ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 04, pp.21287-21292, April, 2022

DOI: https://doi.org/10.24941/ijcr.43370.04.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

MECHANICAL REDUCTION OF ENTEROCOCCUS FAECALIS BY PROTAPER GOLD AND RECIPROC BLUE IN APICAL THIRD OF ROOT CANAL: SCANNING ELECTRON MICROSCOPE AND MICROBIOLOGICAL EVALUATION

[1]Dr. Renu Sroa, [2]Dr. Baljeet Kumar, [3]Dr. Mamta Katal, [4]Dr. Harshveer Kaur, [5]Dr. Parul Chauhan and [6]Dr. Gaurav Sroa

[1]Professor and Head, Dept. of Conservative Dentistry and Endodontics, Punjab Government Dental College and hospital, Amritsar, Punjab, India
[2]Deptt., of Conservative Dentistry and Endodontics, Govt. Dental College and Hospital Amritsar, India
[3,4,5]Post-Graduate Student, Dept. of Conservative Dentistry and Endodontics, Punjab Government Dental College and Hospital, Amritsar, Punjab, India
[6]Intern, Department of Microbiology, Government Medical College, Amritsar, Punjab, India

ARTICLE INFO

Article History:

Received 19th January, 2022
Received in revised form
16th February, 2022
Accepted 29th March, 2022
Published online 30th April, 2022

Key words:

Pro Taper Gold; Reciproc Blue, Enterococcus Faecalis, Rotary, Reciprocating, Microbiological, SEM.

**Corresponding Author:*
Dr. Baljeet Kumar

ABSTRACT

Background: The microorganisms play an important role in pulpal and peri-radicular disease pathogenesis, their eradication by mechanical instrumentation is the ultimate goal of endodontic treatment. **Objective:** To evaluate the mechanical reduction of Enterococcus faecalis by Rotary ProTaper Gold instrument and Reciprocating Reciproc Blue instrument in apical third of root canal by microbiological culture analysis and scanning electron microscopy. **Material and Methods:** Sixty mandibular premolars were selected and randomly divided in two groups i.e., Group A (ProTaper Gold) and Group B (Reciproc Blue). The teeth were decoronated at 14 mm from the apex to standardize the length. All the samples were sterilized using autoclave, followed by contamination with Enterococcus faecalis. Contamination was confirmed by SEM evaluation. Pre-instrumentation samples were taken for microbiological evaluation. Instrumentation was done in Group A by ProTaper Gold and in Group B by Reciproc Blue. Post-instrumentation samples were taken for microbiological evaluation. The root canals were then split longitudinally into two halves and apical third of each specimen was examined under SEM. Thus collected data were statistically analyzed using paired 't' test, unpaired 't' test and Mann-Whitney test. **Results:** Both techniques showed statistically highly significant difference from pre-instrumentation value to post-instrumentation value ($p<0.001$). The instrumentation in Group B resulted in somewhat better bacterial reduction than in Group A, however no statistically significant difference was found ($p>0.05$). Statistical analysis by Mann-Whitney test showed no statistically significant difference in mean of SEM scores for bacteria at 1 mm and 3 mm level in both groups ($p>0.05$). **Conclusion:** Both instrumentation techniques were effective in reducing bacterial load after mechanical preparation with statistically insignificant difference between them.

Citation: Dr. Renu Sroa, Dr. Baljeet Kumar, Dr. Mamta Katal, Dr. Harshveer Kaur, Dr. Parul Chauhan and Dr. Gaurav Sroa. 2022. "Mechanical reduction of Enterococcus faecalis by Protaper Gold and Reciproc Blue in apical third of root canal: Scanning electron microscope and microbiological evaluation", *International Journal of Current Research*,14, (04), 21287-21292.

INTRODUCTION

The mechanical and biological goals of root canal treatment consist of reduction in the number of pathogenic organisms, disorganizing the bacterial biofilm, eradication of residual vital & non vital pulp tissue, facilitating canal disinfection through mechanical instrumentation and giving suitable confirmation for subsequent hermetic seal.

As microorganisms play an important role in pulpal and peri-radicular disease pathogenesis, their eradication predicts the result of endodontic treatment. The endodontic infections are poly-microbial in nature. Out of multiple bacterial species responsible for endodontic infections, Enterococcal faecalis is the most common microorganism found in persistent endodontic infections. Enterococcus faecalis is adaptable to harsh, high pH environment and has the ability to form biofilms within root canals making it the primary organism

responsible for post- treatment failure[1,2]. The chemomechanical preparation to reduce bacterial counts in infected root canals involves mechanical instrumentation, use of different irrigation solutions and antimicrobial medicaments. Although chemical agents in the form of irrigating solutions or intracanal medicaments play important role in chemomechanical preparation, but the core method for bacterial reduction in the infected root canal by mechanical instrumentation should be evaluated[3,4]. The automated mechanical NiTi instruments can be used in either continuous rotary or reciprocating motion. The NiTi rotary files have been developed over the years to improve their properties. One such method to increase their properties is by thermal treatment of alloy. In heat treated alloys, Pro Taper Gold (PTG; Dentsply Maillefer, Ballaigues, Switzerland) have been introduced with advanced metallurgy with gold heat treatment that is claimed by the manufacturer to enhance flexibility, cyclic fatigue, cutting efficiency & safety[5].

In reciprocating motion, single file systems have gained attention because of their simplicity and reduced working time. In reciprocating motion, Reciproc Blue (VDW, Munich, Germany) have been introduced. Reciproc Blue instrument is overall improved version of conventional Reciproc instrument having higher cyclic fatigue resistance, lower microhardness and increased flexibility than conventional Reciproc system[6]. The apical third of root canal system is most difficult to clean due to presence of complex apical morphology and narrow diameter than coronal third.This area has highprevalence of bacteria in necrotic teeth and failure to properly clean this area will be considered as potential cause of persistent endodontic infection leading to endodontic failure[7]. To the best of our knowledge, there is limited literature comparing the mechanical reduction of Enterococcus faecalis with rotary and reciprocating instrument in the apical third of root canal. Therefore, the present ex-vivo study was undertaken to compare the reduction of Enterococcus faecalis with ProTaper Gold and Reciproc Blue in the apical one third of root canals by using microbiological & SEM evaluation.

MATERIALS AND METHODS

Sixty freshly extracted human mandibular premolar teeth with straight and single root canals were collected. Pre-operative radiographs were taken and the teeth having straight root canals and non complicated root canal anatomy were selected. To provide disinfection & organic tissue fixation, teeth were stored in 10 % formalin for 24 hours and teeth were then cleaned thoroughly with curettes (Hu-Friedy, USA) to remove bone, debris, calculus and soft tissue on the root surface. The selected teeth were decoronated at 14mm from the apex to standardize the root length. Apical patency of each canal was determined with # 10 K file and working length was established 1mm short of apical foramen. Root canals were then instrumented to size #25 K file one mm short of apical foramen. Irrigation was done with sterile saline with 5 ml plastic syringe. Root canals were then be filled 17% EDTA for three minutes to remove smear layer & 5 ml of distilled water (Sai Chemical, Pune, India) was added to finally rinse the canal. Sealing of apical foramen was done with composite resin (Ivoclar Vivadent Te econom plus, Schaan, Liechtenstein) & all the external root surface of canals was varnished with nail polish to prevent bacterial leakage. Each root canal was sterilized in autoclave (Sterline, Dental X, Italy) at 120^0 C and 15 psi for 15 minutes.

Roots were divided randomly into two experimental groups viz:Group A and Group B depending on the instrument used, with each group having thirty teeth. Sterilization was confirmed microbiologically as well as under scanning electron microscope by selecting one root randomly from each group. The root canals of remaining fifty-eight teeth were infected using 24-hour pure culture suspension of Enterococcus faecalis (ATCC 29212) cultivated in brain heart infusion (BHI). McFarland standard no.4 was used to evaluate the broth in order to obtain the bacterial count 1.2 X 10^9 colony forming units (CFU) per milliliter. Through cervical aperture 20 ml of E. faecalis suspension was added and sealing of cervical aperture was done with temporary cement. The roots were placed in sterile petri dish & incubated at 37^0 C for 24 hours. Two roots were selected randomly for evaluation under scanning electron microscope to confirm bacterial contamination. Bacterial sampling from remaining fifty-six root canals were performed before instrumentation & after instrumentation. Before instrumentation initial samples were taken by inserting sterile #15 paper points into the canal for 1 minute and stored in tubes containing 500 ml of peptone water. Serial dilutions were prepared and were plated in brain heart infusion agar at 37^0 C for 24 hours for bacterial count in colony-forming units CFU/ml (Figure 1 and 3). After pre-instrumentation sampling, mechanical Instrumentation of canal was done with ProTaper Gold in Group A and with Reciproc blue in Group B.

Group A: With torque controlled endodontic electromotor (X-Smart Plus, Dentsply Maillefer, Ballaigus, Switzerland) the root canals were prepared in crown-down technique with ProTaper Gold (Dentsply Maillefer, Ballaigus, Switzerland) shaping files S_X, S1 and S2 and finishing files F1 and F2 files in sequential order with continuous in and out movement at 300 rpm with torque of 3 Ncm for S_x and S1, 1.5 Ncm for S2 and F1 files, 3 Ncm for F2. The Sx file was used first in the canal up to two-third the working length of canal followed by S1, S2, F1 (size 20,0.07 taper) and F2 (size 25,0.08 taper) at working length of canal. The instruments were removed every time after three gentle in- and -out motion strokes and cleaned. The same procedure was repeated till working length was reached.

Group B: Reciproc blue file R25 (0.25/0.08) was used in 'RECIPROC ALL MODE' at 300 rpm with up and down pecking motion with amplitude of 3mm until cervical and middle third of canal was prepared. After 3 up and down pecking movements file was removed to clean the flutes and then reinserted and the procedure was repeated till it reaches the working length. Irrigation was done in canals during and after completion of instrumentation with 5 ml sterile saline solution with plastic syringe. Post-instrumentation bacterial samples were taken in a similar manner as pre-instrumentation samples by #25 paper points and were plated in brain heart infusion agar at 37^0 C for 24 hours for bacterial count in colony-forming units CFU/ml(Figure 2 and 4). Two longitudinal grooves were given on buccal and lingual aspect of root without perforating the root canal and then splitting of root canal into two halves was done by using mallet and chisel. One half of each sample was selected for examination of apical one-third under scanning electron microscope.These samples were dehydrated by immersion in ethanol solutions (70%, 90% & 100%). The specimens were then be dried in critical point dryer. Roots were mounted on metallic stubs and gold sputtering done.

Examination under scanning electron microscope (SUPRA 55VP, ZEISS, Germany) at 5000 X magnification was done for apical third thereafter and images were taken at 1mm and 3mm level from apical foramen. These images were scored for the presence of bacteria by applying Paranjpe Aetal. (2012) scoring criteria (Figure 5):

Score 1: - No bacteria on the surface of root canal

Score 2:- Isolated bacteria over the surface with no signs of viability/organization (mitoses, biofilm matrix).

Score3: - Agglomeration of bacteria with signs of viability/organization (mitosis, biofilm matrix).

Score 4: - More than 50% of root canal walls was covered with viable bacteria.

Score 5: - Complete or nearly complete root canal wall coverage with viable bacteria. The data thus collected were tabulated and statistically analysed using student's paired 't' test and unpaired 't' test for microbiological evaluation and Mann- Whitney test for scanning electron microscopic evaluation.

RESULTS

The overall results of the microbiological and SEM evaluation are summarized in Table 1 and Table 2, respectively. As per table 1, statistical analysis by paired 't' test and p-value showed that the reduction in number of colony forming units from S1 (pre-instrumentation) to S2 (post-instrumentation) was statistically highly significant in both groups ($p<0.001$).The percentage reduction from pre-instrumentation value (S1) to post-instrumentation value (S2) was somewhat better in group B (Reciproc Blue) than group A (Protaper Gold) with no statistically significant difference ($p>0.05$). As per table 2, statistical analysis by Mann-Whitney test and p-value showed that there was no statistically significant difference ($p>0.05$) in SEM scores at 1 mm between Group A (Protaper Gold) and Group B (Reciproc Blue). Also, at 3 mm level, there was no statistically significant difference ($p>0.05$) between Group A (Protaper Gold) and Group B (Reciproc Blue).

DISCUSSION

The present study was designed to compare the reduction of Enterococcus faecalis with rotary ProTaper Gold and reciprocating single-file system Reciproc Blue in the apical third of root canals using microbiological and SEM evaluation. For the study, sixty human mandibular premolar teeth with straight and single root canals were selected to avoid variations in the root canal system that may cause error in the results[8]. Root canals were pre-enlarged to size #25 k-file for easy bacterial penetration into dentinal tubules and also to standardize the further instrumentation[9]. After irrigation with saline, root canals were filled with 17% EDTA for three minutes to remove smear layer and to open dentinal tubules. Sterilization was confirmed microbiologically as well as under scanning electron microscope by randomly selecting one root from each group. Microbiological culture procedure is used as gold standard in literature to assess bacterial reduction produced by endodontic instruments[10].

Another method to assess bacterial reduction and biofilm removal is by direct observation of surface topography at high magnification by Scanning electron microscope[11]. All the fifty-eight root canal samples were contaminated with pure culture of Enterococcus faecalis (ATCC-29212).Enterococcus faecalis is a non-fastidious, gram-positive facultative anaerobe. It has the ability to tolerate harsh environment like extreme alkaline pH, high temperature, nutritional scarcity, as well as persist in the presence of intracanal medicaments. It remains viable even in extended period of nutritional scarcity, can penetrate deep into dentinal tubules and form biofilms making it resistant to conventional endodontic treatment measures. It is believed that persistence of microorganism in the apical part of root filled teeth is responsible for development of post-treatment disease and Enterococcus faecalis being the most resistant organism amongst intracanal bacteria, making it the focus of present study[12]. The apical size preparation by an instrument is important parameter for effective bacterial load reduction as confirmed by various studies[13]. Therefore, in the present study apical size preparation by both instrumentswas standardized to #25.

In microbiological culture analysis, the results ofpresent study revealed that mean percentage reduction from pre-instrumentation value to post-instrumentation value was 96.80% in ProTaper Gold and 97.41% inReciproc Blue. The results ofpresent study are in concurrence with Martinho FC et al. who evaluated the effectiveness of single-file reciprocating systems Reciproc and WaveOne, and rotary Proaper Universal in removing endotoxins and cultivable bacteria in endodontic retreatment.Both single-file reciprocating systems [WaveOne (98.27%) and Reciproc (99.54%)] and rotary system [ProTaper (98.73%)] were effective in reducing bacterial load ($P > 0.05$), with no statistically significant difference found among the systems tested[14]. It is evident from the above results that both preparation techniques were effective in reduction of bacterial load. However, when comparing the results, the mean percentage reduction of Reciproc Blue was higher than ProTaper Gold. This may be attributed to the S- shaped cross-section with two cutting edges that impart efficient dentin cutting to the instrument. The positive cutting angle give aggressive aspect to the instrument. The two cutting edges provide more chip space so that it can load and eliminate more debris and bacteria coronally with less chances of lateral compaction obstructing the dentinal tubules that might impair the removal of bacteria from inside them[2,15,16]. The results of present study are in accordance with the study by Choudhary D who evaluated the canal cleaning efficacy with Reciproc Blue and found that Reciproc Blue showed better cleaning efficacy than other file systems tested[17]. Carvalho MC et al. also reported that Reciproc Blue was more effective than other rotary file system used in reducing bacterial load[18].

The findings of present study are in agreement with Marinho ACS et al.who evaluated the effectiveness in removal of bacterial load and endotoxins between Reciproc, MTwo, ProTaper Universal and FKG Race and found that all systems were effective in reducing bacterial load and endotoxins from root canals with no statistically significant difference between them[19]. Similar results were reported by Machado MEL etal.whoevaluated the bacterial reduction between WaveOne, Reciproc, ProTaper and MTwo systems and found that no statistically significant difference was found among all instrument systems in terms of bacterial reduction[20].

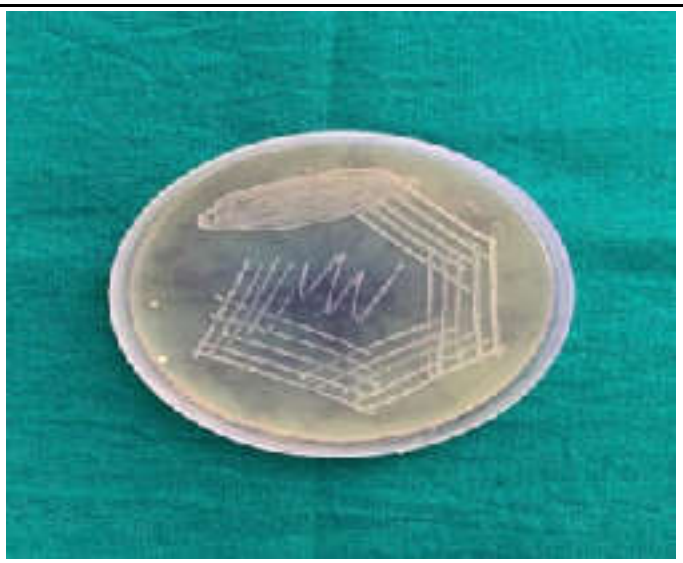

Figure 1

Figure 2

Figure 1 , Figure 2. BHI agar culture plates showing pre & post- instrumentation colonies in Group A (Protaper Gold)

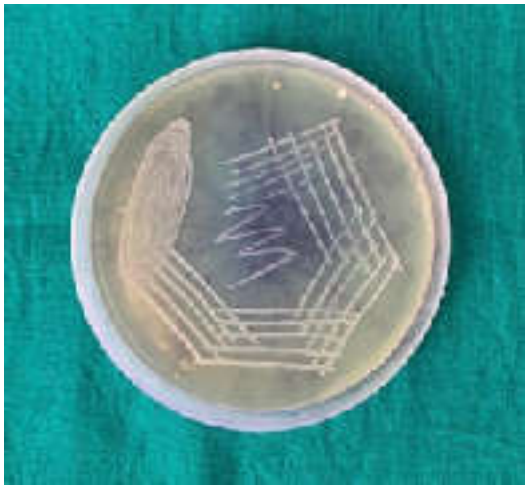

Figure 3

Figure 4

Figure 3 & Figure 4. BHI agar culture plates showing pre & post- instrumentation colonies in Group B (Reciproc Blue)

Table 1. Mean reduction in pre-instrumentation and post-instrumentation values (intragroup) using paired 't' test

Group	S1		S2		Reduction from S1 to S2	p-value	%Reduction from S1 to S2
	Mean	SD	Mean	SD			
Group A (Protaper Gold)	10.12x104	2.20x104	0.31x104	0.11x104	9.81 x104±2.20	<0.001**	96.80±1.42
Group B (Reciproc Blue)	9.90 x104	1.92x104	0.24x104	0.08x104	9.65 x104±1.91	<0.001**	97.41±1.08

*NS: p > 0.05 Non significant; S: *p< 0.05 Significant; **p < 0.001: Highly significant; SD: Standard Deviation*

Table 2. Mean of sem scores at 1 mm and at 3 mm level using Mann-Whitney test

Group	Score at 1 mm			Score at 3 mm		
	Mean ±SD	Mean rank	p-value	Mean±SD	Mean rank	p-value
Group A (Protaper Gold)	1.96±0.69	32.00	0.082^{NS}	1.39±0.62	30.68	0.185^{NS}
Group B (Reciproc Blue)	1.64±0.73	25.00		1.18±0.39	26.32	

*NS: p>0.05 Non significant; S: *p<0.05 Significant; **p<0.001: Highly significant; SD: Standard Deviation*

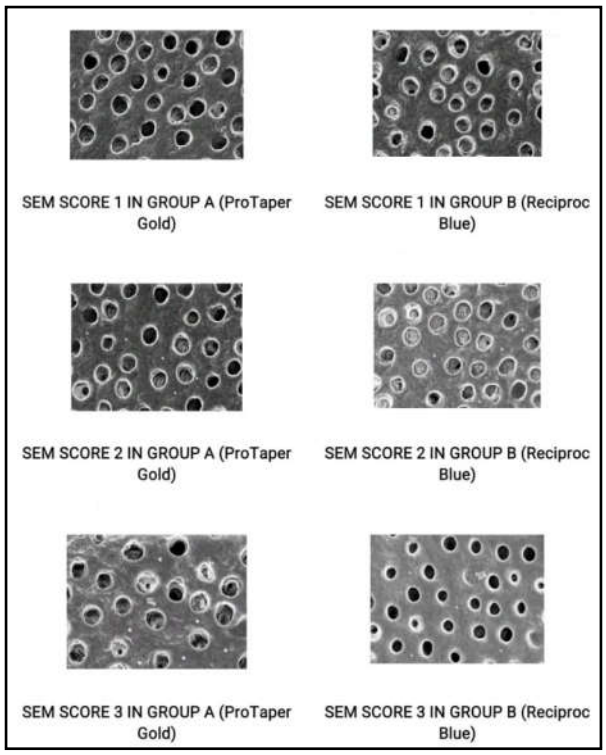

Figure 5. SEM images of the two groups

In Scanning electron microscope examination, both the tested groups removed bacterial load more effectively at 3 mm level as compared to 1 mm level. However, the mean scores for bacteria at both 1 mm and 3 mm level was somewhat less in Reciproc Blue (i.e. 1.64 and 1.18, respectively) as compared to Pro Taper Gold (i.e. 1.96 and 1.39, respectively) with no statistically significant difference ($p>0.05$).The findings of present study are in accordance with study conducted by Paranjpe A et al. who found SEM scores for bacterial load to be higher at 1 mm level as compared to 3 mm level[21]. The results from scanning electron microscopic evaluation were compatible with the microbiological culture analysis findings. From the above discussion, it may be inferred that Reciproc Blue system is somewhat better in reducing bacterial load as compared to ProTaper Gold in the apical third of the canal, however, no statistically significant difference was found between them. Hence, both rotary ProTaper Gold and reciprocating Reciproc Blue may be recommended as a potential instrumentation technique for bacterial reduction in the apical third ofroot canal.

CONCLUSION

It is evident from the above results that both preparation techniques were effective in reduction ofbacterial load. Under the conditions of present study, it may be inferred that both microbiologically and scanning electron microscopically, Reciproc Blue system is somewhat better in reducing bacterial load as compared to ProTaper Gold in the apical third of the canal, however, no statistically significant difference was found between them. This study solely evaluated the bacterial reduction with automated mechanical instrumentation which is not reflective of their overall performance, their additional aspects must be considered. Before drawing any definitive conclusion, clinical evaluation with larger number of samples and more extensive research should be done in terms of bacterial reduction to evaluate both instrumentation techniques in future.

SOURCE OF CONFLICT: None Declared.

FUNDING OF STATEMENT: This research was not funded by any external or government funding sources.

REFERENCES

1. Peciuliene, V, Maneliene, R, Balcikonyte, E, Drukteinis, S & Rutkunas, V 2008, 'Microorganisms in root canal infections: a review', *Stomatologija*, vol. 10, no. 1, pp. 4-9.
2. Halkai, R, Hegde, MN & Halkai, K 2012, 'Enterococcus faecalis can survive extreme challenges-overview', *Journal of Health and Allied Sciences NU*, vol. 2, no. 03, pp. 49-53.
3. Aydin, C, Tunca, YM, Senses, Z, Baysallar, M, Kayaoglu, G & Ørstavik, D 2007, 'Bacterial reduction by extensive versus conservative root canal instrumentation in vitro', *Acta Odontologica Scandinavica*, vol. 65, no. 3, pp. 167-170.
4. Machado, MEDL, Nabeshima, CK, Caballero-Flores, H, Elmadjian-Filho, M, Duarte, MAH, Odinola-Zapata, R & Cai, S 2017, 'Instrument design may influence bacterial reduction during root canal preparation', *Brazilian dental journal*, vol. 28, no. 5, pp. 587-591.
5. Hieawy, A, Haapasalo, M, Zhou, H, Wang, ZJ & Shen, Y 2015, 'Phase transformation behavior and resistance to bending and cyclic fatigue of ProTaper Gold and ProTaper Universal instruments', *Journal of endodontics*, vol. 41, no. 7, pp. 1134-1138.
6. De-Deus, G, Silva, EJNL, Vieira, VTL, Belladonna, FG, Elias, CN, Plotino, G & Grande, NM 2017,'Blue thermomechanical treatment optimizes fatigue resistance and flexibility of the Reciproc files', *Journal of endodontics*, vol. 43, no. 3, pp. 462-466.
7. Sacomani, AC, Cintra, FT, de Jesus Soares, A & Frozoni, M 2021, 'The influence of working length on the reduction of biofilm and planktonic bacteria in oval canals with reciprocating instrumentation', *Research, Society and Development*, vol. 10, no. 5, pp. e9810514765-e9810514765.
8. Vossoghi, M, Vossoghi, M, Shahriari, S, Faramarzi, F, Mashouf, RY & Farhadian, M 2016, 'Efficacy of a novel rotary system in reduction of intracanal bacteria: An in Vitro Study', *Iranian endodontic journal*, vol. 11, no. 3, p.219.
9. Ferrer-Luque, CM, Bejarano, I, Ruiz-Linares, M & Baca, P 2014, 'Reduction in Enterococcus faecalis counts–a comparison between rotary and reciprocating systems', *International endodontic journal*, vol. 47, no. 4, pp. 380-386.
10. Fouad, AF 2017, *Endodontic Microbiology*, Second Edition, John Wiley & Sons, Inc., New Jersey.
11. Paradella, TC & Bottino, MA 2012, 'Scanning electron microscopy in modern dentistry research', *Brazilian Dental Science*, vol. 15, no. 2, pp. 43-48.
12. Sjögren, ULF, Hägglund, B, Sundqvist, G & Wing, K 1990,'Factors affecting the long-term results of endodontic treatment', *Journal of endodontics*, vol. 16, no. 10, pp. 498-504.

13. Martinho, FC, Freitas, LF, Nascimento, GG, Fernandes, AM, Leite, FR, Gomes, AP & Camões, IC 2015, 'Endodontic retreatment: clinical comparison of reciprocating systems versus rotary system in disinfecting root canals', *Clinical oral investigations*, vol. 19, no. 6, pp. 1411-1417.
14. Bürklein, S, Hinschitza, K, Dammaschke, T & Schäfer, E 2012, 'Shaping ability and cleaning effectiveness of two single-file systems in severely curved root canals of extracted teeth: Reciproc and WaveOne versus Mtwo and ProTaper', *International endodontic journal*, vol. 45, no. 5, pp. 449-461.
15. Bucheli, JC, Rios-Osorio, N, Usme, D, Jimenez, C, Pinzon, A, Rincón, J, Azuero-Holguin, MM, Zubizarreta-Macho, A, Gomez-Sosa, JF & Munoz, HR 2021,'Three-dimensional analysis of the root canal preparation with Reciproc Blue, WaveOne Gold and XP EndoShaper: a new method in vivo', *BMC Oral Health*, vol. 21, no. 1, pp.1-10.
16. Aydın, ZU, Keskin, NB & Özyürek, T 2019, 'Effect of Reciproc blue, XP-endo shaper, and WaveOne gold instruments on dentinal microcrack formation: A micro-computed tomographic evaluation', *Microscopy research and technique*, vol. 82, no. 6, pp. 856-860.
17. Choudhary, D 2021,'Comparative evaluation of cleaning efficacy of three different single file systems: an in vitro study', *Journal of Advances in Medicine and Medical Research*, vol. 33, no. 12, pp.26-31.
18. Carvalho, MC, Zuolo, ML, Arruda-Vasconcelos, R, Marinho, ACS, Louzada, LM, Francisco, PA, Pecorari, VGA & Gomes, BPFDA 2019, 'Effectiveness of XP-Endo Finisher in the reduction of bacterial load in oval-shaped root canals', *Brazilian oral research*, vol. 33, p. e021.
19. Marinho, ACS, Martinho, FC, Gonçalves, LM, Rabang, HRC & Gomes, BPFA 2015, 'Does the Reciproc file remove root canal bacteria and endotoxins as effectively as multifile rotary systems?', *International endodontic journal*, vol. 48, no. 6, pp. 542-548.
20. Machado, MEL, Nabeshima, CK, Leonardo, MFP, Reis, FAS, Britto, MLB & Cai, S 2013, 'Influence of reciprocating single-file and rotary instrumentation on bacterial reduction on infected root canals', *International endodontic journal*, vol. 46, no. 11, pp. 1083-1087.
21. Paranjpe, A, de Gregorio, C, Gonzalez, AM, Gomez, A, Herzog, DS, Piña, AA & Cohenca, N 2012, 'Efficacy of the self-adjusting file system on cleaning and shaping oval canals: a microbiological and microscopic evaluation', *Journal of Endodontics*, vol. 38, no. 2, pp. 226-231.

******