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Original Article

Scanning electron microscopic evaluation of the effect of different root-end resection methods in the crack formation in root canals filled with mineral trioxide aggregate or calcium-enriched mixture cement

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ABSTRACT

Background: There is some concern that root resection may alter the surface features and crack formation of the previously set orthograde material. The aim of this *in vitro* study was to evaluate the crack formation in orthograde mineral trioxide aggregate (MTA) and calcium-enriched mixture (CEM) plugs after root resection.

Materials and Methods: This *in vitro* study was conducted on 170 extracted human maxillary anterior teeth. The teeth were randomly divided three experimental (n = 50) and control (n = 20) groups. In Group 1, after root canal treatment, half of the roots were cut with a bur, and the other half with an ultrasonic cutter. In Groups 2 and 3, after the 4-mm CEM and MTA plugs were placed and set, the root ends of half of the samples were cut with a bur and the other half by an ultrasonic cutter. The prevalence of cracks in the dentin and orthograde apical plugs of MTA and CEM was then assessed by scanning electron microscopy. Data were analyzed using the McNemar's, Chi-square, and Fisher's exact tests at $P \leq 0.05$ level of significance.

Results: In general, the prevalence of crack in dentin in Groups 2 and 3 was significantly higher than in the plug ($P < 0.05$). There was no significant difference in the prevalence of dentin crack in the studied groups ($P > 0.05$). The prevalence of crack in dentin was lower when the bur was used to cut off the end of the root, although the difference was not significant. The prevalence of crack in the plug was similar in CEM and MTA.

Conclusion: Based on the results of this study, the prevalence of crack in dentin is always significantly higher than its prevalence in the plug, and the prevalence of crack in the plug was similar in CEM and MTA; then, when there is an orthograde access to the root canal and surgery is likely in future, MTA and CEM can be placed in an orthograde technique and it just resects the root during surgery.

Key Words: Dentine, pulp canal, retrograde obturation, root canal, ultrasonic surgery

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INTRODUCTION

There are several challenges in teeth requiring endodontic treatment, one of which is changes

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in the pulpo-dentinal complex such as pulp canal obliteration or calcific metamorphosis which may commonly occur to young people's teeth due to trauma.[1] Furthermore, degenerative changes in the pulp-dentin complex associated with aging lead to a reduction in the size of the pulp space over time due to secondary and tertiary dentin deposition.[2] Diffuse pulp calcifications may interfere with the usual root canal treatment when they engage canal curvature or block the canal.[3]

Complete blockage of the canal space in radiography does not necessarily mean that there is no canal space, and in most of these cases, the canal space contains pulp tissues.[4,5] Although there is a controversy about the prophylactic root canal treatment of these teeth, it is recommended that teeth that have tenderness to percussion and negative response to sensitivity tests should undergo root canal treatment.[6]

If using micro-instruments, pathfinding instruments, ultrasonic tips, dyes, and microscope, sodium hypochlorite (bubble or champagne test), microscope,[7] "Guided Endodontics,"[8] and radiography from different angles won't let us enter the canal, endodontic microsurgery will be indicated as a treatment option.[9]

However, finding the calcified canal after root resection will be difficult.[10] Since there are no guidelines in previously unprepared calcified canals that direct the retro tip into the canal space, surgery is difficult in these cases.[1] On the other hand, the remnants of necrotic tissues in root-end resection specimens of calcified canals can cause persistent chronic inflammation and treatment failure.[4] Therefore, it is recommended that surgical treatment be performed only in cases where periapical lesions and symptoms remain stable despite nonsurgical treatment.[9]

According to the above points, in cases where the attempt to open the apical area is not successful in a calcified canal, it is possible to fill 3–4 mm of the most apical section of the prepared canal with materials such as mineral trioxide aggregate (MTA) and calcium-enriched mixture (CEM) and obturated the remaining canal with gutta-percha and sealer. In case apical surgery is needed in future, only root-end resection will be performed and there will be no need for retropreparation and retrofill. In this way, the surgical time will be shorter and the surgical procedure will be faster than usual. The only concern is that root resection may change the seal, integrity, and the surface properties of MTA and CEM cement of orthograde.[11]

Various methods have been proposed to assess surface properties and cracks formed in the roots, including magnification with or without dye, histological sections, fluorescent confocal microscopy, stereomicroscopy, and scanning electron microscopy (SEM).[12] Therefore, this study aimed to assess the crack formation in MTA and CEM orthograde during root-end resection with ultrasonic or bur using SEM.

MATERIALS AND METHODS

Tooth preparation

In this *in vitro* study, 170 extracted human maxillary anterior teeth, with closed apices, single and straight root canals, without cracks, caries, restorations, and resorption and previous root canal treatment, were selected. This study was approved by the Ethics Committee of Zahedan University of Medical Sciences (IR.ZAUMS.REC.1399.054). Until experiment time, these teeth were kept in 0.9% normal saline (Samen, Mashhad, Iran) to maintain moisture and prevent the crack formation and bacterial growth. The teeth were randomly divided into two experimental (instrumented [n = 150]) and control (noninstrumented [n = 20]) groups. Of all the samples, digital periapical radiography was performed in mesiodistal and buccolingual dimensions. All specimens were then examined using a dental operating microscope (OPMI Pico, Carl Zeiss, Göttingen, Germany) under the magnification ×10 and ×16, and teeth with crack and dentinal defects were excluded. Samples were kept in a humid environment while working to prevent dehydration.

Mounting samples and simulating periodontium

To restore periodontal tissues, teeth were mounted in a sheep's mandible. For this purpose, a cavity was prepared inside the mandibular bone in the area of the inferior alveolar canal perpendicular to the outer surface of the buccal plate. An artificial apical lesion was simulated, and then, the sheep's tooth sockets were prepared to accommodate human teeth so that they were connected to the apical lesion cavity. To restore the PDL, the Speedex (Asia Chemi Teb Co; Tabriz, Iran, under the license of Coltene, Switzerland) was prepared and poured into the cavity, and the teeth were immediately placed in it so that about 3 mm of teeth apex could be seen in the lower cavity. To prevent dehydration, the sample was regularly moistened with normal saline.

Samples grouping

The samples in experimental groups (*n* = 150) were randomly divided into three groups (*n* = 50) and each group was divided into two subgroups (*n* = 25).

The first experimental group: only endodontic treatment

Subgroup A (*n* = 25) only received root canal treatment and root canal obturated with gutta-percha and sealer and their root end was resected with a bur. They did not receive any other treatments (Endo/RRB).

Subgroup B (*n* = 25) only received root canal treatment and root canal obturated with gutta-percha and sealer and their root end was resected with ultrasonic. They did not receive any other treatments (Endo/RRU).

Second experimental group: Calcium-enriched mixture apical plug and endodontic treatment

Subgroup A (*n* = 25) received CEM apical plug and root canal obturated with gutta-percha and sealer and their root end was resected with a bur (CEM/RRB).

Subgroup B (*n* = 25) received CEM apical plug and root canal obturated with gutta-percha and sealer and their root end was resected with ultrasonic (CEM/RRU).

Third experimental group: Mineral trioxide aggregate apical plug and endodontic treatment

Subgroup A (*n* = 25) received MTA apical plug and root canal obturated with gutta-percha and sealer and their root end was resected with a bur (MTA/RRB).

Subgroup B (*n* = 25) received MTA apical plug and root canal obturated with gutta-percha and sealer and their root end was resected with ultrasonic (MTA/RRU).

Endodontic treatments

Access cavity preparation in experimental samples was performed under standard conditions using high-speed fissure burs with air-water cooling. Working length was determined by inserting #15 K File (Dentsply Maillefer, Ballaigues, Switzerland) within an approximate length of 3 mm of the apical foramen and confirmed with radiography. Each sample was prepared through the crown-down technique using the rotary file SP1V taper (Park, Shenzhen, China) up to F3 (# 30/0.09). The root canals were irrigated with 1 mL of 5.25% sodium hypochlorite (Cerkamed Medical Company, Poland) as an irrigant between each file. To remove the smear layer, each tooth was washed with 1 ml of 17% ethylenediaminetetraacetic acid (EDTA) (Ariadent, Asia Chemi Teb, Tehran, Iran) followed by 1 ml of 5.25% sodium hypochlorite for 1 min. The final wash was carried out with normal saline. All control and experimental samples were kept in a humid environment until the canal obturation.

Root canal obturation

Samples of the experimental group were randomly assigned into three groups of 50. They were subsequently re-evaluated for any possible cracks while the canal got prepared using a dental operating microscope (under magnification ×10 and ×16). In the case of cracked specimens, they were dismissed and a new specimen was replaced. The samples were then placed back in the mandibular socket. Before obturation, the canals were dried with sterile paper points (Aria Dent, Tehran, Iran).

In the first group, the canals were filled only with gutta-percha (GAPADENT Co, Ltd, Germany) and AH26 sealer (Dentsply; DeTrey, Konstanz, Germany). For this purpose, the sealer was mixed according to the manufacturer's instructions[13] and was placed into the canal using a Lentulo spiral (Dentsply Maillefer, Ballaigues, Switzerland). Gutta-percha cone (#30/0.02) was impregnated with sealer and fitted inside the canal. Canal obturation was performed using a spreader and the lateral compaction technique. The access cavities were filled with Cavisol (Golchay, Tehran, Iran).

In the second group, apical part of their canal was enlarged using # 2, 3 Peeso Reamers (Dentsply Maillefer, Ballaigues, Switzerland). Irrigation was done using EDTA and sodium hypochlorite. Subsequently, CEM cement powder (CEM Cement, Yektaz Dandan; Bionique Dent, Tehran, Iran) was mixed according to manufacturers'[14] and placed in the canal in an orthograde direction with a messing gun (needle gauge# 1 mm) (Endogun; Medidenta, Woodside, NY, USA) and applied within the canal with an endodontic plugger (size# B) (Hu-Friedy, Chicago, IL, USA) to create an apical plug measuring 4 mm in thickness, 2 mm shorter than the working length. To check the quality of the plug, digital peripheral radiography was performed. If the quality of the apical plug was confirmed, the samples were kept in a 37°C incubator with 100% humidity for the complete setting of the plug for 24 h. The next day, the coronal section of the canal was obturated and filled similar to the first group.

In the third group, as in the second group, after the epical area was prepared, MTA Angelus powder (Angelus, Londrina, PR, Brazil) was mixed with the liquid according to the manufacturer's instructions[14] and placed in the apical area as a 4-mm plug. Radiography was performed to assess the quality of the apical plug and canal obturation. Samples with unacceptable quality were removed and a new sample was replaced.

All samples were stored in a humid environment until reuse. Again, the teeth were mounted in the sheep mandible.

Root-end resection

In subgroup A, in all groups, 3 mm of the apical root was cut by Tungsten Carbide Surgical bur (H162, Komet, Gebr. Brasseler, Lemgo, Germany) at a high speed with water cooling perpendicular to the longitudinal axis of the root, while in subgroup B of all groups, the roots were cut by an ultrasonic tip, SG1A (Nsk Variosurg, Japan) with TiN coating installed on the handpiece of a surgical ultrasonic device (Nsk Variosurg3, Japan) with medium power and water spray perpendicular to the longitudinal axis of the root. Each tip was used to cut a maximum number of three roots. Following the apicoectomies, the resected surfaces were carefully treated with 15% EDTA solution.

Scanning electron microscope evaluation

The resected teeth were stored at room temperature for drying, and then mounted on metallic stubs, sputter coated with gold, and examined with the SEM (KYKY-EM3900M, CHINA). SEM photomicrographs were taken at ×36 magnifications for assessment of the crack in dentin and orthograde material.

Statistical analysis

The data were analyzed in SPSS software (SPSS version 22, SPSS, Chicago, IL, USA), and the percentage was used to describe the data. Furthermore, McNemar's test, Chi-square test, and Fisher's exact test were used for data analysis. The significance level was set at $P < 0.05$.

RESULTS

Table 1 shows the percentage of cracks in dentin and the orthograde apical plug in the studied groups. The scanning electron micrographs of the studied groups are shown in Figure 1.

Table 1: Comparison of the percentage of crack in dentin and filling material between the studied groups

Group	*n*	Prevalence of crack in dentin (%)	Prevalence of crack in plug (%)	McNemar (*P*)
Control group (bur)	10	90	-	-
Control group (ultrasonic)	10	100	-	-
Endo/RR B	25	88	-	-
Endo/RR U	25	100	-	-
CEM/RR B	25	100	60	8.1 (0.002*)
CEM/RR U	25	100	48	11.08 (<0.001*)
MTA/RR B	25	96	48	10.08 (<0.001*)
MTA/RR U	25	100	40	13.07 (<0.001*)
χ^2 (*P*)	-	8.39 (0.07)$	2.04 (0.62)	-

$Fisher's exact test was used for analysis; **P*-value is significant at 0.05. Endo/RR B: Endodontic/root-end resection with bur; Endo/RR U: Endodontic/root-end resection with ultrasonic; CEM/RR B: CEM Plug/root-end resection with bur; CEM/RR U: CEM Plug/root-end resection with ultrasonic; MTA/RR B: MTA Plug/root-end resection with bur; MTA/RR U: MTA Plug/root-end resection with ultrasonic

As can be seen in Table 1, in Groups 2 and 3, regardless of the type of plug and the method of root cutting, the prevalence of crack in dentin is always significantly higher than its prevalence in the plug ($P < 0.05$). Furthermore, the prevalence of crack in dentin among the studied groups did not have a statistically significant difference ($P = 0.07$). On the other hand, although the prevalence of crack in dentin was slightly lower when the bur was used to cut the roots, this difference was not statistically significant. In this study, comparing the prevalence of cracks in the plug between groups did not show a statistically significant difference ($P > 0.05$). The prevalence of crack in the plug was similar for CEM and MTA ($P > 0.05$). Furthermore, out of the total samples examined, five were completely free of crack.

DISCUSSION

The purpose of root-end resection and preparation during apical surgery is to eliminate the stimuli present in inaccessible parts of the apical root canal system. In some cases, where anatomical access is difficult during periapical surgery, and where it is impossible to access the working length during nonsurgical root canal treatment, obturation materials can be inserted through orthograde. In these cases, if periapical surgery is needed, the clinician can only

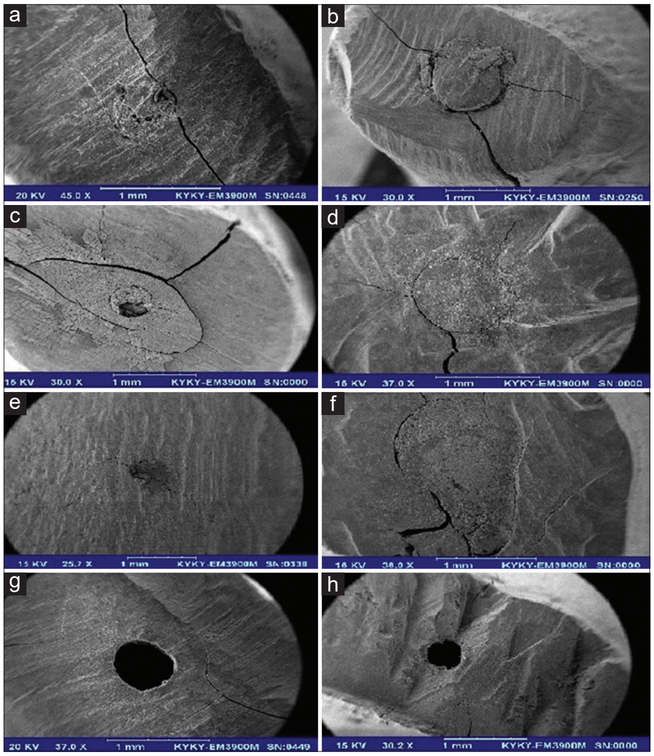

Figure 1: The scanning electron micrographs of the studied groups: (a) Endo/RRB, (b) Endo/RR U, (c) CEM/RRB, (d) CEM/RRU, (e) MTA/RRB, (f) MTA/RRU, (g) Control/RRB, and (h) Control/RRU.

resect the root end instead of inserting a new MTA as retrograde filling. This way, stages of surgery are performed faster than conventional methods.[11] Furthermore, when the complete obturation of the root canal system is performed by using MTA as orthograde filling materials, the emergence of cracks will be less likely after root-end resection due to lack of ultrasonic root-end preparation.[15]

The main concerns of clinicians in these cases include alterations in the sealing ability of orthograde materials previously set[11] and the emergence and expansion of cracks followed by root-end resection and preparation. Cracked and chipped root-end filling materials can lead to perpendicular root fracture, apical leakage, bacterial penetration, and treatment failure in the long term.[16] Microcracks generated and expanded due to the application of sonic and ultrasonic instrumentations for root-end preparation have been a matter of controversy.

The purpose of this study was to investigate cracks created in root dentin, MTA, and CEM orthograde in resected roots using ultrasonic and bur during periapical surgery with an electron microscope.

Disadvantages of MTA such as setting delays, inappropriate handling features, and tendency to be washed out, led to the invention of CEM as a new endodontic cement.[17] In the present study, there was no significant difference in crack frequency in bur resection method between MTA plug (48%) and CEM plug (60%), and between MTA plug (40%) and CEM plug (48%) in ultrasonic resection method. Therefore, the prevalence of cracks in CEM was higher than MTA. In root dentin with bur method, the prevalence of cracks was lower than that of the ultrasonic method, although no significant difference was observed between the groups.

In general, there are limited reports on the use of ultrasonic tips to resect the root end. In one case, carbide bur was shown to have smoother apical surfaces and a shorter apicoectomy time than ultrasonic tips.[18] The results of a study by Ayranci F *et al.* using an SEM microscope also revealed that the prevalence of cracks on root dentin generated due to root-end resection with bur was lesser than that of ultrasonic and Er: YAG laser methods. Furthermore, only 17% of the studied samples had no cracks.[19] Other studies have shown that the use of the ultrasonic method increases the formation of new cracks and the expansion of existing ones.[20,21] The results of the study by Aydemir *et al*. also showed that bur and laser did not have a significant difference regarding the formation of cracks and their types.[22] Rashed *et al*. using a digital microscope showed that root-end resection by bur could lead to the formation of dentin cracks in 47% of the specimens and ultrasonic root-end preparation to 40%.[23]

Differences in the prevalence of dentin cracks in this study as compared to our study can be attributed to the use of an electron microscope in our study that had a higher accuracy and resolution in the identification of more cracks in the dentin. However, dehydration of hard tissues during preparation for SEM can cause defects and artifactual cracks in dentin.[24,25] *In vitro* studies have shown that as a result of conditions such as dehydration of specimens, stress during tooth extraction, and improper maintenance and handling of teeth, the results may be overestimated. This study focuses on extracted teeth, which is one of the most important limitations of this study. However, in the present study, an attempt has been made to use newly extracted teeth with proper storage and preparation conditions as per the recommendations of other authors.[16] Furthermore, at all stages of treatment, the root surface was evaluated with an endodontic surgery microscope, and at each stage, except after root resection, if a crack was observed, the sample was removed from the study and a new sample was replaced.

Two other studies by Saunders *et al*. and Layton *et al*. also showed that ultrasonic root-end preparation is more likely to generate cracks than just root-end resection with a bur. In ultrasonic devices, lower power mode leads to the formation of fewer cracks than the higher power mode.[26,27] Similarly, Waplington *et al*. reported cracks and chipping in all samples prepared by ultrasonic.[28] However, other studies have not shown the formation of cracks after ultrasonic root-end preparation.[29,30] The reasons for the differences in the results of various studies include the different power of the ultrasonic device, the working time of the device, the presence or absence of preexisting microcracks, and the dentin thickness around the cavity. Existing microcracks can also be caused by noniatrogenic factors such as age changes, par functional stresses and occlusal interactions, severe forces during tooth extraction, and the effect of environmental changes on dentin, such as dehydration. Numerous studies have not considered the patient's age as a criterion for entering the study, however, it is clear that with increasing age, the physical and mechanical properties of teeth change.[31] In general, apart from the nature of the analytical method, the differences in results between this study and other studies can be attributed to many effective factors in experiment results, such as sample selection, storage conditions, and test instruments.

On the other hand, comparing the prevalence of cracks in the control group with other groups shows that crack can also be observed in unprepared and unfilled teeth, which leads us to conclude that canal preparation does not affect the occurrence of a crack in dentin. This result was consistent with the study of Beling *et al*., who did not report a significant statistical difference in terms of microcrack prevalence in prepared and unprepared canals.[29] Even De-Deus *et al*. have revealed the occurrence of microcracks before endodontic treatment and attributed it to extraction forces and tooth preservation conditions.[32] However, other studies have demonstrated that contact between the instrument and the canal wall during the preparation stages can cause stress concentration in the dentin and the formation of microcracks, which can become a vertical root fracture in the long term.[33]

Limitations of the study

The most important limitation of this study is its focus on the extracted teeth.

CONCLUSION

The results of this study showed that there is no statistically significant difference in the incidence of crack formation between different root-end resection methods and it seems that the higher prevalence of cracks in some groups is related to the process of preparing samples for SEM. Therefore, root canal orthograde obturation with materials such as MTA and CEM and root resection with bur should be considered as an optional treatment in case future surgery is required. Furthermore, *in vivo* research is needed in future to investigate crack formation after periradicular surgery under magnification and check the outcome.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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