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Original Article

Cleaning of Endodontic Files with and without Enzymatic Detergent by Means of the Manual Method Versus the Ultrasonic Method: An Experimental Study

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ABSTRACT

Aim: The aim of this article is to evaluate the cleanliness level achieved with and without the application of enzymatic detergent for the manual method versus the ultrasonic method, applied to Flexoreamer K-type files No. 25, No. 30, and No. 35. **Materials and Methods:** 192 K-type Flexoreamer files were divided into four categories: A1 (ultrasonic method with enzymatic detergent), A2 (ultrasonic method without enzymatic detergent), B1 (manual method with enzymatic detergent), and B2 (manual method without enzymatic detergent). Each category was randomly distributed in three groups of 16 files each (No. 25, No. 30, and No. 35). The files were used for biomechanical instrumentation of the root canal in premolars. The active part of the files was examined under a stereomicroscope, considering four cleaning levels: 4 (100% cleanliness), 3 (95–99% cleanliness), 2 (85–94% cleanliness), 1 (75–84% cleanliness), and 0 (less than 75% cleanliness). For hypothesis testing, the Mann–Whitney *U*-test was used to differentiate between techniques, and the Kruskal–Wallis multiple comparison test was used to compare pairs of files within each cleaning method. **Results:** When using enzymatic detergents, the manual and ultrasonic methods did not show significant differences when comparing each group of the files analyzed ($P > 0.05$). However, when comparing the cleaning level without enzymatic detergent between the manual and ultrasonic methods, we observed that it obtained a superior result when compared with the manual method for each type of file: No. 25 ($P = 0.021$), No. 30 ($P < 0.001$), and No. 35 ($P < 0.001$). Both methods achieved a significantly higher level of cleaning with the application of the enzymatic detergent ($P < 0.05$) than without applying it. **Conclusion:** The ultrasonic cleaning method proved to be the most effective method for the removal of biologic waste when compared with the manual method using a nylon brush. However, there was no significant difference between these two methods when enzymatic detergent was used.

KEYWORDS: *Biologic waste, endodontic files, enzymatic detergent, nylon brush, ultrasonic waves*

INTRODUCTION

In the field of endodontics, files are used for mechanical root canal instrumentation and are

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considered critical instruments because they come into contact with biological fluids, which may represent a source of infectious disease transmission.[1-9] The function of endodontic files is to remove biological agents and pathogens from the root canal while it is being widened. These endodontic files can be reused in different patients after being disinfected and efficiently sterilized to avoid cross-infection.[3,4] Among the techniques used in various studies to evaluate the cleanliness level achieved in endodontic files,[3,5-7,10-13] we have the manual technique in which enzymatic detergents are applied to endodontic files along with physical brushing for 1 min or less. We also have the ultrasonic technique in which endodontic files are immersed in an enzymatic detergent solution using an ultrasonic unit for varying periods of time. Finally, there is the impregnation method in which endodontic files are only immersed in enzymatic detergents and then rinsed with water. This last technique achieves a low level of cleaning efficiency when compared with the first two techniques mentioned.[5,8]

Several studies claim that use of enzymatic detergent in combination with the manual method applied to endodontic files achieves a similar level of cleaning efficacy as enzymatic detergent baths within an ultrasonic unit.[3,7,8] It should be noted that in several studies there was not the same protocol in terms of application time for both techniques, nor was the same frequency of cycles per second (Hz) applied with the ultrasonic unit.[3-10] However, Masoud *et al.*[9] found no significant differences when comparing the cleanliness level of endodontic files when using ultrasonic equipment with different cycle frequencies per second. Also, Aasim *et al.*[12] state that increasing the time of immersing endodontic files in enzymatic detergent using ultrasonic equipment does not improve the cleanliness level after 10 min. These results differ from those obtained by other authors.[7,10,11]

As is evident, there is no gold standard protocol for the application of various cleaning techniques, such as the manual technique with enzymatic detergent or the technique with ultrasonic application. For example, the authors do not agree on the amount of time to use the manual method with sponge or nylon brushing. There is not enough consistent literature that categorically states how long endodontic files should undergo the ultrasonic method, as there are contradictory studies on this subject. For this reason, several authors recommend further research in this area. In contrast, the methodology used in these studies coincides with the use of dyes and observation with the stereomicroscope to assess the cleanliness level of endodontic files.[5,7,10-12] Removal of the smear layer prior to sterilization is essential as the start of disinfection process. Therefore, many authors recommend the use of enzymatic detergents combined with ultrasonic equipment.[3,5-12,14-19]

Therefore, the aim of this study was to evaluate the cleanliness level achieved with and without enzymatic detergent used for the manual method versus the ultrasonic method, applied to Flexoreamer K-type files No. 25, No. 30, and No. 35. As a null hypothesis, it was proposed that there would be no significant differences in cleanliness level between the manual method and the ultrasonic method when using enzymatic detergent, applied to Flexoreamer K-type files No. 25, No. 30, and No. 35.

Materials and Methods

Bioethical considerations

This research respected the bioethical principles for medical research involving human subjects of the Declaration of Helsinki related to confidentiality, freedom, respect, and non-maleficence. This research was approved by the Ethics and Research Committee of the Faculty of Stomatology of Inca Garcilaso de la Vega University with the resolution No. 012-2020-DFE.

Sample calculation and group allocation

The study had a cross-sectional and comparative experimental design. The sample size per group (n) was 16 Flexoreamer K-type files (Maillefer, Dentsply Sirona, York, PA, USA) and was calculated using a formula for comparison of proportions,[20] based on a pilot test of six files for each group. A Zα: 0.05 (confidence coefficient), Z (1 - β): 0.80 (power coefficient), P1: 0.90, and P2: 0.45 were taken into consideration for the sampling formula. The method used was double-blind, as the laboratory assistant who made the random distribution and the statistician who processed the results were unaware of the allocation of the samples to each group. In addition, the files (No. 25, No. 30, and No.,35) were distributed by simple random sampling without replacement in each of the groups with different cleaning methods (A1, A2 [control], B1, B2 [control]) [Table 1].

Intra-examiner [CC: 0.90, confidence interval (CI): 0.86–0.93] and inter-examiner (CC and BE: 0.82, CI: 0.79–0.84) calibration of the active zone measurements (in millimeters) of the Flexoreamer K-type files with biological residues was performed. Pearson's correlation coefficient was applied and acceptable results were obtained.

The study and its evaluation were carried out in the Basic Science Laboratory at the Faculty of Stomatology

Table 1: Sample size per group, according to the cleaning methods applied

Cleaning method	fi	Category	fi	Group	Sample size (*n*)
Ultrasonic	96	A1: With enzymatic detergent	48	Flexoreamer K-type file No. 25	16
				Flexoreamer K-type file No. 30	16
				Flexoreamer K-type file No. 35	16
		A2: Without enzymatic detergent (control)	48	Flexoreamer K-type file No. 25	16
				Flexoreamer K-type file No. 30	16
				Flexoreamer K-type file No. 35	16
Manual	96	B1: With enzymatic detergent	48	Flexoreamer K-type file No. 25	16
				Flexoreamer K-type file No. 30	16
				Flexoreamer K-type file No. 35	16
		B2: Without enzymatic detergent (control)	48	Flexoreamer K-type file No. 25	16
				Flexoreamer K-type file No. 30	16
				Flexoreamer K-type file No. 35	16
Total					192

fi: absolute frequency; *n*: sample size per group

of the Universidad Inca Garcilaso de la Vega (UIGV), Peru.

Variables used

The study variables were:

- Cleaning methods (independent variable) with four categories: ultrasonic method with enzymatic detergent, ultrasonic method without enzymatic detergent, manual method with enzymatic detergent, and manual method without enzymatic detergent.
- Cleanliness percentage (dependent variable) was grouped into four levels, according to Aasim *et al.*,[12] as follows:

4 = 100% cleanliness on the Flexoreamer K-type file surface.

3 = 95–99% cleanliness on the Flexoreamer K-type file surface.

2 = 85–94% cleanliness on the Flexoreamer K-type file surface.

1 = 75–84% cleanliness on the Flexoreamer K-type file surface.

0 = less than 75% cleanliness on the Flexoreamer K-type file surface.

- File size (intervening variable): Flexoreamer K-type files No. 25, No. 30, and No. 35.

Technique and procedures for obtaining information

The following criteria were taken into account for the selection of the 64 premolars:

Inclusion criteria

- Premolars without dilaceration
- Upper or lower premolars
- Premolars without dental caries

Exclusion criteria

- Premolars with a storage period longer than 6 months.
- Premolars with obstructed or calcified canals.
- Premolars with root resorption.

The premolars were kept in a solution of chloramine trihydrate T (Merck KGaA, Darmstadt, Germany) for 1 week to be disinfected. They were then immersed in distilled water and refrigerated at 4°C with replacement every 7 days, until 24 h before the experiment, since 1 day prior to the experiment they were conditioned in distilled water at 23 ± 2°C.[21]

Before use, the Flexoreamer K-type files were viewed under the binocular stereomicroscope (Leica EZ4, Leica Microsystems, Wetzlar, Germany) with controlled intensity at a magnitude of 16× where it was possible to verify that the active part of each file was completely clean.

For mechanical instrumentation, three Flexoreamer K-type files (No. 25, No. 30, and No. 35) were used for each premolar tooth. These premolar teeth were collected and provided by the Dental Teaching Clinic of the Universidad Inca Garcilaso de la Vega (UIGV), with prior informed consent form of the patients. The researchers had no contact with the patients.

In group A1, after mechanical preparation, the Flexoreamer K-type files were immersed in Alkazyme enzyme solution (Alkapharm, Romainville, France) for 15 min.[3,22] They were then rinsed in distilled water and placed in the ultrasonic cleaner (BAKU BK-3550, Guangdong, China) with enzymatic detergent at 50 W power, 220 V, and 40,000 Hz operating frequency for 60 min.[12] At the end of this process, the Flexoreamer K-type files were rinsed again with distilled water

Figure 1: Equipment used. A: Ultrasonic equipment. B: Stereomicroscope

Figure 2: Removal of Flexoreamer K-type files from the ultrasonic cleaner for washing and ambient air drying

and dried with sterile gauze. In Group A2, the same method was employed with the ultrasonic cleaner but without enzymatic detergent, using only distilled water throughout [Figures 1 and 2].

In Group B1, after mechanical preparation, the 48 Flexoreamer K-type files were brushed with a nylon brush (Oral B Pro Salud, Procter & Gamble, Cincinnati, OH, USA) and enzymatic detergent (Alkazyme, Alkapharm, Romainville, France) for 30 s and then immediately immersed in the enzymatic detergent for 15 min.[3,21] They were then brushed again as before, holding the handle firmly and rotating each Flexoreamer K-type file to brush all its grooves, discarding the brush after every 20 Flexoreamer K-type files. They were then rinsed with distilled water for 15 s and dried with sterile gauze.[7] However, in Group B2, the manual method was used with the nylon brush without enzymatic detergent, using only distilled water at all times [Figure 3].

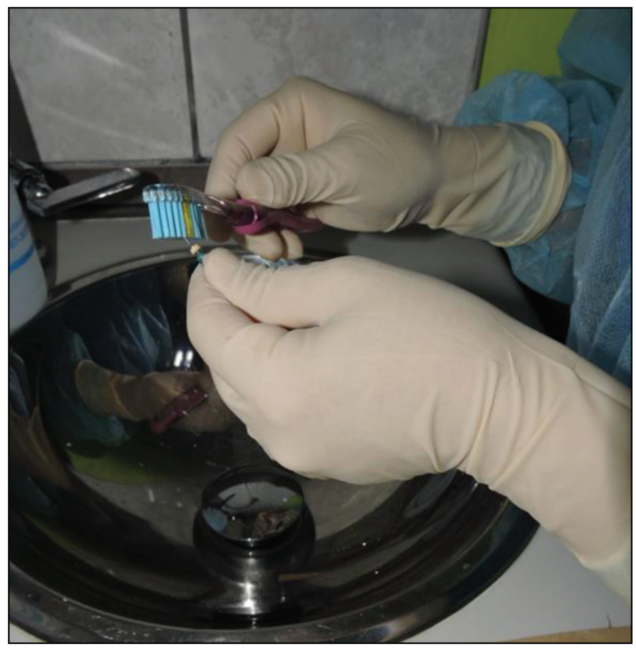

Figure 3: Manual washing with nylon brush

Finally, all Flexoreamer K-type files were immersed in Rhodamine B stain for 24 h. They were then rinsed with distilled water for 5 min and left to dry on gauze at ambient air drying.[5] Subsequently, they were visualized under a stereomicroscope at 16× magnification. The entire active part of each Flexoreamer K-type file was inspected, and the total number of millimeters was recorded to verify the percentage of biologic waste present in the grooves of each Flexoreamer K-type file, in order to calculate the total percentage in relation to the active part [Figure 4].

Statistical techniques for data processing

Data were stored in Microsoft Excel 2016 software and imported into SPSS (Statistical Package for the

Social Sciences) version 24.0 for statistical analysis. The data were summarized in classification tables with descriptive values of central tendency and dispersion. Box and whisker plots were also used to represent the distribution of the data.

For hypothesis testing of difference between groups, the non-parametric Mann–Whitney *U*-test was used to differentiate between techniques and within each group of Flexoreamer K-type files; and the Kruskal–Wallis multiple comparisons test was used for comparison between pairs of Flexoreamer K-type files within each cleaning method. These tests were used based on the Shapiro–Wilk test indicating the absence of normal distribution.

All statistical tests were tested at a 95% confidence level and a 5% significance level.

Results

For the ultrasonic method with enzymatic detergent, the highest median with interquartile range of the cleanliness percentage was 82.73 ± 11.72 for Flexoreamer K-type file No. 25, whereas the lowest median with interquartile range was 62.75 ± 49.31 for Flexoreamer K-type file No. 35. For the manual method with enzymatic detergent, the highest median with interquartile range was 89.06 ± 30.75 for Flexoreamer K-type file No. 30, and the lowest median with interquartile range was 48.13 ± 36.94 for Flexoreamer K-type file No. 35 [Table 2]. Furthermore, only methods with enzymatic detergents showed significant differences when comparing the cleanliness level of the Flexoreamer K-type files. Therefore, a Bonferroni correction was necessary. Thus, we found that the only significant difference between the cleanliness levels of the Flexoreamer K-type files resulted from the manual method, and the Flexoreamer K-type file No. 35 had the lowest level when compared with the Flexoreamer K-type files No. 25 ($P = 0.003$) and No. 30 ($P = 0.001$) [Table 3 and Figure 5].

When comparing the cleaning percentage with enzymatic detergent between the ultrasonic method and the manual method on Flexoreamer K-type files No. 25, No. 30, and No. 35, the analysis showed that

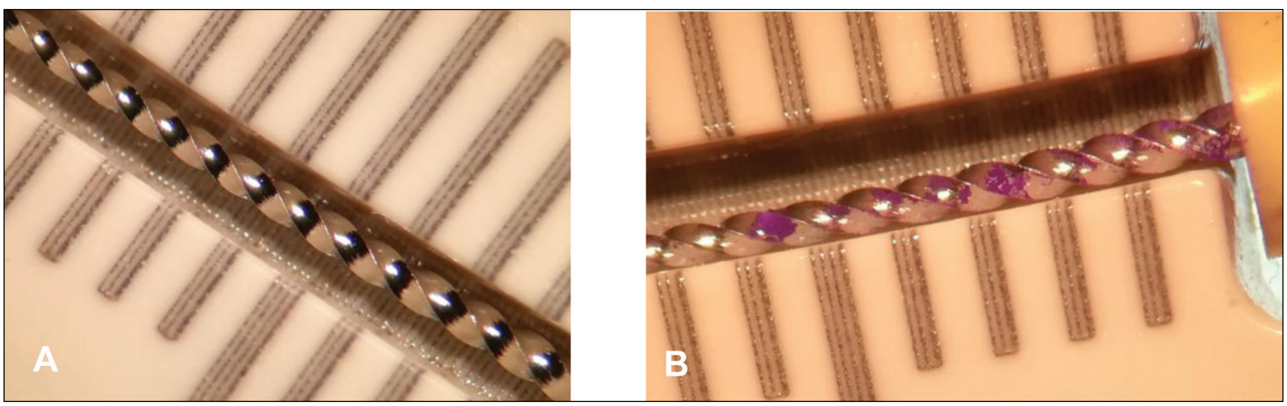

Figure 4: Observation under the stereomicroscope. A: Active part of the Flexoreamer K-type file before mechanical instrumentation. B: Active part of the Flexoreamer K-type file after mechanical instrumentation

Table 2: Comparison of the cleanliness level of Flexoreamer K-type files in each category, according to the method used

Methods	Flexoreamer K-type files	*n*	Median	IQR	*P*-value*
Ultrasonic method with enzymatic detergent (A1)	File No. 25	16	82.73	11.72	0.042*
	File No. 30	16	79.50	27.43	
	File No. 35	16	62.75	49.31	
Ultrasonic method without enzymatic detergent (A2)	File No. 25	16	41.88	8.54	0.171
	File No. 30	16	46.56	11.34	
	File No. 35	16	41.88	12.05	
Manual method with enzymatic detergent (B1)	File No. 25	16	84.37	40.56	0.000*
	File No. 30	16	89.06	30.75	
	File No. 35	16	48.13	36.94	
Manual method without enzymatic detergent (B2)	File No. 25	16	36.87	14.97	0.359
	File No. 30	16	30.81	12.94	
	File No. 35	16	30.63	13.94	

IQR: interquartile range; *based on the Kruskal–Wallis test, significant difference ($P < 0.05$)

Table 3: Comparison of the cleanliness level of type K files, according to the method used with enzymatic detergent

Methods	Ultrasonic		Manual	
Flexoreamer K-type files	File No. 30	File No. 35	File No. 30	File No. 35
File No. 25	$P = 1.000$	$P = 0.095$	$P = 1.000$	$P = 0.003$*
File No. 30	—	$P = 0.081$	—	$P = 0.001$*

*Based on the Bonferroni test, significant difference ($P < 0.05$)

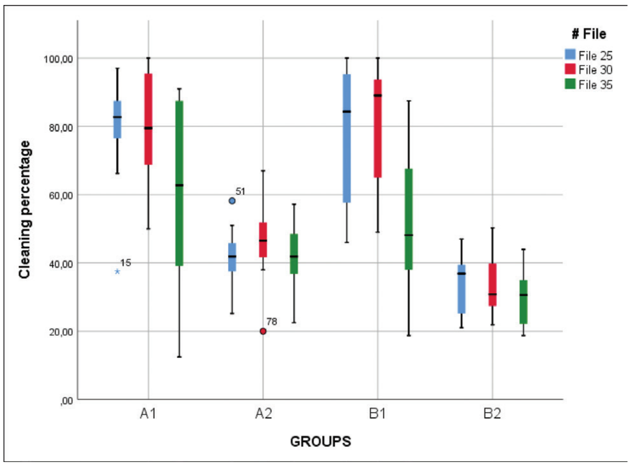

Figure 5: Cleanliness level achieved according to the methods employed: A1 (ultrasonic method with enzymatic detergent), A2 (ultrasonic method without enzymatic detergent), B1 (manual method with enzymatic detergent), and B2 (manual method without enzymatic detergent)

there is no statistically significant differences for each group of Flexoreamer K-type files evaluated ($P > 0.05$). However, when comparing the cleanliness level without the use of enzymatic detergent between the ultrasonic method and the manual method, we observed that the ultrasonic method achieved a significantly higher cleanliness level than the manual method when applied to Flexoreamer K-type files No. 25 ($P = 0.021$), No. 30 ($P < 0.001$), and No. 35 ($P < 0.001$) [Table 4 and Figure 5].

In Table 5, it can be seen that in all Flexoreamer K type files No. 25, No. 30, and No. 35, the ultrasonic method and the manual method achieved a significantly higher cleanliness level ($P < 0.05$) when the enzymatic detergent was used when compared with the results of both methods without enzymatic detergent.

Discussion

This study tested the first step for the removal of biologic waste prior to sterilization of No. 25, No. 30, and No. 35 Flexoreamer K-type files by evaluating two cleaning methods: the manual method with nylon brushing and the ultrasonic method with and without the application of enzymatic detergent. According to the results obtained, the ultrasonic cleaning method with enzymatic detergent achieved a higher cleanliness level than the manual method with nylon brushing, although these differences were not statistically significant, which is consistent with the null hypothesis. However, it is important to note that without the enzymatic detergent, the ultrasonic method achieved a significantly higher cleanliness level of the Flexoreamer K-type files than the manual method.

As mechanical instrumentation was performed on premolar teeth without root dilacerations, it was not necessary to use NiTi files.[22,23] However, other studies showed that when NiTi files were cleaned with different ultrasonic or manual techniques after mechanical instrumentation, no significant cleaning was achieved when compared with stainless steel endodontic files.[24,25]

Dinesh *et al.*[5] concluded that endodontic files cleaned manually with a nylon brush and soaked in enzymatic detergent were more effective than methods using only enzymatic detergents. This statement is supported by the results obtained in the present research, as using the enzymatic detergent in combination with both methods according to the scale of Aasim *et al.*[12] resulted in high cleanliness levels in most of the endodontic files. For this reason, chemical disinfection alone does not seem to be enough to achieve 100% efficacy in removing biologic waste from endodontic files.[6] Hence, the use of enzymatic detergent in combination with manual brushing or ultrasonic equipment is important to obtain greater removal of biologic waste adhering to K-type endodontic files.[3,8,18,19]

Regarding the removal of biologic waste according to endodontic file size when applying both cleaning methods, Nosouhian *et al.*[3] found a higher amount of biologic waste in the smallest K-type size files (No. 15 and No. 25), differing with the results obtained in this research as the Flexoreamer K-type files with the highest level of biologic waste after the application of the cleaning methods were the Flexoreamer K-type files No. 35. However, these results should be taken with caution, as the interquartile range is high for both techniques in all Flexoreamer K-type files, except for the cleaning obtained on No. 25 Flexoreamer K-type files with the ultrasonic method [Tables 2 and 3].

Additionally, regarding the use of ultrasonic equipment, there is evidence that the application of

Table 4: Comparison of the cleanliness level between the methods used with and without enzymatic detergent and according to the Flexoreamer K-type file size

Flexoreamer K-type files	Methods	*n*	Median	IQR	*P*-value*
File No. 25	Ultrasonic method with enzymatic detergent	16	82.73	11.72	0.838
	Manual method with enzymatic detergent	16	84.37	40.56	
	Ultrasonic method without enzymatic detergent	16	41.88	8.54	0.021*
	Manual method without enzymatic detergent	16	36.87	14.97	
File No. 30	Ultrasonic method with enzymatic detergent	16	79.50	27.43	0.897
	Manual method with enzymatic detergent	16	89.06	30.75	
	Ultrasonic method without enzymatic detergent	16	46.56	11.34	0.000*
	Manual method without enzymatic detergent	16	30.81	12.94	
File No. 35	Ultrasonic method with enzymatic detergent	16	62.75	49.31	0.239
	Manual method with enzymatic detergent	16	48.13	36.94	
	Ultrasonic method without enzymatic detergent	16	41.88	12.05	0.000*
	Manual method without enzymatic detergent	16	30.63	13.94	

IQR: interquartile range; *based on the Mann–Whitney *U*-test, significant difference ($P < 0.05$)

Table 5: Comparison between each method used with and without enzymatic detergent according to the Flexoreamer K-type file size

Flexoreamer K-type files	Methods	*n*	Median	IQR	*P*-value*
File No. 25	Ultrasonic method with enzymatic detergent	16	82.73	11.72	0.000*
	Ultrasonic method without enzymatic detergent	16	41.88	8.54	
	Manual method with enzymatic detergent	16	84.37	40.56	0.000*
	Manual method without enzymatic detergent	16	36.87	14.97	
File No. 30	Ultrasonic method with enzymatic detergent	16	79.50	27.43	0.000*
	Ultrasonic method without enzymatic detergent	16	46.56	11.34	
	Manual method with enzymatic detergent	16	89.06	30.75	0.000*
	Manual method without enzymatic detergent	16	30.81	12.94	
File No. 35	Ultrasonic method with enzymatic detergent	16	62.75	49.31	0.029*
	Ultrasonic method without enzymatic detergent	16	41.88	12.05	
	Manual method with enzymatic detergent	16	48.13	36.94	0.000*
	Manual method without enzymatic detergent	16	30.63	13.94	

IQR: interquartile range, *based on the Mann–Whitney *U*-test, significant difference ($P < 0.05$)

a frequency between 50 Hz and 53,000 Hz does not make a significant difference in the removal process of biologic waste in endodontic files. However, the lower the frequency, the longer the application time (not less than 10 min) is recommended.[9,12] For this reason, in the present study, ultrasound was used at its maximum frequency (40,000 Hz) for 60 min after immersing the Flexoreamer K-type files in Alkazyme enzyme solution for 15 min.[3,22] Good but still unsatisfactory results were obtained, as only a few Flexoreamer K-type files reached level 4 (100%) or level 3 (95–99%) of cleanliness, which is similar to the results obtained by different authors.[6,12,19]

In the present study, without the use of the enzymatic detergent, the ultrasonic cleaning method was significantly more effective than the manual method with nylon brushing [Table 3]. This is probably due to the fact that ultrasound generates mechanical sound waves with a frequency of 40,000 cycles per second (Hz). Thus, the waves generated by vibration were able to produce a mechanical phenomenon called cavitation, which consists in the controlled and repetitive generation of vacuum microbubbles within a liquid, followed by an implosion[16,17] which allowed the biologic waste to detach from the active part of the file. This effect was evidently enhanced by the application of the enzymatic detergent Alkazyme, containing proteolytic enzymes (0.6%), calcareous absorbent agents (32%), non-ionic surfactants (8.75%), and didecyldimethylammonium chloride (state-of-the-art quaternary ammonium), which facilitated the emulsification and removal of solid particles impregnated in the Flexoreamer K-type files.[26,27] However, it should be clarified that the enzymatic detergent enhanced the effect of both methods, as there were no significant differences between them, with the ultrasonic method showing a slightly higher cleaning percentage.

A limitation of this study was not using a scanning electron microscope with EDX (energy dispersive X-ray) to verify the percentage of biologic waste atoms present in the Flexoreamer K-type files. Moreover, the complete sterilization process of Flexoreamer K-type files was not compared, and the fresh cadaveric model suggested by De-Deus *et al.*[28] was not used to perform the endodontic mechanical preparations.

Further studies are needed using the protocol described in this research, including the use of disinfecting agents such as 0.12% chlorhexidine or 2% glutaraldehyde[11,19] prior to immersion in enzymatic detergents. Further comparative studies on the timing of ultrasound application in endodontic files immersed in enzymatic detergents are also recommended.

Conclusions

The ultrasonic cleaning method was found to be the most effective method for the removal of biologic waste when compared with the manual method with a nylon brush. However, there was no significant difference between these two methods when using enzymatic detergent. Both the manual method and the ultrasonic method showed significantly higher effectiveness when enzymatic detergent was used for the cleaning of Flexoreamer K-type files, although it is evident that neither method achieved complete removal of biologic waste from all Flexoreamer K-type files, which could lead to improper sterilization of endodontic files and, consequently, be a factor to be considered in clinical cross-infection.

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Nil.

Conflicts of interest

None to declare.

Authors contributions

They conceived the research idea (C. F. C.-R., E. B.-A.), elaborated the manuscript (C. F. C.-R., E. B.-A., E. D. S.-C., M. I. L.-C.), collected, tabulated the information (E. B.-A., A. S. A.-M., C. F. C.-R.), carried out the bibliographic search (E. B.-A., A. S. A.-M., L. A. C.-G.), interpreted the statistical results and helped in the development from the discussion (C. F. C.-R., K. K. H.-C.), he performed the critical revision of the manuscript (C. F. C.-R., L. A. C.-G., M. I. L.-C., A. S. A.-M.). All authors approved the final version of the manuscript.

Ethical policy and institutional review board statement

The study was approved by the Research and Ethics Committee of the Faculty of Stomatology of Universidad Inca Garcilaso de la Vega No. 012-2020-DFE. All the procedures have been performed as per the ethical guidelines laid down by Declaration of Helsinki.

Patient declaration of consent

Not applicable.

Data availability statement

The data that support the study results are available from the author (Dr César F. Cayo-Rojas, e-mail: cesarcayorojas@gmail.com) on request.

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