THE ANTIMICROBIAL EFFICACY OF NANOSILVER MODIFIED ROOT CANAL SEALER

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ABSTRACT

Aim To test the antimicrobial effect of root canal sealer after adding nanosilver particles.
Methodology With the use of Enterococcus faecalis ATCC 29212as a test microorganism, the agar diffusion test (ADT) was performed. Bacteria were grown to the log phase. Then, cells were resuspended and prepared by inoculating colonies for 24 hours on BHI agar plates in controlled incubation conditions. Suspension of 200 µl bacteria colonies were spread on plates with BHI agar. In each plate, five recesses were prepared and different mixes from the tested root-canal sealer material were mixed and filled the recesses. The testes material was sub-grouped to five groups with 0%, 0.5%, 1%, 2% and 4% additive of nanosilver particles to the weighted powder respectively. After incubation period, the diameter of bacterial-inhibition zones the agar plates (n=30) were measured in millimeter. The mean of each sample was calculated and data were statistically analyzed.
Results there was a significant difference among the tested group (P= 0.000). Groups with nanosilver additives shoed significant difference compared to unmodified group (P= 0.000). The highest mean value was recorded with group 5 (4% nanosilver)
Conclusions Antimicrobial activity of the root-canal sealer increased significantly by adding nanosilver particles to the powder of the root canal sealer.

Keywords: agar diffusion test, E. faecalis, root-canal sealer antimicrobial activity, nanosilver.

INTRODUCTION

The presence of the microorganism in the root canal and surrounding apical portion has the main role in the prognosis of endodontic treatment. The practitioner tries to eliminate the microorganism and the related effects through a restrict protocol. Repeated irrigation with good instrumentation may reduce the microorganism in the infected root canal. On the other hand, the presence of microorganism within the dentinal tubules was recorded (Garcez et al., 2007; Sen, Piskin, & Demirci, 1995). Using intra-canal medicaments reduce the count of the microorganism inside the pulp champers and canals during the time of treatment. At obturation of the prepared canals, the anti-microbial effect of the sealer material has the durable effect. To expand the favorable antimicrobial effect, authors examined the destroying effect of sealer against the root canal microbes.(Al-Khatib et al., 1990) Other authors tried additive to sealer to enhance the microbial effect especially with cases suffering from apical periodontitis and/or pulpal necrosis.(Spangberg, Engström, & Langeland, 1973; Weiss, Shalhav, & Fuss, 1996)

Recently, Nanosilver is used in dental field. The material has a recorded antimicrobial effect.(Sotiriou & Pratsinis, 2010) The physicochemical properties of nanosilver make it the

most commercialized nano-materials in health field.(Chen & Schluesener, 2008) Adding nanosilver to dental biomaterials and cement has a positive antimicrobial effect(Corrêa et al., 2015; Magalhães et al., 2012; Masallat, Omar, Khalifa, & Emara, 2016). For endodontic treatment, gutta-percha was covered with nanosilver(Shantiaee, Maziar, Dianat, & Mahjour, 2011) or added to sealer to work as preservative.(Nawal, Parande, Sehgal, Naik, & Rao, 2011) The antimicrobial effect of the dental materials has been studied through different in vitro methods. The direct contact test (DCT) is a quantitative test that allows investigating the water-insoluble materials.(Weiss et al., 1996) While the technique needs more experience and equipments. The agar-diffusion test (ADT) is used for the same experimental purposes. Comparing to DCT, the ADT is simple and commonly used but needs high care at experiment to avoid the variation of incubation temperature and environment.

This study aims to study the antimicrobial effect for endodontic sealer with four different additives of nanosilver concentrations.

METHODOLOGY

Microorganisms incubation

The antibacterial effect of the sealer was evaluated against the Enterococcus faecalis ATCC 29212. The strain was obtained from the Department of Microbiology, Faculty of Medicine, Mansoura University, Egypt. Within anaerobic condition, bacteria were grown to the log phase, when the doubling of cells occurs, from frozen- cultures in brain-heart infusion (BHI) broth at room temperature for 24 hours. Cells were re-suspended by centrifuging and harvested in fresh medium. The test organism was prepared by inoculating colonies for 24 hours on BHI agar plates into 20 ml of BHI broth in a 100-ml Erlenmeyer flask and incubated for 6 hours at 37°C.

Agar diffusion test (ADT)

Suspension of 200 µl bacteria, equals 5 x 10^7 colony-forming units, were spread on plates with BHI agar. In each plate, five circles with 5mm diameter for each one were drawn. A punch with same diameter connected to a contra-angle headpiece for air motor (Ti-Max 205L, NSK, Japan) at 1000 rpm speed was used to make a recess in the agar. In this study, a sealer with powder and liquid form was used (Sultan, Chemists, Inc., Englewood, NJ). Mixed specimens from each tested sealer group with different concentration of nanosilver particles were prepared by pouring into the created recess. The concentrations of the nanosilver to the mixed powder are mentioned in table 1. Each of the prepared plates (n=30) received the five different group to standardize the effect within different bacteria concentration. After incubation room temperature for 24 hours and 7 days in a humid atmosphere, the agar plates were investigated for bacterial-inhibition zones. The diameter of the halo formed was measured in millimeters in two bisected perpendicular locations for each sample. The mean of each sample was calculated and data were statistically analyzed.

RESULTS

Three is a high statistically significant correlation was recorded among antibacterial inhibitory effect within the different additive of nanosilver (p= 0.000). Means and standard deviations of inhibitory zones of the tested groups against *Escherichia coli* were recorded by table 2. The unmodified sealer (group 1) showed the lowest inhibitory zone with significant difference with the lowest nanosilver concentration (group 2) (p= .0001) The halo increased within the increase in the nanosilver concentration. The inhibitory zone showed noticeable increase within 1% nanosilver (group 4) with a noticeable increase in the mean compared to

the 2% nanosilver concentration (p=0.0001). The highest result was recorded with 4% concentration (group 5). While there is a significant difference between group 4 and 5 (p=0.0001), but the means for both groups were closed, fig 1.

Table 1. The weight nanosilver to the powder of sealer within experimental groups

Group no.	Nanosilver concentration
Group 1	0% (unmodified control group)
Group 2	0.5%
Group3	1%
Group4	2%
Group5	4%

Table 2. Result for student t-test.

groups	Mean	Std. Deviation	t	Sig.
unmodified	1.5209	.25426	32.763	.000
0.5% conc	1.9554	.09611	111.439	.000
1% conc	3.7787	.09474	218.449	.000
2% conc	8.3207	.20326	224.221	.000
4% conc	9.0084	.05504	896.418	.000

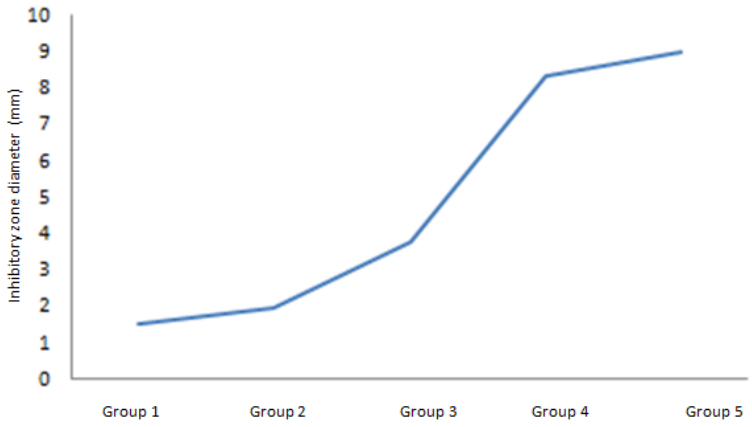

Figure 1. Graphical representation of mean value of sealers within different nanosilver concentrations by agar diffusion test.

DISCUSSION

For endodontic disease, different microbes are the primary predisposing agents. The antibacterial effect of the commercial sealers may vary from a trade name to other. From the clinical findings, the antimicrobial effect for the sealer needs to inhibit bacterial growth over long period. Anaerobic bacteria has the capacity to adapt and survive on the necrotic tissues which has limitation in nutrient and oxygen supplies.(zu Bentrup & Russell, 2001) The interaction between the facultative anaerobic microorganisms with other anaerobes, may alter the nutrition and cause potential oxygen tension, which determine microbial-survival

relationship.(Lai et al., 2001) Antibacterial activity of root-canal sealers with may control microorganism sustained after chemo-mechanical preparation and intra-canal medicament.(Cobankara, Altinoz, Erganis, Kav, & Belli, 2004) In this study, E. faecalis was selected to compare the antibacterial properties of the five groups of the study. The E. faecalis is easily isolated from the infected roots and widely used in previous studies.(16, 17)
Regardless the disadvantages of ADT, it is considered the most often used method to investigate antibacterial activity of dental materials.(Kaplan, Picca, Gonzalez, Macchi, & Molgatini, 1999) Comparing to other methods as direct contact test (DCT), ADT can assess the antibacterial prosperities of freshly mixed sealer.(Chong et al., 1994) Standardization of incubation factors, size and number of microorganisms, agar viscosity and storage conditions lead to parity avoiding the limitation of ADT method.(Lai et al., 2001) To avoid the debate of the variation of bacterial count among the tested agar plates (n=30) and the incubation, we supplied each plate by the five tested groups. The descriptive result of our experiment showed limited standard deviation per tested groups which might an evidence on the controlled experimental factors.

Root-canal sealers should be tested immediately after mixing and also after a period of time when it is assumed that it has reached its final chemical structure. This mimics the clinical situation as the sealer is inserted into the mouth after mixing in incompletely set stage. After the setting period, toxic ingredients may release from the sealer.(Cobankara et al., 2004) Thus, we measured the inhibitory zones after 7 days to record the antibacterial effect of the material after setting.

The result of this study rereleased antibacterial activity for unmodified tested root-canal sealer. This is due the ingredient of the tested sealer. Sultan root-canal sealer contains Zinc-oxide in the powder. ZOE-based Sultan indicated prolonged antibacterial activity. It has been mentioned that eugenol is a potent antibacterial agent and it has the main role within the activity of ZOE-based sealers(Fuss et al., 1997)(Hume, 1986)

After aging the nanosilver particles to the experimented roo-canal sealr powder, there was a significant antibacterial effect (p=0.000). There is a rational thought regarding the role of freeing Ag+ ions from nanosilver and its toxicity towards microorganisms. (Tolaymat et al., 2010) The potential effect of nanosilver its role as a source of Ag+ ions.(Navarro et al., 2008) Miao et al.(Miao et al., 2009) elaborated the effect of nanosilver to the dissolved Ag+ ions toxicity.

In comparing to the group 4 and 5, there was a significant difference after doubling the concentration of the nanosilver particles (p=0.000). on the contrary, the antibacterial effect was not doubled. In other words, the means of both groups were closed in value, (table2) (fig 2). This may be elaborated by the ability of experimental species to diffuse of flow. The increase in particles weight by adding nanosilver particles would affect the solubility and the diffusion of the material. In other study, the authors revealed that the release of Ag+ alone is not significant without the direct contact of nanosilver particles with microorganisms.(Fabrega, Fawcett, Renshaw, & Lead, 2009) This agreed with Kawata et al. (Kawata, Osawa, & Okabe, 2009) who stated the toxicity of nanosilver to bacteria is not only due to the release of Ag+ but to the particles themselves. The antibacterial activity for the nanosilver particles is correlated to the particles prosperities by quantitative measuring. (Lubick, 2008). The sizes of the nanosilver particles control the antibacterial effect to a great grade. The laboratory work examined the effect of the size of nanosilver particles on antimicrobial activity revealed that the fine particles have stronger effect comparing to coarse

particles.(Sotiriou & Pratsinis, 2010) The same conclusion was illustrated by Lok et al. (Lok et al., 2007) Thus, in addition to direct toxicity , the different diffusion rates of the different experimental specimens may influence the results (Sotiriou & Pratsinis, 2010).

The inhibitory zones surrounded the experimental materials had not any collapse or reduction in size. Even after full set of the root-canal sealer, the inhibitory zone progressed to the full capacity representing antimicrobial activity. The sustainable antibacterial effect on the experimental plate may have a role in the significance of result. In a previous study, the antibacterial activities of nanosilver particles and ions were tested. A suspension from Ag/SiO2 are examined at the beginning the suspension was centrifuged and the particles were removed. The remnant ions in the solution had a positive antimicrobial effect in advance.(Sotiriou & Pratsinis, 2010)

CONCLUSIONS

Within the limitations of this in vitro study, adding nanosilver particles to the powder of root canal sealer has antibacterial effect. Other tests, as test of toxicity, are required before depending on this maneuver in ordinary clinical work.

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