

Original Article

Comparison of fluid filtration and bacterial leakage techniques for evaluation of microleakage in endodontics

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ABSTRACT

Background: Apical leakage assessment is a way to compare the efficiency of a filling material to seal the apical region of the tooth. Many microleakage testing techniques have been introduced through the years, but there has been no agreement as to which technique gives the most accurate results. The aim of this study was to compare the accuracy of fluid filtration and bacterial leakage techniques in the assessment of the apical sealing ability of mineral trioxide aggregate (MTA) and calcium enriched mixture (CEM).

Materials and Methods: A sample of 34 extracted single-rooted human teeth were selected and prepared. The samples were divided into 2 experimental groups. The apical 3 mm of each root was resected at 90° to its long axis and root end preparation was done with ultrasonic tips to a depth of 3 mm and filled with MTA and CEM, respectively. Assessment of apical sealing ability was done with fluid filtration technique and bacterial leakage technique along 90 days with *Enterococcus faecalis* bacteria. Mann-Whitney U-test and Chi-square test were used to analyze the data using SPSS (SPSS Inc., Chicago, IL, USA). P less than 0.05 was considered as significant.

Results: There was no significant difference in apical sealing ability between MTA and CEM in bacterial leakage and fluid filtration techniques. Samples which had bacterial leakage showed higher leakage values by fluid filtration technique.

Conclusion: Both techniques showed same results and there was no significant difference between fluid filtration and bacterial leakage techniques in assessment of apical microleakage.

Key Words: Bacterial leakage, fluid filtration, microleakage, root-end filling

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INTRODUCTION

Microleakage is defined as seepage of fluids, debris, microorganisms or ions along the interface between a restorative or a filling material and the wall of the tooth.^[1] Clinical studies have revealed that apical leakage of root filling materials is one of the main causes of endodontic therapy failures.^[2] Through the

years a variety of different apical leakage assessment methods have been introduced and developed.^[3] They estimate the sealing capacity of a filling material or a filling method, generally by measuring the extent and path of penetration of a tracing agent into the filled canal. The most frequent tracers that have been used are dyes, radioactive isotopes and bacteria and its products.^[2,3]

The fluid filtration method was developed by Derkson *et al.*^[4] and modified by Wu *et al.*^[5] for use in endodontics. In the fluid filtration technique, the coronal or apical tip of the sample is attached to a micropipette which has been filled with liquid. A small air bubble is inserted into the pipette. The entire system has been kept under a constant pressure. The

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amount of bubble displacement during a specified period of time is measured, which is a sign of the amount of liquid passage through the sample and present the microleakage of that sample and is calculated in $\mu\text{l}/\text{min}$.^[6]

The bacterial microleakage technique was first used by Fraser in order to evaluate the leakage of glass tubes which were filled with amalgam.^[1] In the bacterial microleakage technique, bacteria are used as markers. Bacterial culture is settled in contact with the coronal part of the tooth and the apical tip of the sample is in contact with the sterile culture medium, as the only path between the bacterial culture (upper chamber) and sterile culture medium (lower chamber) is the root canal filling. This assembly is incubated in 37°C and the samples are controlled every day to assess turbidity in the lower chamber. The period of time that takes the culture medium to become turbid is a marker of root canal contamination. This method was first introduced as Dual Chamber technique by Torabinejad *et al.*^[7] In this technique, it is important to make sure about the vitality and activity of the bacteria, so the bacterial suspension in the upper chamber is refreshed every other day. Moreover, in order to verify the authenticity of the experiment, it is necessary to define the bacteria which caused the culture medium (lower chamber) turbidity.^[8]

Mineral trioxide aggregate (MTA) was introduced specifically as a retrograde filling material.^[9] Various aspects of its properties were compared to other materials in several *in vitro* and *in vivo* studies. Biocompatibility and sealing ability of MTA was better than others materials; in addition, it has cementum sedimentation quality.^[10-12] Recently, calcium enriched mixture (CEM) cement was introduced in endodontics by Asgary *et al.*^[13] The clinical application of CEM is the same as MTA.^[14] The properties of CEM are high alkalinity, release of calcium hydroxide and adequate antimicrobial quality.^[15]

There is an expectation that different microleakage methodologies give similar results when assessing the same material or technique, but since these methodologies are not standardized, different methodologies would lead to conflicting results.^[16,17] Therefore, it seems necessary to compare the accuracy of various techniques and suggest a standard method for determination of the sealing ability of restorative or filling materials and techniques in endodontic therapies. The aim of this study was to compare the

results of fluid filtration and bacterial microleakage techniques in the evaluation of the sealing ability of retrograde MTA and CEM.

MATERIALS AND METHODS

Sample preparation

After approval by the Research Ethics Committee of the Mashhad University of Medical Sciences (protocol number #88160), 34 teeth were selected from a large series of single-rooted anterior teeth which had recently been extracted. The teeth had mature apices, no caries or internal or external resorption, no cracks on root surfaces and no curves (curves less than 20° were included). After sample collection the root surfaces were cleaned with a periodontal curette to remove all calculi and soft-tissue. Then, the samples were disinfected by 5.25% sodium hypochlorite (Naocl) for an hour and were kept in distill water.^[18] The coronal part of the samples was cut in order to standardize 15 mm of each root. Apical foramen diameter of all samples were checked by inserting a #15 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) into the canal. Using this method, the apical foramen of all teeth was standardized. Canal preparation was carried out using K-file #40 (Dentsply, Maillefer, Ballaigues, Switzerland) with step-back technique. Coronal portion of canals were flared by Gates-Glidden drills #2, 3 and 4 (Dentsply, Maillefer, Switzerland). After using each instrument, canals were rinsed by 1 ml of 5.25% Naocl.^[16] Then, samples were randomly divided into two experimental groups, each group containing 15 samples; positive and negative control groups had four samples.

In experimental groups, the apical 3 mm of each root was resected at 90° to its long axis and root end preparation was done with ultrasonic tips (piezo-electric [Spartan, Fenton, MO, USA]) to a depth of 3 mm. In order to provide an intracanal matrix to support the condensation of retrograde filling material, high convergence gutta-percha was adapted into the coronal part of the apical cavity [Figure 1a]. Then, the 3 mm apical cavity was filled with MTA (Dentsply, Tulsa Dental, Tulsa, OK, USA) or CEM (BioniqueDent, Tehran, Iran) using an MTA carrier and filling density was verified by radiographs [Figure 1b]. Samples were kept in 100% humidity and 37°C for retrograde material setting.

External surfaces of the experimental samples, except the apical region, were sealed with two layers of

nail polish and sticky wax. Two samples of positive control group were prepared, but not filled and external surfaces were sealed similar to experimental samples. The outer surface and apical region of 2 samples of negative control group, which were prepared and filled according to the experimental groups, were sealed with two layers of nail polish and sticky wax.

Fluid filtration technique

This system evaluates the passage of liquid through the samples in order to assess the sealing ability of that case. This is done by measuring the bubble displacement which is produced on the path of liquid movement. In order to make the liquid move and assessing the leakage, there was an oxygen gas pressure behind the liquid, which was kept constant throughout the experiment on 0.5 atm using a manometer. A small air bubble was inserted into a 0.1 mL pipette with a syringe. The bubble must include the whole internal diameter of pipette in order to be sure that the bubble displacement is a trustful sign of liquid passage through the tube.

First, the positive control group's samples were attached to the system and rapid movement of the bubble was noticed from beginning to the end of the pipette. Then, negative control group's samples were attached and no bubble movement was observed in 8 min. The system was ready for use after passing these 2 tests. A 10 megapixel digital Camera (Canon powershot G 11, Japan) [Figure 2a] and Adobe Photoshop 7.0 software (Adobe Systems Inc., San Jose, USA) were used to record and analyze the bubble movement [Figure 2b]. The system was allowed to equilibrate for 30 s before measuring the bubble movement. Afterward, the first picture was taken from the initial position of the bubble in pipette. The following 4 pictures were taken at 2 min intervals. After the computation, the amount of the fluid passing through the samples was calculated in $\mu\text{l}/\text{min}$.

Bacterial microleakage technique

First, the bottom of an Eppendorf test micro tubes was cut with the blade. The teeth, which all had a layer of hot sticky wax on their coronal half of the root surfaces, were inserted into the cut micro tubes. The coronal part of the roots and the access cavities were in the tubes and the 2-3 mm of apical part was out. The junction between tubes and teeth were sealed internally again by sticky wax. The assemblies [Figure 3a] were sterilized in ethylene oxide gas for

8 h by Anprolene machine (Anderson products Inc., Haw River, NC, USA).

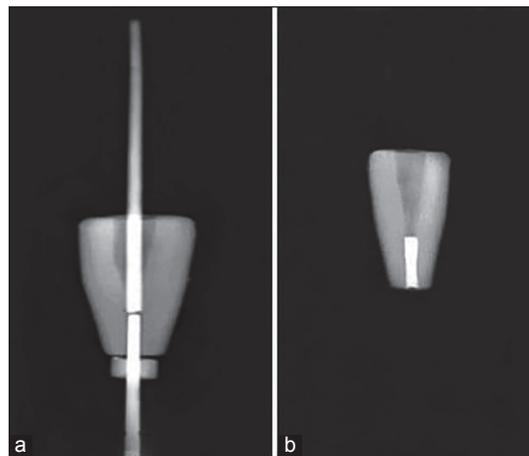


Figure 1: (a) Root end preparation, (b) retrograde filling

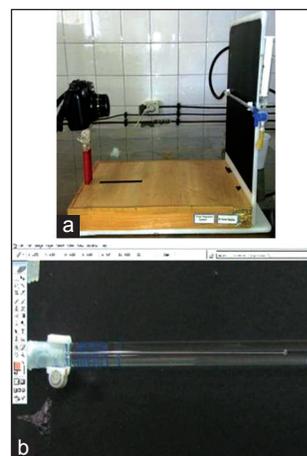


Figure 2: (a) Camera system, (b) small air bubble in 0.1 cc pipette of fluid filtration system

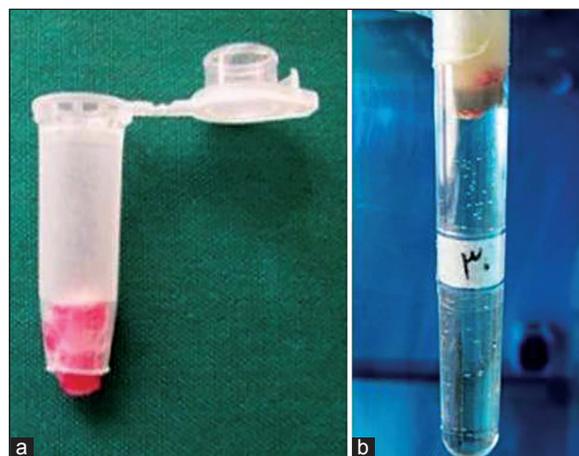


Figure 3: (a) The root-micro tubes assembly, (b) the root-micro tubes assembly and test tube

Muller-Hinton Broth culture medium (Merck, Darmstadt, Germany) was added to test tubes#12 (Pouyanteb, Tehran, Iran), using a syringe and sterile needle (22-gauge), in a way that the 10-15 mm of the tubes remained empty for placing the assemblies. Afterwards, tubes containing culture mediums were sterilized in 116-121°C for 10-15 min by autoclave (Iran Abzarteb company, Tehran, Iran). The root-micro tubes assemblies were settled inside test tubes as the root tips were contacting the culture medium. The junction region between micro tubes and test tubes were sealed using sticky wax and parafilm straps [Figure 3b].

A culture containing 9×10^8 CFU/ml of *Enterococcus faecalis* in 1 ml of Muller-Hinton Broth solution was placed into the micro tubes in contact with the coronal access opening of the roots. The bacterial suspension was refreshed every 3 days. The culture medium in the lower chamber was monitored daily for 3 months for turbidity. In order to be sure about *E. faecalis* presence in turbid mediums, samples were picked from the lower chambers and cultured in bile esculinagar (Merck, Darmstadt, Germany). The black discoloration of the medium, which was amber at first, was a sign of *E. faecalis* presence.

Statistical analysis

Mann-Whitney U-test and Chi-square test were used to analyze the data using SPSS 12 (SPSS Inc., Chicago, IL, USA). P less than 0.05 was considered as significant.

RESULTS

The MTA group showed no statistically significance difference with CEM group in the fluid filtration technique [Table 1]. There was no significant difference in apical sealing ability between MTA and CEM in bacterial leakage technique [Table 2].

In both groups mean fluid filtration values in positive bacterial leakage cases were higher than the negative cases. All negative and positive control groups showed results as expected.

DISCUSSION

In the present study, fluid filtration and bacterial microleakage techniques were compared for assessment of sealing ability of retro-filing materials (MTA and CEM) and consistent results were obtained. MTA was developed in 1995 and its

Table 1: Comparison of bacterial microleakage in CEM and MTA groups

Groups	Bacterial leakage		
	N	Negative	Positive
CEM (%)	15 (100)	2 (13.3)	13 (86.7)
MTA (%)	15 (100)	3 (20)	12 (80)

P = 0.98 CEM: Calcium enriched mixture; MTA: Mineral trioxide aggregate

Table 2: Comparison of mean fluid filtration values in CEM and MTA groups

Groups	N	Min	Max	Mean	SD
CEM	15	0.0000612	0.0011799	0.000388754	0.000359601
MTA	15	0.0000350	0.0013197	0.000442827	0.000403579

P = 0.701 CEM: Calcium enriched mixture; MTA: Mineral trioxide aggregate; SD: Standard deviation

application for the treatments of radicular perforation, root-end retrofilling, apexification and conservative pulp therapy is expected. CEM cement is another retrofilling material with clinical applications the same as MTA.^[9,13] Although the main purpose of this study was to compare the fluid filtration and bacterial microleakage as techniques for evaluation of the sealing ability of materials, another finding of this study was that MTA and CEM have similar sealing abilities. This finding was comparable with the results of studies of Milani *et al.*^[9] and Asgary *et al.*^[13] MTA and CEM are hydrophilic endodontic cements and allow access of cement within gaps and help the entrance of small cement particles into dentinal tubules. Furthermore, MTA and CEM exhibit slight expansion after setting^[14] and provide enhanced adaptation of the biomaterials to the walls. In addition, MTA and CEM form hydroxyapatite and provide an improved seal at the interface of biomaterials and dentin walls.^[13,19,20]

Several articles in literature could be found that investigate the sealing capacity of different materials.^[1-8] The main topics covered by the top-cited articles were microleakage and endodontic microbiology.^[21] However, there are few studies that compared the effectiveness of the techniques for measuring the sealing ability.^[3,16,17] Verissimo and do Vale concluded that there was a real lack of technique standardization even when the same method is used, which may lead to variable results.^[3]

Dye penetration is a common microleakage measuring technique and in spite of the disadvantages, the popularity of this technique has not diminished due

to ease of use and low cost.^[22] One of the major drawbacks of this technique is the small size of the dye molecules and probably false positive results and overestimation of leakage. The reliability, reproducibility and clinical relevance of dye penetration are questionable.^[23] Among microleakage measuring techniques, the bacterial leakage model is considered to be the most clinically and biologically relevant.^[18]

Fluid filtration technique presents many advantages in comparison to dye penetration technique, as the samples are not destroyed^[24] and the results are precise (small volumes can be recorded) and quantitative.^[25] Another reason for selecting fluid filtration was that most other techniques are not suitable under some experimental conditions, such as chemical reactions. In fact, some staining agents used in conventional testing techniques react with dentin, which may lead to unfavorable results. The fluid filtration technique avoids these disadvantages because it does not require any chemical agent for testing.^[26] Pommel and Camps declared that some procedures in this technique were not standardized, as the pressure might range between 1 and 20 psi and the experiment time from 1 min to 3 h.^[27] This would affect the results, as the longer measuring time led to lower filtration values and higher pressure was associated with higher leakage values. They suggested 15 cm H₂O would be efficient, as it was close to physiologic pressure. According to their study, the pressure, bubble size, or micropipette diameter could affect the system sensitivity.^[27]

In the current study, we compared fluid filtration and bacterial microleakage techniques. For bacterial microleakage technique, a single subtype of *E. faecalis* was used. It is one of the most common bacterial causes of endodontic therapy failures, can penetrate through the tubules and it is resistant against irrigation and intracanal medication.^[28]

In their study, Mortensen *et al.*^[29] and Krakow *et al.*^[30] revealed that the bacterial microleakage technique was more accurate in leakage assessment, in comparison to dye penetration or radioisotopes techniques. In contrast to the present study, Karagenç *et al.*^[31] compared 4 leakage testing techniques in assessing the leakage of two root canal filling techniques. They showed there was a lack of correlation between bacterial leakage and fluid filtration techniques. Different methodologic aspects between the present study and Karagenç *et al.* study may be the reason

for different results. For example, in the present study, we used an oxygen gas pressure behind the liquid, which was kept constant throughout the experiment on 0.5 atm, but in Karagenç *et al.*^[31] study fluid pressure was applied with helium gas using a pressure of 250 mm Hg. The lack of standardization in microleakage techniques hinder comparisons of the studies and could explain the divergent results.^[32]

However, according to the results of the present study, there was no statistically significant difference between the sealing ability of MTA and CEM cement using the fluid filtration and bacterial leakage techniques. Samples which had bacterial leakage showed higher leakage values by fluid filtration technique. This study concluded high correlation between the two techniques.

CONCLUSION

Based on the results of this study, there was no significant difference between two techniques. The bacterial leakage technique can be replaced with the fluid filtration technique, because the bacterial leakage method takes more time, the procedure is more complex and it requires a skilled microbiologist.

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