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Original Article

A SEM/EDS evaluation of debris removal of used rotary Ni-Ti endodontic files by four different cleaning solutions

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ABSTRACT

Purpose: The aim of this study was to compare the efficacies levels of four cleaning solutions for removing debris from rotary Nickel-Titanium (Ni–Ti) endodontic instruments.

Materials and methods: Twelve instruments that fractured during ex vivo instrumentation were used. Fractured surfaces were investigated by SEM before and after 3, 6 and 9 min of ultrasonic cleaning in 17 % EDTA.3NaOH (Group A), 2.5 % NaOCl (Group B), Dentasept 3H Rapide (Group C) and ZymeX™ (Group D) solutions. EDS analyses of selected files from all four groups of untreated and ultrasonically cleaned samples were performed to assess the elemental composition of the alloy surfaces.

Results: SEM analysis revealed that after 9 min of ultrasonic agitation, all four investigated solutions had cleaned fractured surfaces. However, some low-atomic-number regions exhibited random distributions on the fractured surfaces. EDS analyses indicated that only C was retained on surface after 9 min of ultrasonic cleaning. This finding was common in all tested groups.

Conclusions: All four investigated solutions substantially removed debris from the surfaces of the Ni–Ti files and were considered appropriate for clinical practice.

1. Introduction

Compared with stainless steel hand files, rotary Nickel-Titanium (Ni–Ti) endodontic files tend to retain more biological debris (Van Eldik et al., 2004; Bryson et al., 2018), thereby reducing the efficiencies of cleaning techniques used before sterilization (Martins et al., 2002; Alapati et al., 2003; Linsuwanont et al., 2004; Parashos et al., 2004; Van Eldik et al., 2004). Researchers have shown that the presence of bioburden does not reduce the efficacy of steam sterilization during microorganism elimination (Johnson et al., 1997; Van Eldik et al., 2004; Aasim et al., 2006; Kocak et al., 2014). However, the unknown effects of debris on the properties of chemical disinfectants and sterilants (Linsuwanont et al., 2004) and the possible transmission risks of very resistant prion diseases, such as Creutzfeldt–Jacob disease (Palacios-Sanchez et al., 2008), via oral tissues both support the principle in which each instrument must be rendered free from bioburden before a sterilization procedure can be performed to prevent cross-infection (Van Eldik et al., 2004).

Additionally, it has been suggested that organic residue that remains on the surfaces of used Ni–Ti endodontic instruments after ultrasonic cleaning may predispose to fracture (Alapati et al., 2003). Debris accumulates mostly on surface defects, such as machining grooves and metal rollovers, that arise from the manufacturing process. It has been hypothesized that debris wedges at these sites, leading to crack propagation (Alapati et al., 2004), although this theory has been questioned (Parashos and Messer, 2006). The accumulation of debris has been theoretically linked to reduced cutting ability (Van Eldik et al., 2004). Copious irrigation is recommended during instrumentation with rotary Ni–Ti instruments (Clauder and Baumann, 2004). Moreover, files are designed to prevent the accumulation of dentin in a root canal by aiding its coronal escape (Clauder and Baumann, 2004; Van Eldik et al., 2004). The revised cleaning protocols include ultrasonication with the instruments usually placed, either loosely in a glass beaker or constrained in a mesh basket (Parashos et al., 2004) or perforated metal container

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Table 1
Manufacturer, active reagent, pH value, and suggested contact time with endodontic files for all cleaning solutions tested.

Cleaning solution	Manufacturer	Active ingredients	pH	Suggested minimum contact time
17 % EDTA.3NaOH	Triplex III, Rhône-Poulenc, Cedex, France	EDTA.3NaOH	4.6*	Not given
2.5 % NaOCl	Digas G. & Co, Thessaloniki, Greece	NaOCl	12*	Not given
Dentasept 3H Rapide	Anios, Lille-Hellemmes, France	Didecyldimethylammonium chloride, chlorhexidine digluconate, polyhexamethylene biguanide, nonionic surfactant**	7**	5 min
ZymeX™	Sultan Chemists, INC. Englewood, NJ	Enzymes and surfactants (isopropyl alcohol and triethanolamine)**	7**	5 min

*Measured with a digital pH meter (Consort P903, Scientific Instrument, Belgium).
**Data provided by the manufacturer.

(Van Eldik et al., 2004) and in an ultrasonic bath containing various solutions such as sodium hypochlorite (Linsuwanont et al., 2004), EDTA (Parashos et al., 2004), and enzymatic or non-enzymatic detergents (Parashos et al., 2004; Van Eldik et al., 2004). However, prolonged immersion times compromises the surface integrity of endodontic files (Uslu et al., 2018).

The aim of this study is to compare the effectiveness of four cleaning solutions in removing debris from rotary Ni-Ti endodontic instruments that have been used in clinically simulated conditions. The null hypothesis is that no differences will be observed among the clinical efficacies of the solutions tested.

2. Materials and methods

This study was initiated after securing approval from the internal review board. Mesial root canals of extracted mandibular molars with 60 ± 10° angles and 2 ± 1 mm radii of curvature according to Pruett (Pruett et al., 1997) were used. Root canal preparation was performed using the ProFile system (Dentsly/Mailleffer, Ballalgues, Switzerland) with a reduction handpiece (Kavo Dental GMBh & Co., Germany) on a torque control motor (ATR Tecnika, Pistoia Italy). For each instrumentation, a 6 file set was used consisting of: OS #3, OS #2, Profile #25 and 20 taper 0.06 and Profile #25 and 20 taper 0.04.

15 % EDTA gel (Root gel, Magriotis, Athens, Greece) was used as a

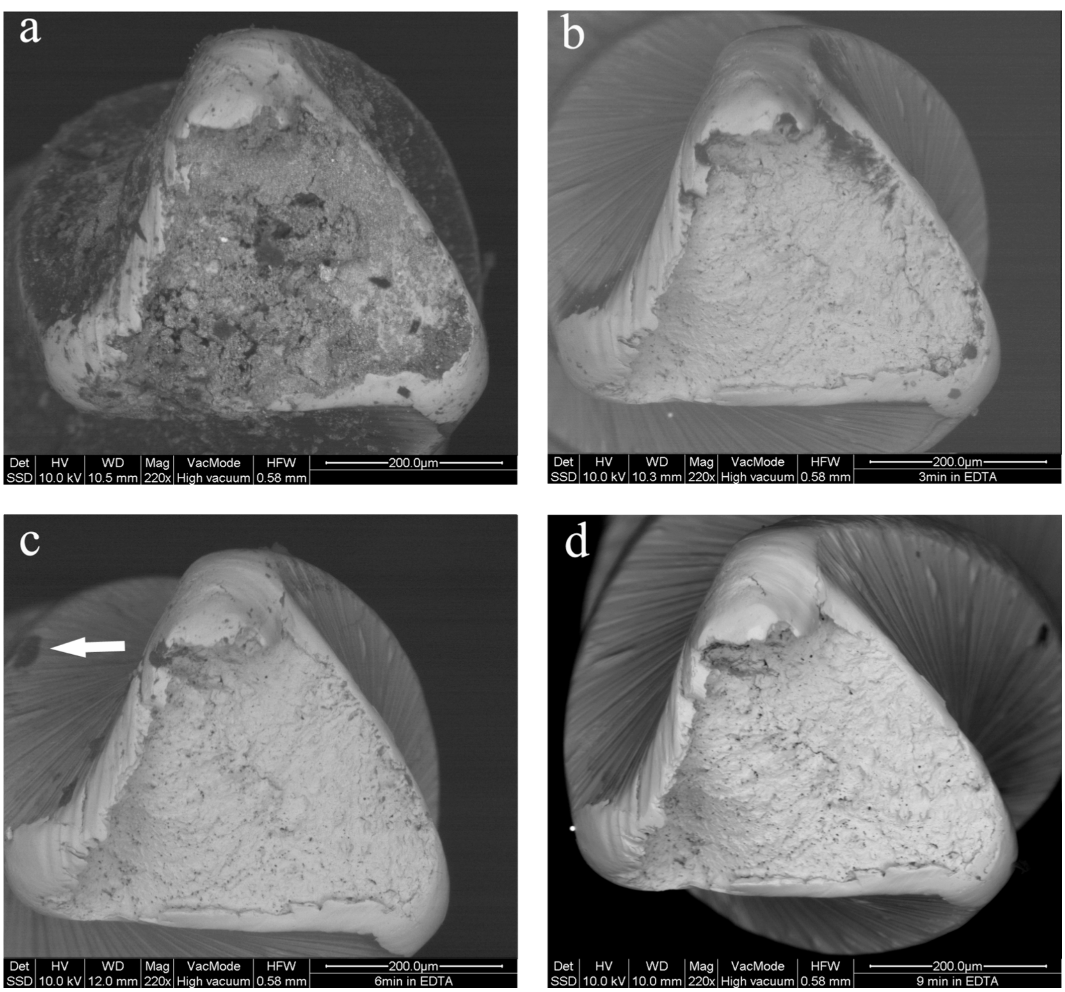

Fig. 1. SEM images of the fractured surfaces and adjacent flutes of a file used as received (a) and after 3 (b), 6 (c) and 9 (d) min of cleaning in 17% EDTA.3NaOH (Group A). The arrow indicates redeposition of debris.

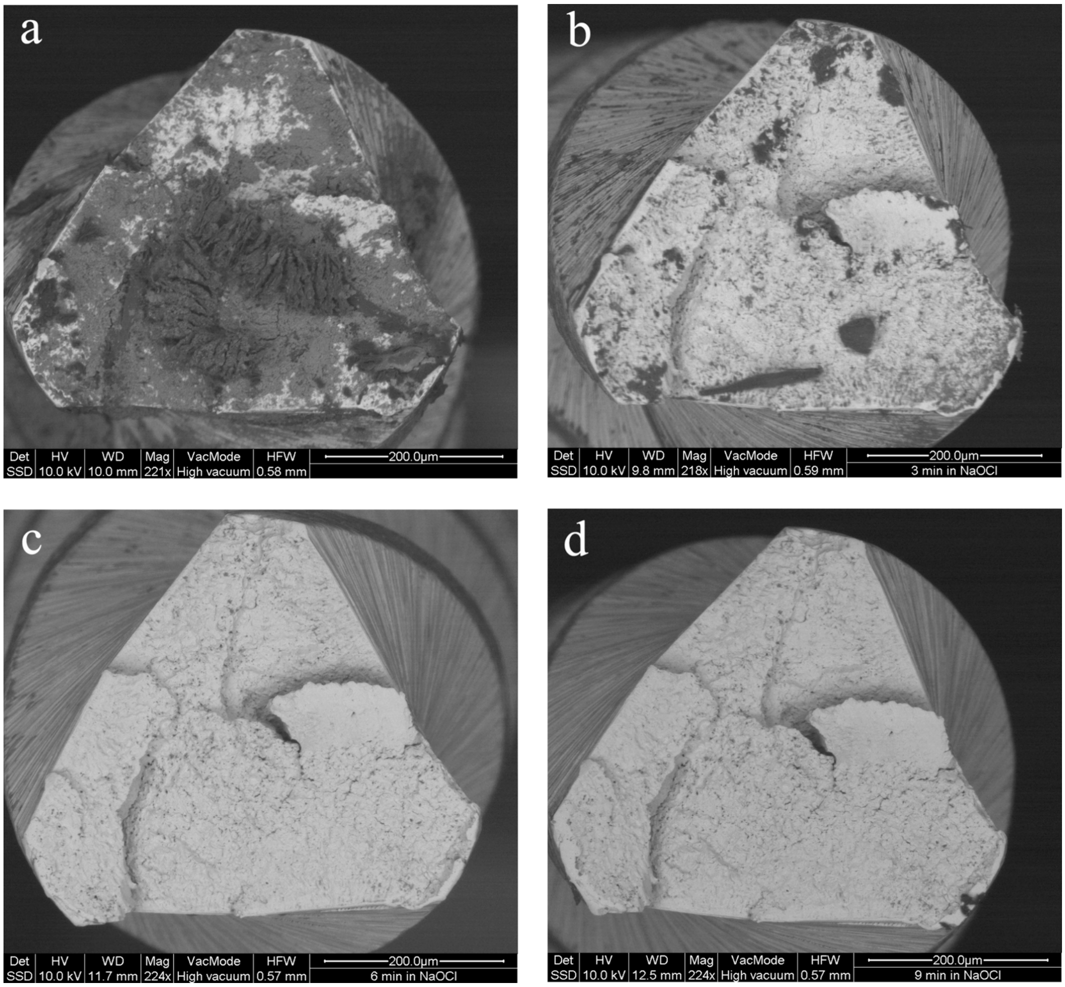

Fig. 2. SEM images of the fractured surfaces and adjacent flutes of a file used as received (a) and after 3 (b), 6 (c) and 9 (d) min of cleaning in 2.5% NaOCl (Group B).

chelating agent with each instrument and was inserted into a canal. Canals were irrigated between successive instruments with 5 mL of 2.5 % sodium hypochlorite (NaOCl) delivered with 27-gauge needle tips placed passively in the canal, with the tip distances reaching 3 mm from the apical foramen without binding.

Each time an instrument was removed from a canal, it was swabbed using gauze moistened with 0.12 % chlorhexidine gluconate aqueous solution (Paroex, Buttler, Sarrono, Italy). Prior to their first use and after the completion of each instrumentation, the instrument set was immersed in a beaker containing Dentasept 3H Rapide (Anios, Lille-Hellemmes, France) cleaning solution, placed in an ultrasonic bath (Biosonic, Coltene – Whaledent Inc., Cuyahoga Falls, Ohio) for 5 min, rinsed in running tap water for 30 sec, and subsequently sterilized in an autoclave (Lisa MB17 Class B, W&H, Sterilization Srl, Pedrengo, BG) at 134 °C for 30 min. Files were subjected to a comprehensive visual inspection after each use with 4.5 × magnification loops to detect signs of plastic deformation and/or fracture. In the case of plastic deformation, the file was discarded and excluded from additional analysis, while in the case of fracture, the file was collected and stored for further analysis.

Twelve fractured ProFile instruments retrieved after performing the clinically simulated chemo-mechanical preparation described above were randomly divided into four groups—A, B, C and D—each consisting of three files. A different cleaning protocol was followed for each group. The files of all groups were mounted on a customized jig and investigated by scanning electron microscopy (SEM) (Quanta 200, FEI, Hillboro, OR, USA), employing backscattered electron imaging (BEI) at an accelerating voltage of 10 kV and a beam current of 90 μA. To assess the elemental composition of the alloy surfaces and detected debris, one instrument from each group was examined with an energy-dispersive X-ray spectrometer (EDS) coupled to an SEM instrument equipped with a liquid N_2-cooled Si(Li) detector (Sapphire CDU; EDAX, Mahwah, NJ, USA) with a super ultrathin window (Be). One EDS spectrum was collected from the central region of each specimen under the following conditions: 20 kV accelerating voltage, 110 μA beam current, 130 × 130 μm area of analysis, 1000 × nominal magnification, 200 s acquisition time and 30–35 % detector dead time. Subsequently, the files were placed in glass beakers containing cleaning solutions of 17 % trisodium EDTA (EDTA.3NaOH) (Triplex III, Rhône-Poulenc, Cedex, France) (Group A), 2.5 % NaOCl (Digas G. and Co., Thessaloniki, Greece) (Group B), Dentasept 3H Rapide (Anios, Lille-Hellemmes, France) (Group C) or ZymeX™ (Sultan Chemists, INC. Englewood, NJ) (Group D) (Table 1) and immersed in an ultrasonic bath. The EDTA.3NaOH solution was prepared by mixing 17 g of trisodium EDTA salt (Triplex III, Phone Pouleny France) with 100 mL of distilled water. Dentasept 3H Rapide and ZymeX™ were prepared at the recommended dilution levels of 8 % and 2 %, respectively, as suggested by the manufacturer. Cleaning cycles were performed for 3, 6 and 9 min in the same solution. Following each cycle of ultrasonic cleaning, the files were rinsed in running tap water for 20 sec (Parashos et al., 2004), air dried, mounted on a customized jig and microscopically studied as previously described. Images of the fractured surfaces were acquired in a reproducible, standardized manner to make comparisons feasible. EDS analysis was performed to assess the elemental composition of the alloy surfaces and the detected debris for untreated specimens and for specimens after ultrasonic

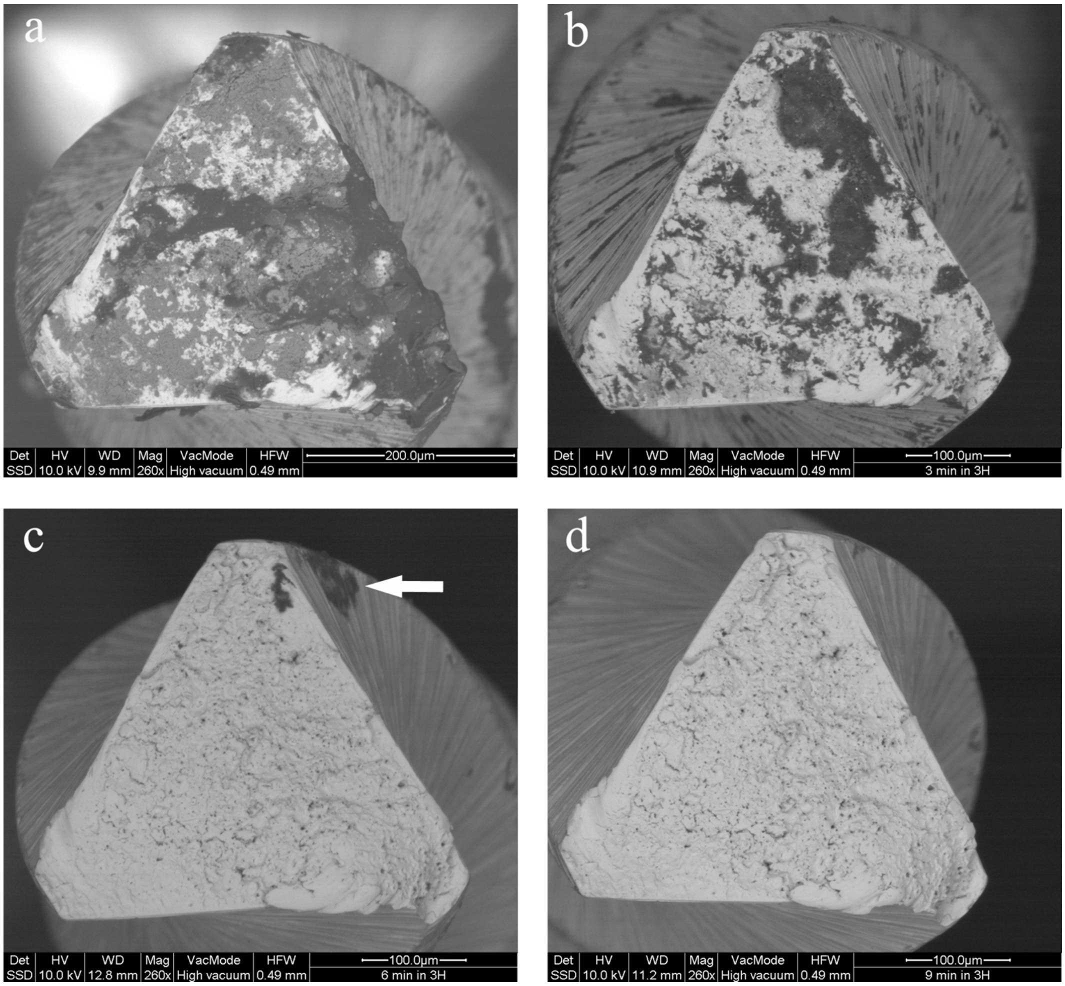

Fig. 3. SEM images of the fractured surfaces and adjacent flutes of a file used as received (a) and after 3 (b), 6 (c) and 9 (d) min of cleaning in Dentasept 3H Rapide (Group C). The arrow indicates the relocation of debris.

cleaning for 3, 6 and 9 min. The EDS spectra were quantified by Genesis software (version 3.5, EDAX) with a nonstandard analysis technique: the atomic number, absorption, and fluorescence (ZAF) correction method.

3. Results

Figs. 1–4 show BE images of the fractured surfaces and adjacent flutes of the Ni–Ti instrument used in all four groups, including samples that were untreated and samples that underwent ultrasonic cleaning for 3, 6 and 9 min, in each solution. In all four cases, a progressive removal of debris from the surface was evident, although the last stage (the last 3 min of ultrasonication in the 6 min and 9 min groups) seemed to have a limited effect on debris removal. In all cases, the surfaces had small black areas with random distributions. With the contrast provided by BE images, the areas were identified as those with compounds with relatively low mean atomic numbers; thus, these compounds were considered to have organic origins. These areas contrasted with the bright areas, which had high-mean-atomic-number (Ni–Ti)

Fig. 5 shows the overlying EDS spectra for untreated surfaces and surfaces after 3 and 9 min of immersion in cleaning solutions. The untreated surfaces revealed that in addition to the alloy components, C, O, Ca, P, Na, Mg, Al, Cl and K were present. After 9 min, only the alloy components and C were traced. C was retained on all the surfaces even after 9 min of cleaning. Table 2 presents the elemental composition of the same files obtained through successive cleaning steps. The results of Group A (trisodium EDTA) samples are representative of all groups tested and should be considered representative of the elemental compositions developed through successive steps.

4. Discussion

Based on the results, the null hypothesis must be accepted. Although the morphological features of the fractured surfaces of Ni–Ti files are different from those of their cutting surfaces, the fractured surfaces are chosen since it is almost unfeasible to place endodontic files in SEM instruments in the same orientation to capture multiple images of the same area.

According to the EDS analysis results, dentin might have been attributed to the presence of C, O, Ca, P, and Mg, and possibly to Na and Cl. The NaOCl solution may have given rise to Na, Cl and O. The EDTA gel, which is used as a chelating agent, is responsible for the traced C, O and N.

The solutions investigated in this study are selected to represent different detergent categories (Table 1). EDTA.3NaOH is a chelating agent that aids in the removal of an inorganic component from a substrate (Scelza et al., 2003) by acting on crosslinked proteins; this agent exhibits antimicrobial activity by producing changes in the outer membranes of gram-negative bacteria (McDonnell and Russell, 1999). NaOCl has a well-documented broad-spectrum antimicrobial effect and a specific organic dissolution capacity (Zehnder et al., 2002). However, the corrosive effect of NaOCl has been a concern. Studies have shown that Ni–Ti instruments are resistant to corrosion when NaOCl is used as an irrigation solution; hence, only the shaft of the instrument comes in contact with the solution (Darabara et al., 2004). When NaOCl is used as

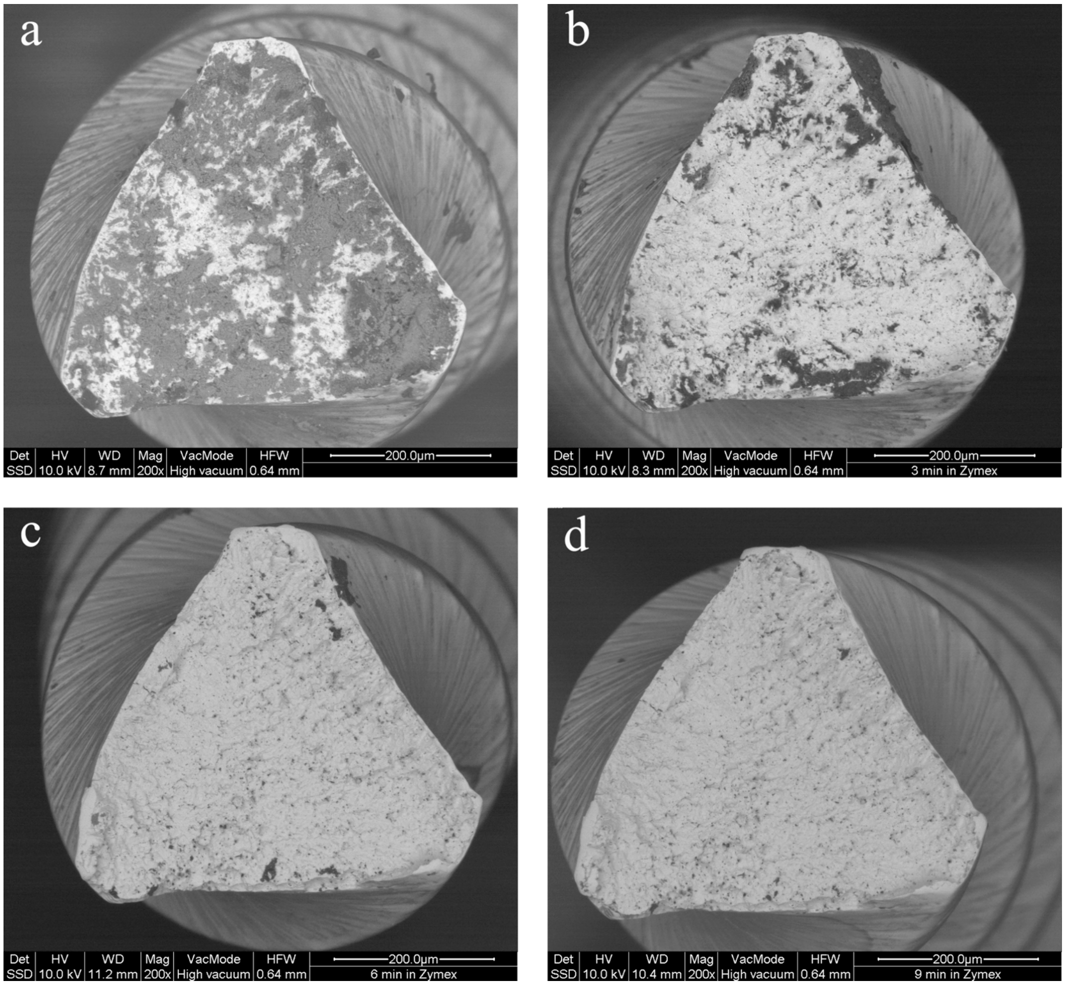

Fig. 4. SEM images of the fractured surfaces and adjacent flutes of a file used as received (a) and after 3 (b), 6 (c) and 9 (d) min of cleaning in ZymeX™ (Group D).

a cleaning solution in an ultrasonic bath and the entire instrument is immersed, corrosion can be detected after 30 min of ultrasonication (Parashos et al., 2004). In contrast, no signs of corrosion are detected in this study after 9 min of ultrasonication, although a simple BE image at a low magnification cannot be conclusive. Dentasept 3H Rapide is a detergent based on quaternary ammonia, a widely used cationic surfactant, and its broad-spectrum bactericidal action is attributed to the inactivation of energy-producing enzymes, denaturation of proteins and disruption of the structural organization and integrity of the cytoplasmic membrane of bacteria (McDonnell and Russell, 1999). This agent contains the biguanide chlorhexidine digluconate, which is known for its broad-spectrum efficacy and its activity that is dependent on pH and greatly reduced in the presence of organic matter. Moreover, polyhexamethylene biguanide, a polymeric diguanide that is active against gram-positive and gram-negative bacteria (McDonnell and Russell, 1999), is present in this agent. ZymeX™ is an enzymatic cleaning solution containing proteolytic enzymes, isopropyl alcohol and triethanolamine. Enzymatic cleansers are widely promoted to disinfect various medical devices because they can remove proteins from surfaces by breaking them down into relatively small parts (amino acids or peptides), which are more soluble in water than large parts (Parashos et al., 2004). Under the conditions of this study all four cleaning solutions, presented similar results (Figs. 1–4).

Under the conditions of this study, 17 % EDTA.3NaOH (pH = 4.6) was as efficient in removing surface debris accumulated during instrumentation as the other solutions examined. In contrast, in an in vivo study (Parashos et al., 2004), 15 % EDTA.2NaOH solution with a pH of 7 was characterized as inefficient for cleaning rotary Ni–Ti endodontic instruments in an ultrasonic bath for a maximum duration of 45 min. In the same study, NaOCl and the enzymatic solution used were found to be equally effective, which is in agreement with the results of this study.

The Dentasept 3H Rapide and ZymeX™ manufacturers suggest a minimum of 5 min of contact (Table 1). The results of this study show that ultrasonic agitation for 9 min is efficient in cleaning Ni–Ti files, and the first 6 min of agitation account for the most significant component of debris removal. This finding is in agreement with other studies where a significant difference is observed within the first 5–10 min of ultrasonic cleaning, whereas no further improvement occurs after 1 h of agitation (Aasim et al., 2006). Exceeding 15 min of ultrasonication has been related to the redeposition of debris and to the dissatisfactory cleaning results (Parashos et al., 2004). Redeposition (Fig. 1, c, arrow) and relocation (Fig. 3, c, arrow) can be observed in this study, which are attributed to the manner in which the instruments are loosely placed in the cleaning solution in the beaker. This positioning allows the removed debris to accumulate in the bottom of the container and recontaminate the instruments. In a comparative study in which different methods of ultrasonication are used, placing the instruments in a supported mesh basket is more effective than placing the files directly in the beaker, probably because it keeps the files away from the debris accumulating in the cleaning solution. No difference in cleanliness is observed when using a basket or a file stand (Parashos et al., 2004). The use of metal containers for ultrasonic cleaning is not recommended since investigators have shown that it results in less satisfactory cleaning than loosely placing the instruments in the beaker, most likely because the

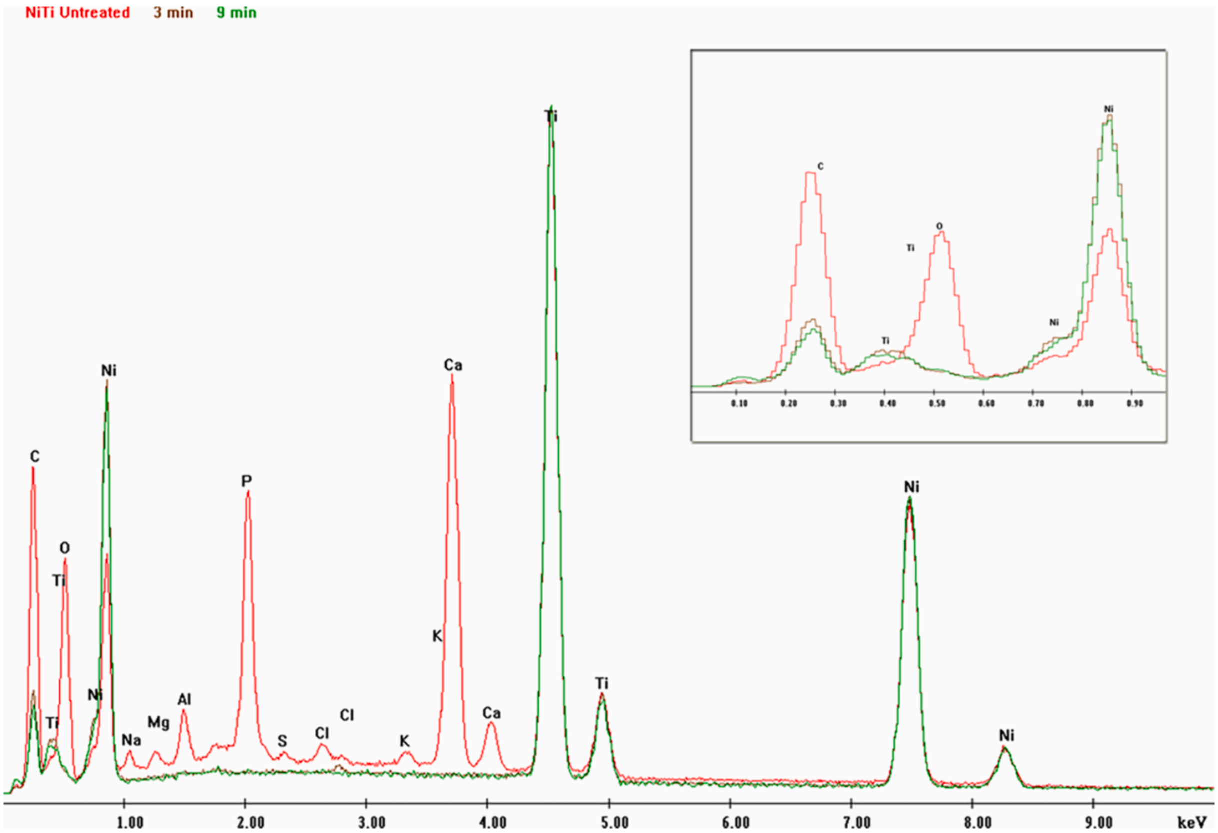

Fig. 5. EDS spectra of the untreated surfaces and the surfaces after 3 and 9 min of ultrasonic cleaning. Apart from Ni and Ti, all the other elements (except for C) were completely removed from the surface. The inset shows the low-energy parts of the spectra with the C peak. Although the intensities of the C peaks decreased, C was retained on the surfaces even after 9 min of cleaning. The spectra after 6 min were deliberately omitted for clarity.

Table 2
Qualitative and quantitative (wt.%) data after EDS analysis though successive cleaning cycles. Only the results of Group A (EDTA.3NaOH) are presented due to space constraints.

Element	Untreated	3 min	6 min	9 min
C	26.02	5.87	2.26	01.83
O	13.29			
Na	0.66			
Mg	0.34			
Al	0.87			
P	4.45			
Cl	0.32			
K	0.29			
Ca	7.85			
Ti	20.31	41.37	42.03	42.22
Ni	25.61	52.76	55.71	55.95

metal container shields the files from the propagation of ultrasonic energy (Van Eldik et al., 2004).

A common finding for all the solutions tested is the presence of small, isolated regions with random distributions on the surfaces of the alloys even after 9 min of immersion in cleaning agents, as indicated by the EDS analysis results in Table 2. However, the quantitative results of light elements such as C cannot be considered valid in nonstandard analyses due to their low photon energies (Goldstein et al., 2003). The X-ray counts and the quantitative C contents can be safely compared among different spectra if collected under the same beam conditions in the same matrix. Analyzing these regions with FTIR and/or Raman spectroscopy and other analytical techniques can provide additional molecular information concerning these C-based compounds, which is essential for comprehending the mechanisms of their strong adherence to alloy surfaces. Moreover, the effect of additional ultrasonication should be tested to explore whether the C-based debris is completely insoluble or whether cleaning agents should be applied for a prolonged period to completely clean the surface.

A potential application of the outcome of this study is related to the use of these cleaning agents during the preparation of Ni–Ti surfaces for fractographic analysis to preserve microstructural features (Zipp and Dahlberg, 1987). To determine the failure modes of rotary Ni–Ti endodontic instruments under SEM, the instruments are usually cleaned in an ultrasonic bath containing water (Eggert et al., 1999) or ethanol (Martins et al., 2002). However, the effectiveness of ultrasonication is questionable since dentinal debris seems to adhere to the surface under study and cannot be completely removed (Marending et al., 1998; Eggert et al., 1999; Tripi et al., 2001; Alapati et al., 2003; Alapati et al., 2004). The results of this study can be applied to cleaning protocols in clinical practice and in fractography studies.

5. Conclusions

Under the conditions of this study, 17 % EDTA.3NaOH, 2.5 % NaOCl, Dentasept 3H Rapide and ZymeX™ were equally efficient in removing surface debris. Six minutes was identified as the minimum time required to achieve a clean surface, while additional time had a limited effect on the cleaning efficacies of all the solutions tested.

CRediT authorship contribution statement

Eleni Kosti: Conceptualization, Investigation, Formal analysis, Data curation, Writing – original draft. **Youssef S. Al Jabbari:** Project administration, Investigation, Resources, Writing – review & editing. **Aref Sufyan:** Investigation, Data curation. **Spiros Zinelis:**

Methodology, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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