



## EVALUATION OF DIFFERENT HISTOLOGIC REACTIONS IN DENTIN-PULP ORGANS AFTER STIMULATION WITH LASER, MTA, AND THERACAL AS PULP CAPPING THERAPIES

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### ABSTRACT

**Objective:** This histological was preformed to assess the different histological reactions in dentin-pulp organs in the pulp of mongrel dogs after simulation with low level laser (LLL), mineral trioxide aggregate (MTA) and TheraCal light cured (LC) and compare it with conventional calcium hydroxide. **Subjects and methods:** Eight male mongrel dogs 12-18 months old were chosen for this study. Pulp exposure was done via the preparation of a class V cavity on the buccal surface of the premolar teeth. In each dog the premolar teeth were assigned to four different groups (group 1: LLL; group 2: MTA; group 3: TheraCal LC; and group 4: Ca(OH)<sub>2</sub>). The histological reactions were recorded at 3 different follow up periods (7, 14 and 42 days). **Results:** Histological findings showed formation of a partial or complete dentine bridge in laser and MTA therapies after 42 days, and the pulp returned to its normal condition. Additionally, TheraCal LC and Ca(OH)<sub>2</sub> showed the formation of granulation tissues. **Conclusion:** MTA specimens form a complete dentine bridge after the end of the follow up period. However laser specimens formed an incomplete dentine bridge after the end 42 days. TheraCal LC has a comparable result when compared with Ca(OH)<sub>2</sub>.

**KEY WORDS:** Dentine pulp organ, Histological reactions, Laser, MTA, TheraCal

### INTRODUCTION

The standard basis of restorative dentistry rely on the conservation of the dentin-pulp complex in a healthy state with proper function which results in the successful healing of injured dental pulp <sup>(1)</sup>. The physiognomies of proper healing of exposed dental pulp comprise restructuring of the injured pulp tissues, differentiation of newly odontoblastic-like

cells (ODLCs) from the sub-odontoblastic cells, and consequently healing of the dentin tissues via formation of what is called “reparative dentin” <sup>(2)</sup>.

The procedure of covering the exposed pulp tissues with capping material is known as “direct pulp capping (or) vital pulp therapy” and implicates the placement of a biocompatible material on the pulpal tissue exposed to accidental traumatic

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injury<sup>(3)</sup>. The goals of this therapeutic expense are to preserve the pulp health through capping and protecting the pulp against the penetration of bacteria, support the pulpal organ to divide at the exposure site and initiate the formation of a dentin-like bridge<sup>(4)</sup>.

The use of  $\text{Ca}(\text{OH})_2$  as a direct pulp capping material during vital pulp therapy is a classic method, however, using  $\text{Ca}(\text{OH})_2$  has numerous shortcomings, comprising its excessive initial solubility and arbitrary treatment sequels<sup>(5,6)</sup>. Therefore, a diversity of newly introduced pulp capping materials has been proposed as alternatives; one such alternative material is MTA<sup>(7)</sup>. In this regard, MTA has been documented as a biocompatible material that induces exceptional induction for the formation of hard dental tissue<sup>(8)</sup>. Primarily, MTA was used in endodontics treatment to close off entirely the communication passageways between the canal system space and the exterior tooth surface<sup>(9)</sup>.

TheraCal LC is another capping material for the exposed pulp in the case of vital pulp therapy<sup>(10)</sup>. TheraCal is a filled calcium-silicate-based liner modified with light-cured resin that is proposed in the dental market for use in diverse vital pulp therapies. It was stated that TheraCal LC was introduced as a low cytotoxic light-cured resin liner<sup>(10)</sup>. Moreover, TheraCal LC has been stated to have inferior solubility, excluding alkaline pH, and superior Ca release when compared with the conventional MTA or traditional  $\text{Ca}(\text{OH})_2$ <sup>(10,11)</sup>. Moreover, in an earlier Vivo study, TheraCal LC has hard tissue formation that has been described to be equivalent to pure Portland cement and better than the conventional  $\text{Ca}(\text{OH})_2$  or glass ionomer cement (GIC) without a significant inflammation for the pulpal tissues<sup>(12)</sup>.

LLL, when used in cells or tissues, is not grounded on the process of heating, i.e. "the absorbed photons energies not altered into heat", but transformed into photophysical, photochemical, and/or photobiological consequences<sup>(13)</sup>. It was

stated that when laser light in a suitable dosage interacts with tissue or cells their functions can be motivated<sup>(14)</sup>. Therefore, nowadays LLLT has been used as a bios-stimulator for tissue healing, as it helps to enhance cell proliferation, local circulation, and synthesis of collagen<sup>(13,14)</sup>.

However, the histologic reaction of the pulpal organ in response to the LLL capping therapy, and capping materials has not been established. Therefore, this current study was directed to assess the healing progress of the dentin-pulp organ in response to various treatment modalities of LLL, MTA, and TheraCal LC in dog pulpotomy models in terms of histologic reactions responses and compare it with  $\text{Ca}(\text{OH})_2$  as the gold standard pulp capping material.

## SUBJECTS AND METHODS

### Animal selection and operative procedures:

Eight male mongrel dogs weighing 10-15 kg and 12-18 months old were chosen for this study. The animals had intact dentition and a healthful periodontium. Animal selection, administration, surgical protocol, and provision were implemented according to routine measures approved by the Faculty of Veterinary, Cairo University. Dogs were exposed to free admission to diet and water.

All operative procedures were achieved under general anesthesia in a sterile operating room under aseptic circumstances with limited isolation. The animals were injected intravenously by tramadol (1 mg/kg) and intramuscularly by xylazine (0.2 mg/kg) and zoletil (5 mg/kg). Subcutaneous injection of enrofloxacin (5 mg/kg) was given before and after treatment and intraoral amoxicillin-clavulanate (12.5 mg/kg) was given for 5 to 7 days postoperatively to counteract infection<sup>(15)</sup>.

### Cavity preparation

The surgical field was disinfected with 0.2% chlorhexidine gluconate and a dry field was attained

readily using cotton rolls and gauze <sup>(16)</sup>. Buccal Class V cavities were prepared on the cervical third of the buccal surface of each tooth roughly (0.5-1 mm) coronal to the gingival margin. To confirm proper cutting efficacy, a new bur was used on each quadrant <sup>(17)</sup>. The deepness of the cavity preparation is assorted rendering to the anatomy of each tooth using a sterile round bur # 2 under cooling with sterile normal saline solution. Pulp exposure was achieved in the middle of the cavity floor. After these procedures, the cavity was irrigated and the dentin debris was removed by the use of 10 ml of sterile saline solution. Finally, hemostasis was attained by employing a cotton pellet over the exposure sites for 10 seconds <sup>(18)</sup>.

#### **Sample grouping and restoration:**

In each doge four premolar teeth were involved and allocated to four different groups based on the treatment method group 1: Laser; group 2: MTA; group 3; TheraCal LC, and group 4; Ca (OH)<sub>2</sub>.

In group 1; the soft laser used was an aluminum gallium diode laser arsenide (Pocket Laser, Orotig. Med, S.r.I, Italy) with energy source of 660-nm and power of 3-mW and 18 J/cm<sup>2</sup>. The light was applied at a 0.5-1 cm distance from the tooth surface. LLLT was standardized at 4-second exposures per point; buccal, palatal, and perpendicular to the tooth axis, however, the sealing of exposed pulp still required so calcium hydroxide was placed on exposure site as classic capping material <sup>(13, 19)</sup>. In group 2; ProRoot MTA (Dentsply, Tulsa, OK) powder was mixed with water (3:1) on a glass slab by using a metal spatula according to manufacture instructions and then condensed immediately to the exposure area <sup>(7)</sup>. In group 3; TheraCal LC (Bisco Inc, Schaumburg, IL, USA) paste in a pre-mixed syringe, was applied from the dispensing tips directly to the dried exposure area in thickness of 1-mm and extended 1-mm beyond the exposure onto sound dentin, and then light-cured with (Woodpecker light curing LED.F 1600W/1800W) for 20 seconds <sup>(11)</sup>. In group

4; A two-paste system Ca(OH)<sub>2</sub> (Dentsply Caulk, Milford, USA, LOT 023407) was mixed following manufacturer instructions on a paper pad with plastic spatulas and applied to the exposure site using a Dycal carrier <sup>(5)</sup>. For all cavities in all groups the GIC (Fuji IX GP) was used as a final coronal restoration.

#### **Samples preparation for histological examination:**

The dogs were sacrificed at 7, 14, and 42 days (to examine dentine bridge formation) after the end of the operative procedures. The samples were fixed in 10% formalin for 2 weeks then the samples were decalcified using ethylene diamine-tetra-acetic acid (EDTA) in a concentration of 125 gm in one liter of distilled water and sodium hydroxide structures as a buffer for 2 weeks. Then, the samples were washed with running water to remove any decalcifying leftovers. After that sample dehydration was performed in ascending grades of ethyl alcohol starting by 70% till 100% and methyl benzoate for 1 day. To remove the alcohol residue, paraffin benzol was used for 2 hours. Samples were then immersed in paraffin wax "in three changes" and then consigned in wax blocks of the appropriate size to be disposed of for cutting. Cutting of the tissue samples was done using microtome for serial sections of 4-6 um thick in longitudinal and buccolingual direction <sup>(18)</sup>.

#### **Histological examination:**

After sample preparation, the staining was done with regular Hematoxylin and Eosin as well as Masson trichrome stains to assess diverse histologic reactions in dentin-pulp organs involving odontogenic zones, pulp cores, and supposed tertiary dentin formation. Masson trichrome stained collagen fibers in dentin-pulp organ discharged by fibroblasts, odontoblasts, and ODLCs. Mature collagen fibers were stained with green color and immature fibers with red color <sup>(20)</sup>.

## RESULTS

### Histologic changes result after 7 days:

At the exposure site, the histologic pulp changes showed comprehensive damage and atrophy to the odontogenic layer (ODL), in some areas vacuolization of odontoblasts was evinced for the four tested pulp therapies. While some residues of capping material existed for MTA and TheraCal only. However, Laser and MTA pulp therapies showed granulation tissue formation with multiple macrophages, fibroblasts, and newly proliferated blood vessels were formed. Also, in the MTA group, undifferentiated mesenchymal cells (UMS) appeared with high mitotic activity was noticed. While for TheraCal and  $\text{Ca}(\text{OH})_2$ , the pulp tissues showed liquefaction necrosis with some areas of clotted blood, and Inflammatory cell infiltrations. The blood vessels were dilated and clotted in response to all materials, and MTA and  $\text{Ca}(\text{OH})_2$  showed distended red blood cells (RBCs). (**Figure 1**).

The mid-region of the pulp tissues showed complete degeneration of the odontoblastic layer in some areas in all tested pulp therapies. Laser, MTA, and  $\text{Ca}(\text{OH})_2$  pulp therapies showed granulation tissue formed of (multiple macrophages, fibroblasts, and newly formed blood capillaries) which replace the odontogenic zone. While TheraCal showed the disappearance of the cell-free zone (CFZ). In all tested pulp therapies, the cell-rich zone (CRZ) showed increased activity through multiple mitotic figures and migration of UDMCs toward the dentinal wall. The rest of the pulp demonstrated Inflammatory cell infiltrations, and dilated arterioles engorged with RBCs. A newly proliferated blood capillaries were formed in response to MTA and  $\text{Ca}(\text{OH})_2$ . In the pulp core, fibroblasts showed a high mitotic activity index and scattered in collagen fiber meshwork in TheraCal and MTA. In  $\text{Ca}(\text{OH})_2$ , the fibroblasts showed high mitotic activity, the periphery showed the disappearance of the ODL which was completely replaced by granulation tissue. (**Figure 2**)

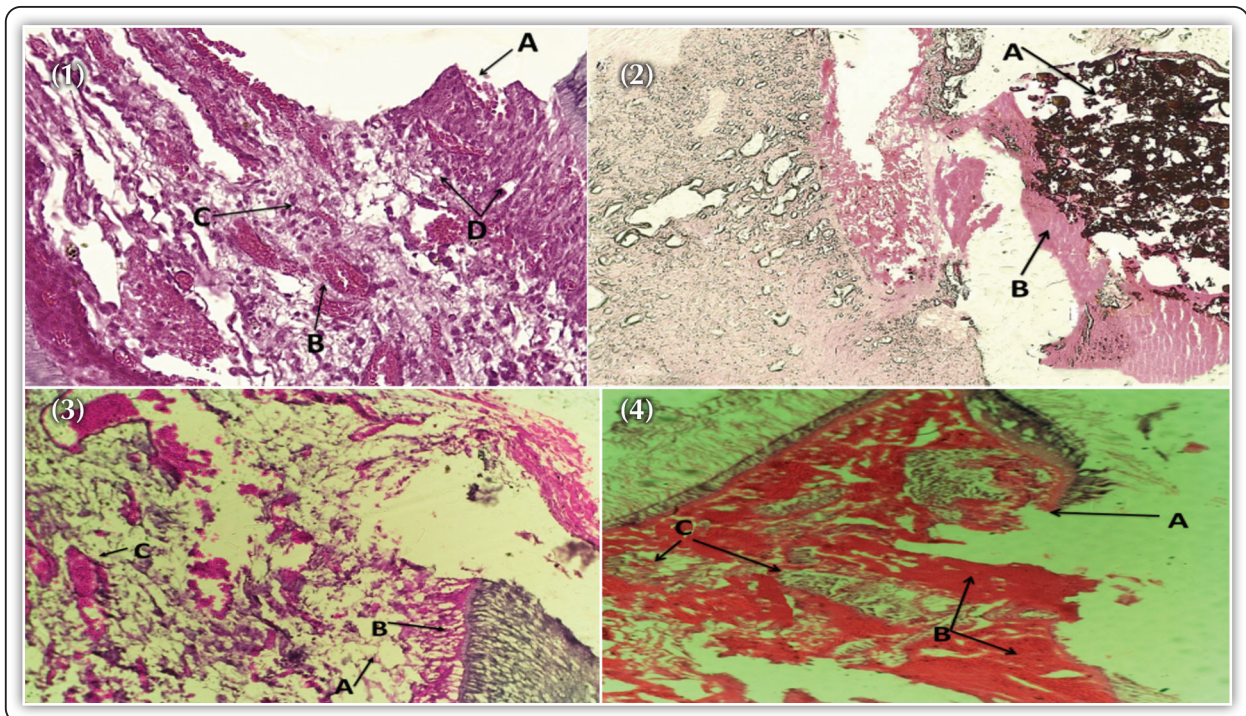


FIG (1) (1) Laser; (A) complete degeneration of odontogenic zone, (B) dilated blood vessels, (C) granulation tissue and (D) proliferating blood capillaries. H&E stain (20x); (2) MTA; (A) remnant of capping material and (B) blood clot. H&E stain (20x); (3) TheraCal; (A) liquefaction necrosis, (B) odontoblasts vacuolization, and (C) dilated, clotted blood vessels. H&E stain (20x); (4)  $\text{Ca}(\text{OH})_2$ ; (A) complete degeneration of odontogenic zone, (B) blood clot and (C) necrosis. H&E stain (20x)

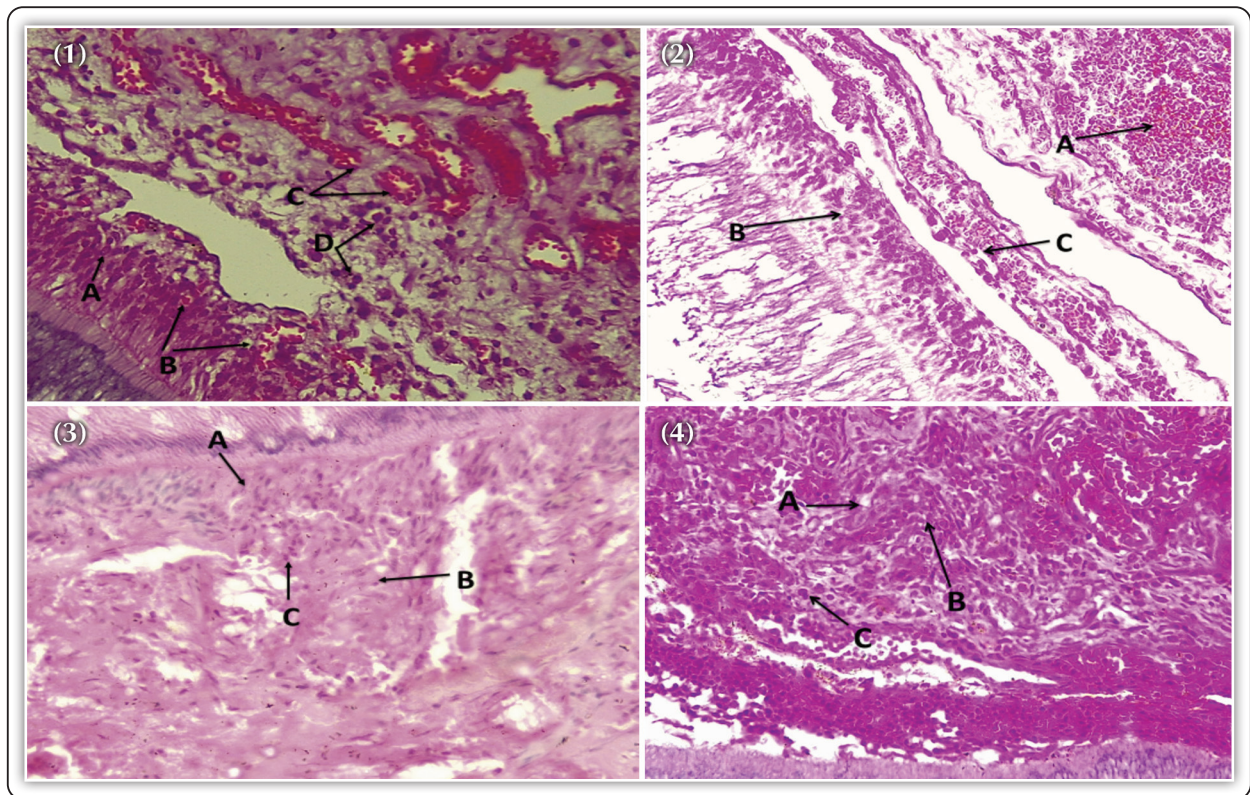


FIG (2) (1) Laser; (A) granulation tissue, (B) dilated blood vessels, and (C) mitotic figures. H&E stain (40x); (2) MTA; (A) inflammatory cell infiltrate, (B) multiple mitotic figures, and (C) multiple macrophages. H&E stain (40x); (3) TheraCal; (A) multiple mitotic figures, (B) dilated blood vessels, (C) newly proliferated blood capillaries and (D) inflammatory cell infiltrates. H&E stain (40x); (4) Ca(OH)<sub>2</sub> (A) proliferating blood capillaries, (B) granulation tissue and (C) inflammatory cell infiltrate. H&E stain (40x)

### Histologic changes result after 14 days:

At the exposure site, the histologic pulp changes followed the laser therapy showed disappearance of odontogenic layer, formation of organized granulation tissue closing exposure site, some specimens showed partial hard tissue formation at the site of exposure, newly formed pre-dentin on each side of the pulp, formation of collagen bundles closely packed to each other, areas of calcification. Masson trichrome stain showed the red color of a partially calcified bridge. Some specimens showed moderate inflammatory cells infiltrate and edema. While in the MTA group showed black areas representing the capping material surrounded by a thin area of hard tissue calcification closing exposure site, collagen fiber bundles (CFBs) closely packed to each other, and mild inflammatory cell infiltrations. The response of the pulp tissues to MTA

capping material showed very high mitotic figures of fibroblasts and UMC, and migration of UDMCs to form ODLCs. Multiple proliferating blood capillaries (PBC) and red-colored calcific tissue formation surrounding capping material stained by Masson trichrome were observed. TheraCal group showed granulation tissue formation beneath parts of capping material, degeneration of ODL, CFBs were closely packed to each other, and inflammatory cell infiltrations. High mitotic index of fibroblasts and UDMCs, many PBC, and no signs of hard tissue formation. Ca(OH)<sub>2</sub> group showed degeneration and complete loss of ODL and formation of granulation tissue at exposure site, CFBs closely packed to each other, and moderate inflammatory cell infiltrations. Multiple dilated blood vessels engorged with RBCs were observed, and odontoblasts in some areas appeared vacuolated, with no signs of hard tissue formation. (Figure 3, 4)

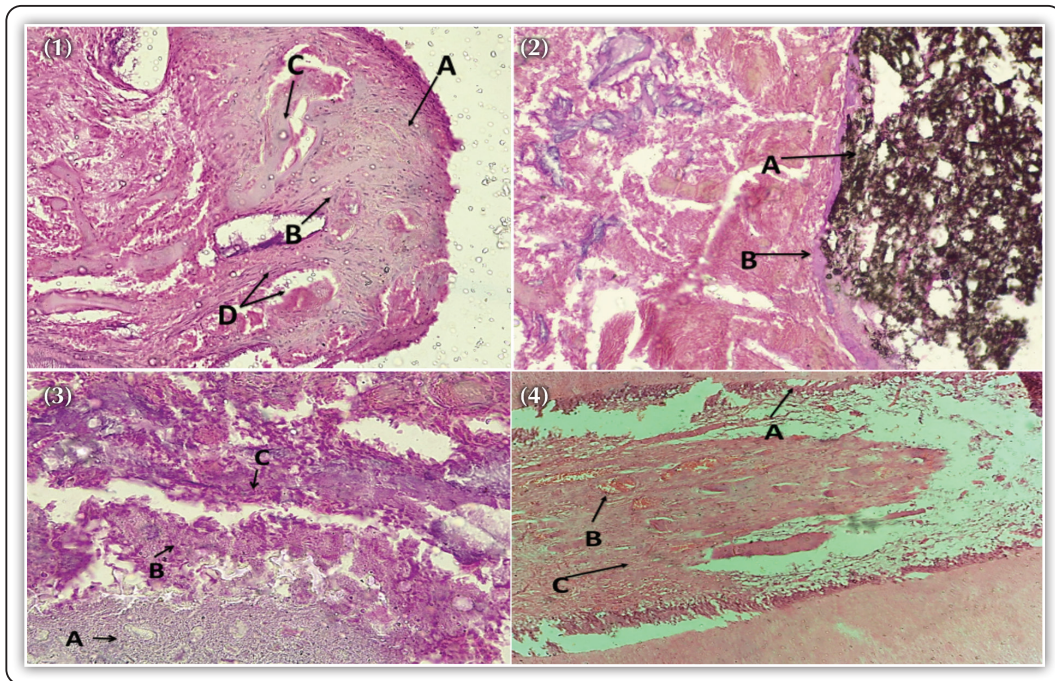


FIG (3) (1) Laser; (A) granulation tissue, (B) odontoblast like cells, (C) newly formed predentin and (D) areas of calcification. H&E stain (20x); (2) MTA; (A) capping material (B) hard tissue calcification. H&E stain (20x); (3) TheraCal; (A) capping material, (C) granulation tissue, and (D) mitotic division of fibroblasts. H&E stain (20x); (4) Ca(OH)<sub>2</sub>; (A) vacuolated odontoblasts, (B) dilated blood vessels, and (C) collagen bundles. H&E stain (20x)

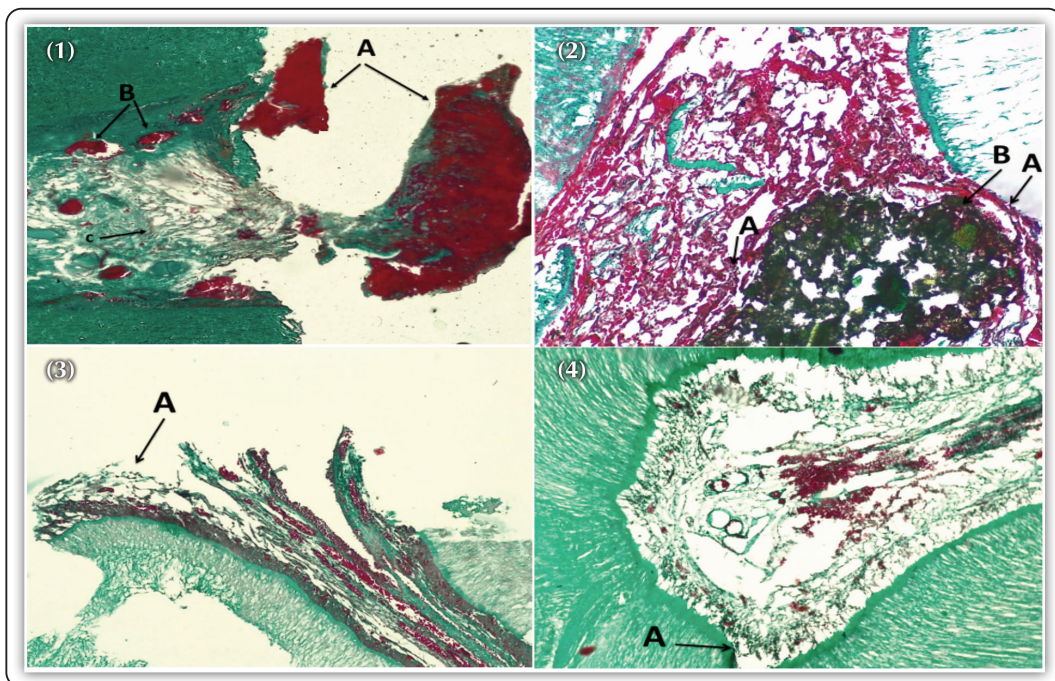


FIG (4) (1) Laser; (A) red color partial calcified bridge, (B) areas of calcification and (C) collagen bundles. Masson trichrome stain (20x); (2) MTA; (A) capping material, (B) calcified hard tissue formation. Masson trichrome stain (20x); (3) TheraCal; (A) green colored collagen fibers. Masson trichrome stain (20x); (4) Ca(OH)<sub>2</sub>; (A) green colored collagen fibers. Masson trichrome stain (20x)

In the mid-region, the examined pulp tissue of this period showed that; in the Laser and MTA groups the ODLCs were prearranged alongside the dentin and intermingled with PBC. Additionally, the CFZ disappeared, and CRZ appeared full of mitotic divisions of UDMCs in the Laser and MTA group. Also, there were multiple dilated blood vessels engorged with RBCs, CFBs appeared closely packed to each other, and some specimens show intense edema. In the TheraCal group; the ODLC completely disappeared and was replaced by organized granulation tissue, fibroblasts showing high mitotic activity, and the rest of the pulp demonstrated inflammatory cell infiltrations and dilated blood vessels engorged with RBCs. Some specimens show edema. In the Ca(OH)<sub>2</sub> group; the ODLC appeared vacuolated, and numerous PBC was formed. In some areas; several mitotic divisions of UDMCs were present. Additionally, there were multiple dilated blood vessels engorged with RBCs, and CFBs appeared closely packed with each other. (Figure 5)

#### Histologic changes result after 42 days:

At the exposure site, the all examined samples showed that; the ODLC disintegrated, atrophied, or disappeared. In the MTA groups, most of the examined specimens showed complete dentin bridge configuration closing exposure site while in Laser specimens most specimens showed incomplete dentin bridge formation. Both of MTA and Laser specimens showed a slight inflammatory reaction. While, in the TheraCal group, the examined pulp tissue at the site of exposure in some areas showed restored normal architecture, and formation of partial and thin fibrous tissue at the exposure site while in some specimen's granulation tissue closing exposure site. Some specimens showed areas of calcification and inter-cellular edema, but no signs of hard tissue formation. However, the samples of the Ca(OH)<sub>2</sub> group, showed that the exposure site was closed by organized granulation and fibrous tissues. areas of liquefaction necrosis were present, there were a little number of extravasated blood vessels under the fibrous tissues. (Figure 6, 7)

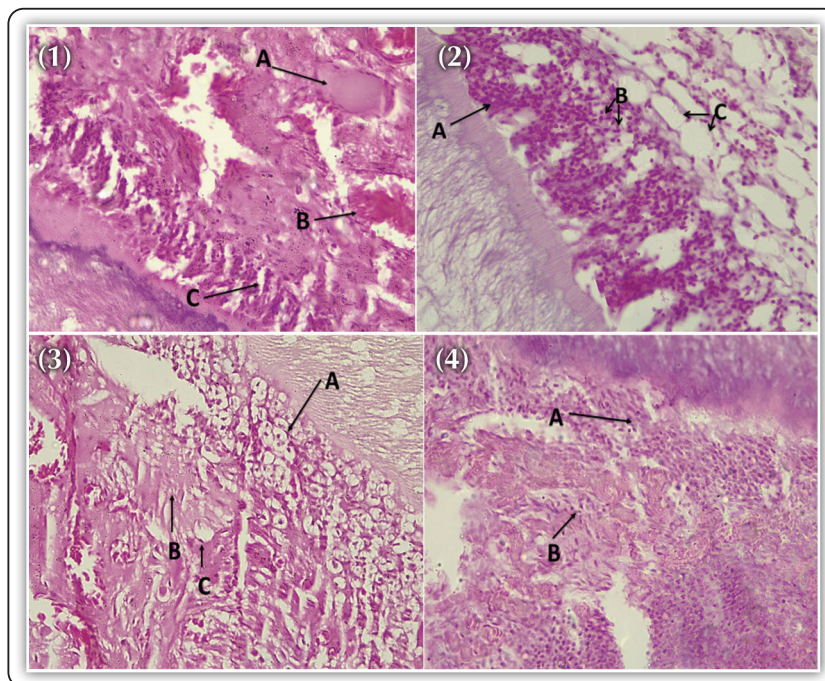


FIG (5) (1) Laser; (A) area of calcification, (B) dilated blood vessels engorged with RBCs, (C) proliferating blood capillaries. H&E stain (40x); (2) MTA; (A) OBLs (B) mitotic divisions of UMCs and (C) edema. H&E stain (40x); (3) TheraCal; (A) organized granulation tissue (B) mitotic division of fibroblasts. H&E stain (40x); (4) Ca(OH)<sub>2</sub>; (A) vacuolated odontoblasts (B) collagen fiber and (C) proliferating blood capillaries. H&E stain (40x)

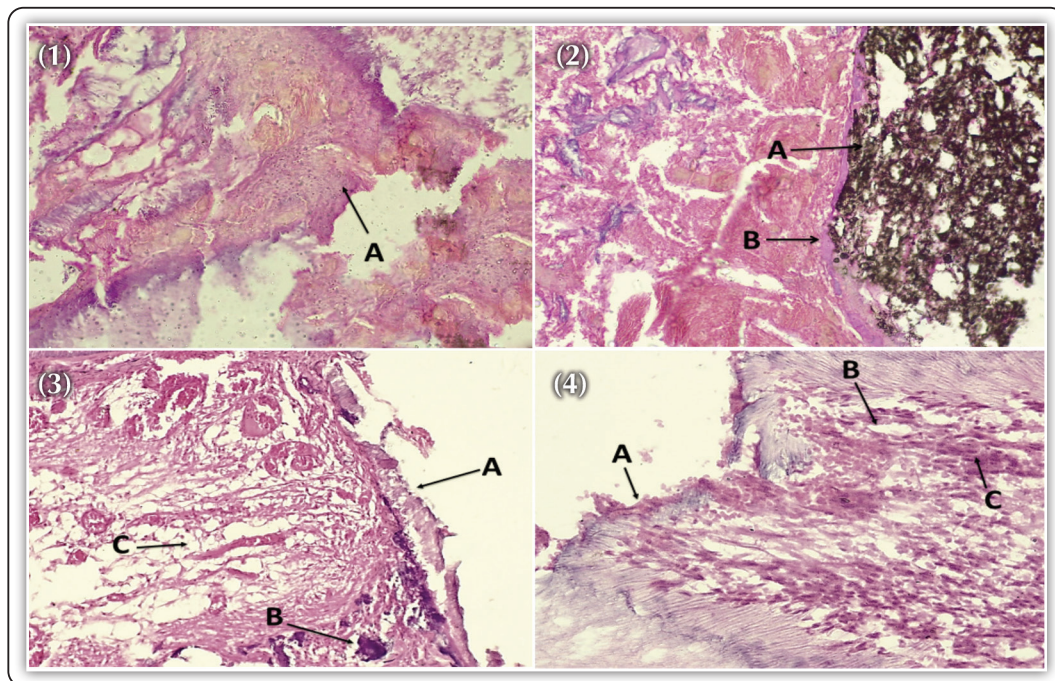


FIG (6) (1) Laser; (A) complete dentin bridge. H&E stain (40x); (2) MTA; (A) capping material (B) hard tissue calcification. H&E stain (40x); (3) TheraCal; (A) granulation tissue, (B) area of calcification dilated blood vessels, (C) edema. H&E stain (40x); (4) Ca(OH)<sub>2</sub>; (A) fibrous tissue, (B) blood capillaries, and (C) inflammatory cell infiltrate. H&E stain (40x)

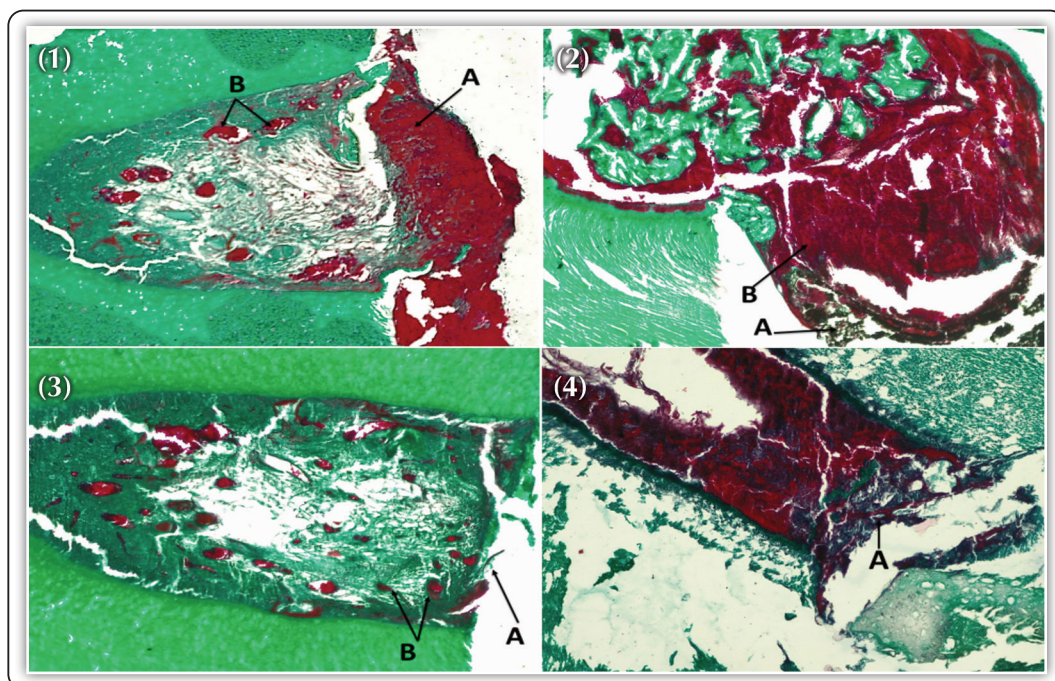


FIG (7) (1) Laser; (A) complete dentin bridge and (B) area of calcification. Masson trichrome stain (20x); (2) MTA; (A) capping material, (B) hard tissue bridge. Masson trichrome stain (20x); (3) TheraCal; (A) no signs of hard tissue formation, (B) area of calcification. Masson trichrome stain (20x); (4) Ca-(OH)<sub>2</sub> (A) fibrous tissues. Masson trichrome stain (20x)



In the mid-region, the examined pulp tissue for the Laser and MTA groups showed that; the odontogenic zone in some specimens return to a normal pseudostratified appearance and normal radicular pulp tissues without inflammatory cell infiltrations in some specimens. Numerous newly formed blood capillaries appear in the odontogenic zone. The blood vessels appeared less dilated and also engorged with RBCs, pulp core appeared with more or less normal architecture, multiple blood vessels, fibroblasts, and fibers. While TheraCal showed loss of odontogenic layer, there were ODLCs with numerous newly formed blood capillaries, organized granulation tissue showed increased activity through multiple mitotic figures,

and migration of UDMCs toward the dentinal wall to form ODLCs. Some specimens showed vacuolated odontoblasts, the blood vessels still dilated and engorged with RBCs, and organized granulation tissue still present in the pulp mid-region. The pulp core appeared more or less close to normal architecture. However, Ca(OH)<sub>2</sub> showed that; the odontogenic layer appeared degenerated, vacuolated and ghost-like cells, underneath there, was granulation tissue formation with multiple fibroblasts and fibers. Area of calcification and edema were noticed, and the blood vessels were still dilated and engorged with RBCs in this group. (Figure 8)

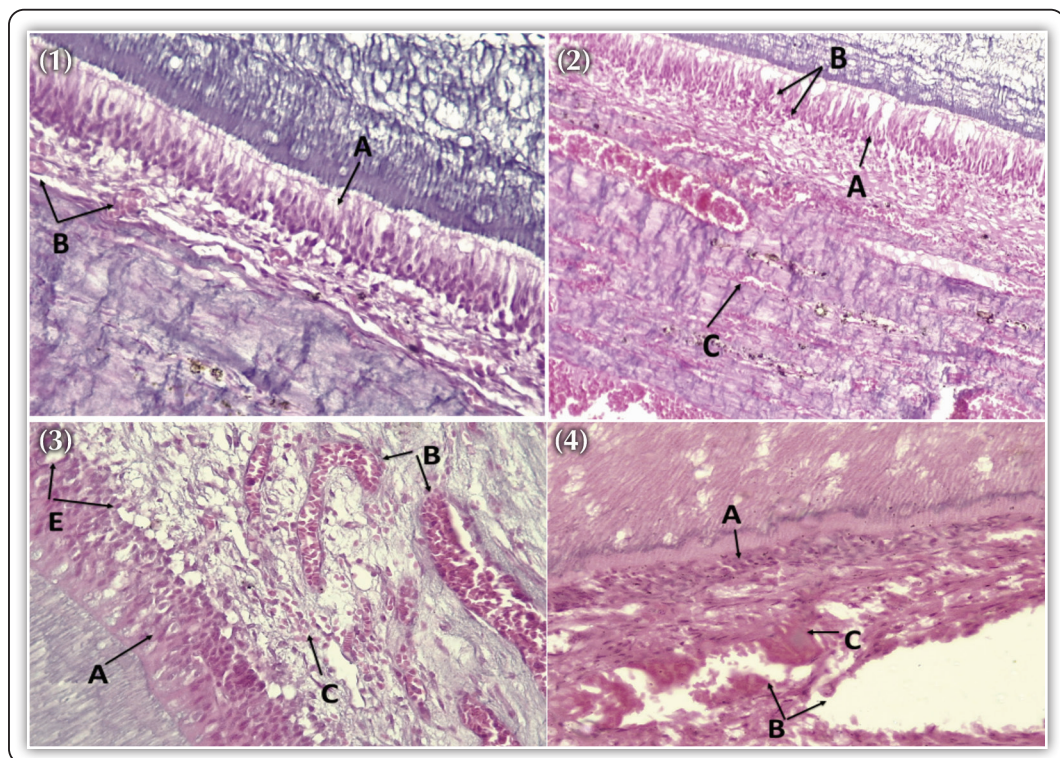


FIG (8) (1) Laser; (A) odontoblasts with normal pseudostratified appearance (B) proliferating blood capillaries. H&E stain (40x); (2) MTA; (A) normal odontoblastic layer, (B) newly formed blood capillaries, and (C) less dilated blood vessels engorged with RBCs. H&E stain (40x); (3) TheraCal; (A) close to normal odontoblastic layer, (B) dilated blood vessels engorged with RBCs, (C) inflammatory cell infiltrate, and (E) newly formed blood capillaries. H&E stain (40x); (4) Ca(OH)<sub>2</sub>; (A) degenerated odontoblasts, (B) edema and (C) area of calcification. H&E stain (40x)

## DISCUSSION

Preserving dental pulp vitality has been one of the primary goals of dental caries management for a long time. If the pulp chamber was accidentally exposed, it is preserved typically by the procedure of direct capping or pulpotomy that depends on the capability of the dentin-pulp complex to repair and form a new dentin layer <sup>(21)</sup>.

A dog model was chosen for this study because this was the most widely used experimental model in biological research. Additionally, it has been stated that the pulpal, apical, and periapical recovery course in dogs resembles that in humans. <sup>(15)</sup> Furthermore, the dogs' teeth are sufficiently large to enable the steps of cavity planning and to allow sufficient room required for placing the capping materials with good screen-ability and accessibility <sup>(22)</sup>.

In the current study, MTA, TheraCal LC, and Ca(OH)<sub>2</sub> were selected as tested materials because they could perform as a scaffold for the formation of reparative dentin. Because the dentinal fluids can engross within it, causing the discharge of Ca and hydroxyl ions (OH), the tooth responds positively to them to form apatite which plays a critical role in pulpal protection <sup>(23)</sup>.

The histological results of the Laser and MTA groups at 7 days' intervals showed granulation tissue formation with multiple macrophages, Russel bodies, and fibroblasts in addition to the formation of newly proliferated blood vessels, and the existence of mild inflammatory cell infiltrates. This is because MTA is less toxic, causes less pulpal inflammation, and can direct the progenitor cells' migration toward the material-pulp interface and stimulates their differentiation to odontoblastic cells secreting reparative dentin <sup>(24)</sup>. Additionally, it was stated that LLL therapy improves the chemotactic and phagocytic activities of human leukocytes. In the process of wound repair, activation of lymphocytes by laser radiation can make them more responsive to stimulatory mediators present in injured tissues <sup>(25)</sup>.

In the mid-portion of the pulp specimens treated with Laser and MTA after 7 days, the CRZ in some areas showed increased activity through multiple mitotic figures and migration of UDMCs toward the dentinal wall, the rest of the pulp demonstrated mild inflammatory cell infiltrates. These findings also coordinated with D'Arcangelo et al <sup>(26)</sup>, who stated that the use of MTA, as a capping material, resulted in infrequent inflammatory cells, and increased the proliferative activity of the odontoblasts after 7 days. Also, it was stated that therapeutic-laser treatment increases the phagocytic activity of macrophages during the tissue repair, facilitating cleaning of the wound and establishing the conditions needed for the subsequent proliferative phase <sup>(25)</sup>.

However, the histological results of the TheraCal and Ca(OH)<sub>2</sub> groups at 7 days' intervals showed liquefaction necrosis with some areas of clotted blood, and high inflammatory cell infiltrate, in the pulp core fibroblasts showed high mitotic index and scattered in collagen fiber meshwork. These results come in agreement with Lee <sup>(27)</sup> who stated that TheraCal specimens had a high extensive inflammation percentage, dilated blood vessels, and disintegration in the superficial cell layers and these changes may be because TheraCal contains resinous components such as hydroxyethyl methacrylate, bisphenol A-glycidyl methacrylate (Bis-GMA), and urethane di-methacrylate (UDMA) monomers. If TheraCal is un-polymerized, the free monomers can be dispersed through the dentinal tubules and reach the underlying pulpal tissues where they can employ their poisonous upshots. Also, Pereira et al, <sup>(28)</sup> and Oguntebi et al, <sup>(29)</sup> stated that the preliminary effect of Ca(OH)<sub>2</sub> on the pulp is destructive, and caused chemical grievance by hydroxyl ions (OH)<sup>+</sup> due to its high initial alkalinity that commanding superficial necrosis of the pulp with mild irritation with the existence of moderate to the severe cellular inflammatory response.

The histological results of the Laser and MTA groups at 14 days' intervals in the exposure site

showed hard tissue calcification closing the exposure site, mild inflammatory cell infiltrates, very high mitotic figures of fibroblasts, and migration of UDMCs to form odontoblast-like cells. These results agreed with Chicarelli et al<sup>(30)</sup> who suggest that The MTA groups showed induction of amorphous mineralized material in the middle of cellular tissue and scattered scanty inflammatory cells. Moreover, many authors<sup>(25,31)</sup> confirmed that LLL therapy stimulates endothelial cell proliferation, hence the formation of frequent blood vessels, and also excites the local microcirculation in combination with the relaxation of vascular smooth muscle, thus paying to the anti-inflammatory and analgesic effects of laser therapy. Other authors have assumed that the angiogenesis process is of prime importance for the renewal of the dentin-pulp complex<sup>(13)</sup>. Furthermore, it was affirmed that endothelial cells are essential for elaborate dentin and that progenitor cells migrate from the pulp in reply to endothelial cell injury<sup>(32)</sup>.

The histological results of the TheraCal and Ca(OH)<sub>2</sub> groups at 14 days' intervals showed granulation tissue formation beneath parts of capping material, with no signs of hard tissue formation. These results agreed with Andrei et al<sup>(33)</sup>, who proved that the TheraCal LC specimens lacked the deposition of hard tissue, most likely due to pulp necrosis. These results also come in agreement with Oguntebi et al<sup>(29)</sup>, who reported moderate to a severe cellular inflammatory response to Ca(OH)<sub>2</sub> due to its initial high alkalinity.

The histological results of the Laser and MTA groups at 42 days' intervals in the exposure site showed partial to complete dentin bridge formation closing the exposure site, a slight inflammatory reaction. The present findings coordinated with Dammaschke et al<sup>(24)</sup> and Lee et al<sup>(27)</sup>, who evident formation of newly mineralized tissue in the area of investigational exposure to a varying extent with nearly free of inflammation. Also, the results of Suzuki et al<sup>(34)</sup>, stated that the use of a laser

resulted in complete dentin bridge formation with the formation of newly odontoblast-like cells seen beneath the dentin bridge connected to the primary odontoblasts at both ends of the exposure.

The histological results of the TheraCal and Ca(OH)<sub>2</sub> groups at 42 days' intervals in the exposure site showed the formation of partial and thin fibrous tissue at the exposure site while in some specimen's granulation tissue closing the exposure site, inflammatory cells infiltrate. This comes in agreement with Lee et al<sup>(27)</sup>, who suggested that TheraCal had low quality for the formation of calcific barriers, with widespread inflammation, and fewer promising odontoblastic layer configurations. This could be explained by the existence of free monomers with their known toxic effects which could be dispersed through the dentin to the underlying pulp tissues. While areas of calcification and edema were noticed in specimens treated with Ca(OH)<sub>2</sub>. These results agreed with and Nakamura et al<sup>(35)</sup>, who observed distinctive liquefaction necrosis in the pulp tissues found close to the capping material in teeth treated with Ca(OH)<sub>2</sub>. However, they observed only small amounts of predentin onto the primary dentin walls underneath the necrotic layer.

Also, the histologic examination results in the current study at the mid-portion showed that the odontogenic zone in some specimens return to normal with its pseudostratified appearance with numerous blood capillaries and some specimens showed odontoblast-like cells. This result was confirmed by Suzuki et al<sup>(34)</sup>, who stated that the pulpal morphology is a return to normal after the ending of the inflammation response.

## CONCLUSIONS

The use of Laser, and MTA as pulp-capping therapies has a considerable positive histological reaction on the dentin-Pulp organs when compared with TheraCal and Ca(OH)<sub>2</sub>. MTA specimens form a complete dentine bridge after the end of the

follow-up period. However, Laser specimens from incomplete dentine bridge after the end 42 days. The pulp tissue in both of MTA and Laser specimens returns to its normal structure by the end of follow-up periods. While the pulp specimens of TheraCal and Ca(OH)<sub>2</sub> form granulation tissues after the end 42 days of follow-up period. TheraCal has a comparable result when compared with Ca(OH)<sub>2</sub>.

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