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Efficacy of photobiomodulation when used with calcium hydroxide for pulp capping of dogs' teeth

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***Abstract*---**Objective: The current study assessed the effect of calcium hydroxide [$Ca(OH)_2$] and Photobiomodulation [PBM] on pulp capping when [$Ca(OH)_2$] is used as a dental pulp capping material on dental pulp exposures of dogs' teeth. Methods: 48 teeth in 3 mongrel dogs were divided in random into two major study groups; group I where $Ca(OH)_2$ was used as a pulp capping agent and group II in which both $Ca(OH)_2$+PBM were used. The groups were equally divided according to the observation period following completion of pulp capping into Subgroup (A) 1week, Subgroup (B) 2 and subgroup (C)16weeks. The teeth were examined for histological inflammatory response as well as dentine bridge formation Results: With regards to inflammatory response at 1 week significantly less intense inflammation was observed in $Ca(OH)_2$+PBM (group II) compared to the $Ca(OH)_2$ (group I) for the same time period with no significant difference for between group I and group II for other time intervals. As for dentin bridge formation PBM+$Ca(OH)_2$ groups showed statistically significant thicker

dentine bridge formation at 16 weeks than $Ca(OH)_2$ alone group for the same time period with no significant difference for between group I and group II for other time intervals. Conclusions: Under the conditions of this study, laser irradiation appeared to be a beneficial adjunct in dental pulp capping procedures in which $Ca(OH)_2$ was the pulp capping material.

***Keywords*---**diode laser, dentinal bridge, direct pulp capping, $Ca(OH)_2$.

Introduction

The dental pulp has crucial defensive, nutritive, formative and sensory functions. Following pulp exposure, direct pulp capping procedures enable the pulp tissue to form a reactionary hard tissue barrier at the exposure site. Various pulp capping materials have been developed and used in dental pulp capping procedures with new materials continually being developed [1]. $Ca(OH)_2$ is the basic material for direct pulp capping because it can induce dentine bridge [2] as well as it has antimicrobial properties[3]. The formation of dentine bridge by $Ca(OH)_2$ has been describe in literature [4,5]

Other pulp capping materials were introduced more recently as substitutes for dental pulp capping procedures, however $Ca(OH)_2$ remains widely used due to its low cost and availability. PBM has can inhibit inflammation and encourage tissues to improve metabolic mechanisms [8]. In systematic review by Sleep et al (2021) it was found that PBM increased the metabolic ability of dental pulp. [9]. Moreover, low power laser of 810 to 903 nm wavelength stimulated repair of dental pulp tissue[10,11].

The use of PBM in conjunction with direct pulp capping [12,13] has been studied[14]. PBM casuses the dental pulp to secrete some proteins that allow dentin bridge formation cycle to happen, in addition to other proteins that initiate dentinogenesis[15-17].This study determined the effects of $Ca(OH)_2$ with and without PBM on exposed pulps of dogs' teeth at observation periods 1 week, 8 weeks, and 16 weeks after pulp capping.

Materials And Methods

Ethical approval

The National Research Centre [Egypt] gave ethical approval for this research 13/144.

Animal Model

Three male mongrel dogs with an estimated weight of 65 pounds and 18 months old, were quarantined for 10 days before pulp capping procedures study. Direct pulp capping was done on 16 teeth per dog and a total of 48 teeth were in this study. All teeth used were of health and no caires was observed. The dogs were followed up every day before any procedure was initiated.

Anesthesia

According to Dominguez et al (2003) [18 general anesthesia was achieved by f Ketamine injected intramuscularly. Local anesthesia was achieved by an infiltration injection of 1.8 ml of 2% lidocaine [18]

Pulp Capping

Two incisors as well as two premolars were treated in each quadrant. One of the incisors was treated with Ca(OH)2 while $Ca(OH)_2$+PBM treated the other. One of the premolars was treated with $Ca(OH)_2$ while Ca(OH)2+PBM treated the other. High speed diamond burs were used to obtain pulp exposure. [18] In all teeth, $Ca(OH)_2$ [Dycal, Dentsply, USA] was mixed and used to cover the exposure.Glass ionomer [Fuji II LC, GC Industrial Co, Tokyo, Japan] was used us a final restoration in all teeth.

Laser irradiation

A therapeutic laser apparatus "soft-laser-SL-202" [Petrolaser, Stachek, 47, Saint-Petersburg, Russia] at 50 mW power and 870 nm wavelength was used as an infrared PBM laser source at a continuous mode. Infra red laser was used to irradiate half of the treated teeth on each dog by consecutive sessions immediately after restoration of the tooth and then at 48 hours and finally at 96 hours with 48 hour span between laser treatments [19]. Only teeth on the right side of each jaw were lased while the contra-laterals were not lased [19]. The full laser application time through the study was 120 Seconds/tooth.

Study Groups

Teeth were grouped as shown in Table 1:

Table 1Showing grouping of all samples used in the present study

Group / Period	Group I ($Ca(OH)_2$)	Group II ($Ca(OH)_2$+PBM)
1 Week (A)	IA	IIA
8 Week (B)	IB	IIB
16 Week (C)	IC	IIC

Animal sacrifice and tissue processing

With accordance to Dominguez et al (2003) [18] the dogs were sacrificed after completing their previously decided dates at 1 week, 8 weeks and 16 weeks. Treated teeth were separated using a special saw and place in 10% formalin solution. Block specimens were then immersed in paraffin wax and sectioned serially at 5.0 with a microtome. Sections were obtaine and were stained with hematoxylin and eosin[18]

Histological assessment

Slides were examined by an oral histology specialist. Asgary et al's (2008) [20] scoring system ,with modification, to give a grade to specimens as seen in table [2]

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

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

Statistical Analysis

Categorical qualitative data of the scoring system were compared using Chi-square test. Inflammatory intensity and dentine bridge thickness quantitative results were compared using Mann Whitney test and Wilcoxon signed rank test. Statistically significant results were set at $P<0.05$.

Results

The outcomes of histological evaluation are detailed according to the inflammatory reponse and dentin tissue formation as follows:

Inflammatory Response Figures [1-3] Table [3][5]

With regards to *inflammatory type*, there was no statistically significant difference between Group I [Ca(OH)2 Only] and Group II [$Ca(OH)_2$+PBM] subgroups [Table 3]. For *inflammatory intensity* grading analysis according to Asgary et al was performed, there was no statistically significant difference between Group I [$Ca(OH)_2$] and Group II [$Ca(OH)_2$+PBM] subgroups. [Table 3] However when numerical analysis of was calculated–Table [5], it showed that at 1 Week Group II [$Ca(OH)_2$+PBM] showed a statistically significant less inflammatory intensity than Group I [$Ca(OH)_2$] [p=0.004]). Coming to the inflammatory extension, all inflammation was localized inflammatory reaction [grade ii] with no statistically significant difference between Group I [$Ca(OH)_2$] and Group II [$Ca(OH)_2$+PBM]. [Table 3]

Table 3 Demonstrating Inflammation Results of all Groups according to Asgary et al scoring system (modified)

Inflammation	Grading	IA	IIA	IB	IIB	IC	IIC
Type	I	6(75%)	5(63%)	0(0%)	0(0%)	0(0%)	0(0%)
	II	2(25%)	3(37%)	2(25%)	2(25%)	2(25%)	0(0%)
	III	0(0%)	0(0%)	6(75%)	6(75%)	6(75%)	8(100%)
	P Value	0.86		1.000		0.318	
Intensity	I	5(63%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	II	3(37%)	8(100%)	2(25%)	2(25%)	1(13%)	0(0%)
	III	0(0%)	0(0%)	6(75%)	6(75%)	7(87%)	8(100%)
	P Value	0.026*		1.000		0.586	
Extension	I	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
	II	8(100%)	8(100%)	2(25%)	2(25%)	1(13%)	0(0%)
	III	0(0%)	0(0%)	6(75%)	6(75%)	7(87%)	8(100%)
	P Value	1.000		1.000		0.586	

Table 4 Demonstrating dentine bridge Results of all groups according to Asgary et al scoring system (modified)

Dentine Bridge	Grading	IA	IIA	IB	IIB	IC	IIC
Continuity	I	8(100%)	7(87%)	1(13%)	0(0%)	0(0%)	0(0%)
	II	0(0%)	1(13%)	7(87%)	6(75%)	1(13%)	1(13%)
	III	0(0%)	0(0%)	0(0%)	2(25%)	7(87%)	7(87%)
	P Value	0.586		0.214		1.000	
Thickness	I	8(100%)	8(100%)	1(0%)	1(0%)	0(0%)	0(0%)
	II	0(0%)	0(0%)	3(37%)	4(50%)	2(25%)	1(13%)
	III	0(0%)	0(0%)	4(50%)	3(37%)	6(75%)	7(87%)
	P Value	1.000		0.866		0.814	

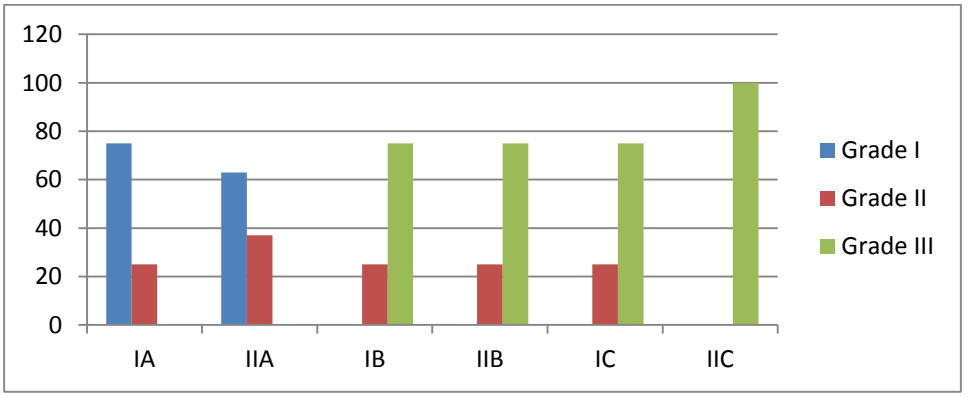

Figure 1 Bar Chart illustrating the frequency of inflammatory cell type in all groups at different observation times

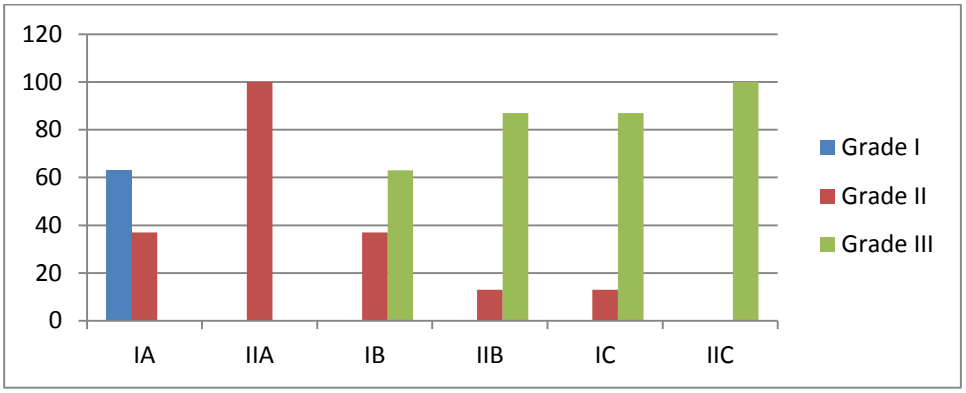

Figure 2 Bar Chart illustrating the frequency of inflammatory intensity in all groups at different observation times

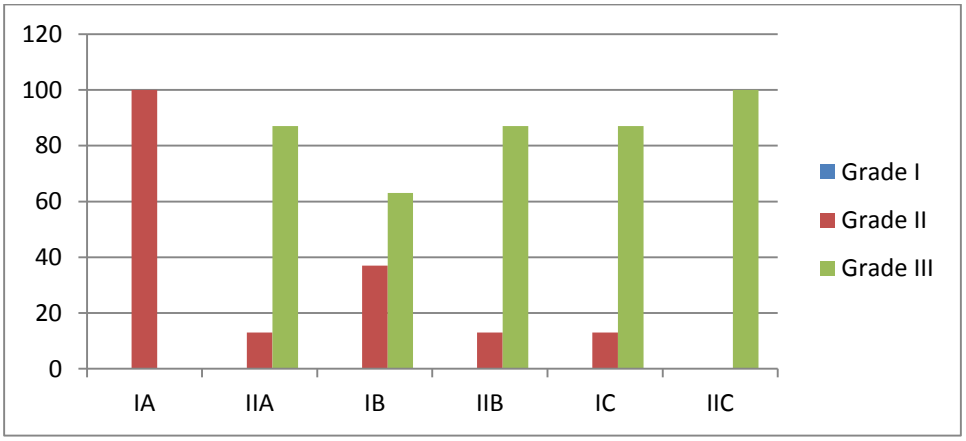

Figure 3 Bar Chart illustrating the frequency of inflammatory Extension in all groups at different observation times

Dentine bridge formation Figures [4-5] Table [4][6]

Regarding *Dentine Bridge Continuity* and *Dentine Bridge thickness* at all three observation times there was no statistically significant difference between Group I and Group II as seen in table [4] However when quantitative analysis of *dentine bridge thickness* represented in table [6] was performed, there was no statistically significant difference between Group I and Group II as seen in table [6]

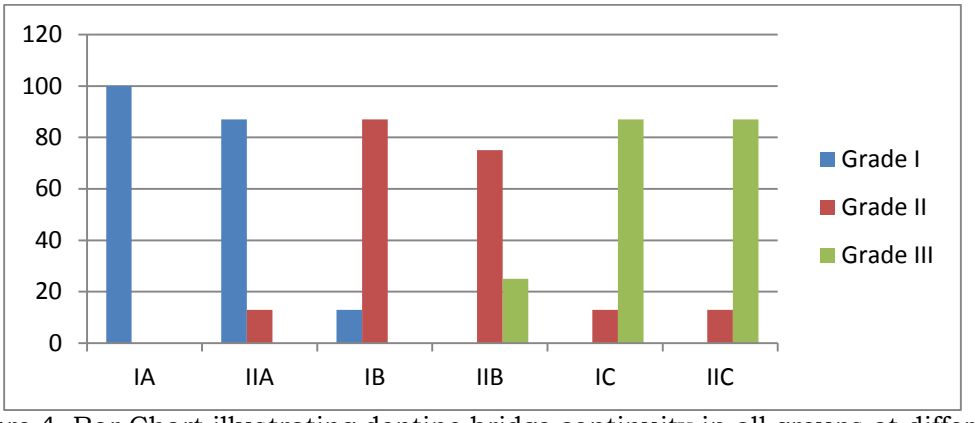

Figure 4 Bar Chart illustrating dentine bridge continuity in all groups at different observation times

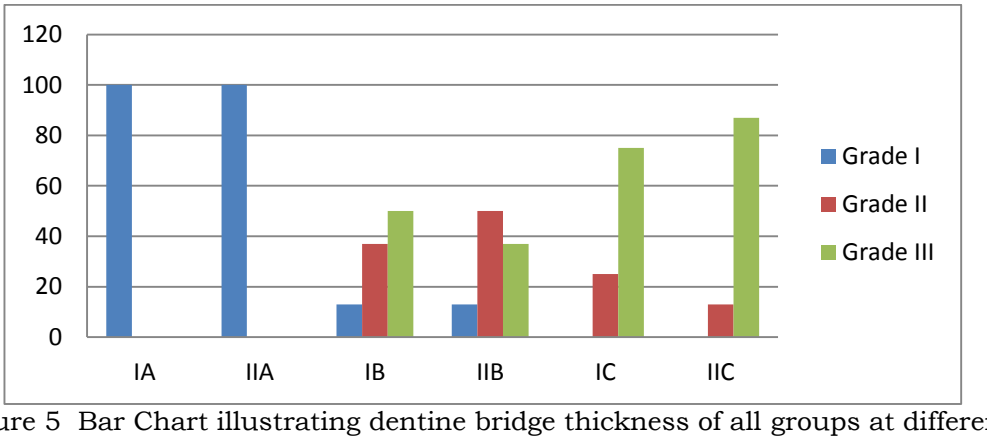

Figure 5 Bar Chart illustrating dentine bridge thickness of all groups at different observation times

Table 5 Demonstrating quantitative statistical analysis of mean inflammatory cell intensity

		Subgroup A	Subgroup B	Subgroup C
Group I				
	Range	73-44	42-0	32-0
	Mean+SD	61.75±12.139	9.25±17.335	4.00±11.314
Group II				
	Range	55-31	14-0	0
	Mean+SD	42.00± 7.616	3.25±6.042	0±0
	P value	0.004*	0.783	0.317

P-value ≤ 0.05 is significant.

Table 6 Demonstrating quantitative statistical analysis of mean Dentine Bridge Thickness

		Subgroup A	Subgroup B	Subgroup C
Group I				
	Range	0.000-0.000	340.3-12.5	488.75-119.75
	Mean+SD	0.000±0.000	203.219±105.439	347±138.03
Group II				
	Range	0.000-0.000	295.3-27.3	521.5-228.00
	Mean+SD	0.000±0.000	201.438±93.371	428.718±89.583
	P value	1.000	0.753	0.115

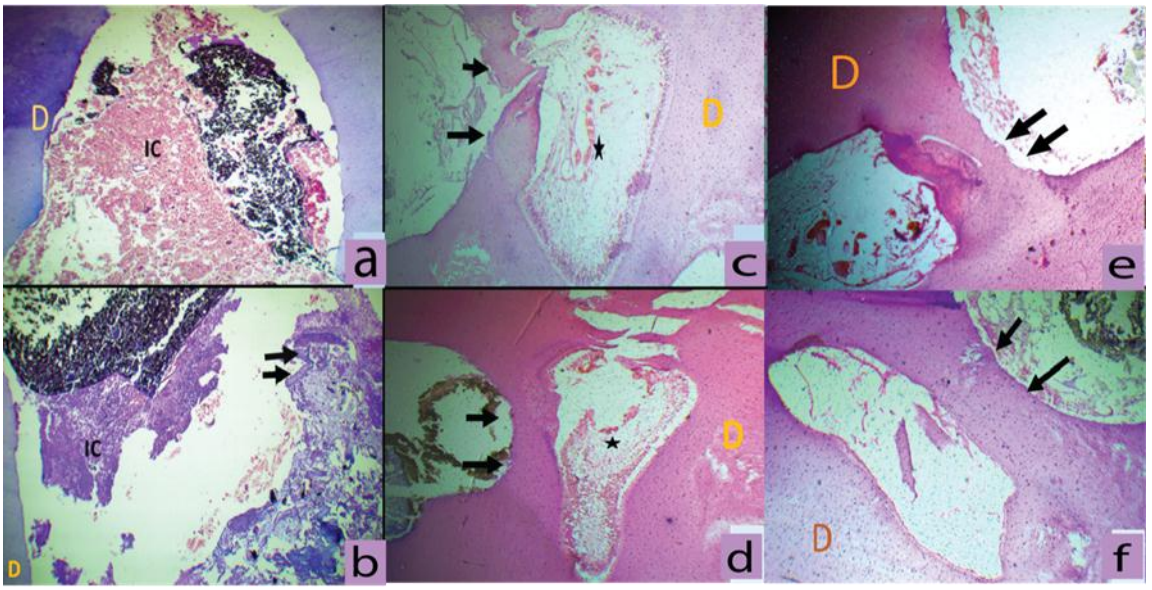

Figure 6 (x40): Photomicrograph of (a)1 week, (c) 8week and (e)16 weeks of Ca(OH)2 only group with gradual dentine bridge formation and inflammatory response decreasing with time Photomicrograph of (b)1 week, (d) 8week and (f)16 weeks PBM/Ca(OH)2 showing the dentin bridge formation and inflammatory response. [Arrow heads: Dentine Bridge. Stars: Inflammatory cells]

Discussion

The main purpose of direct pulp capping is to set an environment for the formation of a hard tissue calcific bridge to isolate the capping material from the dental pulp tissue. More than one direct pulp capping material or technique have been assessed in research to try to point out the advantages and disadvantage of these techniques. [21, 22] Numerous factors dictate the outcomes of direct pulp capping procedures [23,24] including absence of infection and inflammation as well as the dental materials used [25,26].

Despite that previous authors determined only one evaluation time usually 8weeks [27,28,29] or 150 days [18, 20] until the animals were sacrificed, the authors of

the current study chose three different time periods of 1 week, 8weeks and 16 weeks. This allowed the examination of inflammation and dentine bridge formation as a process from start up to 16 weeks.

In 2016 Bidar et al [30] found that the material used in pulp capping was more important than the use of laser irradiation. The authors of this study stated that a of limitation in their research was that the laser application was only done one time before putting the cap material on the pulp exposure [30]. This limitation was escaped in our research in which laser was irradiated in sessions after putting of the capping material at 0, 2 and 4 days.

Only Toomarian et al [19] prior to our research, examined the laser irradiation on dental hard tissue in consecutive sessions. They directed the laser at roots of lower molar teeth at spans of 48 hours to assess its effect on rat tooth root formation. They found that PBM makes the development of the molar rat roots faster with a lower inflammatory cell intensity.

De Santana et al [14] found that 780nm PBM increased number of odontoblastic cels after application towards the root area of incisor teeth of rats. Neiburger [31] showed enhanced healing of mucous membrane uclers following PBM therapy. The reason for using Glass ionomer restoration was because of its true chemical bond sealing [32] which is capable of protecting the pulp capping material and pulp tissue until final healing happens. The significantly less intense inflammatory reaction in the group II [$Ca(OH)_2$+PBM] than group I [$Ca(OH)_2$] can be backed by the research made by Bidar et al [30] who assessed the effect of laser therapy on pulp response of dogs' teeth. In the latter study, PBM groups showed the lowest inflammation if used in addition to a pulp capping material.

Dentine bridge formation is a mark of success of direct pulp capping [33]. In the current study, all pulp capping procedures in all groups were successful. Previously, In Arafa et al's [34] study all teeth pulp capping procedures were successful with no signs of pulp necrosis. A number of studies comparing different materials showed varying dentine bridge thickness and formation[35,36]. Some capping materials can produce more growth factors than $Ca(OH)_2$ [37,38] . These growth factors are crucial conditions for forming of dentine hard tissues [39].

Although not statisticall significant At 1 week and at 8 weeks there was a faster thickening of calcific bridge in the lased groups. Nawam et al [40] found that PBM at diferent energy densities lead to a statistically significant production of odontogenic genes. Moreover, Paschalidou et al [41] found that PBM at increased viability, and calcification of stem cells from primary teeth. In an attempt to elaborate on the effect of PBM on stem cell, Ohbayashi et al [42] concluded that PBM improved alkaline phosphatase related processes and certain molecular expression which is important for calcified tissue formation [43, 44]. The effects of 870 nm PBM on dentin barrier accelerated formation could be due to any of the mentioned mechanisms or a combination of any of them.

Conclusion

Under conditions used in this study, using PBM with 870 nm at 50 μW lead to a less intense inflammatory response with $Ca(OH)_2$ at 1week and is a beneficial adjunct to pulp capping.

Recommendations

Further materials at different laser irradiation parameters should be trialed to understand more the effectgs of PBM.

Conflict of Interests

The authors of this study have no interest or relation in any product, service, and/or company that has been used in this study

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