Efficacy Of Photobiomodulation Using 870 nm Diode Laser, Experimental Nano Calcium Aluminate/Tri Calcium Silicate Material And MTA In Furcal Perforation Repair (Animal Study) (Part Two)

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Abstract

Objective: The purpose of this research was to compare the histological tissue responses to application of diode Laser 870nm, experimental nano calcium aluminate/tricalcium silicate (CA/ C3S) material and MTA after the attempt to repair experimentally induced furcal perforations in dogs' teeth.

Methods: A total of forty-five teeth from five dogs were used. Teeth were divided into three groups according to the evaluation period, each group was subdivided into three subgroups according to the material used and whether the 870nm laser was used or not. Root canal treatment was performed, furcal perforation was created then sealed with MTA or (CA/ C3S). For the lased group, a diode 870 nm laser was applied in addition to (CA/ C3S). Dogs were sacrificed, samples containing the treated teeth fixed, decalcified, sectioned and stained for histological assessment.

Results:

Inflammation decreased by time in all subgroups. This decrease was statistically significant in the lased CA/ C3S subgroup, ($p=0.008$). Bone apposition showed a gradualstatistically significant increase by time. Lased CA/ C3S and MTA subgroups showed enhanced bone apposition at 3 months ($p=0.038$). There was no statistically significant difference between material subgroups regarding cementum formation, periodontal ligament formation and epithelialization; except at three-months, where MTA subgroup revealed significantly higher periodontal ligament formation scores.

Conclusion:

Calcium-aluminate/Tricalcium-Silicate has a bone apposition ability comparable to MTA, enhancing cementum, periodontal ligament formation and reducing epithelialization in furcal perforation area, 870nm diode laser reduced the inflammation and improved bone apposition.

Keywords: Calcium-aluminate/Tricalcium-silicate, MTA, furcal-perforation, Diode- 870nm, Bone-Formation, inflammation.

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Introduction

Endodontic therapy has a long history in management of teeth with pulp diseases and/or periapical lesions; it is concerned with the morphology, physiology, and pathology of the human dental pulp as well as the periradicular tissues[1].

In the interarticular zone of multirooted teeth, furcal perforation is described as a pathological or mechanical communication between both the root canal system and the external tooth surface [2].

Furcal perforations could result in inflammatory reactions that impair periodontal tissue and cause bone destruction if it is not adequately treated. Inflammation may cause epithelial proliferation and the development of granulation tissue, depending on how severe it is[3].

Long-term success of a perforation repair depends on a number of factors, including the time of septic exposure, the overall size and site of the perforation, the degree of material insolubility, and the capacity to seal the defect [4, 5].

The nonsurgical approach in perforation treatment has a success rate of over than 70%[6].

The perforation repair material should have suitable physicochemical characteristics, provide adequate seal, induce periradicular tissue regeneration and be cost effective [7].

MTA stimulates osseous healing, interacts well with periapical and periradicular tissues, and is the material of choice to seal furcal perforations [8].However , it lacks good handling qualities., a low radiopacity, undergoes discoloration when exposed to sodium hypochlorite, high setting time , and there is a possibility that it will dissipate in humid conditions [9].

Calcium Aluminate has excellent mechanical strength, sufficient setting time, and rapid hydration rate and the ability of forming an intimate bond to the opposing tissues[10].

Due of its comparable composition and bioactivity, tricalcium silicate has been proposed as a possible replacement for the cement component of the MTA [11].

Low level laser therapy (LLLT), also termed as photobiomodulation (PBM is a treatment that modifies cell functioning using low-level lasers or light-emitting diodes (LEDs). LLLT controls cell processes by influencing cytokine production and cell proliferation, which has a variety of biological effects. LLLT stimulates bone regeneration, angiogenesis, cell proliferation, osteogenic differentiation, and fracture healing [12].

Therefore, the combination of diode laser with the use of different furcal perforation repairing materials could eventually develop a new technique that promote a better and faster repair process according to the used material.

This study aimed to examine the histological tissue reactions to the application of 870nm diode laser, the experimental nano calcium aluminate/tricalcium silicate material, and MTA following the attempt to repair experimentally produced furcal perforations in dog teeth.

MATERIALS AND METHODS

I. Animals' selection and teeth grouping.

The research protocol was approved by Institutional Animal Care and Use Committee Cairo University, Egypt (CU-IACUC CU I F 24 21)**.** An earlier pilot study was conducted to ascertain the needed surgical techniques, aspects of the animals' clinical evolution, and the characteristics of the perforations. Five healthy adult mongrel male dogs, with average weight of 11 to 17 kg and older than 1 year old were selected from the Animal House Unit (Faculty of medicine, Cairo University, Egypt).

For constant identification, a collar tag with a number was worn by each dog. A total of forty-five teeth (n = 45) from the five dogs were used. All teeth were intact, free from caries or periodontal disease, and had fully developed roots. Both sides of the mandibular third and fourth premolars, and maxillary second and third premolars were used.

The 45 teeth were divided into three groups (n=15) according to the evaluation period. G1: two weeks, G2:one month and G3 three months, then each group was further subdivided according to the used material into three sub-groups. **SG-A:** sealed with the experimental CA/ C_3S material, **SG-B:** sealed with the experimental CA/ C3S material with 870 nm diode laser

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application. **SG-C**: sealed with MTA (Angelus, Brazil).

II. Operative procedures

An intramuscular injection of Ketamine (Sigma Tec, Cairo, Egypt) (8-10 mg/kg) and Atropine (EL Nile Medical, Egypt) (0.02-0.04 mg/kg) was used to generally anesthetized dogs , followed by Xylazine (ADWIA Co. S. A. E. 10th of Ramadan city, Cairo, Egypt) (2.2 mg/kg) injected intravenous, and intubated with a cuffed endotracheal tube before beginning the experimental procedure. In order to reduce salivary secretions, the dog also received an intramuscular injection of atropine sulphate at a dose of 0.04 mg/kg.A prophylactic dose of Penicillin (Cefotax 1gm, Egy Pharma Industries Coop, Egypt) was also intramuscularly given at 30.000 U/Kg .

The molars cusps were reduced until the pulps were exposed using a diamond bur (Mani Inc, Touchigi-Ken, Japan) with the use of a high-speed handpiece with water cooling; coronal access cavity was made using a #2 round bur. After pulpectomy, the canal was instrumented using the Crown-down technique with Revo's rotary files system (Micro Mega, SA, 12 avenue du Tunnel 2500 Besancon, France). In the preceding order, % sodium hypochlorite, % chlorhexidine, and 0.9 % saline solution have been used to clean canals. Gutta-percha cones and AH-Plus sealer (Dentsply/ Maillfer cooperation, Zurich, Switzerland) were used to obturate the canals using a warm vertical condensation technique.

The experimental teeth's pulp chamber floor was perforated in the center using a sterile #2 round bur (Dentsply/Maillfer cooperation, Zurich, Switzerland) at a slow speed combined with water spray coolant. The perforation was 1.4 mm in diameter. The alveolar bone was only perforated only two millimeters deep [13]. Bleeding was controlled and swabbed with sterile cotton pellets.

Materials were prepared as follow; MTA powder was hand mixed with sterile water into homogenous plug. Nano calcium aluminate /tricalcium silicate material composed of a ratio of 1:1 powder of Calcium Aluminate (CA) and Tricalcium Silicate (C_3S) was mixed in room temperature with distilled water till a homogenous plug is formed and applied with metallic condenser in a liquid/ powder ratio of (0.762gm. :0.1ml) [14].

The materials were applied to the site of perforation and the excess materials were removed with a fine stone driven on low-speed hand piece and manual excavator.

III. Irradiation application.

For laser groups, in continuous mode (CW),irradiation was applied in uniformly scanning motion using a diode 870 nm laser with spot size diameter 2mm with the intensity 1.592 mW/cm^2 and the dosage 24 J/cm^2, and power of 50 mW .Laser was applied on the perforation site before sealing with repair material for 60 sec on 2 cycles (each cycle is 30 sec) [15]. After sealing the cavities with final restoration another application for 30 sec on 3 cycles (each cycle 10 sec) was applied perpendicular to buccal bone and the lingual /palatal at the perforation site, the previous step repeated after three days and seven days after the animal was anesthetized.

The dogs were sacrificed according after evaluation period by using a 150 mg/kg overdose of sodium pentobarbital (65 mg/ mL) (Altabarak 10st Alkopa, Cairo, Egypt).

IV. Histological evaluation:

Samples containing the treated teeth were fixed with formalin, decalcified in formic acid for 14 days, and then treated with 17 % EDTA solution for 120 days to prepare histology slides. The samples were washed under running water for continuous 24 hours. Five μm paraffin sections of each block were cut using a microtome through the area of the furcal perforation. Slides were stained with hematoxylin and eosin and examined under light microscopy for qualitative and quantitative analysis

The histological sections at the furcation area were quantitively scored for inflammation severity score and bone apposition. The cementum deposition, periodontal ligament regeneration and epithelium formation at the furcal perforation site were qualitatively scored as following; - (Yes) for presence of tissue formation and (No) for absence of tissue formation, According to the criteria used by **AL Alhadainy et al 1998 and AL Daafas et al 2007**[16, 17] as listed in **Table(1)**.

Table (1): The scoring system used for testing inflammation severity and Bone Apposition

The scoring system used for testing inflammation severity	
Scoring	**Description**
0	(None)No infiltration of inflammatory cells
1	(Mild)Few scattered inflammatory cells
2	(Moderate) Inflammatory cells did not obscure the normal tissues
3	(Severe)Massive infiltration of inflammatory cells replaced normal tissue
The scoring system used for testing bone apposition.	
Scoring	**Description**
0	None
1	Osteoblastic rimming with no osteoid tissue formed
2	Heavy osteoblastic rimming with some osteoid tissue
3	Heavy osteoblastic rimming with abundant osteoid tissue

V. Statistical analysis

Categorical and ordinal data were presented as frequency and percentage values. Categorical data were compared using Fisher's exact test followed by multiple pairwise comparisons. The significance level was set at p≤0.05 within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows[18].

Results

Regarding inflammation severity score, there was no significant difference between the three evaluation time periods in both (SG-A) and (SG-C) (p>0.05). For (SG-B), there was a significant difference between the three evaluation periods, with value recorded at two weeks being significantly higher from that of three months (p=0.008). At each of the three evaluation periods, there was no significant difference between different subgroups (p>0.05). Results of intergroup and subgroup comparisons of inflammatory score are presented in **table (2) and figure (1)**

Regarding bone apposition score, there was a significant difference between different evaluation periods within (SG-A), (SG-B) and (SG-C) with value recorded at two weeks being significantly lower from that recorded at three months (p<0.05). Within two weeks and one-month evaluation period, there was no significant difference between different material subgroups. For three months evaluation period, (SG-A) revealed significantly lower scores compared to other subgroups (p=0.038). Results of intergroup and subgroup comparisons of bone apposition score are presented in **table (3)** and **figure (2).**

Regarding cementum formation, (SG-A) revealed a significant increase in cementum formation by time, with a significantly lower value (0%) recorded at two weeks in comparison to three months observation (60%), (p=0.036). All other intergroup and subgroup comparisons showed no significant difference (p>0.05), **(table 4).**

Regarding epithelialization and periodontal tissue formation, there was no significant difference between different observation times within the same group (p>0.05). Comparing material subgroups revealed no significant difference, except at three months evaluation period, where (SG-C) revealed significantly higher periodontal ligament formation scores compared to other subgroups (p=0.036). Results of intergroup and subgroup comparisons of epithelialization and periodontal tissue formation are presented in **table (4).**

The histological picture of different subgroups at different observation times is illustrated in **Figure (3).**

Table (2): Intergroup and subgroup comparisons of inflammatory score

Subgroup	Score	Two weeks		One month		Three months		p-value within SG
		n	%	n	%	n	%	
(SG-A)	Zero	0^{Aa}	0.0%	0^{Aa}	0.0%	3^{Aa}	60.0%	0.095ns
	Mild	2	40.0%	2	40.0%	2	40.0%	
	Moderate	2	40.0%	3	60.0%	0	0.0%	
	Severe	1	20.0%	0	0.0%	0	0.0%	
(SG-B)	Zero	0^{Aa}	0.0%	0^{ABa}	0.0%	4^{Ba}	80.0%	0.008*
	Mild	1	20.0%	2	40.0%	1	20.0%	
	Moderate	3	60.0%	3	60.0%	0	0.0%	
	Severe	1	20.0%	0	0.0%	0	0.0%	
(SG-C)	Zero	0^{Aa}	0.0%	1^{Aa}	20.0%	2^{Aa}	40.0%	0.155
	Mild	3	60.0%	3	60.0%	3	60.0%	
	Moderate	2	40.0%	1	20.0%	0	0.0%	
	Severe	0	0.0%	0	0.0%	0	0.0%	
p-value bet. SGs		0.392		0.259		0.459		

Values with different upper and lowercase superscript letters within the same horizontal row and
vertical column respectively are significantly different *; significant (p ≤ 0.05)

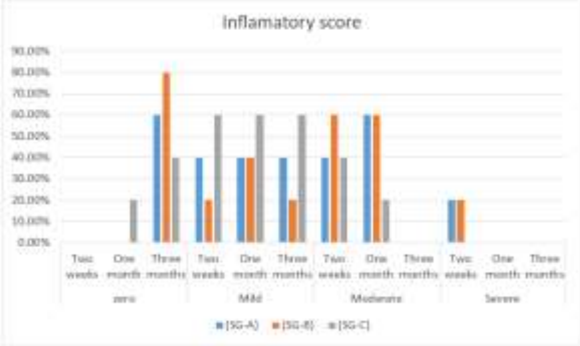

Figure (1) Bar chart showing inter-subgroup comparison of inflammatory score

Table (3): Intergroup and subgroup comparisons of bone apposition score

Subgroup	Score	Two weeks n	Two weeks %	One month n	One month %	Three months n	Three months %	p-value within SG
(SG-A)	0	5^{Aa}	100.0%	3^{ABa}	60.0%	0^{Ba}	0.0%	0.008*
	1	0	0.0%	2	40.0%	4	80.0%	
	2	0	0.0%	0	0.0%	1	20.0%	
	3	0	0.0%	0	0.0%	0	0.0%	
(SG-B)	0	5^{Aa}	100.0%	1^{ABa}	20.0%	0^{Bb}	0.0%	0.006*
	1	0	0.0%	2	40.0%	1	20.0%	
	2	0	0.0%	2	40.0%	1	20.0%	
	3	0	0.0%	0	0.0%	3	60.0%	
(SG-C)	0	5^{Aa}	100.0%	1^{ABa}	20.0%	0^{Bb}	0.0%	0.002*
	1	0	0.0%	4	80.0%	0	0.0%	
	2	0	0.0%	0	0.0%	4	80.0%	
	3	0	0.0%	0	0.0%	1	20.0%	
p-value bet. SGs		1		0.195		0.038*		

Values with different upper and lowercase superscript letters within the same horizontal row and vertical column respectively are significantly different *; significant (p ≤ 0.05)

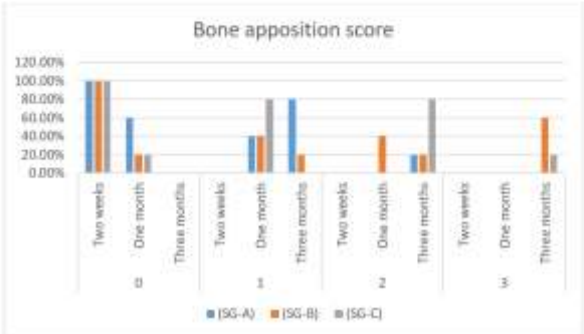

Figure (2): Bar chart showing inter-subgroup comparison of bone apposition score.

Table (4): Intergroup and subgroup comparisons of incidence of cementum, periodontal ligament formation and epithelialization

	Subgroup	Two weeks n	Two weeks %	One month n	One month %	Three months n	Three months %	p-value within SGs
Cementum formation	(SG-A)	0^{Aa}	0.0%	2^{ABa}	40.0%	4^{Ba}	80.0%	0.036*
	(SG-B)	1^{Aa}	20.0%	2^{Aa}	40.0%	4^{Aa}	80.0%	0.153
	(SG-C)	1^{Aa}	20.0%	1^{Aa}	20.0%	4^{Aa}	80.0%	0.082
	p-value bet. SGs	0.562		0.741		1		
Periodontal ligament formation	(SG-A)	1^{A}	20.0%	3^{A}	60.0%	3^{A}	60.0%	0.435
	(SG-B)	1^{A}	20.0%	2^{A}	40.0%	4^{A}	80.0%	0.287
	(SG-C)	0^{A}	0.0%	2^{AB}	40.0%	4^{B}	80.0%	0.036*
	p-value bet. SGs	0.562		0.765		0.711		
Epithelial-ization	(SG-A)	3^{Aa}	60.0%	2^{Aa}	40.0%	1^{Aa}	20.0%	0.435
	(SG-B)	2^{Aa}	40.0%	1^{Aa}	20.0%	1^{Aa}	20.0%	0.287
	(SG-C)	1^{Aa}	20.0%	0^{Aa}	0.0%	1^{Aa}	20.0%	1
	p-value bet. SGs	0.435		0.711		0.562		

Values with different upper and lowercase superscript letters within the same horizontal row and vertical column respectively are significantly different *; significant (p ≤ 0.05)

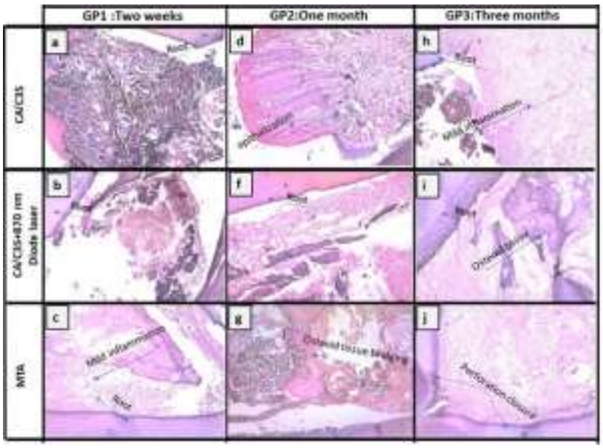

Figure (3) Photomicrograph of Furcal perforation site (H&Ex40). At 2 weeks, mild to severe inflammation is noted in SG-A&B (a,b), with no bone apposition in all subgroups. At one month, mild to moderate inflammation is still noted in all subgroups. Note the epithelialization in SG-A (d) and new bone formation in SG-B&C (f, g). At 3 months, mild inflammation is still noted in all SG-A (h). Note the abundant osteoid tissue in SG-B&C (i, j).

Discussion

The potential of a root perforation repair material to preserve instead of harm tissues should always be considered when selecting one. Depending on the situation, these materials may influence repair process, which is advantageous, or bone destruction and other unfavorable complications, which affect prognosis [19]. Clinical researches play important rule in treating symptoms and assessing repair (or lack of repair) around periodontal supporting tissues[20, 21]. As a result, this experiment employed an in vivo qualitative and quantitative investigation of the histological findings following the use of MTA and the experimental calcium aluminate/tri calcium silicate with and without the use of an 870 nm diode laser to treat furcation perforation.

The high visibility and accessibility of canine dental morphology explains why this species is frequently used in furcal perforation studies [2, 17]. However, dogs' bone margin and furcal area relationships are not directly equivalent to those of human teeth[5] .

When Mineral trioxide aggregate and calcium aluminate /tri calcium Silicate contact oral tissues ,they release calcium ions and induces medium alkalinization associated with the formation of calcium hydroxide [14, 22, 23] The antibacterial activity, biocompatibility, and bioactivity of the materials are significantly influenced by these mechanisms.

Evaluation of induced inflammation in the three groups showed that the specimens had mild to moderate inflammatory reaction during the first two weeks, then the inflammation decreased by time. This finding is corroborated by a number of studies where the degree of inflammation in response to MTA compared to other experimental materials was mild to moderate and decreased over time. [5, 17, 24, 25] .This intense initial inflammation at the start of treatment may be explained by the release of calcium ions and a high pH of the experimental calcium aluminate/Tri calcium silicate (12) and MTA (12.5) [14, 26, 27].For the SG-C that involved application of 870nm diode laser, it has highest improvement between all the subgroups after three months evaluation period with no inflammation in 80% of the samples. That is in agreement with the finding of **Aoki et al (2004)** who reported that, a diode laser with a wavelength of 655nm to 980 nm can accelerate wound healing, promote angiogenesis, increase growth factor release, and decrease inflammation[28]. **Gupta et al (2015)** also applied low level laser therapy on burn wounds in rats using 904nm diode laser and detected faster healing, reduction of histological signs of inflammation, decreased expression of tumor necrosis factor alpha (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and up-regulated expression of Vascular endothelial growth factor (VEGF), fibroblast growth factor receptor 1 (FGFR-1), heat shock protein 60 (HSP-60), heat shock protein 90 (HSP-90), Hypoxia-inducible factor 1-alpha (HIF-1α). They concluded that Laser could involve both pro-inflammatory effects and anti-inflammatory effects[29]. Another report done by **Shimizu et al (1995)** indicated that LLLT inhibit cyclooxygenase-2 expression and decrease the production of the active mediator involved in the inflammatory and painful processes, Prostaglandin E2 (PGE2) [30].

CA/C_3S repair material in SG-A stimulates the formation of hard tissue over time, with osteoblastic rimming noted in 80% of samples and heavy osteoid tissue formation noted in 20% at the end of the evaluation periods. The hypothesis of previous research reports that the new bone apposition might be the result of several properties of the repairing material used in sealing the perforation, i.e., high alkalinity, biocompatibility, antibacterial activity, hydroxyapatite production, and sealing ability [10, 11, 14].

CA/C_3S cement with the combination of 870nm diode laser in SG-B showed the highest bone apposition score among the tested subgroups after three months within 60% of the tested samples showing heavy osteoblastic rimming and abundant osteoid tissue formation. This outcome was better than using CA/C_3S alone in SG-A. The diode laser 870m effect may be responsible for the observed increase in bone apposition through enhancement of fibroblast proliferation, increase in osteoblastic activity, stimulation of ATP production, which is essential for accelerating mitosis, improvement of the host immune response, and formation of connective tissue with more advanced bone formation [31-33].

A high percentage of bone deposition was noted in MTA in all intervals, with heavy osteoblastic rimming and osteoid tissue formation in all samples after 3 months. This is in accordance with previous reports [13, 34, 35].

Previous studies[17, 36] exhibited cementum repair following dog teeth that had been perforated and sealed with MTA, where the newly formed cementum might have derived from either the surviving PDL or the ingrown connective tissue [37]. Due to the role of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue barrier [38] , the reaction of MTA's calcium hydroxide component with the connective tissues contributed to the observation of the calcite crystals. Moreover, the release of calcium hydroxide as by-product form the hydration reaction of CA/C_3S. **Nassar et al (2022)** , supports the hypothesis of cementum formation in all the tested groups with nearly the same rate of 80% of all samples after the end of the three months evaluation period[14]. On the other hand, 870 nm diode laser didn't have a noticeable impact on new cementum formation in the current study.

Concerning the periodontal ligament regenerati on there was no statistically significant difference between the three groups. However, a study by **Aladimi et al (2020)** [39] found a correlation between the MTA cementogenesis activity and the material's ability to promote the regeneration of new periodontal ligament. [39]. Such finding explains the constant increase in periodontal ligament formation after three months along with the cementum formation in all subgroups.

All the tested subgroups showed low epithelial tissue invasion at 3-months interval. This may be due to the high biocompatibility and sealing ability of the used materials as illustrated in a study done by **Holland et al (2001)** . After three months, 20% of all subgroups showed epithelial tissue infiltration that was most likely a result of trauma sustained during the perforation of the furcal area. A disadvantage of employing a dog model to evaluate the furcation perforation may be the presence of epithelium due to the dog's teeth CEJ's proximity to the location of the furcation [39]. In contrast to humans, where the root furcation is deeper and connective tissue development and epithelization are less prevalent, dogs frequently have root furcation that are as close as 1-2 mm to the cementoenamel junction. As a result, given that humans have a wider distance between the furcal area and the cementoenamel junction, procedures that have positive results in dogs may be expected to have an even higher impact on humans [25, 39, 40].

Conclusion

Within the limits of the current study, using Calcium-aluminate/Tricalcium-silicate as perforation repair material found to have a comparable bone apposition ability to MTA. Moreover CA/ C_3S can induce cementum deposition, periodontal ligament formation and reduce epithelialization .870nm diode laser has a high impact in reducing inflammation and increase bone apposition in furcal perforation sites.

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