Original Study

MICROLEAKAGE ASSESSMENT OF A NEW MINERAL TRIOXIDE AGGREGATE-BASED ROOT CANAL SEALER IN THE PRESENCE AND ABSENCE OF SALIVA

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ABSTRACT

Aim: Sealing ability is the one of the primary characteristic of the ideal sealers. This study aims to evaluate the apical microleakage of new Endoseal MTA sealer and compare with AH plus in the absence and presence of saliva.

Materials & Method: Fifty-four human decoronated maxillary central incisors teeth were used and prepared by Mtwo rotary files method. 44 root canals were filled with a single gutta percha cone, using Endoseal or AH plus sealer, under dry and moist root canal conditions (11 teeth in each group). Ten specimens were considered as positive and negative controls (n=5). The orifices of all teeth were sealed by glue wax and The external surfaces of all specimens, except the positive controls, were coated with two layers of nail varnish up to 1 mm from the root apex. After ten days in 100% humidity condition, the teeth were placed in methylene blue for three days and then were cut. Finally, the microleakage of methylene blue was measured via stereomicroscope. The data were analyzed using SPSS software and ANOVA and Bonferroni tests. The $p < 0.05$ was considered statistically significant.

Results: Overall mean apical leakage values of 2.0454 and 2.6932 mm were obtained for Endosael MTA and AH Plus sealers, respectively ($p < 0.05$). The mean apical leakages of Endosael MTA and AH Plus sealers were 1.8909 and 2.5955 mm in dry condition and 2.2000 and 2.7909 mm in wet condition, respectively ($p > 0.05$).

Conclusion: It was observed that mean apical leakage of Endoseal MTA sealer is significantly lower than that of AH Plus, and humidity has no significant effects on the mean apical leakage of the considered sealers.

Key words: *AH Plus, Apical Seal, Endoseal MTA, Microleakage, Moisture, Sealer.*

Introduction

In all root canal filling techniques, dentist must utilize a type of sealer to improve the filling quality.[1] During the filling procedure, sealers act as a lubricant and fill the gap between gutta-percha and canal walls. As a result, the quality of root canal filling strongly depends on the sealing ability of the applied sealer product.[2]

Low quality of the apical seal is known to be the most common cause of root treatment failure.[3]

It is widely recommended to dry the root canal before filling to improve the sealer's adhesion to the canal's dentinal walls and filler material.[4] Many studies have reported that prior presence of blood and moisture in the canal during filling may increase apical microleakage. Recent studies, however, claim that the presence of small amounts of moisture in the root canal does not affect the microleakage.[5]

The possible problems caused by the existence of moisture inside the root canal include adverse effects on the sealer's physical and chemical properties such as setting time and solubility, bubble formation within the sealer, blockage of dentin surface resulting in insufficient sealer penetration into dentinal tubules, and stimulating effects on the periapical region in case of presence of blood in the canal.[5,6]

There are currently several sealer products with different bases and advantages and disadvantages available on the market. Among these, resin-based AH Plus sealer has become a product of choice because of ideal properties such as excellent dentin adhesion and desirable sealing ability and is currently regarded as the gold standard for root canal treatment.[7,8]

However, tricalcium silicate-based cements, commonly referred to as Mineral Trioxide Aggregate (MTA) cements, have also exhibited interesting properties in laboratory tests and are more biocompatible than the conventional endodontic sealers.[9]

A review of the literature shows that given the importance of apical seals and the emphasis on canal drying before filling, these issues have been the subject of many studies. However, there have been some inconsistencies in the results of these investigations depending on the sealer type and research conditions, and continued research is underway to find new sealers with more desirable properties.[4-6,10,11]

Endoseal MTA is a new calcium silicate sealer based on MTA and derived from pozzolan cement. A research effort has shown that the products derived from pozzolan cements have a shorter setting time and better wash-out resistance than other MTA products.[12] Endoseal MTA is available in the form of a premixed and pre-loaded substance packaged within an air-tight syringe, which allows direct application inside the root canal. During the injection, Endoseal MTA absorbs the air moisture and sets without the need for previous powder/liquid or base/catalyst mixing.[13] Other studies on pozzolan cement derived MTA have reported properties such as low cytotoxicity, hard tissue inducing bioactivity, and minimal discoloration.[12,14]

In a study Mijun Kim *et al*. Investigated the microleakage of Endoseal MTA both as a sealer and as a root canal filling agent in comparison to AH Plus. Since the study found no significant difference between experimental groups, it was suggested that Endoseal MTA can be used both as a sealer and as a root canal filler.[12] Hwang *et al*. and Silva *et al*.

also investigated the bacterial microleakage and bond strength of Endoseal MTA and reported positive results.[13,15] The presence and extent of microleakage can be examined by several methods, such as dye penetration, radiography, bacteria infiltration, fluid filtration, spectrophotometry, marginal adaptation, and electrochemical methods.[16-18] Among these, dye penetration is preferable thanks to simplicity, convenience, cost-effectiveness, and the lack of need for special equipment. This method is also convenient for comparative studies.[19,20]

Given the limited number of studies on the microleakage of new Endoseal MTA as compared to other typical sealers and the absence of any study on the impact of pre-filling root canal moisture on the apical microleakage of this sealer, Due to the need for moisture for setting,[14] the present study aimed to expand the literature on these topics. In line with this aim, the study evaluated the apical microleakage of MTA-based Endoseal in comparison with resin-based AH Plus in the presence and absence of saliva using dye penetration technique.

The tow null hypothesis were tested: 1. There is no difference in apical microleakage between sealers 2. moisture does not affect in sealing ability of sealers.

Materials & Method

This experimental trial was approved by the Human Research Ethic Committee of The Mazandaran University of medical science, Iran (IR.MAZUMS.REC.95.2610). Based on previous studies [6,12] 54 extracted human permanent maxillary central teeth with direct conical root and complete apex were selected. There were no sign of internal or external resorption, fracture or crack in the root, and without calcification.

1) Preparation of Specimens

The extracted teeth were immersed for 24 hours in 1000 ml of 5.25 % sodium hypochlorite (Darugar-Iran) for surface sterilization. The teeth were then stored in distilled water at room temperature until the next step.

To homogenize the specimens before the experiment, a diamond bur on a high speed handpiece was used to cut the crown of the teeth from the cementoenamel junction so that about 12 mm of the root length remain. Then, a K-file No.15 was pushed into the root canal until file tip was visible at the apical foramen. The operating length was considered to be 1mm shorter than this length. The prepared teeth were again stored in distilled water at room temperature until the time of experiment.

Root canals were prepared using the Mtwo rotary file system (as per manufacturer's instructions). First, gates drills No. 2, 3 and 4 were used in that order to expand the orifices and one-third of the coronal of canals. Then, the middle and apical third of canals were prepared by the use of files No. 20, 25, 30 and 35, in that order, according to the single length technique. These procedures were performed by means of a standard rotary handpiece (NSK, Japan) and the electric motor operating at a speed of 350 rpm and torque of 1.5 NCm (as per manufacturers' instructions). Following to use of each file, the canal was washed with 2 ml of 5.25% sodium hypochlorite.

The smear layer was removed by applying 2 ml of 17% EDTA solution into the canal for 1 minute and then washing it with 2 ml of 5.25% NaOCl solution. A final rinsing was performed with 2 ml of distilled water to neutralize the effect of sodium hypochlorite and EDTA solutions. The root canals were then dried by paper cones. For this purpose, the root canals were dried with paper cones No. 30 until all remaining moisture was absorbed, and to make sure of canal dryness, three extra paper cones were applied after observing the last moist paper. Until this stage, all specimens underwent similar preparations. But at this stage, specimens were randomly assigned into four experimental groups (two dry and two saliva-exposed) each containing 11 specimens [Table 1] and two positive and negative control groups each containing 5 specimens.

Groups	Root Canal Obturation	No.
1	AH Plus + Gutta Percha	11
2	Endoseal MTA + Gutta Percha	11
3	AH Plus + Gutta Percha + Saliva	11
4	Endoseal MTA + Gutta Percha + Saliva	11

Table 1: Classification of experimental groups.

2) Sealer Application

The specimens in four experimental groups were subjected to following procedures:

Group 1 - Canals were filled with gutta-percha and AH Plus. The composition of sealers is summarized in Table 2. The sealer was used after mixing in accordance with manufacturer's instructions and achieving proper consistency.

Sealer Type	Composition	Manufacurer
AH Plus	PasteA: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments	Dentsply DeTrey, Konstanz, Germany
	PasteB:dibenzyldiamine,aminoadamantane,tricyclodecane-diamine,calcium tungstate,zirconium oxide, silica, silicone oil	
Endoseal MTA	Calcium silicates, calcium aluminates, calcium aluminoferrite, calcium sulfates, radiopacifier, thickening agent	Maruchi, Wonju, Korea

Table 2: Composition of sealers used in this study.

Group 2 - Canals were filled with gutta-percha and Endoseal MTA. In accordance with manufacturer's instructions, the tip of Endoseal MTA needle was placed in the coronal third of the root canal and then the sealer was slowly injected. Immediately, a single gutta-percha cone

was pushed into the root canal down to the operating length.

Group 3 - Canals were filled with gutta-percha and AH Plus in a way similar to Group 1, except that after drying and before applying the sealer, an insulin infusion syringe was used to wet each canal with 0.02 cc of saliva solution.

Group 4 - Canals were filled with gutta-percha and Endoseal MTA in a way similar to Group 2, except that after drying and before applying the sealer, an insulin infusion syringe was used to wet each canal with 0.02 cc of saliva solution.

In all specimens, to fill the canals, first a spreader No. B was used to apply the sealer on canal walls. To ensure the complete filling of the canal's apical section, sealer application was continued until observing the leakage of sealer from the apical end. Then, using a forceps, a gutta-percha No. 35 (Meta, South Korea) with taper size of 0.06 was dipped in sealer and pushed inside the canal down to the depth of 11 mm. Obturation was performed with single cone method. The excess gutta-percha was removed by a hot plugger, and then gutta-percha of the coronal section was flattened. Finally, periapical radiography was performed to evaluate the final filling quality.

Ten of the specimens with prepared canals were left unfilled to serve as positive and negative controls.

Next, the root canal orifices of all specimens were sealed with glue wax. The external surfaces of all specimens, except the positive controls, were coated with two layers of nail varnish up to 1 mm from the root apex. For negative controls, the apical foramen of the teeth was covered with adhesive wax.

The teeth were kept for 10 days in incubator (at temperature of 37°C and humidity of 100%), to make sure that sealer is completely set, and finally they were immersed in 5% methylene blue for 3 days.

3) Microleakage Measurement

To measure the dye penetration into the canals, the teeth were split into halves by a disc (Tizcavan-Iran) on a high-speed handpiece. [Figure 1]

Figure 1: Longitudinal cut of tooth

One of the halves was randomly selected to be examined by a stereomicroscope (at 2x magnification) to measure the maximum dye penetration into the walls. [Figure 2]

Figure 2: Measurement of microleakage and dye penetration from apex (in mm)

Kolmogorov-Smirnov test was used to assess the normality of collected data and ANOVA and Bonferroni tests were used for data analysis.

Results

The positive control group had 100% dye penetration and the negative control group showed no dye penetration. The total means of apical microleakage observed in AH Plus and Endoseal MTA specimens were 2.6932±0.50899 mm and 2.0454±0.47478 mm, respectively. The total mean apical microleakage of Endoseal MTA was found to be significantly lower than that of AH Plus. [Tables 3 and 4 and Figure 3]

Scaler Types	Condition	No.	Average	SD	Max.	Min.
AH Plus	Dry	11	2.6	0.42	3.00	1.70
	Wet	11	2.8	0 52	3.60	1.80
Endoseal MTA	Dry	11	1.9	0 52	2.50	0.60
	Wet	11	2.2	0 50	2.80	1.10

Table 3: Mean apical microleakage of chosen sealers

Groups	Transmission	
	p - Value	Significance*
1 and 2	$p < 0.05$	Significant
1 and 3	$p > 0.05$	Not Significant
2 and 4	$p > 0.05$	Not Significant
3 and 4	$p < 0.05$	Significant
Group 1: AH Plus; Group 2: Endoseal MTA; Group 3L AH Plus + Saliva; Group4: Endoseal MTA + Saliva ** The results of Bonferroni test of different froups with each other*		

Table 4: Inter-group comparison

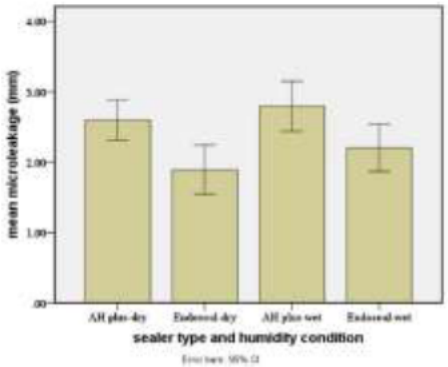

Figure 3: Average apical microleakage of as a function of sealer type and canal humidity condition.

The mean apical microleakage values of Endoseal MTA and AH Plus specimens were 1.8909±0.52049 mm, and 2.5955±0.42098 mm in the dry group and 2.2000±0.49749 mm and 2.7909±0.52859 mm in the saliva-exposed group, respectively. Comparison of apical microleakage of specimens in dry and saliva-exposed groups indicated a higher apical microleakage in saliva-exposed canals, but this difference was not statistically significant. [Tables 3 and 4 and Figure 3]

Discussion

The sealing ability of materials used in endodontic procedures is a determinant of the success of treatment.[12] Although there are many commercial root canal sealer products available on the market, there is no consensus on the substances that make sealers more effective.[5] Each of the existing root canal sealers lacks some characteristics of the ideal endodontic sealer. Consequently, new alternative sealers especially bioceramic or calcium silicate based materials (MTA and BioAggregates) are under constant development so as to achieve better sealing ability and biological properties.[13]

In our experiment, the first null hypothesis was rejected because the mean microleakage of Endoseal MTA was significantly lower than that of AH Plus. But the second null hypothesis was accepted and as expected, humidity did not have a significant effect on the microleakage of two sealers.

This study used maxillary central teeth to minimize the differences between the specimens. In addition, the crown of the teeth was cut such that roots had almost identical lengths. Also, rotary files were utilized to expand the root canals to the same size.

Of several methods available for the evaluation of the seal between root canal filler and root wall, including linear dye penetration,[4,6,12,20] microbial leakage,[21] glucose penetration,[2] fluid filtration [5,22] and tubular penetration.[23] This study chose the methylene blue dye penetration for this purpose. As mentioned, this method is simple, convenient, and inexpensive, does not require any special equipment, and is especially useful for comparative evaluations.[19] Moreover, given the small size of methylene blue molecule and its very high penetration, any filler that is able to block this molecule can also block the microleakage of bacteria and their associated products.[20,24]

In the current study,similar to previous studies,[4,5] root canal was obturated using the single-cone obturation technique. This technique eliminates other factors that may affect microleakage (e.g., interface between secondary gutta-percha) thus allowing a clearer sealing ability measurement to be made.[25] It has been reported that there is no difference in terms of sealing ability between the single-cone and the lateral condensation techniques.[26]

The AH Plus is among the most popular sealers and is widely considered as the sealer product of choice because of ideal properties such as excellent dentin adhesion, desirable flow, and low solubility.[7,8,27] Endodontic treatment with AH Plus and gutta-percha is a conventional method extensively used by many clinicians.

The sealing ability of a root canal sealer product is associated with its powder/liquid mixing requirement, setting time, setting expansion, and solubility. Endoseal MTA is a pre-mixed sealer consisting of hydraulic cement and non-aqueous liquid, therefore, can be used with a constant powder/liquid ratio. Endoseal MTA has a lower setting time and greater expansion than AH Plus, which may lead to better sealing ability.[14,28] Based on the manufacturer, very good flow of Endoseal MTA allows it to provide improved bond strength and sealing ability by penetrating into the dentinal tubules, anatomical faults, and lateral canals. Endoseal MTA also has excellent dentinal wall distribution, which can be due to its injectable nature. Another clinical advantage of this sealer is its self-setting.[15] These characteristics are likely to be the causes of lower apical microleakage of the specimens filled with Endoseal MTA in the present study.

Similar to our study Asawaworarit *et al* concluded that the tricalcium silicate-based sealer (MTA fillapex) compared to AHplus promoted proper sealing ability.[9] Also According to Parwar *et al*. the Endosequence BC sealer (Brasseler), which is a calcium silicate based sealer, leaked less than AH Plus.[29] Hwang *et al*. have reported that bacterial microleakage of Endoseal MTA is similar to that of AH Plus and GuttaCore.[15]

In a study conducted by Mijun Kim *et al*. where the microleakage of Endoseal MTA both as a sealer and as a root canal filler was compared with AH Plus, no significant difference was found between the experimental groups and it was suggested that Endoseal MTA has the potential to be used both as a sealer and as a root canal filler.[12] The design of this study similar to ours, whit the difference that it was used Rhodamine B instead of methylene blue.

In another study, Silva *et al*. compared the push out bond strength of Endoseal MTA with MTA Fillapex and AH Plus. In this study, EndoSeal presents satisfactory bond strength performance for application in endodontic therapy compared with MTA Fillapex but it is not able to improve adhesion compared with AH Plus.[13] the reason for the difference in our result and this study can be related to difference method of the two study.

Hyojin Kim *et al*. examined the sealing ability of Endoseal MTA and its penetration into dentinal tubules in comparison with ProRoot MTA and AH26. Their outcomes showed that, in terms of canal wall sealing and tubular penetration ability, Endoseal MTA is weaker than AH26 and is not significantly different from ProRoot MTA, but it has a higher tubular penetration depth and a shorter setting time than its rivals. Overall, these researchers concluded that despite its lower sealing ability, Endoseal MTA is a viable alternative to resin-based sealers.[20]

The inconsistency between our results and the results of other studies [13,20] may be due to the difference in the

research method, the number of specimens especialy the impact of methylene blue decoloration. This substance has widespread usages in dye penetration tests, but because of instability of its chemical structure in alkaline media, it loses some color in the presence of calcium hydroxide and MTA. It has been suggested that Rhodamine B has a better detection and penetration ability than methylene blue and is preferable in sealing ability evaluation in the presence of materials such as MTA.[12]

The dryness of root canal before filling has long been regarded as an important filling operation detail. In some cases, penetration of liquids such as blood, exudate or pus through the apical foramen into the canal or dentist's inability to completely dry the canal's apical region by paper cones may result in the wetness of root canal.[30] As mentioned before, the possible problems occurred by the moisture inside the canal include adverse effects on the sealer's physical and chemical properties such as setting time and solubility, bubble formation within the sealer, blockage of dentin surface resulting in harder sealer penetration into the dentinal tubules, and stimulating effects on the periapical region in case of presence of blood in the canal. Researchers found that For the tested sealers including AHplus and MTA Fillapex, it may be advantageous to leave canals slightly moist before filling. As a consequence, a continued search for more effective sealers with lower apical microleakage in moisture-exposed canals is absolutely necessary.[5]

Different studies on this subject have utilized different methods for applying moisture to the canal. Various methods used for this purpose include storage under 100% humidity for several days,[6] injection of normal saline or distilled water,[4] exposure to saliva,[30] immersion in blood [5,10] and immersion in blood after filling. In the present study, the inner surface of the canals was initially dried and then wetted with 0.02 cc of saliva solution using an insulin injection syringe.

The use of saline, saliva, distilled water and blood for wetting the root canal have had different results.

Since, so far, no similar research has reported on the effect of moisture on the apical microleakage of Endoseal MTA, the results of our study cannot be directly compared with any other research.

In the present study, dry specimens in both group had a slightly lower apical microleakage, but this difference was not significant.that is similar nagas *et al* and confirming the claims of Endoseal MTA manufacturer.in the setting process, Endoseal absorbs the environmental moisture from atmospheric air.[13]

Ehsani *et al*. reported that in all examined sealers (AH26, Excite DSC, MTA Fillapex and ZOE), the dry groups had lower apical microleakage. But similar to our study for MTA based sealer (MTA Fillapex) , the difference in apical microleakage values of dry and wet groups was not significant.[4]

In a study carried out by Soleymani *et al*.[10] where they investigated the apical microleakage of MTA Fillapex and AH26 in the presence of blood in the canal by measuring the blue dye penetration using a stereomicroscope, it was found that the complete drying of the canal leads to better apical sealing. But Khalilak *et al* identified blood contamination has no significant effect on the apical microleakage of Epiphny and AH26.[5] However, in both of these studies, the type of moisture contamination was different from our study.

Unlike our study, Jang *et al* has been shown that the presence of moisture increases the microleakage of AH plus sealer [2] of course, their study technique was different and based on glucose leakage model. This could be a reason for the different results of two studies.

The possible causes of these contradictory between studies results regarding the impact of moisture on microleakage may be the difference in sealer type, sample size, the amount of moisture within the root canal and research procedures, including the method of microleakage measurement.

This study has the limitation that it used the classical dye penetration method with methylene blue. It is recommended that future studies using dye penetration with Rhodamine-B, dye-extraction, i.e., dissolution method and fluid filtration method on a larger sample and different percentages of root canal moisture.

Conclusion

Under the limitations of this stud, the mean apical microleakage of Endoseal MTA is significantly lower than that of AH Plus. The presence of moisture in root canal prior to filling was found to have no significant influence upon the mean apical microleakage in neither AH Plus nor Endoseal MTA tooth specimens. Further in vivo studies are required for more definitive conclusions.

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