

## Original Article

## Microleakage Evaluation of New Nano Zinc Oxide Eugenol Sealer (COLORADO™) with Conventional Sealers in Root Canal Treatment: An *in vitro* Study

Neda Naghavi <sup>1</sup>; Armita Rouhani <sup>1</sup>; Hosein Bagheri <sup>2</sup>; Saber Babazadeh <sup>3</sup>; Mohammad Sadegh Khadivi <sup>4</sup>; Melika Mohammadi <sup>5</sup>;

<sup>1</sup> Dept. of Endodontics, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>2</sup> Dental Materials Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>3</sup> Dept. of Community Oral Health, Faculty of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>4</sup> Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.

<sup>5</sup> Dept. of Endodontics, Faculty of Dentistry, North Khorasan University of Medical Sciences, Bojnurd, Iran.

### KEY WORDS

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### ABSTRACT

**Background:** One of the key factors in evaluating the success rate of root canal treatment is apical sealing ability. Studies have shown that all sealers may permit some degree of microleakage. Therefore, *in vitro* evaluation of microleakage is essential before the clinical application of any new sealer.

**Purpose:** This study was conducted to assess the microleakage of a novel zinc oxide nanoparticle sealer and to compare its performance with a standard sealer (AH26) and a sealer with a similar base but without nano-particles (Pulpdent root canal sealer).

**Materials and Method:** In this *in vitro* study, 55 human single-rooted mandibular premolars were selected. The specimens were decoronated at the cemento-enamel junction, so that all the root samples were standardized to a length of 15 mm. Root canals were prepared using rotary instruments. The specimens were randomly divided into three groups (AH26, Pulpdent, and new nano zinc oxide-eugenol based sealer, each group containing fifteen specimens). Obturation was performed by cold lateral condensation technique with gutta-percha and the designated sealers in each group. After one week, all teeth underwent fluid filtration test and a second examination was performed 30 days after root canal obturation. Non-parametric statistical analyses were applied. Kruskal Wallis test was used to compare the microleakage between experimental groups and Wilcoxon test was used to compare each group's microleakage over time.

**Results:** The new nano zinc oxide-eugenol based sealer presented the lowest microleakage ( $p$  Value<0.05). The greatest reduction in microleakage between the two measurement intervals (7 days and 30 days), was observed in the AH26 group. The new nano zinc oxide-eugenol based sealer group showed the second highest reduction, which was also statistically significant. The Pulpdent sealer group demonstrated the lowest microleakage reduction between the two measurement intervals, which was not statistically significant.

**Conclusion:** In the present study, the new nano zinc oxide-eugenol based sealer demonstrated a lower level of microleakage compared with the other similar sealers.

**Corresponding Author:** Mohammadi M, Dept. of Endodontics, Faculty of Dentistry, North Khorasan University of Medical Sciences, Bojnurd, Iran. Tel: +98-9151566612 Email: melikamohammadi7293@gmail.com

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### Introduction

The purpose of root canal treatment is to achieve thorough chemo-mechanical cleansing of the contaminated

root canal system, followed by obturation with a root canal filling material [1].

Effective removal of contaminated debris and preve-

tion of intraoperative or postoperative infections are essential for the success of the procedure [2]. Root canal obturation represents the final stage of treatment. However, this step poses a significant challenge in dentistry due to the complex morphology of root canals and the limitations of current techniques and materials [3].

The application of gutta-percha, a semi-solid root filling material, in combination with a sealer, represents the most common method of root canal filling. On its own, gutta-percha lacks sufficient flow and does not adhere to the root canal walls. Therefore, the use of an appropriate sealer is essential [4]. A suitable endodontic sealer forms a microscopic, impermeable barrier that prevents microbes from penetrating the root canal system. An ideal sealer should possess several key characteristics including bacteriostatic properties, the ability to provide hermetic sealing, radio-opacity, an appropriate working time, strong adhesion to root canal dentin, and dimensional stability [5]. The most commonly used sealers in endodontic treatment are zinc oxide-eugenol based sealers (ZOE-based sealer). These sealers consist of zinc oxide powder and eugenol liquid. When mixed and placed in the root canal, the reaction between zinc oxide and eugenol produces an amorphous gel, while the residual zinc oxide powder forms a supporting matrix [6]. Pulpdent root canal sealer, used in this study, is a ZOE-based sealer. The powder component contains zinc oxide, zinc stearate, calcium phosphate, and barium sulfate, while the liquid component consists of eugenol and Canada balsam [7].

AH-26 root canal sealer is another widely used material in endodontics. It is an epoxy resin-based sealer, reported to undergo shrinkage during polymerization and to release formaldehyde. However, these two drawbacks are no longer present in AH Plus, another epoxy resin-based sealer [5].

Nanotechnology is the science of manipulating atoms and molecules at the nanoscale, typically with dimensions ranging from 1 to 100 nanometers [8]. Small particles at the nanoscale can impart advanced properties to materials. Nanotechnology and nanomaterials have become highly active areas of research due to their promising applications across numerous fields, including medicine, dental materials, and information technology. In dentistry, the incorporation of nanotechnology into dental materials results in superior mechanical

properties, enhanced wear resistance, and improved optical characteristics [9].

Recently, an experimental nano ZOE-based sealer known as COLORADO™ (Fannavarani Pishro Sanaat Arad Co, Mashhad, Iran) has been introduced. It is similar to conventional ZOE-based sealers, but incorporates zinc oxide nanoparticles of varying sizes, which distinguishes it from traditional formulations [9]. This nano-sealer has demonstrated significant and advantageous properties [9].

One of the key factors in evaluating the success of root canal treatment is apical sealing ability. Studies have shown that all sealers may permit some degree of microleakage. Microleakage allows microorganisms to re-enter the periapical space, compromises the healing of periapical lesions, and can ultimately lead to treatment failure. Therefore, *in vitro* evaluation of microleakage is essential before the clinical application of any new sealer. Several methods are available for this purpose, including dye penetration, fluid filtration, dye extraction, and bacterial or toxin infiltration techniques. Among these, dye penetration is relatively simple, whereas bacterial infiltration methods are more complex (Figure 1) [10].

Based on the study of Javidi *et al.* [11], fluid filtration method is considered a reliable method and has many advantages; it does not need any special indicator, the possibility of using distilled water or normal saline (which, in addition to its neutrality, simulates the body tissue conditions), the samples are not destructed during the procedure and also the possibility of measurement after certain periods of time.

This study was conducted to evaluate and compare the microleakage of a new nano ZOE-based sealer with AH 26 and Pulpdent sealer in human single-rooted mandibular premolars, assessed at 7 and 30 days after root canal obturation by using fluid filtration method.

## Materials and Method

This study was approved by the Ethics committee of Mashhad University of Medical Sciences (IR.MUMS.DENTISTRY.REC.1401.102).

In this study, 55 human single-rooted mandibular premolars were selected. Each tooth was initially examined using parallel periapical radiographs in both the mesiodistal and buccolingual dimensions to confirm the



**Figure 1:** Fluid filtration test

presence of a single root canal. Teeth with more than one canal, curved roots, internal and external root resorption, root caries, canal calcification, open root apices, and short root canals with initial apical size larger than file # 25, were excluded from the study. The samples were decoronated at the cemento-enamel junction with a carborundum disk (Resista, Omegna, Italy), so that all the root samples were 15 mm in length.

Working length was determined using a #10 or #15 K-file, positioned 1 mm shorter than the root apex. Instrumentation of all of the samples was done with Denco blue rotary files (SX-F3; Shenzhen Denco Medical Co., Shenzhen, China) in conjunction with an E-connect endomotor (Eighteenth, Changzhou, China) set at 300rpm and 2N.cm torque. The root canals were irrigated, using 5mL of 5.25% NaOCl (Morvabon, Tehran, Iran) and normal saline (Darupakhsh, Tehran, Iran) between each file. The smear layer was removed using 2.5 mL of 17% EDTA (Morvabon, Tehran, Iran) for 1 minute, followed by a final rinse with 5 mL of normal saline. All instrumented specimens were dried with #30 paper-point (Meta Biomed, Chungbuk, South Korea), and an F3 master gutta percha (Meta Biomed, Chungbuk, South Korea) was fitted and confirmed using parallel periapical radiographs.

Each specimen was assigned a number, and using the website [www.random.org](http://www.random.org), the specimens were randomly divided in to three groups, each group containing fifteen specimens. The remaining ten teeth were allocated as positive and negative controls.

In the positive control group, obturation was performed using gutta-percha alone. In the negative control group, after obturation with gutta-percha, the teeth were additionally sealed with several paraffin strips. In the first experimental group, the root canals were obturated

with gutta-percha and AH26 root canal sealer (Dentsply, DeTrey, Konstanz, Germany) using the lateral condensation technique. In the second experimental group, obturation was performed with gutta-perch and Pulpdent sealer (Pulpdent Co. Watertown, USA) using the same technique. In the final group, the root canals were obturated with gutta-percha and the new nano ZOE-based sealer (COLORADO™; Fannavaran Pishro Sanaat Arad Co, Mashhad, Iran) according to the manufacturer's instructions [9], also with lateral condensation technique. All studied sealers were mixed according to the manufacturer's guidelines. The new nano ZOE-based sealer was prepared in the dental materials laboratory of the Mashhad Faculty of Dentistry and was mixed according to the manufacturer's instructions as one part eugenol and two parts zinc oxide until a uniform, creamy consistency was achieved.

Following root canal treatment, all the specimens were stored at 100% humidity and 37°C for the next 7 days. After one week, all teeth underwent a fluid filtration test, performed according to the method described in the previous study [11]. In this step, the obturated root was connected to a nitrogen gas source (99.99 %, 0.6 bar) through a graduated test tube (0.5mm internal diameter and 5cm length) containing liquid (double distilled water) [11] (Figure 2). The volume of liquid displaced in the tube over a 10-minute period was recorded as the measure of microleakage [11]. A second evaluation was done 30 days after root canal obturation. The samples were stored under the same incubation condition, between the two examinations. All preparation steps were performed by single operator. Another operator who was blind to the types of root canal sealers, measured the microleakage, and statistical analysis was carried out by an independent analyst who was



Figure 2: Colorado root canal sealer

blind to the procedures. Microleakage measurements were taken at room temperature.

**Statistical analysis and sample size**

Because no previous studies used the fluid filtration method with these root canal sealers, we began with a pilot study including 10 specimens per experimental group and 5 per control group. Based on the pilot results, the final sample size for each experimental group was calculated to be 15.

Non-parametric analyses were used in this study. The Kruskal Wallis test compared the microleakage across experimental groups and Wilcoxon test was used to compare each group's microleakage over time.

**Results**

In this research, 55 human premolar teeth were selected. Initially, two groups of 5 samples each were randomly selected for positive and negative controls, and the rest

of the samples were randomly divided in to 3 experimental groups of 15 specimens each, to assess the microleakage using fluid filtration test.

Through the procedure, any sample which was inappropriate, due to obturation or any other damage during the operation, was removed and replaced with a suitable sample that conforms to the experiment's criteria.

In Table 1, the microleakage measurements of each group of sealers are presented. The lowest microleakage average belongs to the second measurement (after 30 days) in the nano ZOE-based sealer group, while the highest microleakage average belongs to the first measurement (after 7 days) in the AH26 sealer group (Table 1). At the first measurement, the nano ZOE-based sealer group showed the lowest standard deviation, whereas the AH26 group showed the highest. The differences were statistically significant ( $p$  Value< 0.05) (Table 1).

The Kruskal- Wallis test was used to compare micr-

**Table 1:** Statistical information of the experimental groups; (1)\* refers to 7 days evaluation and (2)\*\* refers to 30 days evaluation ( $p$  value< 0.05)

Groups	Sample size	The least microleakage (µL/min)	The most microleakage (µL/min)	Average	Standard deviation
AH-26 sealer (1)*	15	0.45	2.40	0.6960	0.48316
AH-26 sealer (2)**	15	0.20	2.00	0.5433	0.41814
Pulpdent sealer (1)	15	0.50	0.70	0.5940	0.07099
Pulpdent sealer(2)	15	0.40	1.45	0.6087	0.25289
*Nano ZOE-based sealer (1)	15	0.40	0.60	0.4727	0.06777
Nano ZOE-based sealer (2)	15	0.25	0.55	0.4073	0.09075

Nano zinc oxide-eugenol based sealer (Nano ZOE-based sealer)

**Table 2:** 7- day interval microleakage assessment in each group ( $p$  value <0.05)

	Groups	Number of specimens	Ranking based on Kruskal Wallis test
7- day interval microleakage evaluation	AH-26	15	26.53
	Pulpdent	15	29.67
	Nano ZOE-based sealer	15	12.80
Nano zinc oxide-eugenol based sealer (Nano ZOE-based sealer)			

oleakage among the sealer groups for the first and second measurements. The information is illustrated in Tables 2-3.

At the both primary (7 days) and secondary (30 days) measurements, the nano ZOE-based sealer group presented the lowest microleakage, while the Pulpdent group showed the highest. The differences were statistically significant ( $p < 0.05$ ) (Tables 2-3).

The microleakage reduction between two measurements (after 7 days and after 30 days), in each group of sealers, was assessed with the Wilcoxon test. The highest microleakage reduction between two measurements was attributed to AH26 group. In the second place, the nano ZOE-based sealer group was placed, which were both significant. The lowest microleakage reduction was featured with the Pulpdent sealer group, which was not significant (Table 4).

## Discussion

In this study, fluid filtration technique was used to assess the apical microleakage of three different root canal sealers in 7-day and 30-day intervals. Various techniques have been introduced to assess the sealing capability after root canal obturation including dye penetration, teeth clearing, bacterial penetration, glucose microleakage test, and fluid filtration [11-12]. Fluid filtration technique seems similar to the oral condition and samples are not destructed after the experiment, so they can be used in several time intervals. For this reason, this

**Table 3:** 30- day interval microleakage assessment in each group ( $p$  value <0.05)

	Groups	Number of specimens	Ranking based on Kruskal Wallis test
30- day interval microleakage evaluation	AH-26	15	21.57
	Pulpdent	15	31.53
	Nano ZOE-based sealer	15	15.90
Nano zinc oxide-eugenol based sealer (Nano ZOE-based sealer)			

**Table 4:** Comparison of microleakage reduction during 7-day and 30-day follow-up in each group

Groups	AH-26 sealer	Pulpdent sealer	Nano-ZOE based sealer
Microleakage reduction	- 3/297	- 2/965	- 0/ 791
$p$ Value	0/001	0/003	0/429
Nano zinc oxide-eugenol based sealer (Nano ZOE-based sealer)			

method was used to evaluate the sealing ability in this study and other similar studies [9, 12-15].

Khademi *et al.* [15], Raina *et al.*[13], Asawaworarit *et al.*[16], Manjila *et al.*[17] and Kelmendi *et al.* [18], evaluated the specimens in 7-day interval whereas, in the study by Javidi *et al.* [9], the samples were assessed in 3-day, 45-day and 90-day intervals. The current study, conducted fluid filtration technique in 7- day and 30-day intervals.

In order to reproduce as many samples as possible and precise assessment of the samples, human single-rooted premolar teeth were selected. The presence of a smear layer during root canal preparation can obstruct dentinal tubules and impede sealer penetration, leading to a weak bond strength. Shantiaee *et al.* [19] evaluated the effect of different intracanal chelators (RC-Prep, 17% EDTA and Glyde File Prep) on the apical microleakage. They announced that there was not any significant difference among the chelators. However, preparations with RC Prep and Glyde File Prep yielded the highest and the lowest values of apical microleakage, respectively. A review study by Boutsuioukis *et al.* [20] revealed that NaOCl and EDTA delivered by a needle and possibly activated by an ultrasonic file could be the current irrigation method regarding the current evidence. In this study, consistent with the results of other researches [9, 13-15], we decided to use EDTA as a chelator to remove smear layer.

In this report, cold lateral condensation method was used considering its advantages such as, long-term use, predictability, and relative ease of application. ZOE-based sealers are one of the first sealers introduced to the market and extensive studies have been conducted on their properties.

Manjila *et al.* [17] reported that conventional ZOE-based sealers have shown more microleakage, compared to bioceramic- based and resin-based sealers.

Also, in many studies [20-22], resin-based sealers, like Adseal sealer and AH Plus sealer, presented less

microleakage than other investigated sealers.

In the current study, AH 26 was used as the gold standard sealer. Pulpdent, which shares the same zinc oxide eugenol base as the new nano sealer but does not contain nanoparticles, was included to evaluate the effect of nanoparticles.

Our study showed that new nano ZOE-based sealer's microleakage was less than Pulpdent and AH 26 sealer's microleakage, in 7-day and 30-day intervals. Also our results were in consistence with the results yielded by study of Javidi *et al.* [9] which evaluated the sealing properties of nano ZOE-based sealer, AH 26 and micro-sized ZOE-based sealer.

Hajihassani *et al.* [23] investigated the apical sealing ability of AH Plus sealer and Sur-Seal root canal sealer in mandibular second premolars in 7- day and 90-day intervals by fluid filtration method. No significant differences were found in 7-day evaluation. However, after 90 days, the AH Plus group illustrated significantly less microleakage than the Sure-Seal root canal sealer group.

In a research carried out by Kelmendi *et al.* [18], three root canal sealers including Canason, Well-Root ST, and AH 26 were evaluated. The results showed that microleakage of Well-Root ST sealer was the lowest followed by AH 26 and Canason respectively. The results of our study were not consistent with this study, which might be due to the different sealers and methods employed for assessing sealing ability.

Malinja *et al.* [17] compared the root canal sealer microleakage, between four different sealers including ZOE-based, resin-based, calcium-hydroxide based, and bioceramic sealers. According to the study results, bioceramic sealer group presented the least apical microleakage and the highest microleakage occurred in the ZOE group. The difference between the results of this study and our study may be attributed to the differences in the methodology of these studies and the differences in the sealer types.

The limitations of the study can be ascribed to the specimens, which consisted of human mandibular single-root premolars. Since, root canals might have a divergent anatomy. Another limitation is the small sample size. Larger sample sizes generally yield more reliable results. For future studies, a larger sample size, other tooth groups like molars and application of other microleakage measurement methods, are recommended.

## Conclusion

The new nano ZOE-based sealer presented less microleakage in comparison with the other sealers tested, although each sealer has its own advantages and disadvantages. Further studies and formulation changes could improve the properties of this new sealer.

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## Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by the local ethics committee of Mashhad University of Medical Sciences, Iran (IR.MUMS.DENTISTRY.REC.1401.102). Human mandibular premolars extracted due to orthodontic treatment or periodontal diseases were obtained from the Oral and Maxillofacial Surgery clinic of the Mashhad Faculty of Dentistry, and patients' written informed consents for using their extracted teeth, were obtained.

## Conflict of Interests

None declared.

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