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THE EFFECT OF ADDITION OF SILVER NANOPARTICLES ON THE ANTIBACTERIAL EFFECT OF THREE DIFFERENT ROOT CANAL SEALERS (AN IN VITRO STUDY)

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ABSTRACT

Introduction: The aim of this study is to evaluate the effect of addition of silver nanoparticles (SNP) to AD Seal, MTA Fillapex and GuttaFlow 2 in terms of antibacterial effect.

Methods: The method applied was the Direct Contact Test (DCT). Crushed sealers were put in sterile polyethylene tubes. About 18 mg of each sealer were weighed and placed in a tube. The tubes then incubated at 37 degrees Celsius then covered with 400 micro-L of Brain H eart Infusion (BHI) broth and 100 micro-L of bacterial suspension of Enterococcus Faecalis. After 1 min, 1 hour, the bacterial survival was evaluated by culturing on Brain Heart Infusion (BHI) agar plates and the Colony Forming Unit/mL (CFU/mL) were counted.

Results: The results showed that there was no statistically significant difference between AD Seal and MTA Fillapex with or without addition of SNP at 1 min. However, a statistically significant difference was shown between GuttaFlow 2 and GuttaFlow 2 + SNP. At 1 hour, results showed a statistically significant difference between all groups except ADSeal + SNP and GuttaFlow 2 showed no statistically significant difference, with the highest antibacterial effect was for MTA Fillapex + SNP. There was statistically significant difference between all groups at 1 minute and 1 hour with the highest antibacterial effect at 1 hour except for AD Seal which showed the least antibacterial effect at 1 hour and the highest value favoring MTA Fillapex+ SNP.

Conclusion: The addition of silver nanoparticles to all sealers increased their antibacterial effect.

KEYWORDS: Silver nanoparticles, Antibacterial effect, AD Seal, MTA Fillapex, GuttaFlow 2

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INTRODUCTION

The goal of successful endodontic treatment is to obtain a hermetic seal of the root canal system. The process of endodontic treatment includes mechanical shaping of the canals using endodontic files either manual files or rotary files, cleaning of the root canal system through irrigants and finally obturation of the canal. An inadequate filling during obturation may result in reentry of bacteria and irritation of periapical tissues. Obturation of the canal is done through using of gutta percha alongside with different root canal sealers. Root canal sealers should meet high biological, physic-chemical and mechanical properties to ensure obtaining successful sealing of the root canal system.

Many attempts were done to increase the anti-bacterial effect of the root canal sealers. Additives to sealers may be beneficial to obtain this goal. Recently, silver nanoparticles are widely used in dental field due to its recorded high antibacterial and physic-chemical properties. Nanomaterials offer unique physicochemical properties such as large surface area/mass ratio, ultrasmall sizes and increased chemical reactivity in comparison with their bulk counterparts. Silver nanoparticles exert their antibacterial effect through acting on multiple targets as altering the hydrogen bonding/respiratory chain, unwind DNA, interaction with the sulfhydryl groups of proteins and DNA and interference with cell wall synthesis and cell division. They also de-stabilize the bacterial membrane and increase its permeability leading to leakage of cell components

This study compared between different root canal sealers in terms of antibacterial effect after incorporating silver nanoparticles into them to evaluate its effect on this property. Epoxy resin-based sealer (AD Seal), MTA based sealer (MTA Fillapex) and silicon-based sealer (GuttaFlow 2).

MATERIALS AND METHODS

I-Materials

Silver nanoparticles (SNP) preparation:*

Silver nanoparticles have been prepared by chemical reduction method as reported by Turkevich[(1)] and Lakhno[(2)]. A solution of $AgNO_3$ has been used as Ag^{1+} ions precursor. The PVP has been used as stabilizing agent and borohydrate as mild reducing agent. The color of the solution slowly turned into grayish yellow, indicating the reduction of the Ag^{1+} ions to Ag nanoparticles.

SNPs Gel: 0.4gm of Carboxymethyl cellulose (Loba CHIME, india) was sprinkled gently and gradually over the solution of Silver NPs 200ppm under mild temperature with vigorous stirring to get homogenous gel. The gel was mixed with sealer in ration 1:1 to get final sealer of 200ppm AgNPs.

As shown in figure 1, MTA Fillapex** was mixed prepared by mixing base and catalyst according to manufacturer's instructions and was used in group I. MTA Fillapex was prepared by mixing catalyst and base according to manufacturer's instructions. SNP was added to the MTA Fillapex paste to achieve a concentration of 200 ppm .MF-SNP was used in group II. GuttaFlow 2*** was prepared by mixing catalyst and base according to manufacturer's instructions and it was used in group III. GuttaFlow 2 was prepared by mixing catalyst and base according to manufacturer's instructions. SNP was added to the GuttaFlow 2 paste to achieve a concentration of 200 ppm. GF-SNP was used in group IV. AD Seal**** was prepared by mixing catalyst and base according

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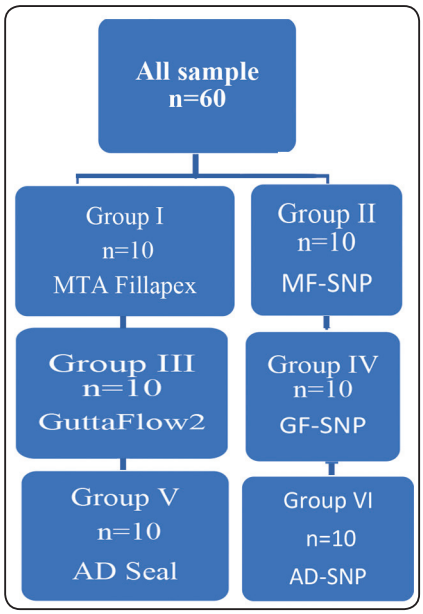

Fig. (1): Sample classification

to manufacturer's instructions and it was used in group V. AD Seal was prepared by mixing catalyst and base according to manufacturer's instructions. SNP was added to the AD Seal paste to achieve a concentration of 200 ppm. AD-SNP was used in group VI.

II-Methods:

Sealers were undergone antibacterial evaluation after 13 hours of mixing considering the period needed for setting of all materials.[3,4]. The antibacterial activity was evaluated against a reference strain of E-faecalis (ATCC 29212) obtained from the microbiology unit at Faculty of Dentistry, Cairo University. The bacteria were grown in brain heart infusion (BHI) broth at 37 degrees Celsius for 18 hours. A bacterial suspension then was made with 0.85% saline to match the turbidity equivalent to 0.5 McFarland standard, corresponding to 3x10^8 colony-forming units (CFU)/mL.(3,4).

The methodology here was adapted from Wainstein et al[4] and Heyder et al.[5] The endodontic sealers were put in sterile polyethylene tubes. About 18 mg of each sealer were weighed and placed in a tube. The tubes then incubated at 37 degrees Celsius simulating clinical conditions of humidity and temperature for setting.

Sealers then covered with 400 micro-L of Brain Heart Infusion (BHI) broth and 100 micro-L of bacterial suspension. As a negative control, same amount of sealer and culture media and 100 micro-L of saline solution (without bacterial suspension) was used. As a positive control, same amount of culture media and 100 micro-L of bacterial suspension without any sealer was used.

The same procedure was made again after 1 hour. After 1 min, 1 hour, the bacterial survival in each tube was evaluated by 10-fold serial dilutions up to 10 ^-7, and three aliquots of 20 micro L from each dilution were cultured on BHI agar plates. Plates were then incubated for 24 hours at 37 degrees Celsius and the CFU/mL were counted.

RESULTS

As shown in Figure 2, The results showed that there was no statistically significant difference between AD Seal and MTA Fillapex with or without addition of SNP at 1 min. However, a statistically significant difference was shown between GuttaFlow 2 and GuttaFlow 2 + SNP. At 1 hour, results showed a statistically significant difference between all groups except ADSeal + SNP and GuttaFlow 2 showed no statistically significant difference, with the highest antibacterial effect was for MTA Fillapex + SNP.

There was statistically significant difference between all groups at 1 minute and 1 hour with the highest antibacterial effect at 1 hour except for AD Seal which showed the least antibacterial effect at 1 hour (P=2.60216 ±0.00017) and the highest value favoring MTA Fillapex+ SNP (P=0.477131 ±0.00012).

TABLE (1): The antibacterial values between the six groups at 1 minute and 1 hour.

	1min	1H	P-Value
ADSeal	2.30104 ±0.00001 [a]	2.60216 ±0.00017 [a]	0.00
ADSeal + SNP	2.30104 ±0.00001 [a]	2.17637 ±0.00033 [b]	0.00
MTA Fillapex	2.30109 ±0.00007 [a]	1.95460 ±0.00071 [c]	0.00
MTA Fillapex + SNP	2.17608 ±0.00003 [a]	0.477131 ±0.00012 d	0.00
GuttaFlow 2	2.27870 ±0.00009 [b]	2.17611 ±0.00005 [b]	0.00
GuttaFlow 2 + SNP	2.30112 ±0.00011 [c]	2.27784 ±0.00143 [e]	0.00
P-Value	0.00	0.00	

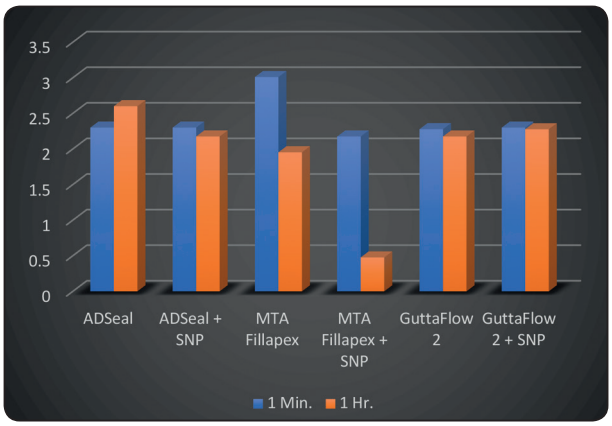

Fig. (2): The effect of addition of silver nanoparticles at 1 minute and 1 hour.

DISCUSSION

Enterococcus faecalis are commonly the only species isolated from obturated root canals indicating having a major role in chronic endodontic treatment failure. This may be attributed to their ability to resist intracanal medication and surviving into root canal system as a single organism without support from other bacteria.[6] Therefore, *E. faecalis* was preferred for antibacterial evaluation in this study.

The Direct Contact Test (DCT) is used for evaluating of antibacterial activity of insoluble materials. The test is based on measuring the effect of direct physical contact between bacteria and test materials. This concept has been suggested to overcome the limitations of Agar Diffusion test (ADT). The major advantages of this method are reproducibility and quantitative assay.[3,7]

Therefore, DCT methods was the one chosen to assess the antibacterial effect of this study. For evaluating the antimicrobial effect using DCT method, the time of 60 mins was used as less than that, the sealers were found to have no time to affect the resisting *E. faecalis*.[8]

In terms of the antibacterial effect, there was a statistically significant difference of the antibacterial effect among all sealers with the sealer having the highest antibacterial effect was MTA Fillapex. This may be explained by the release of calcium hydroxide during setting. These ions increase the environmental pH leaving it unsuitable for microorganisms.[9,10]

The results of this study is consistent with many authors who reported the same results as Garcia et al[11], Baras et al[12], Jafari et al[13] and Rodriguez et al[14]. The dramatic increase of the antibacterial effect of all test sealer after incorporating silver nanoparticles is due to the release of silver ions towards the microorganisms.[15] The silver antibacterial effect is explained by acting on multiple targets of the bacteria.[16] Silver interacts with sulfhydryl groups of DNA and proteins, unwind DNA, alter the hydrogen bonding/respiratory chain and interfere with cell wall synthesis and

cell division.[17,18] Silver nanoparticles are believed to further destabilize the bacterial membrane and increase the membrane permeability leading to leakage of cell constituents and subsequent cell death.[19,20]

CONCLUSION

The addition of silver nanoparticles to all sealers increased their antibacterial effect.

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