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AN IN-VITRO COMPARATIVE ASSESSMENT OF THE SEALING ABILITY OF AH PLUS SEALER WITH DIFFERENT ROOT CANAL NANO SEALERS

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ABSTRACT

Objectives: The aim of the present study was to evaluate the sealing ability of experimental nano sealers (nano calcium hydroxide and nano bioactive glass) and to compare it with the commercial AH plus sealer using a dye penetration method.

Materials and methods: Sixty single-rooted mandibular premolars were selected. The coronal portion of each tooth was removed to standardize root length at 16 mm and the root canals were prepared with NiTi rotary files (ProTaper Next) up to X4 (40/0.06). Teeth were randomly assigned and divided according to the sealer used for obturation into three groups of 20 each. Group I: AH plus, Group 2: Nano calcium hydroxide and Group 3: Nano-bioactive glass. All root canals were obturated using single cone gutta-percha(#40/.06) and one of the tested sealers. Root canal sealing was assessed by a dye penetration test. The data were statistically analyzed by ANOVA test followed by post hoc analysis ($P < 0.05$).

Results: Significant improvement shown by the presented study suggests that nano calcium hydroxide sealer showed significantly less dye leakage than nano bioactive glass sealers and Ah plus sealer.

Conclusion: The results of this study showed that the synthesized nano-powder sealers are suitable for use in root canal therapy to prevent leakage. The root canal can be sealed better by using smaller nano-powder particle sizes. In addition, the two groups exhibited significant differences in leakage in comparison with commonly used AH plus sealer.

KEYWORDS: Nano sealers, AH plus, Sealing ability, Obturation**,** Dye penetration test.

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INTRODUCTION

Sealing the entire root canal system after cleaning and shaping is of outmost importance to prevent ingress of bacteria from the oral environment and entombing any residual microorganisms [1]. The best-known and most commonly used filling material is gutta-percha. However, it cannot hermetically seal the root canal alone[2]. To obtain a hermetic seal, root canal should be obturated with a combination of a central core material, gutta-percha, with sealer[1].

Endodontic sealers serve as a lubricant during obturation process, fill canal irregularities, seal the spaces between the gutta-percha and the dentinal walls ([3]. Root canal sealers should fulfill some requirements, such as dimensional stability, insolubility in oral fluids, biocompatibility, radiopacity, ease of application, antibacterial properties, adaptability to the root canal walls, as well as the ability to produce a hermetic seal [4]. However, none of the currently available sealers have all characteristics of the ideal sealer [5].

AH Plus is an epoxy resin-based sealer commonly used as a gold standard endodontic sealer due to its high bond strength to dentine, adequate radiopacity, flowability, dimensional stability, low solubility and high resistance [1]. However, it cannot totally eliminate leakage [6].

Nanotechnology is the science of evaluating and producing materials in nano-dimensions by re-location and re-arrangement of atoms to prepare materials with better properties. Nanoparticles (Np) are ultrafine particles of insoluble constituents with a diameter less than 100 nm[8-9].

Presence of very small particles leads to superior properties of the material[10]. Because of these valuable properties, utilization of nanoparticles in production of endodontic sealers has become favorable for many researchers.

Recently, nanoparticles sealers have been introduced by the addition of nanoparticles to the endodontic sealers without changing their physical or mechanical properties, but rather a slight change in the flowability of the sealers has been noted. So, their penetration into the dentinal tubules has been increased, improving their sealability and antimicrobial properties [11,12].

Calcium hydroxide nanoparticles may possess several advantages over their conventional counterparts, such as improved depth of penetration, increased surface area contact with pathogens, superior solubility and greater antimicrobial activity[13-15]. Several studies have found that nano-calcium hydroxide showed deeper penetration into dentinal tubules and had superior antibacterial activity against *E. faecalis* compared to conventional calcium hydroxide [13-16].

Bioactive-glass (B-G), one of bioceramics, plays a significant role in promoting hard tissue healing in many clinical situations and has a particular interest for endodontic care because of its biocompatibility, regenerative and antimicrobial properties as well as chemical composition that closely resembles the mineral make-up of human bone and dentine. Nanoparticles of bioactive glass are a modification which enhances all these properties [17].

Recently, the authors of this article prepared new experimental endodontic sealers (nano calcium hydroxide and nano bioactive glass) in the Dental Material Research Center (NanoTech Egypt for Photo-Electronics,Al-Giza, Egypt). These sealers are similar to various calcium hydroxide and bioactive glass sealers, but with different sizes of nano-particles. The sealing ability of our synthesized nano-sized calcium hydroxide and bioactive glass sealers was compared with AH plus sealer.

The recently-introduced nanosealers (nano calcium hydroxide and nano bioactive glass) are known to possess great ability to inhibit biofilm formation within the sealer dentin interface, reduce cytotoxicity, and improve sealability [18, 19]

MATERIALS AND METHODS

In vitro study

Nano Calcium Hydroxide

Manufacture Method in this Study

(NanoTech Egypt for Photo-Electronics, Al-Giza, Egypt). Homogeneous phase precipitation which normally takes place in the aqueous phase following mixing of a strong base (*e.g.*, NaOH) and Ca salt (*e.g.*, $CaCl_2$ or $Ca(NO3)_2$) solutions (20,21). Variations to this route include synthesis in organic liquids (hydrocarbons) using surfactants that adopt a reverse-type micelle configuration around $Ca(OH)_2$ colloidal particles [22] and synthesis in water-in-oil emulsions [23].

Nano Bioactive Glass

1. Manufacture Method used in this Study (NanoTech Egypt for Photo-Electronics, Al-Giza, Egypt)

The production of 45S5 bioactive glass has traditionally been carried out through sol-gel methods. The sol-gel method is a wet-chemical technique that can be used for the fabrication of both glassy and ceramic materials [24]. In the sol-gel process, the precursors are catalyzed and dissolved in the solvent to form a sol. The sol gradually becomes a gel-like diphasic system containing both a liquid and a solid phase.

The morphology ranges from dispersed particles to continuous polymer-like networks, which mainly depends on the pH and solvent. Generally, the sol-gel process includes hydrolysis, polycondensation, drying, and stabilization. To strengthen the gelation and aging network, an aging process is necessary.

Removal of the remaining liquid solvent phase requires a drying process. Finally, a thermal treatment (stabilization) is often carried out to enhance the mechanical properties and to improve structural stability[25].

Samples collection:

Non-carious, intact sixty freshly extracted mandibular premolar teeth with single roots and single canals were collected from the outpatient clinic of the Faculty of Dentistry, Assiut University hospital. Soft tissue deposits and calculus were removed with an ultrasonic scaler. Then, they were washed under running water and immersed in 0.1% thymol solution (Formula e Acao, Sao Paulo, SP, Brazil) till the time of use.

Teeth were optically inspected under stereomicroscope for the presence of any crack or resorption areas. Teeth with root fractures, root caries, evidence of periapical resorptive processes, or multiple canals were excluded from the study.

Samples preparation:

The teeth were decoronated at the level of cemento-enamel junction (CEJ) using a diamond disc (Komet, Gebr. Brasseler GmbH & Co. Germany) mounted on a high speed handpiece under a copious water-cooling to standardize the root length to 16 mm (figure 1). Each root canal was scouted to the apex using a stainless steel K-file ISO size 10 (Dentsply Sirona, Ballaigues, Switzerland) to check patency and those having an apical diameter larger than a size 25 K-file (Dentsply Sirona, Ballaigues, Switzerland) or being non-negotiable were discarded.

Root canal preparation:

All instrumentation procedures were performed by a single experienced endodontist. Working length was determined visually by inserting a stainless steel K-file ISO size 10 till it became visible at the apical foramen under a magnification of 20X using a stereomicroscope to ignore the fault samples and then a 0.5 mm was subtracted from this measurement. ProTaper Next (Dentsply-Maillefer, Ballaigues, Switzerland) rotary instruments mounted on an electric endomotor (TriAuto mini; J. Morita MFG. CORP. Japan) were used with crown down technique up to MAF # X4 (40/0.06) in a continuous rotation motion. The torque and the speed were adjusted according to manufacturer's instructions. During instrumentation, root canal irrigation was accomplished using the conventional syringe irrigation technique with 2 ml of 5.25% sodium hypochlorite (Egyptian company of household bleach, Egypt) delivered via a 30 gauge closed end side-vented needle (Max I probe; Dentsply Maillefer, Switzerland) placed 1mm short of working length. During the biomechanical preparation, the canal patency was maintained by #10 K-file. The final rinse was performed at the end of root canal instrumentation using 5 ml of 5.25% sodium hypochlorite followed by 5 ml of saline solution and finally 5 mL of 17% ethylenediaminetetraacetic acid solution (EDTA) (META BIOMED CO, Republic of Korea) to remove the smear layer.

To remove the remaining NaOCl and EDTA solutions, final irrigation was done using 5 mL of normal saline solution. Thereafter, the roots were dried with corresponding paper points**.** This protocol was employed for all the teeth.

Obturation

After completion of the biomechanical preparation of root canal, samples were assigned and randomly divided into three groups according the sealer type used in obturating the canals with single cone technique as the following (n=20) :

Group A: Canals were obturated with gutta-percha along with AH-plus sealer (Dentsply, Konstanz, Germany).

Group B: Canals were obturated with gutta-percha along with nano calcium hydroxide sealer (NanoTech Egypt for Photo-Electronics**,** Al-Giza, Egypt).

Group C: Canals were obturated with gutta-percha along with nano bioactive glass sealer (NanoTech Egypt for Photo-Electronics**,** Al-Giza, Egypt).

Sealers were dispensed and mixed according to manufacturer's instructions. Before placement of sealers, gutta-percha alone was placed to assess the fit and to determine their extension till working length.

The canals were coated with the sealers with the help of Lentulo spiral # 30 (Dentsply Maillefer) till 2 mm short of apex. Thereafter, single cone technique was employed for root canal obturation with gutta-percha (#40/.06). Excess gutta-percha was removed with heated plugger 1mm below the canal opening. The root canal orifices were sealed with temporary filling material Cavitemp (Ammdent, SARONNO-ITALIA).

Subsequently, all specimens were stored at 37°C temperature and 100% humidity for 1 week in a humidifier (Universal Humidifier, Delhi) to allow setting of the sealers.

Microleakage evaluation

Leakage was estimated by dye penetration test. Teeth were coated with two layers of nail varnish down to the margins of the apical foramen. Specimens were then immersed in 2% methylene blue solution for 7 days at room temperature**.** After being removed from the dye solution, the specimens were washed and the nail varnish scraped away from the root surface (Figure 2). The roots were then sectioned bucco-lingually in a longitudinal direction with a diamond disc under running water.

Roots were sectioned into two equal halves along the long axis, in buccolingual direction with a double-sided diamond disc (Figure 3). Two parallel grooves were established just short from the walls of canal then split into 2 halves by a mallet and chisel. The dye penetration was measured for each sample from the apex to the most coronal extent of the dye penetration under a stereomicroscope at ×10 magnification with digital vernier calliper.

Fig. (1): Extracted teeth after de-coronation

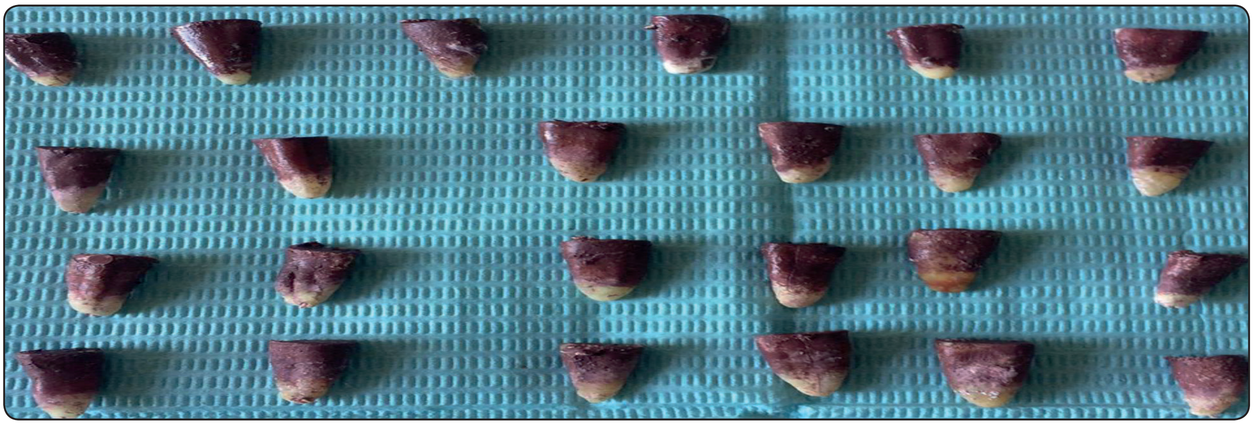

Fig. (2): Extracted teeth after removal from methylene blue dye

Fig. (3): Sectioned teeth bucco-lingually in a longitudinal direction

Statistical analysis

Statistical analysis of the data recorded was performed using SPSS (Statistical Package for Social Science) computer program (version 19 windows, SPSS, Egypt). One-way ANOVA test was used followed by post hoc test. Statistical program for the micro-leakage values with $P < 0.05$ was considered significant.

RESULTS

Comparison between AH plus, nano $Ca(OH)_2$ and nano bioactive glass sealers: **Table (1), Figure (4):**

The mean and standard deviation values of sealability by dye penetration test were 2.14±0.74, 0.53±0.28 and 1.12±0.59 after using AH plus, nano $Ca(OH)_2$ and nano bioactive glass sealers respectively.

Nano $Ca(OH)_2$ showed statistically significantly higher mean sealability(lower mean vertical apical leakage) than AH plus and nano bioactive glass sealers (p<0.02).

TABLE (1): Means, standard deviations and results for comparison between sealability by dye penetration test after AH plus, nano $Ca(OH)_2$ and nano bioactive glass sealers. Leakage of specimens in each group (In mm).

	AH plus	**Nano $Ca(OH)_2$**	**Nano bioactive glass**
	N=20	N=20	N=20
Seal ability			
Range	(0.86-2.613)	(0.24-1.03)	(0.29-2.223)
Mean ± SD	2.14±0.74	0.53±0.28	1.12±0.59

One-way ANOVA test for parametric quantitative data between the three groups followed by post hoc analysis between each two groups. P value <0.002*.

****: Significant difference at P value < 0.05.***

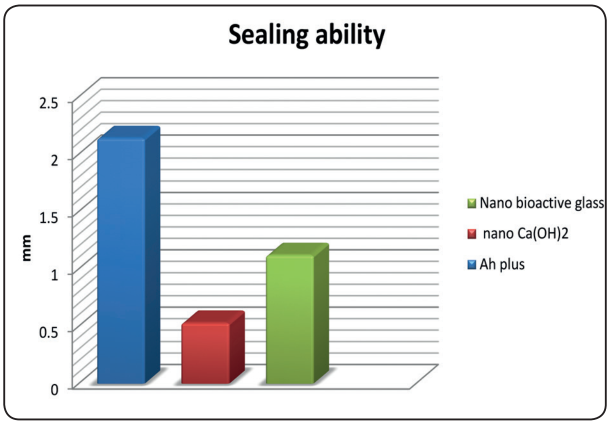

Fig. (4): Bar chart representing comparison between microleakage by dye penetration test after using AH plus, nano $Ca(OH)_2$ and nano bioactive glass sealers.

DISCUSSION

Selection of an appropriate endodontic sealer is a decision for the long term success of conventional endodontic treatment [26]. The primary functions of root canal sealers are sealing off any voids between dentinal walls and gutta percha and filling irregularities in root canal system including accessory canals and ismuthi [27,28]. However, the majority of failure (59%) was found due to incomplete obliteration of the root canal space [29]. So, investigations on the sealing ability must not stop till the ideal sealer capable to seal all the spaces inside the root canal is found.

The aim of the present study was to to evaluate the sealing ability of experimental nano sealers (nano calcium hydroxide and nano bioactive glass) and to compare it with the commercial AH plus sealer using a dye penetration method.

The appearance of nanotechnology improves the properties and sealability of endodontic materials as the active nanoparticles can penetrate the dentinal tubules and enter the accessory canals to ensure that the spaces have been sealed effectively [30].

Nanoparticulate calcium hydroxide sealer is similar to various calcium hydroxide based sealers but it has been recently manufactured to eliminate the shortcomings of conventional calcium hydroxide. Reducing the size of calcium hydroxide particles into nanoparticles enhances the penetration of this medicament into dentinal tubules and increases their antimicrobial efficacy[31,32].

For instance, nanoparticulate calcium hydroxide was recently synthetized for dental applications as described earlier by **Roy and Bhattacharya 2010**[33] with some modifications.

Bioactive glass (BG) which is composed of silicon oxide, calcium oxide and phosphorus pentoxide has higher bioactivity potential comparing to other bioceramic materials accounting for the amorphous structure [34]. The ions exchange between BG and body fluids could induce the formation of hydroxyapatite via biochemical reactions at the interface, forming a firm chemical bond with bones and soft tissues and stimulating tissue repair and regeneration [35]. Recently, BG has been applied in endodontics due to its biocompatibility, regenerative and antimicrobial properties. Nanoparticles of bioactive glass are thought to be a modification which enhances all these properties [17].

AH plus was used as a control as it is considered the gold standard for comparison with other sealers [1].

To evaluate apical microleakage, our study used the dye penetration test as it is considered the most popular, probably because it is a simple and reliable method [36]. In this study, methylene blue dye was used as it thought to have a molecular size similar to or smaller than that of bacterial products [37].

In the present study, all root canals were prepared using rotary NiTi files, ProTaper Next, to reduce the time required for biomechanical preparation and to assure standardization of canals instrumentation [38].

An irrigation protocol of a combination of NaOCl and EDTA was employed as it was found to be more effective in removing the smear layer that could interfere with adaptation and sealing of the obturating materials [39-41]. Also, Baumgartner and Mader in 1987 (42) revealed that the most powerful combination to produce clean root canal walls was the alternation of EDTA solutions with NaOCl to efficiently remove inorganic and organic components of the smear layer.

The full concentration of NaOCl (5.25 %) was used in this study because its concentration affects the dissolution of tissue, debridement of the canal and antimicrobial properties [43-45]. The in vitro effectiveness of 17 % EDTA on smear layer removal was demonstrated with and without ultrasonics by Kuah et al. (2009) [46]. The final rinse was carried out with a saline solution to neutralize the solutions used and to prevent any erosion that can have a negative effect on the integrity of the dentine matrix [40].

A 30-gauge flexible side-vented needle was used to optimize the effectiveness of the irrigation, especially in the apical part while avoiding binding of the needle at the apex [47] and placed 1 mm short of the working length to enhance irrigant replacement[48].

Obturation of the root canals was accomplished by using cold lateral compaction of gutta-percha with the root canal sealers. As the sealer may display a variable level of solubility, depending on its physical and chemical nature, it is important to use a sealer that has a minimum film thickness adjacent to the dentinal wall [49,50]. Moreover, with a thin film thickness, the sealer may also infiltrate deeper into the canal irregularities. Hence, the predictable outcome of root canal obturation is to achieve a great volume of gutta-percha and a least volume of sealer within the root canal space [51,52] with improved permeation into the canal irregularities and dentinal tubules.

To our knowledge, it is the first study to compare the sealing ability of nano $Ca(OH)_2$ and nano bioactive glass sealer with that of AH plus. Our results showed that Nano $Ca(OH)_2$ showed statistically significantly higher mean sealing ability than nano bioactive glass and AH plus sealers ($p<0.02$).

A possible explanation for this significant difference in the sealing ability between nanoparticles and traditional sealers was related to the size of the nanoparticles with a diameter of 100 nm or less which provided them with a greater contact surface area and charge density than bulky powders. This was in agreement with Liang-Jiab BI et al 2006 who stated that hydroxyapatite nanoparticles can effectively seal the exposed dentinal tubules [(53)].

Kishen et al (2007) showed that the addition of nanoparticles did not deteriorate the flow characteristics of the sealer and reduced viscosity, leading to enhanced flow of the sealer [(54)]. Also, Nazila et al (2018) illustrated that using nano-sized materials, the sealer anti-leakage property of can be enhanced [(55)].

Ca(OH)2 based sealer takes nine hours to set fully in either dry or humid environment, it shows very little water sorption. This means that it is quite stable which improves its sealing quality. Another possibility may be that Ca(OH)2 has been transported or mechanically forced into the dentinal tubules and thus occluded the dentinal tubules, blocking them off and decreasing dentinal permeability[(56)].

The cause of sealing ability of nano bioactive glass, is the alkaline nature of bioceramic by-products as it was reported to denature collagen fibers, which facilitates the penetration of sealers into the dentin tubules (Balguerie et al., 2011) [(57)].

CONCLUSION

The results of this study showed that synthesized nano-powder sealers exhibited less microleakage in comparison with AH plus sealer, making them suitable for use in root canal treatment. Nevertheless, further studies should be carried out and limitations and the potential unknown risks involved in the use of nano-powders as a medical material should be considered to verify their safety.

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