ORIGINAL ARTICLE (CC BY-SA)



UDC: 616.31-72 DOI: https://doi.org/10.2298/VSP220913103J

Comparison of the efficiency of cleaning and disinfection protocols for hand endodontic instruments

Upoređivanje efikasnosti protokola čišćenja i dezinfekcije ručnih endodontskih instrumenata

Milica Jovanović-Medojević*, Vanja Opačić-Galić*, Katarina Geler[†], Dušan Pavlica[‡]

University of Belgrade, †Faculty of Dental Medicine, *Department of Restorative Dentistry and Endodontics, †Institute of Microbiology and Immunology, Belgrade, Serbia

Abstract

Background/Aim. There is no standard protocol for cleaning and disinfection of used endodontic instruments before their sterilization and reuse. The aim of this study was to determine the efficiency of the different methods of removing biological debris from different types of used hand stainless steel endodontic instruments. Methods. A total of 120 hand stainless steel endodontic instruments: KerrTM reamers, KerrTM files, and HedströmTM files, each forty ISO 25, used for root canal treatment on extracted teeth, were analyzed. The used instruments were divided into four groups based on different decontamination protocols. The evaluation of the efficiency of the cleaning methods was based on the evaluation of the amount of stained organic residues on the instruments (Van Gieson staining). Samples were analyzed by stereomicroscopy (x40). Statistical analysis was performed using the Mann-Whitney U test for the KerrTM reamers and HedströmTM files, while the One-Way ANOVA/Bonferroni test was used for the KerrTM files, at a significance level of 5% ($\alpha = 0.05$). **Re**sults. Residual biological debris was observed on 93.3% of all the samples taken. The thermal disinfectant cleaning method showed the lowest contamination values for all types of instruments. The method of mechanical cleaning showed that the mean value of maximum biologic contamination (MBC) was 58.5% for the KerrTM reamers and 56.2%for KerrTM files, while for HedströmTM files, the highest MBC (50.2%) was shown by the ultrasonic method of cleaning. **Conclusion**. The use of a thermal disinfectant was the most efficient cleaning method for all three types of hand endodontic instruments.

Key words:

decontamination; dental instruments; endodontics; infection control; root canal preparation.

Apstrakt

Uvod/Cilj. Ne postoji standard za čišćenje i dezinfekciju upotrebljenih endodontskih instrumenata pre njihove sterilizacije i ponovnog korišćenja. Cilj rada bio je da se ustanovi efikasnost različitih metoda uklanjanja ostataka biološkog materijala sa radnih površina upotrebljenih ručnih endodontskih instrumenata od nerđajućeg čelika. Metode. Analizirano je ukupno 120 ručnih endodontskih instrumenata od nerđajućeg čelika: po četrdeset KerrTM proširivača, KerrTM turpija i Hedström™ turpija ISO 25, upotrebljenih za obradu kanala korena na ekstrahovanim zubima. Na osnovu protokola koji je korišćen za dekontaminaciju instrumenata, upotrebljeni instrumenti su podeljeni u četiri grupe. Procena efikasnosti korišćenih metoda čišćenja zasnovana je na proceni količine prebojenih organskih ostataka na instrumentima (bojenje po Van Gieson-u). Uzorci su analizirani stereomikroskopijom (x40). Statistička analiza dobijenih rezultata izvršena je Mann-Whitney U testom za KerrTM proširivače i HedströmTM turpije dok je za KerrTM turpije korišćen One-Way ANOVA/Bonferoni test, na nivou pouzdanosti od 5% ($\alpha = 0.05$). **Rezultati.** Prisustvo rezidualnih ostataka biološkog materijala uočeno je na 93,3% svih analiziranih instrumenata. Metoda čišćenja toplotnim dezinfikatorom pokazala je najniže vrednosti kontaminacije za sve tipove instrumenata. Metoda mehaničkog čišćenja pokazala je da je srednja vrednost parametra maksimum biološke kontaminacije (MBK) bila 58,5% za KerrTM proširivače i 56,2% za KerrTM turpije, dok je za HedströmTM turpije najviša vrednost MBK (50,2%) bila posle ultrazvučne metode čišćenja instrumenata. Zaključak. Primena toplotnog dezinfikatora je najefikasnija metoda čišćenja za sva tri tipa ručnih endodontskih instrumenata.

Ključne reči:

dekontaminacija; stomatološki instrumenti; endodoncija; infekcija, kontrola; zub, korenski kanal, priprema.

Introduction

The success of endodontic therapy depends not only on the correct diagnosis, adequate mechanical and medical treatment of the endodontic space, and hermetic obturation but also on the correct implementation and maintenance of the aseptic work protocol ¹.

Stainless steel hand endodontic instruments [KerrTM reamers (KR), KerrTM files (KF), and HedströmTM files (HF)] are generally accepted as reusable instruments ^{1–3}, although there are literature references that support their single-use ^{4–6}. During instrumentation, organic and inorganic debris (residues of vital and necrotic tissue, dentin chips, bacteria, blood, and its decomposition products) may remain on the threads of endodontic instruments, which may have antigenic, infectious, and nonspecific irritating potential ^{7–9}.

Prevention of the possibility of irritation of periapical tissues, cross-infections between patients, and avoidance of additional introduction of foreign microorganisms into the endodontic space is achieved by sterilization of used, contaminated endodontic instruments before their reuse ¹⁰. About 700 species of microorganisms persist in the oral cavity, and the reuse of endodontic instruments potentially carries the risk of transmitting bacterial and viral diseases (Fusobacterium nucleatum, Porphiromonas Gingivalis, and Streptococcus mutans are the most common bacteria isolated from infected root canals) ^{10–12}.

To be reused, endodontic instruments must undergo a process of cleaning and disinfection because the presence of organic material and debris on the surface can compromise their sterilization ¹⁰. A strict treatment protocol for the instruments used should be followed to eliminate or reduce the risk of cross-contamination between patients as efficiently as possible ⁵. Only clean endodontic instruments can be effectively sterilized, so cleaning is a particularly important step in the cycle of preparing instruments for reuse ⁶. Spaulding's classification according to the potential risk for infection transmission describes three categories of instruments (critical, semi-critical, and non-critical), each of which has specific requirements for reprocessing 12. Infection control and quality management in the dental office classify endodontic instruments as critical and are subject to stricter requirements for processing and reuse ^{7,8}.

Evaluation of the success of different methods of cleaning and disinfection of endodontic instruments before their sterilization has been the subject of numerous scientific studies ^{1–3, 13–24} in the constant need to improve aseptic work and provide the most efficient cleaning and disinfection techniques. Studies by Aslam et al. ²⁵ and Mustafa ²⁶ emphasize the importance of involving dental assistants and dentists in training programs for the preparation of endodontic instruments for sterilization and the application of a successful cleaning and disinfection protocol.

The aim of the study was to check the efficiency of different methods of removing biological debris from work surfaces of used hand stainless steel endodontic instruments.

Methods

This study was conducted with the consent of the Ethics Committee of the Faculty of Dentistry, University of Belgrade (No. 36/2; 2020). A total of 120 new instruments were used in the study: forty KR, forty KF, and forty HF (all of Shenzhen Denco Medical Co., Ltd. Guangdong, China), size #25. Directly from the original packaging, the instruments were subjected to ultrasonic (US) decontamination to be cleaned of inorganic and organic debris that occurs during the production process.

The instruments were used for the manual treatment of root canals on 120 intact single-rooted teeth extracted for orthodontic reasons or advanced periodontitis. After the formation of the access cavity, a certain working length was determined (0.5 mm shorter than the length at which the tip of the instrument appears at the apex). All teeth were processed with manual instruments, KR #10–20 (Dentsply, Sirona, USA), and between each instrument, irrigated with 2 mL of 2% NaOCl (Chloraxid 2%, Cerkamed, Polska). Instruments #10 and #15 were used in a clockwise motion, while instrument #20 was used in a combination of clockwise motion and balanced force motion.

Each #25 instrument was used to process one canal until the working length was reached. KR were used in a combination of clockwise and balanced force motion. KF and HF were activated in the canals by the motion of filing and scraping (up and down). During the instrumentation, NaOCl was used as an irrigant using a plastic syringe and an endodontic irrigation needle with a closed tip and side openings (side-vented needle, SmearClear, SybronEndo) in the amount of 2 mL before applying the instrument to the canal.

After instrumentation, the instruments were stored in closed endodontic boxes. The instruments were randomly divided into four groups of thirty instruments (ten KR, ten KF, and ten KH) and were subjected to different cleaning and disinfection methods/protocols: Method 1 – the instruments were immersed in a 3% solution of Gigasept Instru AF disinfectant (Schulke & Mair GmbH, Nordstedt, Germany) for 15 min and then mechanically cleaned with a brush under running water for two min for each instrument; Method 2 – the instruments were immersed in a 3% solution of Gigasept Instru AF disinfectant for 15 min and then cleaned in a US bath with the same disinfectant for 10 min; Method 3 - the instruments were immersed in a 3% solution of Gigasept Instru AF disinfectant for 15 min, then mechanically cleaned with a brush under running water and finally cleaned in a US bath with 3% solution of the same disinfectant for 10 min; Method 4 - the instruments were immersed in a 3% solution of Gigasept Instru AF disinfectant for 15 min and then subjected to a thermal disinfection treatment with water disinfectant "Miele PG 8582 CD" (Miele & Cie. KG, Gutersloh, Germany) (compliant to EN ISO 15883).

Used instruments were disposed of in a Biohazard Sharps container in disinfectant. Cleaning agent (Neodisher® Mediclean Forte 0.5%) and rinse aid (Neodisher® Mediklar Special 0.03%) were used with a program washer/disinfector

cleaning cycle for 10 min at 93°C completed by hot air drying for 15 min at 110°C.

After the cleaning and disinfection protocol, all instruments were subjected to the Van Gieson solution staining method (1% aqueous solution of acid magenta and a saturated aqueous solution of picric acid, which stains collagen red orange) for three min. After rinsing in distilled water for one min, the instruments were dried on an endodontic stand (at room temperature).

The instruments were placed in a square holder, which enabled the rotation of the instruments by 90°. The working surfaces of the examined instruments were recorded with a stereomicroscope with an integrated digital camera (Boeco BSZ-405, Germany) at x40 magnification. Digital images were saved as JPG format files and then processed and analyzed in Scopeimage 9.0 software (Telescope, Austria).

The evaluation of the efficiency of the applied cleaning and disinfection methods was performed by analyzing saved digital images using the method of Linsuwanont et al. ¹³ (based on the number of residual debris).

The found debris was recognized as a biological risk factor and was evaluated by grades based on the amount of repainted material: Grade 0 – clean surface without any debris; Grade 1 – the presence of an organic film (a thin non-textured layer that covers part of the surface of the instrument and turns red); Grade 2 – slight discoloration in the form of individual, rare particles of debris, scattered on the surface of the working part of the instrument); Grade 3 – medium discoloration, organic particles covering the surface of the instrument in the form of a continuous cover; Grade 4 – pronounced discoloration with fields on the instruments where the grooves of the work surfaces are completely filled with debris.

The instruments were observed at three levels: apical, middle, and coronary. At each level, the samples were tested on four sides, gradually rotating by 90° so that each sample had twelve measurements and thus covered the entire working surface of the instrument. The results of all positions were summed, so the minimum grade value was 0 (without organic material), and the maximum was 48 (all surfaces were heavily contaminated with debris). For each instrument, the mean value was calculated and converted into the percentage mean value of the maximum biological contamination (% MBC).

The assessment of the detected contamination was performed by two independent researchers, and the harmonization of the results was performed by Cohen Kappa analysis. Statistical analysis of the results obtained by the Mann-Whitney U test and the One-Way ANOVA/Bonferroni test was performed.

Results

After analyzing the obtained images, a different degree of % MBC was observed on 112 (93.3%) instruments. A completely clean surface was observed on 8 (6.7%) instruments cleaned with a thermal disinfectant.

The fourth method (method with thermal disinfectant) showed the lowest values of contamination for all types of instruments. After the application of the thermal disinfectant, a completely clean instrument surface was obtained on eight instruments (3 KR, 2 KF, and 3 HF). The highest contamination of KR instruments (Table 1, Figure 1) was shown for the first method (mechanical cleaning) with $58.5 \pm 8.9\%$ MBC value. Comparing all four methods of cleaning for KR, a statistically significant difference at the level of p < 0.005 was observed.

Table 1 Contamination of different types of instruments after different methods of cleaning and disinfection (% MBC)

Type of instrument	Method 1	Method 2	Method 3	Method 4
Kerr reamers	58.5 ± 8.9	49.4 ± 5.9	42.1 ± 7.8	12.9 ± 9.3
Kerr files	56.2 ± 12.3	51.9 ± 8.9	39.8 ± 8.1	14.6 ± 7.9
Hedström file	37.7 ± 5.8	50.2 ± 7.8	38.9 ± 5.8	11.2 ± 7.9

MBC – maximum biological contamination. Description of the methods is given in the paragraph Methods. Results are expressed as mean \pm standard deviation.

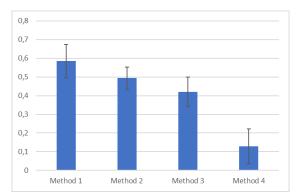


Fig. 1 – Prevalence of Kerr reamers contamination after four different cleaning and disinfection methods (expressed in % MBC).

MBC – maximum biological contamination. Description of the methods is given in the paragraph Methods.

Analyzing the success of different methods of cleaning and disinfection of KF instruments (Table 1; Figure 2), the highest contamination was shown for the first method (mechanical cleaning), with $56.2 \pm 12.3\%$ MBC value. Comparing all four methods of KF cleaning, a statistically significant difference was observed at the level of p < 0.005, except between the first and the second method.

For HF-type instruments, the highest contamination was shown for the second, US method, with a $50.2 \pm 7.8\%$ MBC value (Table 1; Figure 3). Comparing all four methods of cleaning for HF, a statistically significant dif-

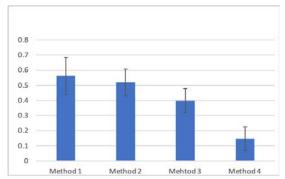


Fig. 2 – Prevalence of Kerr files contamination after four different cleaning and disinfection methods (expressed in % MBC).

MBC – maximum biological contamination. Description of the methods is given in the paragraph Methods.

ference was observed at the level of p < 0.005, except between the first and third methods.

Analyzing the success of different methods of cleaning and disinfection on the apical third of the instruments (Table 2), a statistically significant minimum amount of contamination was observed for all types of instruments after the application of thermal disinfectant compared to all other methods (p < 0.005) (KR $15.6 \pm 13.9\%$, KF $18.1 \pm 10.4\%$, and HF $16.2 \pm 11.8\%$). The highest contamination was observed on the apical third of the KR after the first, mechanical method ($61.9 \pm 19.6\%$) and on the apical surface of the KF ($61.2 \pm 12.4\%$) and HF ($55.0 \pm 9.7\%$) after the US method (Figures 4 and 5).

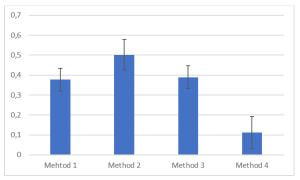


Fig. 3 – Prevalence of Hedström files contamination after four different cleaning and disinfection methods (expressed in % MBC).

MBC – maximum biological contamination. Description of the methods is given in the paragraph Methods.

Table 2

Contamination of different types of instruments after different methods of cleaning and disinfection on the apical, middle, and coronal third of the instruments (% MBC)

	· /			` ,	
Type of instrument	Instrument third	Method 1	Method 2	Method 3	Method 4
	A	61.9 ± 19.6	60.0 ± 9.4	46.2 ± 12.6	15.6 ± 13.9
Kerr reamers	M	58.8 ± 6.7	43.8 ± 7.8	37.5 ± 9.8	13.1 ± 9.5
	C	55.0 ± 8.2	44.4 ± 10.8	42.5 ± 10.5	10.0 ± 9.9
	A	55.6 ± 14.3	61.2 ± 12.4	40.0 ± 12.9	18.1 ± 10.4
Kerr files	M	51.9 ± 12.5	48.8 ± 12.4	38.8 ± 10.5	13.1 ± 9.9
	C	61.2 ± 14.9	45.6 ± 11.4	40.6 ± 7.9	12.5 ± 12.2
Hedström file	A	45.6 ± 13.2	55.0 ± 9.7	40.0 ± 9.4	16.2 ± 11.8
	M	31.9 ± 4.6	51.2 ± 11.3	36.9 ± 6.9	9.4 ± 8.9
	C	35.6 + 6.6	44.4 + 9.1	40.0 + 5.3	8.1 + 8.9

A – apical third, M – middle third, C – crown third; MBC – maximum biological contamination. Results are shown as mean \pm standard deviation.

Description of the methods is given in the paragraph Methods.

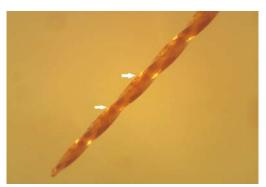


Fig. 4 – Apical third Kerr reamer instrument after combining mechanical and ultrasonic cleaning methods (red spots, white arrows).

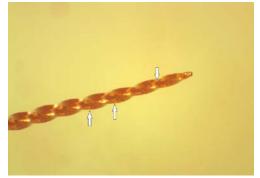


Fig. 5 – Apical third of Kerr file instrument after mechanical cleaning method (red spots, white arrows).

A statistically significant minimum amount of contamination was observed on the middle and coronal thirds for all types of instruments after the application of thermal disinfectant compared to all other methods (p < 0.005) (Table 2; Figure 6).



Fig. 6 – Middle third completely clean in Hedström-type instruments after the application of the thermal disinfectant.

Discussion

Respecting and providing aseptic conditions during endodontic treatment is important, not only in preventing the risk of infection but also in ensuring the success of this therapy. The application of an adequate disinfection and sterilization control protocol prevents and reduces the possibility of the spread of infections, and the greatest risk is represented by blood-borne infections (human immunodeficiency virus/acquired immunodeficiency syndrome, hepatitis C virus, hepatitis B virus) ⁸. Endodontic instruments come into close contact with biological fluids and are applied in a working field that is often bacterially contaminated, so it is important to study and apply all the factors that ensure aseptic work ⁷⁻⁹.

The complex geometry and gracefulness of endodontic instruments, as well as the demanding technical production conditions, affect the appearance of inorganic and organic material on new, unused instruments 14, 16, 27. The presence of metal chips of nickel-chromium and organic material (various lubricants and human material) was noticed on new instruments; possible bacterial contamination was also noticed 14, 16, 27. Therefore, new instruments must also be subjected to cleaning of inorganic and organic debris that has occurred during the production and packaging or storage process, which in this study was done using a US bath with a mild disinfectant. This procedure was applied following the literature recommendations that suggest decontamination with a US bath or thermal disinfectant 18-20. The recommendation is that instruments should not be placed in special containers during the application of the US method to improve the removal of detritus, which was observed in this study.

In cases where the recommendations for single use of endodontic instruments are not followed, and the endodontic instrument is used again on another patient, it is necessary to apply a strict cleaning and sterilization protocol to eliminate or reduce the risk of cross-contamination between patients ¹².

The presence of debris on the instruments can compromise their sterilization due to the impossibility of steam penetration, but also due to the nature of biologically active material which, with minimal moisture content, in its vegetative forms (spores) becomes more resistant to heat ²¹.

The high percentage (93.3%) of contamination on the examined instruments in this study confirms the difficulties in adequate cleaning of endodontic hand instruments and preparation for their sterilization and reuse in clinical conditions ^{3–6, 13–16}. This finding is consistent with the studies of Popović et al. ¹⁴ (84%), Linsuwanont et al. ¹³ (90%), Khullar et al. ¹⁵ (94%), Buchanan et al. ¹⁶ (94%), and Smith et al. ³ (98%), who also noticed a high prevalence of contamination after various methods of cleaning endodontic instruments. The results of a study by Aasim et al. ¹⁷ showed the impossibility of complete decontamination of endodontic instruments (especially the removal of calcium hydroxide medications).

This study aimed to assess which of the applied method is the most effective for a given type of instrument. Examining the contamination of three types of hand endodontic instruments (KR, KF, and HF) after applying four different methods of cleaning and disinfection, the most successful method is convincingly the application of thermal disinfectant for all three types of instruments. Only this method obtained a completely clean surface of the tested instruments.

This result agrees with the results of Vassey et al. 18, who confirmed the effectiveness of thermal disinfectants for cleaning endodontic instruments. The aforementioned authors showed that disinfection devices are significantly more efficient in cleaning endodontic instruments than a combination of manual and US cleaning. The use of thermal disinfectants in a routine procedure can primarily improve the efficiency of cleaning and disinfection and increase productivity, but also reduce the exposure of dental staff to contaminated sharp instruments 19, 20. According to Assaf et al. 20, thermal disinfectants can remove detritus more effectively than other methods (but cannot remove it completely). Furthermore, Assaf et al. 20 and Souza et al. 21 pointed out that the effectiveness of the thermal disinfectant decreases with the decrease in the diameter of the endodontic file. The results of this study also highlighted a lower decontamination efficiency depending on the diameter of the instrument (the apical segment is more difficult to clean and disinfect).

Analyzing the success of the cleaning protocols of KR and KF, the methods of mechanical removal of impurities with a steel brush under running water and US decontamination were singled out as insufficiently successful if performed individually. However, combined, these two methods give statistically better success in removing debris from the surface of these instruments. The explanation for this probably lies in the design of these instruments, where the cutting edge of the reamers is placed more longitudinally in relation to the axis of the instrument (an angle of about 20°), while the cutting edge of the KF is under 40°, which certainly makes it difficult to access the fibers of the cleaning brush. Therefore, the combination of the application of a US bath with a disinfectant after mechanical cleaning with a steel

brush under running water gives the best results for cleaning KR and KF if we are unable to apply a thermal disinfectant. Most chemical methods and the use of strong disinfectants during the decontamination process potentially damage the metal surfaces of endodontic instruments (corrosion, potentiation of existing defects), so the use of mild disinfectants in the US tub is recommended ^{3, 22}. Disinfectants have a double effect, breaking down biological contamination and removing detritus from the blades of endodontic instruments.

The US bath uses vibrational energy that transmits sound waves in the liquid to remove biological material from the surface of the instrument. US cleaning is an efficient method that saves time and saves dental staff, although it is not able to remove all contamination ²⁸. Van Eldik et al. ²³ have also confirmed the harmfulness of using instrument containers during US decontamination, which by their design, can dampen sound waves and reduce the effect of cleaning and disinfection.

The results of the study by Souza et al. ²¹ also indicate that the best results in the decontamination of KR (ISO 25) are achieved by the combined use of the mechanical method of brushing with a US bath.

Although the use of plastic, nylon, and metal brushes for cleaning endodontic instruments is a common and most used method, many researchers have confirmed its ineffectiveness ^{3, 13, 21}. Linsuwanont et al. ¹³ observed that brushing instruments while in the stand restricts access to all surfaces, and often the bristles of the brush are larger than the width of the grooves of the instruments (apical thirds and instruments of small dimensions). In addition, the brushing is performed perpendicular to the longitudinal axis of the instruments while they are held between the fingers of the dental assistant, and the fibers of the metal brush do not move along the blade of the instrument but over them. In addition, the results of research by Souza et al. 21 and Smith et al. 3 point out that the application of this mechanical, manual brush cleaning poses a risk to operators due to possible injury and infection, but also the formation of aerosols during cleaning and decontamination procedures. However, according to the results of these studies, the presence of organic and inorganic detritus does not interfere with the sterilization process by creating a protective layer for bacteria because the heat of the autoclave is able to destroy all microorganisms (except in the case of prions) 3, 7, 8.

Analyzing the cleaning and disinfection protocols of HF, the method of US decontamination is the least successful. Compared to this method, the method of mechanical cleaning-brushing is statistically significantly more successful, as well as the combined method (brushing and US method). The reasons for the difference in the efficiency of different methods of decontamination of HF in relation to KR and KF should be sought in the different designs of these instruments. KR and KF are created by twisting a triangular, i.e., quadrangular wire profile which twists counter-clockwise, while HF are created by milling round wire profiles, which results in spirally twisted blades, so they are one of the most efficient hand tools due to their specific design (blade edges are almost at right angles, an angle bigger than 65°). The re-

sults of this study show that for HF, in the absence of a thermal disinfectant, the dominant method is the mechanical removal of impurities because brush fibers can penetrate better between cutting edges. This finding confirms that the design of the instruments can influence the success of the cleaning method and the selection of the best protocol for their decontamination. The disadvantages of this mechanical method are, in addition to the long-time protocol, the disruption of the surface structure of the instruments as well as the risk of additional contamination and injury to the dental staff performing it ¹².

The result highlighted in this study is the influence of instrument design on the efficiency of the cleaning and disinfection method. While analyzing the influence of the design of the working part of endodontic instruments, it was noticed that the instruments of HF type had the lowest degree of contamination. This finding is consistent with the results of a study by Van Eldik et al. ²³, who showed a cleaner surface of HF compared to rotating instruments after the application of a thermal disinfectant (88.6%). According to the same study, the size and conicity of endodontic instruments have no effect on their cleaning efficiency, unlike the design of cutting surfaces. Due to the specific design of HF (the cutting edges are at right angles), this study showed the necessity of mechanical cleaning of instruments of this type because the application of a US tub only gave the worst result.

Another result highlighted in this study is the different degrees of % MBC observed on different parts of the work surface of hand endodontic instruments (apical, middle, and coronal third). The analysis of the results showed the highest degree of contamination on the apical third of KR and HF after using all four methods and of the KF after using the US and thermal disinfectant methods. Mechanical cleaning (method 1) was the least efficient to remove impurities from the coronal third of KF (maximum % MBC). The combined mechanical US technique (method 3) performed best in terms of cleaning all types of instruments in their middle third. This method showed similar results on the apical and coronal thirds of KF and HF. Although no parameters have been found in the available literature with which the results of this study can be compared, this result can be explained by different designs of examined instruments (different crosssections, different cutting edges, blade depths, different cutting angles).

Plasma cleaning is the most modern method of cleaning and disinfection described by Whittaker et al. ²⁴, involving the use of ionized gases. The advantages of this technique are its nonaggressiveness on the working surface of the instruments and the fact that its application does not release toxic substances (the remaining gases are usually CO₂, H₂O, and N₂).

The reuse of endodontic instruments has the basic goal of reducing material costs ^{4–6, 10, 16}. However, if the cost of disposable instruments is compared to the additional costs arising from reuse, such as the cost of cleaning instruments, sterile storage, and keeping proper records, the projected savings are reduced to a minimum ^{6, 12, 28, 29}. The impossibility of completely removing the biologically active material in this study supports the shortcomings of multiple uses of instruments. Ana-

lyzing mandatory quality management and the possibility of prolonged infections, this important procedure in dental practice is increasingly a topic of clinical discussion.

Conclusion

The most efficient method of cleaning and disinfection was thermal disinfection for all three types of hand endodontic instruments – KR, KF, and HF, and the efficiency of this technique depends on the diameter of the endodontic instrument. Removing biological material was more difficult to perform in the apical portion of manual instruments, regardless of the instrument type.

In the absence of thermal disinfection, the combination of US bath and mechanical cleaning gives the best results for cleaning all types of hand instruments.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

The authors would like to thank Assist. Prof. Đorđe Stratimirović, Ph.D., for his help in the statistical analysis of the results.

REFERENCES

- Parashos P, Linsumanont P, Messer HH. A cleaning protocol for rotary nickel-titanium endodontic instruments. Aust Dent J 2004; 49(1): 20–7.
- Letters S, Smith AJ, McHugh S, Bagg J. A study of visual and blood contamination on reprocessed endodontic files from general dental practice. Br Dent J 2005; 199(8): 522–5; discussion 513.
- Smith A, Letters S, Lange A, Perrett D, McHugh S, Bagg J. Residual protein levels on reprocessed dental instruments. J Hosp Infect 2005; 61(3): 237–41.
- Mirza K. Single Use of Endodontic Instruments. EC Dent Sci 2017; 8(1): 1–7.
- Buchanan GD, Warren N. Single-use of endodontic hand files: perceptions and practice. J Infect Prev 2019; 20(1): 32–6.
- AlYamoor R, Stefanescu S, Al-Saffar H, Chirila M. A Critical Evaluation of the Single-Use Policy of Endodontic Instruments. J Dent Maxillofac Res 2020; 3(3): 1–5.
- Dioguardi M, Crincoli V, Laino L, Alovisi M, Laneve E, Sovereto D, et al. Surface Alterations Induced on Endodontic Instruments by Sterilization Processes, Analyzed with Atomic Force Microscopy: A Systematic Review. Appl Sci 2019; 9(22): 4948.
- Dioguardi M, Sovereto D, Illuzzi G, Laneve E, Raddato B, Arena C, et al. Management of Instrument Sterilization Workflow in Endodontics: A Systematic Review and Meta-Analysis. Int J Dent 2020; 2020: 5824369.
- Cayo-Rojas CF, Brito-Ávila E, Aliaga-Mariñas AS, Hernández-Caba KK, Saenz-Cazorla ED, Ladera-Castañeda MI, et al. Cleaning of endodontic files with and without enzymatic detergent by means of the manual method versus the ultrasonic method: An experimental study. J Int Soc Prev Community Dent 2021; 11(3): 307–15.
- Giovarruscio M, Sauro S, Makeeva I, Foschi F. Strategies to reduce the risk of reinfection and cross-contamination in endodontics. Clin Dent Rev 2019; 3(1): 8.
- Walker JT, Budge C, Vassey M, Sutton JM, Raven ND, Marsh PD, et al. vCJD and the cleanability of endodontic files: a case for single use. ENDO - Endod Pract Today 2009; 3(2): 115–20.
- Australian Dental Association. Guidelines for Infection Prevention and Control. 4th ed. Australia: Australian Dental Association; 2021.
- Linsuwanont P, Parashos P, Messer HH. Cleaning of rotary nickeltitanium endodontic instruments. Int Endod J 2004; 37(1): 19– 28
- Popovic J, Gasic J, Zivkovic S, Petrovic A, Radicevic G. Evaluation of biological debris on endodontic instruments after cleaning and sterilization procedures. Int Endod J 2010; 43(4): 336–41.

- Khullar P, Raisingani D, Gupta S, Bishen KA. Evaluation of biological debris on reusable endodontic instruments subjected to different cleaning methods prior to sterilization. Int J Infect Control 2013; 9(2): https://doi.org/10.3396/ijic.v9i2.10821.
- Buchanan GD, Warren N, Gamieldien MY. Debris contamination of endodontic hand files in dental practice. S Afr Dent J 2018; 73(7): 442–5.
- Aasim SA, Mellor AC, Qualtrough AJE. The effect of presoaking and time in the ultrasonic cleaner on the cleanliness of sterilized endodontic files. Int Endod J 2006; 39(2): 143–9.
- 18. Vassey M, Budge C, Poolman T, Jones P, Perrett D, Nayuni N, et al. A quantitative assessment of residual protein levels on dental instruments reprocessed by manual, ultrasonic and automated cleaning methods. Br Dent J 2011; 210(9): E14.
- 19. Miller CH, Tan CM, Beismanger MA, Gaines DJ, Setcos JC, Palenik CJ. Cleaning dental instruments: measuring the effectiveness of an instrument washer/disinfector. Am J Dent 2000; 13(1): 39–43.
- Assaf M, Mellor AC, Qualtrough AJE. Cleaning endodontic files in a washer disinfector. Br Dent J 2008; 204(10): E17; discussion 562–3.
- Sonza MA, Menin ML, Montagner F, Cecchin D, Farina AP. SEM and microbiological analysis of dirt of endodontic files after clinical use and your influence on sterilization process. Dent Press Endod 2011; 1(1): 82–6.
- Bryson LM, Fernandez Rivas D, Boutsioukis C. Cleaning of used rotary nickel-titanium files in an ultrasonic bath by locally intensified acoustic cavitation. Int Endod J 2018; 51(4): 457– 68.
- Van Eldik DA, Zilm PS, Rogers AH, Marin PD. Microbiological evaluation of endodontic files after cleaning and steam sterilization procedures. Aust Dent J 2004; 49(3): 122–7.
- Whittaker AG, Graham EM, Baxter RL, Jones AC, Richardson PR, Meek G, et al. Plasma cleaning of dental instruments. J Hosp Infect 2004; 56(1): 37–41.
- 25. Aslam A, Panuganti V, Nanjundasetty JK, Halappa M, Krishna VH. Knowledge and attitude of endodontic postgraduate students toward sterilization of endodontic files: A cross-sectional study. Saudi Endod J 2014; 4(1): 18–22.
- Mustafa M. Knowledge, Attitude and Practice of General Dentists towards Sterilization of Endodontic Files: A Cross-Sectional Study. Indian J Sci Tech 2016; 9(11): 1–4.
- Jovanović-Medojević M, Pelemiš M, Nešković J, Popović-Bajić M, Stratimirović D, Živković S. SEM analysis of working surface in new manual endodontic instruments. Stomatol Glas Srb 2020; 67(1): 15–26.

- 28. Morrison A, Conrod S. Dental burs and endodontic files: are routine sterilization procedures effective? J Can Dent Assoc 2009; 75(1): 39.
- 29. Merdad KA, Alghamdi FT. Assessment of the Sterility of New Endodontic Files Received From the Manufacturer Using Mi-

crobial Culture and Scanning Electron Microscopic Analysis: An In Vitro Study. Cureus 2022; 14(8): e28092.

Received on September 13, 2022 Revised on November 19, 2022 Accepted on December 06, 2022 Online First December 2022