Elimination of *E. faecalis* and *C. albicans* Biofilm: A Comparison Between Single and Multiple File Systems in an *Ex Vivo* Study

Verónica Méndez-González MsC¹; Claudia Casillas-Contreras MsC²; Marlen Vitales-Noyola PhD³; Diana Lorena Alvarado-Hernández PhD⁴; Ana María González-Amaro MsC⁵; Antonio Aragón-Piña PhD⁶; Amaury Pozos-Guillén PhD⁷

1. Endodontics Postgraduate Program, Faculty of Dentistry, UASLP, San Luis Potosí, SLP, México. https://orcid.org/0000-0002-0620-6603
2. Endodontics Postgraduate Program, Faculty of Dentistry, UASLP, San Luis Potosí, SLP, México. https://orcid.org/0000-0002-8783-6040
3. Endodontics Postgraduate Program, Faculty of Dentistry, Research Center of Health Sciences and Biomedicine (CICSaB), UASLP, San Luis Potosí, SLP, México. https://orcid.org/0000-0002-1659-7476
4. Research Center of Health Sciences and Biomedicine (CICSaB). Departments of Immunology and Microbiology, School of Medicine, UASLP, San Luis Potosí, SLP, Mexico. https://orcid.org/0000-0001-5986-0011
5. Endodontics Postgraduate Program, Faculty of Dentistry, UASLP, San Luis Potosí, SLP, México. https://orcid.org/0000-0002-6375-9642
6. Institute of Metallurgy, Faculty of Engineering, UASLP, San Luis Potosí, SLP, México. https://orcid.org/0000-0003-2246-9152
7. Basic Sciences Laboratory, Faculty of Dentistry, UASLP, San Luis Potosí, S.L.P., México. https://orcid.org/0000-0003-2314-8465

Correspondence to: PhD. Amaury Pozos-Guillén - apozos@uaslp.mx

ABSTRACT: To evaluate whether the WaveOne Gold and Reciproc single file instrumentation systems, are effective in reducing the microbial load of a mixed biofilm and the cleaning of apical third compared to the Twisted File Adaptive system (multiple-file system). Seventy mesial roots of the first and second molars were included and randomly divided into three experimental groups (n=20, n=10 controls). Biofilms were formed inside canals over 31 days. After instrumentation with the unique file systems, WaveOne Gold and Reciproc and the multiple file system Twisted File Adaptive, using 2.25% sodium hypochlorite as an irrigant in all cases, a count of colony forming units was performed using serial dilutions, cleaning of the apical third was evaluated using scanning electron microscopy. Comparisons amongst groups were made by using parametric and non-parametric statistics, according to a normal or non-normal data
distribution, respectively. No significant differences in the reduction of the microbial load after employing a single-file system in comparison to the multiple-file system were found; in addition, the cleaning of the apical third was similar for the three different instrumentation systems. The single-file system is equal in effectiveness compared with the multiple-file system in reducing the microbial load.

KEYWORDS: Candida albicans; Enterococcus faecalis; Reciproc; TFA; WaveOne Gold.

INTRODUCTION

The key role of endodontic therapy relies on its ability to decrease the presence of microorganisms. The complete disinfection of root canals guarantees the success of this therapy. Essential factors for achieving this aim are the use of ideal chemical irrigants and an adequate instrumentation system; however, there are some factors that make the total disinfection of root canal systems impossible (1). The root canal is a unique environment where biological selection drives the type and course of infection. In an anaerobic environment, microbial interactions and nutrient availability are factors that define the composition of the root canals' microbial flora (2). Some microorganisms present in the root canals can survive after the endodontic therapy, for instance, Candida albicans and Enterococcus faecalis. In fact, these microorganisms have been strongly correlated with endodontic failure or secondary infections (3-5).

C. albicans is a yeast that has been reported to colonize the dentine, causing infection in root canals system; it can compete for space and nutrients with other microorganisms of the endodontic flora (6) and form hyphae, allowing invasion through pores and incomplete walls (7). E. faecalis is a Gram-positive coccus and facultative anaerobe able to survive in extreme environments,
such as alkaline pH and temperatures ranging from 10-60°C, allowing it to cause periradicular infection including persistent endodontic infections. It can also interact with other strict anaerobes such as Porphyromonas, Prevotella, and Fusobacterium (8-10).

Indeed, there is a wide range of instrumentation systems whose objective is to achieve the best conditions for root canals, decrease the bacterial concentration in a minimal amount of time, and provide adequate characteristics of endodontic files, such as greater resistance and flexibility as well as decrease the incidence of fractures to the instrumentation systems. The WaveOne Gold system has a variable taper in the active portion that aids to improve flexibility and allows a more conservative preparation of the root canal system at the coronal zone. In addition, this system has a reciprocal movement of 150 degrees of rotation in an anti-clockwise direction, serving to cut dentine and then rotate the file 30 degrees in a clockwise direction to release the instrument (11). Reciproc is a single instrumentation system for the preparation of root canals that employs reciprocal rotational movements. This instrument cuts in an anti-clockwise direction with approximately 120 degrees of movement and then relieves the tension of the instrument with clockwise movement (12). Twisted File Adaptive (TFA) is an instrumentation system that employs unique motion technology that automatically adapts to the pressure of the instruments. This system uses interrupted movements both clockwise and anti-clockwise in a continuous rotation, thus allowing better removal of debris in oval root canals. Additionally, when the canal is modeled due to greater pressure in the metal, the movement of the instrument changes to an oscillating mode (13).

The aim of this study was to evaluate whether the WaveOne Gold and Reciproc single-file instrumentation systems produce a reduction in the microbial load of the apical third compared to the Twisted File Adaptive system (multiple-file system). The proposed null hypothesis was that there would be no difference between the single and multiple-file systems in reducing the microbial load of mixed biofilm.

MATERIALS AND METHODS

SAMPLES

First and second lower molars were extracted from patients with dental caries, periodontal disease, and/or for orthodontic reasons at the Oral Surgery Clinic of the Faculty of Dentistry. All patients signed an informed consent form, and this study was approved by the Ethics Committee in accordance with Helsinki Declaration. Teeth without previous endodontic treatment, without internal, external, or apical resorption, and without calcified canals were included in this study. The crown of each tooth was cut using a low-speed saw (Isomet, Buehler, Lake Bluff, IL), and an X-ray of the dental roots was taken to measure the curvature using Autocad software (Autodesk, San Rafael, CA). Dental roots measuring less than 25 degrees were accepted for the study. Overall, seventy mesial dental roots were included in this study (n=20 per group and 10 controls). The standardization of the roots to 15mm was done with a K #10 endodontic file (Dentsply Sirona, York, PA), and afterwards, the roots were instrumented serially with a K #20 file (Dentsply Sirona). The roots were cleaned as described in the protocol by Haapasalo & Orstavik (13) which includes 5.25% NaOCl, 17% ethylene diamine tetra-acetic acid (EDTA) and distilled water. Then, the roots were sterilized with moist heat in an autoclave (KitLab USA, Chicago, IL) for 15 minutes at 121°C.

In vitro formation of mixed biofilm: C. albicans and E. faecalis were isolated from the root canals of patients with endodontic secondary infections at the endodontic clinic, and the biochemical identification of the microorganisms
was made using the API test (bioMérieux sa, Marcy l’Etoile, France) according to the manufacturer’s instructions. In vitro formation of the mixed biofilm on the dental roots was made in static form over 31 days with Brain Heart Infusion (BHI) culture medium (BD Bioxon, Estado de México, México) and Dextrosa-Sabouraud (DS) (BD Bioxon) (75% and 25%, respectively); additionally, 50μl of human serum was added at the start of biofilm formation for *C. albicans* growth (14). The culture medium was changed every two days, and Gram stain was used for monitoring the microbial growth.

**IN VITRO INSTRUMENTATION OF ROOT CANALS**

After 31 days of biofilm formation, the dental roots were placed in a device filled with silicone-based impression material (Speedex putty, Coltene, Alstätten, Switzerland) and the operative field disinfection protocol was performed using these solutions: 5.25% NaOCl, 10% Na₂SO₃ and 30% H₂O₂. The pre-instrumentation samples inside root canals were taken with sterile paper tips #20 and were placed in BHI/DS culture medium for 24h at 37°C. Then, the instrumentations were made by one operator according to the manufacturer’s instructions. The Twisted File Adaptive (TFA) system (SybronEndo, Coppell, TX) was performed with adaptive movement by an electric motor (Elements Motors, Axis/SybronEndo, Coppell, Texas), at 500rpm with #15 file up to work length, diameter D₁ of 0.25mm and 6% conicity, while the Reciproc (VDW, Munich, Germany) and WaveOne Gold (Dentsply Sirona Endodontics, Ballaigues, Switzerland) systems were performed by alternative reciprocal movement using the X Smart plus electric motor (Dentsply Mailiefer, Switzerland). For Reciproc system was employed #15 file up to work length with diameter D₁ of 0.25mm and 8% conicity and for Wave One Gold system the root canals were permeabilized up to apical zone by using #10 file with diameter D₁ of 0.25mm and 7% conicity and later the glide path was performed with ProGlyder file (Dentsply) up to work length. Later of instrumentation with each system, the root canals were irrigated with two milliliters of 2.25% NaOCl using the Endoeze syringe (Ultradent Products Inc, South Jordan, Utah) with the simultaneous aspiration of the content of the root canal. The apical permeability was maintained using the #10 K file, and the final irrigation was made with passive ultrasonic irrigation with 17% EDTA and 5.25% NaOCl. Immediately after the root canals instrumentations, the post-instrumentation samples were taken with #35 K file inside the root canal and placed in BHI/DS culture medium for 24h at 37°C. Controls were roots without instrumentation and roots without biofilm formation. In addition, distilled water was used as irrigation control.

**COUNT OF COLONY-FORMING UNITS**

After 24h of incubation of the pre-and post-instrumentation samples, the McFarland scale was measured for all samples, and serial dilutions were made with sterile distilled water and spread on BHI/DS agar plates through a massive spread with an “L” shape loop. Then, the plates were incubated for 24h at 37°C, and the CFU was counted in a semiautomatic counter (Felisa, Jalisco, Mexico). The total CFU count was made with this formula: CFU= (number of CFU) (dilution factor)/milliliters of culture. In addition, the percent of elimination of microbial load was calculated for the 3 employed systems, using distilled water as control and sodium hypochlorite as irrigant at 2.25% and 5.25%.

**SCANNING ELECTRON MICROSCOPE**

After post-instrumentation samples were taken, all teeth were submitted to the evaluation of the cleanliness of the apical zone of root canals using the different instrumentation systems through the Scanning Electron Microscope (SEM) (Jeol JSM-6610LV, Peabody MA). First, the mesial roots were longitudinally cut in sense lingual-vestibular and were dehydrated with alcohol at different concentrations (10% until absolute alcohol). Then,
the roots were totally dehydrated in a critical point dryer (Leica EM CPD030, Wien, Austria). Later, gold coating was added to all the dental roots using the JEOL JFC-1100 equipment (Jeol), and the samples were processed until 10,000 magnifications using SEM. Data of cleanliness of the apical zone was reported as permeability of dental tubules by using the next formula: percent of permeability=100-percent of microbial presence. The presence of microbial load in root canals was evaluated by 2 observers to simple blind using the modified scale Rome, at 1 and 3 millimeters in apical zone.

STATISTICAL ANALYSIS

The data regarding the decrease of microbial load and cleanliness of apical zone are expressed as the mean±standard deviation or as the median and interquartile range, according to their normal or non-normal distribution, respectively. The Kolmogorov-Smirnov test was employed for calculating the normality of data. Comparisons among the groups were performed by using Student’s t-test, the U Mann-Whitney test, the Kruskal-Wallis test, or one-way analysis of variance (ANOVA), as required. All data were analyzed by using GraphPad Prism software v5.0 (GraphPad, San Diego, CA), and p<0.05 was considered significant.

RESULTS

Decreasing of microbial load by using the different instrumentation systems. After 31 days of biofilm formation inside root canals, the samples were instrumented and irrigated, as indicated in the material and methods section. There was a significant diminution of CFU at the three different files systems after instrumentation in comparison to that before instrumentation (Wave One Gold=8.31x10^7±4.37x10^7 vs 71.50±213.5, mean±standard deviation, before and after instrumentation, respectively. Reciproc=3.98x10^7±3.64x10^7 vs 255.0±710.0, mean±standard deviation, before and after instrumentation, respectively. TFA=5.35x10^7±6.42x10^7, vs 351.0±776.5, mean±standard deviation, before and after instrumentation, respectively) (P<0.0001) (Figure 1. A-C); in addition, after comparing the diminution of the microbial load in the three systems after instrumentation of a single file using Wave One Gold or Reciproc and multiple files by TFA, there were no statistically significant differences among the three files systems (71.50±213.5, 255.0±710.0, 351.0±776.5, mean±standard deviation, respectively) (P=0.54) (Figure 1. D). The percent of elimination of the microbial load was calculated for each instrumentation system using different concentrations of NaOCl and distilled water as a control, and there was a significant difference in the percent elimination of microbial load with NaOCl in comparison to distilled water (WaveOne Gold=0.6±0.54 vs 99.9±0.04, mean±standard deviation, Reciproc=0.6±0.0547 vs 99.98±0.044, mean±standard deviation, TFA=0.06±0.54, vs 99.9±0.047, mean±standard deviation (P<0.0001) in all instrumentation systems (Figure 2. A-C), but there were no significant differences employing 2.25% NaOCl in comparison with 5.25% in all cases (100±0.00015, 100±0.01, 100±0.002, mean±standard deviation) (P>0.05) (Figure 2. A-C). When comparing the elimination percent of the microbial load with the three different systems using 2.25% NaOCl as an irrigant, there was no statistically significant difference (p=0.34) (Figure 2.D). In controls without instrumentation, in vitro formation of E. faecalis and C. albicans biofilms was observed; cocci and yeast are observed at different magnifications along the root canal (Figure 3. A-B).
Figure 1. Diminution of colony forming units after instrumentation. The counts for the diminution of the bacterial load of the mixed biofilm was performed as indicated in the methods for each different instrument system employed. A) Number of colonies forming units (CFU) before and after instrumentation with the WaveOne Gold system. B) Number of colonies forming units (CFU) before and after instrumentation with the Reciproc system. C) Number of colonies forming units (CFU) before and after instrumentation with the Twisted File Adaptive (TFA) system. D) Number of colonies forming units (CFU) after instrumentation with WaveOne Gold, Reciproc and Twisted File Adaptive (TFA) systems. Horizontal lines (D) correspond to the median and the first and third quartiles. *P=0.05; **P=0.01; ***P=0.005.

Figure 2. Percent of elimination of the microbial load. The percentage of elimination of the microbial load was calculated for each instrumentation system employed. A) Percent of elimination of the microbial load for the Wave One Gold system. B) Percent of elimination of the microbial load for the Reciproc system. C) Percent of elimination of the microbial load for the Twisted File Adaptive (TFA) system. D) Percent of elimination of the microbial load for the WaveOne Gold, Reciproc and Twisted File Adaptive (TFA) systems. Horizontal lines (A-D) correspond to the mean. *P=0.05; **P=0.01; ***P=0.005.
Figure 3. *In vitro* formation of the biofilm of *E. faecalis* and *C. albicans*. Controls without instrumentation were employed to evaluate the *in vitro* formation of the biofilm on root canals and root canals without biofilm formation was employed as negative controls. A) Root canals without biofilm formation, 400X, 1000X and 2000X of magnification. B) Cocci and yeast corresponding to *E. faecalis* and *C. albicans* inside root canals at 2500X, 7500X and 10000X of magnification. C) Inside of a root canal with a biofilm at 55X, 4000X and 10000X.

Evaluation of dentinal permeability. The dentinal permeability of the apical third was evaluated by scanning electron microscope as indicated in material and methods. There were no significant differences in the percent permeability of the dentinal tubules evaluated at 1mm in the different instrumentation systems, WaveOne Gold, Reciproc and Twisted File Adaptive (35.71±28.35, 17.86±27.82 and 46.43±39.34, mean±standard deviation) (p=0.285) (Figure 4. A). Similar results were observed at 3mm, where no significant differences in the percent of dentinal permeability in the different instrumentation systems was found (35.71±31.81, 46.43±39.34 and 50.0±40.82, mean±standard deviation) (P=0.753) (Figure 4. B).
**DISCUSSION**

There are several endodontic pathogenic microorganisms capable of causing pulp pathology; one of the aims of root canal treatment is to decrease these opportunistic pathogens and maintain the ideal shape and anatomy of the root canals (15). Indeed, there are many instrumentation systems that allow the reduction of the microbial load in the minimal amount of time and maintain the root canals in the best possible conditions (16). However, in some cases, it is not possible to eliminate all microorganisms present inside root canals, driving to an endodontic failure or endodontic secondary infections. The microorganisms commonly found in secondary infections are *E. faecalis*, with an incidence of 24-77%, which is associated more frequently with asymptomatic cases (17). Nevertheless, there are many studies that have demonstrated the presence of fungi in endodontic infections, not only bacteria. Thus, fungi are also of interest in the development of periradicular infections since they can form biofilms alone or with other bacterial species (18). The most common fungus that causes infection in root canals is the yeast *C. albicans*, as well as its pseudohyphae, which can penetrate dentinal tubules (19). Thus, we decided to employ these two microorganisms in this study. In addition, there are some studies indicating that *E. faecalis* has the
capacity to invade the dentinal tubules and survive endodontic treatment, staying viable ex vivo in root canals for at least 12 months (20). Sen et al. (7) have demonstrated that the presence of the smear layer increases the adherence of C. albicans in the dentine. They raised the hypothesis that an increase in adhesion is due to the disintegrated organic structure of dentine and the availability of calcium ions as sources for growth and adherence. These microorganisms form biofilms, which make it difficult to eliminate microbes from root canals. In this study, the formation of a mixed biofilm was performed using a static system over 31 days, as has been reported in other studies where maturation of the biofilms of these microorganisms is carried out over at least 20-30 days (7).

The decrease of endodontic microorganisms can be achieved by indirect methods, for example, by not eliminating the endodontic microorganisms but rather by only altering their environment. It has been demonstrated that a change in the internal environment where the infection is produced can destabilize bacterial metabolism and thus reduce the number of bacteria and the severity of pathology (21,22). This is an important factor that should be considered in future in vitro evaluations.

Recently, it has been confirmed that single file instrumentation systems can prepare and completely clean the root canals (23) and decrease the global time of work by 40% in comparison with traditional rotatory techniques using continuous movement, but the downside to using these NiTi instruments is fracture risk associated with an increase of fatigue caused by repetitive use and the possibility of cross contamination (24). Therefore, it is recommended to use multiple file instrumentation systems of different diameters with the aim of gradually widening the root canal. For this reason, in the present study, the evaluation of the cleaning and disinfection efficiency of this system was performed.

Bürklei et al. (25) studied curved root canals in vitro using a single file instrumentation system such as WaveOne Gold and Reciproc in comparison to serial instrumentation systems such as Mtwo and Protaper, and they compared the time of work and the cleaning using 2.25% NaOCl as an irrigant; in addition, the evaluation of the cleaning was performed using a numeric scale, and the presence of a smear layer was evaluated by SEM of the coronal, medium and apical third of the root canals. In the four systems, they found similar results with partially no instrumented areas. In this study, no significant differences in the presence or absence of the smear layer or dentinal permeability among the different instrumentation systems studied were found; similar results have been observed in other studies (16,26-28). Thus, the single file instrumentation systems are as effective in the cleaning of root canals as the multiple files instrumentation systems. In addition, these single file systems (WaveOne Gold and Reciproc) showed shaping ability in severely curved canals (29). However, opposite results have been observed in other studies, which mention that multiple file systems (as Hyflex EDM and XP-endo shaper) show significantly greater bacterial reduction in comparison to single file systems such as WaveOne Gold (30). These authors concluded that the limited shaping ability of single file systems in wider canals could compromise disinfection.

Other authors have reported that a reduction in the bacterial inner root canals does not depend on the instrumentation system employed; they conducted a study comparing different instruments and concluded that the key factors for bacterial reduction are the extension and irrigation of root canals, in addition to the total time of instrumentation and irrigation (31-33).

In this study, we evaluated the elimination of the intraradicular biofilm of three different instrumentation systems in vitro; however, in vivo
studies evaluating the microbial load before and after the instrumentation process in patients who underwent endodontic treatment and comparing different single and multiple file systems are necessary to support these results. In addition, it would also be interesting to perform in vitro studies evaluating the reduction of biofilm in teeth with major curvatures, since the decrease represents a greater challenge by the endodontist, and more technical ability is required in those cases.

The relevance of this study relies in the fact that in endodontic practice, one of the main objectives is the cleansing of root canals; this objective is achieved, among others, by using the instrumentation systems. The single file systems have the advantage of reducing work time and eliminating the endodontic microorganisms in the same form as the multiple file systems; in addition, the shown simplicity and reduction of crossed contamination, and the fact that every time there is a better acceptance of the single files instrumentation by Endodontists. Although there are several studies focusing on the evaluation of the ability of cleansing and disinfecting different instrumentation systems, this study has data with a mixed biofilm of endodontic treatment resistant microorganisms, since biofilm usually consists of different species in root canals.

CONCLUSIONS

The use of single file instrumentation systems is comparable in the reduction of bacterial concentration and the cleaning of the apical third with multiple file instrumentation systems.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: V.M.G. and C.C.C. Literature review: M.V.N. and A.H.D.L. Methodology and validation: V.M.G., A.M.G.A. and A.A.P. Formal analysis: M.V.N. Investigation and data collection: A.P.G., M.V.N. and D.L.A.H. Resources: C.C.C. Data analysis and interpretation: A.A.P. and M.V.N. Writing-original draft preparation: A.P.G., M.V.N. and D.L.A.H. Writing-review & editing: A.P.G. Supervision: A.P.G. Project administration: V.M.G. Funding acquisition: V.M.G.

REFERENCES

1. Mohammadi Z., Jafarzadeh H., Shalavi S., Palazzi F. Recent advances in root canal disinfection: A review. Iran Endod J. 2017; 12 (4): 402-406. doi: 10.22037/iej.v12i4.17935
2. Olcay K., Ataoglu H., Belli S. Evaluation of related factors in the failure of endodontically treated teeth: A Cross-sectional Study. J Endod. 2018; 44 (1): 38-45. doi: 10.1016/j.joen.2017.08.029
3. Adams N., Tomson P.L. Access cavity preparation. Br Dent J. 2014; 216 (6): 333-339. doi: 10.1038/sj.bdj.2014.206
4. Dewhirst F., Chen T., Izard J., et al. The human oral microbiome. J Bacteriol. 2010; 192 (19): 5002-5017. doi: 10.1128/JB.00542-10.
5. Sakko M., Tjäderhane L., Rautemaa-Richardson R. Microbiology of root canal infections. Prim Dent J. 2016; 5 (2): 84-89. doi: 10.1308/205016816819304231.

6. Siqueira J.F. Jr., Sen H. Fungi in endodontic infection. Oral Surg Oral Med Oral Pathol. 2004; 97 (5): 632-641. doi: 10.1016/S1079210404000046

7. Sen B.H., Chugal N.M., Liu H., Fleischmann J. A new method for studying the adhesion of Candida albicans to dentin in the presence or absence of smear layer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 96 (2): 201-206. doi: 10.1016/s1079-2104(03)00165-3

8. Siqueira J.F. Jr. Endodontic infections: Concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002; 94 (3): 281-293. doi: 10.1067/moe.2002.126163

9. Stuart H., Schwartz A., Beeson J., Owatz B. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006; 32 (2): 93-99. doi: 10.1016/j.ijo.2005.10.049

10. Kapil J., Parolia A., Shetty K., Mehta K. Biofilm in endodontic: a Review. J Int Soc Prev Community Dent. 2015; 5 (1): 1-12. doi: 10.4103/2231-0762.151956

11. Johnson M., Flannagan E., Sedgley E. Coaggregation interactions between oral and endodontic enterococcus faecalis and bacterial species isolated from persistent apical periodontitis. J Endod. 2006; 32 (10): 946-952. doi: 10.1016/j.ijo.2006.03.023

12. Dioguardi M., Troiano G., Laino L., et al. ProTaper and WaveOne systems three-dimensional comparison of device parameters after the shaping technique. A micro-CT study on simulated root canals. Int J Clin Exp Med. 2015; 8 (10): 17830-17834. eCollection 2015.

13. Marinho A.C., Martinho F.C., Goncalves L.M., Rabang H.R.C., Gomes B.P.F.A. Does the Reciproc file remove root canal bacteria and endotoxins as effectively as multifile rotary systems? Int Endod J. 2015; 48 (6): 542-548. doi: 10.1111/iej.12346

14. Gonzalez A.M., Corpus E., Pozos-Guillén A., Silva-Herzog D., Aragon-Piña A., Cohenca N. Continuous drip flow system to develop biofilm of E. faecalis under anaerobic conditions. ScientificWorldJournal. 2014; 2014: 1-5. doi: 10.1155/2014/706189

15. Vaishnavi N. Endodontic microbiology. J Dent Clin. 2010; 13 (4): 15-19. doi: 10.4103/0972-0707.73386

16. Yared G. Canal preparation using only one Ni-Ti rotary instrument: preliminary observations. Int Endod J. 2008; 41 (4): 339-344. doi: 10.1111/j.1365-2591.2007.01351.x

17. Gambarini G., Piasecki L., Di Nardo D., et al. Incidence of deformation and fracture of twisted file adaptive instruments after repeated clinical use. J Oral Maxillofac Res. 2016; 7 (4): e5. doi: 10.5037/jomr.2016.7405

18. Tennert C., Fuhrmann M., Wittmer A., et al. New bacterial composition in primary and persistent/Secondary endodontic infection with respect to clinical and radiographic findings. J Endod. 2014; 40 (5): 670-677. doi: 10.1016/j.joen.2013.10.005

19. Nair P.N. Endodontic biofilm, technology and pulpal regenerative therapy: where do we go from here? Int Endod J. 2014; 47 (11): 1003-1011. doi: 10.1111/iej.12287

20. Chandra J., Kuhn D., Mukherjee P., Hoyer L.L., McCormick T., Ghannoum M.A. Biofilm formation by the fungal pathogen Candida albicans: Development, architecture, and drug resistance. J Bacteriol. 2001; 183 (18): 5385-5394. doi: 10.1128/JB.183.18.5385-5394.2001

21. Yang Y., Shen Y., Wang Z., et al. Evaluation of the susceptibility of multispecies biofilms in dentinal tubules to disinfecting solutions. J Endod. 2016; 42 (8): 1246-1250. doi: 10.1016/j.joen.2016.05.011
22. Van der Waal S., de Almeida J., Krom B.P., de Soet J.J., Crielaard W. Diffusion of antimicrobials in multispecies biofilms evaluated in a new biofilm model. Int Endod J. 2017; 50 (4): 367-376. doi: 10.1111/iej.12634

23. Ghivari S.B., Bhattacharya H., Bhat K.G., Pujar M.A. Antimicrobial activity of root canal irrigants against biofilm forming pathogens- An in vitro study. J Conserv Dent. 2017; 20 (3): 147-151. doi: 10.4103/JCD.JCD_38_16

24. Dhingra A., Ruhal N., Miglani A. Evaluation of single file systems Reciproc, Oneshape, and WaveOne using cone beam computed tomography -an in vitro study. J Clin Diagn Res. 2015; 9 (4): ZC30-ZC34. doi: 10.7860/JCDR/2015/12112.5803

25. Bürklein S., Hinschitza K., Dammaschke T., Schäfer E. 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. Int Endod J. 2012; 45 (5): 449-461. doi: 10.1111/j.1365-2591.2011.01996.x

26. De Oliveira B.P., Aguiar C.M., Câmara A.C., et al. Evaluation of microbial reduction in root canals instrumented with reciprocating and rotary systems. Acta Stomatol Croat. 2015; 49 (4): 294-303. doi: 10.15644/asc49/4/4

27. Carvalho M. de S., Junior E.C., Bitencourt Garrido A.D., Roberti Garcia L.F., Franco Marques A.A. Histological evaluation of the cleaning effectiveness of two reciprocating single-file systems in severely curved root canals: Reciproc versus WaveOne. Eur J Dent. 2015; 9 (1): 80-86. doi: 10.4103/1305-7456.149648

28. Ahlquist M., Henningsson O., Hultenby K. The effectiveness of manual and rotary techniques in the cleaning of root canals: a scanning electron microscopy study. Int Endod J. 2001; 34 (7): 533-537. doi: 10.1046/j.1365-2591.2001.00429.x

29. Bürklein S., Flüch S., Schäfer E. Shaping ability of reciprocating single-file systems in severely curved canals: WaveOne and Reciproc versus WaveOne Gold and Reciproc blue. Odontology. 2018; 107 (1): 96-102. doi: 10.1007/s10266-018-0364-3

30. Üreyen Kaya B., Erik C.E., Sesli Çetin E., Köle M., Maden M. Mechanical reduction in intracanal Enterococcus faecalis when using three different single-file systems: an ex vivo comparative study. Int Endod J. 2018; 52 (1): 77-85. doi: 10.1111/iej.12984

31. Siqueira J.F. Jr., Rôças I.N., Favieri A., Lima K.C. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5% and 5.25% sodium hypochlorite. J Endod. 2010; 26 (6): 331-334. doi: 10.1097/00004770-200006000-00006

32. Rôças I.N., Lima K., Siqueira J.F. Jr. Reduction in bacterial counts in infected root canals after rotary or hand nickel titanium instrumentation-a clinical study. Int Endod J. 2013; 46 (7): 681-687. doi: 10.1111/iej.12045

33. Topçuoğlu H.S., Düzgün S., Aktı A., Topçuoğlu G. Laboratory comparison of cyclic fatigue resistance of WaveOne Gold, Reciproc and WaveOne files in canals with a double curvature. Int Endod J. 2017; 50 (7): 713-717. doi: 10.1111/iej.12674