ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 3, June - 2021, Page No. : 90 - 97

Nano fiber incorporated intra canal medicaments and its antibacterial effect against enterococcus faecalis: An Invitro study

[1]Sanjay Kumar.P, [2]Sebeena Mathew, [3]Karthick Kumaravadivel, [4]Boopathi, [5]Deepa N T

[1-5]KSR Institute of Dental Science and Research, Tiruchengode, Tamilnadu

Corresponding Author: Sanjay kumar.P, KSR Institute of Dental Science and Research, Tiruchengode, Tamilnadu

Citation of this Article: Sanjay kumar.P, Sebeena Mathew, Karthick kumaravadivel, Boopathi, Deepa N T, "Nano fiber incorporated intra canal medicaments and its antibacterial effect against enterococcus faecalis: An Invitro study", IJDSIR- June - 2021, Vol. – 4, Issue - 3, P. No. 90 – 97.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Aim: The aim of this in vitro study was to evaluate and compare the antibacterial effect of calcium hydroxide and triple antibiotic paste with and without nanofiber against enterococcus faecalis biofilms.

Objectives: To compare the antibacterial effect of calcium hydroxide and triple antibiotic paste with and without nanofiber against enterococcus faecalis biofilms using confocal laser scanning microscope

Introduction: The success of endodontic therapy depends on complete disinfection of root canal system which can be accomplished by thorough chemomechanical preparation and three dimensional obturation of the root canal system.[1] A Triple antibiotic mixture of metronidazole, ciprofloxacin, and minocycline is used for root canal disinfection. When using a mixture of metronidazole, ciprofloxacin, and minocycline bacterial elimination is seen in the deep root canal dentin. Metronidazole being bactericidal, Minocycline being bacteriostatic and Ciprofloxacin being a bactericidal broad spectrum synthetic quinolone, triple antibiotic paste is able to eradicate both gram positive aerobes and anaerobic bacteria. Antibiotic ineffectiveness in systemic route of administration has led to the intra canal application to increase its efficacy.[2] Calcium hydroxide is the most commonly used intracanal medicament for disinfection. Calcium hydroxide is most widely used because of its alkaline Ph.[3.] Intracanal medicaments are used within the canal because of their antimicrobial activity, their ability to neutralize the tissue remnants within the canal and because of their ability to prevent and control pain after treatment.[4] In this present study, we have used the triple antibiotic paste and calcium hydroxide with and without nanofiber as intracanal medicaments to compare their antimicrobial property against enterococcus faecalis.

Materials and methods

Triple antibiotic nanofiber fabrication: A 10 wt% Poly vinyl pyrrolidone polymer solution was prepared in hexafluoro- 2-propanol. The 3 antibiotics (metronidazole, ciprofloxacin and minocycline) were added to the Poly

vinyl pyrrolidone solution at 30 wt% concentration (relative to the total PVP [600 mg] weight; ie, 180 mg of each antibiotic) and mixed together via stirring. Antibiotic-free Poly vinyl pyrrolidone (control) and the triple antibiotic–containing polymer solutions were spun into fibers at 2 mL/h, 18-cm distance, and 15–19 kV. After processing, the fibers were dried for 2 days under a vacuum to eliminate any residual solvent and stored at room temperature until it was used.

Calcium hydroxide nanofiber fabrication: A 10 wt% Poly vinyl pyrrolidone polymer solution was prepared in hexafluoro- 2-propanol. Calcium hydroxide powder was added to the Poly vinyl pyrrolidone solution at 10 wt% concentration (relative to the total PVP [600 mg] weight; i.e, 180 mg of calcium hydroxide) and mixed together via stirring. Medicament free Poly vinyl pyrrolidone (control) and the Calcium hydroxide containing polymer solutions were spun into fibers at 2 mL/h, 18-cm distance, and 15–19 kV. After processing, the fibers were dried for 2 days under a vacuum to eliminate any residual solvent and stored at room temperature until it was used.

Sample preparation: The coronal portions were cut with a 0.3mm diamond disc and the root canal length was standardized at 4× 4×1 mm radicular dentin specimens

A biofilm was established on dentin. Gram positive, facultative anaerobic bacteria, Enterococcus faecalis, were selected, because Enterococcus faecalis is a cocci bacterium responsible for secondary infections in necrotic teeth after treatment. 70 human,caries-free, nonrestored lower premolar were used to obtain 4× 4×1 mm radicular dentin specimens. To remove the smear layer, all the specimens were placed in an ultrasonic bath containing 2.5% sodium hypochlorite followed by 17% EDTA solutions for 3 minutes each. All specimens were rinsed in saline solution for 10 minutes and autoclaved at 121°C. Next, the specimens were randomly placed into the wells of a 24-well plate containing 800 mL sterile brain-heart infusion broth and bacterial suspensions Enterococcus faecalis were inoculated into each well and allowed to grow for 7 days at 37°C in an incubator for biofilm development. The broth was changed every other day. Scanning electron microscopy (SEM) was performed to qualitatively evaluate the biofilm formation at 5th day to confirm the biofilm formation. After 1 week, all specimens were rinsed for 1 minute with phosphate buffer saline solution to remove loosely bound bacterial cells. They were inoculated in all the specimens. They were randomly divided into 5 groups based on the intracanal medicaments used

GROUP1 (N=10)-control group, GROUP2 (N=15)-Calcium hydroxide paste, GROUP3 (N=15)-Calcium hydroxide incorporated Nanofiber, GROUP4 (N=15)-Triple antibiotic paste, GROUP5 (N=15)-Triple antibiotic incorporated nanofiber. After 15 days, they were stained with the fluorescent dye to check Bacterial Viability (Live/dead bacteria). A total of 70 specimens were scanned and viewed using confocal laser scanning microscopy.

Discussion: Eradication of bacteria is essential for successful outcome of endodontic treatment. Thorough sterilization of the root canal and periradicular region is essential as it promotes good healing of periapical diseases in adults. The application of antibacterial medicaments to endodontic lesions is a part of the clinical procedures that may be used to sterilize lesions[6.] .Enterococcus faecalis present in dentinal tubules is resistant to intracanal dressings of calcium hydroxide for over 10 days. Enterococcus faecalis and candida which are commonly found in diabetic patients, are found to be greatly resistant to calcium hydroxide[5] . This organism is able to survive when calcium hydroxide is used alone as an intracanal medicaments only when the pH is lesser than 11.5. It is

able to survive by maintaining pH haemostasis. It is very unlikely that a pH of 11.5 is maintained as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity. Therefore Calcium hydroxide is ineffective at killing enterococcus faecalis on its own. At a pH of 11.5 or greater Enterococcus faecalis is unable to survive. However, as a result of the buffering capacity of dentin, it is very unlikely that a pH of 11.5 can be maintained in the dentinal tubules with current calcium hydroxide utilization techniques. Enterococcus faecalis has a proton pump that provides an additional means of maintaining pH homeostasis. This is achieved by "pumping" protons into the cell to lower the internal pH.[7]

Root canal infections are poly microbial in nature. As these infections are complex in nature, a combination of antibiotics is required to address the diverse microbial flora. Triple antibiotic paste is a combination of three antibiotics namely minocycline, ciprofloxacin and Metronidazole. Metronidazole is a nitro imidazole compound; selectively toxic and effective against anaerobic organisms. The Presence of redox protein reduces the nitro groups of this compound and generates free radicals that results in DNA damage and lysis of cell.

Minocycline which is primarily bacteriostatic, hinders protein synthesis by binding to a 30S ribosome in susceptible organisms. Ciprofloxain is a synthetic Fluoroquinolone with quick bactericidal action. It inhibits the enzyme bacterial DNA gyrase. Hoshino et al recommended the use of metronidazole (500 mg) minocycline (100mg) and ciprofloxacin (200mg) at 1:1:1 ratio for 3mix formulation. Even though Triple antibiotic paste is an effective medicament, it has its own disadavantage. When used as an intra canal triple antibiotic paste medicament is shown to be most cytotoxic to human periodontal ligament fibroblasts. It promotes exacerbated inflammatory reaction in subcutaneous connective tissue, and the minocycline component causes discoloration.[8] Pereira et al conducted a study on cellular and molecular tissue response to triple antibiotic intracanal dressing and he concluded that compared to calcium hydroxide, triple antibiotic paste induced a profuse angiogenic and inflammatory response, higher vascular area, and more inflammatory cells.[9] As an intra canal medicament calcium hydroxide is not as effective as triple antibiotic paste.[10] Electrospinning or electrostatic spinning, a textile technology, has been utilized to fabricate antibiotic containing polymer-based nanofibers for drug delivery applications in dentistry. This mode of drug delivery is used to ablate periodontal and endodontic infections. The idea behind the use of antibiotic-containing nanofibers as a three-dimensional (3D) tubular drug delivery construct that can be placed inside the root canal system of necrotic teeth rests on the fact that the addition of low antibiotic concentrations and the slow drug release rendered by these nanofibrous constructs will be able to eradicate infection and thus generates a bacteria-free environment favorable to tissue regeneration. However in this strategy, the antimicrobial agents could be used at a much lower concentration as they are delivered in a predictable fashion onto the dentinal walls where microbial biofilms have been detected. In electrospinning, a polymer solution/melt containing the preferred concentration of antibiotics is prepared to produce nanofibers. A high-voltage source is used to produce an electrical potential difference between the metallic needle tip and the grounded collector fixed at a set distance, which overcomes the surface tension of the fluid droplet, creating a jet. The fluid jet experiences whipping instabilities and tends to dry and produce nano to micron sized polymeric fibers. The chosen polymer solution can be incorporated with one or a combination of antibiotics, making it possible to fabricate fibers with a

narrow or wide spectrum of action (e.g., ciprofloxacin, metronidazole, and minocycline, among others) that have been shown to inhibit the growth of endodontic pathogens.[11] In this study it has been observed that all the four intra canal medicaments do not consistently and completely eradicate the enterococcus faecalis biofilm in the root canal. The control Group (nanofiber) without medicament showed more live cells than the other four groups. Enterococcus faecalis showed successful biofilm formation when viewed under field emission scanning electron microscope. Confocal laser scanning electron microscope showed that a dense population of viable bacteria adhered to dentin. Triple antibiotic incorporated nanofibers (Group 5) showed nearly complete elimination of viable bacteria on the dentin surface when observed under the confocal laser scanning microscope. Triple antibiotic incorporated nanofiber group showed significantly higher eradication of Enterococcus faecalis biofilm, when compared with the triple antibiotic paste group, calcium hydroxide paste group, and calcium hydroxide incorporated nanofiber group. On inter group comparsion control group showed lesser eradication of Enterococcus faecalis biofilm than the calcium hydroxide paste group, calcium hydroxide incorporated nanofiber group, triple antibiotic paste group, triple antibiotic incorporated nanofiber group. When comparing the calcium hydroxide group with the other groups, calcium hydroxide showed greater erradication of Enterococcus faecalis than control group and lesser eradication of Enterococcus faecalis than calcium hydroxide incorporated nanofiber group, triple antibiotic paste group, triple antibiotic incorporated nanofiber groups. When comparing the calcium hydroxide incorporated nanofiber group with the other groups, the calcium hydroxide incorporated nanofiber group showed greater eradication of Enterococcus faecalis than control group and calcium hydroxide group and smaller than triple antibiotic paste group, triple antibiotic incorporated nanofiber group. When comparing the triple antibiotic paste group with other the groups, triple antibiotic paste group showed greater erradication of Enterococcus faecalis biofilm than the control group, calcium hydroxide group, calcium hydroxide incorporated nanofiber group. When compared with the triple antibiotic incorporated nanofiber group, triple antibiotic paste group showed lesser erradication of Enterococcus faecalis. The results of the present study coincides with studies conducted by Madhubala et al and DeLucena et al in which the triple antibiotic paste group showed higher antibacterial effects than calcium hydroxide on Enterococcus faecalis. When comparing the triple antibiotic incorporated nanofiber group with the other groups,the triple antibiotic incorporated nanofiber group showed greater eradication of Enterococcus faecalis than control group, calcium hydroxide paste group, calcium hydroxide incorporated nanofibergroup and triple antibiotic paste group.

Result

Statistical Analysis

Post Hoc Test

Group	Total Number	Mean Value	Standard Deviation	P Value
Control (Group I)	10	94.8000	3.55278	.000
Calcium hydroxide paste (Group II)	15	85.7333	7.04543	.000
Calcium hydroxide Nanofiber (Group III)	15	72.0000	10.29563	.000

Tripleantibiotic paste (Group IV)	15	25.9333	10.13809	.000
Triple antibiotic nanofiber (Group V)	15	11.2000	4.75395	.000

Table 1: Mean standard deviation for live bacteria

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Control Group (Group I)	Calcium hydroxide paste (Group II)	9.06	3.22	.049	.0272	18.1061
	Calcium hydroxide Nanofiber group (Group III)	22.8	3.22	.000	13.7606	31.8394
	Triple antibiotic paste (Group IV)	68.8	3.22	.000	68.8667	3.22167
	Triple antibiotic Nanofiber (Group V)	83.6	3.22	.000	83.60000	3.22167

Table 2: Tukey HSD for multiple comparisons of Group-I versus Group-II, Group-III, Group-IV, Group-V.

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Calcium hydroxide paste (Group II)	Control (Group I)	9.06667	3.22167	.049	18.1061	.0272
	Calcium hydroxide Nanofiber (Group III)	13.73333	2.88155	.000	5.6482	21.8184
	Triple antibiotic paste (Group IV)	59.80000	2.88155	.000	51.7149	67.8851
	Triple antibiotic Nanofiber (Group V)	74.53333	2.88155	.000	66.4482	82.6184

Table 3: Tukey HSD for multiple comparisons of Group-II versus Group-I, Group-III, Group-IV, Group-V

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Calcium hydroxide Nano fiber (Group III)	Control (Group I)	-22.8000	3.22167	.000	-31.8394	-13.7606
	Calcium hydroxide paste(Group II)	-13.73333	2.88155	.000	-21.8184	-5.6482
	Triple antibiotic paste (Group IV)	46.06667	2.88155	.000	37.9816	54.1518
	Triple antibiotic Nanofiber (Group V)	60.80000	2.88155	.000	52.7149	68.8851

Table 4: Tukey HSD for multiple comparisons of Group-III versus Group-I, Group-II, Group-IV, Group-V

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Triple antibiotic paste (Group IV)	Control (Group I)	-68.86667	3.22167	.000	-77.9061	-59.8272
	Calcium hydroxide paste (Group II)	-59.80000	2.88155	.000	-67.8851	-51.7149
	Calcium hydroxide Nanofiber (Group III)	-46.06667	2.88155	.000	-54.1518	-37.9816
	Triple antibiotic Nanofiber (Group V)	14.73333	2.88155	.000	6.6482	22.8184

Table 5: Tukey HSD for multiple comparisons of Group-IV versus Group-I, Group-II, Group-III, Group-V

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Triple antibiotic Nano fiber (Group V)	Control (Group I)	-83.60000	3.22167	.000	-92.6394	-74.5606
	Calcium hydroxide paste (Group II)	-74.53333	2.88155	.000	-82.6184	-66.4482
	Calcium hydroxide Nanofiber (Group III)	-60.80000	2.88155	.000	-68.8851	-52.7149
	Triple antibiotic paste (Group IV)	-14.73333	2.88155	.000	-22.8184	-6.6482

Table 6: Tukey HSD for multiple comparisons of Group-V versus Group--I, Group-II, Group-III, Group-IV

Group I: Control group (Nanofiber without medicament) Enterococcus faecalis seen in 20x magnification

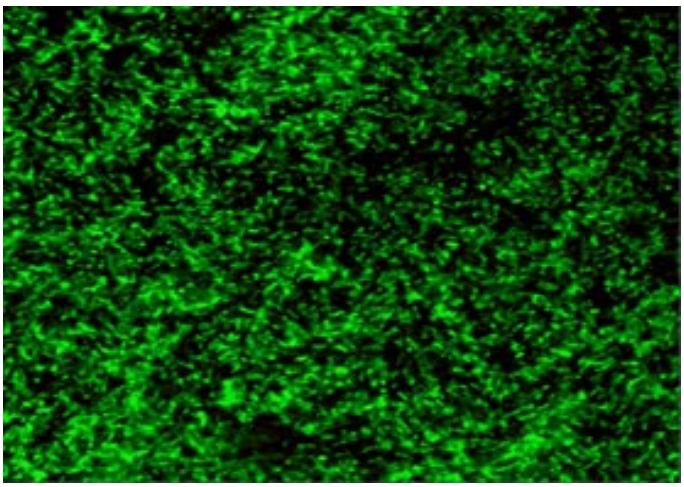

Figure 1: Live Enterococcus faecalis in control group (Group I)

Group II: Calcium hydroxide paste group, Enterococcus faecalis seen in 20x magnification

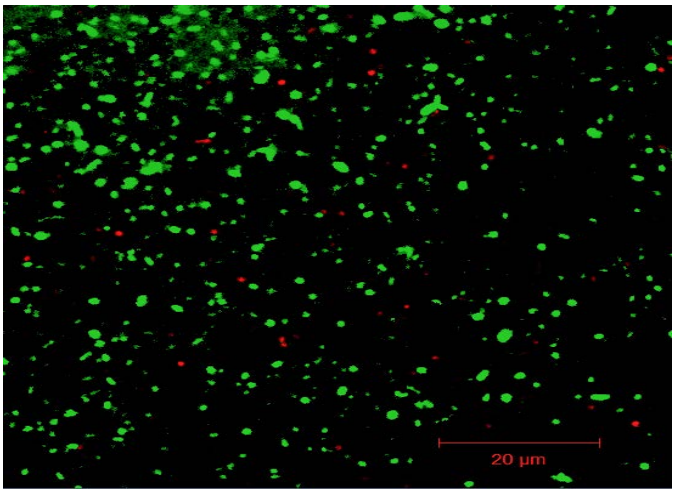

Figure 2: Live Enterococcus faecalis in calcium hydroxide paste group (Group II)

Group III: Calcium hydroxide incorporated nanofiber, Enterococcus faecalis seen in 20x magnification

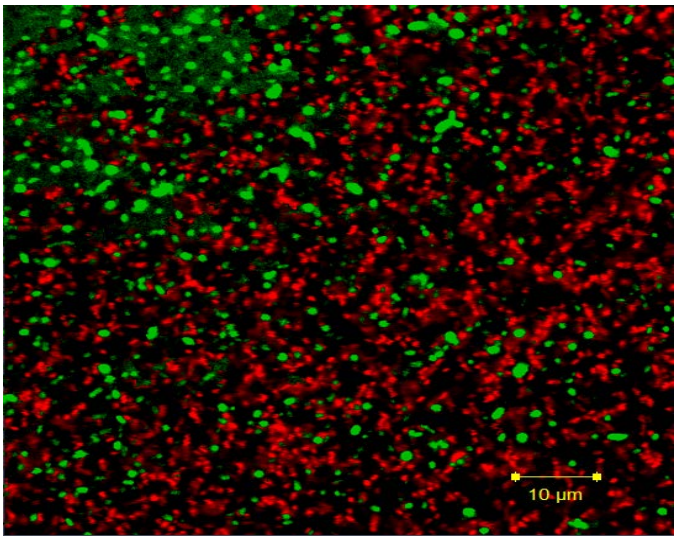

Figure 3: Live Enterococcus faecalis in calcium hydroxide nanofiber group (Group III)

Group IV: Triple antibiotic paste, Enterococcus faecalis seen in 20x magnification

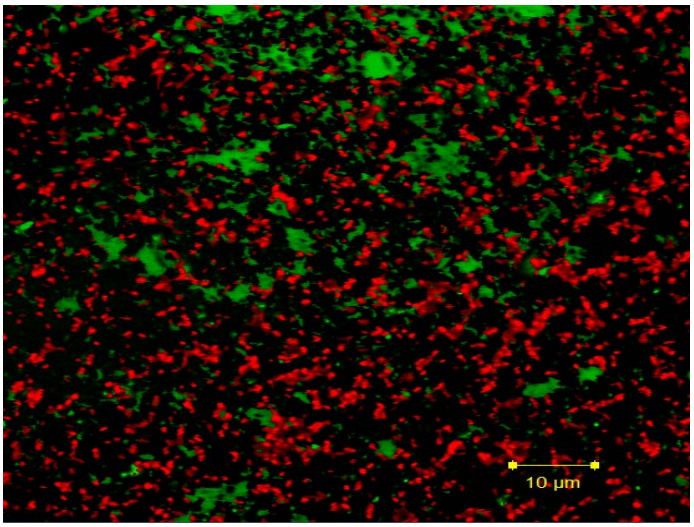

Figure 4: Live Enterococcus Faecalis in Triple antibiotic paste group (Group IV)

Group V: Triple antibiotic incorporated nanofiber, Enterococcus faecalis seen in 20x magnification

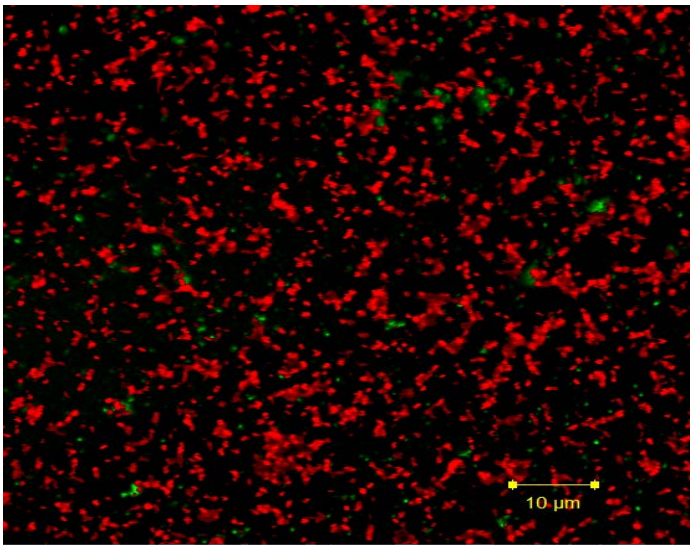

Figure 5: Live Enterococcus faecalis in Triple antibiotic nanofiber group (Group V)

Conclusion

Within the limitations of the study, it was concluded that triple antibiotic nanofibers are more effective against enterococcus faecalis followed by triple antibiotic paste, calcium hydroxide nanofiber and calcium hydroxide. Clinically, Triple antibiotic paste is more effective than calcium hydroxide and the nanofibers increase the efficacy of the intracanal medicament used by its effective drug delivery property

References

1. Azhar Iqbal. The factors responsible for endodontic treatment failure in the permanent dentitions of the patients reported to the college of dentistry, the university of aljouf, kingdom of Saudi arabia. JCDR. 2016;10(5):146-148.
2. Ashley Karczewski,BS, Sabrina Feitosa A, Ethan Hamer I, Divya Pankajakshan, Richard Gregory L, Kenneth Spolnik J, Marco Bottino C. Clindamycin-modified triple antibiotic nanofibers: A stain free antimicrobial intracanal drug delivery system. J Endod 2018;44(1):155-162.

3. Nikhil Vineeta, Sachin Gupta, Aditi Chandra. Retrievabilty of calcium hydroxide intracanal medicament with Chitosan from root canals: An in vitro CBCT volumetric analysis. J Conserv Dent 2014;17(5):454-457.
4. Armita Rouhani, Mahbobe Erfanzadeh, Hamid Jafarzadeh, Elham Najafi. Comparison of residual triple antibiotic paste, propolis and calcium hydroxide on root canal walls in natural open apex teeth: An in vitro study. Iran Endod J 2018;13(1):25-29.
5. Faruk Gokmese, Ibrahim Uslu, Arda Aytimur. Preparation and characterization of PVA/PVP nanofibers as promising materials for wound dressing. Polymer Plast Tech Eng 2013;52(12):1259-1265.
6. Hoshino.e, Kurihara-ando.N, Sato.I, uematsu.H, Sato.M, Kota.k, Iwaku.M. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. J Endod 1996;29:125-130.
7. Charles H. Stuart, , Scott A. Schwartz, , Thomas J. Beeson, and Christopher B. Owatz. Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment. J Endod 2006;32(2):93-98.
8. Ramaprabha Balasubramaniam , Srilekha Jayakumar. Antibiotics in endodontics - A concise review. IJADS 2017; 3(4): 323-329.
9. Zahed Mohammadi , Hamid Jafarzadeh, Sousan Shalavi , Shapour Yaripour , Farid Sharifi , JunIchiro Kinoshita. A review on triple antibiotic paste as a suitable material used in regenerative endodontics. Iran Endod J 2018;13(1): 1-6.
10. Swathi Pai, A. R. Vivekananda Pai , Manuel S. Thomas , Vishal Bhat. Effect of calcium hydroxide and triple antibiotic paste as intracanal medicaments on the incidence of inter-appointment flare-up in diabetic patients: An in vivo study. J Conserv Dent 2014;17(3):208-211.
11. Maria T. P. Albuquerque , Juliana, Nagata. Y, Anibal R, Diogenes , Asma A. Azabi, Richard L. Gregory, Marco C. Bottino. Clinical Perspective of Electrospun Nanofibers as a Drug Delivery Strategy for Regenerative Endodontics. Curr Oral Health Rep (2016);3(3):209-220.