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IMPACT OF A NEW SILICATE-BASED CAPPING MATERIAL ON HEALING POTENTIALITY OF TRAUMATICALLY EXPOSED HEALTHY DOG'S DENTAL PULP

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ABSTRACT

Objectives: The aim of the current study was to evaluate the impact of a new silicate-based capping material on healing potential of traumatically exposed healthy dog's dental pulp.

Materials and Methods: Two pulp capping materials were evaluated in this study; Mineral trioxide aggregate (MTA), and Five mineral oxides (5MO). Glass ionomer as overlying restorative material. Six adult healthy dogs with intact permanent dentition were selected for the study. A total of sixteen anterior teeth were used in each dog. Both pulp capping materials were used in the same dog. Class V cavities were prepared on the teeth. After pulp exposure, the pulp capping materials were applied directly to the exposed pulp. All cavities were sealed with glass ionomer restorative material. The used six dogs were divided into two groups; three dogs each, according to the capping periods to 7 days and 90 days. At the end of each period the dogs were sacrificed by injecting an overdose of pentobarbital sodium. Teeth were separated from bone with immediate sectioning of their roots in the mid-way between cementoenamel junction and apex. The teeth were fixed in 10% neutral buffered formalin. Then the teeth were demineralized in Morse's solution (50% formic acid and 20% sodium citrate) for 3 months. In the last, the teeth were embedded in paraffin wax and serially sectioned buccolingually and stained with hematoxylin-eosin. The specimens were examined under optical light microscope to estimate the amount of pulp inflammation and dentin bridge formation at the interface of the capping material.

Results: There was no significant difference between the MTA and 5MO materials in terms of pulp response, hard tissue formation and normal pulp tissue preservation in terms of criteria mentioned above ($P > 0.05$). Histological evaluation after 7 days of direct pulp capping, both group have severe and moderate inflammation with no dentin bridge formation. After 90 days of direct pulp capping, a calcified bridge was formed directly underneath the capping material at the injury site in both the MTA and 5MO groups; both groups showed no signs of inflammation.

Conclusions: There is no significance difference between MTA and 5MO when used as direct pulp capping material

KEYWORDS: Mineral Trioxide Aggregate, Five Mineral Oxides, Direct Pulp Capping

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INTRODUCTION

Through-out the significant developments occurred in dentistry, it was very important to preserve the vitality of the dental pulp as possible to ensure a long life to tooth in oral cavity. The dental pulp has a high capacity to repair itself after traumatic exposure, either in human or in animals. The use of appropriate material to cover the exposed pulp increases rate of success and contributes to maintain the vitality of the dental pulp. [1]

The aim of vital pulp therapy is to protect the vital exposed pulp during cavity preparation or during the removal of last traces of deep caries. Preserving the pulp vitality, function and formation of reparative dentin are the ultimate goals of this line of treatment. The healing criterion of the exposed pulp is the deposition of reparative dentin in the exposure site and formation of Hard Tissue Barrier (HTB). [2]

For the success of pulp capping procedures the exposed pulp must have a good blood supply, as well as, being free from symptoms of irreversible pulpitis. The exposure site must be less than 1.5 mm in diameter. The bleeding at the site of exposure should be controlled. Optimal quality of the formed reparative dentine requires a pulpal tissue specific response treatment, referred to as reparative dentinogenesis and seems to be a critical pre-requisite for the long term success of vital pulp therapy. The repair process starts by inflammation which induces a specific cell response by both activation of old odontoblasts or differentiation of new odontoblast-like cells. [3]

Materials used in Direct Pulp Capping (DPC) procedure should be biocompatible and have high capacity of healing. Many successive researches and studies on other materials used in the same context have been made, such as Calcium Hydroxide (CH), Tricalcium phosphate, dentine fragments, enzymes, collagen, glass inomer cement, bonding systems and other materials.[4] Mineral trioxide aggregate (MTA) was introduced for pulp capping due to its biocompatibility and excellent therapeutic efficacy, also it can be useful in endodontic and operative treatment, including retro-filling material, direct pulp capping, treatment of fractures and treatment of chronic absorption lesion. Mineral Trioxide Aggregate considered as one of the best capping materials, so comparing it with new silicate based material in direct pulp capping procedure is of value. [5]

Mineral Trioxide Aggregate (MTA), was introduced as a retrograde filling material in a way to overcome the disadvantages of the filling materials used in sealing the pathways of communication between the root canal system and the external tooth surface.[6] It is used in slurry form by its mixing with water, when it gradually hardens in the oral environment, hydration of the powder result in a colloidal gel that solidified to a hard structure in less than four hours.[7] MTA seals the exposed pulp more tightly than calcium hydroxide and is also more stable, which might be relevant for restorative success.[8] Recent systematic review and studies found evidence from both randomized and non-randomized trials that supporting MTA for direct pulp capping, with a significantly reduced risk of failure when using MTA instead of calcium hydroxide.[9, 10]

The MTA has expanded into many other surgical and non-surgical applications of pulp protection, root repair and bone healing. These applications include direct pulp capping, pulpotomy, repair of lateral, furcation and strip root perforation, apexogenesis, apexification and chronic absorption lesion.[11] The advantages of this material are biocompatibility, sealing ability and the ability to set in moisture, thus, making it near to an ideal endodontic material. MTA is composed of complex compounds,

constituents of tri-calcium silicate, di-calcium silicate, tri-calcium aluminate and tetra-calcium aluminoferrite.[12, 13] To increase radio-opacity, bismuth oxide was added in MTA. The tri-calcium aluminate was known to reduce the setting time of MTA.[14] White MTA (WMTA) contained significantly lesser amount of aluminum oxide (Al2O3), magnesium oxide (MgO) and ferric oxide (Fe2O3) than grey MTA (GMTA).[15]

Five Mineral Oxides is a modified version of MTA. It contains Titanium oxide (TiO2) in addition to silicon, calcium, aluminum and magnesium oxides. Although there are no available informations about the direct effect of titanium oxide, present in 5MO, several researches demonstrated that pure titanium is a nontoxic material and shows excellent biocompatibility. This behavior is due to a titanium surface oxide layer[16, 17]

Heravi et al.[18] reported lower toxicity of nano-scale TiO2 containing orthodontic adhesives, when compared with conventional adhesives. A study carried out on animals demonstrated that resin denture base when coated with TiO2 does not cause irritation or sensitization of the oral mucosa, skin or intracutaneous tissue. ***Dechsakulthorn et al***.[19] indicated that higher toxicity of zinc oxide nanoparticles compared to TiO2 nanoparticles was reported. However, In vivo experiments reported adverse effects of TiO2 nano-powder in mice with pulmonary toxicity after inhalation of TiO2 nanoparticles in rats. [20] Inhalation is the most common route of exposure among these studies which is not a case in dental material applications.

Aim of the study:

This study was designed to compare between MTA and 5MO histologically, to evaluate the impact of this new silicate-based capping material on healing potential of traumatically exposed healthy dog's dental pulp.

MATERIALS AND METHODS

Materials:

Two pulp capping materials (Mineral Trioxide Aggregate and Five Mineral Oxides) and a Glass-ionomer cement restorative material were used in the current study. Composition, manufacturers and batch numbers are listed as follow:

Materials	Composition	Manufacturer	Batch no
Mineral Trioxide Aggregate (MTA)	**Powder :** a mixture of refined portland cement, bismuth oxide and trace amounts of SiO_2,CaO,MgO,$K_2So_4$the major component , Portland cement, is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, and tetracalciumaluminoferrite. **Liquid:** distilled water	DENTSPLY caulk, Milford, DE, USA	**+J003A04050000011001+**
Five Mineral Oxides (5MO)	**Powder:** a mixture of refined portland cement, bismuth oxide and trace amounts of SiO_2,CaO,MgO,Al_2O_3,TiO_2 the major component , Portland cement, is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum, and tetracalciumaluminoferrite **Liquid:** distilled water	Golden Yatti LLC Muscat, Oman	120150000001
Glass Ionomer Cement (GIC)	**Powder:** Fluoroaluminosilicate glass **Liquid:**Polyalkenoic acid	VOCO, Germany	7893628 018846

Methods:

Experimental procedures of this study were performed in the Medical Experimental Research Center (MERC) at Faculty of Medicine, Mansoura University. Six healthy male mongrel dogs aged between 2 and2.5 years old with intact dentitions and free from any systemic disease were included in this study. Grouping of the teeth; both pulp capping materials were used in each dog. Group I: MTA capping material was applied to half of the included teeth in each dog, with a total of 48 teeth. Group II: 5MO capping material was applied to half of the included teeth in each dog, with a total of 48 teeth. Each group was subdivided into two subgroups according to the capping periods; 7 days of capping and 90 days

Experimental procedures:

All dogs were injected intravenously with ketamine in a dose of 1 mg/kg for starting and maintaining sedation. General anesthesia and muscle relaxation were induced with an intravenous injection of 6 mg/kg thiopental sodium. Trachea was intubated before the operation, and general anesthesia was maintained by using halothane in oxygen, conducted through a semiclosed ventilator loop.

Permanent maxillary and mandibular 4 canines and 12 incisors were used as most suitable teeth. All teeth were scaled and polished with a rubber cup on the day of operative procedures. Quadrants of teeth were isolated by using sterile cotton rolls and saliva ejector and coolant water were controlled through high-speed evacuation

Non-retentive half-moon shaped class V cavities (approximately 2.5 mm wide, 3 mm long, 1.5–2 mm deep) were prepared on the cervical third of the buccal surfaces of teeth by using a tungsten carbide rose head bur 1.2mm at ultra-high speed, with a copious oil free air/water spray. A new bur was used on every fourth cavity to avoid dullness of the bur and excessive heating. The preparations were cut 1-2 mm above the free gingiva, parallel to cementoenamel junction. Pulp exposure was performed in the middle of the cavity floor by using a sterile round tungsten carbide bur 0.8mm at high speed and oil free air/water cooling, without excessive pressure and with repeated light strikes fig.(1). The size of pulp exposure produced was about 0.8–1.0 mm (pinpoint exposure) according to the size of the bur. The cavities were washed with sterile normal saline and dried with sterile cotton pellets. Light pressure was applied by cotton pellets so that hemorrhage could be controlled fig.(2). In each animal of the first and second groups, exposed pulps of teeth were capped either with white MTA or 5MO capping materials according to manufactures' instructions

Using a sterile metal spatula, MTA powder was mixed with distilled water in a 3:1 ratio (according to manufactures instructions), and then placed over the exposure site with a sterile amalgam carrier and sterile metal condenser. Five Mineral Oxides powder was mixed with distilled water by sterile metal spatula according to manufactures instructions, and then placed over the exposure site using a sterile amalgam carrier and sterile metal condenser fig. (3). In all groups, Glass Ionomer cement was used to restore the cavities; Glass ionomer powder and liquid were mixed by sterile metal spatula then applied over capping material fig.(4)

The pulpal tissue responses to capping materials were assessed at post-operative periods of 7 and 90 days. After post-operative periods, the animals were sacrificed by injecting an overdose of pentobarbital sodium. The teeth were separated from bone, and their roots were immediately sectioned midway between the cementoenamel junction and the apex to be sure of fixation of the pulp

The teeth were fixed in 10% neutral buffered formalin solution for 10 days. Mesial and distal surfaces of teeth were reduced by high speed diamond stone with oil free water spray coolant. Then the specimens were demineralized in Morse's

Fig. (1) Class V cavitites on buccal surfaces of teeth.

Fig. (2) Class V cavities with pinpoint exposures

Fig. (3) Application of capping material

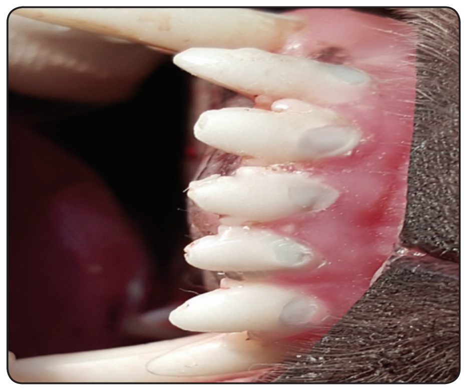

Fig. (4) Sealing of cavities with Glass Ionomer as final restoration

solution (50% formic acid, 20% sodium citrate) for 3 months. Finally, the teeth were embedded in paraffin wax and serially sectioned in buccolingual direction at an average thickness of 6 μm and stained with hematoxylin-eosin. With an optical light microscope, specimens were examined as coded slides to avoid possible bias.

RESULTS

Pulp tissues were mainly infiltrated with moderate to severe inflammatory cells in both MTA and 5MO treated groups 7 days post operatively (fig. 5,6), with no signs of inflammation in 90 days post operatively in both groups, except only one specimen from 5MO shows mild inflammation only (fig. 7, 8) (table 1). Mann Whitney test showed that there is no statitically significance difference between teeth treated with MTA and 5MO groups ($p>0.05$) (table 3,4). The Wilcoxon signed ranks test showed that, regarding to the inflammatory cells infiltration, there is a significant difference between 7 and 90 days in groups treated by MTA ($P<0.001$). Capping with 5MO also showed a significant difference between groups after 7 days and 90 days ($P<0.001$) (table 5, 6)

TABLE (1) Inflammatory cell response 7 and 90 days after direct pulp capping with MTA and 5MO

groups	None		Slight		Moderate		Severe	
	7	90	7	90	7	90	7	90
MTA	0	17	0	0	8	0	12	0
5MO	0	16	0	1	9	0	11	0

Pulp tissues revealed absence of signs of hard tissue (Dentin Bridge) formation, at the exposure site after 7 days post-operatively (fig. 5,6). After 90 days there is complete thick hard tissue (Dentin Bridge) formation in most of the cases except one case capped with MTA, and two cases capped with 5MO were there is partial hard tissue formation (fig. 7,8) (table 3). The Mann Whitney test showed that there was no significant difference between groups treated by MTA and groups treated by 5MO, ($p>0.05$) (table 4, 5). The Wilcoxon signed ranks test showed that there was significance difference between 7 and 90 days groups treated by MTA ($P<0.001$). Five Mineral Oxides also showed significance in difference when compared teeth treated 7 days and other treated 90 days with 5MO capping material ($P<0.001$) (table 6,7)

TABLE (2) Hard tissue formation after 7 and 90 days after direct pulp capping with MTA and 5MO

groups	None		Partial		Complete	
	7	90	7	90	7	90
MTA	20	0	0	1	0	16
5MO	20	0	0	2	0	15

TABLE (3) Result of differences between MTA and 5MO 7 days post-operative regarding inflammation and hard tissue formation

Parameters of evaluation	Material	Median (min-max)	P value
Inflammation	MTA	4.0(3.0-4.0)	0.75
	5MO	4.0(3.0-4.0)	
Hard tissue formation	MTA	0.0(0.0-0.0)	1.0
	5MO	0.0(0.0-0.0)	

TABLE (4) Result of differences between MTA and 5MO 90 days post-operative regarding inflammation and hard tissue formation

Parameters of evaluation	Material	Median (min-max)	P value
Inflammation	MTA	0.0(0.0-0.0)	0.78
	5MO	0.0(0.0-2.0)	
Hard tissue formation	MTA	2.0(1.0-2.0)	0.75
	5MO	2.0(1.0-2.0)	

TABLE (5) Result of differences between 7 and 90 days post-operative of MTA capping material regarding inflammation and hard tissue formation

Parameters of evaluation	Material (MTA)	Median (min-max)	P value
Inflammation	7 days	4.0(3.0-4.0)	0.001
	90 days	0.0(0.0-0.0)	
Hard tissue formation	7 days	0.0(0.0-0.0)	0.001
	90 days	2.0(1.0-2.0)	

TABLE (6) Result of differences between 7 and 90 days post-operative of 5MO capping material regarding inflammation and hard tissue formation

Parameters of evaluation	Material (5MO)	Median (min-max)	P value
Inflammation	7 days	4.0(3.0-4.0)	0.001
	90 days	0.0(0.0-0.0)	
Hard tissue formation	7 days	0.0(0.0-0.0)	0.001
	90 days	2.0(1.0-2.0)	

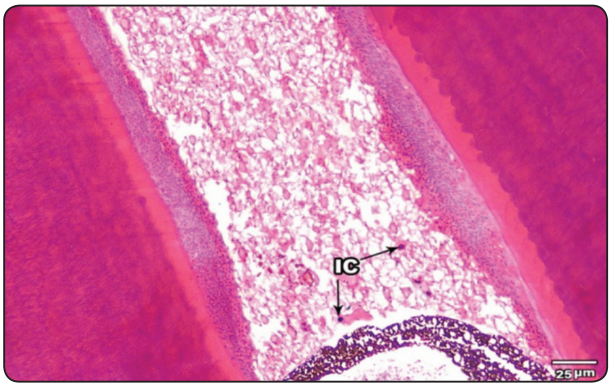

Fig. (5) Photomicrograph of decalcified section at the site of mechanical pulp exposure capped with 5MO for 7 days showing increased in number of inflammatory cells (IC) with moderate inflammation and no dentin bridge formation. (H&E X 400)

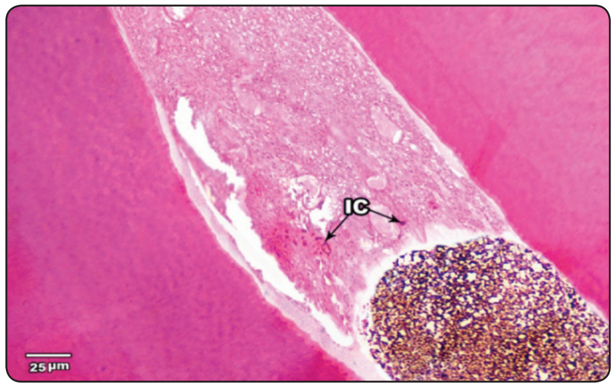

Fig. (6) Photomicrograph of decalcified section at the site of mechanical pulp exposure capped with MTA for 7 days showing mild inflammation with few number of inflammatory cells (IC) and no dentin bridge formation (H&E X 400)

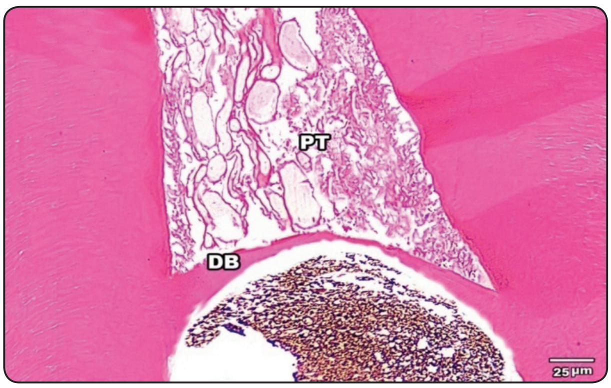

Fig. (7) Photomicrograph of decalcified section at the site of mechanical plup exposure capped with MTA for 90 days showing complete dentin bridge (DB) formation with normal pulp tissue organization (PT) with no inflammatory cells. (H&E X 400)

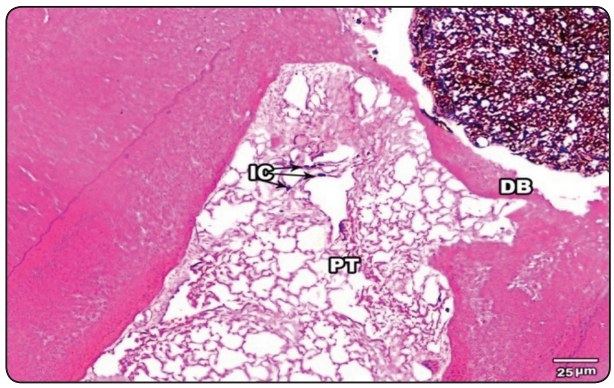

Fig. (8) Photomicrograph of decalcified section at the site of mechanical plup exposure capped with 5MO for 90 days showing complete dentin bridge (DB) formation with normal pulp tissue organization (PT) and few number of inflammatory cells (IC). (H&E X 400)

DISCUSSION

In the present study, periods of 7 and 90 days intervals were used to evaluate the effect of capping materials on the pulp tissues. Most of published study on dogs used intervals of 4 and 8 weeks, some investigations found thin layer of calcified bridge after 2 weeks. Several other studies evaluated pulp tissue at different time intervals up to 150 days. In our study, anterior teeth of dogs were used to maintain more visibility and accessibility, also to preserve isolation and prevent contamination. Incisors, canines and premolars were used by ***Menezes et al***. [21]. Some studies used canines only. Other studies did not mention the type of teeth used. [22, 23] in 2007, a modified MTA material Five Mineral Oxides (5MO) which consist of five mineral oxides was developed by Dr. Maysour Ala-Rachi (Syrian Patent number: 5770). This material was compared to calcium hydroxide as a pulp capping material. ***Alarachi et al***. compared clinical and radiographical success rate between 5MO and calcium hydroxide. Twenty two vital premolars and molars with deep caries were treated with direct pulp capping after pulp exposure, pulp was capped with 5MO and calcium hydroxide randomly. Then teeth restored with amalgam over resin modified glass ionomer cement. After 7, 30 and 180 days, clinical and radiographical test showed that there is no difference between 5MO and calcium hydroxide in success rate. [24]

CONCLUSION & RECOMMENDATIONS

Both 5MO and MTA can act similarly as capping materials in terms of pulp response, dentin bridge formation and preservation the vitality of the pulp.

Further biochemical tests and immune - histochemical stains data were recommended to characterize completely the nature and specificity of the direct pulp capping outcome.

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