IEJ
Iranian Endodontic Journal

Histological Evaluation of Periapical Tissues after Root Canal Treatment with or without Coronal Seal in Dogs for Six Months

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ARTICLE INFO

Article Type: **Original Article**

Received: 13 Feb 2020
Revised: 18 May 2020
Accepted: 08 Jun 2020
Doi: *10.22037/iej.v15i4.26811*

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ABSTRACT

Introduction: Preventing recontamination of the obturated root canal is important for successful root canal treatment (RCT). The main purpose of this study was assessing the histological health of periradicular tissue in obturated root canals with or without coronal seal between two and six months. **Methods and Materials**: Sixty roots from five dogs with vital pulps were randomly assigned to one of the following five groups (n=12): Group 1, RCT and six months oral exposure; Group 2, RCT and immediate amalgam restoration; Group 3, RCT and amalgam restoration after two months exposure to the oral cavity; Group 4, RCT and amalgam restoration after four months exposure to the oral cavity; Group 5, RCT with two months exposure to oral cavity. The teeth were prepared and filled with gutta-percha and sealer using lateral condensation technique. Two intact root canals of each animal were regarded as the negative control group (n=10), and the two root canals exposed to the oral cavity constituted the positive one (n=10). After six months the animals were euthanized. The upper and lower jaws were removed and submitted for histological processing. Longitudinal sections were obtained from each root. After staining the sections, periradicular regions were examined histologically under light microscope. The Kruskal-Wallis and Mann-Whitney tests was used to analyze the data (P<0.05). **Results:** The results showed a significant difference between all groups (P<0.05). The negative control group was free of any inflammation. Two-by-two comparison revealed that the positive control group, Group 1 and Group 4 displayed the most intense inflammation. Groups 2, 3 and 5 showed similar results without developing any significant inflammation. **Conclusion**: Based upon the findings of this animal study, it can be recommended that the obturated root canals which are exposed to oral cavity for around four months or more should be retreated before crown restoration.

Keywords: Coronal Leakage; Inflammation; Periapical Tissue

Introduction

The primary purpose of endodontic therapy, once any pain is resolved, is prevention or treatment of periodontitis [1], which is commonly caused by microorganisms and their by-products in the root canal system [2, 3]. Elimination of the canal's microorganisms and avoiding reinfection are essential for successful root canal treatment [4]. However, it has been definitely proved that sealer and gutta-percha alone cannot prevent recontamination of the canal [5-7].

Several conditions might result in recontamination of obturated root canals. Examples of such conditions include delay in coronal seal placement, poor quality of coronal filling, and any crown fracture or caries [8]. Many studies have been performed to assess the maximum acceptable interval between root canal therapy and permanent coronal reconstruction [4-10].

Torabinejad *et al.* [8] examined coronal microleakage of root-filled teeth. Fifty percent of their extracted samples were recontaminated with bacteria after 19 or 42 days, depending on the type of microorganism. Khayat *et al.* [4], in an *in vitro* survey, evaluated human saliva penetration to the coronally unsealed obturated root canals. They have stated that these canals might be recontaminated in less than 30 days after obturating. Yavari *et al.* [11] recommended to apply intra-orifice barriers to minimize the canal recontamination. In another study they found that, MTA and CEM cement could make a better coronal seal against the invasion

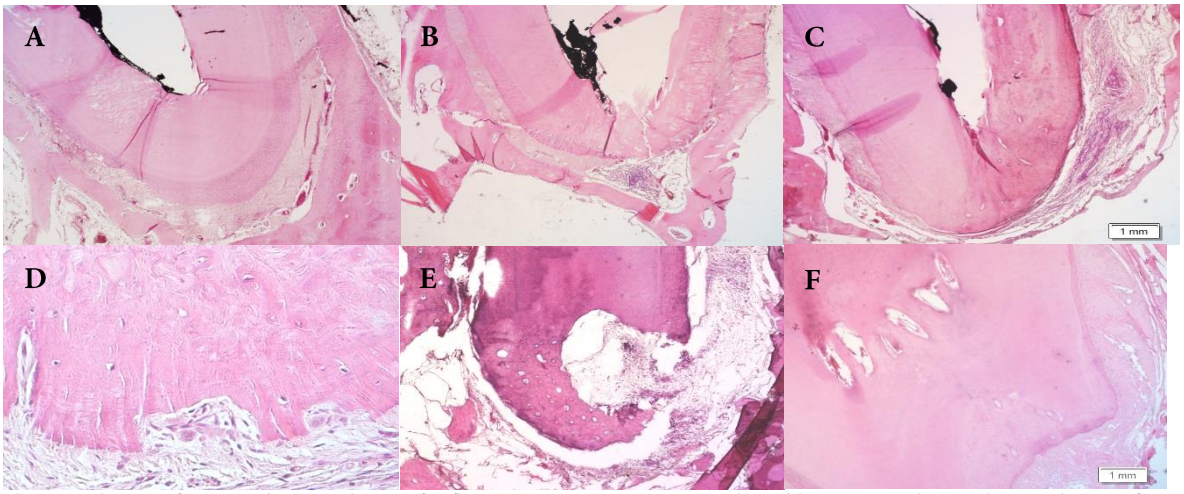

Figure 1. *A)* Normal view of apex with no evidence of inflammatory reaction or periodontal ligament widening (original magnification 25×); *B)* Severe inflammatory cell infiltration localized to the apical area (original magnification 25×); *C)* A root with inflammatory reaction beyond half of the apical periodontal ligament is shown (original magnification 25×); *D)* Osteoclast cells present in Howship's lacunae, causing resorption on apical cellular cementum (original magnification 4×); *E)* Severe root and bone resorption (original magnification 25×); *F)* Multiple foramina at the apex of canine teeth are shown (original magnification 25×)

of bacteria in to the canals in comparison with amalgam and resin composites [12]. Naseri *et al.* [13] compared three temporary filling materials as intra-orifice barriers, they concluded that the Zonalin showed more leakage than the other materials.

Based on the results of past studies, the obturated canals' recontamination might happen between one to three months after oral exposure [4-10, 14, 15]. Magura *et al.* [16] stated that salivary leakage at three months was clinically noticeable and significantly more intense than that seen before one month.

Most of these examinations have been done under *in vitro* conditions. However, the outcomes might have been influenced by the dynamic oral environment and the host defensive response. It is impossible to correlate *in vitro* coronal leakage with *in vivo* periradicular disease [7]. Furthermore, it is still unknown how long after coronal-seal breakage periradicular disease might happen in endodontically treated teeth. The present study aims to obtain a more practical guideline in this regard.

Materials and Methods

All the procedures utilized in this study were in compliance with the ethics guidelines recommended by the International Organization for Standardization (ISO 7405-1997 [E]: Biological evaluation of dental materials) [17]. It was also approved by the Animal Research Committee of Shiraz University (No 4965). Five 1 to 1.5 year-old healthy hybrid dogs weighing between 20-25 kg were selected. The specimens consisted of 80 vital root canals by using premolars from both jaws. All the teeth were intact. Any sign of caries, wear, or fracture would exclude the specimen from the study. Before root canal therapy, the animals were anesthetized by intravenous administration of 10% Ketamine (20 mg/kg) (Ketamin; Alfason, Woerden, Holland) and 2% Xylazine (0.15 mg/kg) (Alfasan, Woerden, Holland). 1-2% Halothane was also applied to maintain the anesthesia. The jaws were stabilized with a bite block, and periapical radiographs were taken using parallel technique. The regional nerve was blocked by injecting local anesthetic (2% Lidocaine with 1:100000 epinephrine).

The specimens were divided into five experimental groups, one negative, and one positive control group. For each animal, the teeth related to test groups were scattered in four quadrants. Two intact root canals of each animal were considered as the negative control group (n=10), and the two root canals exposed to the oral cavity without any root or crown filling constituted the positive one (n=10). Treatment procedure in test groups (n=12) was as follows: Group 1: Root canal filling without any crown restoration for six months, Group 2: Root canal filling and immediate crown reconstruction with amalgam for six months, Group 3: Root canal filling with two months of exposure to the oral cavity and then amalgam reconstruction of the crown for four months, Group 4: Root canal filling with four months of exposure to the oral cavity and then amalgam reconstruction of the crown for two months and finally Group 5: Root canal filling exposed to the oral cavity for two months (for sacrificing all the dogs at six months these teeth were received root canal treatment after four months delay in comparing to the other groups).The teeth related to the first four

groups were isolated with rubber dam. After disinfecting the subject area with 2% chlorhexidine gluconate, access to the pulp chambers was made. The root canal pulp was removed with H-file #25 (Dentsply Maillefer, Ballaigues, Switzerland). The working length was estimated 1 mm shorter than the apex, applying K-file #25 (Dentsply Maillefer, Ballaigues, Switzerland) and apex locator Raypex5 (Bayerwaldstr, Munchen, Germany). This had been confirmed by using periapical radiography.

Biomechanical cleaning of the root canals was done with K-files (Dentsply Maillefer, Ballaigues, Switzerland) up to #35 in the working-length limit, and canal preparation was completed with ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) by manufactory order. 2.5% sodium hypochlorite was used as root canal irrigation during instrumentation. After apical enlargement with K-file #40 and final irrigation with normal saline, the root canals were dried by paper points. The roots were obturated by lateral condensation technique using gutta-percha cones (Dentsply-Herpo, Petropolis, Brazil) and AH-26 sealer (Dentsply Maillefer, Ballaigues, Switzerland). Dental radiographs were taken to evaluate the quality of root canal fillings.

The coronal access cavities of Group 2 were immediately restored with at least 3 mm amalgam (Velvalloy, SS White Ltd, Rio de Janeiro, Brazil) and the other groups were left exposed to the oral environment. Over the course of study, the dogs were fed with soft diet. Two months later, the dogs from Group 3 were anesthetized again, and crown amalgam reconstruction was done for obturated roots. Four months after the first treatment session, samples from Group 4 were restorated with amalgam and Group 5 underwent root canal therapy with the crowns exposed to the oral environment. After a six-month study period, the animals were euthanized by anesthetic overdose. The upper and lower jaws were removed and sectioned into segments, each containing one root with at least 2 mm of the surrounding apical tissues. The segments were fixed with 10% phosphate-buffered formalin, decalcified in 10% nitric acid for 10 to 15 days, and then embedded in paraffin.

The blocks were serially sectioned in longitudinal orientation to an average thickness of 5 µm. Five sections per root were selected, which showed the full length of the canal and at least 2 mm of periradicular tissue. In this situation the apical foramen located somewhere in the samples.

After staining the sections with hematoxylin-eosin, histological evaluation of periradicular tissues were done by two blind skilled examiners independently (one endodontist and one oral pathologist). The evaluation has been performed under a light microscope (Zeiss, Olympus, Tokyo, Japan) under 25× and 40× magnifications. The histological analysis was based on the five parameters of Leonardo's studies (11, 15) (Table 1). The first four parameters have been scored from 1 to 4, and the last one from 1 to 3. A higher score indicates worse conditions of periradicular tissue.

Statistcal analysis

Kruskal-Wallis nonparametric test was used for evaluating the difference between groups. The significance level was set at $P<0.05$. In case of noticing any significant difference, two-by-two comparison of Mann-Whitney was done for the groups ($P<0.05$).

Table 1. Parameters and scores used for evaluation

Parameters	Scores
Extension of the inflammatory reaction	1: Absent
	2: Restricted to the apical foramen
	3: Up to half of the apical periodontal ligament
	4: Beyond half the apical periodontal ligament
Intensity of the inflammatory infiltration	1: Absent
	2: Slight
	3: Moderate
	4: Severe
Apical periodontal ligament thickness (mm)	1: Normal (0.36 mm)
	2: Moderately enlarged (0.37–046 mm)
	3: Intensity enlarged (0.47–0.55 mm)
	4: Severely enlarged (more than 0.56 mm)
Cementum-Dentin resorption	1: Absence
	2: Discrete
	3: Moderate
	4: Severe
Bone tissue resorption	1: Absence of resorption
	2: Few areas of resorption
	3: Large areas of resorption

Results

The negative control samples were entirely normal with score 1 for each parameter and have been regarded as normal references. The results obtained from the other groups are reported in Table 2. Based on the outcomes of Kruskal-Wallis test, all five experimental groups and the positive control group had significant difference ($P<0.05$). Therefore, two-by-two comparisons were performed to obtain more detailed results (Table 3).

Extension of the inflammatory reaction

The positive control group had the highest extension of inflammatory reaction with significant difference from the other groups. Groups 1 and 4 showed the high extension of inflammatory reaction. Group 1 showed significant difference from Groups 2, 3 and 5. Group 4 demonstrated significant difference from Groups 2 and 3. The lowest extension of inflammatory reaction was found in Group 2 (Figure 1A).

Intensity of the inflammatory infiltration

The positive control group, Group 1 and 4 revealed the highest levels of inflammatory infiltration respectively (Figure 1B). These three groups were significantly different from the other groups in this regard. The intensity of inflammatory infiltration in Group 2 was at a minimal level without significant difference from Groups 3 and 5.

Apical periodontal ligament thickness

The periodontal ligament width was measured by an eyepiece micrometer with accuracy of 0.01 mm. The average width of 5 specimens in each group was consider as this entity.

The positive control group had the maximum number of samples with widening in apical periodontal ligament thickness. This occurred in a few cases of Group 1 and 4. Periodontal ligament widening was a rare finding in Groups 2, 3, and 5 (Figure 1C). The difference of the positive control group with the other groups is significant.

Cementum-dentin resorption

The highest incidence of cementum and dentin resorption was displayed in the positive control group (5/8 samples), with significant variance from Groups 2, 3, and 5 (Figure 1D). Neither of the samples in Groups 2, 3, or 5 demonstrated any score of resorption.

Bone tissue resorption

The few areas of bone tissue resorption were a common finding in the positive control group as well as Group 1 and 4. Groups 2, 3, and 5 indicated no sign of resorption. Only one sample in positive control group (1.8%) revealed notable resorption of bone during the six months, (Figure 1E). Radiographic observations were absolutely in compliance with the histological findings. Multiple foramina at the apex of canine teeth was seen (Figure 1F).

Table 2. Numbers of teeth in each score and mean rank

Parameters evaluated in inflammatory reaction	Scores	1 (*n*=12)	2 (*n*=12)	3 (*n*=12)	4 (*n*=12)	5 (*n*=12)	Control+ (*n*=10)	Total (*n*=70)	*P*-value
Extension	**1**	0	10	7	2	8	0	27	0.000
	2	9	2	5	8	2	2	28	
	3	2	0	0	2	2	3	9	
	4	1	0	0	0	0	5	6	
	Mean Rank	54.54	23.25	31.13	47.25	31.50	66.25		
Intensity	**1**	0	10	7	2	8	0	27	0.000
	2	4	1	3	4	1	2	15	
	3	4	1	2	3	2	2	14	
	4	4	0	0	3	1	6	14	
	Mean Rank	56.33	23.25	30.54	48.79	30.79	63.19		
Periodontal ligament thickness (mm)	**1**	9	12	11	9	12	1	54	0.000
	2	3	0	1	3	0	6	13	
	3	0	0	0	0	0	2	2	
	4	0	0	0	0	0	1	1	
	Mean Rank	39.1	31.70	34.1	39.1	31.70	65.9		
Cementum-Dentin resorption	**1**	7	12	12	9	12	4	56	0.000
	2	5	0	0	2	0	3	10	
	3	0	0	0	1	0	2	3	
	4	0	0	0	0	0	1	1	
	Mean Rank	47.21	32.00	32.00	41.71	32.00	56.38		
Bone tissue resorption	**1**	7	12	11	9	12	2	53	0.000
	2	5	0	1	3	0	7	16	
	3	0	0	0	0	0	1	1	
	Mean Rank	46.13	30.50	33.63	39.88	30.50	64.31		

Discussion

It is well-documented that gutta-percha together with sealers cannot inhibit the corono-apical entrance of bacteria to the canal [5-7]. A proper coronal seal has been proved to be significantly influential on periapical tissue health [14, 18]. The required time span for the appearance of apical periodontitis after exposure of the obturated root to the oral environment is still controversial [19].

Canine teeth are commonly used in order to simulate human teeth. They have been regarded as a good experimental model [14].Each dog has 14 premolar teeth, most of which are two-rooted. In sum, 24 roots per dog were available for this study. The main difference between canine and human teeth is the apical anatomy of the root. Multiple foramina are a common finding at the apex of canine teeth [14]. Radiographic examination is the most common technique for evaluating the result of root canal therapy[18]. This kind of test is highly subjective, and can be influenced by multiple factors [20]. The outcome of endodontic therapy is depending on cure or healing of apical periodontitis, which is a histological status. Therefore, histological method was selected for evaluating the results. This technique seems to have a minimal amount of variations.

Previous investigations [4, 8, 15] showed that coronally unsealed obturated canals will be recontaminated with saliva and microorganisms in a short period of time after root canal therapy. In the present study, 100% of obturated roots without coronal seal (Group 1) showed signs of inflammation at the apex. After six months, the inflammation amounts of Group 1 and the positive control group were similar. Yamauchi *et al.* [1] obtained the same results for groups with eight months of oral exposure in dogs. Comparing the sealing quality of Epiphany with Sealapex in obturated canine teeth, Leonardo *et al.* [14] found the inflammation in most of the coronally unsealed samples after three months.

Magura *et al.* [16] reported in an in vivo study that the obturated roots might need retreatment after three months of exposure to the oral environment. They also stated that one month of oral exposure is not clinically significant, but they could not predict what would happen between one month and three months. so experimental model of the present study was designed to answer this question and compare the periradicular health of root canal filled teeth with or without coronal seal after two, four and six months. We have one group that left open for 2 months and sealed after that period. In this group after six months follow, we could not find any significant inflammation at the apical area of teeth and it possibly showed that if the tooth sealed after two months of root canal therapy it may remain safe even if some bacteria invaded the canal.

Groups 3 was designed to investigate if any changes would occur in the periradicular tissue of the root canal treated teeth with two months exposure to oral cavity before crown restoration. Most of the specimens were completely free of inflammation and none of them suffered any hard tissue resorption. The same results were obtained from specimens in group 5 which the obturated root canals were exposed to oral cavity for two months before sacrificing the animals. The most noticeable finding of this study is that a two-month delay for crown restoration of filled roots might not threaten the treatment outcome.

Table 3. Results of two-by-two comparisons of tested groups. (*P*-Value obtained by Mann-Whitney U Test)

Parameters evaluated in inflammatory reaction	Extension	Intensity	Periodontal ligament thickness (mm)	Cementum-Dentin resorption	Bone tissue resorption
Groups 1-2	0.000*	0.000*	0.070	0.014*	0.014*
Groups 1-3	0.001*	0.001*	0.284	0.014*	0.065
Groups 1-4	0.248	0.334	1.000	0.506	0.397
Groups 1-5	0.008*	0.004*	0.070	0.014*	0.014*
Groups 1-Control+	0.021*	0.185	0.006*	0.199	0.031*
Groups 2-3	0.187	0.206	0.317	1.000	0.317
Groups 2-4	0.001*	0.002*	0.070	0.070	0.070
Groups 2-5	0.286	0.305	1.000	1.000	1.000
Groups 2-Control+	0.000*	0.000*	0.000*	0.002*	0.000*
Groups 3-4	0.023*	0.022*	0.248	0.070	0.284
Groups 3-5	0.946	0.920	0.317	1.000	0.317
Groups 3-Control+	0.000*	0.001*	0.001*	0.002	0.001*
Groups 4-5	0.061	0.036	0.070	0.070	0.070
Groups 4-Control+	0.005*	0.050	0.006*	0.113	0.006*
Groups 5-Control+	0.001*	0.002*	0.000*	0.002*	0.000*

**: Statistically significant*

Group 4, with four months coronal leakage before crown restoration, showed significant levels of preradicular inflammation. Root or bone resorption was a rare finding in all samples of this study. Only one sample in positive control group demonstrated severe bone resorption. This outcome was similar to the findings of Sabeti *et al.* [21] and Mah *et al.* studies [19].

The present survey has been performed *in vivo*, and the findings are based on the histological evaluation of peri-radicular tissue. Additionally, the current design of the experimental groups in this study makes it different from the other studies so it is difficult to compare the results with previous ones. We would recommend further studies to determine the exact time taken for periapical inflammation to occur.

Conclusion

The results of this animal study indicated that the obturated canals which were exposed to oral cavity for two months may be restored safely without retreatment as there was no evidence of any significant histological periapical inflammation. The obturated root canals with 4 and 6 months exposure to oral cavity should be retreated due to findings of this study.

Acknowledgement

Acknowledgement: The authors thank the Vice-Chancellory of Shiraz University of Medical Sciences for supporting this research (Grant#91-01-03-4617). This manuscript is based on the thesis by Dr Atefeh Hoseini. The authors also thank Dr M Vosough of the Center for Research Improvement of the School of Dentistry for the statistical analysis.

Conflict of Interest: 'None declared'.

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Please cite this paper as: Moazami F, Mirhadi H, Hoseini A, Sahebi S, Torabinejad M. Histological Evaluation of Periapical Tissues after Root Canal Treatment with or without Coronal Seal in Dogs for Six Months. Iran Endod J. 2020;15(4): 211-6. Doi: 10.22037/iej.v15i4.28611.