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RESEARCH ARTICLE **OPEN ACCESS**

Different Disinfection Protocols for Pulp Revitalization: An *In Vitro* Study

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Abstract:

Objectives: Pulp revitalization is a procedure indicated for immature teeth with pulp necrosis. This study aimed to investigate the microbial load reduction of four cleaning protocols for pulp revitalization: Prophylactic Brush for canal (MK Life), microbrush Aplik Extrafine (Angelus), n. 50 K file (Dentsply) and XP Endo Finisher (FKG).

Materials and Methods: Fifty single-rooted mandibular premolars were standardized in canal size and diameter. Contamination by *E. faecalis* was induced for 21 days in 50 specimens, where 48 were used for the experimental groups (n=12) and 2 were used as a negative control to validate the sterilization process before contamination. Irrigation with saline solution at 36.5°C was performed, where the mechanical resource for cleaning was varied (n=12). Colony counting (CFU) was performed before (S1) and after (S2) the cleaning procedure. Kruskal Wallis accounted for and analyzed the differences between S2 and S1.

Results & Discussion: Prophylactic Brush for the canal reduced 99.27% of the microbial load, followed by XP Endo Finisher (99.13%), Aplik microbrush (98.71%) and K file (98.66%). (p=0.3616). There was no statistical difference in the reduction of microbial load between the groups tested.

Conclusion: The mechanical cleaning methods tested alone were effective in substantially reducing the microbial load of *E. faecalis* on lower premolars by simulating open apex teeth and showed that bristle instruments such as Prophylactic Brush for Canal and microbrush Aplik Extrafine could be used to accomplish mechanical debridement of large canals.

Keywords: Pulp revitalization, Regenerative endodontics, Disinfection protocol, XP Endo Finisher, Microbial load, Pulp necrosis.

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1. INTRODUCTION

The highest prevalence of trauma and the presence of caries are in the age group of 6 to 13 years old [1]. The endodontic management of immature permanent teeth is a challenge for clinicians and a public health problem [2].

The paralization of the root formation process leaves,

as a consequence, an expressive structural limitation due to the thin remaining root walls and fragile to resist the masticatory efforts. Reactivating the root development process is of great importance in increasing the lifespan of teeth, making their roots gain structure, strength and resistance both in terms of adequate length and wall thickness. Such thinking greatly impacts the restorability and longevity of immature permanent teeth affected by pulp necrosis [2-6].

Regenerative Endodontics or Pulp Revitalization are a set of procedures that aim to promote the biological replacement of necrotic pulp tissue. It was developed based on the principles of tissue engineering, which considers three factors: stem cells, nutritional matrix, and environment [2, 7]. In general, in order to be successful, the procedure must carry out three important steps: 1- maximum possible disinfection of the root canal area; 2- the formation of a nutritional matrix within the canal system; 3- sealing the canal entrance area with bioceramic material and subsequent coronal restoration [7-9].

Disinfection is vital for the success of therapy, as the microbial presence alone is an impediment to the establishment and differentiation of stem cells from the new tissue [10-14]. However, the management of such teeth offers two important challenges: 1- the impossibility of carrying out a vigorous mechanical preparation to disorganize and neutralize organic matter and bacterial biofilm; 2- the size of the foraminal opening, which may favor the extrusion of irrigants [15, 16]. In a deeper analysis, it is observed that the concern with the first factor ends up overlapping the care with the second. It is observed in the literature that disinfection protocols focus much more on irrigation regimes of high volume of disinfectant substances and the use of intracanal medication based on compounds that can have toxic effects on the mesenchymal cells of the apical papilla [17, 18].

Several studies have shown that irrigation effectively eliminates the surface cells of the bacterial biofilm, as well as those that are free in the root canal in their planktonic form. However, the major concern is the bacterial presence inside the dentinal tubules and in the deeper and adherent layers of the biofilm [8, 19-22].

Thus, seeking mechanical methods to favor cleaning the root canal walls that do not wear the surface or weaken it is important to ensure greater efficiency and safety for the procedure, and it may favor using smaller volumes of irrigants. In view of this, we hypothesized that instruments with bristles can be effective resources for the mechanical disinfection of such wide canals. Thus, the present study aimed to analyze the microbial load reduction of four mechanical root canal preparation techniques, two of which are traditional in the endodontic field: K file n° 50 (Denspty Syrona) and XP Endo Finisher® (FKG Dentaire), with two experimental ones with bristles: microbrush Aplik Extrafino® (Angelus) and Prophylactic Brush for canal® (MK Life).

2. MATERIALS AND METHODS

The present research was submitted and approved by the Research Ethics Committee of the University of Taubate, Brazil (CEP – CAAE 45245820.7.1001.5501). The sample size was determined based on studies that used a similar methodology, followed by sensitivity power analysis (G*Power software, version 3.1.9.2; Heinrich-Heine- Universitat) with fixed main effects and interaction with a power of 80% and a significance level of 95%. Fifty human lower premolar teeth, with straight and formed roots and only one canal, were selected from the University of Taubate Tooth Bank. Teeth with fractures or cracks on the root surface of more than one canal or curvature were excluded. Radiographs were taken in the orthoradial and mesioradial direction to confirm the presence of a single canal.

2.1. General Sample Preparation and Microbial Load Reduction Analysis

Selected teeth were submerged in a 2.5% sodium hypochlorite solution for 2 hours. Then, the root surfaces were scraped with a No. 15 scalpel blade to remove eventual organic remains, washed with 5% sodium thiosulfate solution, and stored in saline solution. The crowns were preserved, and the root apices were cut with a diamond disc. The tooth segments were standardized to 15 mm long, with the apical portions removed. After access surgery, the teeth were initially explored with a No. 10 K-file, and then a Protaper Gold SX nickel-titanium rotary instrument was used to promote initial canal widening. Subsequently, a Gates Glidden No. 5 drill was activated to standardize the diameters of the canals at 1.3 mm (ISO 130) throughout their length and simulate immature teeth with an open apex. Subsequently, the teeth were sterilized by 2 cycles of autoclaving process at 131°C. Two specimens out of the selected 50 were immersed in sterile Brain-Heart Infusion (BHI) broth and incubated for 48 hours to function as a negative control, attesting to the effectiveness of the sterilization method. A McFarland level 1 suspension was prepared in BHI broth and diluted 30 times to obtain an initial 1 x 107 CFU/mL suspension. The 48 remaining teeth were accessed and individually immersed in 1mL of BHI broth of pure culture suspension of *Enterococcus faecalis* (CCT 14494) in 1.8 mL polypropylene cryogenic tubes. These samples were incubated in an oven at 37 °C, with 5% CO_2 and 95% humidity for 3 weeks, with the BHI broth being replenished every 48 hours. Confirmation of bacterial growth was obtained by the intense turbidity of the medium during the incubation period, and the purity of the cultures was attested by the Gram stain method and observation under an optical microscope.

At the end of the incubation period, the teeth were externally washed in saline to remove free cells, and samples were collected from each canal before (S1) and after (S2) endodontic preparation (to be explained later) with n. Forty sterile papers point to the full length of the specimen for 1 minute in circular motions to allow maximum contact with the canal wall. Paper points were

transferred to test tubes containing 1 mL of 0.85% saline. Each tube was vortexed for 1 minute, and its contents were diluted in 9 mL of sterile saline. Aliquots of 0.1 mL of each sample from the 10^{-1} dilution were seeded in triplicate on Petri dishes with BHI agar medium and incubated for 24h at 37 °C. At the end of the period, the plates were removed from the oven to count colonies in CFU. The same procedure was performed before and after the application of the endodontic procedure, and the data collected were recorded and tabulated for posterior analysis [23]. The irrigant used was 5 mL of sterile saline solution at 36.5 °C and only the mechanical cleaning method employed was varied.

2.2. The Endodontic Procedure

Forty-eight specimens were randomly divided into 4 groups (n=12).

The groups were randomly divided as follows, according to the endodontic instrument to be used:

Group 1 - K file No. 50 (n=12).

Group 2 – Microbrush Aplik Extrafino® (n=12).

Group 3 - Prophylactic Brush for Canal® (n=12).

Group 4 – XP Endo Finisher® (n=12).

At the end of the incubation period in culture broth with *E. faecalis*, each specimen was individually removed from the cryogenic tube and washed in saline solution. The irrigation regimen adopted for all groups was 5 mL of saline solution at 36.5 °C in a syringe with a silicone plunger and a side-vented needle. An immersion thermometer was used to measure the temperature. A workbench was set up in the laboratory to perform the endodontic procedure inside a laminar flow chamber.

2.3. New Instruments were Used for Each Specimen of Each Group

In group 1, the canal was filled with saline solution and a K file n. 50 (Dentsply Syrona, Ballaigues, Switzerland) was gently introduced in clockwise and counterclockwise oscillating movements, associated with gentle forward and backward movement until reaching a working length of 14 mm. Scrape movements were done to promote the disorganization of organic matter and biofilm. The time for the procedure was standardized at 1 minute and 20 seconds for all specimens, and the saline solution was renewed every 20 seconds of instrument preparation. At the end of the procedure, a sterile paper point was introduced for 1 minute to collect the post-procedure sample (S2), and this step was repeated in the same way in all the experimental groups.

In group 2 (Applik® Extrafino microbrush (Angelus, Londrina, Paraná), the teeth were filled with a saline solution at 36.5°C and the microbrush was introduced in back-and-forth movements seeking to brush the walls until a working length of 14 mm was reached. The solution was renewed every 20 seconds of the instrument's action. The procedure was repeated until a total time of 1 minute and 20 seconds. At the end of the step, sample collection was performed (S2).

In group 3 (Prophylactic Brush for Canal® - MK Life, Porto Alegre, Rio Grande do Sul), the instrument was coupled to a contra-angle with 1:1 reduction and driven by an endodontic electric motor (VDW Silver, Dentsply Syrona, Ballaigues, Switzerland), with 800 rpm and a torque of 1 N/cm^2, in back-and-forth and circular movements to seek contact with the walls of the canals, for 1 minute and 20 seconds. Every 20 seconds of instrument activation, the solution was renewed. At the end of time, the sample was collected (S2).

Finally, in group 4, the teeth were filled with saline solution at 36.5 °C, and the XP Endo Finisher® instrument (FKG Dentaire, La Chauxdes Fonds, Switzerland) was activated at the parameters of 800 rpm speed and torque of 1 N/cm^2, according to the manufacturer's instructions. Inlet and outlet movements were used to allow agitation of the irrigant throughout the entire length of the canal. The procedure was performed on each specimen for 1 minute and 20 seconds, with intervals for renewing the irrigating solution every 20 sec. At the end, sample collection (S2) was performed.

2.4. Statistical Analysis

Data were analyzed using the BioEstat 5.3 software. The sample distribution was not normal, so a non-parametric Kruskal Wallis test was used.

3. RESULTS

Table **1** shows the results found in the microbial load reduction analysis in each specimen according to the method that was tested. In S1, the CFU count is before the cleaning procedure. In S2, the count after mechanical preparation with saline as an irrigant, then the difference between post and before the procedure. Table **2** summarizes the results found in each group. Fig. (**1**) presents the microbial load reduction experiment based on the averages of the differences found between S1 and S2 and transformed into percentages.

Table 1. Distribution of colony counts according to each specimen in each group.

-	50 K File			Microbrush			Prophylactic Brush			XPEndo		
Sample	**S1**	**S2**	**S2-S1**	**S1**	**S2**	**S2-S1**	**S1**	**S2**	**S2-S1**	**S1**	**S2**	**S2-S1**
1	**6793**	**3.8**	**-6789**	**5228**	**16**	**-5211.7**	**24600**	**8**	**-24592**	**10175**	**20.5**	-10155
2	**5040**	**5.5**	**-5034**	**26300**	**5.6**	**-26294**	**5698**	**411**	**-5287**	**22600**	**2**	**-22598**
3	**3819**	**13**	**-3805**	**10143**	**9.1**	**-10133**	**9955**	**1.5**	**-9953**	**6986**	**688**	**-6297**
4	**5729**	**14**	**-5715**	**3756**	**413**	**-3342**	**22893**	**4.8**	**-22888**	**29200**	**4**	**-29196**

(Table 1) contd.....

-	50 K File			Microbrush			Prophylactic Brush			XPEndo		
Sample	S1	S2	S2-S1	S1	S2	S2-S1	S1	S2	S2-S1	S1	S2	S2-S1
5	8793	407	-8386	9576	105	-9471	4598	77	-4521	7634	97	-7537
6	18753	261	-1849	4379	26	-4353	8738	34	-8704	14364	58	-14306
7	9502	59	-9443	9777	381	-9396	15741	145	-15596	11279	62	-11217
8	24172	175	-2399	6116	479	-5637	17562	201	-17361	12980	193	-12787
9	23505	101	-2340	10924	59	-10865	3971	16	-3955	4352	28	-4324
10	21525	422	-2110	4928	105	-4823	24825	42	-24783	10290	87	-10203
11	7448	329	-7119	9480	80	-9400	12091	235	-11856	20277	279	-19998
12	5802	18	-5784	25654	8	-25646	16934	35	-16899	26300	9	-26291
Mean	11740	151	-1158	10521	141	-10381	13967	101	-13866	14703	127	-14576
DesvPad	7824	163	-	7645	176	-	7618	126	-	8031	195	-
Mean Reduction (%)	98.71%	-	-	98.66%	-	-	99.27%	-	-	99.13%	-	-

Table 2. Colony count before (S1) and after procedure (S2) and percentage of microbial load reduction – CFU.

-	50 K File			Microbrush			Prophylactic Brush			XPEndo		
-	S1	S2	S2-S1	S1	S2	S2-S1	S1	S2	S2-S1	S1	S2	S2-S1
Mean	11740	151	-11589.6	10522	141	-10381.2	13967	101	-13866.4	14703	127	-14576
DesvPad	±7825	±163	-7661.78	±7646	±176	-7469.51	±7619	±126	-7493.34	±8031	±195	-7835.9
Red. %	98.71%			98.66%			99.27%			99.13%		

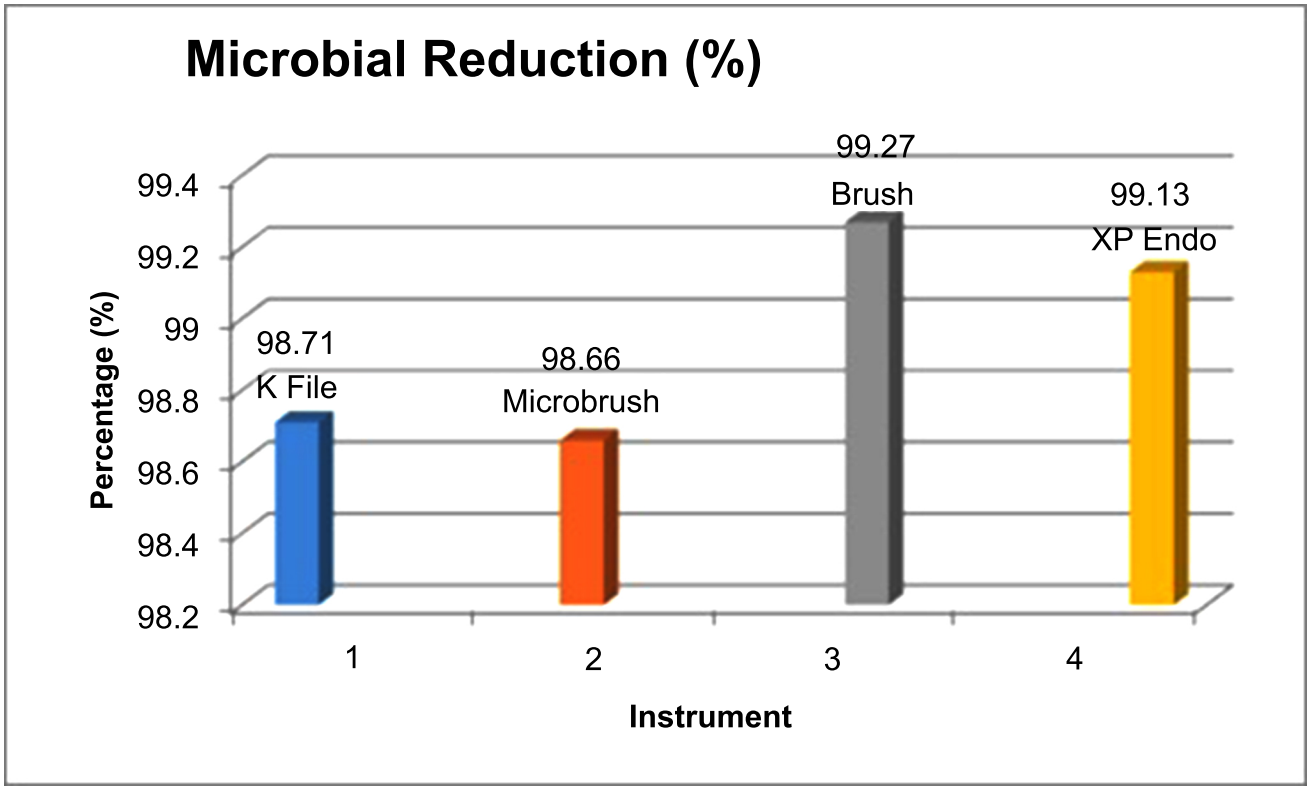

Fig. (1). The means' values of differences between S1 and S2 in percentage (non-significant, p=0.3616).

Mechanical cleaning alone promoted a considerable reduction in the microbial load. The instruments used were equivalent to each other, and such confirmation was clear in the statistical analysis by the Kruskal Wallis test,

where there was no significant difference (p=0.3616)

4. DISCUSSION

The challenges involving Regenerative Endodontics are many. In particular, expectations involve meeting the patient's wishes and seeking clinical and radiographic evidence that supports the restoration of health and the root development process. The researcher's focus is on identifying and offering solutions that make processes more predictable and controllable [2]. Therefore, an important focus is on improving Regenerative Endodontics processes to induce tissue regeneration. Several studies have suggested that disinfection plays an important role in its good conduction [8, 12-14, 24].

The microbial presence within the root canal system produces inflammation of the apical tissues, altering the circulatory and cellular dynamics, which may divert an originally regenerative process to a reparative one. The bacterial biofilm adhered to the walls can cause damage to the dentin microstructure and its collagen fibers, leaving the surface eroded and irregular, hindering or preventing the adhesion, proliferation, and differentiation of stem cells from the new tissue [24]. Preserving predentin with minimal mechanical preparation can cause higher levels of growth factors to be released, favoring the quality of the cell differentiation process [25, 26].

A common point in the different regenerative therapy protocols is the recommendation to use large volumes of irrigating solution passively, given the concern with wall thickness [27]. To improve the mechanical displacement provided by irrigation, recently, methods of agitation of such solutions have been suggested, such as using sonic and ultrasonic currents, low-power lasers (PIPS), and rotating instruments made of different materials. The agitation and vortexing of the liquid are capable of causing small currents that exert shear forces on the organic mass adhered to the root canal walls, breaking and displacing its contents, favoring its removal [23, 24, 28-30].

The concern of not promoting wear of root structures is well defined when evaluating data from work by Kontakiotis *et al*. (2015) [21], who surveyed the endodontic literature on the various regenerative protocols used, found that of 32 articles analyzed, 25 (78%) do not recommend mechanical resources, focusing disinfection on irrigants and intracanal medication. Only seven studies (22%) mentioned the use of instrumentation but with the description “minimal” or “mild.” Hristov *et al*. (2020) demonstrated that the use of stainless steel hand files removed 200% more dentin structure in immature permanent teeth when compared with XP Endo Finisher and a bristle device (GentleBrush) [31].

Keir *et al*. (1990) introduced the concept of using brushes to improve the degree of cleaning of dentin walls after mechanical preparation [32]. Salman *et al*. (2010), Gorduysus *et al*. (2012), and Markovic *et al*. (2015) evaluated the performance of a polypropylene-based brush (CanalBrush) and observed good results in the degree of post-preparation cleaning [33-35]. Therefore, our objective was to evaluate the performance of instruments with bristles in the mechanical cleaning of large canals simulating immature permanent teeth.

Elnaghy *et al*. (2017), Turkaydin *et al*. (2017), and Kaya *et al*. (2018) [23, 28, 29] demonstrated better performance of a resource with bristles (Irrigation needle Navitip FX - Ultradent) and XP EndoFinisher®, either to reduce the microbial load of canals contaminated with *E. faecalis* or to remove calcium hydroxide paste when compared with K file. These results were in agreement with the results of our study. The idea of thinking about instruments that have non-invasive action, i. e., not wearing down the dentin, led to the Aplik Extrafino® microbrush (Angelus) and the Prophylactic Brush (MK Life). Analyzing their dimensions, it has been verified that they could fit in cleaning large ducts since their tuft diameter is around 1.5 mm (equivalent to an ISO n° 150 instrument) and 2 mm (ISO n° 200), respectively. The XP Endo Finisher® instrument has been successfully tested in similar situations as it has the characteristic of deforming when in contact with body temperature. Such deformation, in the shape of a spoon, promotes greater drag of the liquid and even contact of the instrument's surface with the canal wall, helping to displace the organic mass. In our study, bristle resources achieved similar results to XP Endo Finisher®, which is very interesting to use as an alternative. Our results showed that the microbrush performance was similar to that of traditional resources, and that of the Brush was superior [23, 28, 29].

Although a single operator carried out the operative steps of the present experiment with almost 30 years of clinical experience in Endodontics, a fact that drew attention was the ease with which the brush and microbrush techniques were performed. It was decided that only one operator would perform the procedure in order to minimize bias related to operator skills. The XP Endo Finisher® needs a specific resource, the electric endodontic motor, as it demands specific parameters of torque and speed for its safe use without fractures. Although the Prophylactic Brush was used in the same way as a matter of logistics due to its use in the microbiology laboratory, it was designed to be used in a conventional micromotor. The use of the microbrush was performed manually in brushing movements of the walls, which certainly reduces its operational costs even more, as well as the training of professionals for its proper use.

The time was standardized at 1 minute and 20 seconds to simulate the clinical conditions of chemical solution agitation protocols that prescribe 20 seconds of instrument action at each liquid renewal. An extra 20 seconds was added to compensate for the irrigant suction moments every 20-second cycle, which required a change in handling the instrument with the aspirator and syringe.

The investigation was aimed to analyze which instrument promotes a greater reduction of microbial load. Thus, to have a real evaluation of the impact of mechanical resources in the disinfection of root canals, it was decided to use irrigation with saline solution only to eliminate a possible influence of the antimicrobial activity of irrigants, such as sodium hypochlorite. If, on the one hand, using

instruments associated with disinfectant-acting irrigants can simulate conditions closer to the clinical reality, in the specific case of the performance evaluation of new proposals, such use could mask eventual differences and point to wrong directions. Kaya *et al*. (2018) performed a study comparing the performance of XP Endo Finisher® with other rotary instruments, using the induction of E. faecalis biofilm formation in extracted teeth and saline solution [23]. Carvalho *et al*. (2018) also induced *in vitro* biofilm of the same bacterial species and compared two rotary instruments regarding the reduction of bacterial presence, varying the irrigating solution (2.5% sodium hypochlorite or saline solution). The results showed that even with the use of the saline solution, the mechanical preparation alone produced a decrease in the microbial population, as was also verified in our findings. Yet, the use of XP Endo Finisher® at the end potentiated the antimicrobial effect, even in the specimens in which saline solution was used [36]. However, Sasanakul *et al*. (2019) performed an experiment in which they also induced the formation of *E. faecalis* biofilm for analysis of microbial load reduction in extracted teeth and tested the passive irrigation of 1.5% and 2.5% NaOCl solutions with their associations with PUI, Navitip FX, XP Endo Finisher®, Self Adjustment File (SAF), circumferential file with N. 50 K file and preparation sequence with K files n. 90 to 110. The results showed that the presence of a solution at 2.5% improved microbial reduction due to all resources. However, the Navitip NF needle, with its bristles and brushing the walls with each irrigation, was the one that provided the best results, with the advantage of not causing wear on the dentin [37].

As one of the instruments under test depended on a specific temperature to achieve its best performance, the saline solution used in our experiment was heated to 36.5 °C. In the same way, it was done for all groups. The choice of *Enterococcus faecalis* as a microbial agent was made due to the objective of seeking maximum similarity with clinical conditions. Its use as an experimental model in endodontic research is widely accepted and established [23, 37].

In the colony count in the second sample collected, the following percentage reductions were observed after mechanical preparation: Prophylactic Brush for Canal (99.27%), XP Endo Finisher® (99.13%), n. 50 K file (98.71%) and Aplik Extrafino® microbrush (98.66%). The results showed that the resources are equivalent when comparing the microbial reduction provided by the different instruments. The use of a mechanical resource had a great impact on disinfection, even without the use of an antimicrobial irrigant substance. There was a small advantage in the numbers related to the Prophylactic Brush. With regard to microbrush, this is a promising result when it comes to an experimental resource not originally designed for this purpose. Of special interest was that both the Prophylactic Brush and the microbrush, at the end of the procedure, had their bristles with a brownish color, as if some dirt had been impregnated. No deformation of the Prophylactic Brush bristles was observed after use. However, every Aplik microbrush showed deformation of its bristles at the end of its use.

In general, the performance of the tested instruments was similar in the laboratory environment. Using mechanical resources with bristles to debride the walls of infected root canals in teeth with pulp necrosis and incomplete root development may allow a reduction in the volumes of irrigants used, thus reducing any extravasated volumes.

Comparing such instruments with the best results in the endodontic literature, the XP Endo Finisher®, the alternatives proposed by this work appear to be promising, and future modifications can be developed to improve the performance and contribution of such experimental resources to the disinfection process in Regenerative Endodontics. The results of this laboratory study can not be extrapolated to clinical situations. Nevertheless, they point out a new direction in the investigation of cleaning resources in Regenerative Endodontics.

CONCLUSION

- No significant difference in the reduction of microbial load promoted by the four methods tested.
- The mechanical cleaning methods tested alone effectively reduced the microbial load of *E. faecalis* on lower premolars by simulating open apex teeth.
- Bristle instruments (Prophylactic Brush for Canal and microbrush Aplik Extrafine) were effective in eliminating *E. faecalis* from large canals.

ABBREVIATION

PIPS = Low-power Lasers

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The present research was submitted and approved by the Research Ethics Committee of the University of Taubate, Brazil (CEP – CAAE 45245820.7.1001.5501).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committees and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was obtained from all participants.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article are a part of Dr. Barroso's PhD degree thesis in Endodontics at University of Taubaté, Brazil (1st author).

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Bukhari S, Kohli MR, Setzer F, Karabucak B. Outcome of revascularization procedure: A retrospective case series. J Endod 2016; 42(12): 1752-9. http://dx.doi.org/10.1016/j.joen.2016.06.021 PMID: 27726882

[2] Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endod Topics 2013; 28(1): 2-23. http://dx.doi.org/10.1111/etp.12040

[3] Iwaya S, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001; 17(4): 185-7. http://dx.doi.org/10.1034/j.1600-9657.2001.017004185.x PMID: 11585146

[4] Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod 2004; 30(4): 196-200. http://dx.doi.org/10.1097/00004770-200404000-00003 PMID: 15085044

[5] Shah N, Logani A, Bhaskar U, Aggarwal V. Efficacy of revascularization to induce apexification/apexogensis in infected, nonvital, immature teeth: A pilot clinical study. J Endod 2008; 34(8): 919-25. http://dx.doi.org/10.1016/j.joen.2008.05.001 PMID: 18634921

[6] Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: Comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: A retrospective study. J Endod 2012; 38(10): 1330-6. http://dx.doi.org/10.1016/j.joen.2012.06.028 PMID: 22980172

[7] Galler KM, Krastl G, Simon S, *et al*. European society of endodontology position statement: Revitalization procedures. Int Endod J 2016; 49(8): 717-23. http://dx.doi.org/10.1111/iej.12629 PMID: 26990236

[8] Lin J, Zeng Q, Wei X, *et al*. Regenerative endodontics *versus* apexification in immature permanent teeth with apical periodontitis: A prospective randomized controlled study. J Endod 2017; 43(11): 1821-7. http://dx.doi.org/10.1016/j.joen.2017.06.023 PMID: 28864219

[9] Chrepa V, Joon R, Austah O, *et al*. Clinical outcomes of immature teeth treated with regenerative endodontic procedures – A san antonio study. J Endod 2020; 46(8): 1074-84. http://dx.doi.org/10.1016/j.joen.2020.04.008 PMID: 32560972

[10] Wang X, Thibodeau B, Trope M, Lin LM, Huang GTJ. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. J Endod 2010; 36(1): 56-63. http://dx.doi.org/10.1016/j.joen.2009.09.039 PMID: 20003936

[11] Gomes-Filho JE, Tobias Duarte PC, Ervolino E, *et al*. Histologic characterization of engineered tissues in the canal space of closed-apex teeth with apical periodontitis. J Endod 2013; 39(12): 1549-56. http://dx.doi.org/10.1016/j.joen.2013.08.023 PMID: 24238445

[12] Lin LM, Shimizu E, Gibbs JL, Loghin S, Ricucci D. Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: A case report. J Endod 2014; 40(2): 291-5. http://dx.doi.org/10.1016/j.joen.2013.08.024 PMID: 24461421

[13] Vishwanat L, Duong R, Takimoto K, *et al*. Effect of bacterial biofilm on the osteogenic differentiation of stem cells of apical papilla. J Endod 2017; 43(6): 916-22. http://dx.doi.org/10.1016/j.joen.2017.01.023 PMID: 28416302

[14] Cameron R, Claudia E, Ping W, Erin S, Ruparel NB. Effect of a residual biofilm on release of transforming growth factor β1 from dentin. J Endod 2019; 45(9): 1119-25. http://dx.doi.org/10.1016/j.joen.2019.05.004 PMID: 31277901

[15] Nosrat A, Li KL, Vir K, Hicks ML, Fouad AF. Is pulp regeneration necessary for root maturation? J Endod 2013; 39(10): 1291-5. http://dx.doi.org/10.1016/j.joen.2013.06.019 PMID: 24041394

[16] Soares AJ, Lins FF, Nagata JY, *et al*. Pulp revascularization after root canal decontamination with calcium hydroxide and 2% chlorhexidine gel. J Endod 2013; 39(3): 417-20. http://dx.doi.org/10.1016/j.joen.2012.10.005 PMID: 23402520

[17] Fouad AF. Microbial factors and antimicrobial strategies in dental pulp regeneration. J Endod 2017; 43(9): S46-50. http://dx.doi.org/10.1016/j.joen.2017.06.010 PMID: 28778502

[18] Ayoub S, Cheayto A, Bassam S, Najar M, Berbéri A, Kazan FM. The effects of intracanal irrigants and medicaments on dental-derived stem cells fate in regenerative endodontics: An update. Stem Cell Rev Rep 2020; 16(4): 650-60. http://dx.doi.org/10.1007/s12015-020-09982-9 PMID: 32394343

[19] Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: Study of prevalence and association with clinical and histopathologic findings. J Endod 2010; 36(8): 1277-88. http://dx.doi.org/10.1016/j.joen.2010.04.007 PMID: 20647081

[20] Becerra P, Ricucci D, Loghin S, Gibbs JL, Lin LM. Histologic study of a human immature permanent premolar with chronic apical abscess after revascularization/revitalization. J Endod 2014; 40(1): 133-9. http://dx.doi.org/10.1016/j.joen.2013.07.017 PMID: 24332005

[21] Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: A data analysis of clinical protocols. J Endod 2015; 41(2): 146-54. http://dx.doi.org/10.1016/j.joen.2014.08.003 PMID: 25301351

[22] Latham J, Fong H, Jewett A, Johnson JD, Paranjpe A. Disinfection efficacy of current regenerative endodontic protocols in simulated necrotic immature permanent teeth. J Endod 2016; 42(8): 1218-25. http://dx.doi.org/10.1016/j.joen.2016.05.004 PMID: 27372159

[23] Kaya UB, Erik CE, Çetin SE, 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 2019; 52(1): 77-85. http://dx.doi.org/10.1111/iej.12984 PMID: 29985531

[24] Kim S. Infection and pulp regeneration. Dent J 2016; 4(1): 4. http://dx.doi.org/10.3390/dj4010004 PMID: 29563446

[25] Gonçalves LF, Fernandes AP, Cosme-Silva L, *et al*. Effect of EDTA on TGF-β1 released from the dentin matrix and its influence on dental pulp stem cell migration. Braz Oral Res 2016; 30(1): e131. http://dx.doi.org/10.1590/1807-3107bor-2016.vol30.0131 PMID: 28001241

[26] Barroso L, Khurshid Z, Baroudi K. Biomolecules and growth factors for endodontic regeneration. In: Khurshid Z, Zafar M, Najeeb S, Eds. Biomaterials in Endodontics Duxford. Woodhead Publishing 2021.

[27] Chaniotis A. Treatment options for failing regenerative endodontic procedures: Report of 3 cases. J Endod 2017; 43(9): 1472-8. http://dx.doi.org/10.1016/j.joen.2017.04.015 PMID: 28712637

[28] Elnaghy AM, Mandorah A, Elsaka SE. Effectiveness of XP-endo finisher, EndoActivator, and file agitation on debris and smear layer removal in curved root canals: A comparative study. Odontology 2017; 105(2): 178-83. http://dx.doi.org/10.1007/s10266-016-0251-8 PMID: 27206916

[29] Turkaydin D, Demir E, Basturk FB, Övecoglu SH. Efficacy of XP-endo finisher in the removal of triple antibiotic paste from immature root canals. J Endod 2017; 43(9): 1528-31. http://dx.doi.org/10.1016/j.joen.2017.04.017 PMID: 28712635

[30] Yanes PJ, Provenzano JC, Alves MMF, *et al*. Distribution of sodium hypochlorite throughout the mesial root canal system of mandibular molars after adjunctive irrigant activation procedures: A micro-computed tomographic study. Clin Oral Investig 2020; 24(2): 907-14. http://dx.doi.org/10.1007/s00784-019-02970-5 PMID: 31240405

[31] Hristov K, Gateva N, Ishkitiev N, Doitchinova L, Doitchinova L. Comparative analysis of root dentin loss when using modern mechanical clinical instruments in immature permanent teeth. Folia Med 2020; 62(2): 352-7. http://dx.doi.org/10.3897/folmed.62.e39515 PMID: 32666763

[32] Keir DM, Senia ES, Montgomery S. Effectiveness of a brush in removing postinstrumentation canal debris. J Endod 1990; 16(7): 323-7. http://dx.doi.org/10.1016/S0099-2399(06)81942-4 PMID: 2081946

[33] Salman MI, Baumann MA, Hellmich M, Roggendorf MJ, Termaat S. SEM evaluation of root canal debridement with sonicare canalbrush irrigation. Int Endod J 2010; 43(5): 363-9. http://dx.doi.org/10.1111/j.1365-2591.2009.01675.x PMID: 20518928

[34] Yilmaz Z, Gorduysus O, Atila B, Karapinar SO, Gorduysus M. Effectiveness of a new canal brushing technique in removing calcium hydroxide from the root canal system: A scanning electron microscope study. J Conserv Dent 2012; 15(4): 367-71. http://dx.doi.org/10.4103/0972-0707.101909 PMID: 23112486

[35] Markovic L, Booth F, Zimmer S. Use of the CanalBrush improves removal of calcium hydroxide paste from instrumented straight root canals. J Dent Sci 2015; 10(3): 233-9. http://dx.doi.org/10.1016/j.jds.2015.04.003

[36] Carvalho MC, Zuolo ML, Vasconcelos AR, *et al*. Effectiveness of XP-Endo finisher in the reduction of bacterial load in oval-shaped root canals. Braz Oral Res 2019; 33: e021. http://dx.doi.org/10.1590/1807-3107bor-2019.vol33.0021 PMID: 31508722

[37] Sasanakul P, Ampornaramveth RS, Chivatxaranukul P. Influence of adjuncts to irrigation in the disinfection of large root canals. J Endod 2019; 45(3): 332-7. http://dx.doi.org/10.1016/j.joen.2018.11.015 PMID: 30803542