

Comparative Evaluation of the Efficacy of Different Irrigation Systems for Removal of Triple Antibiotic Paste from Root Canals - An *In Vitro* Study

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Abstract

The main goal of endodontics is the complete elimination of bacteria from the root canal system [1]. Because of the complex root canal anatomy that includes the presence of apical deltas, oval extensions, accessory canals and isthmus, with the routine endodontic procedures followed, it is difficult to shape and clean the root canal completely [2,3]. The success of root canal therapy depends on the complete elimination of these diverse varieties of pathologic micro-organisms. While mechanical preparation of the root canal serves as a primary mechanism for the elimination of the bacteria, irrigants and intra canal medicaments serve as a valuable adjunct. The irrigants can enhance the mechanical debridement by aiding in removal of smear layer, manual flushing of the debris out of the root canal, dissolving the necrotic and vital pulp tissues which serve as a harbour of micro-organisms and by having an effective anti-microbial activity. Various irrigants have varied action to aid in debridement of the root canal space. Even though irrigation plays a vital role in elimination of bacteria from the root canal system, the wide spectrum of micro-organisms demands additional antibiotic agents for their complete elimination. The use of systemic antibiotics is considered invaluable for use in teeth undergoing root canal treatment, hence use of local delivery of antibiotics is suggested [4]. Thus, the combination of instrumentation associated with irrigation and intracanal medicament has been proposed to be effective against the polymicrobial flora of the root canal. The introduction of triple antibiotic paste was aimed at providing a combination that could possibly be effective against all of the micro-organisms in the root canal pathology. With this background, the aim of the study was to evaluate the effectiveness of few irrigation techniques and irrigating solutions for the removal of the most commonly used intracanal medicament TAP from root canal system of single rooted teeth.

Keywords: Endodontics; Triple Antibiotic Paste (TAP); Irrigation

Aim

The aim of this study was to comparatively evaluate the efficacy of different irrigation techniques using different irrigation solution in the removal of triple antibiotic paste (TAP) from root canal walls.

Materials and Methods

48 mandibular premolars extracted for orthodontic and periodontal reason were collected from Department Oral and Maxillo-

facial Surgery of Ahmedabad Dental College and Hospital, Ahmedabad (Image). The teeth were stored in normal saline until used. Later these 48 teeth were sectioned into halves, thus total sample size was 96 divided into 8 experimental groups with 12 samples (n = 12) in each group.

- **GROUP 1 (positive control group)** - n = 12 in which Triple Antibiotic Paste was not removed.
- **GROUP 2 (negative control group)** - n = 12 in which Triple Antibiotic Paste was not placed in the root canals.

- **GROUP 3** - n = 12 in which Triple Antibiotic Paste was removed using Endoactivator irrigation system, irrigated with 3% Sodium Hypochlorite.
- **GROUP 4** - n = 12 in which Triple Antibiotic Paste was removed using EndoVac irrigation system, irrigated with 3% Sodium Hypochlorite.
- **GROUP 5** - n = 12 in which Triple Antibiotic Paste was removed using 27 gauge vented needle, irrigated with 3% Sodium Hypochlorite
- **GROUP 6** - n = 12 in which Triple Antibiotic Paste was removed using Endoactivator irrigation system, irrigated with 2%w/v Chlorhexidine.
- **GROUP 7** - n = 12 in which Triple Antibiotic Paste was removed using EndoVac irrigation system, irrigated with 2%w/v Chlorhexidine.
- **GROUP 8** - n = 12 in which Triple Antibiotic Paste was removed using 27 gauge vented needle, irrigated with 2%w/v Chlorhexidine. Decoronation of sample was done with diamond disc attached to the micromotor under water coolant with a length of 14mm in all the samples.

Access cavity was modified in the decoronated samples with a rotor using a no.4 high speed round carbide bur along with water spray. Later, the teeth were irrigated with 3% Sodium Hypochlorite to remove the pulp remnants.

All the procedures were performed by the same operator. Working length was determined by viewing the tip of #10 K-file at the apical foramen and was established 1mm short of this length. Apical preparation upto file size #15 and #20 was done using Mani hand file. A proTaper SX file (Dentsply) was used to flare the orifice. ProTaper Next files were used sequentially as per manufacturer's instructions using crown down technique up-to X4. Between each instrument, the canals were conventionally irrigated with 5 ml of 3% sodium hypochlorite (NaOCl) and finally rinsed with 5 ml 17% EDTA, followed by a final rinse of 5 ml normal saline. During the irrigation, the needle was used in an up-down motion within the apical third. Throughout the cleaning and shaping procedures, apical patency was maintained by recapitulation with a number 10 K-file. After drying the canals with paper points all the 48 decoronated teeth received a paste of TAP. Triple antibiotic paste was prepared using 400 mg Metronidazole (Tablet I.P Flagyl) 100mg Minocycline

and 500mg Ciprofloxacin (Tablet BP Cifaur) in the ratio of 3:1:3 by wt% respectively by using commercially available tablets. This powder was then mixed with propylene glycol in the ratio 3:1 to obtain the paste form. This paste was placed into the root canals with the help of a lentulo spiral. The samples were stored in normal saline at room temperature for a period of 7 days.

For evaluation of removal of TAP, the samples were sectioned bucco-lingually using a diamond disc with copious water avoiding penetration into the root canal lumen and was split into two halves with a spatula. Each sample was then sub-divided in 3 sections, each of 4mm (coronal 4 mm, middle 4 mm and apical 4 mm). A total of 12 samples were obtained for each group. All the samples were analysed for the remaining TAP using stereomicroscope. Digital images of the root halves were obtained. The images were then transferred to the computer. The images were evaluated with DIGIMIZER image analysis software for the amount of TAP remaining in the canal. The amount of TAP remaining in the canal was measured in mm scored according to percentage. A scoring system was used to score the obtained samples for remaining TAP in the canals as:

- Score 0 - The canal was totally empty
- Score 1 - TAP was present in less than 25% of the canal surface.
- Score 2 - TAP was present in 50% of the canal surface.
- Score 3 - TAP was present in less than 75% of the canal surface.
- Score 4 - TAP was completely filled in the canal surface.

Results and Discussion

The success of the endodontic therapy is dependent on various factors that mainly govern the complete debridement of the root canal system of the pathological microorganisms. Though there is no general agreement defining the term "success" in endodontics, various studies have been carried out regarding the cause for the failure of the endodontic treatment. The goals of non-surgical endodontic re-treatment are to remove materials that are present in the root canal space, address deficiencies and repair defects and most importantly elimination of the persistent micro-organisms. It helps in identification of mechanical failures, previously missed canals and radicular subcrestal fractures. Additionally, it also allows the clinicians to three dimensionally clean and obturate the

root canal system. In case of regeneration TAP is placed within the canal for 7-21 days and later removed, but as we have to evaluate the removal of TAP, we have placed the ICM for 7 days within the root canal.

This study has an aim for comparing the efficacy of the different irrigation systems and solutions in the removal of TAP from the entire root canal wall. An *in vitro*, closed-ended canal model was used to simulate the clinical conditions. Various methods have been used to check the amount of residues on the canal walls, such as the use of digital photographs, stereomicroscopes, scanning electron microscopes, micro-computed tomographic imaging, and spiral computed tomographic imaging. The use of digital photography requires ample amount of practice and training to develop such skills. The clinician must know the photographic resources and have technical and operational expertise to improve the performance and outcomes, also for the beginners.

The scanning electron microscopes, micro-computed tomographic imaging studies give good observational results but are expensive hence, their use has not that drastically increased yet. In spiral computed tomographic imaging the costs, availability and the higher ionizing radiation exposure are always the area of concern. Considering the main advantages of stereo microscopes are that they can examine opaque specimens and provide a 3-D view of the sample. They also offer a large working distance allowing users to manipulate the specimens viewed by the scope [52]. Some stereo-microscopes are used at low magnification (4-40X) but there are some stereo microscopes that are hybrids between a light and stereo microscope capable of up to 2500X magnification [53]. In the present study stereomicroscope was used at 40x magnification because of easy availability and accuracy of result and economical factor.

Here single rooted mandibular premolars were used for study to avoid the complexities and variation in root canal anatomy. These samples were extracted for orthodontic or periodontal purposes and were used within 2 months of extraction. Early use of these samples is advised due to cracks formation in later stages. Also, there are chances of the tooth structure to get brittle and get fracture if kept for a longer duration of time. These samples were decoronated to achieve a working length of 14 mm due to ease of accessibility. After decoronation it becomes easier for the clinician to place any intracanal medicament in the root canal surface completely due to better visibility and ease of operation. All these samples were prepared upto F4 size to receive the freshly prepared

Triple Antibiotic Paste. The apical region has maximum colonization of bacteria hence it becomes important to attain a size where ICM and irrigants can reach and their proper mode of action is delivered. Many authors have suggested the apical preparation of size F4 for a tooth to receive any ICM. Various methods have been adopted for placing intracanal medicaments in the root canal. In the present study removal of Triple Antibiotic Paste from the samples was done using the irrigation systems such as Endoactivator (Dentsply, Tulsa, OK), Endovac (Kerr Endo Vac Starter Kit) and 27 Gauge (SS White) side vented needle along with 3% sodium hypochloride and 2% chlorhexidine gluconate irrigating solutions.

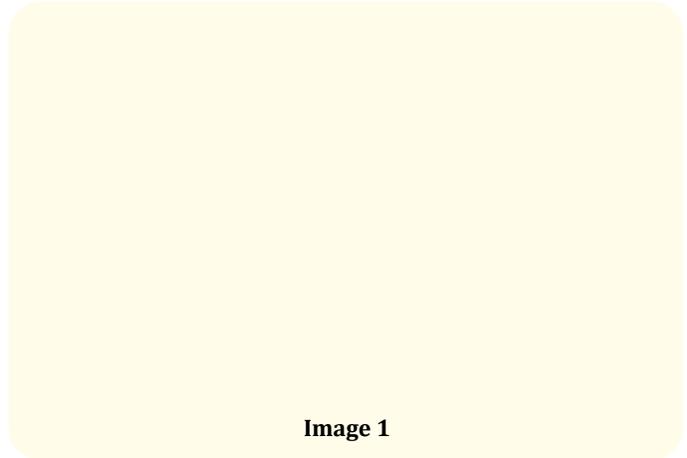


Image 1

Images of evaluation and digimizer system usage.

The amount of Triple Antibiotic Paste remaining in the root canal after the irrigation procedure in group 1, group 2, group 3, group 4, group 5, group 6, group 7 and group 8 were measured in mm and were recorded as score 0 to 4 of overall surface area using a Digimizer software. The results are tabulated in table 1 based on the scoring criteria.

Group	Coronal	Middle	Apical
Group 1	4.00 ±0.0	4.00 ± 0.0	4.00 ±0.0
Group 2	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0
Group 3	1.58 ±0.67	1.58 ± 0.90	1.33 ±0.65
Group 4	2.00 ± 1.13	2.25 ± 0.75	2 ± 0.60
Group 5	3.33 ± 0.65	2.58 ± 0.51	2.08 ± 0.67
Group 6	1.58 ± 0.67	1.58 ± 0.79	1.75 ±0.62
Group 7	2.5 ± 0.80	2.83 ± 0.39	1.91 ± 0.90
Group 8	2.41 ± 0.51	2.16 ± 0.58	1.91 ± 0.51

Table 1: Descriptive statistics calculated for all the 8 groups for coronal, apical and middle third. The prefix showing the mean and suffix showing the standard deviation.

Here the results show that group 3 and group 6 gave better results in coronal, middle and apical third as compared to group 4,5,7 and 8.

The scoring criteria was as follows

- Score 0 - The canal surface was totally empty.
- Score 1 - TAP was present in less than 25% of the root canal surface.
- Score 2 - TAP was present in 50% of the root canal surface.
- Score 3 - TAP was present in less than 75% of the root canal surface.
- Score 4 - TAP was completely filled in the root canal surface.

Group 2 showed p significant value < 0.05 as compared to any other group as it was the positive control group where no TAP was placed and was the most effective when compared to all the other groups.

	Coronal	Middle	Apical
Group 3 - Group 4	>0.05	>0.05	>0.05
Group 3 - Group 5	<0.001	0.065	>0.05
Group 3 - Group 6	>0.05	>0.05	>0.05
Group 3 - Group 7	0.198	0.002	>0.05
Group 3 - Group 8	0.336	>0.05	>0.05
Group 4 - Group 5	0.032	>0.05	>0.05
Group 4 - Group 6	>0.05	0.66	>0.05
Group 4 - Group 7	>0.05	0.747	>0.05
Group 4 - Group 8	>0.05	>0.05	>0.05
Group 5 - Group 6	<0.00>0.05	0.033	>0.05
Group 5 - Group 7	0.557	>0.05	>0.05
Group 5 - Group 8	0.34	>0.05	>0.05
Group 6 - Group 7	0.198	0.001	>0.05
Group 6 - Group 8	0.336	>0.05	>0.05
Group 7 - Group 8	>0.05	0.282	>0.05

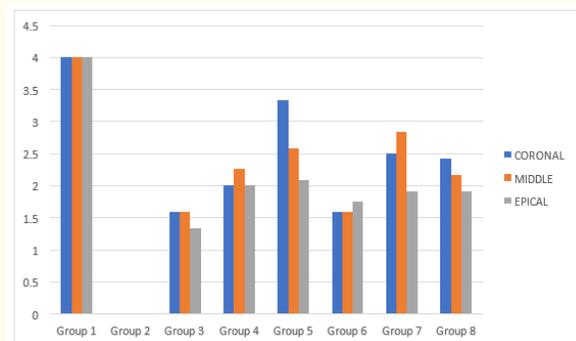
Table 2: Descriptive statistics calculated for 6 Groups (Group 3 to Group 8) for removal of TAP in coronal, apical and middle third. The results are intercompared within to find the best results for removal of TAP.

Here the results show that group 3 (at coronal middle and apical region) and Group 6 (at coronal middle and apical region) showed significant difference in removal of TAP, followed by Group 4 (at coronal third region) and group 8 (at middle and apical third region).

Intergroup statistical analysis		P significant value (0.05)
Group 1	Group 2	<0.001
	Group 3	<0.001
	Group 4	<0.001
	Group 5	.015
	Group 6	<0.001
	Group 7	<0.001
	Group 8	<0.001
Group 1	Group 2	<0.001
	Group 3	<0.001
	Group 4	<0.001
	Group 5	<0.001
	Group 6	<0.001
	Group 7	<0.001
	Group 8	<0.001
Group 1	Group 2	<0.001
	Group 3	<0.001
	Group 4	<0.001
	Group 5	<0.001
	Group 7	<0.001
	Group 8	<0.001

Table 3: Descriptive statistics calculated for all the 7 Groups for coronal, apical and middle third in comparison to positive control group i.e. Group 1 using the ANNOVA test.

Here the results show that all the groups showed significant removal of TAP from the root canal surface when compared to the positive control group which was totally filled of the intracanal medicament TAP.



Graph 1: Distribution of mean value for the remaining TAP after different irrigation protocols in Group 1 to Group 8 at coronal middle and apical third respectively.

Graph 2: Distribution of scores for the remaining TAP using 3% NaOCl irrigating solution in Group 3,4 and 5 at coronal middle and apical third respectively.

Graph 3: Distribution of scores for the remaining TAP using 2% CHX in group 6,7 and 8 at coronal middle and apical third respectively.

The distribution of scores among the eight groups is as follows.

Group 3 had the highest number of samples with score 1 followed by group 6. This distribution shows that higher numbers of samples with least amount of remaining TAP in the root canals were seen with group 3 and group 6.

Group 4 and group 7 showed maximum score 2 in coronal and apical third. This distribution shows that higher numbers of samples with 50 % of remaining TAP in the root canals were seen with group 4 and group 7.

Whereas group 5 and 8 showed maximum presence of TAP in coronal, middle and apical third regions of root canals.

The above results indicate that group 3 and group 6 had the lowest amount of TAP i.e. less than 25% of TAP when compared to other groups all over the root canal.

Image 2: Indicates the null hypothesis summary.

Conclusion

In spite of acceptable results in comparison to one another, no irrigation system and irrigating solution was able to completely remove the TAP from the root canal surface. Hence, within the limitations of the present study it can be concluded that, none of the proposed irrigation methods resulted in complete elimination of TAP from the root canals. But a combination of two systems may be effective [5-61].

Bibliography

1. Ricucci D and Bergenholtz G. "Bacterial status in root-filled teeth exposed to the oral environment by loss of restoration and fracture or caries - a histobacteriological study of treated cases". *International Endodontic Journal* 36 (2005): 787-797.
2. Peters OA. "Current challenges and concepts in the preparation of root canal systems: a review". *Journal of Endodontics* 30 (2004): 559-567.
3. Nar'r PNR., *et al.* "Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after one visit endodontic treatment". *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* 99 (2005): 231-252.

4. Mohammadi Z and Abbott PV. "On the local applications of antibiotics and antibioticbased agents in endodontics and dental traumatology". *International Endodontic Journal* 42.7 (2009): 555-67.
5. Chong BS and Pitt Ford TR. "The role of intracanal medicaments in root canal treatment". *International Endodontic Journal* 25.2 (1992): 97-106.
6. Iwaku M, et al. "Lesion sterilization and tissue repair (LSTR) therapy: new pulpal treatment. How to conserve infected pulps". Tokyo, Japan: Nihon-Shika Hyoron (1996).
7. Hoshino E., et al. "In vitro antibacterial susceptibility of bacteria from infected root dentin to a mixture of ciprofloxacin, metronidazole and minocycline". *International Endodontic Journal* 29 (1996): 125-130.
8. Sain S., et al. "Lesion Sterilization and Tissue repair-Current Concepts and Practices". *International Journal of Clinical Pediatric Dentistry* 11.5 (2018): 446-450.
9. Lima KC., et al. "Susceptibilities of Enterococcus faecalis biofilms to some antimicrobial medications". *Journal of Endodontics* 27.10 (2001): 616-619.
10. Abbott PV. "Selective and intelligent use of antibiotics in endodontics". *Australian Endodontic Journal* 26.1 (2000): 30.
11. Kim JH., et al. "Tooth discoloration of immature permanent sincipisor associated with triple antibiotic therapy: a case report". *Journal of Endodontics* 36 (2010): 1086-1091.
12. Van der Sluis LW., et al. "The evaluation of removal of calcium hydroxide paste from an artificial standardized groove in the apical root canal using different irrigation methodologies". *International Endodontic Journal* 40.1 (2007): 52-57.
13. Taşdemir T., et al. "Efficacy of several techniques for the removal of calcium hydroxide medicament from root canals". *International Endodontic Journal* 44.6 (2011): 505-509.
14. Ahmetoğlu F., et al. "Efficacy of self-adjusting file and passive ultrasonic irrigation on removing calcium hydroxide from root canals". *Dental Material Journal* 32.6 (2013): 1005-1010.
15. Chou K., et al. "Effectiveness of different intracanal irrigation techniques in removing intracanal paste medicaments". *Australian Endodontic Journal* 40.1 (2014): 21-25.
16. SA Türker, et al. "Comparison of calcium hydroxide removal by self-adjusting file, EndoVac, and CanalBrush agitation techniques: An *in vitro* study". *Journal of Conservative Dentistry: JCD* 16.5 (2013): 439.
17. Adl A., et al. "A Comparison between the Antimicrobial Effects of Triple Antibiotic Paste and Calcium Hydroxide against Enterococcus Faecalis". *Iranian Endodontic Journal* 7.3 (2012): 149-155.
18. Arslan H., et al. "Efficacy of Needle Irrigation, EndoActivator, and Photon-initiated Photoacoustic Streaming Technique on Removal of Double and Triple Antibiotic Pastes". *Journal of Endodontics* 40.9 (2014): 1439-1442.
19. Berkhoff JA., et al. "Evaluation of triple antibiotic paste removal by different irrigation procedures". *Journal of Endodontics* 40.8 (2014): 1172-1177.
20. Capar ID., et al. "Effect of Different Final Irrigation Methods on the Removal of Calcium Hydroxide from an Artificial Standardized Groove in the Apical Third of Root Canals". *Journal of Endodontics* 40.3 (2014): 451.
21. Chua., et al. "Antifungal effectiveness of various intracanal medicaments against *Candida albicans*: an *ex-vivo* study". *BMC Oral Health* 14 (2014): 53.
22. Arslan H., et al. "Efficacy of various irrigation protocols on the removal of triple antibiotic paste". *International Endodontic Journal* 47.6 (2014): 594-599.
23. Shaik J., et al. "Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and calcium hydroxide using chitosan as carrier against *Candida albicans* and *Enterococcus faecalis*: An *in vitro* study". *Journal of Conservative Dentistry* 17 (2014): 335-339.
24. Silva LJ., et al. "Micro-CT evaluation of calcium hydroxide removal through passive ultrasonic irrigation associated with or without an additional instrument". *International Endodontic Journal* 48.8 (2016): 768-773.
25. Topçuoğlu HS., et al. "Effectiveness of conventional syringe irrigation, vibringe, and passive ultrasonic irrigation performed with different irrigation regimes in removing triple antibiotic paste from simulated root canal irregularities". *Journal of Conservative Dentistry* 19.4 (2014): 323-327.
26. Khademi AA., et al. "Removal efficiency of calcium hydroxide intracanal medicament with RinsEndo system in comparison with passive ultrasonic irrigation, an *in vitro* study". *Dental Research Journal (Isfahan)* 12.2 (2015): 157-160.

27. Alturaiki S., et al. "Efficacy of 3 Different Irrigation Systems on Removal of Calcium Hydroxide from the Root Canal: A Scanning Electron Microscopic Study". *Journal of Endodontics* 41.1 (2015): 97-101.
28. Ok E., et al. "Effectiveness of Different Irrigation Solutions on Triple Antibiotic Paste Removal From Simulated Immature Root Canal". *Scanning* 37.6 (2015): 409-413.
29. Abbas Ali k., et al. "Removal efficiency of calcium hydroxide intracanal medicament with RinsEndo system in comparison with passive ultrasonic irrigation, an *in vitro* study". *Dental Research Journal (Isfahan)* 12.2 (2015): 157-160.
30. Akman M., et al. "Comparison of different irrigation activation regimens and conventional irrigation techniques for the removal of modified triple antibiotic paste from root canals". *Journal of Endodontics* 41.5 (2015): 720-724.
31. Thakur DA., et al. "Comparative scanning electron microscopy evaluation of Canal Brushing technique, sonic activation, and master apical file for the removal of triple antibiotic paste from root canal (*in vitro* study)". *Contemporary Clinical Dentistry* 6.4 (2015): 517-521.
32. Kuştarıcı A., et al. "Efficacy of Laser Activated Irrigants in Calcium Hydroxide Removal from the Artificial Grooves in Root Canals: An *Ex Vivo* Study". *Photomedicine, and Laser Surgery* 34.5 (2016): 205-210.
33. Wigler R., et al. "Efficacy of XP-endo finisher files in the removal of calcium hydroxide paste from artificial standardized grooves in the apical third of oval root canals". *International Endodontic Journal* 50.7 (2017): 700-705.
34. Görkem Can., et al. "Comparison of EndoVac, CanalBrush, EndoActivator and Syringe Irrigation on Removal of Triple Antibiotic Paste". *Journal of Dental and Medical Sciences* 16.12 (2017): 41-45.
35. Vishal Pai Swathi., et al. "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". *Journal of Conservative Dentistry* 17.3 (2014).
36. Amrita Chawla and Vijay Kumar. "Evaluating the efficacy of different techniques and irrigation solutions for removal of calcium hydroxide from the root canal system: A scanning electron microscope study". *Journal of Conservative Dentistry* 21.4 (2018): 394.
37. Kfir A., et al. "Efficacy of self adjusting file, XP-endo finisher and passive ultrasonic irrigation on the removal of calcium hydroxide paste from an artificial standardized groove". *Australian Endodontic Journal* 44.1 (2018): 26-31.
38. Santosh Kumar., et al. "Comparison of the efficacy of CanalBrush, EndoActivator, and Passive Ultrasonic Irrigation on the removal of triple antibiotic paste from root canal walls: An *in vitro* study". *International Journal of Preventive and Community Dentistry* 10.4 (2020): 424-430.
39. Mehmet Adigüzel and Koray Yilmaz. "Comparison of the efficacy of sonic irrigation and conventional syringe irrigation in the removal of curcumin and triple antibiotic paste from root canals". *Journal of Dental Research, Dental Clinics, Dental Prospects* 15.3 (2021): 157-162.
40. Sundqvist G., et al. "Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment". *Oral Surgery, Oral Medicine and Oral Pathology* 85 (1998): 86-93.
41. Hoshino E., et al. "*In vitro* antibacterial susceptibility of bacteria from infected root dentin to a mixture of ciprofloxacin, metronidazole and minocycline". *International Endodontic Journal* 29 (1996): 125-130.
42. Mozayeni MA., et al. "Antimicrobial effects of four intracanal medicaments on enterococcus faecalis: an *in vitro* study". *Iranian Endodontic Journal* 9.3 (2014): 195-198.
43. Oktay EA., et al. "Effect of intracanal medicaments used in endodontic regeneration on the push-out bond strength of a calcium-phosphatesilicate-based cement to dentin". *Pakistan Journal of Medical Sciences* 34.2 (2018): 310-315.
44. Galvão T., et al. "Efficacy of three methods for inserting calcium hydroxide-based paste in root canals". *Journal of Clinical and Experimental Dentistry* 9.6 (2017): e762-e766.
45. Heilborn C., et al. "Cleaning efficacy of an apical negative pressure irrigation system at different exposure times". *Quintessence International* 41 (2010): 759-767.
46. Parente JM., et al. "Root canal debridement using manual dynamic agitation or the endovac for final irrigation in a closed system and an open system". *International Endodontic Journal* 43 (2010): 1001-1012.
47. Ghivari S and Kubasad G. "Root canal debris removal using different irrigating needles: an SEM study". *Indian Journal of Dental Research* 22.5 (2011): 659-663.

48. Velayutham Gopikrishna, *et al.* "An *in vivo* assessment of the influence of needle gauges on endodontic irrigation flow rate". *JCD* 19.2 (2016): 189193.
49. Leighton TG. "The Acoustic Bubble, Chapters 1 and 2". New York: Academic Press (1994).
50. Manzur A., *et al.* "Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial". *Journal of Endodontics* 33 (2007): 114.
51. Okino LA., *et al.* "Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel". *International Endodontic Journal* 37 (2004): 38-41.
52. R Berdan. "Focus Stacking Comparing Photoshop, Helicon Focus and Zerene". *Motic America* (2020).
53. Schnitzler and K Zimmer. "Advances in Stereomicroscopy". Optical Design and Engineering III edited by L. Mazuray *et al.* Proc. of SPIE Vol. 7100. 7100P-1. Leica Microsystems Heerbrugg, Switzerland (2008).
54. Chow TW. "Mechanical effectiveness of root canal irrigation". *Journal of Endodontics* 9 (1983): 475-479.
55. Tay FR., *et al.* "Effect of vapor lock on root canal debridement by using a side-vented needle for positive pressure irrigant delivery". *Journal of Endodontics* 36 (2010): 74550.
56. Siqueira JF Jr., *et al.* "Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals". *Journal of Endodontics* 23 (1977): 499-502.
57. Rödiger T., *et al.* "Comparison of ultrasonic irrigation and RinsEndo for the removal of calcium hydroxide and Ledermix paste from root canals". *International Endodontic Journal* 44.12 (2011): 1155-1161.
58. McGill S., *et al.* "The efficacy of dynamic irrigation using a commercially available system (Rinsendo) determined by removal of a collagen "biomolecular film" from an *ex vivo* model". *International Endodontic Journal* 41 (2008): 602-608.
59. Mitchell RP., *et al.* "Comparison of apical extrusion of NaOCl using the endovac or needle irrigation of root canals". *Journal of Endodontics* 36 (2010): 338.
60. Gu LS., *et al.* "Review of contemporary irrigant agitation techniques and devices". *Journal of Endodontics* 35 (2009): 791-804.