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Antibacterial Efficacy of Chlorhexidine Irrigant Loaded with Nano Silver particles and its Effect on Root Canal Filling Adaptability: An In vitro study

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Abstract

Introduction: The aim of the current study was to evaluate the antibacterial efficacy of Chlorhexidine/ Silver nanoparticles combination against *E. faecalis* biofilm as well as their effect on adaptation of filling materials on dentin.

Methods: 60 extracted premolars were divided into two groups (n=30). Group1 to assess the antimicrobial efficacy; was subdivided into three subgroups (n=10). Subgroup 1A: 2% Chlorhexidine (CHX), subgroup 1B: 100ppm silver nanoparticles (AgNPs) solution and subgroup 1C: combination of both. Group2 (n=30) to assess the adaptation of filling materials; was subdivided into three subgroups (n=10) in the same manner as group1. The antibacterial efficacy was determined by counting the number of colony-forming units (CFU/mL) before and after irrigation and the adaptation of filling materials was evaluated by scoring the presence of gaps between the filling materials and the root dentine.

Results: The CHX/AgNPs combination was the most effective solution against *E. Faecalis*. No significant difference was found in filling materials adaptation.

Conclusion: Irrigation with CHX/ AgNps combination results in significantly less CFU/ml of *E. faecalis*.

Introduction

Bacteria play a major role in the pathogenesis of apical periodontitis; therefore, success of endodontic treatment is mainly dependent on its eradication before root canal obturation[1] The success rate of endodontic treatment was claimed to be

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approximately 10-15% lower for teeth which yield a positive culture before filling than for teeth which yield a negative culture[1].

Chlorhexidine (CHX) has a broad-spectrum antimicrobial activity comparable to that of Sodium hypochlorite (NaOCl). Several studies showed it to be superior to NaOCl[4]La Chaux-de-Fonds, Switzerland, some concluded that it showed no added effect but hypochlorite often achieved more negative cultures than CHX[2]. While other studies proved that both are similarly effective[3].

Recently, Different Nano-particle types were used due to their known antimicrobial capabilities as endodontic irrigants [4,5]. They possess bactericidal activity against Gram-positive and Gram-negative bacteria. AgNPs proved to be effective against oral pathogens as shown in a report suggesting that AgNPs of 10 nm particle size were as effective as 2.25% Sodium hypochlorite at eradication of E faecalis6.The bactericidal potential of AgNPs especially against gram negative and multi drug resistant bacteria such as E. faecalis was found to be more efficient when used in addition to CHX for root canal disinfection[7].

Bacterial recontamination would jeopardize healing of periapical tissues[8]. The use of different irrigants during root canal preparation affects the adaptation of the final obturation, maybe du to effects of those solutions on dentin surface and their ability to remove smear layer[9]. The aim of the current study was to evaluate the antibacterial efficacy of CHX/AgNPs combination against *E. faecalis* biofilm as well as their effect on adaptation of filling materials on dentin surface.

Materials and Methods

2.1 Silver Nano particles preparation:

Silver Nanoparticles suspension was prepared by the chemical reduction method described by **Turkevichet al**[10] and **Lee et al**[11]. A silver nitrate (AgNO3) solution was used as silver (Ag) precursor. Then Polyvinylpyrrolidone (PVP) was used as stabilizing agent and Sodium Borohydride as a reducing agent. The resultant nanoparticles were spherical in shape with an average size of 20 ±5 nm.

Chlorhexidine loading with Silver Nano particles.

AgNPs were dispersed in the required amount of CHX to give a final concentration of 100ppm then mixed overnight by hot plate and stirring in a Stuart heat-stir UC152 device to obtain a uniformly mixed solution as described by **Charannya et al**[12].

2.3 Preparation of samples:

A total of 60 extracted single rooted – single canaled mandibular premolars were included. Access cavity was prepared using #2 round bur and Endo-Z bur. Working length determination was done by extruding a #15 K-file beyond the apex followed by its withdrawal to be one mm shorter than the apex. Mechanical preparation was done using rotary ProTaper Universal instruments up to size F4. During instrumentation canals were irrigated using 2.5% NaOCl. Smear layer was removed using 5ml of 2.5% NaOCl and 5 ml 17 % EDTA. After chemo mechanical preparation, teeth were randomly divided into two main groups of 30 teeth each.

2.4 Antibacterial efficacy:

Group1 (n=30): Teeth were autoclaved to achieve sterility before induction of *E. Faecalis* biofilm. Teeth were filled with a 24-hour pure culture suspension of *E. faecalis* grown in Brain Heart Infusion (BHI) broth and incubated at 37°C in sealed vials for 21 days. Root canals where then divided equally and randomly to three subgroups according to irrigant used as follows: subgroup1A: 2% CHX group, subgroup1B: AgNPs group and subgroup 1C: combination group. Activation of irrigants was done in all subgroups by manual dynamic agitation. After irrigation, the root canals were flushed with 5 ml saline solution. Initial samples (S1) were collected before flushing the canals with the tested irrigants to estimate the CFU/mL before irrigation and final samples (S2) were collected after the final flush using

sterile paper points to obtain CFU/ mL after irrigation.

2.5 Root canal filling adaptation:

Group2 (n=30) canals were subdivided into 3 subgroups (n=10) subgroup 2A subgroup 2B and subgroup 2C where rrigation and activation was done in the same manner as in group 1. All canals were dried, obturated using the lateral compaction with Gutta percha and AH plus sealer. Teeth were stored for one week to ensure complete setting of the root canal sealer before evaluation then sectioned perpendicular to the long axis at apical and middle parts using a sterile diamond disc. Sections were evaluated under Scanning Electron Microscope (SEM) at 200x and the presence of gaps between the filling material and dentinal walls was scored according to the criteria described by **Tran et al**[13] as follows: *Score 1* (well adapted) no gaps in >70% of the circumference of the canal. *Score 2* (moderately adapted) gaps observed in 30–60% of the circumference of the canal. *Score 3* (poorly adapted): significant gaps in >60% of the circumference of the canal.

Results

3.1 antibacterial efficacy:

There was no statistically significant difference between the three subgroups (group 1A, group 1B and group 1C) regarding the number of CFU/ml before irrigation (P value=0.549). The CHX/ AgNPs combination had the highest antibacterial efficacy against *E. faecalis* biofilm as shown in table 1 and figure 1, where A highly significant statistical difference was found between the three subgroups regarding the number of CFU/ml after irrigation with the tested solutions (P value <0.001).

Table 1: Mean, Standard deviation (SD), minimum, maximum and P-value for the CFU/ml number after irrigation.

		Group 1 A	Group 1 B	Group 1 C	P value
Colony count after (CFU/ml)	Mean (SD)	2350 (1504.99) a	2170 (3467) a	120 (122.93) a	<0.001
	Minimum	1000	500	0	
	Maximum	5000	12000	300	

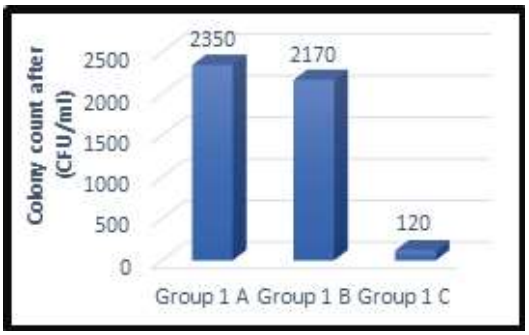


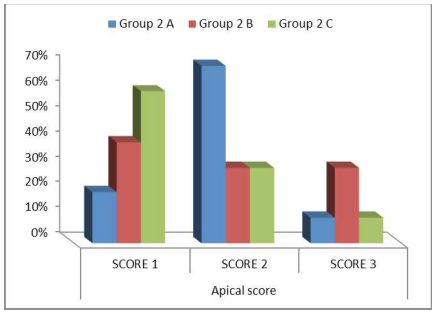
Fig 1: Number of colony forming units per milliliter (CFU/ml) across groups after irrigation.

Adaptation of root canal filling materials:

There were no statistically significant differences between the three groups regarding the apical third (P =0.255) and middle third (P value=0.119) adaptation scores as shown in table 2 and figures 3 and 4.

Table 2: Count and percentage of different adaptation scores in apical & middle thirds in the 3 groups.

		Group 2 A		Group 2 B		Group 2 C		
		Count	**%**	**Count**	**%**	**Count**	**%**	**P value**
Apical scores	**SCORE 1**	2[a]	20.0%	4[a]	40.0%	6[a]	60.0%	0.255[ns]
	SCORE 2	7[a]	70.0%	3[a]	30.0%	3[a]	30.0%	
	SCORE 3	1[a]	10.0%	3[a]	30.0%	1[a]	10.0%	
Middle scores	**SCORE 1**	2[a]	20.0%	4[a]	40.0%	6[a]	60.0%	0.119[ns]
	SCORE 2	7[a]	70.0%	2[a]	20.0%	3[a]	30.0%	
	SCORE 3	1[a]	10.0%	4[a]	40.0%	1[a]	10.0%	



Fg3: Bar chart representing the percentages of different apical third scores in each group.

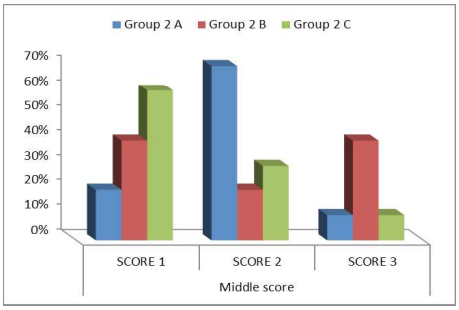


Fig4: Bar chart representing the percentage of different middle third scores in each group.

Discussion

One of the critical points in root canal therapy is chemical disinfection of the root canals along with mechanical preparation, due to inability of endodontic files to remove all inaccessible microorganisms and organic debris, also due to the major role of bacteria and their byproducts in both initiation and aggravation of pulpal and periapical diseases[14].

Due to *E. faecalis* major role in primary and secondary endodontic infections, it was incubated for 21 days as reported by **Guerreiro-Tanomaru et al** [15] inside the root canals. In this study, 2% CHX was used due to its broad-spectrum antimicrobial activity and substantivity. It's been proved to be as effective as NaOCl [16,17,18] without its drawbacks including irritation of the periapical tissues and burning of surrounding tissues[19]. Moreover, **Ercan et al**[20] reported that a 2-min rinse of 2% CHXcan be used to remove *E. faecalis* from the dentinal tubules up to 100 μ.

In the current study Silver Nanoparticles of a size 20±5 nm at a concentration of 100 ppm was used. It has been suggested that Silver Nanoparticles solution has a positive bactericidal effect against *E. Faecalis* and the same effect occurred when compared with 2.25% NaOCl[6]. Other studies proved that AgNPs with size ranges from 10–100 nm showed powerful bactericidal action against both gram positive and gram negative bacteria[21]. Previous studies concluded that AgNPs at a low concentration of 100 ppm was proved to be effective as an antimicrobial agent[22] and safe regarding cytotoxicity and cell viability[23].

Regarding the antimicrobial efficacy of the tested irrigants; the CHX/ AgNPs combination was

significantly better than both 2% the CHX ($p < 0.001$) and AgNPs ($P = 0.005$), this could be explained by a synergistic effect that enhanced the efficacy of both solutions. This agreed with the findings of **Charannya et al** [12] who evaluated the effects of 2% CHX, AgNPs and their combination on *E. faecalis* by measuring the zones of inhibition and found the combination to be the most effective solution.

Regarding the adaptation of filling materials, there was no statistically significant difference among groups at the apical and the middle thirds scores yet the best adaptation was observed in the CHX/ AgNPs group were 60% of specimens had good adaptation in both apical and middle thirds. The differences in the adaptation of root canal filling materials could be explained by the capability of AgNPs to remove the smear layer as suggested by **González et al**[6]**.** It also known that CHX significantly improves resin-dentin bond stability as it is known to be a protease inhibitor, that can suppress the action of dentin MMPs [24].

conclusions

Within the limitations of the current study; it can be concluded that Irrigation with CHX/ AgNPs combination results in significantly less CFU/ml of E. faecalis and that it doesn't significantly improve root canal filling adaptation.

References

1. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J. 1997;30(5):297-306.

2. Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM. In vivo evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. J Endod. 1982;8(5):200-204.

3. Zandi H, Rodrigues RCV, Kristoffersen AK, et al. Antibacterial Effectiveness of 2 Root Canal Irrigants in Root-filled Teeth with Infection: A Randomized Clinical Trial. J Endod. 2016;42(9):1307-1313

4. Del Carpio-Perochena A, Kishen A, Felitti R, et al. Antibacterial Properties of Chitosan Nanoparticles and Propolis Associated with Calcium Hydroxide against Single- and Multispecies Biofilms: An In Vitro and In Situ Study. J Endod. 2017;43(8):1332-1336.

5. Shrestha A, Kishen A. Antibacterial Nanoparticles in Endodontics: A Review. J Endod. 2016;42(10):1417-1426.

6. Gonzalez-Luna PI, Martinez-Castanon GA, Zavala-Alonso NV, et al. Bactericide Effect of Silver Nanoparticles as a Final Irrigation Agent in Endodontics on Enterococcus faecalis: An Ex Vivo Study. J Nanomater. 2016;2016

7. Zheng T, Huang X, Chen J, et al. A liquid crystalline precursor incorporating chlorhexidine acetate and silver nanoparticles for root canal disinfection. Biomater Sci. 2018;6(3):596-603.

8. Mazotti D, Sivieri-Araujo G, Berbert FL, Bonetti-Filho I. In vitro evaluation of the obturation ability, adaptation and compaction of gutta-percha in the root canal system employing different filling techniques. Acta Odontol Latinoam. 2008;21(1):3-9.

9. Penaz BJ. Effect of Endodontic Irrigation Protocols on Dentin Wettability and Tubule Penetration of Calcium Silicate Sealer. 2018;(August).

10. Peter, John HJT, Cooper. A study of the nucleation and growth process in the synthesis of colloidal gold. Discuss Faraday Soc. 1951;55(c):55-75.

11. Lee PC, Meisel D. Adsorption and surface-enhanced Raman of dyes on silver and gold sols - The Journal of Physical Chemistry (ACS Publications). J Phys Chem. 1982;60439(50 mL):3391-3395.

12. Charannya S, Duraivel D, Padminee K, Saravanan P, Nishanthine C, Srinivasan M. Comparative Evaluation of Antimicrobial Efficacy of Silver Nanoparticles and 2% Chlorhexidine Gluconate When Used Alone and in Combination Assessed Using Agar Diffusion Method: An In Vitro Study. Vol 9.; 2018.

13. Tran D, He J, Glickman GN, Woodmansey KF. Comparative Analysis of Calcium Silicate – based Root Filling Materials Using an Open Apex Model. J Endod. 2016:1-5.

14. Silva EJ, Herrera DR, Souza-Junior EJ, Teixeira JM. Influence of irrigation and obturation techniques on artificial lateral root canal filling capacity. Acta Odontol Latinoam. 2013;26(2):112-115.

15. Guerreiro-tanomaru JM, Ordinola-zapata R. Comparative Analysis of Enterococcus faecalis Biofilm Formation on Different Substrates. 2013;39(3):346-350.

16. Rôças IN, Provenzano JC, Neves MAS, Siqueira JF. Disinfecting Effects of Rotary Instrumentation with Either 2.5% Sodium Hypochlorite or 2% Chlorhexidine as the Main Irrigant: A Randomized Clinical Study. J Endod. 2016;42(6):943-947.

17. Estrela C, Ribeiro RG, Estrela CRA, Pécora JD, Sousa-Neto MD. Antimicrobial effect of 2% sodium hypochlorite and 2% chlorhexidine tested by different methods. Braz Dent J. 2003;14(1):58-62.

18. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of long-term exposure to endodontic disinfecting solutions on young and old Enterococcus faecalis biofilms in dentin canals. J Endod. 2014;40(4):509-514.

19. Jaiswal N, Sinha DJ, Singh UP, Singh K, Jandial UA, Goel S. Evaluation of antibacterial efficacy of Chitosan, Chlorhexidine, Propolis and Sodium hypochlorite on Enterococcus faecalis biofilm: An in vitro study. J Clin Exp Dent. 2017;9(9):e1066-e1074.

20. Ercan E, Ozekinci T, Atakul F, Gul K. Antibacterial Activity of 2% Chlorhexidine Gluconate and 5.25% Sodium Hypochlorite in Infected Root Canal: In Vivo Study. J Endod. 2004;30(2):84-87.

21. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod. 2014;40(2):285-290

22. Afkhami F, Akbari S, Chiniforush N. Entrococcus faecalis Elimination in Root Canals Using Silver Nanoparticles, Photodynamic Therapy, Diode Laser, or Laser-activated Nanoparticles: An In Vitro Study. J Endod. 2017;43(2):279-282.

23. Takamiya AS, Monteiro DR, Bernabé DG, et al. In Vitro and in Vivo Toxicity Evaluation of Colloidal Silver Nanoparticles Used in Endodontic Treatments. J Endod. 2016;42(6):953-960.

24. Tjäderhane L, Tay FR. Subclinical Degradation of Dentin Hybrid Layers in vivo. 2005:741-746.rupting: A Case report Wael A. Hussein* , Dina Y. Fouad** , Tamer A. Nasr***

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