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ANTIBACTERIAL EFFICACY OF NANO PARTICLE MODIFIED SEALERS

Dental Science

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ABSTRACT

Aim: The aim of the study was to assess, in vitro, the antimicrobial effectiveness of conventional endodontic sealers containing chitosan nanoparticles or silver nanoparticles, on E.coli, C.albicans, E.faecalis using colony forming units. **Methodolgy:** Silver and Chitosan nanoparticles as well as Blast-X were synthesized. The nanoparticles were subjected to XRD analysis and incorporated into the various sealers used, namely, AH plus, ZOE, MTA fillapex and the laboratory processed Blast-X, which was used as the fourth sealer. The sealers were then mixed according to the manufacturer's instructions and CFU count was done to assess the antibacterial efficacy of the four modified sealers aginst E.coli, E.faecalis and C.albicans. **Results:** From colony forming unit count it was observed that, by combinations of Blast+Chitosan, ZOE+Chitosan, ZOE+Silver and MTA+Chitosan there was complete inhibition of Candida albicans, while the specimens treated with Blast+Silver showed least reduction. The combination of MTA+Silver and ZOE+Silver showed complete eradication E.coli, while AH+Silver and Blast+Chitosan showed least reduction. The combinations of Blast+Silver, ZOE+Chitosan, ZOE+Silver and MTA+Chitosan showed complete eradication of E.faecalis, while MTA+Silver showed the least reduction. Statistical analysis was performed using Kolmogorov-Smirnov and Kruskal Wallis tests and a p value <0.05 was considered statistically significant. **Conclusion:** The incorporation of various nanoparticles into sealers or root filling materials significantly improved the antibacterial efficacy by inhibition of bacterial biofilm formation on the surface

KEYWORDS

Chitosan, C.albicans, E.coli , E.faecalis, Silver, Nanoparticles.

INTRODUCTION:

The main objective of endodontic therapy is thorough shaping and cleaning of the canal, followed by hermetic sealing with the elimination of microorganisms from the root canal system and the prevention of subsequent reinfection [8].

The spectrum of the microorganisms detected in the primarily or secondarily infected root canal may be relatively broad [1]. A success rate of 86% is observed in root canal treated with apical periodontitis, compared to 96% of success rate in root canal teeth without apical periodontitis. Predominantly facultative anaerobes are responsible for causing apical periodontitis observed in root canal failure [2].

Enterococcus faecalis is a pathogenic organism detected in about 77% of the cases resistant to root canal treatment. The virulence factor of this organism allows its adherence to host cells as well as to the extracellular matrix that favours tissue invasion and allows aggregation with other bacteria to produce biofilms [7]. The ability of E. faecalis to penetrate dentinal tubules, sometimes to a deep extent, can enable it to escape from the action of endodontic instruments and irrigants used during chemo-mechanical preparation. Moreover, its ability to form biofilms in root canals can be important for its persistence after intracanal antimicrobial procedures, including calcium hydroxide medicament and it has the ability to thrive in anaerobic environment [23].

Although fungi are found in primary infections, Candida species have been detected in root canal treated teeth in up to 18% of the cases. Fungi gain access to root canals via contamination during endodontic therapy (secondary infection) or they overgrow after inefficient intra-canal antimicrobial procedures. Among the fungal species, C.albicans is by far the most commonly detected fungal species in root canal treated teeth. This species has several properties that can be involved in persistence following treatment, including its ability to colonize and invade dentin and resistance to calcium hydroxide [23].

Escherichia coli, a gram-negative facultative anaerobe, often found in infected root canals, but, research literature on this particular organism is very sparse [10].

Persistent apical periodontitis after an endodontic treatment causes refractory infections and this could be due to poor sealing of accessory canals and presence microorganisms [9]. Also, the complexity of the anatomy of the root canal space harbours bacteria in places where the endodontic sealers cannot reach, such as, canal irregularities or in proving a seal to minor spaces between the dentinal wall and the core filling material [11].

A possible approach for this would be to improvise the existing sealers by adding nanoparticles or any other chemical components, which could help in eradicating the microorganisms, improving prognosis and endodontic success. Chitosan nanoparticles (CsNPs) and silver nanoparticles (AgNPs) are two of the most important nano materials advocated for application in the biomedical field due to their excellent physicochemical and biological properties [12,13].

Recently, chitosan nanoparticles have emerged as prospective carriers for delivery of drugs which include small organic molecules to proteins and nucleic acids. These nanoparticles can be easily dispersed in distilled water or aqueous buffers of neutral pH and exhibit higher antibacterial efficacy in comparison to chitosan. They are non-toxic, inexpensive, and highly biocompatible. They can be easily biodegraded through different hydrophilic enzymes and produce actions such as bactericidal, anti-inflammatory, antioxidant, antitumor, and healing properties [14-16,21].

Silver nanoparticles are one of the most commonly used metallic nanomaterials for the control of several types of microorganisms. They are recognized antimicrobial properties and are also effective in drug-resistant microorganisms, including the E. faecalis strain. These nanoparticles show multiple antibacterial mechanisms such as adherence and penetration into the bacterial cell wall causing loss of integrity of bacterial cell membrane and cell wall permeability. Previous studies suggested that AgNPs with size in the range of 10–100 nm showed powerful bactericidal potential against both gram-positive and gram-negative bacteria, including the multidrug resistant bacteria.

Blast-X is an antimicrobial wound gel patented by Next Science. It is based on a non-toxic biofilm-disruption Xbio™ Technology. It deconstructs the bacteria biofilm extracellular polymetric substance (EPS) matrix, destroys bacteria within the gel, and defends from recolonization while maintaining a moist wound environment. However, its use in dentistry is still under research as it is more popularly used in medicinal field.

Counting the colony forming units is considered to be one of the most valid methods to detect viable bacteria. CFU counting is considered to be a reliable method to evaluate the potential of the antimicrobial efficacy of different agents [22]. Based on the previous studies done on colony forming units (CFU) by Ehsani et.al, Tanomaru et.al, Arabi et.al and Jafari et.al, this method was preferred to overcome the shortcomings of agar diffusion test. This agar diffusion test is dependent on the diffusion ability of materials across the medium, physical properties of tested materials and the affinity of the material in the culture [25].

Although a number of studies have been conducted to determine the antimicrobial efficacy of chitosan and silver nanomaterials against E. faecalis, they have mainly been used in combination with irrigating agents or intra canal medicaments. There are very few studies that have evaluated the antimicrobial efficacy of modified endodontic sealers against the three micro-organism commonly responsible for reinfection of an already treated tooth.

Thus, the aim of the study was to assess, in vitro, the antimicrobial effectiveness of three conventional endodontic sealers along with experimental preparation of BlastX used as a sealer, each containing CsNPs or AgNPs, on E.coli, C.albicans, E.faecalis using CFU.

MATERIALS AND METHODOLOGY:

A)Synthesis of nano particles:

1.Procedure for the preparation of Ag Nano particles

Silver nitrate (Sigma, Aldrich) was dissolved in purified water. Polyvinylpyrrolidone (PVP) (Sigma, Aldrich) was further dissolved in water and mixed with silver nitrate solution. The reaction mixture was kept under ice bath (15 degrees C). Sodium borohydride solution (Sigma, Aldrich) was further added drop by drop to the silver nitrate solution using a glass pipette. Black precipitate was obtained. The precipitate was further washed with deionised water for several times and dried under vacuum for 2 hours at 80 degrees C. The nanoparticles thus obtained were confirmed using XRD analysis [Figure 1].

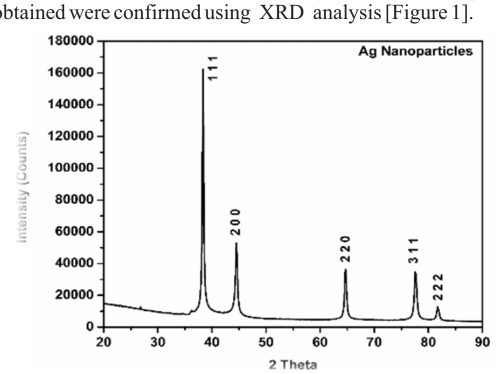

Figure 1: XRD image of silver nano particles.

2.Procedure for th e Preparation of Chitosan Nano particles:

1% w/v Chitosan (Sigma, Aldrich) was dissolved in 5% acetic acid. To this 2% Thiamine pyrophosphate (TPP) Sodium solution (Sigma, Aldrich) was added drop wise under stirring for 4 hours at 500 rpm. The chitosan nanoparticles were washed and re-dispersed. The nanoparticles were then freeze dried. The nanoparticles thus obtained were confirmed using XRD analysis [Figure 2].

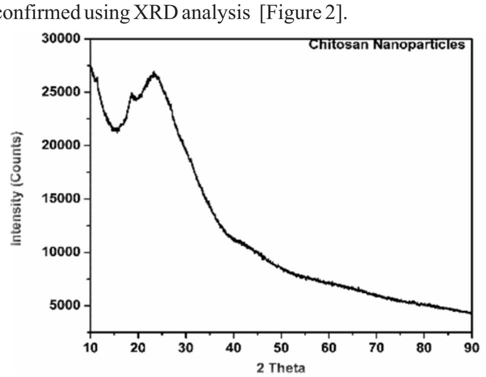

Figure 2: XRD image of chitos an nano particles

B.) Preparation of Lab processed Blast-X:

This gel was processed using Benzalkonium Chloride 0.13%, Polyethylene Glycol 400, Polyethylene Glycol 3350, Sodium Citrate, Citric Acid, and deionized Water.(Sigma, Aldrich)

C.) Preparation of the sealers:

Sealers were mixed according to the manufacturer's instructions. In all the groups, Chitosan and Silver nanoparticles were added to the sealer powder to a ratio of 2% (wt/wt) and prepared by manually mixing the powder and liquid components. Any excess sealer present, was removed with a size 15 scalpel blade under sterile conditions.

D.) Specimen preparation and Aging

The sterilized petri plates were incubated with 0.6 OD adjusted active culture from 18h grown in Mueller Hinton broth (Himedia, Mumbai, India) in sterile micro-centrifuge tube containing 2 ml of sterile Mueller Hinton broth autoclaved under 121°C at 15 psi for 20 minutes. The inoculated specimens were incubated under a bacteriological incubator for 24 h at 37°C. The specimens were stored for 7 days at 37°C and 100% humidity and were subjected to aging at 37°C for 1 week.

E.) Grouping of samples:

The specimens were divided into 4 groups namely, **Group 1:** ZINC OXIDE EUGENOL (n=30), **Group 2:** AH PLUS (n=30), **Group 3:** MTA FILLAPEX (n=30), **Group 4:** BLAST-X (n=30). These groups were further subdivided into Silver nanoparticles (n=15) [a) E.coli b) C.albicnas c) E.faecalis] and Chitosan nanoparticles (n=15) [a) E.coli b) C.albicnas c) E.faecalis].

F.) CFU count:

Colony forming unit for treated and untreated samples were evaluated using Mueller Hinton agar plate count method. Surface sterilized samples were carefully taken and added with 5-10 µl of fresh Mueller Hinton broth. The remaining dead/alive bacteria were siphoned and drained at least 5 times, about 5 µl of the pipette out and poured on to the sterile Mueller Hinton agar plate and incubated for 24 h at 37°C. After 24 h of incubation the cultured plates were examined, photographed and counted for colony forming unit.

RESULTS:

The results obtained from CFU count on C.albicans showed that the combinations of Blast+Chitosan, ZOE+Chitosan, MTA+Chitosan and MTA+Silver showed complete eradication of C.albicans, while the combination of Blast+Silver showed least eradication [Table 1].

Table 1: Colony for ming units for C.albicans

Sl. No.	Group Name	Parameters	Method	CFU/ tooth	Reduction (%)
1	No medicament	CFU	Agar Plate	3240	0.00
2	AH+Chitosan	CFU	Agar Plate	248	92.35
3	AH+Silver	CFU	Agar Plate	2	99.94
4	Blast+Chitosan	CFU	Agar Plate	0	100.00
5	Blast+Silver	CFU	Agar Plate	2450	24.38
6	ZOE+Chitosan	CFU	Agar Plate	0	100.00
7	ZOE+Silver	CFU	Agar Plate	0	100.00
8	MTA+Chitosan	CFU	Agar Plate	0	100.00
9	MTA+Silver	CFU	Agar Plate	10	99.69

The results obtained from CFU count on E. coli showed that the combinations of ZOE+Silver and MTA+Silver showed complete eradication of E.coli, while the combination of AH+Chitosan and Blast+Silver showed the least eradication [Table 2].

The results obtained from CFU count on E.faecalis showed that the combinations of Blast+Silver, ZOE+Chitosan, ZOE+Silver and MTA+Chitosan showed complete eradication of E.faecalis and the combination of Blast+Chitosan and AH+Silver showed least reduction[Table 3]

Table 2: Colony for ming units for E.coli

S. No.	Group Name	Parameters	Method	CFU/ tooth	Reduction (%)
1	No edicament	CFU	Agar Plate	3890	0.00
2	AH+Chitosan	CFU	Agar Plate	2460	36.76
3	AH+Silver	CFU	Agar Plate	2180	43.96

4	Blast+Chitosan	CFU	Agar Plate	2070	46.79
5	Blast+Silver	CFU	Agar Plate	2380	38.82
6	ZOE+Chitosan	CFU	Agar Plate	1970	49.36
7	ZOE+Silver	CFU	Agar Plate	0	100.00
8	MTA+Chitosan	CFU	Agar Plate	3	99.92
9	MTA+Silver	CFU	Agar Plate	0	100.00

Table 3: Colony for ming units for E .faecalis.

S. No.	Group Name	Parameters	Method	CFU/tooth	Reduction (%)
1	No medicament	CFU	Agar Plate	3230	0.00
2	AH+Chitosan	CFU	Agar Plate	2640	18.27
3	AH+Silver	CFU	Agar Plate	2840	12.07
4	Blast+Chitosan	CFU	Agar Plate	2950	8.67
5	Blast+Silver	CFU	Agar Plate	0	100.00
6	ZOE+Chitosan	CFU	Agar Plate	0	100.00
7	ZOE+Silver	CFU	Agar Plate	0	100.00
8	MTA+Chitosan	CFU	Agar Plate	0	100.00
9	MTA+Silver	CFU	Agar Plate	1820	43.65

Statistical analysis:

Descriptives of all the medicaments and their inhibition percentage on the three micro-organisms were tabulated [Table 4] Kolmogorov-Smirnov Test was used for statistical analysis. Difference between efficacy of chitosan and silver on Candida albicans was checked. For one-tailed hypothesis if z value is greater than .6 at 95% confidence interval there is significant difference between chitosan and silver. Since z value was greater than .6 (.707) there is significant difference between chitosan and silver nano particles. Chitosan has more efficacy on inhibition of candida albicans CFU in 7days. There is no signifant difference between silver and chitosan nano particles on inhibition of **Enterococcus faecalis** [Table 5].

Table 4: Comparison between groups

S. No.	Sealer with Nano particles	Inhibition% OnCandida albicans after 7 days	Inhibition% OnEnterococcus faecalis after 7 days	Inhibition% On E.Coli after 7 days
1	No medicament	0.00	0.00	0.00
2	AH+Chitosan	92.35	18.27	36.76
3	AH+Silver	99.94	12.07	43.96
4	Blast+Chitosan	100	8.67	46.79
5	Blast+Silver	24.38	100.00	38.82
6	MTA+chitosan	100	100.00	99.2
7	MTA+Silver	99.69	43.65	100.00
8	ZO+Chitosan	100.00	100.00	49.36
9	ZO+Silver	100.00	100.00	100.00

Table 5: Kolmogorov-Smirnov Z to measure test statistics for candida

Test Statistics		
		candida
Most Extreme Differences	Absolute	.500
	Positive	.000
	Negative	-.500
Kolmogorov-Smirnov Z		.707

Difference between efficacy of chitosan and silver on E.coli was also checked. For one-tailed hypothesis if z value was greater than .6 at 95% confidence interval, there is significant difference between chitosan and silver. Since z value was greater than .6 (.707) there is significant difference between chitosan and silver nano particles. Silver has more efficacy on inhibition of E.coli CFU in 7days [Table 6]. Kruskal Wallis test was test was performed to check if there is significant difference sealers used in inhibition of microbial growth in CFU.

If p value is less than .05 and chi square value is greater than tabled value of degree of Freedom then there significant difference in efficacy of sealers in inhibition of microbial growth in 7 days. Since p value is greater than .05 and chi square value is lesser than the tabled value of degree of freedom there is no difference in efficacy of sealers in inhibition of microbial growth in 24 hours [Table 7]

Table 6: Kolmogorov-Smirnov Z to measure te s t statistics for E.coli.

TestStatistics		
		E.coli
MostExtreme Differences	Absolute	.500
	Positive	.500
	Negative	-.250
Kolmogorov-SmirnovZ		.707

Table 7: Chi-square test to measure te st statistics

TestStatistics	
	Candida
Chi-Square	2.601
df	3
Asymp.Sig.	0.457

DISCUSSION:

For an endodontic procedure, complete shaping, cleaning and obturation of the root canal system is crucial. Thus, the key objective of any endodontic therapy is to eliminate the microorganisms from the root canal space and to prevent any subsequent re-infection.

It is impossible to eliminate the bacteria from the root canal during treatment; which is why, it is essential for an endodontic material to have specific ingredients that have the capability to release substances to produce antibacterial activity [8]. An endodontic sealer is a material that is used to fill the gap between the root canal wall of the teeth and the obturating material. Inadequate seal due to the presence of accessory canals and microorganisms within the root canal or any persistent apical periodontitis after an endodontic treatment can result in refractory infections [9].

This problem encountered by persistant and antibiotic resistant micro-organisms can be overcome by modifying the composition of the existing endodontic materials in an attempt to enhance their antimicrobial efficacy. Chitosan (CsNPs) and silver nanoparticles (AgNPs) are two of the most commonly used nanomaterials for applications in the biomedical field due to excellent physicochemical and biological properties [12, 13].

Blast-X is an antimicrobial wound gel patented by Next Science. It is based on a non-toxic biofilm-disruption Xbio™ Technology. It deconstructs the bacteria biofilm extracellular polymetric substance (EPS) matrix, destroys bacteria within the gel, and defends from recolonization while maintaining a moist wound environment. However, its use in dentistry is still under research as it is more popularly used in medicinal field.

Counting the colony forming units is considered to be one of the most valid methods to detect viable bacteria. CFU counting is considered to be a reliable method to evaluate the potential of the antimicrobial efficacy of different agents [22]. Based on the previous studies done on colony forming units (CFU) by Ehsani et.al, Tanomaru et.al, Arabi et.al and Jafari et.al, this method was preferred to overcome the shortcomings of agar diffusion test. This agar diffusion test is dependent on the diffusion ability of materials across the medium, physical properties of tested materials and the affinity of the material in the culture [25].

Although a number of studies have been conducted to determine the antimicrobial efficacy of chitosan and silver nanomaterials against *E. faecalis*, they have mainly been used in combination with irrigating agents or intra canal medicaments. There are very few studies that have evaluated the antimicrobial efficacy of modified endodontic sealers against the three micro-organism commonly responsible for reinfection of an already treated tooth. The aim of the study was to assess, in vitro, the antimicrobial effectiveness of three conventional endodontic sealers along with experimental preparation of BlastX used as a sealer, each containing CsNPs or AgNPs, on E.coli, C.albicans, E.faecalis using CFU.

Silver and Chitosan nanoparticles as well as BlastX were synthesized. The nanoparticles were subjected to XRD analysis and incorporated into the various sealers used, namely, AH plus, ZOE, MTA fillapex and the laboratory processed BlastX, which was used as the fourth sealer. The sealers were then mixed according to the manufacturer's instructions and CFU count was done to assess the antibacterial efficacy of the four modified sealers aginst *E.coli, E.faecalis* and *C.albicans.*

The results obtained from CFU showed complete inhibition of Candida albicans by combinations of Blast+Chitosan, ZOE+Chitosan, ZOE+Silver and MTA+Chitosan, while the specimens treated with Blast+Silver showed least reduction.

The combination of MTA+Silver and ZOE+Silver showed complete eradication E.coli, while AH+Silver and Blast+Chitosan showed least reduction.

The combinations of Blast+Silver, ZOE+Chitosan, ZOE+Silver and MTA+Chitosan showed complete eradication of E.faecalis, while MTA+Silver showed the least reduction.

Shrestha, et al., conducted a study and proved that the chitosan and zinc oxide nanoparticles reduced and disrupted the biofilm structure using confocal microscopy techniques;and also proved that the antibacterial property of these nanoparticles was retained even after aging for 90 days. Aldo del Carpio-Perochena et.al demonstrated the capacity of chitosan to resist aging, its ability to inhibit collagen degradation and the threw light on the hydrophilic nature of chitosan. He concluded that, the above mentioned properties were the reason to chitosan having a low bacterial colonization at the sealer-dentin interfaces. He further states that the large swelling of chitosan matrices is also a factor responsible for the low adhesion of biofilm bacteria.

Wu et al. assessed the efficacy of silver nanoparticles in a concentration of 0.1% as an endodontic irrigant in the form of a solution as well as a gel in two different concentrations (0.02% and 0.1%) against Enterococcus faecalis. He found that the solution did not produce any major change to the structure of E. faecalis, however, gel form had shown to disrupt the structural integrity of the E. faecalis and thus resulting in a decrease in the number of viable bacteria. Afkhami et.al, found that Ag-NPs enhance the antibacterial properties of calcium hydroxide in eradication of E.faecalis.

Statistical analysis was performed using Kolmogorov-Smirnov and Kruskal Wallis tests. According to the analysis that was done to assess the difference between efficacy of chitosan and silver on C. albicans and E.coli, showed that since z value is greater than 0.6 (.707) there is significant difference between chitosan and silver nano particles on C.albicans and E.coli. Chitosan has more efficacy on inhibition of C.albicans CFU in 7 days. Silver has more efficacy on inhibition of E.coli CFU in 7 days. There is no signifcant difference between silver and chitosan nano particles on inhibition of E. faecalis.

The amount of nanoparticles added to each specimen was 2% (wt/wt) which is similar to previous studies done by DaSilva et.al, Tanomaru et.al and Barros et.al who studied the antibacterial properties after addition of nanoparticles with concentrations of 2% (wt/wt).

Silver and chitosan nanoparticles have been drawing increased attention in the biomedical field due to their bactericidal activity against some bacteria and fungi. The physicochemical properties of these nanoparticles have been developed to analyze the biodegradability, biocompatibility, aggregation capability, bacteriostatic and bactericidal activity, precipitation range and solubility. Nonetheless, more studies need to be conducted in order to gain more reliability on the antibacterial efficacy of nanoparticle modified endodontic sealers under simulated clinical conditions.

CONCLUSION:

The incorporation of various nanoparticles into sealers or root filling materials significantly improved the antibacterial efficacy by inhibition of bacterial biofilm formation on the surface. Among the two nanoparticles, chitosan was more effective against C.albicans CFU in 7 days and silver was more effective against E.coli CFU in 7days. There is no significant difference between silver and chitosan nano particles on inhibition of E.faecalis.

REFERENCES:

1. Markus Heyder, Stefan Kranz, Andrea Völpel, Wolfgang Pfister,David C. Watts, Klaus D. Jandt, Bernd W. Sigusch. Antibacterial effect of different root canal sealers on three bacterial species. Dental materials 2013, 542–549
2. Dr.Amer A. Mohammed ,Dr.Firas Saddam Oglah, Dr.Sundus Hussein Naser. In vitro evaluation of the Antimicrobial Activity of four resin based Endodontic Sealers on threebacterial species. MDJVol.:11 No.:1 2014
3. Annie Shrestha and Anil Kishen. Antibacterial Nanoparticles in Endodontics: A Review. Endod 2016;42:1417–1426
4. Luis DaSilva,Yoav Finer, Shimon Friedman, Bettina Basrani, and Anil Kishen. Biofilm Formation within the Interface of Bovine Root DentinTreated with Conjugated Chitosan and Sealer Containing Chitosan Nanoparticles. J Endod 2013;39:249–253
5. Jay Kumar ,Rohit Sharma , Madhurima Sharma , V Prabhavathi , John Paul , Chava Deepak Chowdary. Presence of Candida albicans in Root Canals of Teeth with Apical Periodontitis and Evaluation of their Possible Role in Failure of Endodontic Treatment. Journal of International Oral Health 2015; 7(2):42-45
6. Bashayer H. Baras , Mary Anne S. Melo , JirunSunc , Thomas W. Oates , Michael D. Weir , XianjuXie , YuxingBai , HockinH.K. Xu. Novel endodontic sealer with dual strategies of dimethylaminohexadecyl methacrylate and nanoparticles of silver to inhibit root canal biofilms. Dental materials August 2019, Pages 1117-1129
7. NoopurKaushik, UshaRehani, Abhay Agarwal, MayurKaushik, VivekAdlakha. Antimicrobial Efficacy of Endodontic Irrigants against Enterococcus Faecalis and Escherichia Coli: An in vitro study. IJCPD September-December 2013;6(3):178-18
8. Juan Pablo Loyola-Rodríguez, Fernando Torres-Méndez, León Francisco Espinosa-Cristobal , José ObedGarcía-Cortes, Alejandra Loyola-Leyva, Francisco Javier González, Uriel Soto-Barreras4, Renato Nieto-Aguilar and Guillermo Contreras-Palma. Antimicrobial activity of endodontic sealers and medications containingchitosan and silver nanoparticles against Enterococcus faecalis. Journal of Applied Biomaterials &Functional Materials July-September: 1–9, 2019
9. Haapasalo M, Udnaes T and Endal U. Persistent, recurrent and acquired infection of the root canal system post-treatment. Endod Top 2003; 6: 29–56.
10. Henderson IR and Nataro JP. Virulence functions of autotransporter proteins. Infect Immun 2001; 69: 1231– 1243.
11. Guimarães BM, Amoroso-Silva PA, Alcalde MP, et al. Influence of ultrasonic activation of 4 root canal sealers on the filling quality. J Endod 2014; 40: 964–968.
12. Verlee A, Mincke S and Stevens CV. Recent developments in antibacterial and antifungal chitosan and its derivatives. CarbohydrPolym 2017; 164: 268–283.
13. Pazos-Ortiz E, Roque-Ruiz JH, Hinojos-MárquezEA, et al. Dose-dependent antimicrobial activity of silver nanoparticles on polycaprolactone fibers against Gram-positive and Gram-negative bacteria. J NanomaterEpub ahead of print 6 November 2017.
14. Xia W, Liu P, Zhang J, et al. Biological activities of chitosan and chitooligosaccharides. Food Hydrocoll 2011; 25: 170–179.
15. Ravindran A, Singh A, Raichur AM, et al. Studies on interaction of colloidal Ag nanoparticles with bovine serum albumin (BSA). Colloids Surf B Biointerfaces. 2010; 76: 32–37.
16. Espinosa-Cristóbal LF, Martínez-Castañón GA, LoyolaRodríguez JP, et al. Bovine serum albumin and chitosan coated silver nanoparticles and its antimicrobial activity against oral and nonoral bacteria. J Nanomater 2015; 16: 366.
17. Jayash SN, Hashim NM, Misran M, et al. Formulation and in vitro and in vivo evaluation of a new osteoprotegerin-chitosan gel for bone tissue regeneration. J Biomed Mater Res A 2017; 105: 398–407.
18. Shen R, Xu W, Xue Y, et al. The use of chitosan/PLAnanofibers by emulsion electrospinning for periodontal tissue engineering. Artif Cells NanomedBiotechnol 2018; 46:419–430.
19. VaroniEM, Vijayakumar S, Canciani E, et al. Chitosan-based trilayer scaffold for multitissue periodontal regeneration. J Dent Res 2018; 97: 303–311.
20. Zhou T, Liu X, Sui B, et al. Development of fish collagen/ bioactive glass/chitosan composite nanofibers as a GTR/ GBR membrane for inducing periodontal tissue regeneration. Biomed Mater 2017; 12: 055004.
21. Shilpa Sharma. Enhanced Antibacterial efficacy of Silver Nanoparticles immobilized in a Chitosan Nanocarrier. International Journal of Biological Macromolecules, 2017
22. Vianna ME, Horz H-P, Conrads G, Feres M, Gomes BPFA. Comparative analysis of endodontic pathogens using checkerboard hybridization in relation to culture. Oral MicrobiolImmunol 2008: 23: 282–290
23. Hargreaves, K. M., **Cohen, S.**, & Berman, L. H. (2016). **Cohen's pathways** of the **pulp**. St. Louis, Mo: Mosby Elsevier.
24. DamingWu , Anil Kishen, James L. Gutmann and Bing Fan. Evaluation of the Antibacterial Efficacy of Silver Nanoparticles against Enterococcus faecalis Biofilm. J Endod 2014;40:285–290
25. Hala F. Mohammed, Mohammed M. Ibrahim, Abd El-Fattah H. Abd El-Fattah, Thanaa I. Shalaby. Antibacterial effect of two types of nano particles incorporated in zinc oxide based sealers on enterococcus faecalis (in vitro study). Alexandria Dental Journal. (2016) Vol.41 Pages:169-175