Histological Evaluation of Inflammation after Sealing Furcating Perforation in Dog's Teeth by Four Materials

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ABSTRACT

Introduction: The materials used in sealing furcating perforation can have considerable effects on controlling the ensuing inflammation and periodontal repair. The objective of the present study was to carry out a histological comparison between the effects of pro-root, cold ceramic, glass-ionomer cement, and root MTA on the healing of periodontal tissues after furcal perforation in dog's teeth.

Methods and Materials: One-hundred premolar teeth of one-year old dogs were used in this experimental/animal study. After anesthetizing the dogs and the premolar teeth, the access cavities were prepared at the occlusal level and the root canals were instrumented and filled with gutta percha and AH26 sealer, using the step-back technique. Furcations were perforated to a size of 3×3 mm2, using long burs. These areas were then randomly filled with aforementioned four test materials (a total number of 84 premolar teeth) while the access cavities were filled with amalgam. The remaining 16 teeth were selected to serve as positive and negative controls. Biopsy samples were taken from the perforated areas at 1, 2, and 3-month intervals and were transferred to laboratory for pathological examination. The results were statistically analyzed, using the Kruskal-Wallis and Mann Whitney tests.

Results: The statistical analysis revealed that under similar conditions, periodontal tissues surrounding Pro-root, show less inflammatory response than the other three materials. However, no significant differences were observed among the four studied materials during 1 and 2months as evidenced by the biopsy samples ($P>0.05$). For longer period (three month), however, samples surrounding cold ceramic and Root MTA showed decreasing inflammatory responses.

Discussion: From the findings of the present study, it may be concluded that although tissues adjacent to Pro-root showed less inflammatory response than other three test materials, all of them (Pro-root, Glass-ionomer cement, cold ceramic, and Root MTA) may be considered to be suitable materials for sealing furcal perforation providing. They receive approval by other tests including micro leakage, cytotoxicity, tissue analysis, and etc.

Key words: Cold Ceramic, Root MTA, Furcal Perforation, Periodontal Tissues, Sealing.

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Introduction

In root canal therapy, like in all other areas of dental treatment, there is imminent risks and unexpected conditions that may influence prognosis and treatment [1, 2]. One of such risks during root treatment is furcal perforation as a result of drilling for access cavity preparation which will negatively influence tooth long-term prognosis [1]. The first objective in creating an access cavity in root canal therapy is to create a direct route to the apical area, free of any obstacles.

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Incidents such as over instrumentation of the tooth tissue and perforation of the pulp chamber floor during efforts to search for the canal opening may occur. Factors affecting the long-term prognosis of furcal perforation include: perforation site, size of damage, setting time of the perforation, and the type of sealing material [1]. As the type of material used in sealing perforation has drastic impact on periodontal tissue repair, this study was designed to investigate the reaction of periodontal tissue in perforated teeth using four different materials of Pro-root, glass-ionomer cement, Root MTA, and cold ceramic.

Given that the type of material used for sealing the furcal perforated site plays an important role in controlling the inflammation and repair of the debrided periodontal tissues, due to perforation, it follows that the material selection must be based on its favorable reaction with the surrounding tissue, its sealing ability, and its availability and cost. Pro-root was introduced in 1993 by Torabinejad from Loma Linda University, USA. Its constituents include calcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide [3]. Pro-root comes in powder and includes mineral trioxide aggregate (MTA) with hydrophilic particles which crystallize in the presence of moisture. Initially, the substance was used in retrofillic treatments but, nowadays, it has found wide application in root treatment including apexogenesis, apexification, direct pulp capping, and in all types of perforation repair [3].

More recently, Lotfi embarked on manufacturing a tooth filling material called Root MTA which is very similar to Pro-root and Iranian Organization of Science and Technology (IROST) has also evaluated and compared it with Pro-root to approve its quality. The advantages of the newly-manufactured material include its availability and lower price.

In our present study, we also evaluated cold ceramic. This is a bicalcium hydroxide manufactured in Yazd University of Medical Sciences and is presently used in the process of test operations [4].

In the light of these findings and given the high price of Pro-root in Iran, the authors embarked on investigating the behavior of the four materials; Pro-root, cold ceramic, glass-ionomer cement, and Root MTA after setting in furcal perforations in dogs' teeth to identify the most suitable material available in Iran.

Methods and Materials

It was an interceptive (experimental) parallel study, used animal as a model. In this study, 100 premolars from 10 mature, healthy, one-year-old dogs weighing around 30 Kgs each, were used. The dogs were all selected from same locality, were fed on almost similar diets, and were kept at Professor Torabinejad's Research Laboratory at the faculty of dentistry, Isfahan University of Medical Sciences.

The cases were under veterinary care, kept in separate cages, and quarantined for 10 days prior to the experiment. They were subjected to tests one week after vaccination and prescrib0.ing anti-parasitic drugs. Anesthesia was administered by respiratory anesthetics. 10mg/kg of Ketamine HCL (Alfasan-Holland) with 0.15 mg/kg of Xylasine (Alfasan-Holland) was used intramuscularly, followed by 0.5 mg/kg of Ketamine and 0.04 mg/kg of Xylasine injected intravenously together with respiratory administration of halothane and N_2O volatile gases.

Sulfonamide drop (Abidi-Iran) was used to prevent desiccation of dogs' cornea. The combination of the two anesthetic drugs provided almost one hour of anesthesia. An additional injection of 2 ml of Ketamine was administered when life reflexes were detected in cases. After complete anesthesia, a mouth gag was fitted on the molars opposite to the working site. Prior to initial radiography, premolars were examined to make sure of safe enamel and healthy periodontal tissues.

It must be mentioned that as the first premolar in the upper jaw is single-rooted, only two premolars were used. Microbial flora was controlled using chlorhexidine disinfectant.

Also the premolar teeth were locally anesthetized by half a carpule of 2% Lidocaine with 1/80000 Adrenalin (Darou Pakhsh-Iran) was infiltrated. Using # 2 turbine carbide fissure bur accompanying by water spray, tooth crown was cut off 2-3 mm from the gingival edge. In order to gain better access to canal orifice, the orifice was enlarged using a fine diamond and turbine bur fissure accompanying by water and air spray.

The cavity was then irrigated with normal saline to remove debris and the teeth were prepared for root canal therapy. Using a file, the root canals were searched. The teeth were x-rayed, using endodontic files to measure canal length.

Canals in each tooth were filed up to # 25 while they were being irrigated with normal saline to remove debris. Then Gates Glidden burs and Peeso reamers of sizes 2 and 3 were used to enlarge the canals. After irrigating the canals, they were dried with sterile absorbent paper cones and each canal was filled with gutta-percha (Dentsply, USA) and AH26 sealer, using lateral condensation technique. Then teeth furcations were perforated to an approximate size of $3\times3mm^2$ using a fissure bur accompanying by air and water sprays. The perforated areas were irrigated with normal saline in order to control bleeding and then dried with a sterile cotton pellet. In 84 premolars, the perforated areas in each quadrant were randomly sealed with one of the four materials, namely, Pro-root (Dentsply, USA), glass ionomer cement (Fuji, Japan), Root MTA (Tabriz University, Iran), or cold ceramic (Yazd University, Iran) that were prepared according to the manufacture's directions. These powders were mixed with saline or its solution on glass slab to produce paste like materials which can be packed in the perforated cavities. Two were separately mixed on glass slabs and a paste with sealer viscosity was obtained which had the capacity to be packed onto the cavity floor. The amalgam (Sinalux, Iran) was carried to the area in an amalgam carrier and was packed using a cotton pellet until the access cavity was filled and burnishers were used for marginal adaptation. Finally, perforated areas were radiographed to ensure proper sealing of the areas. Sixteen premolars were used as positive and negative controls.

Positive controls:

These included 8 premolars from mature dogs which had their crowns cut off up to 3 mm from the gingival edge. After root canal therapy and furcal perforation, using the method described above, these preparations had not been sealed but only the crowns had been slightly filled with cotton and amalgam.

Negative controls:

These included 8 safe and healthy teeth from mature one-year-old dogs, without any intervention.

After recovery from anesthesia, the dogs were transferred to cage. Biopsy samples were taken at one, two, and three-month intervals after vital perfusion of dogs. After coding, the samples were transferred to lab for pathological investigations where histological sections were prepared and the lamellas were submitted for examination. It must be mentioned that all samples were cut along the longitudinal section after decalcification, so that in addition to the longitudinal section of the tooth, periodontium tissues including PDL, cementum, and alveolar bones were included in the histological sections (figures 1, 2, 3, and 4). All results from evaluation of periodontal tissue repair were recorded (table 1). These included tissue reactions at perforation sites and repair trend against the repair evaluation table. Maximum repair consisted in the formation of cementum and bone with the presence of mature fibroblasts and PDL regeneration while minimum repair included the formation of abscesses and necrotic areas with PDL degeneration. The pathologist knew nothing about the used coding system. The data was analyzed by Kruskal-Wallis and Man-Whitney tests.

Table 1: Tissue response table.

Stage I	StageII	StageIII	Stage IV
No inflammatory cells	<10 Macrophage & plasmacell	10-25 Macrophage & plasmacell	>25 macrophage & plasmacell
>30 Fibroblast	10-30 fibroblast	5-9 fibroblast	1-4 fibroblast
Mature Fibrous Tissue with many collagen	Immature Fibrous Tissue with little collagen	Granulation Tissue	Focal areas of necrosis
PDL Regeneration	PDL Regeneration	PDL Degeneration	PDL Degeneration
Cement Formation	Cement Formation	Cement Resorption	Abcess
Bone Formation	Bone Formation	Bone Resorption	Abcess

Results

In one, two, and three months period of time, maximum inflammation was observed in the positive control group (stage 4), while minimum inflammation was seen in the negative control group (stage 1).

Results of this study are shown in tables 2-4, pictures 1-4, and diagram 1.

Table 2: Results of histological evaluation of samples taken after one month, according to the used material.

Material	n	Degree			
		1	2	3	4
Pro-root	7	4	1	2	0
Cold ceramic	6	0	4	0	2
Glass ionomer	7	2	2	3	0
Root MTA	8	1	4	3	0

Table 3: Results of histological evaluation of samples taken after two months, according to the used material.

Material	n	Degree			
		1	2	3	4
Pro-root	7	2	4	1	0
Cold ceramic	8	2	3	3	0
Glass ionomer	8	2	3	3	0
Root MTA	7	2	4	1	0

Table 4: Results of histological evaluation of samples taken after three months, according to the used material.

Material	Quantity	Degree			
		1	2	3	4
Pro-root	7	2	3	2	0
Cold ceramic	6	2	3	1	0
Glass ionomer	7	1	3	3	0
Root MTA	6	1.	3	2	0

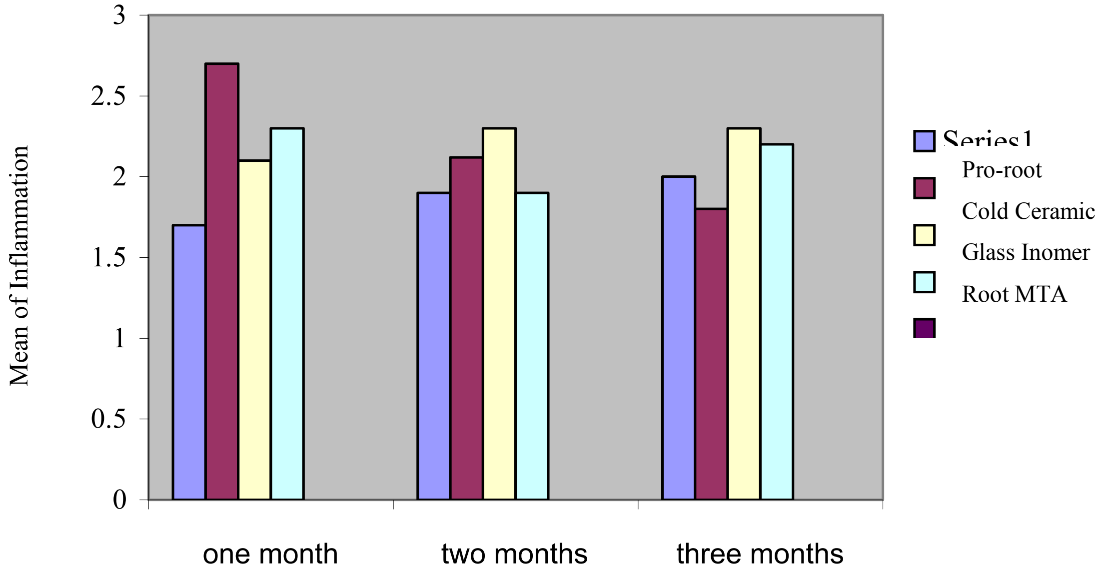
Over a one-month period, excluding the control groups, minimum and maximum severities of inflammation were seen in the Pro-root and the cold ceramic groups, respectively (table 1).

Over the one-month period, average values of inflammation severity in the perforated areas for the four materials of Pro-root, cold ceramic, glass ionomer, and Root MTA showed slight differences but they were not significant ($P>0.05$) (diagram 1 and table 1).

The difference in average values of inflammation severity over the one-month study among cold ceramic, glass ionomer, Root MTA, and negative control samples were significant; however, they did not show any significant differences with positive control samples. Comparison of the Pro-root and the negative control groups showed no significant differences ($P>0.05$) but showed a significant difference when compared with the positive control group ($P<0.05$). These results are in favor of that the Pro-root material indicates its superiority in dental repair when is compared to the other three test materials (diagram 1).

In the two-month period, maximum inflammation severity belonged to the positive control group while the minimum one belonged to the negative control group. No significant differences were observed among the four groups of Pro-root, cold ceramic, glass ionomer, and Root MTA ($P>0.05$) (table 2 and diagram 2). Slight differences were observed among the cold ceramic, Root MTA, and the negative control groups but the difference was significant between the Root MTA and the positive control groups in the two-month period ($P<0.05$) which indicates the improvement of cold ceramic and Root MTA with increased time. Comparison of the Pro-root and both negative and positive control groups was similar to that of the one-month period (diagram 1).

In the three-month period, the minimum and maximum inflammation severities belonged to the negative and positive control groups, respectively. The average values of inflammation among the four test groups showed s



significant (P>0.05). However, under similar conditions, the Pro-root samples showed better repair capacity as compared to other groups, but this difference was not significant. The periodontal tissues surrounding cold ceramic and Root MTA showed better repair and less inflammation through the time (diagram 1).

Diagram 1: Average values of inflammation severity as a result of applying different materials after 1, 2, and 3 months.

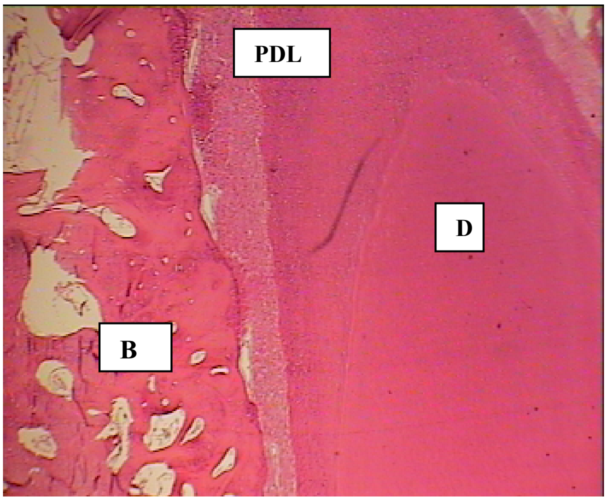


Figure 1: Microscopic View of Pro-root tissue specimen after one month (x40).

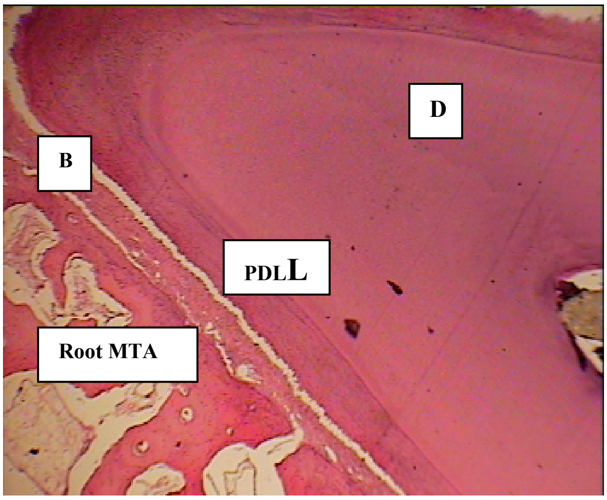


Figure 2: Microscopic View of Root MTA tissue specimen after one month (x40).

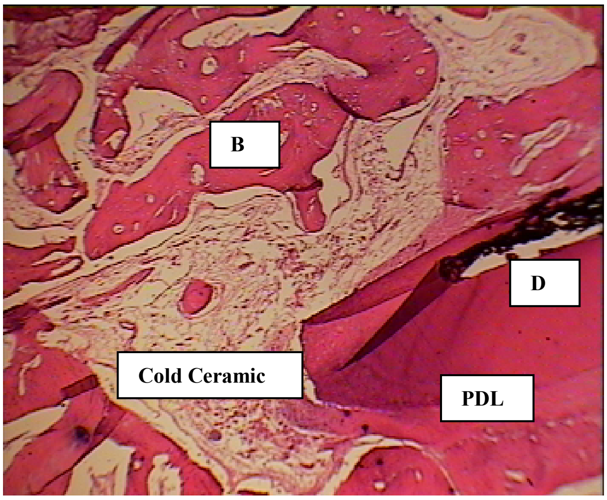


Figure 3: Microscopic View of cold ceramic tissue specimen after one month (x40).

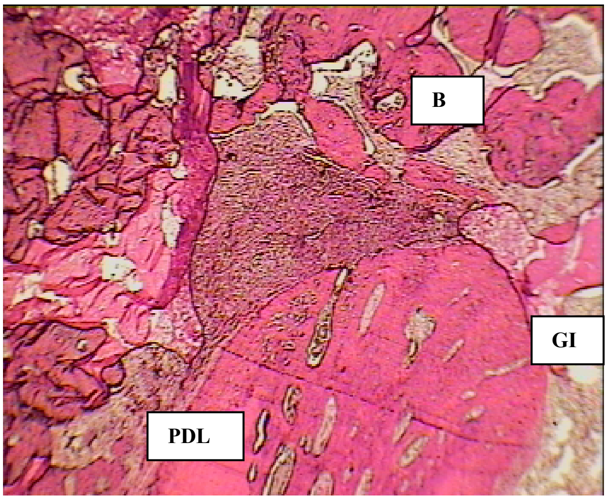


Figure 4: Microscopic View of glass ionomer tissue specimen after one month (x40).

Discussion

In this study, biopsy samples were taken from perforated teeth at one, two, and three-month intervals. In all three study time periods, the periodontal tissues surrounding Pro-root had the lowest severity of inflammation. This finding is in agreement with those of Torabinejad, Pittford, and Holland [1, 4, 5]. Researchers have recently found Pro-root to be a better and more suitable material for sealing furcal and lateral perforation, compared with glass ionomer cement, amalgam, IRM, and composite resins [3-5, 9].

Periodontal tissues in contact with cold ceramic and Root MTA showed relatively severe inflammation after one month but the inflammation reduced as time passed. These findings match those of Modarressi and Asna Asharai [6, 7]. They had found that the severity of inflammation in the merging tissues with cold ceramic and Root MTA reduce with time. Glass ionomer cement samples had only slight inflammation in all three study periods but showed no variation with time. Thus, periodontal tissues of perforated areas surrounding the four test materials showed various degrees of inflammation during all three study periods but the differences were not statistically significant. In a different experiment, all the samples were compared over a three-month period and it was observed that none of the four materials showed any statistically significant differences of microscopic variations over one, two, or three-month periods of time. However, the average values favor the Pro-root samples such that no improvement takes place in their repair over the time, whereas in cold ceramic and Root MTA time improves the repair trend.

Lee et al compared the sealing ability of Pro-root in repair of root lateral perforation with amalgam and IRM. They found Pro-root to be a far more suitable material as compared to IRM and amalgam [8].

Holland et al in their experimental study of furcal perforations repaired with amalgam and Pro-root observed that amalgam caused more inflammation and bone debridement as compared to Pro-root [5.] Pittford et al created furcal perforation in 28 premolars of 7 mandibles of dogs. They immediately filled half of the perforations with amalgam and the other half with Pro-root. The histological examination revealed that Pro-root was better suited for sealing furcal perforations than amalgam [4].

Glass-ionomer cement is another material used in sealing perforations. Himel and Hatem studied 60 extracted teeth. They created access cavities and furcal perforations in these extracted teeth. Perforations were repaired with glass-ionomer cement and composite resin with and without etching the enamel making up four groups. The results revealed that regardless of etching the enamel, glass-ionomer cement provided a better seal compared with composite resins [8].

More recently, Lotfi embarked on manufacturing a tooth filling material called Root MTA which is very similar to Pro-root. Iranian Organization of Sciences and Technology (IROST) has also compared and evaluated it against Pro-root to approve its quality. The advantages of the newly-manufactured material include its availability and lower price. Asna Ashari and Bolourchei studied this new material and compared its tissue reaction with that of Pro-root. They found that the two materials had similar favorable tissue reaction and that with passage of time, the tissue reaction against Root MTA reduced at a faster rate compared to Pro-root [6].

In their study on cold ceramic, Mozaeyeni et al investigated the bacterial seal of the three materials of amalgam, Pro-root, and cold ceramic and found that Pro-root had a lower micro leakage than cold ceramic, while cold ceramic was more advantageous than amalgam in this regard. The study also revealed that at longer periods of time, cold ceramic gained better sealing ability [10]. Modaressi investigated subcutaneous tissue compatibility of Pro-root and cold ceramic in rats. He showed that one-week specimens of cold ceramic caused higher inflammation than similar

Pro-root specimens, while one-month specimens of cold ceramic and Pro-root were not different in their inflammatory response [11]. However, tissues surrounding Pro-root showed less inflammation and higher repair capacity. According to Douglas and Baumgartner idea, the better tissue compatibility of Pro-root may be due to its more sealing ability [12].

Conclusion

The results of the present study showed that the four study materials of Pro-root, cold ceramic, Root MTA, and glass ionomer cement provide favorable conditions for periodontal tissue repair after perforation. However, tissues surrounding Pro-root showed less inflammation and higher repair capacity. The two materials of cold ceramic and Root MTA had a diminishing effect on the inflammation of the surrounding tissues through time, although the inflammation was rather high in the first month. As these two materials are being manufactured in Iran and, therefore, they are readily available and less costly, they can be recommended as appropriate substitutes for Pro-root, if and when they receive approval after other necessary tests. The fourth material, glass ionomer cement, caused rather high degrees of inflammation in the adjacent tissues which even increased with time. Thus, it ranks the fourth and the least recommendable material.

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