Original Article

Comparison of Tissue Reaction of Pulp Chamber Perforations in Dogs' Teeth Treated with MTA, Light-Cured Glass Ionomer and Amalgam

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Abstract:

Statement of Problem: Perforations are significant complications that can occur during root canal therapy and may result in the destruction of adjacent periodontal tissues. An ideal material for repairing a perforation should be biocompatible and have a high sealing ability.

Purpose: The aim of this study was to compare histologic tissue responses of experimentally induced pulp chamber perforations in dogs' teeth repaired with amalgam, light-cured glass ionomer and Mineral Trioxide Aggregate (MTA).

Materials and Methods: Fifty-four lower premolars of 9 dogs were used for this interventional study. Access cavities were prepared and perforations were created on the floors of the pulp chambers. The samples were divided into three experimental groups of 12 teeth and positive and negative control groups consisted of 12 and 6 teeth, respectively. The perforations in the study groups were sealed with amalgam, light-cured glass ionomer and MTA. All access cavities were filled with light-cured glass ionomer. Five dogs were sacrificed after seven days and 4 dogs were put to death after 28 days. The premolars along with the surrounding alveolar bone were cut in block sections and histologically evaluated for inflammation, bone formation and epithelial proliferation. The data were analyzed by Kruskal-Wallis and Mann-Whitney tests .

Results: A statistically significant difference was observed in inflammation and bone regeneration, between amalgam and MTA at both time periods.

Conclusion: It appears that MTA and GI are more suitable materials for perforation repair, as compared to amalgam

Key Words: Pulp chamber Perforation; Amalgam; Light-cured glass ionomer; MTA

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INTRODUCTION

Root perforations are significant complications of endodontic treatment [1], which can often result in the loss of periodontal tissue [2]. A perforation can be described as an accidental opening on the crown or root that may create an artificial communication between the tooth and its supporting tissues. Perforations can result from a resorptive process or can be produced iatrogenically throughout the course of root canal therapy due to an incorrectly directed bur, during filing and post-space preparation, or when trying to locate calcified pulp chambers and canals [1]. The subsequent inflammation may rapidly produce a comm-unication with the gingival sulcus and an

irreversible periodontal lesion resulting in tooth loss. Considering the serious clinical consequences following perforation, intervention would be necessary [3].

When a perforation has occurred, the initial attempt at correction should be an internal repair. Corrective surgery could be reserved for cases in which internal repair is not a treatment option or when internal repair has failed [4]. An important factor in both methods is to use an ideal repair material which should have the ability to seal and to induce osteogenesis and cementogenesis [5]. In addition, substances that come in direct contact with vital tissues should have precise standards of tissue compatibility as well as having the capability of satisfying the treatment and/or mechanical needs [1]. It should be noted that all materials used for restoring a perforation may also have disadvantages [6]. Different materials are used for repairing perforations of chamber surfaces.

The objective of the present study was to compare tissue reaction to amalgam, light-cured glass ionomer (GI) and MTA, used as materials to repair experimentally induced pulp chamber perforations in dogs' teeth. The evaluated histologic tissue responses included inflammation, bone formation and epithelial proliferation

MATERIALS AND METHODS

A total of 54 lower premolars of 9 mature, healthy 1-3 year-old dogs of mixed breeds were used for this interventional study. The experimental protocol was approved by the Tehran University of Medical Sciences animal ethics committee. Each dog was anesthetized with an intramuscular injection of 0.25-0.5mg/kg Acepromazine (Aveco Co., Inc., Fort Dodge, IA), followed by an intravenous injection of 20mg/kg sodium thiopental (Pentotal, Abbot, Madrid, Spain). An access cavity was prepared and a perforation was created on the floor of the pulp chamber by a 2-mm-diameter diamond fissure bur using a high-speed handpiece. In order to control the bleeding, pressure was applied for 5 minutes on the perforation sites by cotton pellets moistened with normal saline. The samples were divided into three experimental groups of 12 teeth each and two control groups. The perforations in the 1st and 2nd dogs were sealed with amalgam (Luxalloy, Fagihi Co., Iran), the 3rd and 4th dogs with light-cured glass ionomer (Fuji II Lc-GC corporation, Japan) and the 5th and 6th dogs with MTA (PRO Root, Dentsply Tulsa Dental, Tulsa, OK, USA). All access cavities were filled with light-cured glass ionomer. In the 12 teeth selected for the positive control group (7th and 8th dogs), the perforations and access cavities were left open to salivary contamination without repair. The negative control group consisted of six teeth (9th dog) with no perforations. Dog numbers 1, 3, 5, 7, and 9 were sacrificed after seven days and dog numbers 2, 4, 6, and 8 were put to death after 28 days using an increased amount of sodium thiopental anesthetics (30mg/kg maximum). Immediately after death, the respective premolar teeth along with the surrounding alveolar bone were cut in block sections using a hand saw and placed in labeled containers with 10% buffered formalin for 24 hours. All specimens were processed and embedded in paraffin. Cross-sections of each block, approximately 5-7μm thick, were obtained and stained with Hematoxylin-Eosin (H&E), followed by examination under a light microscope. The histologic sections were assessed for inflammation, bone formation and epithelial proliferation. The severity of inflammation was classified as none where there was no infiltration of inflammatory cells, mild where a few scattered inflammatory cells were seen, moderate where inflammatory cells did not obscure the normal tissues, and severe when massive infiltration of inflammatory cells replaced normal tissue. Presence and absence of bone regeneration and epithelial

proliferation were scored as 1 and 0, respectively. The data were analyzed by Kruskal-Wallis and Mann-Whitney.

RESULTS

Histologic examination of the negative control group revealed normal alveolar bone and no inflammatory changes. In the positive control group, chronic inflammation was observed under the proliferating epithelium that extended into the surrounding bone.

Results of bone regeneration, inflammation, epithelium reproduction of experimental groups after 7 and 28 days are summarized in Table I and II respectively. Comparison of the histologic tissue responses in the amalgam group at 7 and 28 days, revealed a statistically significant difference only in inflammation (Fig.1). Inflammation was significantly higher at 7 days as compared to 28 days. In the light-cured GI group, bone regeneration showed a significant difference between the two time periods. Bone regeneration was significantly higher at 28 days as compared to 7 days (Fig. 2). A significant difference was not observed between any of the histopathologic criteria in the MTA group (Fig. 3).

Microscopic tissue responses were compared between the three groups and the following parameters showed statistical significance:

1) Inflammation, between amalgam and light-cured GI at 7 days.

2) Inflammation, between amalgam and MTA at both time periods and bone regeneration at 28 days.

3) Inflammation and epithelial proliferation between amalgam and positive controls at both time periods.

4) Inflammation, between GI and positive controls at 7 and 28 days; bone regeneration and epithelial proliferation both at 28 days.

5) Inflammation, between MTA and positive controls at 7 and 28 days; bone regeneration and epithelial proliferation both at 28 days.

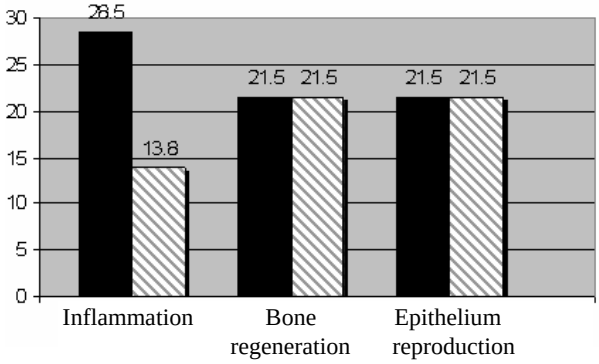

Fig. 1: Comparison of the histologic tissue responses in the amalgam group at 7 and 28 days.

DISCUSSION:

A variety of in vivo and in vitro methods have been suggested for the evaluation of dental materials [7]. Previous studies have indicated that dog lower premolars are suitable for investigation of tissue responses following pulp chamber perforations [8]. Dog premolars have two roots which often diverge, 1-2 mm

Table I: The results of histologic assessment in experimental groups after 7 days.

Materials	Inflammation (%)				Total	Bone Regeneration (%)		Total	Epithelium Reproduction (%)		Total
	0	1	2	3		0	1		0	1	
Amalgam	0 (0)	0 (0)	13 (61.9)	8 (38.1)	21 (100)	22 (100)	0 (0)	22 (100)	22 (100)	0 (0)	22 (100)
GI	0 (0)	10 (47.6)	11 (52.4)	0 (0)	21 (100)	21 (100)	0 (0)	21 (100)	21 (100)	0 (0)	21 (100)
MTA	2 (2.3)	13 (54.2)	9 (37.5)	0 (0)	24 (100)	23 (95.8)	1 (4.2)	24 (100)	24 (100)	0 (0)	24 (100)
Non	0 (0)	0 (0)	7 (33.3)	14 (66.7)	21 (100)	21 (100)	0 (0)	21 (100)	21 (100)	0 (0)	21 (100)
Total	2 (2.3)	23 (26.4)	40 (46.0)	22 (25.3)	87 (100)	87 (98.9)	1 (1.1)	88 (100)	88 (100)	0 (0)	88 (100)

Table II: The results of bone regeneration, inflammation, epithelium reproduction in experimental materials after 28 days.

Materials	Inflammation (%)				Total	Bone Regeneration (%)		Total	Epithelium Reproduction (%)		Total
	0	1	2	3		0	1		0	1	
Amalgam	0 (0)	12 (60)	8 (40)	0 (0)	20 (100)	20 (100)	0 (0)	20 (100)	20 (100)	0 (0)	20 (100)
GI	1 (5)	13 (65)	6 (30)	0 (0)	20 (100)	12 (80.0)	3 (20)	15 (100)	21 (100)	0 (0)	20 (100)
MTA	6 (25)	13 (54.2)	5 (20.8)	0 (0)	24 (100)	7 (29.2)	17 (70.8)	24 (100)	24 (100)	0 (0)	24 (100)
Non	0 (0)	0 (0)	14 (58.3)	10 (41.7)	24 (100)	24 (100)	0 (0)	24 (100)	21 (100)	24 (100)	24 (100)
Total	7 (8)	38 (43.2)	33 (37.5)	10 (11.4)	88 (100)	63 (75.9)	20 (24.1)	83 (100)	88 (100)	24 (27.3)	88 (100)

short of the CEJ [9].

The micro-trauma resulting from perforation causes inflammation in the tooth supporting tissues, which in turn may produce an irreversible periodontal lesion. It has been shown that smaller perforations cause less infection, and closing them with highly sealable materials would be faster and can improve the prognosis [10,11]. The size of perforations in the present investigation was standardized by using a fissure bur to penetrate the alveolar bone without any lateral movement during the procedure in all animals. After controlling the bleeding, the perforation was repaired immediately in all cases to minimize the possibility of microbial infection. Therefore the sealing capability and tissue compatibility of the studied materials were the only factors affecting the results [12,13].

In the amalgam and light-cured GI groups, the intensity of inflammation decreased over time and the bleeding caused by the perforations was organized. The level of inflammation in the MTA group did not change significantly [3,14]. The lower amount of inflammation in this group may be due to the favorable properties of MTA such as: increasing the pressure strength through time and in humid situations, ability to set in the presence of blood, being hydrophilic, low cellular toxicity of freshly mixed cement, anti-microbial effect on certain bacteria, and high pH levels [5,15].

Perforation repair is considered ideal when regeneration of the surrounding bone and periodontium occurs [7]. Bone regeneration has been demonstrated in both MTA and GI, but it developed faster and more pronounced in MTA-repaired teeth [16]. Glass ionomers aggregate (MTA) has been widely utilized in have many favorable properties including

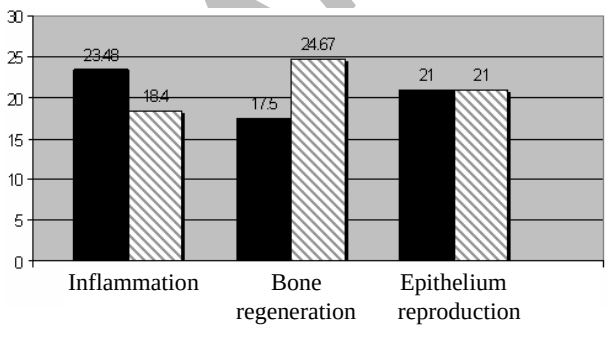

Fig. 2: Comparison of the histologic tissue responses in the glass ionomer group at 7 and 28 days.

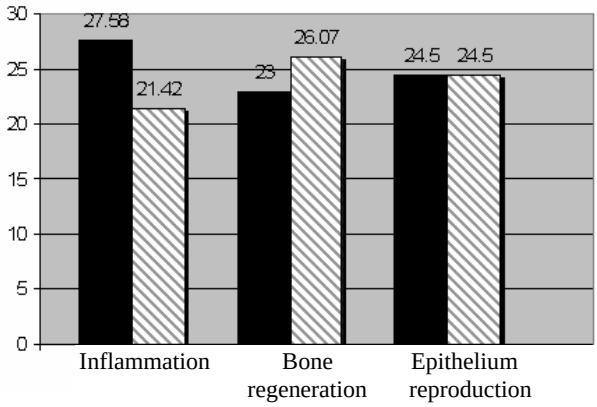

Fig. 3: Comparison of the histologic tissue responses in the MTA group at 7 and 28 days.

fluoride release and rapid setting rate which makes them suitable for application in humid setting conditions [17]. Mineral trioxide endodontic treatment and has shown good results when used as a repair material. Koh et al [18] studied the cytomorphology of osteoblasts and cytokine production in the presence of MTA. They reported that MTA offers a biologically active substrate for bone cells and stimulates interleukin production. Scanning electron microscopy revealed healthy cells in contact with MTA after 1 to 3 days. The stimulating effect of MTA on osteoblasts and cementoblasts makes it a suitable material for the treatment of root perforations with the goal of regenerating a periodontal attachment and inducing osteogenesis and cementogenesis. Repair of the perforated defect is usually complicated by the fact that the size of the defect may allow extrusion of the material into the periodontal ligament space and surrounding structures (9). Deposition of hard tissue over MTA and fusion of newly formed cementum to the original cementum on the root surface has been reported and may compensate for the presence of a foreign material in vital tissues [19].

In the present study, MTA showed less inflammation than amalgam which was similar to the results obtained by Torabinejad et al [7]. In addition, MTA and GI were found to be biocompatible. This was in accordance with a study conducted by Holland et al [20] who also demonstrated superior biologic qualities of MTA compared to GI. Amalgam treated specimens showed the highest score of inflammation in the current investigation which was almost equal to the positive control samples.

Previous studies have shown a high probability of pocket formation subsequent to furcal perforation which can increase with time [4,21]. A layer of epithelium is usually observed immediately beneath the perforation site along with mild inflammation [22]. Similar results were obtained in the present study. All positive controls revealed epithelial proliferation and chronic mild inflammation at 28 days.

CONCLUSION

Based on the conditions of this study, the following conclusions could be proposed:

1. Perforation of the pulp chamber may have serious clinical consequences including epithelial proliferation and possible periodontal pocket formation. Treatment of these defects with MTA and light-cured GI showed better healing responses compared to amalgam. Considering that the histological findings regarding amalgam were similar to the control group, use of amalgam is suggested as a control material in similar future studies.
2. Inflammatory infiltration changed and decreased from acute to chronic during the study period. Bone regeneration increased from 7 to 28 days in the MTA and GI groups which are both considered as biocompatible materials.
3. Application of MTA for repairing perforations is superior to GI and amalgam due to the high moisture resistance.
4. In future studies, evaluation of tissue response to MTA during a shorter time period (less than 7 days) is suggested.

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