



THE EFFECT OF INCORPORATING NANOSILVER PARTICLES ON ANTIBACTERIAL ACTIVITY OF ENDODONTIC SEALERS

Ahmed D Abo Gabal *, Mohammad SH Aly **, Mahmoud H Mohamed *** and Saad-Eldin S Al-Ghazawy****

ABSTRACT

Objectives: The adding of nanosilver particles to endodontic sealers may be considered a method to improve the antibacterial properties. This study is directed to evaluate the antibacterial properties for modified zinc oxide eugenol and resin based endodontic sealers. **Methods:** a total number of 72 samples were used. They were divided into two main groups (n=36) according to the type of endodontic sealer (zinc oxide eugenol sealer {Endofil} and resin sealer {Adseal}), each group was subdivided into four subgroups (n=9) according to concentration of adding nanosilver [control, concentration A, B&C]. Each subgroup were also divided for three periods of treatment (one day, one week and one month). The antibacterial test was done by using CLSM (confocal scanning microscope). T-test was used to evaluate the antibacterial properties for the dental sealers. **Results:** It was found that the Endofil concentration C had the highest value in antibacterial activity (0.75 volumes of dead cells) and the lowest value for Adseal control group (0.16 volumes of dead cells). **Conclusions:** By increase the concentration of nanosilver particles into endodontic sealers, the antibacterial activity was significantly increased.

INTRODUCTION

The main goal of endodontic treatment is disinfection of the root canal system for ensuring successful, long-lasting root canal therapy. The contamination of root canals with bacteria is considered one of the main reasons for failure in endodontic treatment. Teeth that give a negative culture for bacterial growth at the time of a root canal filling have a higher success rate than teeth that are culture positive⁽¹⁾.

Complete sealing of the root canal system after cleaning and shaping is critical to prevent oral pathogens from colonizing, re-infecting the root and periapical tissues. Although gutta percha (GP) is still the most commonly used root canal filling material, a number of new techniques and materials with different physicochemical properties have been developed⁽²⁾.

Nanoparticles (NPs) as one of the novel strategies have been at the center of attention in the past few decades owing to their innovative and functional properties. According to the literature, nano based formulations provide better penetration and allow slow and controlled release of active ingredients at target sites⁽³⁾.

Nanosilver particles (NAgPs) have been known to have inhibitory and bactericidal effects. Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and is a major health problem. The AgNps are one of the most commonly used nano particles because of their ductility, electrical and thermal conductivity, and antimicrobial activity. They have shown antimicrobial effects on many microorganisms; therefore, it seems that using AgNps induces antimicrobial property⁽⁴⁾.

*Assistant lecturer, Dental Bio-Materials Department, Faculty of Dental Medicine, Al-Azhar, University. Assuit branch.

** Professor and Head of Dental Bio-Materials Department, Faculty of Dental Medicine, Al-Azhar, University. Cairo, (boys).

*** Professor and Head of Endodontic Department, Faculty of Dental Medicine, Al-Azhar, University. Assuit branch.

**** Lecturer, Dental Bio-Materials Department, Faculty of Dental Medicine, Al-Azhar University. Cairo, (boys).

Success of a root canal treatment mainly depends on the disinfection and adequate seal of the root canal system. Disinfection is achieved by removing the persisting microorganisms and infected organic and inorganic debris. Proper apical seal prevents microleakage and subsequent reinfection. While doing biomechanical preparation, during canal instrumentation, fine dentinal debris gets deposited over the intracanal dentin surface in the form of smear layer and obliterates the dentinal tubule openings⁽⁵⁾.

The purposes of root canal sealers are to prevent recolonization by bacteria and recontamination of the canal system, to prevent the growth of residual bacteria in the root canal system and to eliminate gaps between the core filling material and the canal walls⁽⁶⁾.

MATERIALS AND METHODS

I. Materials

Two types of endodontic sealers were used in this study (zinc oxide eugenol-based sealer [Endofil] and resin-based sealer [Adseal]). The prepared nanosilver particles were also used as additives with different concentrations of NAgPs (0.01%, 0.1% and 1% by weight).

II Methods

II.1 Teeth selection:

Thirty-six single rooted freshly extracted sound non-carious human permanent lower first premolar teeth free from cracks or any developmental defects by using stereomicroscope were used in this test. Exclusion criteria included teeth with more than one root canal, root canal curvature of more than 15°, the root surface's decay and the teeth with calcified canals. After extraction of teeth, their debridement was done by hand instruments and they were kept in 0.01% NaOCl (sodium hypochlorite) solution before use.

II.2. Samples grouping:

A total number of (72 samples) were used in this test. Samples were divided into two main groups (n=36 each) according to the type of sealer [Endofil and Adseal]. Each main group was subdivided into four subgroups (n=9 each) according to the incorporation of NAgPs:

Subgroup 1: control [sealer without adding nanosilver particles].

Subgroup 2: concentration A subgroup [adding 0.01% by weight nanosilver particles].

Subgroup 3: concentration B subgroup [adding 0.1% by weight nanosilver particles].

Subgroup 4: concentration C subgroup [adding 1% by weight nanosilver particles].

Each subgroup was further classified into three times (n=3 each) according to the duration of treatment for bacteria (one day, one week and one month).

II.3. Dentin preparations:

All teeth crowns were cut and canals were prepared to obtain the prepared root canals. Each root was cemented to an acrylic block. Then longitudinally split the roots into halves using a 0.6mm-thick precision diamond saw at 1000 rpm under water cooling. Roots were cut either at margins to obtain rectangular blocks of dentin about 4mm length × 4 mm width × 1 mm thickness. Each tooth was given two dentin blocks, seventy-two samples were obtained. The smear layer was removed with 10 mL of 17% EDTA for 1 min, followed by irrigation with 10 mL of 5.25% NaOCl according to the method introduced by Goldman et al⁽⁷⁾.

Samples were rinsed in sterile water for 1 minute after smear layer removal. Each prepared dentin specimen was placed in a tube with the canal (pulpal) side up. Any gaps between the dentin specimen and

the inner wall of tube were carefully sealed by resin composite (A2 shade) that was light cured for 20 seconds by using light emitting diode (LED) with intensity of 1000 mw/cm². The intensity of light was frequently tested with curing radiometer. Then, all samples were autoclaved at 134 °C for 15 min.

II.4. Dentin infection with enterococcus faecalis:

Standard suspension of *E. faecalis* (ATCC 29212) containing [1.5 × 10⁸] bacteria per ml was prepared, as number [0.5] McFarland standard and samples were placed into the suspension for 21 days so the bacteria can penetrate fully into the dentinal tubules and form biofilm. To meet the needs of growing bacteria, culture medium was replaced every 3 days.

II.5. SEM examination:

Pulpal surface of dentin blocks of two samples without disinfection treatment were fixed by 2.5% glutaraldehyde for 30 minutes and 1% osmium tetroxide (OsO₄) for 1 hour. Specimens were prepared to observe the pulpal surface of dentin blocks.

II.6. Sealer placement:

Two sealers without adding were used as control groups. All sealers were mixed according to manufacture instructions; they were mixed on a sterile glass slab with a plastic spatula according to the manufacturer's instructions. Each freshly prepared sealer was placed on the dentin surface of the root canal wall to achieve an approximate thickness of 0.5 mm. All dentin samples were placed at 37°C in 98% relative humidity for one day, one week and one month to survive the *E. faecalis* in dentinal tubules⁽⁸⁾. Three samples of each group were examined at each time point by CLSM to determine the proportions of live and dead bacteria.

For the CLSM test, the sealer was scraped off from the root canal wall dentin. The samples were rinsed in distilled water for one minute and vertically fractured into two halves to expose a fresh surface of

longitudinally fractured dentinal tubules for CLSM examination. The fluorescent Live/dead bacterial viability stains containing [Syto 9 and propidium iodide] was used for staining all fractured dentin specimens following the manufacturer's instruction. CLSM was used to view the fluorescence from the stained cells

The specimens were dehydrated by increasing concentrations of ethanol, dried by using a drier and sputter-coated with gold-palladium in a vacuum evaporator. The presence of bacteria in the dentinal tubules was observed by SEM at magnification of 3000–6000 operating at 15–20 kV.

Statistical analysis

Analysis of variance was applied to analyze the differences between the proportions of dead cell volume by different solutions by using SPSS 16.0 software, considering the proportion of dead cell volume as dependent variable and antibacterial agents and treatment time as fixed factors. Post hoc multiple comparisons were used to isolate and compare the results at a significance level of P < 0.05.

RESULTS

ZOE sealer: The results of statistical analysis showed that; Control group (unmodified sealer) was recorded the highest dead cell mean value (vol%) of *E. faecalis* at one month (0.38 ± 0.09) , while at one day recorded the lowest dead cell mean value (vol%) of *E. faecalis* (0.24 ± 0.03) with no statistical significant difference between observation times (P= 0.247) as indicated by one way ANOVA test followed by pair-wise Tukey,s post-hoc test (P<0.05). At 0.01% NAgPs modified ZOE sealer exhibited the highest dead cell mean value (vol %) of *E. faecalis* at one month (0.48± 0.11), while at one day was recorded the lowest dead mean value (vol %) of *E. faecalis* (0.34± 0.06) with no statistically significant difference between observation time (P= 0.104) as indicated by one-way ANOVA test.

At 0.1% NAgPs modified ZOE sealer recorded the highest dead cell mean value (vol %) of *E. faecalis* at one month (0.61 ± 0.08), while at one day recorded the lowest dead cell mean value (vol %) of *E. faecalis* (0.42 ± 0.04) with statistical significant difference between observation time ($P=0.027$) by one way ANOVA test followed by pair-wise Tukey's post-hoc test ($P>0.05$). At 1% NAgPs modified ZOE sealer recorded the highest dead cell mean value (vol %) of *E. faecalis* at one month (0.83 ± 0.13), while at one day recorded the lowest dead cell mean value (vol %) of *E. faecalis* (0.68 ± 0.05) with statistical non-significant difference between observation time ($P=0.21$) by one way ANOVA test followed by pair-wise Tukey's post-hoc test ($P>0.05$).

Adseal sealer: The results of statistical analysis showed that; Control group (unmodified sealer) was recorded the highest dead cell mean value (vol%) of *E. faecalis* at one month (0.23 ± 0.05), while at one day recorded the lowest dead cell mean value (vol%) of *E. faecalis* (0.11 ± 0.04) with no statistical significant difference between observation times ($P=0.09$) as indicated by one way ANOVA test

followed by pair-wise Tukey's post-hoc test ($P<0.05$). At 0.01% NAgPs modified Adseal sealer exhibited the highest dead cell mean value (vol %) of *E. faecalis* at one month (0.37 ± 0.01), while at one day was recorded the lowest dead mean value (vol %) of *E. faecalis* (0.24 ± 0.03) with statistically significant difference between observation time ($P=0.006$) as indicated by one-way ANOVA test.

At 0.1% NAgPs modified ZOE sealer recorded the highest dead cell mean value (vol %) of *E. faecalis* at one month (0.49 ± 0.02), while at one day recorded the lowest dead cell mean value (vol %) of *E. faecalis* (0.39 ± 0.05) with no statistical significant difference between observation time ($P=1.13$) by one way ANOVA test followed by pair-wise Tukey's post-hoc test ($P>0.05$). At 1% NAgPs modified ZOE sealer recorded the highest dead cell mean value (vol %) of *E. faecalis* at one month (0.68 ± 0.14), while at one day recorded the lowest dead cell mean value (vol %) of *E. faecalis* (0.51 ± 0.10) with statistical non-significant difference between observation time ($P=0.28$) by one way ANOVA test followed by pair-wise Tukey's post-hoc test ($P>0.05$).

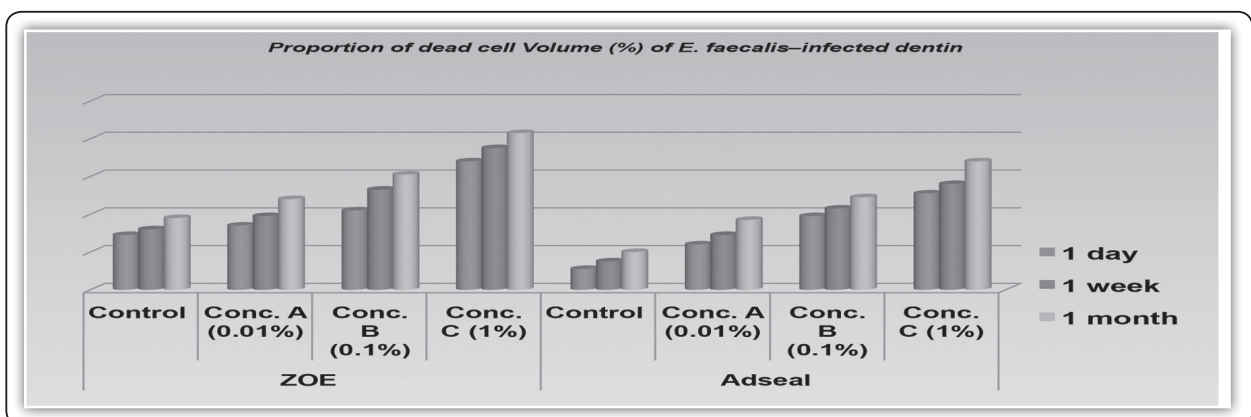


Figure [1]: Column chart showing mean proportion of dead cell Volume (%) of *E. faecalis*-infected dentin in each concentration with different observation times.

DISCUSSION

Enterococcus faecalis was chosen as the target microorganism because of its high presence in persistent endodontic infections, ranging from 24% to 77%. This microorganism is resistant to several irrigants and intracanal medicaments used in endodontics. Therefore, the antibacterial activity of endodontic sealers against *E. faecalis* is important in clinical practice⁽⁹⁾. Zinc oxide and eugenol-based sealer such as [Endofil], and epoxy resin root canal sealer such as [Adseal] were shown good antibacterial activity when compared with other sealers. They have been used as positive control groups⁽¹⁰⁾. In moving towards antibacterial sealers, IABN (Insoluble antibacterial nanoparticles) were incorporated into two commercial products. The antibacterial effect between different sealers, depending on the material used and the IABN concentration⁽¹¹⁾. Furthermore, previous studies were shown that incorporation of nanosilver particles up to 1% could significantly inhibit the bacteria, without sacrificing the mechanical properties such as flexural strength, flexural modulus, compressive strength and micro-shear bond strength⁽¹²⁾.

Recently, a new dentin infection protocol was introduced to screening; a standardized, dense and deep penetration of bacteria into dentinal tubules⁽¹³⁾. This model has since been successfully used in other studies as a strategy to measure the effectiveness of various endodontic disinfectants and irrigating solutions in dentin by using CLSM and bacterial viability staining^(14,15). The dentin block model was one of the first models that aimed to standardize the *in vitro* testing of the effectiveness of root canal disinfecting agents. However, quantitative standardization of bacterial viability before and after disinfection remains a challenge in endodontic research⁽¹⁶⁾.

The unmodified resin sealer exhibited limited antibacterial properties, in relation to the Endofil that had the greatest antimicrobial effect against *E. faecalis*. The eugenol is a potent antimicrobial agent

therefore the activity of ZOE based sealers may be attributable to the free eugenol released from the set materials⁽¹⁷⁾.

In this study, the resin sealers have lower antibacterial effect than ZOE sealer against *E. faecalis*, perhaps because only a small amount of formaldehyde was released over a brief period, so Adseal showed less antibacterial activity. The present study also showed that fresh sealers have antibacterial effect, whereas their antimicrobial activity decreased with time. When 1-week samples of Adseal sealer were tested, in fact; very limited antibacterial activity was found⁽¹⁷⁾. On the contrary to our results; after 1 week from mixing, the Endofil still exerted antibacterial activity to a lesser extent than 1-day samples⁽¹⁸⁾.

Matching with our results some authors reported that only fresh Adseal possessed antibacterial activity, whereas 1 week and 1-month old samples did not show any antibacterial effect against *E. faecalis*⁽¹⁹⁾. The results of the present study are consistent with the previous study, showed that the ZOE-based sealer such as Endofil and demonstrated the highest antimicrobial activity than epoxy resin-based sealer⁽²⁰⁾.

Conflicting results have been reported by some researchers who found (epoxy resin sealer) had the largest inhibition zone in comparison with (ZOE sealers). Difference in microorganism strains used may be the main reasons of these controversies⁽²¹⁾.

Recently, the subject of IABN cytotoxicity when incorporated in endodontic sealers was discussed previously, compared the cytotoxic effect of three commercially available sealers before and following incorporation of IABN. The results showed that IABN incorporation did not impair the sealers' biocompatibility. These showed that bacteria which come in contact with the modified sealers surface are eradicated within one hour⁽²²⁾.

In conclusion, the present findings indicate that dentin extends the antibacterial effect of endodontic

sealers against *E. faecalis* biofilms in dentinal tubules. However, in the clinical root canal infection, the more realistic situation is with the presence of multispecies biofilm containing different size of bacteria.

The outer side (cement side) of the semi-cylindrical dentin pieces was closed again by nail varnish before exposure bacteria to simulate the difficulty these bacteria in penetrating dentin from the outer side. After exposure to the antibacterial solutions and the viability stain, the dentin specimens were fractured to reveal a fresh dentin surface that was used to start scanning for living and dead bacteria. The massive presence of bacteria in the tubules made it possible to obtain representative data from all randomly selected areas with excellent signal tones ratio of the fluorescence. CLSM also better visualized the presence of bacteria in the tubules than SEM because CLSM penetrates 1 mm below the surface of the specimen and includes also those dentinal tubules that are not open to the surface⁽²³⁾.

Interestingly, some images showed areas deep in the dentin with killed bacteria, while dentin closer to the main canal (surface) appeared green. It is possible that the dentinal tubules in each scan were not in the same level, but other explanations related to bacterial density cannot be excluded. The proportion of killed bacteria inside dentin was dependent on the time of exposure and type/concentration of nanosilver. Concentration 1% nanosilver was significantly more effective than control groups⁽¹⁷⁾.

CONCLUSIONS

- 1- Modified sealers with nanosilver particles possess different degree of antibacterial activity.
- 2- By increase the concentration of nanosilver particles into endodontic sealers, the antibacterial activity was significantly increased.
- 3- CLSM was better test than old measures for evaluation the antibacterial activity.

REFERENCES

1. Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population .Oral Surg. Oral Med. Oral Pathol Oral Radiol Endod. 2001; 91: 579-86.
2. Resende LM, Rached FJ, Versiani MA. A comparative study of physicochemical properties of AH Plus, Epiphany, and Epiphany SE root canal sealers. Int Endod J. 2009; 42: 785-93.
3. Lotfipour F, Abdollahi S, Jelvehgari M, Valizadeh H, Hassan M, Milani M. Study of antimicrobial effects of vancomycin loaded PLGA nanoparticles against enterococcus clinical isolates. Drug Res. 2014; 64: 348-52.
4. Chen LS, Huang ZM, Xue C. Nanofiber reinforced PMMA transparent composites incorporated with TiO₂ nanoparticles. J Inorg Mater. 2009; 24: 469-74.
5. Vivan RR, Bortolo MV, Duarte MA, Moraes IG, Tanomaru-Filho M, Bramante CM. Scanning electron microscopy analysis of Resin Endo system and conventional irrigation for debris removal. Braz Dent J. 2010; 21: 305-9.
6. Mickel AK, Nguyen TH, Chogle S. Antimicrobial activity of endodontic sealers on *Enterococcus faecalis*. J Endod. 2003; 29:257-68.
7. Goldman LB, Goldman M, K ronman JH, Lin PS. A scanning electron microscopic evaluation of the debridement capability of edta/ sodium hypochlorite at elevated temperature with ultrasonic agitation-an in vitro study. Oral Surg Oral Med Oral Pathol. 2005; 48:79-83.
8. Saleh IM, Ruyter IE, Haapasalo M, Orstavik D. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers in vitro. Int Endod J 2004; 37:193-8.
9. Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. Int Endod J. 2009; 42: 277-87.
10. Pinheiro CR, Guinesi AS, Pizzolitto AC, Bonetti-Filho I. In vitro antimicrobial activity of Acroseal, Polifil and Epiphany against *Enterococcus faecalis*. Braz Dent J. 2009; 20: 107-11.
11. Kesler Shvero D, Abramovitz I, Zaltsman N, Perez Davidi M, Weiss EI, Beyth N. Towards antibacterial endodontic sealers using quaternary ammonium nanoparticles. Int Endod J. 2013; 46: 747-54.

12. Tavassoli S, Alaghemand H, Hamze F, Ahmadian F, Kaviani M, Atai M. Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. *Dent Mater.* 2013; 29:495-505.
13. Zapata RO, Moraes IG, Bernardineli N, et al. Confocal laser scanning microscopy is appropriate to detect viability of *Enterococcus faecalis* in infected dentin. *J Endod.* 2008; 34:1198–201.
14. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. *J Endod.* 2012; 38:1376–9.
15. Wang Z, Shen Y, Ma J, Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. *J Endod.* 2012; 38:948–53.
16. Jingzhi M, Zhejun W. A New Noninvasive Model to Study the Effectiveness of Dentin Disinfection by Using Confocal Laser Scanning Microscopy. *J Endod.* 2011; 37:1380–5.
17. Shakouie S, Eskandarinezhad M. Antimicrobial efficacy of AH-Plus, adseal and endofill against *Enterococcus faecalis*- An in vitro study. *Afric J Microbio Res.* 2012; 6: 991-4.
18. Smadi L, Khraisat A, Mahafzah A, Salem A. In vitro evaluation of the antimicrobial activity of nine root canal sealers: direct contact test. *Odontostomatol Trop.* 2008; 31: 11-8.
19. Pizzo G, Giammanco G, Cumbo E, Nicolosi G, Gallina G. In vitro antibacterial activity of endodontic sealers. *J Dent.* 2006; 34: 35-40.
20. Kayaoglu G, Erten H, Alacam T, Orstavik D. Short-term antibacterial activity of root canal sealers towards *Enterococcus faecalis*. *Int Endod J.* 2005; 38: 483-8.
21. Gomes B, Pedroso J, Jacinto R, Vianna M, Ferraz C, Zaia A. In vitro evaluation of the antimicrobial activity of five root canal sealers. *Braz. Dent J.* 2004; 15: 30-5.
22. Mohammadi Z, Yazdizadeh M. Evaluation of the antibacterial activity of new root canal sealers. *J Clin Dent.* 2007; 18: 70-6.
23. Abramovitz I, Beyth N, Weinberg G. In vitro biocompatibility of endodontic sealers incorporating antibacterial nanoparticles. *J Nanomat.* 2012; 7:8