Evaluation of immunohistochemical activity of dentin-pulp organs of dog's teeth after stimulation with laser, MTA, and theracal therapies

Ehab Hammed Mustafa Elwardany
Assistant lecturer, Oral and Dental Biology, Faculty of Dental Medicine, (Boys-Assiut), Al-Azhar University, Egypt.
Corresponding author email: drehab.elwardany@gmail.com

Hany Abd elhamid Sherif
Professor, Department of Oral and Dental Biology, Faculty of Dental Medicine (Boys-Cairo), Al-Azhar Universitv, Egypt.

Mohamed Mounir Ali
Lecturer, Department of Oral and Dental Biology, Faculty of Dental Medicine, (Boys-Cairo), Al-Azhar Universitv, Egypt.

Hany Mahmoud Mahmoud
Lecturer, Department of Oral and Dental Biology, Faculty of Dental Medicine, (Boys-Cairo), Al-Azhar Universitv, Egypt.

Abstract---Objectives: This immunohistochemical study was performed to assess the different immunohistochemical activities in dentin-pulp organs in the pulp of mongrel dogs after stimulation with low-level laser (LLL), mineral trioxide aggregate (MTA), and TheraCal light-cured (LC) as pulp therapy and compare their effect with the conventional calcium hydroxide (Ca(OH)) cement material. Materials and Methods: A total of 8 male mongrel dogs 12-18 months old and weighing 10-15 kg were selected to be enrolled in this study. Animal choice, administration, surgical protocol, and provision were completed according to regular techniques standards by the Faculty of Veterinary, Cairo University, Egypt. All surgical techniques were accomplished under general anesthesia (GA) in a sterile operating room under aseptic circumstances with restricted isolation. The pulp exposure procedure was carried out through the preparation of a Class V cavity on the buccal surfaces of the animal premolar teeth. In each animal four premolar teeth were involved and allocated to four different groups grounded on the pulp therapy procedure (group I:

LLL; group II: MTA; group III: TheraCal LC; and group IV; Ca(OH). The final cavity restoration was glass ionomer cement (GIC). The immunohistochemical analysis was completed following the established methods. The transforming GF "growth factor" (TGF-β1), and vascular endothelial growth factor (VEGF) were recorded as area percentage (area%) at the site of exposure and the mid-region at three distinctive follow-up intervals; 7, 14, and 42 days. Results: The statistical results of the immunohistochemical analysis showed that the MTA had a significantly higher response regarding TGF-β1, while LLL had a significantly higher response regarding VEGF when compared with each other and when compared with TheraCal LC and Ca (OH) at different follow-up periods. Also, the results revealed that the lower significant results were recorded with Ca(OH) when compared to TheraCal LC regarding TGF-β1 and VEGF. However, the results showed that these immunohistochemical activities decreased significantly with time in all pulp therapies. Conclusion: The use of Laser and MTA as pulp dressing therapies have the higher and more significant positive immunohistochemical activities regarding VEGF and TGF-β1 respectively when compared with TheraCal and Ca (OH). While TheraCal LC has a significantly higher positive result when compared with Ca (OH).

***Keywords*---**Dentin-Pulp Organ, Dogs, Immuno-histochemical, Laser, MTA, TheraCal.

Introduction

The main core of conservative dentistry depends on the preservation of the dentin-pulp complex in a healthy condition with appropriate function which consequences in the efficacious healing of offended dental pulp. [1] The features of precise healing of exposed dental pulp encompass reorganization of the injured pulp tissues, differentiation of de-novo odontoblastic-like cells (ODLCs) from the pre-odontoblastic cells, and accordingly remedial of the tissues of dentin through the creation of what is called "reparative dentin". [2,3]

The process of lining the unprotected pulpal tissues with biocompatible capping therapies is known as "direct pulp capping" and involves the placement of these therapies on the pulpal tissue exposed because of an accidental traumatic injury. [4,5] The targets of this therapeutic expanse are the realm of the health of pulpal tissues by protecting the pulp against the invasion of bacteria, encouraging the pulpal organ to divide at the site of exposure, and originating the formation of a dentin-like bridge and is called "vital pulp therapy (VPT)". [6,7]

The usage of Ca(OH) as a direct capping material during VPT is a gold standard method, however, using Ca(OH) has frequent weaknesses, comprising its undue initial solubility and arbitrary treatment sequels. [8-10] Therefore, an assortment of newly introduced capping materials has been projected as alternatives; one such alternative material is MTA. [11] In this regard, MTA has been standard as a biocompatible material that persuades incomparable induction for the

development of hard dental tissue. [12,13] Predominantly, MTA was used in endodontics therapy to entirely close off the communication passageways between the space of the root canal system and the exterior surfaces of the tooth. [14]

TheraCal LC is another biocompatible material used for capping the exposed pulp in the case of VPT. [15] TheraCal is a calcium-silicate-based liner modified with light-cured resin (LCR) that is anticipated in the dental market for use in miscellaneous vital pulp therapies. It was affirmed that TheraCal LC was introduced as a soothing cytotoxic LCR liner. [15,16] Moreover, TheraCal LC has been stated to have inferior solubility, high alkaline pH, and grander Ca release when compared with the traditional types of MTA or Ca(OH). [15,17] Moreover, in an earlier Vivo study, TheraCal LC has hard tissue formation capability that has been labeled to be correspondent to pure Portland cement and better than the conventional Ca(OH) or GIC without a substantial inflammation for the pulpal tissues. [18]

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. [19] It was stated that when laser light in a suitable dosage interacts with tissue or cells their functions can be motivated. [20,21] Therefore, nowadays low-level Laser therapy (LLLT) has been used as a bio-stimulator for tissue healing, as it helps to enhance cell proliferation, local circulation, and synthesis of collagen. [19-21]

Additionally, it was stated that the different soluble proteins like TGF-β1can be extorted from the dentine matrix and can employ an orthodox biological effect on the stem cells of the pulp and encourage the repair mechanisms of the mineralized hard tissues. [22,23] Moreover, VEGF is a signal heparin-binding protein known as vascular permeability factor and it is released from the protein GFs, therefore, it indicates the amount of these protein GFs. [24]

However, the quantity of TGF-β1 and VEGF that are liberated from the dentin matrix in response to the capping materials, has not been established. Therefore, this current study was directed to assess the immuno-histochemical activities of the dentin-pulp organ in response to various treatment modalities of MTA, and TheraCal LC in dog pulpotomy models in terms of TGF-β1 and VEGF and compare it with Ca(OH) as the gold standard pulp capping material.

Materials and Methods
Animal selection and operative procedures

A total of 8 male mongrel dogs aged between 12-18 months and weighing 10-15 kg were selected to be enrolled in this study. The enrolled dogs had intact dentition and a healthy periodontium. Animal assortment, management, surgical protocol, and provision were fulfilled following the regular measures permitted by the Faculty of Veterinary, Cairo University, Egypt. The involved animals were exposed to free admission to diet and water.

All operative measures were accomplished under GA in a sterile operating room under aseptic situations with partial isolation. The dogs were intravascularly (IV)

injected by tramadol (1 mg/kg) and injected intramuscularly (IM) by xylazine (0.2 mg/ kg) and zoletil (5 mg/kg). Subcutaneous (SC) injection of enrofloxacin (5 mg/kg) was given before and after management and intraoral (IO) amoxicillin-clavulanate (12.5 mg/kg) was given for 5 to 7 days postoperatively to frustrate infection. [25]

Cavity preparation

The operative field was sterile with 0.2% chlorhexidine (CHX) and a dry field was readily gotten using gauze and cotton rolls. [26] Buccal Class V cavities were made on the gingival third of the buccal surface of each tooth roughly coronal to the gingival margin with 0.5 to 1-mm. A new bur was used on each quadrant to endorse suitable cutting efficacy. [27] The deepness of the cavity preparation is assorted rendering to the anatomy of each tooth using a sterile round bur # 2 under cooling with saline solution (SS). Pulp exposure was attained in the central of the cavity floor. After these procedures, the cavity was irrigated and the dentin debris was removed by the use of 10 ml of SS. Finally, hemostasis was managed by hiring a cotton pellet over the exposure sites for 10 seconds. [28] (Figure 1)

Figure (1): Pulp exposure of dog premolar teeth.

Sample grouping and restoration:

Four premolar teeth in each doge were involved and assigned to four distinct groups based on the therapeutic method as follows; group I: LLL; group II: MTA; group III; TheraCal LC, and group IV; Ca(OH).

In group 1; The LLL used was a soft diode laser (Pocket Laser, Orotig. Med, S.r.I, Italy) based on an aluminum gallium arsenide (AlGaAs), with a light source of 660 nm, and power intensity of 3-mW and 18 J/cm^2. LLL therapy was harmonized at 4-second exposures per point; buccal, palatal, and perpendicular to the tooth axis at a 0.5-1 cm distance from the tooth surface then calcium hydroxide was placed on exposure site. [29] In group 2; MTA (ProRoot, Dentsply, Tulsa, OK) powder was mixed with distilled water at 3:1 by volume by using a metal spatula on a glass

slab following the manufacture instructions and then placed to the exposure area immediately after mixing. [11] In group 3; TheraCal LC paste (Bisco Inc, Schaumburg, IL, USA) in a pre-mixed syringe, was placed directly from the dispensing tips to the exposure area in 1-mm thickness and extended for 1-mm onto sound dentin outside the exposure, and then light-cured for 20 seconds. [16] In group 4; Ca (OH) (Two-paste system, Dycal, Dentsply Caulk, Milford, USA, LOT 023407) was mixed with plastic spatulas trailing manufacturer instructions on a paper pad and placed directly to the exposure site using a special carrier. [8] For all cavities in all groups the GIC (Fuji IX GP) was used as a closing restoration.

Samples preparation

The dogs were sacrificed at 7, 14, and 42 days respectively after the completion of the operative procedures. The tooth samples were fixed in formalin (10%) for two 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 as a buffer for additional two weeks. Then, the samples were washed with running water to remove any decalcifying leftovers. After that sample dehydration was executed in ethyl alcohol (EA) in ascending concentration grades starting at 70% till 100% and methyl benzoate (MB) for one day. To remove the EA residue, paraffin benzol (PB) was used for 2 hours. Samples were then immersed in paraffin wax "in three successive waves" and then transferred in wax blocks of the apposite size to be predisposed for cutting. The tissue sample cutting was completed using microtome for serial sections of 4-6 um thick. [30]

Immunohistochemical examination

TGF-β1 examination:

After sample preparation, the sections were deparaffinized with xylene, hydrated in a series of downward grades of EA, and then washed briefly with tap water and phosphate-buffered saline (PBS) at pH 7.4. They were incubated in 0.3% H_2O_2 in methanol alcohol for 10 min. The normal serum was used for 10 min to block nonspecific protein binding. For the primary antibodies, the sections were incubated for 12 h at 4°C with polyclonal anti-TGF-β1 (TGF-β1 (V) sc-146; Lot #F2306, Santa Cruz Biotechnology, Santa Cruz, CA, USA). They were then rinsed with PBS three times for 5 minutes each time and incubated with biotinylated secondary antibodies for additional 10 minutes and with streptavidin–enzyme conjugate solution for another 5 minutes. The antibody-localized antigen (ALA) was then detected by peroxidase activation of 3, 3′-diaminobenzidine for 10 min to give it brownish discoloration. [31] The area percentage (% area) was detected by a special microscope (Leica Q Win V3).

VEGF examination

The process of immunostaining for VEGF was performed using the alkaline phosphatase-anti-alkaline-phosphatase method (APAAP) with polyclonal antibody (Santa Cruz, CA). Sections of 4 μm were cut and mounted on Poly-L-lysine-coated slides. Paraffin sections were dewaxed by xylene, rehydrated, and finally washed in Tris buffer (pH 7.6) for 10 min. VEGF required proteinase K predigestion in a

working solution of 0.4 mg/ml (Dako Corporation, Carpinteria, CA) for 10 min at room temperature. The following steps were optimized by automatic staining (Dako, TechMate 500). Sections were incubated with primary antibody solution for VEGF at a dilution of 1:400 at room temperature. Slides were rinsed in buffer (Buffer Kit, Dako), and immunoreaction was completed with the APAAP kit (Dako). VEGF was assessed in the vessels, in the cells of the inflammatory infiltrate (INCIs) (mainly lymphocytes, plasma cells, and neutrophils), and fibroblasts. [32] The area percentage (% area) was detected by a special microscope (Leica Q Win V3).

Statistical analysis

A one-way ANOVA test was used to compare the efficacy of different materials at different follow-up periods. The post hoc Tukey test was used for multiple comparisons within every two groups. The significance level was set at $P < 0.05$.

Results

TGF-β1 Results:

After 7 days, immunoreactivities were observed on extracellular matrices underneath, subjacent, and close to pulp exposure sites, walls of blood vessels (BVs), dentinal tubules, pulp cells, and odontoblastic layers. Immunohistochemical examinations revealed weak and mild positive immunoreactivities for TGF-β1 in TheraCal and calcium groups. In MTA and laser groups there was a marked increase of positive immunoreactivities for TGF-β1 after pulp exposure. According to image and data analysis which investigated the area % of TGF-β1 presented in dentin-pulp organ, 7 days' MTA treated dental pulp showed the highest immunoreactivity expressed in dentin-pulp organ overall experimental periods and groups. (Figure 2)
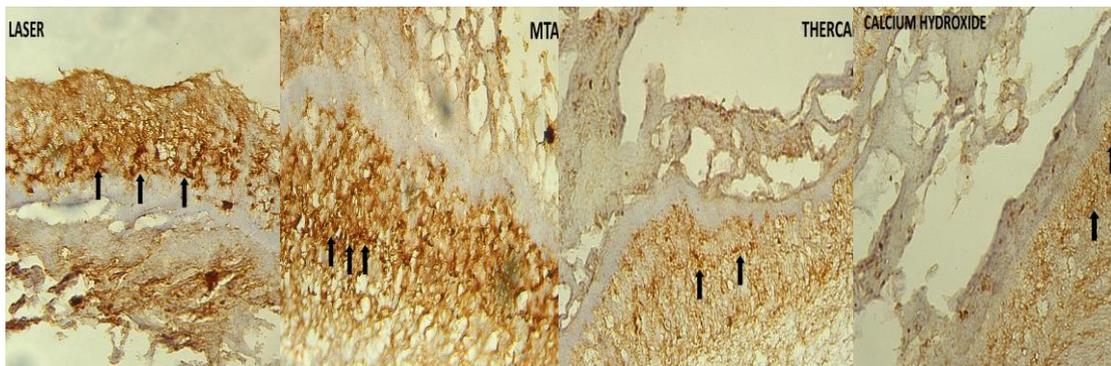
Figure 2: Immunohistochemical slides of four groups after 7 days showing: mild positive and weak immunoreactivity for TGF-β1 sequestrated in TheraCal and Ca(OH) groups, increase positive immunoreactivity in MTA and laser groups at 40x.

After 14 days, the immunohistochemical examinations of four groups showed positive expressions of TGF-β1 in the newly secreted matrix and collagen with

weak and mild immunoreactions detected in other dental pulp areas. Also mild to moderate positive immunoreactivity for TGF-β was distributed inside dentin-pulp tissue. The newly secreted matrix by odontoblast-like cells, nearby odontoblastic layers, and dentinal tubules showed positive reactions. (Figure 3)
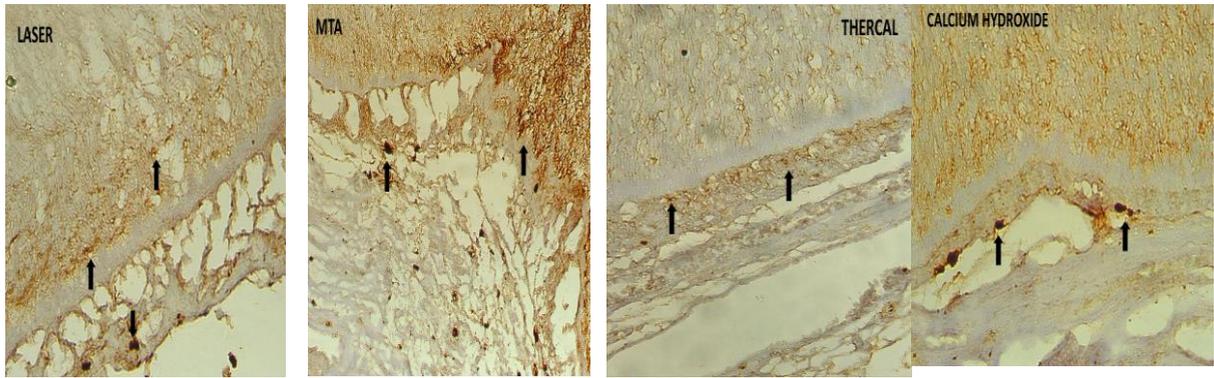
Figure 3: Immunohistochemical slides of four groups after 14 days showing: weak positive immunoreactivity for TGF-β1 in TheraCal and Ca(OH) groups, moderate positive immunoreactivity in MTA and laser groups at 40x.

While, after 42 days, the results showed a marked decrease of positive immunoreactivity for an antigen-antibody complex of TGF-β1 of four groups sequestrated on an extracellular matrix secreted by pulp cells. (Figure 4)
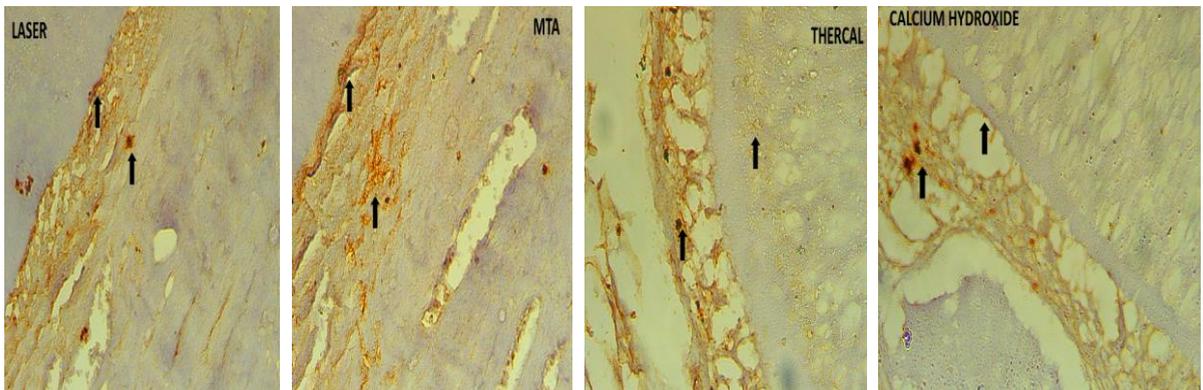
Figure 4: Immunohistochemical slides of four groups after 42 days showing: weak positive immunoreactivity for TGF-β1 in TheraCal and Ca(OH) groups, moderate positive immunoreactivity in MTA and laser groups at 40x.

VEGF results

After 7 days, immunoreactivities were perceived on the walls of BVs and PBC. Immunohistochemical examinations revealed weak and mild positive immunoreactivities for VEGF in TheraCal and calcium groups. MTA and laser groups marked an increase of positive immunoreactivities for VEGF after pulp exposure was seen. (Figure 5)
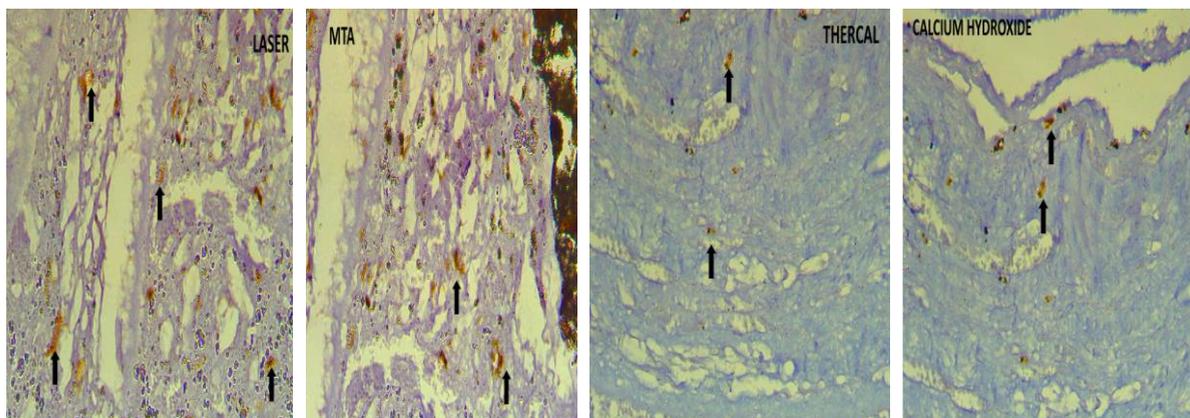
Figure 5: Immunohistochemical slides of four groups after 7 days showing: mild positive and weak immunoreactivity for VEGF in TheraCal and Ca(OH) groups, increase positive immunoreactivity in laser and MTA groups at 40x.

After 14 days, immunoreactivities were observed on the walls of BVs and proliferating blood capillaries. Immunohistochemical examinations revealed weak and mild positive immunoreactivities for VEGF in TheraCal and calcium groups, while in MTA and laser groups moderate to high positive immunoreactivities for VEGF after pulp exposure was observed. (Figure 6)
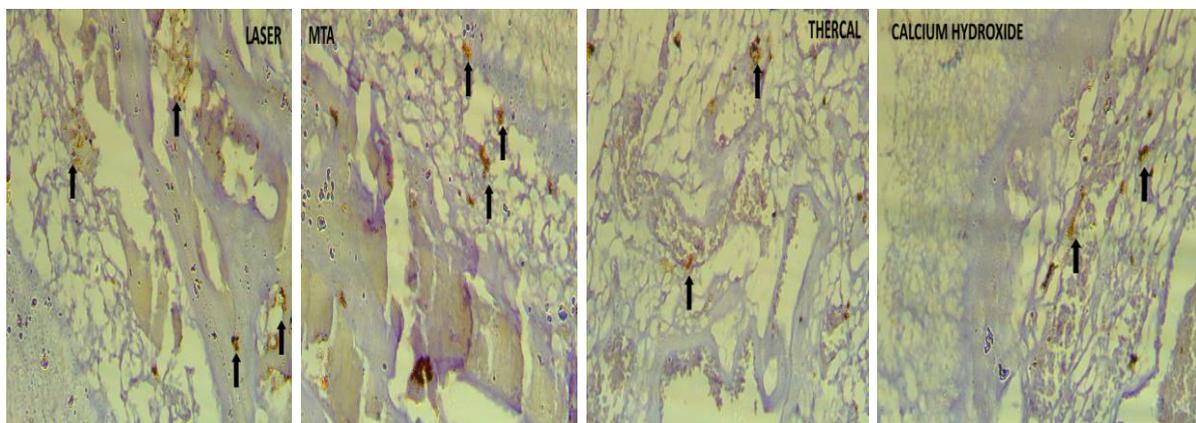
Figure 6: Immunohistochemical slides of four groups after 14 days showing: mild positive and weak immunoreactivity for in TheraCal and Ca(OH) groups, moderate positive immunoreactivity in MTA and laser groups at 40x.

While, after 42 days, the results showed a distinct reduction in the positive immunoreactivity for VEGF in four groups. (Figure 7)
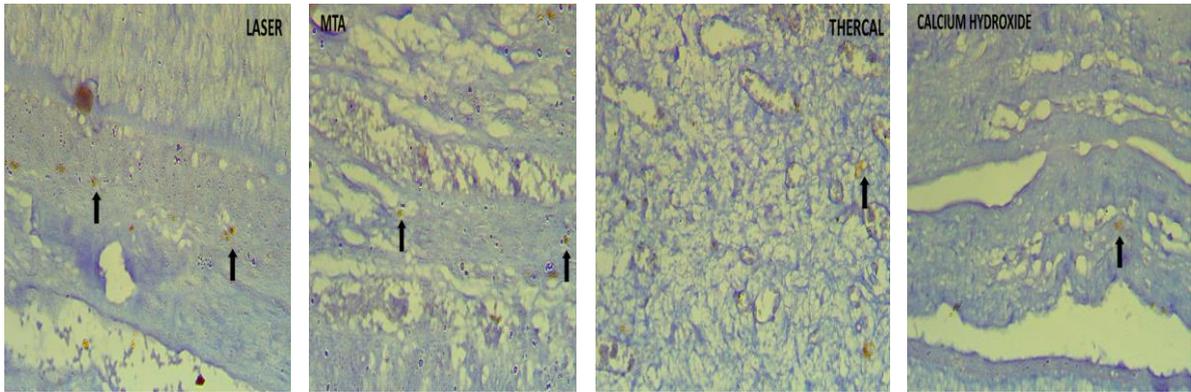
Figure 7: Immunohistochemical slides of four groups after 42 days showing: mild positive and weak immunoreactivity for in TheraCal and Ca(OH) groups, moderate positive immunoreactivity in MTA and laser groups at 40x.

Statistical results:

According to immunohistochemical examinations and statistical analysis, LLL and MTA-treated samples showed the significantly higher immunoreactivity expressed in dentin-pulp organs than TheraCal and calcium groups at all follow-up periods. Also, the results showed a decrease significant decrease in the results of immunoreactivities in order with the periods of scarification in the current study. According to data and statistical analysis, 42 days experimental period among all tested groups showed the lowest concentration of TGF-β1 and VEGF in the dentin-pulp organ. Also, the results presented that the MTA showed significantly higher TGF-β1 concentration results when compared to the other groups. While Laser presented significantly higher VEGF concentration results when compared to the other groups. (Tables 1 - 4)

Table (1): Comparison of TGF-β1 at different follow-up periods among the tested groups

Variable	Laser (mean± SD)	MTA (mean± SD)	TheraCal (mean± SD)	Ca(OH) (mean± SD)	P-value
7 days	40.51±1.21	43.68±1.56	28.56±0.79	22.51±0.74	<0.00001*
Sig. Level per-groups	P1=0.00048*; P2=0.00000*, P3=0.00000*; P4=0.00000*; P5=0.00000*; P6=0.00000*				
14 days	20.01±1.01	20.07±1.01	15.58±0.78	12.93±0.46	<0.00001*
Sig. Level per-groups	P1=0.9993ns; P2=0.00000*, P3=0.00000*; P4=0.00000*; P5=0.00000*; P6=0.0014*				
42 days	9.37±0.51	10.20±0.36	7.16±0.38	5.98±0.27	<0.00001*
Sig. Level per-groups	P1=0.00727*; P2=0.00000*, P3=0.00000*; P4=0.00000*; P5=0.00000*; P6=0.0023*				
*; significant at P < 0.05. ns; non-significant P > 0.05. – P1; Sig Level between Laser and MTA. – P2; Sig Level between Laser and TheraCal. – P3; Sig Level between Laser and Ca(OH). – P4; Sig Level between MTA and TheraCal.					

– P5; Sig Level between MTA and Ca(OH).	
– P6; Sig Level between TheraCal and Ca(OH).	

Table (2): Comparison of TGF-β1 of different materials at different follow-up periods

Variables	7 days (mean± SD)	14 days (mean± SD)	42 days (mean± SD)	P-value
Laser	40.51±1.21	20.01±1.01	9.37±0.51	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			
MTA	43.68±1.56	20.07±1.01	10.20±0.36	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			
TheraCal	28.56±0.79	15.58±0.78	7.16±0.38	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			
Ca(OH)	22.51±0.74	12.93±0.46	5.98±0.27	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			
*; significant at P < 0.05. ns; non-significant P > 0.05. – P1; Sig Level between 7 days and 14 days. – P2; Sig Level between 7 days and 42 days. – P3; Sig Level between 14 days and 42 days.				

Table (3): Comparison of VEGF at different follow-up periods among the tested groups

Variable	Laser (mean± SD)	MTA (mean± SD)	TheraCal (mean± SD)	Ca(OH) (mean± SD)	P-value
7 days	42.23±0.84	39.15±1.02	27.67±1.24	19.87±1.63	<0.00001*
Sig. Level per-groups	P1=0.00153*; P2=0.00000*, P3=0.00000*; P4=0.00000*; P5=0.00000*; P6=0.00000*				
14 days	20.68±1.21	18.42±0.67	14.52±0.88	12.42±0.49	<0.00001*
Sig. Level per-groups	P1=0.00095*; P2=0.00000*, P3=0.00000*; P4=0.00000*; P5=0.00000*; P6=0.00205*				
42 days	10.22±0.68	9.20±0.42	6.13±0.33	5.10±0.23	<0.00001*
Sig. Level per-groups	P1=0.00420*; P2=0.00000*, P3=0.00000*; P4=0.00000*; P5=0.00000*; P6=0.00390*				
*; significant at P < 0.05. ns; non-significant P > 0.05. – P1; Sig Level between Laser and MTA. – P2; Sig Level between Laser and TheraCal. – P3; Sig Level between Laser and Ca(OH). – P4; Sig Level between MTA and TheraCal. – P5; Sig Level between MTA and Ca(OH). – P6; Sig Level between TheraCal and Ca(OH).					

Table (4): Comparison of VEGF of different materials at different follow-up periods

Variables	7 days (mean± SD)	14 days (mean± SD)	42 days (mean± SD)	P-value
Laser	42.23±0.84	20.68±1.21	10.22±0.68	<0.00001*

	P1=0.00000*; P2=0.00000*, P3=0.00000*			
MTA	39.15±1.02	18.42±0.67	9.20±0.42	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			
TheraCal	27.67±1.24	14.52±0.88	6.13±0.33	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			
Ca(OH)	19.87±1.63	12.42±0.49	5.10±0.23	<0.00001*
	P1=0.00000*; P2=0.00000*, P3=0.00000*			

*; significant at P < 0.05. ns; non-significant P > 0.05.
  − P1; Sig Level between 7 days and 14 days.
  − P2; Sig Level between 7 days and 42 days.
  − P3; Sig Level between 14 days and 42 days.				

Discussion

Stabilizing the vitality of tooth pulp has been one of the chief goals of carious tooth management. [1,2] If the pulp was exposed accidentally, it is conserved classically by the direct capping technique or pulpotomy that is contingent on the capability of the dentin-pulp organ complex to renovation and formation of a new mineralized dentin layer. [1-4]

The tooth pulp is composed mainly of connective tissue which is a highly vascular tissue. [3] Typically, the cells of the dental pulp can differ into protective and formative cells. [3,33] The protective cells include lymphocytes, macrophages, and mast cells while the formative cells are UDMCs, fibroblasts, pericytes, and odontoblasts. [4,33] The odontoblasts are accountable for the process of dentinogenesis, they are post-mitotic cells "i.e. once differentiated they can no longer divide and can only be replaced from the sub-odontoblastic cells layer". The fibroblasts are the commonest cells in dental pulp, they are accountable for discharging collagen and ground-like substances. [3,4,33]

In tooth pulp, the route of cell repair commences later to the control of the inflammation, with the replacement of the damaged or necrotic region by the UDMCs. [2-4] After differentiation, these mesenchymal cells developed tissues alike the earlier undamaged cells. [3,34] There are three consecutive stages of cell regeneration: slight inflammation supplementary with cell enlistment, and cell multiplying to fill the injury site, followed by cell differentiation to generate de-novo odontoblasts for the creation of reparative dentin. [34]

A dog model was elected 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. [25] Furthermore, the dogs' dentitions 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. [35]

In this study, MTA, TheraCal LC, and Ca(OH) were elected as tested materials because they could implement as a scaffold for the creation 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. [36] However, LLL therapy helps in the process of wound repair via activation of lymphocytes by laser radiation can make them more responsive to stimulatory mediators present in injured tissues. [37]

The exposure site was chosen to study the immunohistochemical activities in this study, because, at this site the vascular endothelial injury consequences in the stimulation of the complement cascade, platelet accumulation, and liberation of its granular contents. This platelet degranulation liberates GFs and elicits chemotactic signals. Then polymorphonuclear leukocytes (PMNs), tissue macrophages, lymphocytes, and blood monocytes, are drawn toward the injury site and are triggered to liberate cytokines that can excite angiogenesis. [21,38]

The GFs specifically TGF-β1; are considered an imperative in cellular signaling for guided stem cell migration, odontoblasts differentiation, and secretion of dentin matrix, therefore, TGF-β1 was selected as the tested GF. [34,39] Moreover, VEGF was elected as GF signaling in the present study because it is pondered to perform a substantial role in the processes of inflammation, angiogenesis, and pulp injury healing through various mechanisms, comprising deposition of collagen, and angiogenesis. [40]

The results of the present study revealed that MTA had a higher significant effect on the secretion of TGF-β1 GF followed by LLL therapy when compared to the other stimulators. This could be attributed to the previous finding which stated that the application of MTA has an express effect on the renewal potential of the tooth pulp and is correlated with an exceptional increase in TGF-β1 exudation from pulpal cells. [34] Additionally, the results of Arany P et al [41] stated that the LLL therapy had been established to increase truthfully the secretion of TGF-β.

However, the finding of the current study stated that LLL therapy had a higher significant effect on the immunoreactivities of VEGF followed by MTA when compared to TheraCal and Ca(OH) stimulators. This is because LLL therapy is able to stimulate the endothelial cells proliferation, and hence the formation of frequent BVs, in addition to stimulating local microcirculation and the process of angiogenesis which is essential in the process of tissue repair. [34,37] This result coordinates with Jiang et al, [42] who reported that VEGF levels in the laser-treated samples were significantly higher than in control samples 3 days after laser treatment.

However, the results of this study reported that TheraCal had limited immunoreactivities compared to MTA and LLL. This could be related to the cytotoxicity of the resin of TheraCal which could inhibit the cell metabolic activity, especially during the first week after its application according to Jeanneau et al, [43]. Additionally, it is the results of this study found that the Ca(OH) treated specimens had lower immunoreactivity compared to other treated groups. This could be attributed to the presence of OH+ which is blamable for the chemical trauma which resulted in the preliminary arresting of cellular activity of pulp tissues due to initial enzyme suppression of the pulpal tissues. [44] However, this cytotoxicity of TheraCal and Ca(OH) decreased significantly after the first week according to Buonavoglia et al, [44,45]. This could explain the marked increase in immunoreactivities of TheraCal after the first week of the present study.

Conclusions

Based on the finding of this animal model study it could be concluded that the use of LLL and MTA as pulp dressing therapies have the higher and more significant positive immunohistochemical activities regarding VEGF and TGF-β1 respectively when compared with TheraCal and Ca (OH). While TheraCal LC has a significantly higher positive result when compared with Ca (OH). However, the results showed that these immunohistochemical activities decreased significantly with time in all pulp therapies.

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