and chitosan did not improve the quality of dentin bridge produced by the MTA alone.

In the present study, the MTA was used in both groups either alone or with chitosan because the MTA is the gold standard for pulp capping due to its high sealing ability, antibacterial action, and biocompatibility.<sup>[2-4,22]</sup>

**Table 1: Distribution of the dentin bridge score of the mineral trioxide aggregate and mixture of the mineral trioxide aggregate and chitosan within each subgroup and at different subgroups within each tested material**

Subgroups	Scores	Control group, n (%)	Experimental group, n (%)	P
		Group I (MTA)	Group II (MTA + Chitosan)	
A (7 days)	0	10 (100.0)	10 (100.0)	1.00 (NS)
	1	0 (0.0)	0 (0.0)	
	2	0 (0.0)	0 (0.0)	
B (21 days)	0	8 (80.0)	8 (80.0)	0.998 (NS)
	1	2 (20.0)	0 (0.0)	
	2	0 (0.0)	2 (20.0)	
C (60 days)	0	8 (80.0)	6 (60.0)	0.926 (NS)
	1	0 (0.0)	4 (40.0)	
	2	2 (20.0)	0 (0.0)	
P		0.581 (NS)	0.360 (NS)	

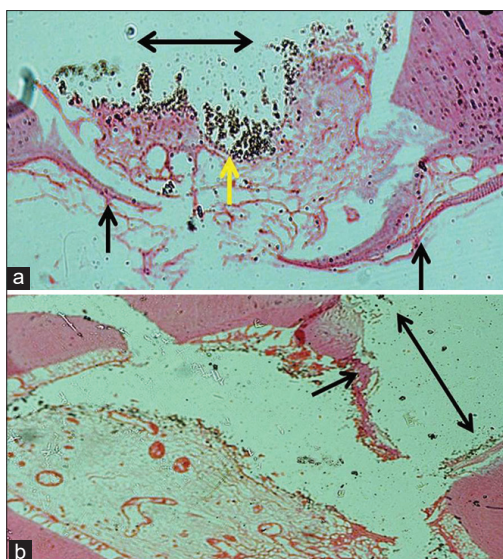
NS: Nonsignificant, MTA: Mineral trioxide aggregate

**Table 2: Mean, standard deviation, maximum, and minimum of dentin bridge thickness ( $\mu\text{m}$ ) for the mineral trioxide aggregate and mixture of the mineral trioxide aggregate and chitosan within each subgroup and at different subgroups within each tested material**

Subgroups	Control group	Experimental group	P
	Group I (MTA)	Group II (MTA + Chitosan)	
B (21 days)	Mean	6.15	0.157 (NS)
	SD	8.42	
	Maximum	15.87	
	Minimum	0	
C (60 days)	Mean	19.84	0.655 (NS)
	SD	44.3	
	Maximum	99.18	
	Minimum	0	
P	0.951 (NS)	0.517 (NS)	

NS: Nonsignificant, MTA: Mineral trioxide aggregate, SD: Standard deviation





**Figure 2:** (a) A photomicrograph of the mineral trioxide aggregate group after 21 days (H and E,  $\times 20$ ) showing interrupted thin hard-tissue formation of 15.4  $\mu\text{m}$  in thickness (black arrows), exposure site (double arrow), and the pulp capping material (yellow arrow). (b) A photomicrograph of the mineral trioxide aggregate + chitosan group after 21 days (H and E,  $\times 20$ ) showing the exposure site (double arrow), and new partially formed dentine bridge of 7.3  $\mu\text{m}$  in thickness (arrow)

This study was conducted *in vivo* using mechanically exposed vital pulps of dog's teeth as mentioned before.<sup>[12-15]</sup> The dog model was selected for this experiment due to its similar dental repair compared with that of the humans over a short period.<sup>[11-15,23]</sup>

The current study depended on the histological assessment in terms of the quality of dentin bridge formation after pulp capping. Histologic investigation is the traditional method used to evaluate the hard-tissue formation following pulp capping as mentioned by several earlier workers.<sup>[7,11-16]</sup>

In this study, the MTA induced a reparative dentinogenic process with partial to nearly complete dentin bridge according to the evaluation period. The calcified dental bridge was not seen in the samples of subgroup A (7 days) in both groups due to the short time for dentinogenesis while it was observed in the samples of subgroups B (21 days) and C (60 days) in both groups. A common finding in most of the samples was the highly organized intact odontoblastic layer. Similarly in humans, the MTA was used successfully for pulp capping in several studies.<sup>[24,25]</sup> Thus, the primary process of reparative dentinogenesis after MTA capping may involve the natural pulpal wound-healing mechanism.<sup>[26]</sup> Furthermore, Ford *et al.*<sup>[27]</sup> recorded dentin bridge formation with no inflammation in nearly all dental pulps evaluated after capping with the MTA.



**Figure 3:** (a) A photomicrograph of the mineral trioxide aggregate group after 60 days (H and E,  $\times 20$ ) showing the newly formed dentinal bridge of 22.9  $\mu\text{m}$  in thickness (black arrow), the exposure site (double arrows), and well-organized pulp tissue with slight hyperemia (blue arrow). (b) A photomicrograph of the mineral trioxide aggregate + chitosan group after 60 days (H and E,  $\times 20$ ) showing the formation of dentine bridge of 19.8  $\mu\text{m}$  in thickness (black arrow), exposure site (double arrow), and pulp capping material (yellow arrow)

In this study, chitosan was added to the MTA because it is a suitable biomedical agent with a good biodegradability, biocompatibility, and osteoconductivity.<sup>[28-30]</sup> Also chitosan monomer (D-glucosamine hydrochloride) promotes the dental pulp regeneration in both *in vivo* and *in vitro* studies.<sup>[21]</sup>

The addition of chitosan to the MTA resulted in nearly similar dentin bridge to that produced by the MTA alone. These histopathological findings are in full agreement with those reported by Li *et al.* who found that chitosan can induce dentin bridge formation after pulp capping procedures in dogs.<sup>[30]</sup> No significant difference was reported between both groups regarding the dentin bridge thickness at 21 days or 60 days. This means that the addition of chitosan to the MTA did not improve the quality of the dentin bridge produced by the MTA alone.

Further studies are recommended for the investigation of any toxic materials produced at the early setting of cement and for detection of any effect of chitosan on the physicochemical properties of the MTA that may in turn affect the thickness and quality of dentin bridge and degree of pulp inflammatory response. Furthermore, future studies are suggested for assessment of the synergistic odontogenic effect of chitosan when added to the MTA after longer periods (3–6 months) of pulp capping.

The main limitations of this study are the small number of used dogs and relatively short periods of evaluation.

## CONCLUSION

The addition of chitosan to the MTA as a direct pulp capping material has no synergistic odontogenic effect in dog's teeth.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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