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Histological Evaluation of Photobiomodulation and Calcium Aluminosilicate on Direct Pulp Capping of Dogs' Permanent Teeth

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Abstract

Introduction: Photobiomodulation (PBM) is beneficial to biological tissues; depending on the optical dose that is absorbed by tissues, it can function as a biostimulative, analgesic, and anti-inflammatory mediator. Thus, the current research aimed to assess the impacts of PBM and Calcium aluminosilicate-based material on direct pulp capping (DPC) of dogs' permanent teeth through histological analysis.

Methods: To study DPC of dogs' teeth, we separated 24 canines and premolars obtained from mature, healthy mongrel dogs into four equal groups: group 1, which served as the control (exposed pulp was covered with a sterile polytetrafluoroethylene, Teflon tape); group 2, which received PBM treatment using a 980 nm diode laser with a 100 mw output power for one minute; group 3: Calcium aluminosilicate-based material; group 4: Calcium aluminosilicate + PBM. In accordance with the assessment period, each group was divided into three equal subcategories: (A) 1 week; (B) 2 months; (C) 3 months. The teeth were evaluated histologically for inflammatory response and dentine bridge formation.

Results: Statistical analysis detected that there was a significant difference between PBM, Calcium aluminosilicate cement, and the combination group of PBM and Calcium aluminosilicate related to the control group in variant evaluation periods regarding the inflammatory response and dentine bridge thickness through the histological analysis.

In relation to the inflammatory response after one week, the combined group (Calcium silicate cement + PBM) exhibited a significantly decreased intensity of inflammation compared to other groups at an identical time. As for dentin bridge creation, the PBM + calcium aluminosilicate group detected thicker dentine bridge creation at three months than other studied groups.

Conclusion: Combined with calcium aluminosilicate-based material, PBM using a 980 nm diode laser with output power of 100 mw for one minute decreased the initial inflammatory response and enhanced a complete thick dentine bridge formation.

Keywords: Calcium aluminosilicate-based material; Photobiomodulation; Direct pulp capping (DPC).

Introduction

Preservation of dental pulp is mandatory during conservative dental treatments.[1,2] Nowadays, various dental treatment approaches used for pulp healing depending on stimulation of the dental pulp cells.[3] Prevalent method of preserving the health and functionality of recently exposed dental pulp structures is through DPC.[2,4] The DPC procedure of permanent teeth can manage hemostatic iatrogenic tiny pulpal exposures by capping the recently exposed pulp tissue with a sealing material before filling the tooth with a restoration to prevent bacterial microleakage from an oral cavity to allow the formation of reparative dentin and pulp healing[5,6] There are different materials used in pulp capping like; Ca(OH), mineral trioxide aggregate (MTA), Ca aluminate cement, Ca phosphate-based material, Ca aluminosilicate cement, and Flavonoids,[2,7-12] which can be used for capping the exposed pulpal tissue. However, ideal pulp capping material does not exist yet.[11,12]

Lately, Well-Root™ PT (Gangwon-Do, Vericom, Korea) which is premixed calcium aluminosilicate-based material has been suggested for many dental treatments such as vital pulp therapies, root perforation repairing procedures, and different surgical applications.[9] Calcium aluminosilicate-based material can exhibit favorable physiochemical properties in the presence of water,[10] and it has excellent insolubility. In addition, it is radiopaque and does not shrink during the hardening phase. Moreover,

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some studies reported that it has encouraging biological properties. They found that it is able to stimulate mineralization by means of inducing bioactive reactions. Furthermore, it does not provoke pulpal or periapical tissue's inflammatory responses.[9-12]

Since the discovery of the photobiomodulatory impact of low-power laser irradiation, a multitude of dentists, surgeons, and physicians have applied low-level laser therapy in different clinical settings.[13] Photobiomodulation (PBM) exerts a beneficial impact on pathological biological compositions and, when administered in a targeted area via optical dose absorption, can function as an analgesic, anti-inflammatory, and biostimulative mediator.[14] Thus, much research has been performed to assess the impact of PBM on reversible inflamed DPC.[13-18] It was observed that PBM therapy can induce favorable responses during vital pulp therapies due to its capacity to regulate cell behaviors, thereby facilitating tissue repair processes.[16] Furthermore, it was observed that capped teeth exposed to PBM showed enhanced hard tissue deposition and reduced inflammatory responses.[18,19]

The current research highlighted the impact of PBM and calcium aluminosilicate-based material on direct pulp capping (DPC) of dog teeth through histological analysis.

Patients and Methods

Experimental Animal Selection

Aged between 1–2 years, twenty-four healthy mature mongrel dogs with a weight of 20–25 kg were employed in this experimental study. The clinical examination of the selected dogs' teeth was performed before starting the study. They showed that all teeth had intact structures and appeared free from any carious lesions or periodontal diseases. The selected dogs for this study were randomly labeled, distributed, and inhabited at Cairo University Animal House, following the regulations and guidelines. To identify the selected dogs, we tagged each one. These dogs were kept in the animal house for a period of 2 weeks for acclimatization before the experiment. During housing, each dog was resided in a daily-cleaned private cage and nourished on a nutritional regime of typical laboratory meals and water ad libitum. Animal care was provided by a certified veterinarian before and after the surgical intervention of the dogs. This veterinarian was responsible for the vaccination and medication of the animals during the pre- and postoperative phases. All steps, procedures, and notes related to each dog were recorded on its worksheet.[20-22]

Anesthetic Procedure

At first, the dogs were subcutaneously injected with 0.05 mg/kg atropine sulphate (Atropine sulphate 1%R; ADWIA, Egypt), and then each dog was cannulated by 20-gauge wings pink cannula (made in India) and was intravenously injected with 1 mg/kg body weight-xylazine (Amoun Pharmaceutical Co, El-Obour City, Egypt) and 5 mg/kg body weight-ketamine HCl (Ketamine; EIMC Pharmaceu ticals Co., Egypt). To ensure anesthetic effect during dental operation, we intramuscularly injected increments of 2.5% solution of thiopental sodium (Egyptian International Pharmaceutical Industries Co, Tenth of Ramadan City, Egypt) at a dose of 25 mg/kg body weight into the dogs.[22-24]

Experimental Design

Twenty-four permanent canines and premolars from healthy mongrel dogs were separated into four equal groups: group 1, which served as the control (exposed pulp was covered with a sterile polytetrafluoroethylene, Teflon tape); group 2, which received PBM treatment using a 980 nm diode laser with a 100 mw output power for one minute; group 3, which received calcium aluminosilicate-based material; group 4, which received calcium aluminosilicate-based material + PBM. Each group was divided into three identical subgroups regarding the assessment periods: (A) 1 week, (B) 1 month, and (C) 3 months (Table 1).

Direct Pulp Capping Procedure

Aseptic preparation of the oral cavity was accomplished before the operation via cleaning all aspects of the dog's dentition with normal saline irrigation (sodium chloride IV, EIPICO, Cairo, Egypt), and then the entire dog's oral cavity was disinfected by using gauzes filled with 0.2% chlorhexidine digluconate (Listermix; Sigma Pharmaceutical Industries, Quesna, Egypt).[4]

As recommended in ISO 7405:2018,[25] under copious saline irrigation, Class V cavities were equipped on the cervical third of the buccal surface of disinfected dogs' teeth, parallel to the cemento-enamel junction, 0.5–1 mm overhead the gingival margin, using a size #2 high-speed sterilized round carbide bur (Lemgo, Komet, Germany), with dimensions of 2.5 mm in width, three mm in length, and 1.5–2 mm in depth. The pulp tissue was standardizedly exposed in the center of the cavity floor after cavities were deepened by utilizing a sharp, sterile endodontic explorer (DG16, Dental USA Inc., McHenry, IL, USA).[2]

After pulp exposure, sterilized saline was employed for rinsing the surroundings and exposure in order to eliminate any debris. One to two minutes were spent with a cotton pellet saturated with NaOCl (JK Dental, A.R.E.)

Table 1. Grouping of the Samples Utilized in the Current Research

Groups	Group 1 (Control)	Group 2 (PBM)	Group 3 (Calcium Aluminosilicate)	Group 4 (Calcium Aluminosilicate + PBM)
Evaluation period				
One week	A1	A2	A3	A4
One month	B1	B2	B3	B4
Three months	C1	C2	C3	C4

solution over the exposure to achieve total hemostasis.[22-24]

After physiologic hemostasis and cavity dryness, the treatment protocols of the direct dental pulp capping procedure were carried out as follows: Group 1 (control) , the exposed pulp left without any treatment. To avoid direct contact among pulp and glass-ionomer cement restoration, we applied a sterile piece of polytetrafluoroethylene, Teflon tape, to the exposed site instead of the capping material. Group 2: The exposed pulp of the teeth was irradiated by a 980-nm diode laser (Wiser II, Doctor Smile, Italy) with output power of 100 mW for 30 seconds in a continuous wave, repeated two times. Pulpal exposures of group 3 were capped with calcium aluminosilicate cement *(*well root PT, Vericom, Gangwon-Do, Korea*)*. However, pulpal exposures of group 4 were capped by calcium aluminosilicate cement, followed by laser irradiation.

Finally, all cavities of all the groups were completely restored by glass ionomer capsules restoration (ketac fill). Glass ionomer capsules were mixed in an amalgamator (Fomos I-Mix, Zhenhai District Ningbo, China) at approximately 4000 rpm for 8 seconds according to the manufacturer's instructions. Upon its immediate removal from the amalgamator, the combined capsule was transferred to a glass ionomer applicator before being injected into the cavity. The restorative material was condensed gently in order not to apply pressure on the capping material. Consequently, contouring was done by using a sterile Hollenpack carver (Martain, Germany). Light curing was done for 20 seconds by using a light curing device (Eliper™ 2500, 3M, ESPE) with an output of 600 mW/cm^2.[22-24]

Laser Irradiation Technique

Laser irradiation was done for group 2 and group 4 with a 980 nm diode laser (Wiser II, Doctor smile, Italy) with output power of 100 mW for 30 seconds, repeated two times with total irriadaition time of one minute in the session. The irradiation of the pulp was repeated three time in one week (day on laser irradiation and day off laser irradiation)[24,26,27] (Table 2).

Following this, each group was divided into the following three subgroups based on assessment periods: (A) 1 week, (B) 1 month, and (C) 3 months.

Tissue Processing for Histological Analysis

Demineralization was achieved by immersing specimens in 0.5 M EDTA at 4 °C for 4–8 months following fixation in ten percent formalin. The block specimens were submerged in paraffin wax and serially sectioned with a microtome at intervals of 5.0–7.0. Five slides were obtained from each block and subsequently subjected to qualitative and quantitative analyses after being stained with hematoxylin and eosin by using a computer system consisting of color video digital camera ((Nikon Eclipse Soft Imaging Solutions, pol), mounted on a light microscope (Nikon Eclipse LV100 pol) which was in turn connected to the computer where the images were viewed by using LC micro–Imaging Software (Nikon Eclipse).[24,26-27]

Table 2. Laser Parameters Utilized in This Research

Parameters	Diode Laser
Wavelength	980 nm (Wiser II, Doctor Smile, Italy)
Mode	Continuous wave
Power	100 mW
energy density	6 J/cm^2
power density	0.2 W/cm^2
Tip	Biostimulation tip
Position	2 mm from the tooth surface
Exposure time	30 seconds for 2 times with a full exposure time of 1 minute
No. of laser sessions	3 times (day on & day off)

Histological Assessment

Histopathological assessment for inflammation and dentine bridge formation was conducted according to Asgary and colleagues'[28] scoring system, as outlined in Table 3.

Statistical Analysis

Data were assessed by Statistical Package for Social Sciences (SPSS) version 26. The results of inflammatory cell number and dentin bridge thickness were expressed as mean ± standard deviation (M ± SD). Analysis of variance (ANOVA) was employed in conjunction with Tukey's multiple-comparison test to identify *P* values. 0.05 was established as the level of statistical significance.

Categorical data as scoring of the inflammatory type and extension as well as continuity of the dentin bridge were accessible as incidence and percentage values, they were analyzed by utilizing chi square. The significance level was set at $P \leq 0.05$ within each test.

Results

Inflammatory Response

The combination of calcium aluminosilicate and PBM significantly reduces pulpal tissue inflammation regarding the inflammation extension, type, and number of inflammatory cells when compared with the other study group, with a statistically significant difference ($P < 0.5$) (Tables 4 & 5; Figure 1).

The Kruskal-Wallis test showed that each group had a high significant variance between the three evaluation periods ($P \leq 0.00013$). There was a significant difference between group 4 and other treatment groups ($P \leq 0.00012$) (Tables 4 and 5).

The mean number of inflammatory cells decreased significantly from one week to 3 months in all experimental

Table 3. Grading System Used for the Assessment of Inflammatory Response and Dentine Bridge Thickness in This Research

Feature	Category	Grading		
		I	II	III
Pulp Inflammation	Type	Chronic and acute inflammation	Chronic inflammation	No visible inflammation
	Intensity	Severe>60 inflammatory cells	Mild [0-30] to moderate [0-60] inflammatory cells	No visible inflammation
	Extension	All coronal pulp	Localized below the exposure area	No visible inflammation
Hard tissue formation	Continuity	No hard tissue formation	Incomplete dentine bridge	Complete dentine bridge
	Thickness	<100 µm	Among 100 µm & -250 µm	Further than 250 µm

Source: Asgary et al.[28]

Table 4. Qualitative Analysis of the Inflammatory Type of the Pulp in Different Groups According to Asgary et al[28]

Groups	Grading	One Week	One Month	Three Months	*P* Value*
Group 1	I	10 (100.0%)	2 (20.0%)	0 (0.0%)	<0.001 (HS)
	II	0 (0.0%)	8 (80.0%)	10 (100.0%)	
	III	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Group 2	I	2 (20.0%)	3(30.0%)	0 (0.0%)	0.012 (S)
	II	8 (80.0%)	7 (70.0%)	5 (50.0%)	
	III	0 (0.0%)	0 (0.0%)	5 (50.0%)	
Group 3	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	10 (100.0%)	6 (60.0%)	0 (0.0%)	
	III	0 (0.0%)	4 (40.0%)	10 (100.0%)	
Group 4	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	10 (100.0%)	5 (50.0%)	0 (0.0%)	
	III	0 (0.0%)	5 (50.0%)	10 (100.0%)	
P value		<0.001 (HS)	<0.001 (HS)	<0.001 (HS)	

P>0.05: Non significant (NS); *P*<0.05: Significant (S); *P*<0.01: Highly significant (HS).
* Chi-square test.

Table 5. Comparisons of Inflammatory Intensity in Different Groups by the Grading System According to Asgary et al[28]

Groups	Grading	One Week	One Month	Three Months	*P* Value*
Group 1	I	10 (100.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	0 (0.0%)	10 (100.0%)	10 (100.0%)	
	III	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Group 2	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000 (NS)
	II	10 (100.0%)	10 (100.0%)	10 (100.0%)	
	III	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Group 3	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	10 (100.0%)	10 (100.0%)	0 (0.0%)	
	III	0 (0.0%)	0 (0.0%)	10 (100.0%)	
Group 4	I	0 (0.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	10 (100.0%)	10 (100.0%)	0 (0.0%)	
	III	0 (0.0%)	0 (0.0%)	10 (100.0%)	
P value		<0.001 (HS)	1.000 (NS)	<0.001 (HS)	

groups (Figure 2). The Kruskal-Wallis test showed that every group had highly significant differences between the three evaluation periods (*P*< *0.001*). (*P*< *0.001*) (Figure 2).

Dentin Bridge Continuity and Thickness

With regard to dentin bridge Continuity, at one week, all groups had no hard tissue formation. At one month no complete bridge formation was observed, while at three months all groups except group one had complete bridge formation (Table 6, Figure 1).

The thickness of the dentin bridge increased significantly

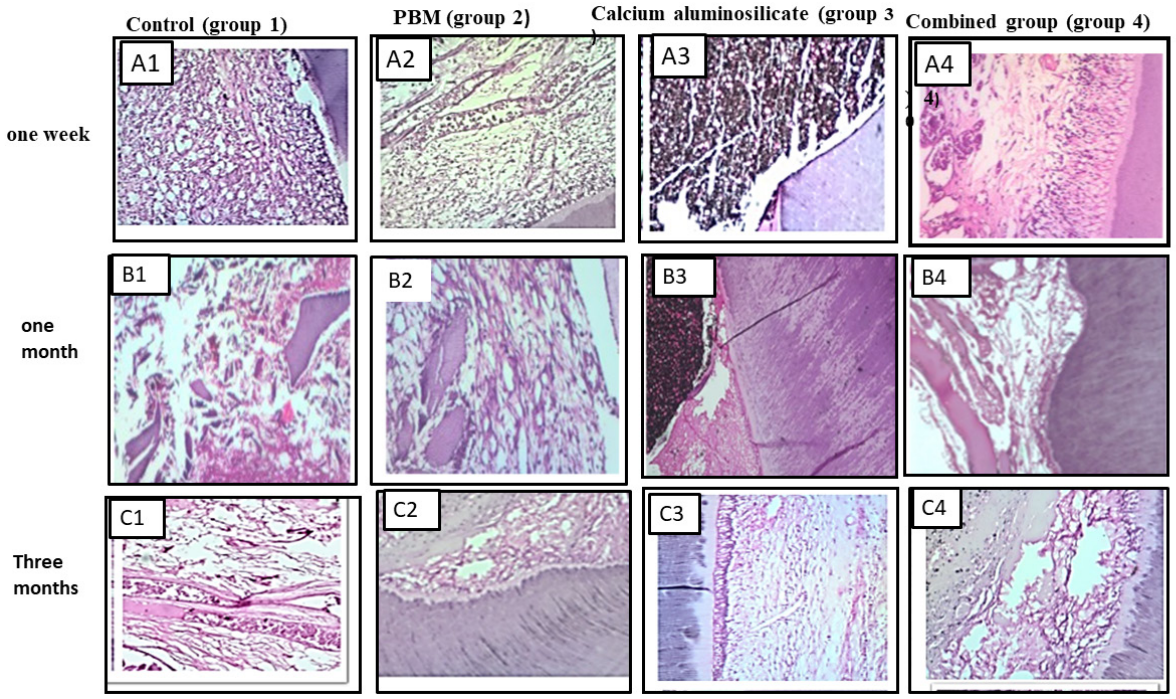

Figure 1. Histological photomicrographs of specimens after one week in different groups; (A) showing high inflammatory cell infiltration with no dentin-like hard bridge creation after one month; (B) showing less inflammatory cell infiltration with incomplete dentin-like hard bridge formation; (C) after three months with complete dentine formation with different thicknesses in different groups

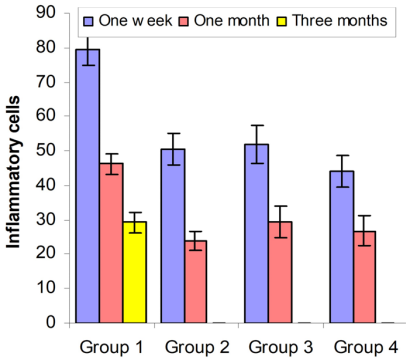

Figure 2. Comparisons Between the Studied Groups According to the Number of Inflammatory Cells at One Week, One Month, and Three Months

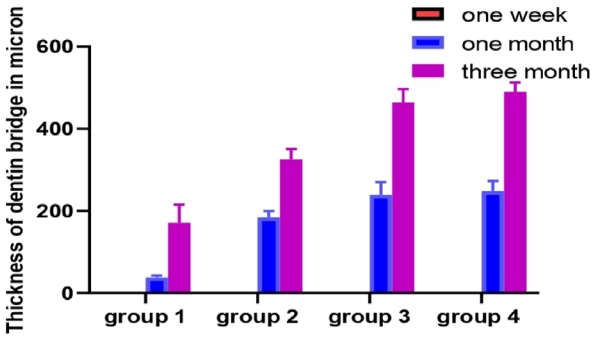

Figure 3. Dentin Bridge Thickness in Micron in Different Groups After Different Observation Periods (One Week, One Month, & Three Months)

in all experimental groups from group 2 to group 4 from one month to three months (Figures 1 and 3). The Kruskal-Wallis test showed that every group had a high significant variance at the one month ($P \leq 0.05$) and three months ($P \leq 0.05$). The Mann-Whitney test demonstrated that group 4 (combination of calcium aluminosilicate and PBM) had the highest thickness and strongly significant difference from all the other groups ($P \leq 0.05$) (Figures 1 and 3).

Discussion

Pulp-dentin regeneration technology represents the most advanced approach to recovering tooth functionality and preventing tooth loss. The DPC procedure is conducted to save the freshly exposed pulp structures to retain the vitality of dental pulp tissue and allow the formation of the reparative dentin bridge. DPC includes teeth that have mechanical or traumatic "pinpoint" exposure of regular pulps through cavity preparation as in the present study, and pulp exposure is induced in the teeth by a using sharp probe in the center of the class V cavity by the same operator.[26,27]

Thus, the current research aimed to measure the histological response of calcium aluminosilicate cement, PBM, and the combination of calcium aluminosilicate cement and PBM as DPC techniques of dogs' permanent teeth.

Dogs were chosen as animal models in the present study because of their anatomical and physiological similarity to humans, suitable pulp-dentin response, and

Table 6. Qualitative Analysis of Dentin Bridge Continuity by the Grading System

Groups	Grading	One Week	One Month	Three Months	*P* Value
Group 1	I	10 (100.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	0 (0.0%)	10 (100.0%)	10 (100.0%)	
	III	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Group 2	I	10 (100.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	0 (0.0%)	10 (100.0%)	2 (20.0%)	
	III	0 (0.0%)	0 (0.0%)	8 (80.0%)	
Group 3	I	10 (100.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	0 (0.0%)	8 (80.0%)	0 (0.0%)	
	III	0 (0.0%)	2 (20.0%)	10 (100.0%)	
Group 4	I	10 (100.0%)	0 (0.0%)	0 (0.0%)	<0.001 (HS)
	II	0 (0.0%)	7 (70.0%)	0 (0.0%)	
	III	0 (0.0%)	3 (20.0%)	10 (100.0%)	
P value		1.000 (NS)	0.097 (NS)	<0.001 (NS)	

identical healing process.[19] Furthermore, in accordance with ethical considerations, the sufficient number of teeth on dogs permits the comparison of various techniques on similar animals while reducing the number of animals that must be sacrificed.[19]

Class V cavities were created on the labial surface of the teeth by using an excessive water spray and a high-speed motor in order to reduce the amount of heat produced and the amount of pressure applied through the procedure. Mechanical pulp exposure was executed by utilizing a sharp, sterilized probe in order to avoid the potential for significant pulp damage that may arise from bur exposure.[22-24]

As in previous research, the present histological research utilized intact teeth from healthy canines in a controlled environment to prevent confounding variables from interfering. The DPC procedure adhered to a strict protocol, involving the isolation of the operative field with a rubber dam, the decontamination of the operational field with two percent chlorhexidine-gluconate, and the utilization of a standardized pulp exposure size.[3]

During the present study, three assessment periods were chosen, with the initial one-week period being designated for assessing the response of pulp tissue in relation to the biocompatibility of the material. Over the course of one month, the differentiation process, the growth of new odontoblasts, and the recently formed calcified bridge were all assessed. The subsequent three-month duration was assigned to assess the formation of the rigid tissue barrier.[3-5]

At one-week and one-month intervals, the number of inflammatory cells was significantly greater in calcium aluminosilicate cement and PBM groups in comparison with the combination group of calcium aluminosilicate cement and PBM. Whilst, at 3 months, a significant decrease in the number of inflammatory cells was detected in every group, this may be the result of the anti-inflammatory properties of PBM and calcium aluminosilicate on the pulp.[27-36]

The favorable outcomes observed at three months showed the effectiveness of the calcium aluminosilicate and PBM group. These outcomes included the absence of inflammation entirely, pulp tissue that was well-organized, the formation of a thicker and more continuous complete dentin bridge, and the existence of an intact odontoblast-like cell layer in nearly all of the samples. Within the calcium aluminosilicate group, the gradual reduction in irritating calcium hydroxide release subsequent to cement hardening improves pulp healing and yields pulp tissue that is more systematically organized. In addition, the outcomes of the current study suggested that calcium aluminosilicate might efficiently stimulate the synthesis of reparative dentin. This is due to the strong biocompatibility and bioactivity of cement, as well as its capacity to provide a suitable surface for odontoblast-like cells to adhere to, recruit, and set up prior to the process of cell differentiation and the creation of novel dentin bridges. These outcomes approve previous research studies[30-36] which examined the response of DPC with calcium aluminosilicate in dog teeth. These studies showed that at 3 months the DPC with calcium alumino-silicate demonstrated a healing process by forming a complete dentin bridge. This may be due to the fact that calcium aluminosilicate cement can improve the osteogenic and odontogenic capacities of odontoblast-like cells as well as osteogenic differentiation and mineralogenic potential of human dental pulp stem cells.[30,31] In addition, PBM has bio-stimulatory effects on the growth and differentiation of odontoblasts, and thus it promotes cell regeneration, increases dentinogenesis, improves cellular metabolism, and encourages tissue actions.[33-36]

The primary restriction of the research is that the current study was carried out in an ideal environment, wherein every tooth was intact and the DPC was in a

good state when exposed. The preoperative condition of the irritated or non-irritated pulp was not taken into consideration. Further data are required in order to evaluate the consequences of pulp inflammation prior to surgery. Consequently, additional research employing longer observation periods and incorporating carious teeth accompanied by pulpal inflammation is necessary to validate the present findings.

Conclusion

Photobiomodulation using 980 nm diode laser with output power 100 mw for one minute irradiation with output power of 100 mw for one minute, combined with calcium aluminosilicate-based material, decreased initial inflammatory response and enhanced complete thick dentine bridge formation.

Authors' Contribution

Conceptualization: Latifa Mohamed Abdelgawad, Mariam Hassan Nghnughi, Dalia El Rouby, Marwa Abdelgwad
Data curation: Latifa Mohamed Abdelgawad.
Formal analysis: Latifa Mohamed, Mariam Hassan Nghnughi, Dalia El Rouby, Marwa Abdelgwad.
Methodology: Latifa Mohamed Abdelgawad, Mariam Hassan Nghnughi, Dalia El Rouby, Marwa Abdelgwad.
Resources: Latifa Mohamed Abdelgawad, Mariam Hassan Nghnughi.
Software: Dalia El Rouby.
Supervision: Latifa Mohamed Abdelgawad, Dalia El Rouby, Marwa Abdelgwad.
Validation: Latifa Mohamed Abdelgawad, Dalia El Rouby, Marwa Abdelgwad.
Visualization: Latifa Mohamed Abdelgawad, Mariam Hassan Nghnughi, Dalia El Rouby, Marwa Abdelgwad.
Writing–original draft: Latifa Mohamed Abdelgawad, Mariam Hassan Nghnughi.
Writing–review & editing: Latifa Mohamed Abdelgawad.

Competing Interests

There are no competing interests.

Ethical Approval

This animal research was accepted by the Institutional Animal Care , Cairo University, Egypt. (Protocol No: CU/I/F/75/ 20). This study was carried out according to the regulations and guidelines of international Animal Care , Cairo University.

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