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ANTIMICROBIAL ACTIVITY OF AH PLUS AND CEASEAL SEALERS WITH AND WITHOUT THE ADDITION OF SILVER NANOPARTICLES AGAINST *ENTEROCOCCUS FAECALIS*

Nehal Nabil Roshdy*, Adel AbdelWahed** *and* Hisham Elshishtawy***

ABSTRACT

Aim: The aim of the present study was to investigate the antimicrobial effect of AH Plus and Ceraseal sealers with and without the incorporation of silver nanoparticles against *Enterococcus faecalis* using direct contact assay.

Methodology: Silver nanoparticles were prepared to be used in a gel form using the chemical reduction method. Antibacterial activity was assessed using the direct contact test (DCT) against *Enterococcus faecalis* ATCC 35550). Fifteen tubes were set for each sealer in duplicate as follow: Group (I): Ceraseal (Meta Biomed Co., Cheongju, Korea), Group (II): Ceraseal& Nano-silver gel, Group (III): AH Plus (Dentsply/Maillefer, Konstanz, Germany) and Group (IV): AH Plus & Nano-silver gel. Colony counts were detected for all groups at different time intervals (1, 24, 168 h).

Results: DCT results revealed that AH Plus had higher antibacterial activity against *Enterococcus faecalis* at different time intervals than Ceraseal sealer unaffected by silver nanoparticles gelincorporation. But the incorporation of silver nanoparticles gel significantly enhanced their antibacterial effect. All sealers had lost much of their antibacterial effects at 7-day intervals.

Conclusion: AH Plus sealer had higher antibacterial activity against *Enterococcus faecalis* than CeraSeal sealer. Adding Silver nanoparticles gel to both sealers improved their antibacterial activity.

KEYWORDS: AH Plus sealer, CeraSeal sealer, Silver nanoparticles, Direct contact test, *Enterococcus faecalis*.

* Associate Professor, Endodontic Department, Cairo University

** Endodontic Department- Faculty of Oral And Dental Medicine- Future University

*** Associate Professor, Department of Microbial Genetics, Agricultural Genetic Engineering Research Institute, Agriculture Research Center, Cairo, Egypt.

INTRODUCTION

Post-endodontic treatment failures are frequently increasing and present a high risk of complicated infections in patients. Research stated that up to 10% of root canal treatments fail because of residing bacteria in the root canal [1]. *Enterococcus faecalis* represents the most resistant species responsible for failed root canal treatment. Thus, elimination of bacteria from the root canal is crucial to ensure the success of the root canal treatment [2].

Multiple improvements have been achieved in the protocols of endodontic chemo-mechanical disinfection used today, yet they are still not able to provide 100% sterility of the root canal complex. Thereby, the antimicrobial activity of root canal sealer aid in the elimination of the residual bacteria left in the root canal system unaffected by various chemo-mechanical preparation protocols [3]. This leads to continuous improvement in the products of endodontic sealers to exhibit a high antimicrobial activity, reduce or inhibit the growth of microorganisms and enhance the repair process of periapical tissues.

Lately, nanotechnology has been utilized in creation of novel bio-materials. Silver nanoparticles have received considerable attention because of their good biocompatibility with human cells[4], low bacterial resistance, broad spectrum antibacterial nature due to sustained ion release, and inhibiting bacterial growth at lower concentrations than antibiotics [5].

Variety of sealers are currently available; epoxy-resin based sealers are considered the most widely used due to their biological, physicochemical and sealing abilityproperties[6], together with the probability of chemical bonding to dentin collagen [7].

Recently, bioceramic-based sealers (calcium silicate and /or calcium phosphate) became favorable because of their biological and physical properties; i.e. alkaline pH, lack of shrinkage and chemical stability [8]. Above all, bioceramic sealers shows enhanced setting properties due to deposition of crystalline structure similar to that of the tooth and bone apatite materials that improves the sealer to root dentin bonding [9].

Thus, the aim of the present study was to investigate the antimicrobial effect of AH Plus (Dentsply/Maillefer, Konstanz, Germany) and Ceraseal sealer (Meta Biomed Co., Cheongju, Korea) with and without the incorporation of silver nanoparticles against *Enterococcus faecalis* using the direct contact assay. The null hypothesis was that there would be no significant difference among the test groups.

METHODOLOGY

1. Silver Nanoparticles(SNP) Preparation and characterization

Silver nanoparticles were prepared by the chemical reduction method as reported by Turkevich 10 and Lee and Meisel 11. A solution of $AgNO_3$ had been utilized as Ag1+ ions precursor. PVP was used as a stabilizing agent and borohydrateacted as a mild reducing agent. When the color of the solution turned slowly into grayish yellow, this indicated the reduction of the Ag1+ ion to Ag nanoparticles.

SNP Gel

0.4gm of Carboxymethyl cellulose (Loba CHIME, india) was sprinkled gently and gradually over the solution of Silver nanoparticles 200ppm under mild temperature with vigorous stirring to get homogenous gel. The gel was mixed with sealer in ratio 1:1 to get final sealer of 100ppm Silver nanoparticles with an average size of less than 20nm and Spherical shape under a transmission electron microscope.

2. Antibacterial test

Direct Contact Test (DCT) was conducted by placing 50mg of freshly mixed sealer in sterile flat-

bottom, screw-capped test tubes. Fifteen tubes were used for each group in duplicate as follow:

- Group (I): Ceraseal (Meta Biomed Co., Cheongju, Korea)
- Group (II): Ceraseal& Nano-silver gel
- Group (III): AH Plus (Dentsply/Maillefer, Konstanz, Germany)
- Group (IV): AH Plus & Nano-silver gel

After that, 50 µL of 0.5 ml McFarland standard suspension (1.5 x 10^6 CFU/ ml) of *Enterococcus faecalis* ATCC 35550 was pipetted and applied over each sealer. Tubes were incubated at 37°C to confirm direct contact between the bacteria and the sealers. The tubes were divided into three equal sub-groups according to the tested time interval. *E. faecalis* suspension was allowed to be in contact with the tested sealer for 1, 24, and 168 h.

Colony Count:

The bacterial suspension was diluted by adding 600 µL of sterile nutrient broth to the screw-capped tubes. Then, 50µL of suspension was drawn from each vial and spread over Mac Conkey agar to detect the colony count manually. Colony counts of all the tested groups at different time intervals (1, 24 and 168 h) were conducted in the same manner to detect the immediate and delayed antimicrobial efficacy against *E. faecalis*. A suspension of *E.faecalis* ATCC 35550 in normal saline was taken as the positive control, sub cultured and colony count was detected at 1, 24, 168 h interval. Another set of test tube containing tested material without bacterial inoculates served as negative control.

Statistical analysis

Numerical data were presented as mean and standard deviation (SD) values. Data were explored for normality by checking the data distribution and using Kolmogorov-Smirnov and Shapiro-Wilk tests, it showed a parametric distribution. One-ANOVA followed by Tukey's post hoc test was used for intergroup comparisons, while one-way repeated measures ANOVA followed by a comparison of main effects utilizing Bonferroni correction was used for intragroup comparisons. The significance level was set at $P \leq 0.05$ within all tests. Statistical analysis was performed with IBM® SPSS® Statistics Version 26 for Windows.

RESULTS

Mean and standard deviation (SD) values for bacterial count in different groups were presented in Table (1) and Figure (1)

Intergroup comparison:

1- Intergroup comparison for all time intervals showed significant difference between

2- Different groups ($p<0.001$). Control group showed the highest value of bacterial count,

3- Followed by Ceraseal group, Ceraseal&Nano group, AH Plus group and AH Plus & Nano

4- Group while negative control group had the lowest mean value. Pairwise comparisons showed

5- Different groups to be significantly different from each other except AH plus & Nano group

6- and Negative control group ($p<0.001$)

For all the time intervals (1, 24, 168 h), there was significant difference among different groups ($p<0.001$). The control group showed the highest value of bacterial count, followed by Group (I): Ceraseal group, Group (II): Ceraseal & Nano-silver gel group, Group (III): AH Plus group and Group (IV): AH Plus & Nano-silver gel while the negative control group had the lowest mean value. Pairwise comparisons showed different groups to be significantly different from each other except for Group (IV): AH plus & Nano group and the negative control group ($p<0.001$).

TABLE (1): Mean and standard deviation (SD) values for bacterial count (CFU/mL)in different groups

Time	(CFU/mL) (Mean±SD)						p-value
	Negative control	Control	Ceraseal	Ceraseal &Nano	AH Plus	AH Plus & Nano	
1h	0.00 ± 0.00^{E}	342.00 ± 23.87^{Ab}	216.20 ± 9.86^{Bb}	163.20 ± 12.56^{Cb}	39.60 ± 6.27^{Db}	1.20 ± 1.30^{Eb}	**<0.001***
24h	0.00 ± 0.00^{E}	473.40 ± 14.93^{Aa}	218.60 ± 11.26^{Bb}	160.40 ± 13.92^{Cb}	45.60 ± 4.83^{Db}	1.60 ± 1.67^{Eb}	**<0.001***
168h	0.00 ± 0.00^{E}	492.00 ± 10.95^{Aa}	256.60 ± 11.24^{Ba}	180.80 ± 10.71^{Ca}	81.40 ± 9.56^{Da}	10.80 ± 4.38^{Ea}	**<0.001***

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical column respectively*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

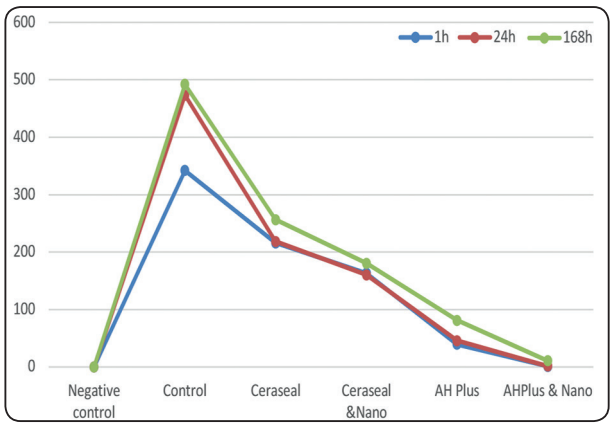

Fig. (1) Line chart showing average bacterial count (CFU/mL) in different groups.

Intragroup comparison

All the tested groups showed a significant difference between different time intervals ($p<0.001$). Pairwise comparisons showed value recorded at168 h to be significantly higher than values recorded at other intervals ($p<0.001$) and there was no significant difference between values recorded in 1 h and 24 h ($p>0.05$). For Group (I): Ceraseal, Group (III): AH Plus and Group (IV): AH Plus and Nano-silver gel; The highest value of bacterial count was recorded at 168 h (256.60±11.24), (81.40±9.56) and (10.80±4.38) respectively followed by 24 h (218.60±11.26), (45.60±4.83) and (1.60±1.676) respectively, while the lowest value was found at1 h (216.20±9.86), (39.60±6.27) and (1.20±1.30) respectively. On the other hand, Group (II): Ceraseal& Nano-silver gel. The highest value of bacterial count was found at 168 h (180.80±10.71), followed by 1 h interval (163.20±12.56) while the lowest value was found at 24 h interval (160.40±13.92).

DISCUSSION

Failure of root canal treatment is usually attributed to the persistence of bacteria especially *E.faecalis* in the root canal system [12]. Thus, using an endodontic sealer with antibacterial properties is required to eliminate residual microbial infections that have survived the chemo-mechanical instrumentation and irrigation protocols [13].

E. faecalis (Gram positive, facultative anaerobic microbe) is usually associated with failed root canal treatment cases. It has the ability to survive in the root canal either alone or with other microbes14. *E. faecalis* is difficult to be eradicated from the root canal as it can penetrate the dentinal tubules and adhere to dentinal collagen[15].

This study aimed to investigate the antimicrobial effect of AH Plus and Ceraseal sealers with and without the incorporation of Silver nanoparticles against *E.faecalis* using the direct contact assay.

The direct contact test is considered the most accepted antimicrobial test for endodontic sealers 16.The test is considered quantitative and reproducible in mimicking the contact between microorganisms and endodontic sealers inside

the canal space, providing information about the bactericidal effect[17], thus providing reliable and relevant results[18,19]. Though, Agar diffusion test (ADT) has been widely used to investigate the antimicrobial activity of sealers, the Editorial Board of the Journal of Endodontics 2007 showed that its results are considered questionable due to agar viscosity, the lack of standardization of inoculum density, plate-storage condition and dependency on the solubility and diffusion characteristic of both the test material and media.

Our results showed that the highest antibacterial effect was reported to Group (IV): AH plus & Nano silver gel group followed by Group (III): AH Plus group followed by Group (II): Ceraseal& Nano silver gel group and the least antibacterial activity was related to Group (I): Ceraseal group. Pairwise comparisons showed significant differences among the groups ($p<0.001$).

Previous studies demonstrated a superior antibacterial efficiency of AH Plus sealer against *E. faecalis* when compared to bioceramic sealer (SureSeal) 20 and (Endosequence BC) against *E. faecalis* [21, 22].

AH Plus presents high antibacterial effectiveness due to its release of bisphenol-A-diglycidyl ether during polymerization 23. While, the antibacterial effect of the bioceramic sealer is related to the combination of high pH, hydrophilicity and active calcium hydroxide diffusion[13, 22].

Our results showed that the addition of silver nano particles to both sealers (AH Plus and CeraSeal) enhanced the antibacterial effect both sealers significantly when compared with conventional sealer ($P<0.001$). Incorporation of nanoparticulate drugs to endodontic sealers aims at deeper penetration into the dentinal tubules and into the microbial cells. Adding nanosilver to dental biomaterials and cement shows a positive antimicrobial effect [24, 25]. The antimicrobial effect of silver nanoparticles could be attributed to the high surface area to volume ratio and unique physiochemical properties of silver ions[26]. Moreover, silver ions are positively charged nanoparticles that interact electrostatically with negatively charged bacterial cells causing altered cell permeability [27]. Furthermore, silver nano particles interact with multiple targets in the microbial cell; cell membrane, plasmids and enzymes. Thus, it is unlikely for microorganism to develop resistance to silver when compared with antibiotics[28].Our findings are consistent with Krishnan et al in 2015 [27] who reported that silver nanoparticles have potential bactericidal effects on *E.faecalis*.

All the tested groups showed significant difference between different time intervals ($p<0.001$). Pairwise comparisons showed mean values of bacteria recorded at168 h to be significantly higher than values recorded at other intervals ($p<0.001$). There was no significant difference between values recorded at 1 h and 24 h ($p>0.05$).

The endodontic sealers have shown to provide the highest antimicrobial effectiveness immediately after spatulation, followed by a gradual loss in the antimicrobial effect over time [29]. The results were consistent with Pizoo et al 30 and Shakya et al [31], who showed that fresh sealers have antibacterial effects that decrease with time. AH Plus presents good flow, thereby diffusing into the dentinal tubules and creating microbial inhibition by means of entombment [32]. It has been reported that material-released formaldehyde in the polymerization process, resulting in the sealers antibacterial property [33]. On the other hand, Kayaoglu et al. (2005) [34] reported that fresh AH-Plus sealer had antibacterial activity against *E. faecalis* that diminished at 24 h and 7-day old samples. This could be attributed to the ease of diffusion of the antibacterial component into the surrounding environment before setting of the material [35].

In contrast, bioceramic sealers exhibit hydrophilic properties. On contact with dentinal moisture, calcium silicates undergo a hydration reaction which results in the formation of calcium silicate

hydrogel and calcium hydroxide 36. Calcium hydroxide partially reacts with calcium phosphate forming hydroxyapatite and water. The water formed, in turn, re-initiates the cycle to produce more calcium silicate hydrogel and calcium hydroxide, resulting in an increase in pH (> 12.5). By the time the sealer sets, its pH also reduces to about 9.14, consequently lessen its antibacterial efficacy. It had been reported that their antimicrobial properties are greatly diminished 7 days after mixing [37]. Zhang et al. (2009) [13] reported the antimicrobial effect of bioceramic sealer after 24 h that greatly decreased 7 days after mixing.

CONCLUSION

AH Plus sealer had higher antibacterial effect than Ceraseal sealer. The incorporation of silver nanoparticles can improve the antibacterial activity of AH Plus and Ceraseal sealer at different time intervals. All the sealers showed the highest antibacterial efficacy when freshly mixed.

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