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The influence of working length on the reduction of biofilm and planktonic bacteria in oval canals with reciprocating instrumentation

A influência do comprimento de trabalho na redução de biofilme e bactérias plantônicas em canais ovais com instrumentação reciprocante

La influencia de la longitud de trabajo en la reducción de biopelículas y bacterias plantónicas en canales ovalados con instrumentación recíproca

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Arieth Cristina Sacomani
ORCID: https://orcid.org/0000-0002-2176-6801
Faculdade de Odontologia São Leopoldo Mandic, Brazil
E-mail: sacomani.endodontics@gmail.com
Fernanda Tessaro Cintra
ORCID: https://orcid.org/0000-0002-7576-1328
Faculdade de Odontologia São Leopoldo Mandic, Brazil
E-mail: nandatess@gamil.com
Adriana de Jesus Soares
ORCID: https://orcid.org/0000-0002-8078-1606
Universidade Estadual de Campinas, Brazil
E-mail: ajsoares.endo@uol.com.br
Marcos Frozoni
ORCID: https://orcid.org/0000-0001-8001-4063
Faculdade de Odontologia São Leopoldo Mandic, Brazil
E-mail: marcosfrozoni@gmail.com

Abstract
To evaluate the influence of reciprocating single-file instrumentation with different working lengths (WL) on the reduction of planktonic bacteria and bacterial biofilm in Enterococcus faecalis-contaminated oval root canals. Methodology: Fifty-five human single-rooted canines were used. Fifty were inoculated with *E. faecalis* for 21 days for biofilm formation. To confirm the formation of biofilm adhered to the root canal wall, 5 contaminated samples from positive control group were analyzed by SEM. Samples were assigned into 3 groups (n = 15) according to working length determined, G+1 root canal preparation 1 mm beyond the apical foramen, G0 root canal preparation at the major foramen, and G-1 root canal preparation 1 mm short of the major foramen. Five roots were not inoculated to serve as a negative control. Bacteriological samples were collected prior to preparation, initial collection (S1), and after reciprocating instrumentation (S2) by disaggregating biofilm to quantify the reduction of planktonic bacteria and intracanal biofilm at different WL. Bacterial quantitation was performed using colony-forming units per milliliter (CFU / mL) count. Statistical analysis was performed at the significance level of 0.05. Results: No bacterial growth was observed in the negative control. All positive controls demonstrated bacterial growth; S1 from all teeth were positive for bacteria with no significant difference. The post-hoc analysis showed G+1 promoting a significantly higher disinfection than G-1 ($p<0,05$) and G-1 similar disinfection to G0 (P=962). Conclusion: Instrumentation as close as possible to major foramen or beyond it improves decontamination in oval root canals with reciprocating instrumentation.
Keywords: Enterococcus faecalis; Apical foramen; Disinfection; Root canal therapy.

Resumo
Avaliar a influência da instrumentação reciprocante com diferentes comprimentos de trabalho (CT) na redução de bactérias planctônicas e biofilme bacteriano em canais ovais contaminados com Enterococcus faecalis. Metodologia: Foram utilizados 55 caninos humanos unirradiculares. Cinqüenta foram inoculados com E. faecalis por 21 dias para a formação de biofilme. Para confirmar a formação de biofilme, 5 amostras contaminadas do grupo controle positivo foram analisadas por MEV. As amostras foram divididas em 3 grupos (n = 15) de acordo com o CT, preparo do canal radicular G + 1, 1 mm além do forame apical, preparo do canal radicular G0 no forame principal e preparo do canal radicular G-1m 1 mm antes de o forame principal. Cinco raízes não foram inoculadas para (controle negativo). Amostras bacteriológicas foram coletadas antes do preparo (S1) e após instrumentação (S2) quantificar a redução de bactérias planctônicas e biofilme intracanal em diferentes WL. A quantificação bacteriana foi realizada usando unidades formadoras de colônias por mililitro (UFC / mL) contagem. A análise estatística foi realizada com

significância de 0,05. Resultados: Nenhum crescimento bacteriano foi observado no controle negativo. Todos os controles positivos demonstraram crescimento bacteriano; S1 de todos os dentes foram positivos para bactérias sem diferença significativa. A análise post-hoc mostrou que o G + 1 promoveu uma desinfecção maior do que o G-1 (p <0,05) e o G-1 desinfecção semelhante ao G0 (P = 962). Conclusão: A instrumentação o mais próximo possível do forame principal ou além melhora a descontaminação em canais ovais com instrumentação reciprocante.
Palavras-chave: Enterococcus faecalis; Forame apical; Desinfecção; Tratamento do canal radicular.

Resumen
Evaluar la influencia de la instrumentación recíproca con diferentes longitudes de trabajo (LT) en la reducción de bacterias planctónicas y biofilm bacteriano en conductos ovalados contaminados con Enterococcus faecalis. Metodología: Se utilizaron 55 caninos humanos; 50 se inocularon con E. faecalis durante 21 días para la formación de biopelículas. Para confirmar la formación de biopelícula se analizaron por SEM 5 muestras contaminadas del grupo de control positivo. Las muestras se asignaron en 3 grupos (n = 15) de acuerdo con la LT determinada, preparación del conducto radicular G + 1 1 mm más allá del foramen apical, preparación del conducto radicular G0 en el foramen mayor y preparación del conducto radicular G-1 1 mm antes de la el foramen mayor. No se inocularon cinco raíces (control negativo). Se recolectaron muestras bacteriológicas antes de la preparación (S1) y después de la instrumentación recíproca (S2) para cuantificar la reducción de bacterias planctónicas y biofilm intracanal en diferentes WL. La cuantificación bacteriana se realizó utilizando unidades formadoras de colonias por mililitro (CFU / mL) de recuento. El análisis estadístico se realizó al nivel de significancia de 0.05. Resultados: No se observó crecimiento bacteriano en el control negativo. Todos los controles positivos demostraron crecimiento bacteriano; El S1 de todos los dientes fue positivo para bacterias sin diferencias significativas. El análisis post-hoc mostró que G + 1 promueve una desinfección mayor que G-1 (p <0,05) y G-1 una desinfección similar a G0 (P = 962). Conclusión: La instrumentación lo más cerca posible del foramen mayor o más allá de este mejora la descontaminación en los conductos radiculares con instrumentación recíproca.
Palabras clave: Enterococcus faecalis; Foramen apical; Desinfección; Tratamiento de conducto.

1. Introduction

The success of root canal therapy in teeth presenting with necrotic pulps is based on the proper disinfection of the entire root canal system. The persistence of the infection after the treatment is completed might risk the healing or lead to the appearance of apical periodontitis (RIcucci et al. 2009).

Bacterial infection within the root canal system involves planktonic bacteria and biofilm-organized bacteria. While planktonic bacteria are easily removed during root canal instrumentation and irrigation, the removal of biofilm is of great challenge. The bacteria organized in a biofilm are harbored in a polysaccharide layer, protecting them from regular irrigation. In addition, root canal ramifications and isthmuses prevent the root canal instrumentation from reaching all of the root canal walls. It is known that the apical third is the region with more incidence of ramifications (De Deus et al 1975). In addition, previous studies demonstrated that oval canals cannot be properly instrumented regardless of the root canal instrumentation system used (Versiani et al 2013).

The enlargement of the apical third of the canals is an attempt to overcome the challenges of cleaning this region. Apical patency aims to passively pass a thin instrument through the main foramen, preventing the clogging of the area. Meanwhile, apical enlargement aims the cleaning of the apical third.

The apical third of the root canal, including the cemental canal, the space between the minor and major foramen, has been shown to have a high prevalence of bacterial biofilms in necrotic teeth. Failure to properly clean this area will serve as a potential cause of persistent infection, compromising the endodontic treatment outcome (Nair et al 2005, Ricucci et al 2008, Ricucci et al 2010). Instrumentations at or beyond the apical foramen can optimize the effectiveness of the irrigation solutions in the apical area and also promote mechanical debridement, improving root canal decontamination (Lin et al 2011, Brandão et al 2019, De Souza Filho et al 1987).

Enterococcus faecalis are frequently found in necrotic root canals, contributing to the perpetuation of the infection. These bacteria can survive in the absence of nutrients and come back to activity when the conditions are better (Sedgley et al 2005). Therefore, these bacteria are of special interest in the field of endodontics (Carvalho et al 2019).

While different studies have assessed the impacts of foraminal enlargement on postoperative pain, debris extrusion, and foraminal deformation, few studies evaluated the influence of different WL determination on bacterial reduction (Silva et al 2013, Silva Santos et al 2018, Frota et al 2018). In this context, this study aimed to assess the effect of different working length – 1 mm short, at the MF, or 1 mm beyond the MF – on the removal of both planktonic and biofilm formation of *E faecalis* in oval canals. The null hypothesis tested is that different WL do not impact root canal disinfection.

2. Methodology

This study was reviewed and approved by the Ethical Committee of our Institution (#3.831.456). The sample number used in this work was based on a previous work consolidated in the current literature. Considering the minimum difference (7.90) between the groups and the standard deviation of the difference (5.08) to compare before and after instrumentation, a minimum of 3 sample units was calculated to achieve the test power of 80 % and significance of 5%. For the comparison between the 3 groups, the minimum calculated was 5. The results, therefore, have power above 80%. This study encompassed 55 human canines presenting oval canals that were extracted for reasons not related to this study. The teeth were kept in 0.1 % thymol solution stored at 4 ° C.

The teeth were radiographed in both mesio-distal and bucco-lingual incidences in order to select canals in which the bucco-lingual length was at least 2.5 times larger than the mesio-distal (Coldero et al 2002). Also, the teeth should present mature apices and straight-single root canals (curvature < 5°) (Schneider 1971). The exclusion criteria removed teeth that were presenting any signs of cracks or fractures assessed under a dental operating microscope, presence of internal or external root resorption or pulp canal obliteration, and any sign of previous root canal intervention.

The crowns of the teeth were removed at the cementoenamel junction (CEJ) and the roots standardized in 16 mm. A size 15 k-file (Dentsply Maillefer, Ballaigues, Switzerland) was introduced in the canal and observed under magnification of a dental operating microscope under 40x magnification (Alliance, São Paulo, Brazil) until the tip of the instrument was visible, being now considered the main foramen. At this point the diameter of the MF was measured and the ones larger than 0.30 mm were excluded. The included teeth had their MF standardized in 0.30 mm by means of a size 30 k-file under irrigation of 5 mL 2.5% sodium hypochlorite (NaOCl, Biodinamica, São Paulo, Brazil). The canals were irrigated with 17% EDTA (Biodinamica) that remained inside the canal for 3 minutes, and were then agitated with a plastic device (Easy Clean, Bassi Endodontics, Belo Horizonte, Brazil) rotating at 950 RPM, 2 N/cm² torque, and amplitude of 5 mm. Finally, the canals were irrigated with 5 mL of 0.9% saline solution.

After the specimens' standardization, all of the roots were inserted in impression material (Zetaplus, Zhermack, Rovigo, Italy) and in 24-well plates (Costar, New York, USA). The set of roots, impression material, and well plates were sterilized in autoclave for 30 minutes at 121° C.

Root Canal Inoculation

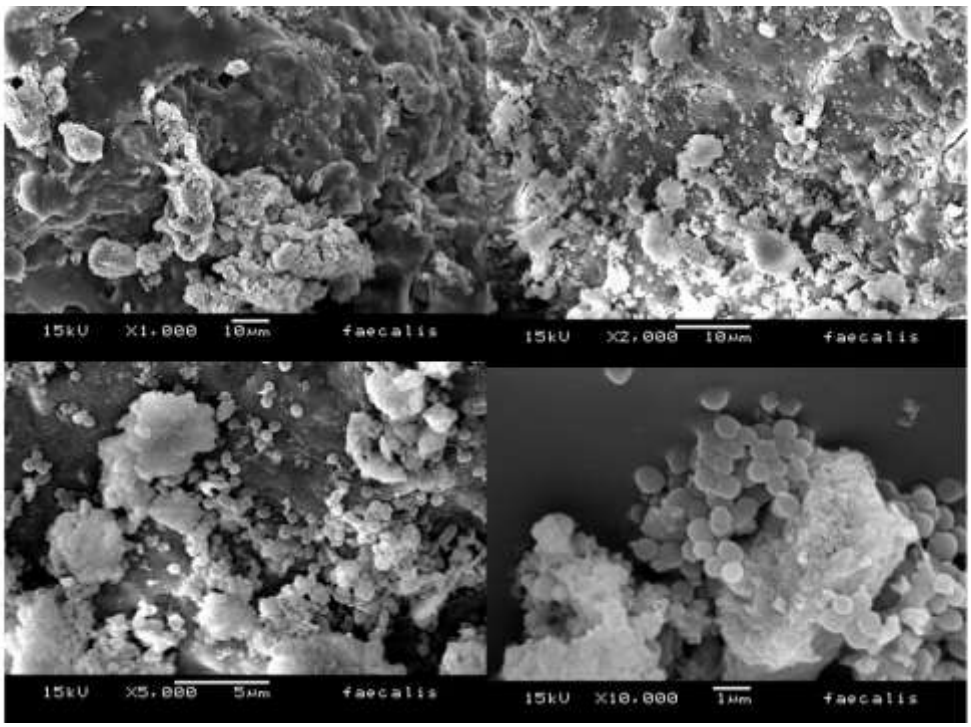
The sterilized roots were inoculated with 20 µL of *E. faecalis* (ATCC 29212), and then cultivated and stored in brain-heart infusion (BHI) at 3.0 x 10^8 UFC/mL. This led to a 1.0 McF suspension according to the McFarland nephelometric scale. After the root canal was filled with the *E. faecalis* solution, a cotton pellet imbibed with the bacterial solution was placed in the cervical third of the root canal. Two samples were assessed weekly by inserting a paper point that was incubated at 37° C for 24 hours (Nabeshima et al 2014). All of the mentioned procedures were performed in a laminar airflow chamber at atmosphere 5% CO_2. Every 48 hours, 20 µL of the BHI infusion were placed inside the root canal to assure its inoculation.

After 4 weeks, the positive inoculation was confirmed as follows: root canal was filled with 0.9% saline solution, and a size 25 Hedstrom file was inserted in the root canal and slightly used against root canal dentin walls to disorganize the

biofilm (Machado et al 2013). Then, a size 20 paper point was placed inside the root canal for 1 minute and transferred to an Eppendorf tube filled with 0.9% saline solution. The tube was then agitated for 30 seconds. Following this, the solution was diluted at 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} and used for bacterial counting (CFU/mL) (Valverde et al 2017). The values obtained at this step were registered as S1 values.

Five samples were then washed in buffered saline solution with 1% phosphate (SSTF), and longitudinally split, and fixed in 2.5% glutaraldehyde for 24h and again washed in buffered saline solution. Finally, the specimens were golden spruted (Denton Vaccum Desk II, Moorestown, NJ, USA) and mounted in metallic stubs. Finally, the specimens were scanned in a Scanning Electron Microscope (Quanta 250 FEG; FEI Ltd, Brno, Czech Republic) for the confirmation of the presence of bacteria and the biofilm formation. The images were taken in the apical third at 1,000x, 2,000x, 5,000x and 10,000x magnification (Figure 1).

Figure 1. Images of the apical third at 1,000x; 2,000x; 5,000x and, 10,000x magnification.



Source: Authors.

Sample Allocation

The inoculated samples were divided into 3 experimental groups (n=15): G+1 instrumented 1 mm beyond the major foramen; G0, instrumented at the major foramen; and G-1, instrumented 1 mm short of the major foramen. Five samples (n=5) were left unprepared (negative control) and five samples (n=5) were not inoculated with *E. faecalis* (negative control).

The instrumentation protocol was performed with an R50 Reciproc Blue (Dentsply Sirona, Munich, Germany) instrument size 50.05. The instrumentation was performed in the "Reciproc" mode of a VDW Silver Motor (VDW, Munich, Germany). An in-and-out movement with 3 mm of amplitude was used for the instruments in all groups. After 3 movements were achieved, the instrument was removed and cleaned with gauze. These movements were repeated until the instrument could reach the pre-determined WL. Each specimen was irrigated with 0.9% saline solution at 5 mL/min., delivered by a peristaltic pump (LPD 101-3, MS Tecnopon, Piracicaba, Brazil).

Post-Instrumentation Bacterial Counting

After the root canal instrumentation procedures, S2 counting was performed using the same protocol applied in the S1 counting.

The Kolmogorov Smirnov tested was used to assess the normality of data. The Wilcoxon test was used to assess the statistically significant differences between the paired groups. Due to the abnormal distribution of data, the Kruskall-Wallis (Post-hoc Tukey/Bonferroni) was used to assess bacterial counting before and after root canal instrumentation. The statistical analysis was conducted in the Statistical Package for the Social Science (SPSS) version 19.0. The statistically significant level was set at $P < 0.05$.

3. Results and Discussion

Results

In the positive control group, the bacterial counting showed no bacteria in the S2 counting. The negative control group showed $18.214 \times 10^5 \pm 8.868 \times 10^5$, which was similar to the bacterial counting in the experimental groups.

SEM analyses showed the presence of intraradicular apical biofilm-like structures harboring bacterial cells attached to dentin walls (Figure 1).

There was no difference in the bacterial counting in the S1 among the 3 experimental groups (p=0.092). Meanwhile, the bacterial counting in the S2 was statistically different among the 3 experimental groups (p=0.024). The post-hoc analysis showed G+1 with lower bacterial counting than G-1, yet similar to G0. The G0 S2 counting was similar to G-1 in regard to bacterial counting (Table 1).

Table 1. Bacterial counting UFC/ml ($x10^{5)}$ in the experimental groups (n=15), Positive Control (n=5) and Negative Control (n=5) before (S1) and after (S2) root canal preparation.

Group	S1				S2				
	Mean	Median	SD	Range	Mean	Median	SD	Range	p* (intragroups)
G+1	8.25	8.40	6.19	0.60 – 8.13	0.18 [a]	0.097	0.28	0.04 –1.11	0.001
G 0	9.27	9.47	7.01	0.34 – 1.07	0.27 [a,b]	0.13	0.25	0.03 –0.77	0.001
G-1	8.76	6.00	8.10	0.09 – 2.80	0.86 [b]	0.31	1.28	0.01 –0.60	0.002
Positive Control	18.21	17.73	8.868	5.2-28	0	0	0		
Negative Control	0	0	0	0	0	0	0		
p** (intergroups)	0.962				0.024				

The different superscript letter shows differences that were statistically significant ($P < 0.05$). SD, Standard deviation. Source: Authors.

Discussion

The complete disinfection of the root canal system presents a great challenge for endodontics using the current technology. By using oval canals, this study aimed to mimic the challenges that clinicians face during ordinary root canal treatment (Versiani et al 2013, Alves et al 2011).

The S.E.M. images showed the presence *of E. faecalis* colonization in the apical third. Successful colonization of the root canal was further confirmed by bacterial load in all S1 samples. Analysis of S1 samples from all groups showed no significant difference (P=0.962), indicating that the experimental method of contamination yielded a homogeneous biofilm structure and bacteria contamination.

The use of a single-file reciprocating system under saline irrigation was used in order to limit the length of instrumentation is the only variable in the present study. As the foramen was standardized with a size 30 k-file the R50 instrument was used. It is worthwhile to mention that a previous study claimed that foraminal enlargement leads to foraminal deformation (Silva Santos et al 2018). Therefore, the characteristics of the alloy used in the R50 Reciproc Blue instrument seem to be able to preserve the position of the foramen even when it is cleaned (Frota et al 2018).

Analyses of samples in S2 counting indicated that all apical limits of instrumentations showed a significant reduction in intracanal biofilm and bacterial populations. This result is in agreement with previous reports on the antibacterial efficacy of chemo-mechanical procedures (Brito et al 2009, Siqueira et al 2007).

In the present study, the instrumentation 1 mm beyond the apex showed better results than instrumentation 1 mm short of the apex; therefore, the null hypothesis cannot be accepted. However, no benefits were found when instrumentation 1 mm beyond the MF is compared with instrumentation at the MF. These findings are in agreement with Yadav et al. (2014), who found apical cleaning to result in less remaining debris and bacterial counting with larger apical preparation and foraminal cleaning.

The instrument herein used presents a taper 0.05 in its first 3 mm. By increasing the length of instrumentation by 1 mm, the enlargement achieved is comparable with the increase of 0.05 mm in diameter. Therefore, it can be concluded that in the G+1 group, the instrumentation at the level of 1 mm beyond the major foramen is equivalent to an instrument size 60. In agreement with previous in vivo and in vitro studies (Card et al 2002, Mickel et al 2007), this enlargement might explain the better disinfection when G+1 group is compared to G-1 group.

In contrast with our results, a previous study showed that apical enlargement has no impact on bacterial load of *E. faecalis* biofilm (Coldero et al 2002). However, in that study, 4.4% NaOCl was used for root canal irrigation; meanwhile, in the present study, 0.9% saline solution was used. Moreover, the NaOCl was kept inside the root canals for 15 minutes, which might have reduced the bacterial load regardless of the WL used. It is our understanding that NaOCl is not the most appropriate irrigant to be used when foraminal enlargement is performed due to the risk of increasing pain and/or accidents (Cruz Jr. et al 2016, Ghivarc'h et al 2017).

The overall result of this study might be explained by the fact that the instrumentation at or beyond the apical foramen promotes foraminal cleaning, and allows better disinfection and removal of debris, improving the apical disinfection (Borlina et al 2010, Coldero et al 2002). Instrumentation as close as possible to the MF or beyond it in teeth with chronic periapical lesions disorganizes the remains of necrotic tissues and biofilm structures, leading to disinfection of the cemental canal and apical foramen, achieving periapical apical healing (Borlina et al 2010).

4. Conclusion

Instrumentation as close as possible to major foramen or beyond it improves reduction of biofilm formation and apical decontamination in oval root canals with reciprocating instrumentation.

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The authors deny any conflicts of interested related to this study.

References

Alves, F. R., Almeida, B. M., Neves, M. A., Moreno, J. O., Rocas, I. N., & Siqueira, J. F. (2011) Disinfecting oval-shaped root canals: effectiveness of different supplementary approaches. *J Endod*.37, 496-501.

Brandao, P. M., de Figueiredo, J. A. P., Morgental, R. D., Scarparo, R. K., Hartmann, R. C., Waltrick, S. B. G., et al (2019). Influence of foraminal enlargement on the healing of periapical lesions in rat molars. *Clin Oral Investig*, 23, 1985-1991.

Borlina, S. C., de Souza, V., Holland, R., Murata, S. S., Gomes-Filho, J. E., Dezan Junior, E., et al (2010). Influence of apical foramen widening and sealer on the healing of chronic periapical lesions induced in dogs' teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.*109, 932-940.

Brito, P. R., Souza, L. C., Machado de Oliveira, J. C., Alves, F. R., De-Deus, G., Lopes, H. P., et al. (2009). Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: an in vitro study. *J Endod*, 35, 1422-1477.

Card, S. J., Sigurdsson, A., Orstavik, D., & Trope, M. (2002). The effectiveness of increased apical enlargement in reducing intracanal bacteria. *J Endod*, 28, 779-783.

Carvalho, M. C., Zuolo, M. L., Arruda-Vasconcelos, R., Marinho, A. C. S., Louzada, L. M., Francisco, P. A., et al (2019). Effectiveness of XP-Endo Finisher in the reduction of bacterial load in oval-shaped root canals. *Braz Oral Res*.33:e021.

Coldero, L. G., McHugh, S., MacKenzie, D., & Saunders, W. P. (2002). Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. *Int Endod J*,35, 437-446.

Cruz Junior, J. A., Coelho, M. S., Kato, A. S., Vivacqua-Gomes, N., Fontana, C. E., Rocha, D. G., et al (2016). The Effect of Foraminal Enlargement of Necrotic Teeth with the Reciproc System on Postoperative Pain: A Prospective and Randomized Clinical Trial. *J Endod*, 42, 8-11.

De Deus, Q. D. (1975). Frequency, location, and direction of the lateral, secondary, and accessory canals. *J Endod*, 1, 361-366.

de Souza Filho, F. J., Benatti, O., & de Almeida, O. P. (1987). Influence of the enlargement of the apical foramen in periapical repair of contaminated teeth of dog. *Oral Surg Oral Med Oral Pathol*, 64, 480-484.

Frota, M. M. A., Bernardes, R. A., Vivan, R. R., Vivacqua-Gomes, N., Duarte, M. A. H., & Vasconcelos, B. C. (2018). Debris extrusion and foraminal deformation produced by reciprocating instruments made of thermally treated NiTi wires. *J Appl Oral Sci*, 26:e20170215.

Guivarc'h, M., Ordioni, U., Ahmed, H. M., Cohen, S., Catherine, J. H., & Bukiet, F. (2017). Sodium Hypochlorite Accident: A Systematic Review. *J Endod*, 43, 16-24.

Lin, L. M., & Rosenberg, P. A. (2011). Repair and regeneration in endodontics. *Int Endod J*, 44, 889-906.

Machado, M. E., Nabeshima, C. K., Leonardo, M. F., Reis, F. A., Britto, M. L., & Cai, S. (2013). Influence of reciprocating single-file and rotary instrumentation on bacterial reduction on infected root canals. *Int Endod J*, 46, 1083-1087.

Mickel, A. K., Chogle, S., Liddle, J., Huffaker, K., & Jones, J. J. (2007). The role of apical size determination and enlargement in the reduction of intracanal bacteria. *J Endod*, 33,21-23.

Nabeshima, C. K., Caballero-Flores, H., Cai, S., Aranguren, J., Borges Britto, M. L., & Machado, M. E. (2014). Bacterial removal promoted by 2 single-file systems: Wave One and One Shape. *J Endod*, 40, 1995-1998.

Nair, P. N., Henry, S., Cano, V., & Vera, J. (2005). Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 99, 231-252.

Ricucci D, & Siqueira JF, Jr. (2008). Anatomic and microbiologic challenges to achieving success with endodontic treatment: a case report. *J Endod*,34, 1249-1254.

Ricucci, D., Siqueira, J. F., Jr., Bate, A. L., & Pitt Ford, T. R. (2009). Histologic investigation of root canal-treated teeth with apical periodontitis: a retrospective study from twenty-four patients. *J Endod*, 35, 493-502.

Ricucci, D., & Siqueira, J. F. (2010). Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. *J Endod*, 36, 1277-1288.

Schneider, S. W. (1971). A comparison of canal preparations in straight and curved root canals. *Oral Surg Oral Med Oral Pathol*, 32, 271-275.

Sedgley, C. M., Lennan, S. L., & Appelbe, O. K. (2005). Survival of Enterococcus faecalis in root canals ex vivo. *Int Endod J*, 38, 735-742.

Shuping, G. B., Orstavik, D., Sigurdsson, A., & Trope, M. (2000). Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. *J Endod*, 26, 751-755.

Silva, E. J., Menaged, K., Ajuz, N., Monteiro, M. R., & Coutinho-Filho, T. de S. (2013). Postoperative pain after foraminal enlargement in anterior teeth with necrosis and apical periodontitis: a prospective and randomized clinical trial. *J Endod*, 39, 173-176.

Silva Santos, A. M., Portela, F., Coelho, M. S., Fontana, C. E., & De Martin, A. S. (2018). Foraminal Deformation after Foraminal Enlargement with Rotary and Reciprocating Kinematics: A Scanning Electronic Microscopy Study. *J Endod*, 44, 145-148.

Siqueira, J. F., Jr., Rocas, I. N., Paiva, S. S., Guimaraes-Pinto, T., Magalhaes, K. M., & Lima, K. C. (2007). Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 104, 122-130.

Valverde, M. E., Baca, P., Ceballos, L., Fuentes, M. V., Ruiz-Linares, M., & Ferrer-Luque, C. M. (2017). Antibacterial efficacy of several intracanal medicaments for endodontic therapy. *Dent Mater J*, 36, 319-324.

Versiani, M. A., Leoni, G. B., Steier, L., De-Deus, G., Tassani, S., Pecora, J. D., et al (2013). Micro-computed tomography study of oval-shaped canals prepared with the self-adjusting file, Reciproc, WaveOne, and ProTaper universal systems. *J Endod*, 39, 1060-1066.

Yadav, S. S., Shah, N., Naseem, A., Roy, T. S., & Sood, S. (2014). Effect of "apical clearing" and "apical foramen widening" on apical ramifications and bacterial load in root canals. *Bull Tokyo Dent Coll*, 55, 67-75.