Original Article

Comparison of Tubular Penetration of Nano ZnO, AH26 and kerr Pulp Canal Sealer EWT Using Scanning Electron Microscope

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Received 10 April 2020 and Accepted 18 August 2020

Abstract

Introduction: The Aim of endodontic treatment is to remove infection and prevent recontamination of root canal system. Endodontic sealers are used to fill irregularities that are inaccessible for core filling material. Their penetration into dentinal tubules is of great importance to obtain a better seal. Sealers with nanoparticles may have a deeper penetration because of their smaller particles. The purpose of this study was to compare the penetration ability of a new nanoparticle sealer with two other sealers. **Methods:** Twenty single-rooted premolars were decoronated and prepared using NeoNiTi rotary files. Then the smear layer was removed and canals were randomized in three groups and filled with gutta-percha. Nano-ZnO sealer, AH26, and Pulp Canal Sealers were used for each group. Teeth were sectioned at two levels (4 and 8 mm from the apex) and examined under a scanning electron microscope. **Results:** The results showed the deepest penetration for AH26 Penetration depth in the coronal section was deeper than apical. There was no significant difference between penetration depth of AH26 and Nano-ZnO sealer in the apical region, but AH26 showed significantly more penetration in coronal section. **Conclusion:** According to the results of this study, Nano-ZnO sealer showed less penetrability compared to AH26 in coronal region of the roots.

Keywords: AH26, Nano-ZnO sealer, Pulp Canal Sealer, Scanning electron microscope, Tubular penetration

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zarei M, Kazemi Z, Javidi M, Shayani Rad M, Karimpour S, Forghani M. Comparison of tubular penetration of Nano ZnO, AH26 and kerr Pulp Canal Sealer EWT using Scanning Electron Microscope. J Dent Mater Tech 2020; 9(3): 147-151.

Introduction

The aim of endodontic treatment is to eliminate microorganisms and their byproducts from tooth root canal system and also establish a durable seal all along the canals to prevent recontamination. Gutta-percha is the most commonly used material to fill the canals. It is a solid material and cannot provide a full adaptation to root canal walls. Therefore, gutta-percha is used as a core and endodontic sealers are used to fill the irregularities and penetrate into dentinal tubules to provide proper fluid-tight (1). Sealer penetration to dentinal tubules has three major benefits: first, it increases the contact surface between filling material and dentin improving sealing ability (2). Second, this interlocking may enhance mechanical retention of the filling material (3-5). And third, it can prevent bacteria from colonizing within the tubules by separating them from nutritional resources (4, 6, 7). Measuring dentinal tubule penetration of endodontic sealers is used to evaluate their performance and sealing ability (4). Endodontic sealers ability to enter tubules is affected by their physiochemical properties (8, 9). There are evidences showing that smear layer plays a negative role in sealer penetration by occlusion of dentinal tubules (3, 10, 11). So, a sealer with good flow rate, film thickness, surface tension, solubility and viscosity may be able to penetrate into dentinal tubules if they are open (12).

Nano-ZnO sealer is a ZnO-based endodontic sealer that has nanoparticles in its composition. Nanoparticles are of interest in endodontics because of their tubular penetration, antimicrobial activity, and reduced leakage plus enhanced physiochemical properties (13). As far as we know, there is no study on penetration depth of this new nanoparticle sealer. Therefore, the purpose of this study was to evaluate the tubular penetrability of Nano-ZnO sealer in comparison with AH26 (a commonly used resin-based sealer) and Pulp Canal Sealer EWT (another ZnO-based sealer without nanoparticles) by means of scanning electron microscopy. The null hypothesis (H_0) was that none of these sealers penetrate into dentinal tubules.

Materials and Methods

Twenty human single-rooted premolars were selected and immerged in NaOCl 5.25% for 1 hour to remove all organic debris from root surface. Mesio-distal radiographs were taken to ensure all teeth have one canal. Teeth were decoronated by a diamond disk (Jota AG-Ruthi Switzerland) to standardize the root length to 12 mm. A #15 k-file (Dentsply/Maillefer, Ballaigues, Switzerland) was inserted through the canal until it was seen at the apical foramen. Working length was determined 1 mm shorter than this length. Root canals were prepared by a crown-down technique using NeoNiTi rotary files (Neolix, châtres-la-Forêt, France) according to the manufacturer's instructions: the coronal one-third was prepared by a NeoNiTi C1 file (25/.12) and the middle and apical one-thirds were prepared by a NeoNiTi A1 file (25/.08). The electric motor (NSK, Japan) was used at the speed of 350 rpm and torque of 1.5 NCm according to the manufacturer's instructions. Canals were irrigated between files using 1 ml of NaOCl 5.25%. In order to remove the smear layer, 1 ml of EDTA 17% was applied for 1 minute followed by 3 ml of NaOCl 5.25% at the end of the instrumentation. 3 ml of normal saline was used as final irrigation and canals were dried using sterile paper points (Meta Biomed, Chungbuk, Korea).

Then, teeth were randomly divided into three groups of 6 teeth in each group as follows:

Group 1. Teeth were obturated with gutta-percha and the resin-based sealer AH26 (Dentsply, DeTrey, Germany).

Group 2. Teeth were obturated with gutta-percha and Pulp Canal Sealer (Sybron Kerr, Romulus, MI, USA).

Group 3. Teeth were obturated with gutta-percha and Nano-ZnO sealer.

Two teeth were sliced longitudinally and examined under the microscope to ensure the removal of the smear layer.

All 3 sealers were prepared according to the manufacturer's instruction on a glass slab with a metal spatula until a homogenous consistency was obtained. A lateral obturation technique was used in a way that the canal walls were coated with sealer by using a size B spreader (Dentsply/Maillefer, Ballaigues, Switzerland). A #25 master gutta-percha cone with 0.02 taper (Sure Dent, Korea), coated with sealer was inserted into the canal followed by sealer coated accessory cones until the entire canal was filled. The excess gutta-percha was removed by a heat-carrier and then orifices were sealed by 2-3 mm of Cavit (Coltosol, Ariadent, Tehran, Iran). All specimens were incubated for 72 hours at 37℃ and 100% humidity to ensure sealers setting were completed.

A low-speed, water-cooled, automatic cutting machine CNC (Nemo, Mashhad, Iran) was used to section teeth at 2 levels (4 and 8 mm from the apex) horizontally. Specimens were then sonicated in distilled water to remove smear layer. In order to prepare specimens for SEM examination, we used Perdigao et al. (14) method:

1. Specimens were placed in a composition of glutaraldehyde 2.5%, paraformaldehyde 2% and phosphate buffer (pH=7.4) for 12 hours and dried with hexamethyldisilazane.
2. Then specimens were embedded in epoxy resin (AXON, France) with mixing ratio of 100 to 32.
3. After 48 hours, the surfaces of specimens were polished with 180 to 3000 grit sandpapers followed by a polishing wool pad
4. After placement in ethanol 100% for 5 minutes, specimens were placed in HCl 6% for 30 seconds to get demineralized and then placed in NaOCl 1% for 10 minutes to remove all organic debris.
5. Specimens were kept in ultrasonic cleaner to remove surface contaminations.
6. All specimens were mounted on aluminum stubs and coated with gold and palladium by using an Emitech SC7620 Sputter Coater (Quorum Technologies, Laughton, East Sussex, UK) for 3 minutes.

At the end, specimens were evaluated under a scanning electron microscope (LEO1450VP, Carl Zeiss AG, Oberkochen, Germany) at 20 kv and 205 nm resolution.

First, specimens were examined at ×200 magnification to see the whole sample surface. Then the area with the deepest sealer penetration was selected and examined at ×500 and ×5000 magnifications.

Results

The normal distribution of data was assessed using the Shapiro-Wilk tests. The data only in AH26 group in Coronal and Apical had a normal distribution (P>0.05).

Table I shows penetration depth of three sealers.

There was no statistically significant difference between sealers penetration depth in apical section (P-value = 0.302)

Table I. Comparison of penetration depth (µm) of 3 sealers in 2 different zones

Groups	Coronal		Apical		P-value
	Mean±SD	Median(IQR)	Mean±SD	Median(IQR)	
AH26	986.00 ±349.05	960.5(624.85)	292.60±340.84	190(665.4)	0.026
Nano-ZnO	282.41±450.72	19.87(703.03)	6.54±10.76	0(16.73)	0.144
Pulp Canal Sealer	37.47±78.51	0(70.62)	19.32±47.31	0(28.98)	0.593
P-value	P = 0.006		P = 0. 302		

IQR :Inter Quartile Range, SD: Standard Deviation

Discussion

This study evaluated dentinal tubule penetration depth of 3 endodontic sealers by means of a scanning electron microscope. Most of studies on tubular penetration have been done on single-rooted teeth, for their simple anatomy and ease of application (1, 8, 11). Therefore, we choose to study human single-rooted premolars. AH26 is a common resin-based sealer. Resin-based sealers have shown better penetrability among endodontic sealers (15, 16) and can be used as gold standard. Pulp Canal Sealer has a similar chemical composition with Nano-ZnO sealer but without nanoparticles. Javidi et al. (17) found that Nano-ZnO sealer exhibited less microleakage in comparison with AH26 and ZOE. In order to see the effect of nanoparticles on sealer penetrability, we decided to compare Nano-ZnO sealer with AH26 as a gold standard and Pulp Canal Sealer as another ZnO-based one. Different studies have reported various incubation periods (2, 8, 18). In this study, incubation for 72 hours after obturation was selected to ensure sealers setting have been completed. Setting time for AH26, Pulp Canal Sealer and Nano-ZnO sealer are 14, 2, and 48 hours, respectively. Most studies have shown that smear layer inhibits sealers to penetrate into tubules as it occludes the dentinal tubule entries (3, 10, 11). Therefore, we removed smear layer to neutralize this negative effect.

Scanning electron microscope has been used in many studies to evaluate endodontic sealer penetration depth (3, 11, 18-20). Dentinal tubule observation and exact penetration depth measurement at high magnifications is the main advantage of this technique but it also has disadvantages like inability to provide a detailed view in low magnifications (11).

Overall, within the limitation of this study and in rejection of the null hypothesis all 3 sealers penetrated into dentinal tubules. The results showed deeper penetration in coronal section that was in accordance with many studies showing regional variations in sealer penetration depth (1, 2, 8, 15, 18). Deeper penetration in coronal region can be due to greater diameter and higher number of tubules in the coronal third or root (15, 21). Moreover, apical dentin has more irregular and sclerosed tubules or even no tubules in some areas (2, 8).

Based on our observation, AH26 showed significantly deeper penetration in the coronal section compared with Pulp Canal Sealer and Nano-ZnO sealer. Nano-ZnO sealer had better penetrability than Pulp Canal Sealer (mean penetration depth of 282.41 µm and 37.47 µm respectively), but the difference was not statistically significant. AH26 sealer deeper penetration could be due to smaller particles (30 nm) that is much smaller than the tubules diameter. Similarly, Mammotil et al. (11) reported a significant difference between penetration depth of AH26 and Pulp Canal Sealer. They reported a mean penetration depth of 1337 µm for AH26 that is close to our results (986 µm as mean penetration depth for AH26). Also, Balguerie et al. (2) reported a mean penetration depth of 40.2 µm for a ZnO-based sealer that is quite similar to our results (37.47 µm as mean penetration depth for Pulp Canal Sealer). Kokkas et al. (3) reported deeper tubular penetration for resin-based sealer AH-Plus than ZnO-based sealer Roth 811. Resin sealers have shown deeper penetration owing to their adequate flow and thin film structure (22).

In the apical section, Nano-ZnO sealer showed least depth of penetration. Although there was no statistically significant difference between sealers penetration depth. Vasiliadis et al. (20) showed that the deepest penetration of sealers was in upper part of the middle third of roots, where greatest forces would be expected during lateral compaction of gutta-percha. It can be concluded that due to low compaction pressure in apical regions, tubular penetration of the sealer is affected by its physical properties such as flow. Versiani et al. (23) showed ZnO nanoparticles improved physicochemical properties of Grossman sealer, however reduced the flow characteristics. So, the lower penetration of Nano-ZnO sealer in the apical region may be due to its lower flow.

Conclusion

Within the limitations of this in-vitro study, Nano-ZnO sealer had less mean penetration depth compared to AH26 in coronal section of the roots. Tubular penetration depth in coronal region had the highest values and decreased as it approached toward the apex. Further studies should evaluate new nanoparticle sealers.

Conflict of interest

The authors deny any conflict of interest.

Acknowledgments

This article is based on a postgraduate thesis (No. 589). The authors would like to thanks the vice chancellor for research of Mashhad University of Medical Sciences.

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