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Evaluation of Antimicrobial Effect of Nano-impregnated MTA in Dentin Blocks (An in Vitro Study)

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ABSTRACT

Background: Perforations either pathologic or iatrogenic can result in inflammatory reaction to the periodontium, bone resorption, and the formation of granulomatous tissue. If not sealed properly, reinfection in the root canal space can occur leading to periapical lesions, bone loss and eventually tooth loss. ***Objectives***: To evaluate the antimicrobial effect of nano-silver and nano-chitosan impregnated MTA compared to conventional MTA by confocal scanning laser microscopy. ***Materials and Methods***: Forty eight samples were divided into three groups according to the tested material: group A was nanosilver impregnated MTA, group B was nanochitosan impregnated MTA and group C was conventional MTA. After bacterial inoculation to the dentin blocks and the addition of the tested materials to each dentin specimen, the samples were stained by Acridine orange and Proprium iodine. Image analysis was done using Zeiss blue software to count the number of dead and living bacterial cells. The percentage of living bacteria was calculated by dividing the number of live bacteria by the total number of bacteria then multiplied by hundred. ***Results:*** Nanosilver impregnated MTA showed a significantly higher antibacterial property followed by the nanochitosan impregnated MTA and finally the conventional MTA. ***Conclusion***: The antibacterial property and solubility of MTA was enhanced by the addition of nanoparticles. The addition of Nanosilver to MTA presented a greater antibacterial effect than nanochitosan.

Keywords: Perforation repair, Mineral trioxide aggregate (MTA), Nano-particles, Chitosan, Silver.

INTRODUCTION

Perforations are defined as pathologic or iatrogenic communications between the root canal space and the attachment apparatus. Pathologic perforations can happen due to extensive dental caries or inflammatory external or internal resorption. Iatrogenic perforations are the result of undesirable incident that can occur at any stage of the root canal therapy such as access cavity, mechanical preparation or post space preparation.[1] The occurrence of perforations in the literature has been reported between

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three and ten percent. Perforations cause a sequala of undesirable events starting with acute inflammatory response followed by destruction of periodontal fibers, bone resorption and the formation of granulomatous tissue and eventually tooth loss.[2]

Hence, the ideal endodontic repair material should provide a hermetic seal, be biocompatible, dimensionally stable, radiopaque, set in moisture, not affected by tissue fluids, non-resorbable, bactericidal or bacteriostatic, inexpensive and easily manipulated. Different materials have been used to repair such defects. Recently, bioceramic materials such as mineral trioxide aggregate, Biodentin, Endosequence and Bioaggregate have been used to treat perforations and their performance was significantly better that other conventional materials.[3]

MTA was originally used as a retrograde filling material in endodontic surgeries. Then its uses have expanded to repair root perforations, as an apical barrier during obturation of teeth with open apices, a pulp capping material, for pulpotomies, apexifications, and regenerative procedures.[4]

In these procedures, moisture contamination occurs and most of the properties of the filling materials are negatively affected by the presence of water. However, MTA sets in moisture, and it, therefore, provides an excellent hermetic seal in the presence of water. MTA is a hydraulic cement which is composed of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, mineral oxide and bismuth oxide. The powder gets hardened in the presence of water to form a colloidal gel which then solidifies in approximately four hours.[5]

Torabinejad et al.[6] evaluated the antibacterial properties of amalgam, zinc oxide eugenol, Super EBA and MTA. It has been shown that MTA had an antibacterial effect on some facultative anaerobes; however it had no effect on strict anaerobes.

Jardine et al.[7] evaluated the survival of a multispecies microcosm biofilm after it was exposed to NeoMTA Plus, Biodentine, and MTA Angelus using confocal scanning laser microscopy. There was no substance that could eliminate all biofilm cells; all groups had more than 50% alive bacteria. It was concluded that all tested materials were ineffective against multispecies bacterial biofilm. Since bioceramic cements are only for use in contaminated clinical settings, it was argued that prior to filling with these materials, additional disinfection measures should be carried out.

Nanomaterials are natural, accidental, or synthetic materials that contain particles in unbound states, aggregates, or agglomerates, with diameters of 1 nm to 100 nm for 50% or more of the particles. They differ from the original material in terms of physical properties. This is due to its larger surface area to volume ratio, increasing its chemical activity, which results in a higher number of atoms near the surface as compared to its macrostructure.[8]

Silver nanoparticles have great antibacterial and antifungal effects. They disrupt cell walls and metabolic processes, inactivate bacterial enzymes, enhance cell permeability, and create reactive oxygen species via electrostatically interacting with cell membranes, and affecting the hydrogen bonds of the proteins. It has been shown that silver nanoparticles act by targeting certain proteins in the cells causing their denaturation, and unwinding the DNA by affecting the hydrogen bonds which stabilizes the DNA.[9-10]

Bahador et al.[11] compared the antimicrobial effect of MTA, and MTA impregnated with nanosilver particles against Fusobacterium nucleatum using agar diffusion and broth dilution methods. The results showed that mixing MTA with a concentration greater than 6% of the solution of nanosilver instead of water significantly increased the material's antibacterial effectiveness.

Liu et al.[12] tested the antibacterial activity of a silver nanoparticles poloxamer thermoreversible gel against Enterococcus faecalis in the root canal using agar counting plate, scanning electron microscope observations, and confocal laser scanning microscope analysis. It was found that silver nanoparticles poloxamer thermoreversible gel exhibits strong antimicrobial activity against the Enterococcus faecalis and is easy to produce, with a continuous release of silver ions making the gel a great candidate for a new root canal disinfection.

Hemmanur, and Nasim,[13] evaluated the antimicrobial effect of MTA based sealer when combined with nanosilver particles at different concentrations. The antibacterial effectiveness was assessed using an E. faecalis well diffusion test. It was found that the greater the concentration of the nanosilver particles added, the greater was the zone of inhibition. It was concluded that MTA based sealers containing nanosilver particles have greater antibacterial effect and can effectively control the growth of Enterococcus faecalis.

Chitosan is a natural, organic biopolymer made from chitin, which is one of

the most available natural polymers forming the main component of the exoskeleton of crustaceans such as crab and shrimp shells. Because chitosan is a cationic molecule, it works as a broad-spectrum antibacterial by interacting with negatively charged bacterial cell membranes, increasing their permeability and causing leaking of intracellular components, ultimately leading to cell death. Furthermore, chitosan is believed to bond to the surface of dentin through binding to calcium ions via its phosphate group, resulting in the formation of calcium phosphate on the dentin surface. Bacterial adhesion to the dentin can be considerably reduced as a result of this.[14]

Beshr and Abdelrahim[15] evaluated the antibacterial effect of the addition of chitosan on MTA fillapex against Enterococcus faecalis strain using the Agar diffusion test. It was found that adding chitosan to MTA fillapex improves its antibacterial efficiency against E. faecalis, and its antibacterial activity increases as the chitosan concentration increases.

Hiremath et al.[16] evaluated the antibacterial properties of adding chitosan gel to Mineral trioxide aggregate (MTA), MTA plus and Biodentine. Gram positive Enterococcus faecalis were used to produce a 3-day biofilm. Results showed that the combination of MTA, MTA Plus, and Biodentine with Chitosan had better antibiofilm capabilities than their individual equivalents. It was concluded that chitosan can be used as a novel material in dentistry to enhance the antibacterial properties of present materials.

MATERIALS AND METHODS

A total of 48 mandibular molars were selected from the MIU teeth bank. All teeth were cleaned, disinfected and examined under magnifying lens for cracks, extensive decay, root caries, vertical fracture, the presence of perforations, and resorption. The selected mandibular teeth were the ones lacking all these, with separate roots and moderate root curvature up to 20^{o} according to Schneider.[17]

Preparation of the dentin samples:

A total of 48 non identified dentin blocks were fabricated from the collected mandibular molars. Coronal and apical parts of the teeth were cut off using a double-sided diamond disk (Extec, Enfield, CT, USA) under refrigeration in Isomet 2000 precision cutting machine **(Figure 1)**. The diamond discs were used to section the middle portions of the roots vertically. A central groove was made in each dentin section using a diamond stone. Final dimension of each dentin block was 4 mm in length, 5 mm in width and 4 mm

Figure (1): showing the Isomet precision cutting machine.

in height, with a central groove 1 mm in width and 3 mm in depth to allow for the formation and growth of biofilm during the experiment **(Figure 2)**. For disinfection, each block was submerged in a 1% sodium hypochlorite solution for 15 minutes which was replenished every 5 minutes, then in 17 percent EDTA for 5 minutes. After that, sterilization was carried out at 121°C, 1atm, for 15 minutes.[18]

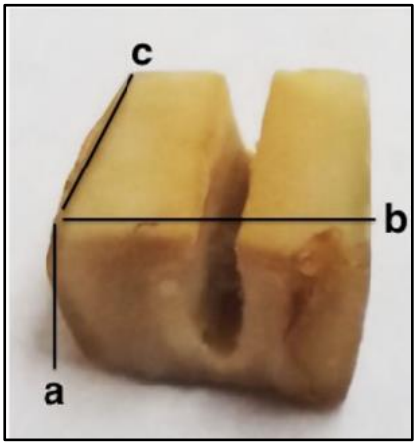

Figure (2): The dimensions of the dentin block (a) height = 4 mm, (b) width = 5 mm, and (c) length = 4 mm.

Bacterial inoculation of the specimens:

Enterococcus faecalis bacteria was cultured in Brain Heart Infusion and incubated anaerobically at 37°C for 24 hours **(Figure 3)**.

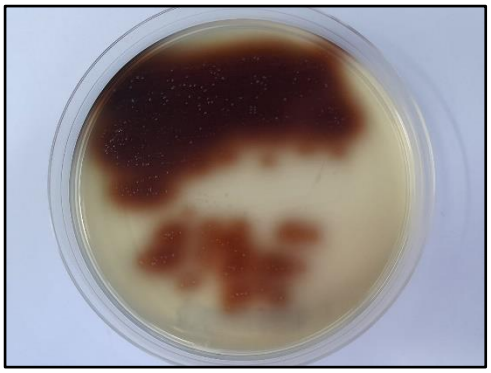

Figure (3): E. faecalis growth on the agar plate.

A single colony of E. faecalis from the BHI agar plate was collected and suspended in sterile BHI broth at 37°C. The sterilized dentin blocks were placed in sterile centrifuge tubes containing 2mL E. faecalis **(Figure 4)**. Then the specimens were

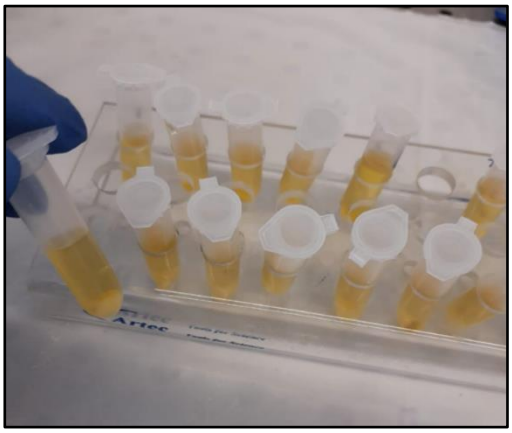

Figure (4): Sterile centrifuge tubes containing 2mL E. faecalis and the dentin blocks.

incubated at 37°C for 21 days under anaerobic conditions. Fresh BHI was

replaced every two days for removal of dead cells and to ensure the viability of the bacteria. After the incubation period, the specimens were removed from the tubes under aseptic conditions, and rinsed with saline to remove any non-adherent bacteria.

Treatment of the root canal surfaces and Confocal microscopy:

Tested materials were manually mixed according to the manufacturer's instructions with a powder to liquid ratio of 1:3. A glass slab and a metal spatula were used to mix the materials to obtain a putty like consistency while ensuring full wetting of the powder particles. Mixing time was less than 4 minutes as prolonged time can cause dehydration of the material.

The specimens were divided into 3 groups according to the material used.

Group A (n=16) was filled using a mixture of MTA with a suspension of silver nanoparticles (200 ppm)

Group B (n=16) was filled with a mixture of MTA with a suspension of chitosan nanoparticles (10 mg per ml)

Group C (n=16) was filled with MTA mixed with distilled water.

All materials were handled according to the manufacturer's instructions. They were manually mixed using a metal spatula on a glass slab with a powder to liquid ratio of 1:3.

Each loaded dentin block with the cement was labelled and incubated separately in well cell culture plates containing BHI at 37 °C for 7 days under aerobic conditions **(Figure 5 and 6)**.

Figure (5): The loaded dentin block.

Figure (6): The loaded dentin block in BHI.

All specimens were examined using confocal laser scanning microscopy. The specimens from each group were stained using fluorescent Live/Dead bacterial viability stain (acridine orange and propidium iodide) to determine the viability of the biofilm. Images of the live and dead bacteria were obtained. The live bacteria present on the biofilm were stained green and the dead ones were stained red.

The percentage of live bacteria was calculated by dividing the number of live bacteria by the total number of bacteria then multiplied by hundred.[18]

Statistical Analysis:

Numerical data were explored for normality by checking the distribution of data and using Shapiro-Wilk test of normality. All data showed normal (parametric distribution). Data were presented as mean and standard deviation (SD) values. One-way ANOVA test was used to compare between the three groups. Tukey's post-hoc test was used for pair-wise comparisons when ANOVA test is significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

The antibacterial effect of each material was tested as a measure of the living bacteria to the total number of bacteria.

Group A showed the lowest mean percentage of live bacteria (34.3% ± 8.7). Meanwhile group C showed the highest mean percentage of live bacteria (70.4% ± 8). Group B stands midway with mean percentage of live bacteria (52.4% ± 10.6) as shown in **(Figure 7)**.

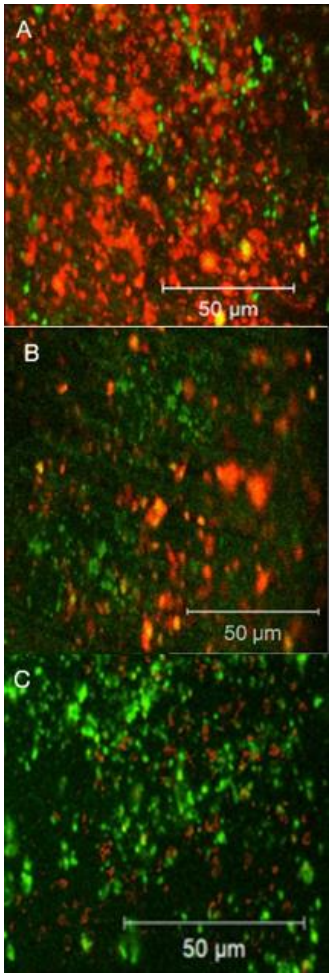

Figure (7): CLSM image representing mean percentage of the bacterial viability in the different groups. (A) Nanosilver group (34.3%), (B) Nanochitosan group (52.4%), (C) MTA group (70.4%).

Applying one-way ANOVA test, there was a statistically significant difference between the percentages of live bacteria in the three groups observed with P-value <0.001) **(Table 1 and Figure 8).**

DISCUSSION

Nanoparticles are natural or artificial substances containing particles in an

Table (1): Descriptive statistics and results of one-way ANOVA test for comparison between percentages of live bacteria (%) in the three groups.

(Group A) Nanosilver + MTA (n = 16)		(Group B) Nanochitosan + MTA (n = 16)		(Group C) MTA (n = 16)		*P*-value	*Effect size (Eta squared)*
Mean	34.3^{C}	Mean	52.4^{B}	Mean	70.4^{A}		
SD	8.7	SD	10.6	SD	8	<0.001*	0.739

**: Significant at P ≤ 0.05*
Different superscripts letters indicate statistically significant difference.

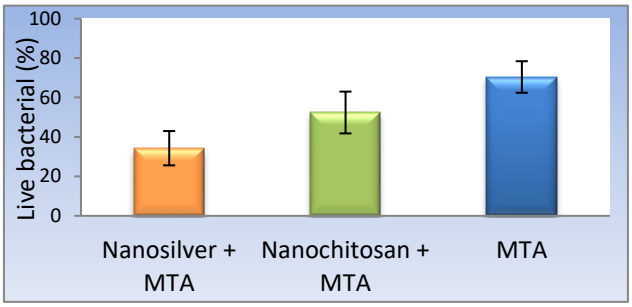

Figure (8): Bar chart representing mean and standard deviation values for percentages of living bacteria in the three groups.

unbound state or agglomerates in which at least half of these particles are in the size range of 1-100 nm. They have characteristic properties due to their significant small size giving them a larger surface area which provides them with a different chemical reactivity compared to their bulk counterparts.[19]

The increased surface area to volume allows the nanoparticles to interact at the molecular and the subcellular level. This allows the antimicrobial effect of the nanoparticles to be significantly better as they can infiltrate bacterial biofilms better. This allows them to interact electrostatically with the bacterial cell wall causing damage to the bacterial plasma membrane, and leading to increased permeability, interference with cellular functions, protein degradation, DNA damage and eventually cell death.[20]

Silver nanoparticles have been demonstrated to have antibacterial and antibiofilm activity against E. faecalis in endodontic infections.[21] The antibacterial activity of silver nanoparticles was comparable to that of traditional endodontic irrigants such as 2% chlorhexidine, 1% NaOCl, and 5% NaOCl; however, NaOCL demonstrated superior tissue dissolving action. It also demonstrated antibacterial efficacy against E. faecalis comparable to ampicillin and 2% chlorhexidine.[22]

It has been found that nanochitosan particles have antibacterial effect against E. faecalis and were able to inhibit the development and growth of the bacterial biofilm. It was also reported that the application of nanochitosan before obturation inhibited bacterial adhesion to the dentin walls and enhanced disinfection.[23]

However, according to another research, its antibacterial efficiency may be dependent on the bacterial form, as bacteria in the planktonic form were completely destroyed whereas biofilm bacteria remained viable after 72 hours.[24]

Mineral trioxide aggregate (MTA) is a biocompatible, hydrophilic endodontic cement that promotes healing and osteogenesis. It can release calcium ions for cell growth when it comes into close contact with human tissues. It also regulates cytokine production by creating an antimicrobial environment with its alkaline pH. As a result, it promotes the migration and differentiation of hard tissue-producing cells, resulting in the formation of hydroxyapatite on the MTA surface and creating a biological seal. Because of its biocompatibility, antibacterial qualities, marginal adaptability, and sealing capabilities, and ultimately, because of its hydrophilic nature, this cement is distinguished from all other conventional materials available.[25]

E. Faecalis was the chosen microorganism to be tested in this study as it is considered a resistant strain, and frequently isolated from persistent infections after root canal treatment. In addition, E. faecalis is relatively easy to culture and was successfully used in previous studies.[26-28]

The prevalence of E. faecalis in failed endodontic cases ranged between 24 and 70%, when culture-based techniques were used. However, studies using polymerase chain reaction (PCR) as the detection method reported a prevalence of 67–77%. As a result, PCR detection methods are widely used to test the antibacterial effectiveness of irrigating solutions, intracanal medicines, and preparation processes.[29]

E. faecalis have the ability to infiltrate the root canal and stay in a dormant state for a long time before becoming an infectious organism without the aid of other bacteria. E. faecalis have a unique ability to survive in alkaline environments with pH as high as 9.6 and high salt concentrations. They can succeed in competing with other microorganisms, by invading the dentinal tubules and resisting nutritional deprivation. They can colonize inside infected root canals in a biofilm structure in heterogeneous aggregation of microbial cells that are embedded in an extracellular polymeric substance matrix rather than being free floating.[29]

The antimicrobial effects of nanosilver impregnated MTA and nanochitosan impregnated MTA were compared to conventional MTA on 21 days old E. faecalis. Confocal scanning laser microscopy was

used to calculate the percentage of live/dead bacteria after exposure to the materials for one week. Confocal scanning laser microscopy requires that the organisms should be stained with specific fluorescent stains which can emit light at certain wavelengths. These stains allow the quantification of cells and the assessment of the percentage of its viability. Formerly, the agar diffusion test was used to test the antimicrobial properties of the materials. However, due to the lack of control over a large number of factors, comparing the data acquired was difficult as the diameter of the microbial inhibition zone was dependent on the solubility and the diffusion ability of the tested material.[30]

In the antibacterial study comparison of the viability of bacterial population was measured by confocal microscopy. The nanosilver group showed the lowest mean of live bacteria by 34.3%, followed by the nanochitosan group by 52.4%, and finally the MTA by 70.4%. The high antibacterial effect was in agreement with Samiei et al.[31,] Jafari et al.[32], Bahador et al.[33], Hemmanur, and Nasim.[13]

A previous study[34] showed that the antimicrobial properties of endodontic sealer was improved by the addition of nanosilver and nanochitosan against E. faecalis on monolayers on agar plates and collagen membrane surface assays. Nanochitosan particles showed lower bactericidal effect against E. faecalis; however, it was suggested that nanochitosan can be used as a carrier for bactericidal substances due to its biocompatibility. It was suggested that a combination of nanosilver and nanochitosan can be useful in in vitro and in vivo tests.

According to Waly[35], the incorporation of silver nanoparticles was also found to increase the antibacterial effect of MTA against E. coli, S. aureus and E. faecalis using disc diffusion method. It was suggested that silver nanoparticles have the potential to bind to the bacterial cell wall, causing structural damage in the cell membrane, resulting in the creation of pits, increasing cell membrane permeability, and finally causing cell death. Prabhu and Poulose[36] suggested that the production of free radicals by the nanosilver particles in interaction with the bacterial cell membrane causes cell membrane damage. It has also been proposed that ionic silver interacts aggressively with the thiol groups of bacterial enzymes, resulting in enzyme deactivation.

Bedier[37] also showed that MTA mixed with silver nanoparticles had higher antibacterial effect than MTA with saline using the direct contact test. It was suggested

that the higher antibacterial effect is attributed to the large surface area of the nanosilver particles allowing better contact with microorganism, attachment and penetration, inhibiting the DNA function, attacking the respiratory chain in the bacterial mitochondrial, and eventually leading to its death.

Waly[35] showed that mixing the MTA with the chitosan solution resulted in a significant increase in the antibacterial activity using a disc diffusion method. However, after five days of incubation, the bacteria experienced a repelling action which means that regrowth of the bacteria in the culture occurred. This suggested that chitosan has a bacteriostatic rather than a bactericidal effect, and that this bacteriostatic effect lasts less than five days.

Another study[38] showed that the addition of chitosan to MTA provided no enhancement against the biofilm models after 24 h of incubation in contrast to the addition of chitosan to biodentine using quantitative microbiological analysis on bovine dentin. It was also found that adding chitosan to MTA did not result in a significant change in pH after 24 hours. The addition of chitosan to biodentine, on the other hand, resulted in a considerable increase in pH. It was suggested that the surrounding acidic media is thought to cause protonation of chitosan amino groups, which favors electrostatic interactions between the generated positively charged chitosan molecules and negative residues at biological locations. These differences could be related to differences in antibacterial activity evaluation methods or differences in the concentrations of the materials used.

CONCLUSION

Within the limitations of this study, it was concluded that:

- The antibacterial property and solubility of MTA was enhanced by the addition of nanoparticles.
- The addition of nanosilver to MTA presented a greater antibacterial effect than nanochitosan.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. American Association of Endodontists. Glossary of endodontic terms. American Association of Endodontists; 2003.
2. Clauder T, SHIN SJ. Repair of perforations with MTA: clinical applications and mechanisms of action. Endodontic Topics. 2006 Nov;15(1):32-55.

3. Madfa AA, Al-Sanabani FA, Al-Kudami NH. Endodontic repair filling materials: A review article. J Adv in Med Med Res. 2014 Mar 13:3059-79.
4. Torabinejad M, Ford TP. Root end filling materials: A review. Dent Traumatol. 1996 Aug;12(4):161-78.
5. Lee YL, Lee BS, Lin FH, Lin AY, Lan WH, Lin CP. Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. Biomaterials. 2004 Feb 1;25(5):787-93.
6. Torabinejad M, Hong CU, Ford TP, Kettering JD. Antibacterial effects of some root end filling materials. J Endod. 1995 Aug 1;21(8):403-6.
7. Jardine AP, Montagner F, Quintana RM, Zaccara IM, Kopper PM. Antimicrobial effect of bioceramic cements on multispecies microcosm biofilm: a confocal laser microscopy study. Clin Oral Investig. 2019 Mar;23(3):1367-72.
8. Kishen A.S. Nanotechnology in Endodontics current and potential clinical applications. Cham, Switzerland: Springer Science + Business Media; 2015
9. Shrestha A, Kishen A. Antibacterial nanoparticles in endodontics: A review. J Endod. 2016 Oct 1;42(10):1417-26.
10. Melo MA, Guedes SF, Xu HH, Rodrigues LK. Nanotechnology-based restorative materials for dental caries management. Trends in Biotechno. 2013 Aug 1;31(8):459-67.
11. Bahador A, Esmaeili D, Khaledi A, Ghorbanzadeh R. An in vitro assessment of the antibacterial properties of nanosilver Iranian MTA against Porphyromonas gingivalis. J Chem Pharm Res. 2013;5(10):65-71.
12. Liu T, Aman A, Ainiwaer M, Ding L, Zhang F, Hu Q, Song Y, Ni Y, Tang X. Evaluation of the anti-biofilm effect of poloxamer-based thermoreversible gel of silver nanoparticles as a potential medication for root canal therapy. Sci Rep. 2021 Jun 15;11(1):1-6.
13. Hemmanur S. and Nasim I. Antibacterial Efficacy of Nanoparticle-Incorporated Root Canal Sealer against Common Endodontic Pathogens - An in vitro Study. J Pharm Res Int. 2020;32(15): 68-74.
14. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbio. 2010 Nov 15;144(1):51-63.
15. Beshr KA, Abdelrahim RA. Antibacterial efficacy of Allium sativum (garlic) and chitosan incorporated into two root canal sealers against Enterococcus faecalis:

Comparative study. Tanta Dent. J. 2019 Apr 1;16(2):94.

16. Hiremath G, Singh M, Kulkarni B, Potdar R, Naik B. Antibiofilm efficacy of root-end filling materials against Enterococcus faecalis-An in vitro study. Endod. 2020 Apr 1;32(2):86.
17. Schneider SW. A comparison of canal preparations in straight and curved root canals. Oral Surg, Oral Med, Oral Pathol. 1971 Aug 1;32(2):271-5.
18. Jardine AP, Montagner F, Quintana RM, Zaccara IM, Kopper PM. Antimicrobial effect of bioceramic cements on multispecies microcosm biofilm: A confocal laser microscopy study. Clin Oral Investig. 2019 Mar;23(3):1367-72.
19. Aggarwal S. Nanotechnology in endodontics: Current and potential clinical applications. Endod. 2016 Jan 1;28(1):78-.
20. Venugopal J, Prabhakaran MP, Low S, Choon AT, Zhang YZ, Deepika G, Ramakrishna S. Nanotechnology for nanomedicine and delivery of drugs. Curr Pharm Des. 2008 Aug 1;14(22):2184-200.
21. Halkai KR, Mudda JA, Shivanna V, Rathod V, Halkai R. Evaluation of antibacterial efficacy of fungal-derived silver nanoparticles against Enterococcus faecalis. Cont ClinDent. 2018 Jan;9(1):45.
22. Halkai KR, Mudda JA, Shivanna V, Rathod V, Halkai R. Antibacterial efficacy of biosynthesized silver nanoparticles against Enterococcus faecalis biofilm: An in vitro study. Cont Clin Dent. 2018 Apr;9(2):237.
23. Ionescu A, Harris D, Selvaganapathy PR, Kishen A. Electrokinetic transport and distribution of antibacterial nanoparticles for endodontic disinfection. Int Endod J. 2020 Aug;53(8):1120-30.
24. Shrestha A, Zhilong S, Gee NK, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J. Endod. 2010 Jun 1;36(6):1030-5.
25. Cervino G, Laino L, D'Amico C, Russo D, Nucci L, Amoroso G, Gorassini F, Tepedino M, Terranova A, Gambino D, Mastroieni R. Mineral trioxide aggregate applications in endodontics: A review. Eur J Dent. 2020 Jul 29.
26. Estrela C., Bammann L.L., Estrela C.R., Silva R.S., Pe´cora J.D. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz DentJ. 2000;11:3–9.

27. Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Antibacterial effect of two mineral trioxide aggregate (MTA) preparations against Enterococcus faecalis and Streptococcus sanguis in vitro. J Endod. 2006 Nov 1;32(11):1053-6.
28. Elbahary S, Haj Yahya S, Koç C, Shemesh H, Rosen E, Tsesis I. Bacterial colonization and proliferation in furcal perforations repaired by different materials: A confocal laser scanning microscopy study. ApplSci. 2021 Jan;11(8):3403.
29. Hussein HH, Abood FM, Alhelal AG. Some virulence factors of Enterococcus Faecalis isolated from root canal infections combined with effect of some irrigation solution against E. Faecalis. Sys Rev Pharm. 2020;11(6):742-8.
30. Fuss Z, Charniaque O, Pilo R, Weiss E. Effect of various mixing ratios on antibacterial properties and hardness of endodontic sealers. J Endod. 2000 Sep 1;26(9):519-22.
31. Samiei M., Aghazadeh M., Lotfi M., Shakoei S., Aghazadeh Z., Pakdel S.M.V. Antimicrobial efficacy of mineral trioxide aggregate with and without silver nanoparticles. Iran Endod J. 2013;8:166-170.
32. Jonaidi-Jafari N, Izadi M, Javidi P. The effects of silver nanoparticles on antimicrobial activity of ProRoot mineral trioxide aggregate (MTA) and calcium enriched mixture (CEM). J Clin and Exper Dent. 2016 Feb;8(1):e22.
33. Bahador A, Pourakbari B, Bolhari B, Hashemi FB. In vitro evaluation of the antimicrobial activity of nanosilver-mineral trioxide aggregate against frequent anaerobic oral pathogens by a membrane-enclosed immersion test. Biomed J. 2015 Jan 1;38(1):77-83.
34. Loyola-Rodríguez JP, Torres-Méndez F, Espinosa-Cristobal LF, García-Cortes JO, Loyola-Leyva A, González FJ, Soto-Barreras U, Nieto-Aguilar R, Contreras-Palma G. Antimicrobial activity of endodontic sealers and medications containing chitosan and silver nanoparticles against Enterococcus faecalis. J of ApplBiomater & Func Mater. 2019 Jul;17(3).
35. Waly GH. Effect of Chlorhexidine Gluconate, Chitosan and Silver Nanoparticles on the setting time, solubility and antibacterial activity of Mineral Trioxide Aggregate. Dent J.

2013 Jul;59(2567):2578.

36. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett. 2012 Dec;2(1):1-0.
37. Bedier M.M.A. Antibacterial efficacy of mineral trioxide aggregate combined with nanosilver additives. Egypt Dent J. 2017;63:1833-1841
38. Abusrewil S, Brown JL, Delaney C, Butcher MC, Tiba M, Scott JA, Ramage G, McLean W. Chitosan enhances the anti-biofilm activity of biodentine against an interkingdom biofilm model. Antibiotics. 2021 Nov;10(11):1317.