Measurement of Tubular Penetration Depth of Three Types of Nanoparticles Mixed With Endodontic Sealer Using Scanning Electron Microscope (An *In Vitro* Study)

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Abstract: Recent advances in the field of nanotechnology have proven successful in enhancing the properties of the endodontic materials especially sealers to overcome their limitations, specifically against resistant bacteria, however further studies were needed to determine whether these sealers modified with nanoparticles will provide better penetration into the complex minute anatomy of the dentinal tubules. **Aim** of this study, was to measure the depth of tubular penetration of Zinc oxide based Pulp Canal Sealer(PCS) (EWT) (Kerr Corporation, USA)when mixed with zinc oxide nanoparticles, silver nanoparticles and doped zinc oxide-silver nanoparticles respectively. **Methods**: Forty mandibular premolars with single canals and mature roots were instrumented using Protaper Next rotary system (Dentsply, Maillefer, Switzerland), then randomly divided into four equal groups, three groups according to nanoparticles incorporated with the sealer used for obturation and a control group as follows, (I):Pulp canal Sealer (PCS) modified with zinc oxide nanoparticles, (II): Pulp Canal Sealer(PCS) modified with silver nanoparticles, (III): Pulp Canal Sealer PCS modified with doped zinc oxide and silver nanoparticles and (IV) : control group with unmodified Pulp Canal Sealer(PCS). In all groups, specimens were obturated using the corresponding Protaper Next gutta-percha and accessory cones (Dentsply, Maillefer, Switzerland). Specimens in each group were sectioned longitudinally in a buccolingual direction. Depth of sealer penetration was measured using scanning electron microscope. **Results**: Kruskal Wallis test showed that incorporation of nanoparticles with the Pulp Canal Sealer (PCS) have significantly improved the penetration depth of the sealer into the dentinal tubules when compared to the control group. (p<0. 05). Sealer mixed with silver nanoparticles showed the deepest penetration. Mann Whitney test have shown that there was a statistically significant difference in the depth of sealer penetration between groups I and II (sealer mixed with Zinc oxide and silver nanoparticles respectively) compared to control group IV. Wilcox on signed rank test showed deepest sealer penetration in the coronal third of all groups while least sealer penetration is at the apical third. **Conclusion:** Incorporation of nanoparticles improved the flow properties of the endodontic sealer materials; this was influenced by the nanoparticles` size which translated in better sealer penetration into the dentinal tubules.

[ElKateb Walid M., Massoud Ahmed G., Mokhless Nayera A, Shalaby Thanaa I. **Measurement of Tubular Penetration Depth of Three Types of Nanoparticles Mixed With Endodontic Sealer Using Scanning Electron Microscope (An In Vitro Study).** *J Am Sci*2015; 11(11):111-122]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 12. doi:10.7537/marsjas111115.12.

Key words: Dentinal tubules, Doped, Flow, Nanoparticles, Particle size, Penetration depth, Pulp canal sealer, Silver, Zinc oxide.

1. Introduction

Recent advances in the field of Nanotechnology have proved to be beneficial in several fields of science and medicine including dentistry. In Endodontics, treatment of infected teeth aims to reduce the microbial load within the root canal system to allow healing, and to prevent microbial re-entry. However several dilemmas exist, including the complexity of the root canal anatomy, the resistant bacteria and the limitations of the obturation materials. The clinician needs to deal with, and try to overcome these dilemmas to reach the ultimate goal of proper cleaning, shaping and obtaining a 3dimensional hermetic seal of the root canals.

Root canal sealer is required to seal the space between the core filling material and the dentinal wall and thus expected to possess good antimicrobial and flow properties with good penetration ability to aid in controlling infection deep within the root canal system. Though most sealers possess antimicrobial constituents, unfortunately recent researches indicated that sealers that had antibacterial activity eventually lost it after setting (Slutzky-Goldberg *et al.,* 2008 and Morgental *et al.,*2011) and even if it doesn`t, these constituents have to be released from the sealer matrix (Kishen *et al.,*2008) in order to diffuse into the dentinal tubules. On the other hand the release of these antibacterial constituents from a set sealer is associated with disintegration of the material (Kayaoglu *et al.,* 2005), which may compromise the sealer-core and sealer-dentin interfaces, making them vulnerable to bacterial re-colonization. This necessitates the use of materials with inherent antibacterial property; i.e. antibacterial property is part of their nature not an added feature.

Nanoparticles with their unique small size provides them with inherent antibacterial properties which disrupts the bacterial cell wall, thus can be used to overcome the problem of the resistant bacteria, with several studies proving the effectiveness of these nanoparticles even against the resistant strain *E. faecalis* responsible for the majority of the failures of the endodontic treatment (Wu *et al,* 2014) and so overcoming one of the main drawbacks of endodontic sealers.

Yet another dilemma remains, which is the complexity of the anatomy of the root canal system, which requires the use of sealers that are better able to penetrate & carry their action deep within the dentinal tubules where the resistant bacteria is lodged.

In this case, the small size of the nanoparticles can also prove to be beneficial, Although recent researches have shown that the addition of nanoparticles to the endodontic sealers does not change its physical or mechanical properties, yet a slight change in the flowability of the sealer was noted (Barros *et al.,* 2014). This can result in improving the penetration of this modified sealer into the minute dentinal tubules and better ability to interact with the bacteria in those hard to reach areas, where they remain viable & flourishing, threatening the success of the endodontic treatment, compared to their regular counterparts.

2. Materials and Methods

Nanoparticles preparation

Zinc oxide, silver and doped zinc oxide with silver nanoparticles were prepared in the nanotechnology laboratory of the biophysics department of the Medical Research Institute (Alexandria University, Egypt)

Zinc oxide nanoparticles wasprepared by chemical reduction (Fazilati, 2013) 0. 1 M Zinc nitrate (Zn $(NO_3)_2$ in 40 ml distilled water and 0. 2 M Sodium hydroxide (NaOH) in 40 ml distilled water were prepared separately. Then, NaOH solution was heated at the temperature of 550C. The Zn $(NO_3)_2$ solutions was added drop wise (slowly for one hour) to the heated (NaOH) solution under high-speed stirring. The beaker was sealed for two hours. Finally, precipitated zinc oxide nanoparticles were cleaned with deionized water and ethanol then dried in air atmosphere at about 60 ^{0}C. Zinc oxide nanoparticles were collected and calcination was done at 600^{0}C for 3 hours.

Silver nanoparticles was prepared by using chemical reduction method (Zhou and Wang, 2012)All solutions of reacting materials were prepared in distilled water. 5 ml silver nitrate (1µM) aqueous solution was heated to boil, to this solution 10 mL of 0. 1gm trisodium citrate was added drop by drop. During the process, solutions were mixed vigorously and heated until change of color was evident (pale yellow). Then it was removed from the heating device and stirred until cooled to room temperature, then freeze dried to obtain powder form.

Zinc oxide doped with silver nanoparticles was synthesized by Coprecipitation method (Chauhan *et al.,* 2010). A solid mixture of 1 -5% of silver nitrate (1µM) in aqueous solution of zinc sulphate ($ZnSO_4$) was dissolved separately in distilled water and then mixed with buffer solution of sodium carbonate and sodium hydroxide, followed by the same procedure as for undoped ZnO. The collected powder was calcined at a temperature of 700^{0}C for 3 hours

Modified Sealer preparation

Each type of nanoparticle powder was added at 1. 5% wt/wt to the powder of endodontic sealers in each of the designated group using a digital analytical balance Kern ACS/ACJ 220-4M (KERN & SOHN, Balingen- Germany) with accuracy (d=0. 1 mg) as follows:

1. Group I: (Pulp Canal Sealer PCS) + Zinc Oxide (ZnO) nanoparticles
2. Group II: (Pulp Canal Sealer PCS) + Silver (Ag) nanoparticles
3. Group III: (Pulp Canal Sealer PCS) + Doped Zinc oxide-Silver (ZnO-AG) nanoparticles
4. Group IV (Control Group): (Pulp Canal Sealer PCS) unmodified.

Characterization

Morphological and structural properties was characterized using scanning electron microscopy (SEM)(JEOL JSM-5300-JEOL Inc USA), Fourier transform infrared spectroscopy (FTIR) and zeta potential (ζ) measurement by Zeta potential analyzer (Zetasizer Nano-ZS, Malvern, UK)to measure the prepared nanoparticle size and to verify no change occurred in the physical property of the sealer after the addition of nanoparticles.

Specimen preparation

40 freshly extracted mandibular first molar teeth extracted for periodontal or orthodontic reasons with fully developed root apices were used for this study. The teeth were stored in saline after the tissue remnants present on the root surface of these teeth were cleaned. Teeth were radiographed mesiodistally to confirm presence of a patent single canal. For standardization teeth were sectioned with a diamond disc (Diatech, Swiss Dental Instruments, Heerbrugg, Switzerland) with water spray cooling to ensure a uniform sample length of 12 mm to eliminate the length as a variable. Working length determination was done by penetration of a K-file (Micro Mega SA,

Besancon, France) size #10 into the root canal till it just appears from the apical foramen then pulled back 1 mm. ***Instrumentation*** was done using rotary system ProTaper Next. K-file size #15 was used to create a patent reproducible smooth glide path. All specimens were instrumented with X1 file (17/04) in a brushing motion following the glide path till the working length is reached, followed by X2 file (25/06) then X3 (30/07) for canal size standardization. During instrumentation files were removed from the canal and cleaned with sterile gauze and apical patency was checked with K-file size # 15. Canals in all groups were irrigated with a standardized volume of 3ml NaOCl 2. 5% using a 27 gauge needle (KerrHawe SA, Biggio, Switzerland) between each file.

Specimens in all groups were flushed with a standardized volume of 10ml of 17% EDTA followed by a standardized volume of 10ml of 5. 25% Na hypochlorite to remove the smear layer & expose the dentinal tubules, followed by final rinse with 10ml distilled water then dried with paper points.

Obturation:

Powder and liquid were manually mixed on a glass slab with a spatula according to manufacturer's instructions, then applied manually into the canals.

In all groups obturation was performed by size F3 Protaper Next gutta-percha cone that matches the taper and size of the X3 file and fitted to the working length with tug back. The selected cone was then lightly coated with sealer and placed into the canal to the working length. Accessory cones were added and laterally condensed using hand spreader till no more space was encountered.

Roots were stored for seven days at 37C^o and 100% humidity to allow complete setting of the sealer.

Scanning Electron Microscope

Specimens were longitudinally sectioned in a buccolingual direction by making a thin slot along the buccal and lingual aspect of the root using diamond disk (Diatech Swiss Dental Instruments, Heerbrugg, Switzerland) at low speed then split using a chisel & mallet. The specimens were subjected to scanning electron microscope (JEOL JSM-5300-JEOLInc USA)where the central beam was directed to the surface area of each sample under x1000 magnification.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20. 0 (IBM, New York, USA). Kruskal Wallis test was used to compare between different groups, pair wise comparison was assessed using Mann-Whitney test and Wilcoxon signed ranks test to compare within the groups. Significance of the obtained results was judged at the 5% level. (Kotz *et al.,* 2006)

3. Results

Characterization

Results of scanning electron microscope revealed that the nanoparticle size ranged from 34 - 55nm for silver nanoparticles, 40-60 nm for Zinc Oxide nanoparticles and 60-75 for zinc oxide doped with silver nanoparticles. Size of the pure Pulp Canal Sealer (PCS) ranged from 350 to ≥500 nm. (Figure 1-2) FT-IR and Zeta potential measurement showed no change in the physical property of the sealer after addition of each type of nanoparticles.

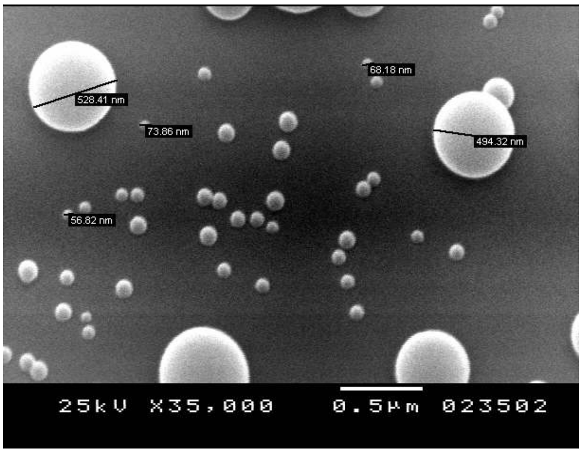
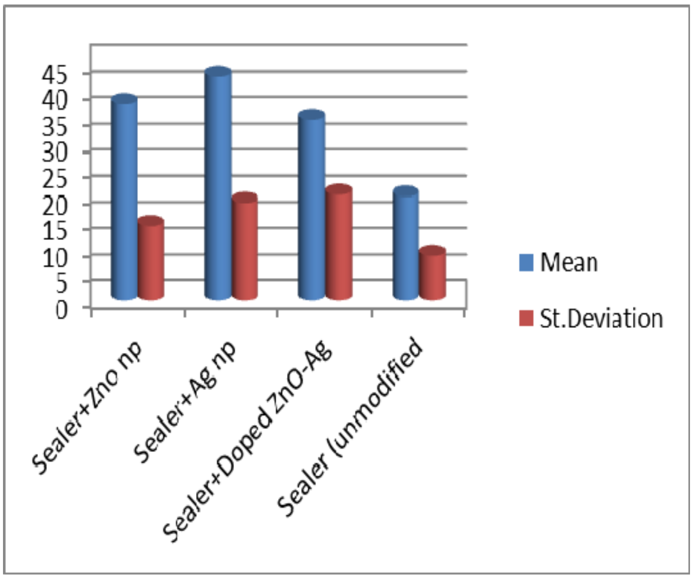


Figure 1. Figure 1. SEM image of sealer mixed with nanoparticles.

Sealer penetration

Kruskal Wallis test showed that there was a statistically significant difference in the sealer penetration depth between the four tested groups, with the control group showing a significantly lower sealer penetration than the other groups. The deepest sealer penetration was found in group II with a mean on average penetration in all sites 42. 76 ±18. 56. The least sealer penetration was found in group IV (control) with a mean 19. 78 ±8. 51 (P≤0. 05). Table1. Graph1.

Multiple pair wise comparisons between the four groups in pairs using the Mann Whitney test have shown that there was a statistically significant difference in the depth of sealer penetration between group 1 (sealer mixed with Zinc oxide nanoparticles) and group 2(sealer mixed with Silver nanoparticles) when compared with the control group 4. However No statistical difference was found between the three groups with modified sealers. No significance was found between group III (sealer mixed with Doped Zinc oxide- silver nanoparticles) and the control group, though the significance value P= 0. 07 was close. (P≤0. 05). **Table 1**



Graph 1:Comparison between the four groups according to the mean of the average sealer penetration in all thirds.

Table 1: Multiple pair wise comparison between the studied groups according to mean of average sealer penetration in all sites

Average	Sealer				$^{KW}\chi^2$	*p*
	Sealer+ ZnO Np (n=10)	**Sealer+ Silver Np (n=10)**	**Sealer+Doped ZnO – Ag Np (n=10)**	**Sealer (unmodified) (n=10)**		
					10. 461*	0. 015*
Min. – Max.	17. 01 – 56. 40	14. 0 – 70. 21	15. 27 – 66. 33	9. 64 – 38. 27		
Mean ± SD.	37. 35 ± 14. 11	42. 76 ± 18. 56	34. 51 ± 20. 13	19. 78 ± 8. 51		
Sig. bet. groups	p_1= 0. 450, p_2=0. 345, p_3=0. 008*, p_4=0. 226p_5=0. 007*p_6=0. 070					

$^{KW}\chi^2$: Chi square test value for Kruskal Wallis testSig. between Groups was done using Mann Whitney test
p_1 : p value for comparing between Sealer + ZnO np andSealer + Silver np
p_2 : p value for comparing between Sealer + ZnO np and Sealer + Doped ZnO - Ag np
p_3 : p value for comparing betweenSealer + ZnO np andPure Sealer (un modified)
p_4 : p value for comparing between Sealer + Silver np and Sealer +Doped ZnO - Ag np
p_5 : p value for comparing between Sealer +Silver npandPure Sealer (un modified)
p_6 : p value for comparing betweenSealer + Doped ZnO– Ag np andPure Sealer (un modified)
*: Statistically significant at $p \leq 0.05$

Comparison of the sealer penetration depth in the coronal, middle and apical third within each experimental group showed that Coronal third had the deepest sealer penetration, and the apical had the least sealer penetration. Wilcoxon signed rank test showed significance between all thirds only in group 2, while significant difference was found between the middle and apical thirds in groups 3 and 4. Group one showed significance between coronal and middle thirds only. ($P \leq 0.05$)**Table 2**

Comparison between pairs using Mann Whitney test showed significance between the group I (sealer +ZnO np) and control group IV (unmodified sealer). Also between Group II (sealer + Ag np) and control group IV (unmodified sealer). $P \leq 0.05$. Comparison of the coronal third of all groups using Kruskal-Wallis test showed a statistically significant difference and no statistical differences for the middle thirds or the apical thirds of all groups**. Table3**

Table 2: Multiple comparison within the studied groups according to mean of average sealer penetration in all sites

	Coronal (n=10)	Middle (n=10)	Apical (n=10)
Sealer + ZnO np			
Min. – Max.	0. 0 – 91. 60	0. 0 – 64. 60	0. 0 – 33. 70
Mean ± SD.	60. 49 ± 32. 77	36. 08 ± 25. 95	15. 46 ± 13. 96
Sig. bet. stages	p_1= 0. 139, p_2= 0. 011*, p_3= 0. 114		
Sealer + Silver np			
Min. – Max.	0. 0 – 110. 83	0. 0 – 61. 60	0. 0 – 38. 40
Mean ± SD.	71. 83 ± 40. 10	40. 41 ± 22. 56	16. 05 ± 14. 71
Sig. bet. stages	p_1= 0. 047*, p_2 = 0. 011*, p_3 = 0. 022*		
Sealer +Doped ZnO - Ag np			
Min. – Max.	0. 0 – 96. 94	0. 0 – 76. 73	0. 0 – 37. 14
Mean ± SD.	54. 73 ± 40. 14	34. 68 ± 31. 07	14. 13 ± 13. 34
Sig. bet. stages	p_1= 0. 203, p_2 = 0. 017*, p_3 = 0. 036*		
Pure Sealer (control)			
Min. – Max.	0. 0 – 58. 38	0. 0 – 49. 02	0. 0 – 18. 65
Mean ± SD.	32. 72 ± 23. 37	20. 13 ± 18. 78	6. 47 ± 8. 43
Sig. bet. stages	p_1= 0. 333, p_2 = 0. 017*, p_3 = 0. 036*		

Sig. within groupswas done using Wilcoxon signed ranks test

p_1 : p value for comparing between Coronal and Middle

p_2 : p value for comparing between Coronal and Apical

p_3 : p value for comparing between Middle and Apical

*: Statistically significant at $p \leq 0.05$

Table 3: Significance between sealer penetration in the coronal, middle and apical third of the four groups

	Sealer +			Pure Sealer (unmodified) (n=10)	$^{KW}\chi^2$	*p*
	ZnO Np (n=10)	Silver Np (n=10)	Doped ZnO – Ag Np (n=10)			
Coronal					10. 015*	0. 018*
Sig. bet. groups	p_1=0. 111, p_2=0. 879, p_3=0. 012*, p_4=0. 158 p_5=0. 012* p_6=0. 072					
Middle					4. 620	0. 202
Apical					4. 663	0. 198

$^{KW}\chi^2$: Chi square test value for Kruskal Wallis test   Sig. between groups was done using Mann Whitney test

p_1 : p value for comparing between Sealer + ZnO np and Sealer + Silver np

p_2 : p value for comparing between Sealer + ZnO np and Sealer + Doped ZnO – Ag np

p_3 : p value for comparing between Sealer + ZnO np and Pure Sealer (un modified)

p_4 : p value for comparing between Sealer + Silver np and Sealer + Doped ZnO- Ag np

p_5 : p value for comparing between Silver np and Pure Sealer (un modified)

p_6 : p value for comparing between Sealer + Doped ZnO - Ag and Pure Sealer (unmodified)

*: Statistically significant at $p \leq 0.05$

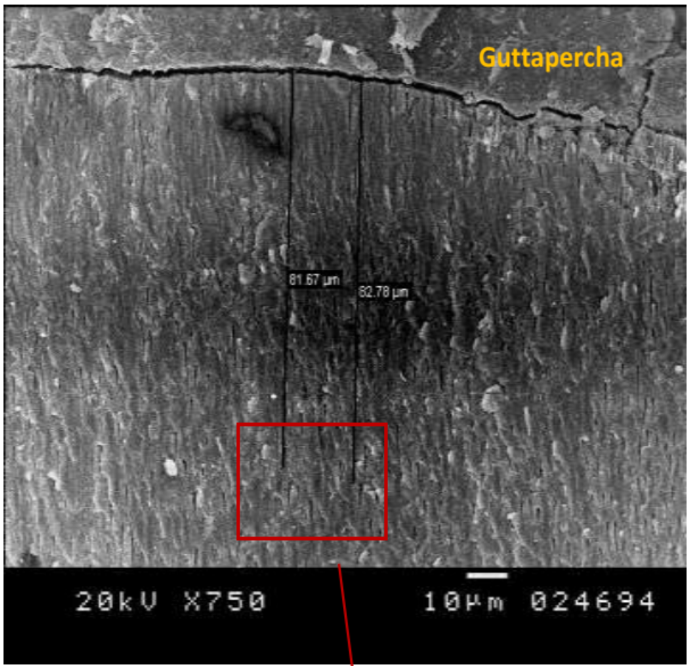


Figure 2. SEM image of sealer penetration at the Coronal third of group I

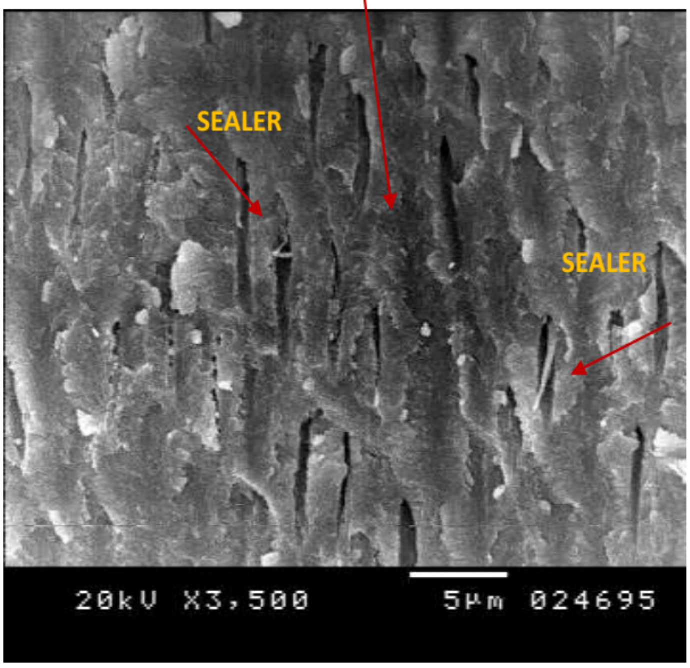


Figure 3.High magnification SEM image of sealer penetration at the Coronal third of group I

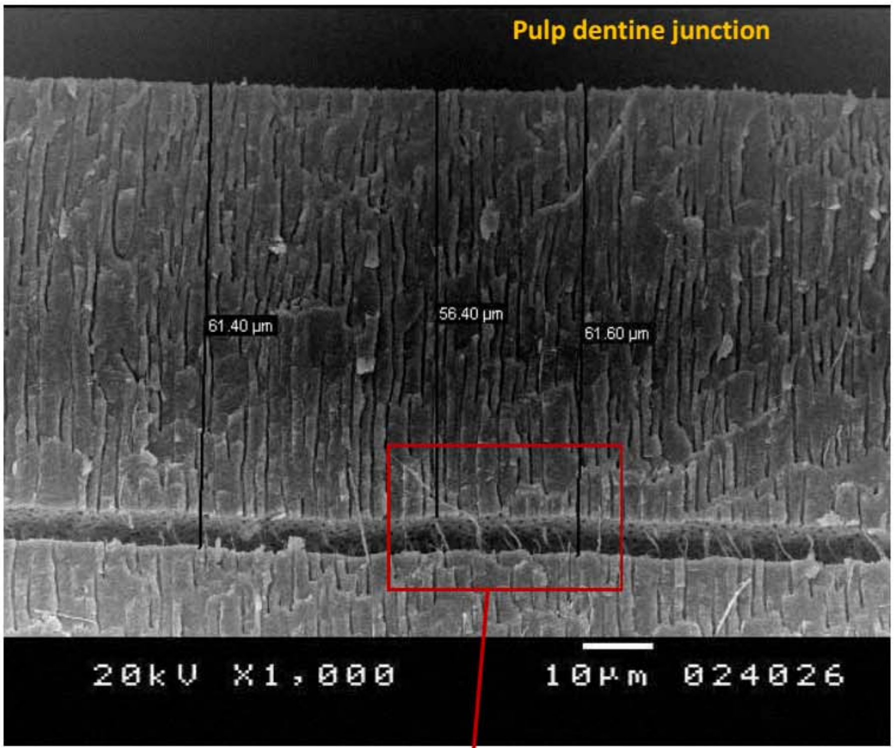


Figure 4. SEM image of sealer penetration at the middle third of group II

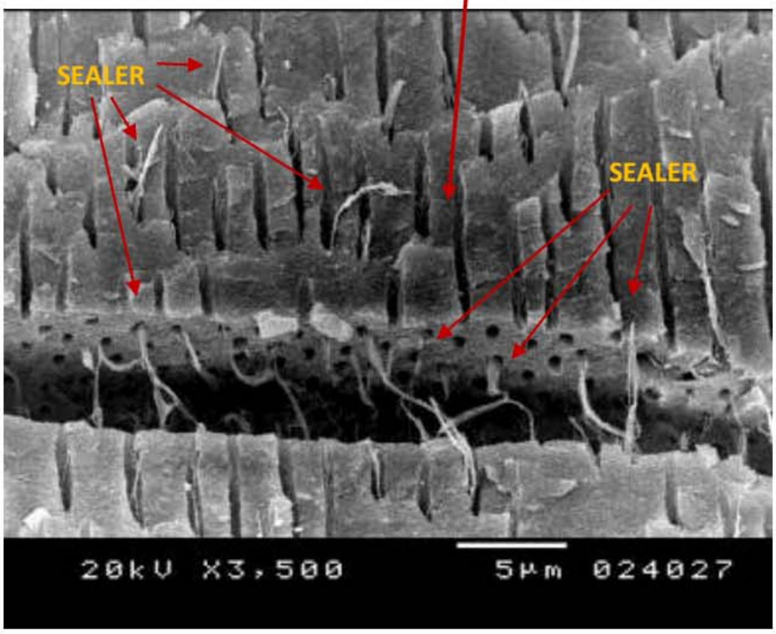


Figure 5.High magnification SEM image of sealer penetration at the middle third of group II

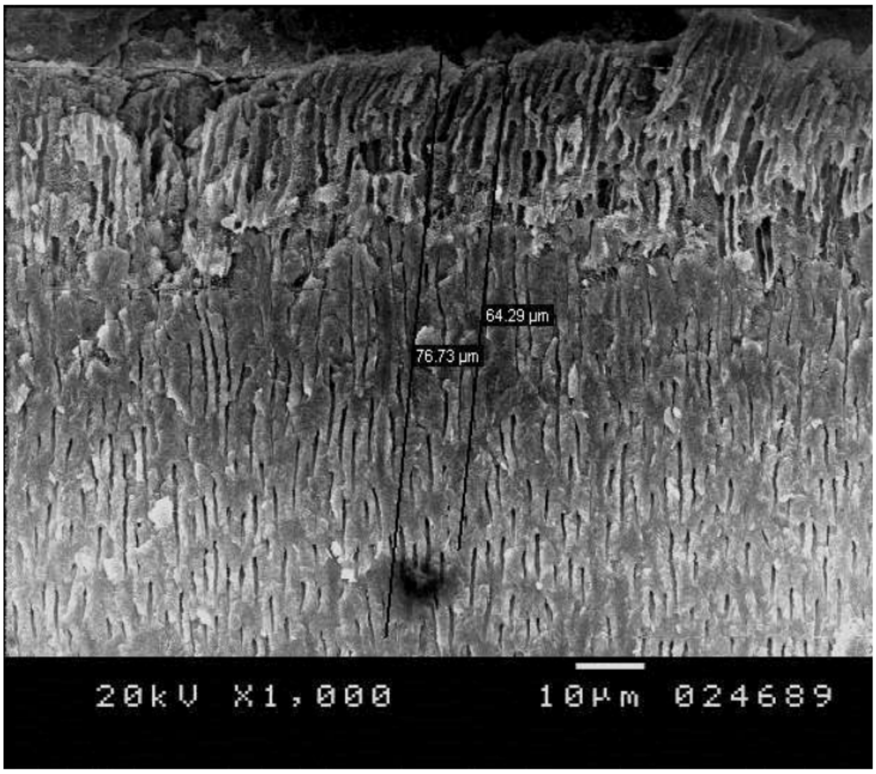


Figure 6. SEM image of sealer penetration at the middle third of group III

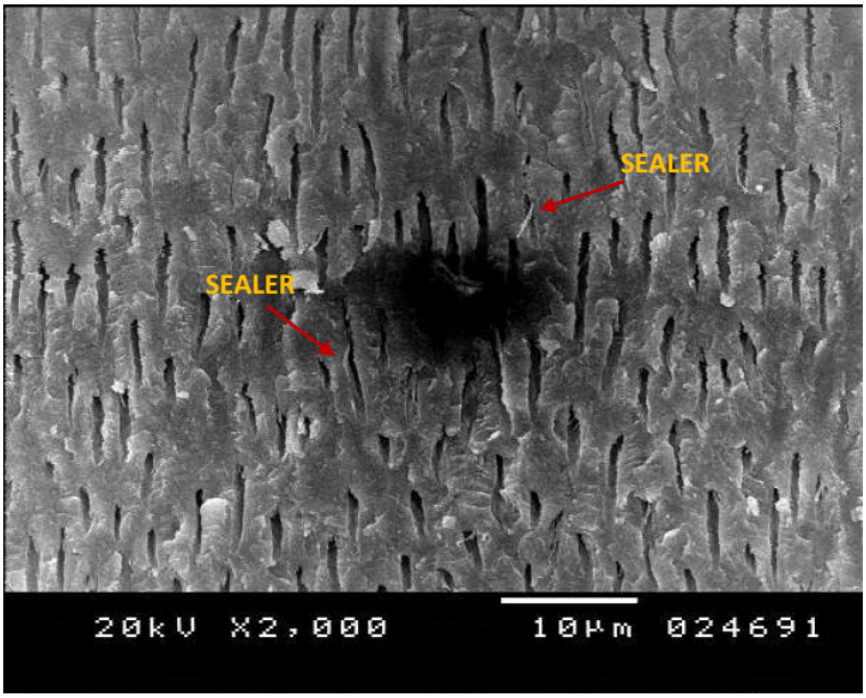


Figure 7. SEM image of sealer penetration at the middle third of group III

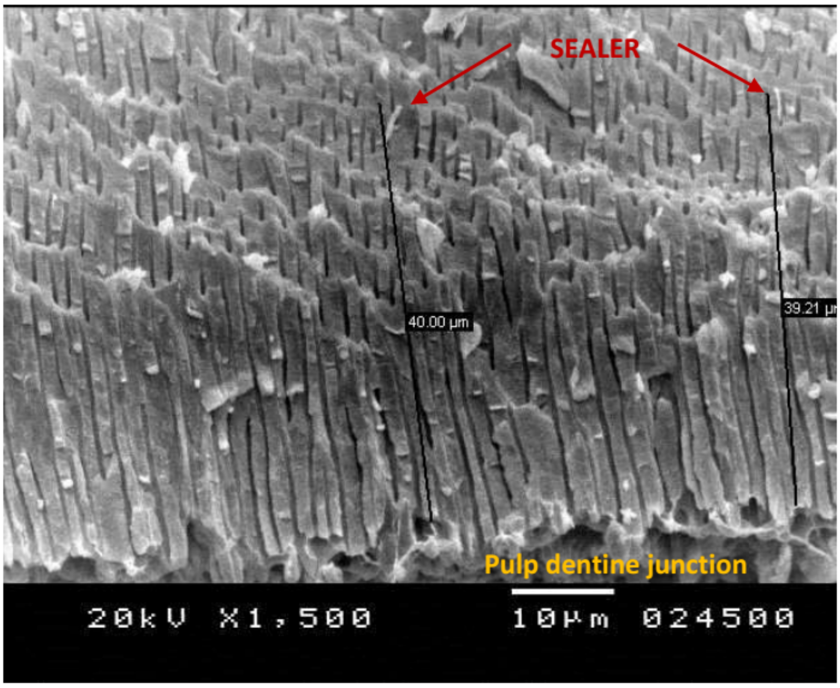


Figure 8. SEM image of sealer penetration at the coronal third of group IV

4. Discussion

Endodontic sealers play an important role in the success of the endodontic treatment. The effect of root canal sealers on bacterial combat and entombment may be influenced by its ability to penetrate into inaccessible areas also sealer penetration into the root canal may enhance sealing ability of root filling material and improve retention of root filling material (Sae-ung *et al.,* 2012)

The future use of nanoparticles appears bright because they can be conjugated with other agents to further enhance their spectrum of use.

Size of the nanoparticles is considered to be their unique characteristic, with a diameter of 100 nm or less, it is responsible for them having a greater contact surface area and charge density than bulky powders. It is also responsible for its antibacterial role as it allows it to have a significantly greater degree of interaction and contact between the positively charged nanoparticles and the negatively charged bacterial cell surface (Kishen *et al.,* 2008)

Their small size also allows them to readily penetrate and extend its action into dentin microporosities, and areas of the root canal that are typically inaccessible to the commonly used endodontic irrigants (Kishen A, 2010). This was taken into consideration during the preparation of the nanoparticles for this study, as a balance was maintained between preservation of the small size of the nanoparticles to harvest its benefit yet at the same time ensuring its biocompatibility. Calcination was thus done at a temperature of 500 C for 3 hours for the Zinc Oxide nanoparticles and 600 C for 6 hours for Doped Zinc oxide with silver. The size of the nanoparticles was thus maintained at a size between 30-100 nm to avoid toxicity to the fibroblasts and macrophages (Abramovitz *et al.,* 2010) for future *in vivo* use. Figure 2-3.

Penetration of sealer inside the tubules is potentially useful because it increases the interface between the core material and dentinal walls, which may improve the mechanical retention of the material via sealer plug interlocking inside the tubules (White *et al.,* 1986). The improved penetration allows the sealer with its antibacterial property to better reach the bacteria deeply dislodged inside the DT and the rest will be entombed (Helung *et al.,* 1996)

However sealer penetration in the dentinal tubules depends on many factors like smear layer removal (De Deus *et al.,*2002), dentinal permeability(the number and the diameter of tubules), root canal dimension and the physical and chemical properties of the sealer (De Deus *et al.,*2004, Weis *et al.,*2004 and Ørstavik, 2005).

The flow is one of the main chemical/physical factors to influence the tubular penetration and is defined as the ability of a sealer to penetrate in irregularities, lateral canals, or dentinal tubules of the root canal system (Bernardes *et al.,* 2010). The flow is determined primarily by the consistency and particle size among other factors (as internal diameter of the root canal, and the rate of insertion (Ørstavik, 2005). It was proven that the addition of nanoparticles did not deteriorate the flow characteristics of the sealer but at the same time decreased viscosity, leading to enhanced flow of the sealer (Kishen *et al.,*2008) However to obtain this result a compatibility between the powder of the sealer and the nanoparticles must exist. This was taken into consideration when choosing both the type of sealer and the nanoparticles used.

Zinc Oxide Eugenol sealer in spite of its popularity as being a sealer with decades of clinical and laboratory application and its antibacterial properties, yet is considered one of the sealers with the least penetration ability (Patel *et al.,*2007, Mamootil *et al.,*2007 and Balguere *et al.,*2011). Thus the choice of Pulp Canal Sealer was made in this study to test if the enhanced flow of the sealer after the addition of the nanoparticles may improve the penetration depth.

To ensure compatibility, the nanoparticles used are the main constituents of the powder of Pulp Canal Sealer (PCS) which are zinc oxide and silver. Another benefit was the antibacterial effect of Zinc Oxide and silver. Zinc Oxidehave demonstrated a potential to generate singlet oxygen asreactive oxygen species (ROS), such as hydrogen peroxide and superoxide anion radicals (Tiller *et al.,*2001 andKlepac-Ceraj *et al.,*2011) and its penetration in either the cytoplasm or the outer membranes may explain such capacity so that the antimicrobial activity of zinc oxide is inversely proportional to the size of its particles (Raghupathi *et al.,*2011 and Seil *et al.,*2012). Silver ions inactivate protein and prevent DNA replication of the bacteria (Feng *et al.,* 2000 and Kim *et al.,* 2007). Doped Zinc oxide with silver nanoparticles will combine the effect of both those types of nanoparticles while at the same time be compatible with the sealer powder.

In this study, depth of penetration of sealers mixed with nanoparticles in groups I, II and III were higher than penetration of the unmodified sealer in the control group IV. This can be attributed to the fact that the addition of smaller particle size to the sealer mix has improved the flow property of the otherwise viscous pulp canal sealer (PCS). Table1, Graph1.

This finding is in agreement with the finding of Beyth *et al.,*2013 who performed a flow test according to ISO 6878:2001 (E) specification (The International Standard Organization specification for the flow of the sealer is based on the diameter of a film of sealer between two glass plates at room temperature) after adding nanoparticles 1. 5%wt/wt to the sealer and found a significant increase in the flow property, yet it was within the clinically acceptable range.

Kishen *et al.,*2008 also reported an improvement in the flow property, where the diameter of the original sealer disc was 24. 2 mm which exhibited less flow than the Zinc Oxide-nanoincorporated sealer disc which was 28. 6 mm, this can be attributed to the fact that an improvement in the flow property of the sealer occurs with a decrease in the particle size.

This was further proved when no significant difference was found in the sealer penetration depth between groups I, II and III (groups with sealer modified with nanoparticles) as the improvement of the flow property occurred in all these groups inspite of the slight differences in the nanoparticle size, Table2, however this was against Kaplan *et al.,* 2003 who concluded that the flow of the sealer was mainly affected by final consistency and setting reaction not on the particle size.

In this study, group II (sealer + silver nanoparticles) showed maximum penetration depth in all thirds (highest in the coronal third at 110. 83µm) while least penetration depth was in the control group (lowest in the apical third at 14. 2µm). Table2. Figure 6, 7.

The mean depth of sealer penetration in group IV (control) with unmodified sealer was 32. 72 ± 23. 37 in the coronal third, Table1, Figure 10, which is similar to De Deus *et al.,*2004 findings at 6 mm from the apex. However this was in contrast to Patel *et al.,*2007 who found the mean penetration depth at 139µm. This was also in contrast to Singh *et al.,* 2012 who found that mean penetration of ZnOE sealer was as low as 4±0. 5µm

It was found that there was a regional variation of the sealer penetration along the root length. It was highest in the coronal third in all groups followed by the middle then finally the apical third. Table3. Thus a significant difference was found between the sealer penetration depth in the coronal third (where the smear layer is best removed and so the tubules are most patent) and the apical third in all groups. This could be attributed to the decrease in the effectiveness of the smear layer removal technique closer to the apex, thus affecting the depth of penetration. This agrees with O`Connell *et al.,* 2000

This was also in agreement with Balguerie *et al.,* 2011 who found little or no penetration of zinc oxide eugenol based sealer in the apical third of his specimens.

And was in accordance with the findings of Ravi et al, 2014 who though used a sealer of different nature (Epiphany) observed a higher percentage of sealer penetration in the coronal section 89. 23%, followed by middle section 84. 19% and the apical section 64. 9%

However these findings were in contrast to Saunders *et al.,* 1992 and Vassilliadis *et al.,* 1994 who observed that the sealer penetration was higher at the middle third.

Significant difference between depth of penetration in the middle and apical thirds was found within groups II, III and IV this can be attributed to the large size of dentinal tubules in the middle third when compared to the apical third, which contains less tubules, and when present, their diameter is smaller or they are more often closed (Carrigan, 1984, Vasiliadis, 1994 and Mjeor, 2001)

Furthermore, the apical portion of roots shows a pronounced variation in structure (Mjeor, 2001), for example, primary dentinal tubules are irregular in direction and density; some areas are devoid of tubules. Also, cementum-like tissue can line the apical root canal wall, occluding any tubules.

It was interesting to note that the only group that showed a significant difference between the depth of sealer penetration in the coronal and the middle thirds was group II which was also the group that showed the deepest depth of sealer penetration at 110. 83 µm coronally with a mean of 42. 76 ± 18. 56. This can be due to the fact that the sealer in this group was more flowable when compared to the other groups due to the smaller particle size of the silver nanoparticles when compared to the other nanoparticles size. This shows that the particle size had a direct effect on the flow property and thus on the sealer penetration ability in the dentinal tubules.

This was in accordance with Balguerie *et al.,* 2007 who concluded that one of the factors influencing the sealer penetration other than the diameter of the dentinal tubules is the particle diameter. However this was not in accordance with Nikhil and Singh *et al.,* 2015 who concluded that sealer penetration was affected by type of placement technique rather than the particle size.

On studying the sealer penetration depth in the coronal third between all tested groups it was found that there was a significant difference between group I (sealer + ZnO np) and control group IV (unmodified sealer). Also between Group II (sealer + Ag np) and control group IV (unmodified), this can be attributed to the comparatively small size of the added nanoparticles, On the other hand, no significant difference was found between group III and group IV, even though the significance value $P= 0.07$ was close to $P\leq 0.05$ this could be due to the slightly larger nanoparticle size of the doped Zinc oxide with silver, also in agreement with Balguerie *et al.,* 2007 about the relevance of the particle size.

Comparison of the middle and apical thirds of all groups showed no significant difference, this can be attributed to the decrease in the effectiveness of the smear layer removal technique (O`Connell *et al.,* 2000) in addition to the limitation of the obturation technique (De Deus *et al.,* 2004). Table3

In conclusion, incorporation of nanoparticles improved the flow properties of the endodontic sealer materials. Among the different types of nanoparticles used, Sealer mixed with Silver nanoparticles showed the deepest penetration, while when mixed with Doped Zinc oxide with silver nanoparticles showed the least depth. This was influenced by the nanoparticles` size which translated in better sealer penetration into the dentinal tubules.

Acknowledgment

The authors thank Dr. A. Massoud, Dr. N. Mokhless and Dr. T. Shalaby, for their valuable time, effort and priceless contribution.

The authors declare no other conflict of interest.

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11/10/2015