Journal of Pharmaceutical Research International

32(15): 68-74, 2020; Article no.JPRI.59659
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antibacterial Efficacy of Nanoparticle-Incorporated Root Canal Sealer against Common Endodontic Pathogens - An *in vitro* Study

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Authors' contributions

This work was carried out in collaboration between both authors. Author SH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IN guided through the entire process. Both the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i1530627
Editor(s):
(1) Dr. Olorunjuwon Omolaja Bello, University of Medical Sciences, Nigeria.
Reviewers:
(1) Abdel Haleem Mostafa Hussein, Al-Azhar University, Egypt.
(2) Carpinelli Assunta, Italy.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59659

Original Research Article

Received 28 May 2020
Accepted 03 August 2020
Published 24 August 2020

ABSTRACT

Infection caused by microorganisms play a crucial role in the induction of inflammation of pulpal as well as the periapical tissues. Factors like improper disinfection or obturation of the root canal space contributes to the failure of the root canal treatment. The aim of the study was to modify the contents of MTA-based sealer with nanoparticles and check for its antibacterial efficacy against *E. faecalis*. Silver nanoparticles (10 nm) were incorporated in MTA based sealer at various concentrations to form the test product. The antibacterial efficacy of the modified sealer was tested by well diffusion test on *E. faecalis*. The zone of inhibition (mm) was checked for each test product. Analysis of the results showed significant diameters of zones of inhibition (mm) as compared to sealer without nanoparticles. The zone of inhibition increased with the increase in the concentration of silver nanoparticles. The silver nanoparticle incorporated sealer can be used in the clinical setup to prevent reinfection of the root canal system and ensure the success of the root canal treatment.

Keywords: Antibacterial; MTA; nanoparticles; root canal treatment; sealer.

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1. INTRODUCTION

Dental caries and trauma are the leading cause of dental pain that indicates the pulpal and/or periapical inflammation and infection [1]. Endodontic management is the treatment of choice in such cases [2].The success or failure of a root canal therapy depends on the complete elimination and disinfection of the root canal system. Inability to prevent reinfection lead to the failure of root canal therapies sooner or later [3,4]. Hence, a three dimensional seal of the canal system that includes the root canal, its accessory canals and abnormal anatomy, if any is extremely important and forms a goal of the root canal therapy. Obturation is the process where the disinfected and prepared root canals are filled with suitable materials as close to the Cemento-enamel junction is possible [5]. Root canal sealers in conjunction with solid core filling materials aid in the sealing of the canal system three dimensionally [6]. Sealers help overcome the limitations of Gutta percha by filling minute microscopic space between the dentinal wall and gutta percha. Major functions of a root canal sealer are to seal canal systems against bacterial in growth from oral cavity, entombment of leftover viable microorganisms and complete obturation of the canal system at a submicroscopic level to prevent stagnant fluid from accumulation and serving as bacterial nutrition [7].

The ideal properties of sealers include establishment of hermetic seal, minimal cytotoxicity to periodontal ligament cells, tackiness when mixed to provide adhesion, radiopacity to be seen on a radiograph, lack of shrinkage and staining of tooth structure [8]. Root canal sealers that possess superior sealing ability and antimicrobial activity would hence be beneficial in clinical aspects [9] as they can not only prevent bacteria from re-entering and re-infecting the canal system but also inactivate the remaining viable bacteria of the canal system post-obturation.

Root canal sealers commonly used belong to the categories of zinc oxide eugenol (ZOE), epoxy resin (ER) and calcium hydroxide (CH), based on the basic composition of the sealers [10]. Calcium silicate based cement consisting of added metal oxides lead to the development of Mineral Trioxide Aggregate (MTA) that has bioactive properties [11]. Apart from being extremely biocompatible, it is seen to stimulate tissue repair and induce mineralization [12,13]. These reasons make MTA a suitable material to be used as a root canal sealer as it fulfills almost of the criteria for a material to be called an ideal root canal sealer.

Silver has gained fame of being an antimicrobial agent and is being incorporated to check for its antimicrobial efficiency against various species of microorganisms important as per the dental perspective [14].

We have numerous highly cited publications on well-designed clinical trials and lab studies [15–30]. This have provided the right platform for us to pursue the current study. Our aim was to evaluate the antimicrobial efficacy of a MTA-based sealer against the common pathogen associated with the failure of root canal therapy when incorporated with silver nanoparticles *In vitro.*

2. MATERIALS AND METHODS

2.1 Test Product

Silver nanoparticles (10 nm) purchased from Sigma Aldrich Company was used in this study. Silver nanoparticles 20 μg/mL solution in aqueous buffer, contains sodium citrate as stabilizer. The endodontic sealer MTA-Fillapex, developed by Angelus (Londrina/Parana/ Brazil) and launched commercially in 2010 was mixed with 0.5%, 1.0%, 2.5%, 5%, and 10% of silver nanoparticles.

2.2 Bacterial Strain

Bacterial strain Gram positive *Enterococcus faecalis* ATCC 29212, was chosen based on their clinical and pharmacological importance. The bacterial strain was cultured on nutrient agar by using spread plate technique on Mueller-Hilton Agar (MHA) plates and was incubated for 24 hours at 37°C. The stock cultures were maintained on nutrient agar slants at 4°C. The test organism was prepared by inoculating colonies into 20 ml of nutrient broth in a 100 ml Erlenmeyer flask and incubated for 24 hours at 37°C.

2.3 Testing Groups

The testing groups included negative control only the medium without any test compound; sealer control, only the sealer without nanoparticles; sealer+silver nanoparticles at different concentrations (0.5, 1.0, 2.5, 5, 10%) of silver nanoparticles mixed with the sealer.

2.4 Antimicrobial Susceptibility Test

2.4.1 Well diffusion test

Seven uniform wells of size 6 mm were prepared on Mueller-Hilton Agar inoculated with E. faecalis. The experimental solutions were added to the respective wells on each plate. These plates were incubated for 24 hours at 37°C in an incubator. After the incubation period, plates were checked for zones of inhibition of bacterial growth and diameters of the zones achieved by each group against *E. faecalis* were recorded in millimeters (mm). All tests were carried out three times to ensure reliability, and the average of the three replicates for each test sample was calculated.

2.5 Statistical Analysis

Multiple comparisons were performed using one-way analysis of variance (ANOVA). Statistical significance was accepted at a level of $p<0.05$. Data were analysed using SPSS (Version 11).

3. RESULTS AND DISCUSSION

MTA Fillapex, developed by Angelus (Londrina/Parana/ Brazil) and launched commercially in 2010, is seen to comprise natural resin, salicylate resin, diluting resin, bismuth trioxide, nanoparticulated silica, MTA and pigments [31]. There have been claims that MTA Fillapex has a good antimicrobial efficiency [32–36]. This claim is in consistency with the findings of the present study as the unmodified test product was seen with a little zone of inhibition against the growth of *E. faecalis*. The test product was shown to have significant antimicrobial efficiency against *E. faecalis* with increasing concentration of silver nanoparticles. The incorporation of silver nanoparticles increased the zone of inhibition of the test products against *E. faecalis*. The diameter of the zone of inhibition is directly proportional to the percentage of silver nanoparticles added (Table 1 and Plate 1). The original test product without silver nanoparticles is the least. The silver ion was tested and found to be effective against a broad range of microorganisms. Silver ions have been used to control bacterial growth in a variety of medical applications. With the discovery of antimicrobial drugs, the use of silver as an antimicrobial substance was reduced. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups and it has a deadly effect on bacterial enzymes, bacterial growth and cell division and results in damage of bacterial cell wall and contents [37]. The emergence of antibiotic resistant species, revived the use of silver as an antimicrobial agent. These silver particles are extremely efficient when delivered in particles of nano ranges. The nano range comprises particles with a size of 1 to 100 nm. The silver nanoparticles are stored in a liquid medium to prevent agglomeration and entrapment of the particles within the matrix. Lara et al. implied that the particle shape, size, distribution and agglomeration are important characteristics of nanoparticles [38]. These factors determine the distribution of the particles *in-vivo*, their biological fate, toxic effects, and targeting ability.

Silver nanoparticles are non-toxic at low concentrations and have a broad spectrum of antibacterial activity that includes many Gram positive, Gram negative and antibiotic drug resistant species like MRSA [39]. The mechanism of action of the silver nanoparticles could be attributed to the ability of the silver ion to bind to the negatively charged part of the bacterial cell wall. The basic function of the bacteria was disturbed causing a leak in the cytoplasmic contents and rupture of the cell membrane [40]. Further, the silver particles infiltrate within the cytoplasm causing interaction with the nuclear content and bacterial cell deaths. The MIC and MBC of silver nanoparticles against *Enterococcus faecalis* as determined is 5 mg/ml [41].

Correa et al [42]. suggested the antimicrobial effectiveness of silver nanoparticles incorporated in various dental material. Silver nanoparticles at varying concentrations have been incorporated in a variety of materials like composite resin, adhesive system, acrylic resin, tissue conditioners, intracanal irrigants and medicaments, gutta percha, root canal sealers, MTA and titanium implants.

The root canal sealers play an important role in overcoming the limitations of the root canal filling materials. Various kinds of nanoparticulate matter are constantly being used to modify the conventional canal sealers so that the chemical, physical or antimicrobial properties of these sealers can be improved. Chitosan and zinc oxide nanoparticles added to sealers were seen

Table 1.Shows the inhibitory zone of the microorganism (in mm)

Samples	Concentration	Zone of inhibition (in mm.)
Negative Control	100µl	NI
Sealer Control	1%	2.6 ± 0.11
Sealer+ AgNP	0.5%	4.6 ± 0.28
Sealer+ AgNP	1.0%	7.8 ± 0.42
Sealer+ AgNP	2.5%	9.3 ± 0.35
Sealer+ AgNP	5.0%	12.4 ± 0.85
Sealer+ AgNP	10.0%	16.7 ± 0.71

NI means no inhibition zone. Each value is expressed as mean ± SD (n = 3). It can be inferred that the zone of inhibition increases proportionally with the concentration of the silver nanoparticles

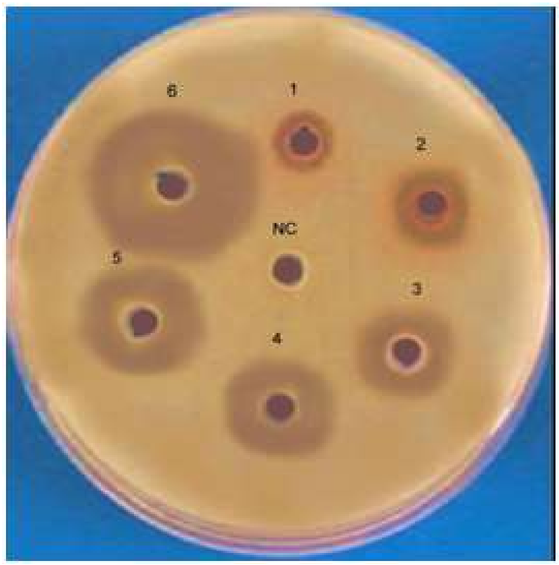

Plate 1. Zone of inhibition against *E. faecalis*

NC-Negative control; 1 – Sealer control; 2 - Sealer + AgNP(0.5%); 3- Sealer + AgNP(1.0%); 4- Sealer + AgNP(2.5%); 5- Sealer + AgNP(5.0%); 6-Sealer + AgNP(10.0%).

to effectively eliminate the bacterial biofilm and disrupts the biofilm structure. It was seen to be retaining this property through aging [43,44]. The antimicrobial efficiency of zinc oxide and silver nanoparticles incorporated zinc oxide eugenol based sealer proved silver nanoparticles have a superior antibacterial effect [45]. Also, silver nanoparticles incorporated within a zinc oxide based sealer with increasing concentration presented with results comparable and in consistency with the present study [46]. Zirconium oxide incorporated MTA seemed to have greater strength and radiopacity but mild antibacterial effect on *E.faecalis* [47]. Amongst other nanoparticles that have been incorporated in the root canal sealers, quarternary ammonium polyethylenimine and bioactive glass [48]. The former nanoparticles improvised the antibacterial efficacy, while the latter promotes closure of the interfacial gap between root canal walls and core filling material. However, the antimicrobial effect of bioactive incorporated canal sealers is still under study.

4. CONCLUSION

The present *In vitro* study gives us a perspective of silver nanoparticles-modified MTA-based sealers to be efficient in controlling the growth of *E. faecalis* in laboratory environments. Also, the periodontal cell viability is seen to increase as the proportion of silver nanoparticles to sealer increases. However, more clinical oriented studies need to be done to check for other aspects like toxicity, discoloration or microleakage in the canal of this silver nanoparticles-modified sealer. Also, the antimicrobial efficiency over time needs to be checked.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nair PNR. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004;15:348–81.
2. Berman DDS L, Hargreaves KM. Cohen's Pathways of the pulp expert consult. Elsevier Health Sciences; 2015.
3. Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13:29–39.
4. Lin LM, Skribner JE, Gaengler P. Factors associated with endodontic treatment failures. J Endod. 1992;18:625–7.
5. Schilder H. Filling root canals in three dimensions. 1967. J Endod. 2006;32:281–90.
6. Kumaravadivel MS, Pradeep DS. Recent advancements of endodontic sealers- a review; IJPT. 8(2):4060-75
7. Orstavik D. Essential endodontology: prevention and treatment of apical periodontitis. John Wiley & Sons; 2020.
8. ØRstavik DAG. Materials used for root canal obturation: technical, biological and clinical testing. Endodontic Topics. 2005;12:25–38.
9. Branstetter J, von Fraunhofer JA. The physical properties and sealing action of endodontic sealer cements: a review of the literature. J Endod. 1982;8:312–6.
10. Shin J-H, Lee D-Y, Lee S-H. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. J Dent Sci. 2018;13:54–9.
11. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod. 1999;25:197–205.
12. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TRP. The constitution of mineral trioxide aggregate. Dent Mater. 2005;21:297–303.
13. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature Review—Part III: Clinical Applications, drawbacks, and mechanism of action. J Endod. 2010;36:400–13.
14. Noronha VT, Paula AJ, Durán G, Galembeck A, Cogo-Müller K, Franz-Montan M, et al. Silver nanoparticles in dentistry. Dent Mater. 2017;33:1110–26.
15. Ramarao S, Sathyanarayanan U. CRA Grid - A preliminary development and calibration of a paper-based objectivization of caries risk assessment in undergraduate dental education. J Conserv Dent. 2019;22:185–90.
16. Poorni S, Srinivasan MR, Nivedhitha MS. Probiotic strains in caries prevention: A systematic review. J Conserv Dent. 2019;22:123–8.
17. Manohar MP, Sharma S. A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. Indian J Dent Res. 2018;29:716–20.
18. Azeem RA, Sureshbabu NM. Clinical performance of direct versus indirect composite restorations in posterior teeth: A systematic review. J Conserv Dent. 2018;21:2–9.
19. Jenarthanan S, Subbarao C. Comparative evaluation of the efficacy of diclofenac sodium administered using different delivery routes in the management of endodontic pain: A randomized controlled clinical trial. J Conserv Dent. 2018;21:297–301.
20. Nandakumar M, Nasim I. Comparative evaluation of grape seed and cranberry extracts in preventing enamel erosion: An optical emission spectrometric analysis. J Conserv Dent. 2018;21:516–20.
21. malli sureshbabu n, selvarasu k, v jk, nandakumar m, selvam d. concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: A Report of Two Cases. Case Rep Dent. 2019;2019:7046203.
22. Siddique R, Nivedhitha MS, Jacob B. Quantitative analysis for detection of toxic elements in various irrigants, their combination (precipitate), and para-chloroaniline: An inductively coupled

plasma mass spectrometry study. J Conserv Dent. 2019;22:344–50.
23. Teja KV, Ramesh S, Priya V. Regulation of matrix metalloproteinase-3 gene expression in inflammation: A molecular study. J Conserv Dent. 2018;21:592–6.
24. Rajakeerthi R, Ms N. Natural Product as the Storage medium for an avulsed tooth – A Systematic Review. Cumhuriyet Dental Journal. 2019;22:249–56.
25. Siddique R, Nivedhitha MS. Effectiveness of rotary and reciprocating systems on microbial reduction: A systematic review. J Conserv Dent. 2019;22:114–22.
26. Janani K, Sandhya R. A survey on skills for cone beam computed tomography interpretation among endodontists for endodontic treatment procedure. Indian J Dent Res. 2019;30:834–8.
27. Siddique R, Sureshbabu NM, Somasundaram J, Jacob B, Selvam D. Qualitative and quantitative analysis of precipitate formation following interaction of chlorhexidine with sodium hypochlorite, neem, and tulsi. J Conserv Dent. 2019;22:40–7.
28. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. Pesqui Bras Odontopediatria Clin Integr. 2019;19:1–10.
29. Govindaraju L, Neelakantan P, Gutmann JL. Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements. Clin Oral Investig. 2017;21:567–71.
30. Khandelwal A, Palanivelu A. Correlation Between Dental Caries And Salivary Albumin In Adult Population In Chennai: An In Vivo Study. BDS. 2019;22:228–33.
31. Bin CV, Valera MC, Camargo SEA, Rabelo SB, Silva GO, Balducci I, et al. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. J Endod. 2012;38:495–500.
32. Faria-Júnior NB, Tanomaru-Filho M, Berbert FLCV, Guerreiro-Tanomaru JM. Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J. 2013;46:755–62.
33. Kuga MC, Faria G, Weckwerth PH, Duarte MAH, Campos EAD, Só MVR, et al. Evaluation of the pH, calcium release and antibacterial activity of MTA Fillapex. Rev Odontol UNESP. 2013;42:330–5.
34. Rahman H, Chandra R,Chowdhary D, Singh S, Tripathi S, Anwar SZ. Antimicrobial Activity of MTA Fillapex, Real Seal SE, Acroseal and zinc oxide eugenol sealers against *Enterococcus faecalis* and *Candida albicans*. IOSR Journal of Dental and Medical Sciences. 2017;16(1):66-69.
35. Hasheminia M, Razavian H, Mosleh H, Shakerian B. *In vitro* evaluation of the antibacterial activity of five sealers used in root canal therapy. Dent Res J. 2017;14:62–7.
36. Ustun Y, Sagsen B, Durmaz S, Percin D. *In vitro* antimicrobial efficiency of different root canal sealers against *Enterecoccus faecalis*. European Journal of General Dentistry. 2013;2:134.
37. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Appl Environ Microbiol. 2008;74:2171–8.
38. Lara HH, Garza-Treviño EN, Ixtepan-Turrent L, Singh DK. Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. J Nanobiotechnology. 2011;9:30.
39. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16: 2346–53.
40. Ibrahim AIO, Moodley DS, Petrik L, Patel N. Use of antibacterial nanoparticles in Endodontics. S Afr Dent J. 2017;72:105–12.
41. Krishnan R, Arumugam V, Vasaviah SK. The MIC and MBC of silver nanoparticles against Enterococcus faecalis-a facultative anaerobe. J Nanomed Nanotechnol. 2015;6:285.
42. Corrêa JM, Mori M, Sanches HL, Cruz AD da, Poiate E, Poiate IAVP. Silver nanoparticles in dental biomaterials. Int J Biomater. 2015;2015.
43. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod. 2010;36:1030–5.
44. Kishen A, Shi Z, Shrestha A, Neoh KG. An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod. 2008;34:1515–20.

45. Hala F, Mohammed M, Thanaa I, Others. Antibacterial effect of two types of nano particles incorporated in zinc oxide based sealers on Enterococcus faecalis (in vitro study). Alex Dent J. 2016;41:169–75.
46. Alzaidy FA, Khalifa AK, Emera RMK. The antimicrobial efficacy of nanosilver modified root canal sealer. European Journal of Research in Medical Sciences. 2018; 6.
47. Guerreiro-Tanomaru JM, Trindade-Junior A, Costa BC, da Silva GF, Drullis Cifali L, Basso Bernardi MI, et al. Effect of zirconium oxide and zinc oxide nanoparticles on physicochemical properties and antibiofilm activity of a calcium silicate-based material. Scientific World Journal. 2014;2014:975213.
48. Kishen A, Shrestha A. Nanoparticles for Endodontic Disinfection. In: Kishen A, editor. Nanotechnology in endodontics: current and potential clinical applications, Cham. Springer International Publishing. 2015;97–119.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59659