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A SEM evaluation of debris removal from endodontic files after cleaning and steam sterilization procedures

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Abstract

Background: In recent times, it has been proposed to classify endodontic files as single-use items due to a perceived inability to adequately clean the instruments. The purpose of the present study was to quantify the surface debris on files removed from the manufacturer's packaging, and after cleaning using an ultrasonic bath or a thermal disinfector.

Methods: Stainless steel and rotary nickel-titanium files were examined after removal from the manufacturer's packaging, after instrumentation in broth-contaminated human teeth, and after various cleaning procedures. The cleaning procedures consisted of either a thermal disinfector cycle, ultrasonication with the files placed in a perforated container or ultrasonication with the files loosely placed in a beaker. The presence of manufacturing debris and biological debris was evaluated using scanning electron microscopy and quantified using image analysis software.

Results: The effectiveness of cleaning was not affected by variation in the size or taper of the files when an effective cleaning procedure was used. Cleaning the files in a thermal disinfector or by ultrasonication within a container did not consistently achieve complete removal of biological debris. Placing the files loosely in the ultrasonic bath achieved the most effective cleaning, an average of 98.33 per cent of the file surface area was freed of any biological debris.

Conclusions: A conventional cleaning method is capable of effectively removing biological debris from endodontic files. The efficacy of ultrasonic cleaning was impaired when the files were placed within a perforated container.

Key words: Infection control, cleaning, biological debris, endodontic files.

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INTRODUCTION

Infection control procedures have contributed to some major changes in modern dental practice. These procedures are regularly updated to accommodate advancing knowledge and the emergence of transmissible diseases. Endodontic files are generally considered to be re-usable instruments. In recent times, the re-use of endodontic files has been scrutinized due to the uncertainty of achieving complete removal of biological debris (the organic and inorganic tissue retained on the surface of the files after being used to instrument a root canal) after cleaning procedures. Protocols for the re-use, cleaning and sterilizing of endodontic files are currently being reviewed.[1]

There has been very little evaluation of the efficacy of cleaning procedures used for contaminated endodontic files. Segall *et al*.[2] examined the manual cleaning of files with gauze or sponges and found these cleaning procedures to be ineffective in producing completely clean files. However, the quantification methods used in this study were subjective and relatively inaccurate. Murgel *et al*.[3] demonstrated no significant difference in the amount of manufacturing debris or biological debris found on used or unused files that had been cleaned in an ultrasonic bath. The authors also stated that none of the cleaning methods for the experimental groups consistently removed all of the biological debris. Smith *et al*.[4] used a light microscope to examine files provided by general practitioners and a hospital dental clinic. Files received from the general practitioners were cleaned by hand-brushing and 76 per cent of these retained debris. Files received from the hospital dental clinic were cleaned in an ultrasonic bath and 14 per cent of these retained debris. There were no details of the number of times the instruments had been re-used and if the files were placed in a container or with other instruments in the ultrasonic bath

Contrary to current tenets of sterilization,[5] it has been demonstrated that effective elimination of microorganisms from endodontic files can be achieved by steam sterilization and is not affected by the presence of biological debris.[6,7] However, there is a theoretical risk that residual biological debris may allow transmission of Creutzfeldt-Jakob disease (CJD),

especially the new variant CJD (vCJD), but more research is required to assess this risk.[8] Accurate assessment of the efficacy of modern cleaning procedures to remove biological debris from contaminated endodontic files is required to evaluate the risks of disease transmission when files are re-used.

The purposes of the present study were to quantify the manufacturing debris on Hedström and rotary files on removal from the manufacturer's packaging, to evaluate the effect of file size, taper or type (Hedström or rotary files) on the efficacy of biological debris removal, and to evaluate the efficacy of various modern cleaning methods to remove biological debris from these files.

MATERIALS AND METHODS

Ethical approval and informed consent was obtained for each tooth used in this study. Thirty premolar teeth extracted for orthodontic reasons were stored in phosphate-buffered saline. Standard endodontic access cavities were prepared in the teeth using a high speed Jet #330 bur. Initial instrumentation of the canals was performed using Hedström ISO size 10 files to facilitate further instrumentation by the larger files.

Sixty Hedström files of ISO sizes 15, 25 and 35, and 60 rotary files of tapers 0.04 (ProFiles, Dentsply, Maillefer, Ballaigues, Switzerland), 0.06 and 0.08 (GT files, Dentsply, Tulsa Dental, Tulsa, Oklahoma, USA) were used in the present study. For each of the following procedures, sample groups of four Hedström files of each size and four rotary files of each taper were examined by scanning electron microscopy after one of the following procedures:

Procedure I. Unused

Twenty-four files were examined directly after removal from the manufacturer's packaging.

Procedure II. No cleaning

Twenty-four files were used to instrument root canals. Hedström files were examined after 60 strokes of the file in a coronal direction. Rotary files were examined after use in a canal for 15 seconds at a speed of 300rpm. Files in the 'No cleaning' group served as the positive controls to which the files from the cleaning groups were compared.

Fig 1. Perforated container (Miele & Cie, Gütersloh, Germany) used to hold files while cleaning in either the ultrasonic bath or the thermal disinfector. Dimensions: L-80mm, W-30mm, H-25mm.

Procedure III. Ultrasonication with a container

Twenty-four files were used to instrument contaminated root canals (as in Procedure II) and then were placed on a gauze square after instrumentation for a maximum of 10 minutes. The files were then placed in a perforated metal container (Fig 1) that was placed in a beaker containing the ultrasonic cleaning solution solution (BioSonic Enzymatic Ultrasonic Cleaner, Whaledent, New Jersey, USA), which was placed in the ultrasonic cleaner (BioSonic Jr, Whaledent, New Jersey, USA) for five minutes. For every cycle, a maximum of 12 files was placed in the container and only one container was placed in the ultrasonic bath at a time.

Procedure IV. Ultrasonication without a container

Twenty-four files were used to instrument contaminated root canals (as in Procedure II) and cleaned in an ultrasonic bath for five minutes. Files were handled in a similar manner to Procedure III but rather than being placed in a container, they were loosely placed in the beaker of cleaning solution.

Procedure V. Thermal disinfector

Twenty-four files were used to instrument contaminated root canals (as in Procedure II) then placed on a gauze square for a maximum of 10 minutes. Files were then placed in a perforated metal container (as in Procedure III), which was then placed in a wire mesh basket in the thermal disinfector (Miele Professional G 7781 TD, Miele & Cie, Gütersloh, Germany). Thermal disinfectors use streams of hot water to physically clean debris from instruments and equipment, and utilize heat as an antibacterial mechanism. The thermal disinfector cycle included a main wash at a temperature of 45-60°C for three minutes and a final rinse at a temperature of 80-93°C for 10 minutes.

For Procedures II-V, a cooked meat broth (Oxoid Pty Ltd, Heidelberg, Victoria, Australia) was used to intentionally contaminate the root canals. Batches of the cooked meat broth were processed in a blender and then sterilized. The blended meat broth was injected into the root canals and transported along the entire length of the canals using finger spreaders in a pumping action. The cooked meat was used to imitate pulp tissue and create a greater challenge for the cleaning procedures. After instrumentation or a cleaning procedure, the files were placed on gauze squares, packaged in sealed sterilization pouches and sterilized by steam sterilization at a temperature of 134°C and a pressure of 27psi for at least 12 minutes (Validator Plus, Siemens, Pelton & Crane, Charlotte, South Carolina, USA). During all procedures, the files were handled with care so that disruption of the biological

Table 1. Mean percentages of clean surface area demonstrating the effect of rotary file taper for each cleaning procedure and comparing the efficacies of the cleaning procedures for each file taper

Cleaning group	Taper of rotary file			p value
	0.04	0.06	0.08	
Unused	93.50*†	85.84*‡	84.18*§	0.00
No cleaning	13.09†	7.94‡	17.73§	0.33
Ultrasonic with container	92.42†	91.50‡	80.20§	0.09
Ultrasonic without container	98.13†	98.61‡	98.63§	0.36
Thermal disinfector	85.11†	82.28‡	87.96§	0.47
p value	0.00	0.00	0.00	

*ANOVA test demonstrated the 0.04 taper ProFile files had significantly greater clean surface areas than the GT 0.06 and 0.08 files.
†ANOVA test demonstrated significant differences between the procedures for ProFile 0.04 taper files: No cleaning < Thermal disinfector < Unused, Ultrasonic with a container, Ultrasonic without a container.
‡ANOVA test demonstrated significant differences between the procedures for GT 0.06 taper files: No cleaning < Unused, Thermal disinfector < Ultrasonic with a container, Ultrasonic without a container.
§ANOVA test demonstrated significant differences between the procedures for GT 0.08 taper files: No cleaning < Unused, Ultrasonic with a container, Thermal disinfector, Ultrasonic without a container.

debris was minimized. Tweezers were used to remove the files from the manufacturer's packaging or the sterilization packaging, avoiding contact with the fluted sections.

After sterilization, the files were mounted on a customized jig and viewed with a scanning electron microscope (SEM) (Philips XL30 Field Emission Gun Scanning Electron Microscope, FEI Electron Optics, Eindhoven, Netherlands). Three 2mm sections (apical, middle and coronal) of each file were examined. For each 2mm section, two sequential 1mm lengths of the file were examined at 235x magnification. Shaft sections of GT 0.06 and 0.08 taper files required 190x magnification to ensure that the entire diameter of the file could be viewed. Images were viewed using the backscatter detector to maximize the contrast between the metal surface of the file and surface debris. Using this detector, the metal surface appears white and the debris appears black. The analySIS® software (Soft Imaging Systems GmbH, Münster, Germany) was used to calculate relative proportions of the clean metal surface for each section of each file. For each 1mm section of file, a rectangular area was chosen for analysis. The size of the rectangular analysis area was identical for all corresponding sections of files of the same size or taper. The position of the analysis area for each file was chosen to minimize any interference by background objects or shadows.

Table 2. Mean percentages of clean surface area demonstrating the effect of Hedström file size for each cleaning procedure and comparing the efficacies of the cleaning procedures for each taper

Cleaning group	Size of Hedström file			p value
	15	25	35	
Unused	94.24‡	90.50§	91.64‖	0.06
No cleaning	53.21*‡	56.29*§	24.44*‖	0.03
Ultrasonic with container	88.47‡	86.92§	44.50‖	0.00
Ultrasonic without container	97.89‡	98.02§	98.73‖	0.06
Thermal disinfector	92.72‡	93.49§	93.07‖	0.66
p value	0.00	0.00	0.00	

*ANOVA test demonstrated significantly less clean surface area for size 35 files after intentional contamination and no cleaning.
†ANOVA test demonstrated significantly less clean surface area for size 35 files after intentional contamination and cleaning in an ultrasonic bath with a container.
‡ANOVA test demonstrated size 15 Hedström files have less clean surface area after intentional contamination and no cleaning than all other procedures.
§ANOVA test demonstrated size 25 Hedström files have less clean surface area after intentional contamination and no cleaning than all other procedures.
‖ANOVA test demonstrated size 35 Hedström files have less clean surface area after intentional contamination and cleaning in an ultrasonic bath with a container than the other procedures.

Table 3. Comparison of mean percentages of clean file surface of rotary and Hedström files for each cleaning procedure and comparison of the efficacies of the cleaning procedures for each type of file

Cleaning group	Rotary files	Hedström files	p value
Unused	88.51*†	92.13*‡	0.02
No cleaning	13.09*†	44.65*‡	0.00
Ultrasonic with container	88.04*†	73.29*‡	0.04
Ultrasonic without container	98.46†	98.21‡	0.06
Thermal disinfector	84.04*†	93.09*‡	0.66
p value	0.00	0.00	

*t tests demonstrated significant differences between the clean surface areas of the rotary and Hedström files for the Unused, No cleaning, Ultrasonic with a container, and Thermal disinfector procedures.
†ANOVA test demonstrated significant differences between the cleaning procedures for the rotary files: No cleaning < Unused, Ultrasonic with a container, Thermal disinfector < Ultrasonic without a container.
‡ANOVA test demonstrated significant differences between the cleaning procedures for the Hedström files: No cleaning < Ultrasonic with a container < Unused, Thermal disinfector, Ultrasonic without a container.

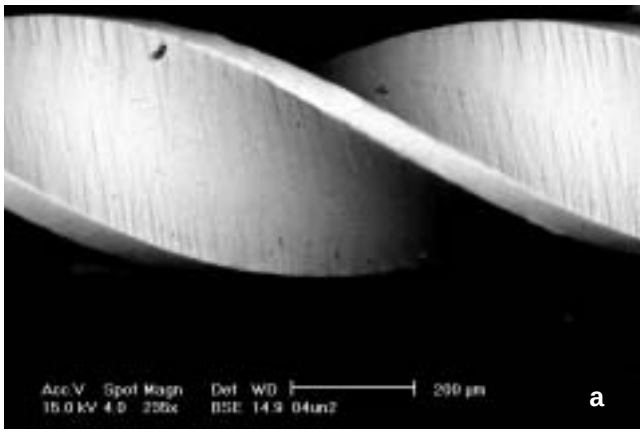
Statistical analysis

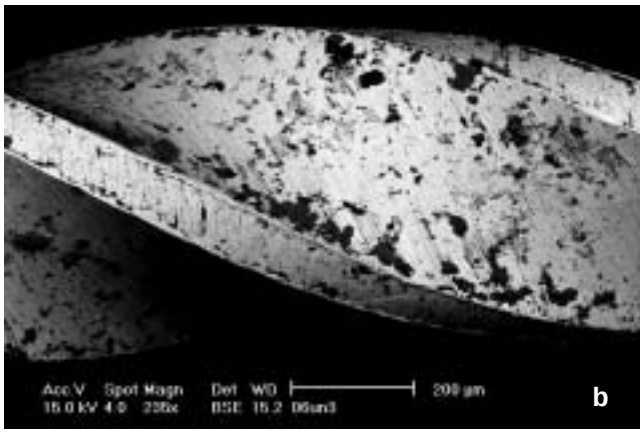
Mean percentages of clean surface area were calculated for each file in each sample group. Analysis of variance tests were used to detect significant differences between groups of varying size or taper, and the various cleaning methods. Tukey post-hoc tests were performed to determine significant differences

Table 4. Comparison of the efficacies of the cleaning procedures for all of the files combined

	Ultrasonic with a container	Thermal disinfector	Ultrasonic without a container
All files	80.67*	88.57*	98.33*

*ANOVA test demonstrated a significant difference between each of the cleaning procedures (p value 0.00).





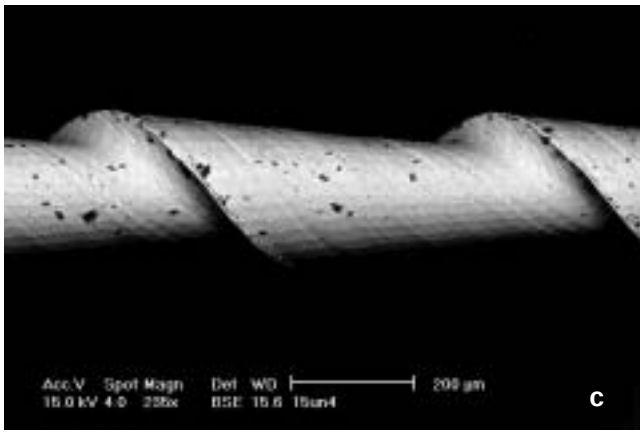


Fig 2a. Scanning electron micrograph of an unused Profile 0.04 taper file. **2b.** Scanning electron micrograph of an unused GT 0.06 taper file. **2c.** Scanning electron micrograph of an unused Hedström ISO size 15 file.

between the individual groups. Student *t* tests were used to determine significant differences between Hedström and rotary files. The level of significance was set at $p<0.05$.

RESULTS

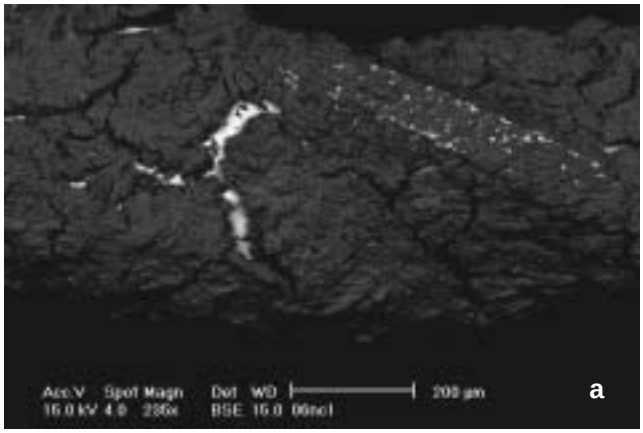
Mean percentages of clean surface area for each file size or taper and for each file type are presented in Table 1-4.

Files removed from manufacturer's packaging (Unused files)

All files removed from the packaging demonstrated manufacturing debris on the fluted sections. A significant difference in the amount of clean surface area was demonstrated within the unused rotary file group (Table 1). The ProFile 0.04 taper files had significantly less manufacturing debris than the GT 0.06 and 0.08 taper files (Fig 2). There was no significant difference between the three sizes of unused Hedström files (Table 2). When comparing the types of files, unused rotary files had a significantly lower mean percentage of clean surface area compared to unused Hedström files (Fig 2).

Effect of file size or taper

Variation of the taper of rotary files did not have a significant effect on the percentage of clean surface area



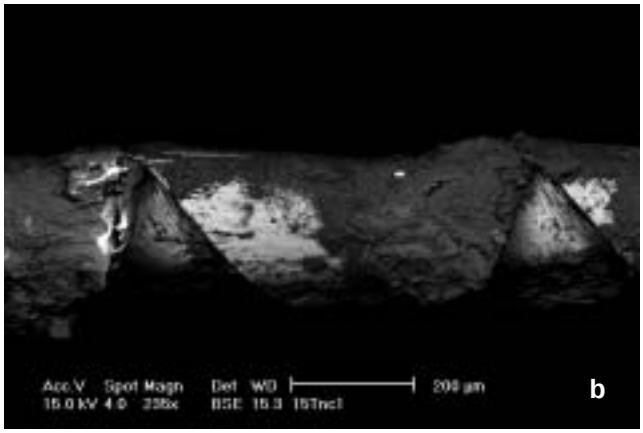


Fig 3a. Scanning electron micrograph of a GT 0.06 taper file after intentional contamination. **3b.** Scanning electron micrograph of a Hedström ISO size 15 file after intentional contamination.

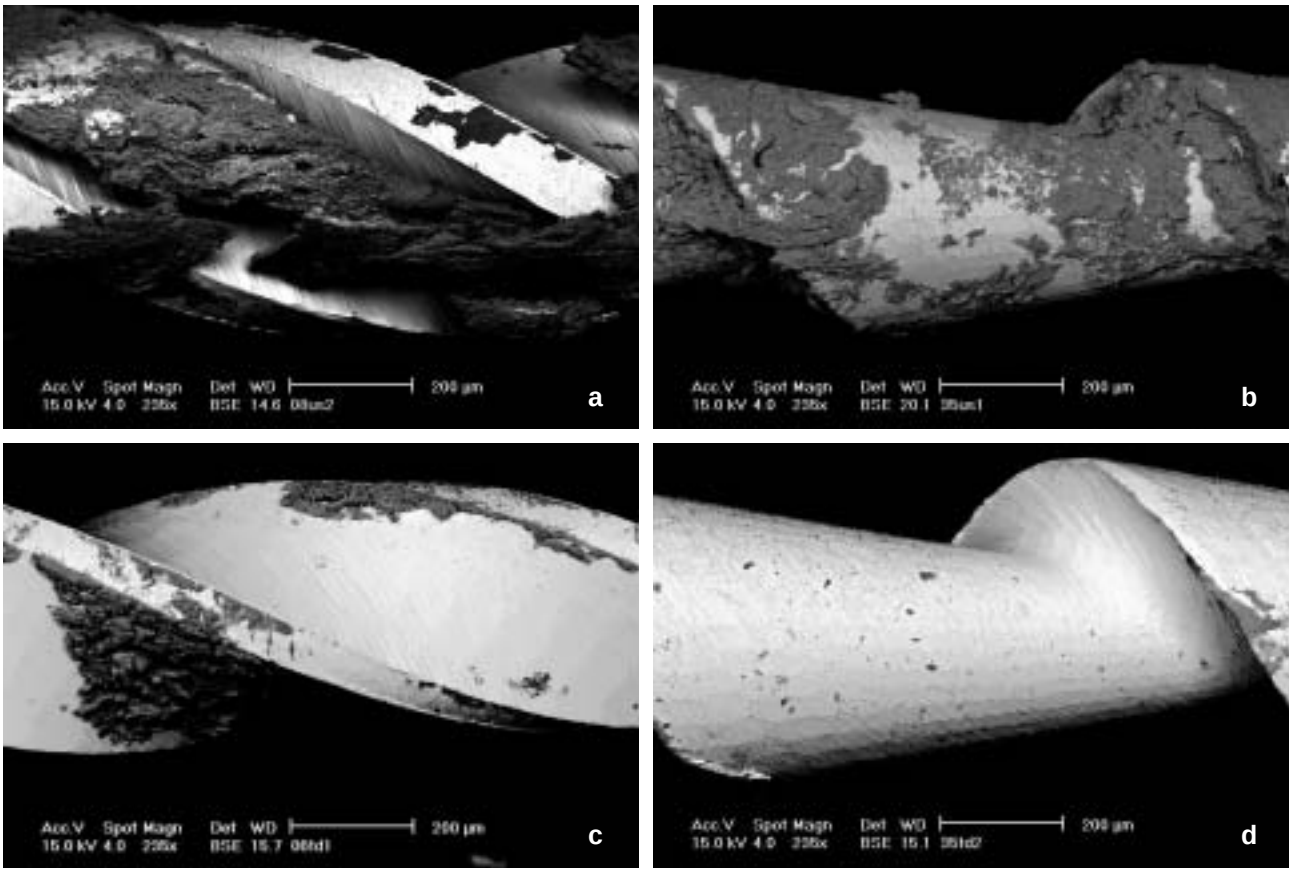


Fig 4a. Scanning electron micrograph of incomplete bioburden removal from a GT 0.08 taper file after cleaning by ultrasonication with a container. **4b.** Scanning electron micrograph of a Hedström ISO size 35 file after cleaning by ultrasonication with a container. **4c.** Scanning electron micrograph of incomplete bioburden removal from a GT 0.06 file after cleaning by a thermal disinfector. **4d.** Scanning electron micrograph of a Hedström ISO size 35 file after cleaning by a thermal disinfector.

of files after intentional contamination with or without a subsequent cleaning procedure (Table 1). Variation of the size of Hedström files had a significant effect on the percentage of clean file surface after the 'No cleaning' and 'Ultrasonication with a container' procedures (Table 2). After these procedures, the ISO size 35 files demonstrated significantly larger areas of biological debris compared with both of the smaller files. All sizes of unused Hedström files and those cleaned by the thermal disinfector or ultrasonication without a container demonstrated no significant difference in clean surface area.

Effect of file type (Table 3)

Rotary files had greater amounts of biological debris after the files were used to instrument the root canals and then not cleaned (Fig 3). Hedström files demonstrated greater proportions of clean surface area when cleaned by the thermal disinfector but the rotary files had greater proportions of clean surface area when cleaned by the ultrasonic with a container. However, effective removal of biological debris was demonstrated for both file types when they were cleaned by the ultrasonic without a container.

Comparison of cleaning methods

Complete removal of biological debris from files was not consistently achieved when they were cleaned by either ultrasonication with a container or a thermal disinfector cycle (Fig 4). There were no significant differences in mean percentages of clean surface area for rotary files that were cleaned by either of these methods (Table 3). However, rotary files that were cleaned by ultrasonication without a container demonstrated significantly greater proportions of clean surface areas (Fig 5).

Hedström files that were cleaned by either a thermal disinfector or ultrasonication without a container demonstrated significantly larger areas of clean file surface compared to ultrasonication with a container (Fig 4 and 5). There was no significant difference between the clean surface areas after cleaning with the thermal disinfector and ultrasonication without a container (Table 3). However, the latter method consistently resulted in higher percentages of clean file surfaces.

When the cleaning procedures were compared for the total number of files, a significant difference was demonstrated between each group (Table 4). Ultrasonication without a container demonstrated the most effective removal of biological debris; an average of 98.33 per cent of the surface of the files was free of any biological debris. Cleaning with the thermal disinfector, showing an average of 88.57 per cent clean surface area, was the next most effective cleaning

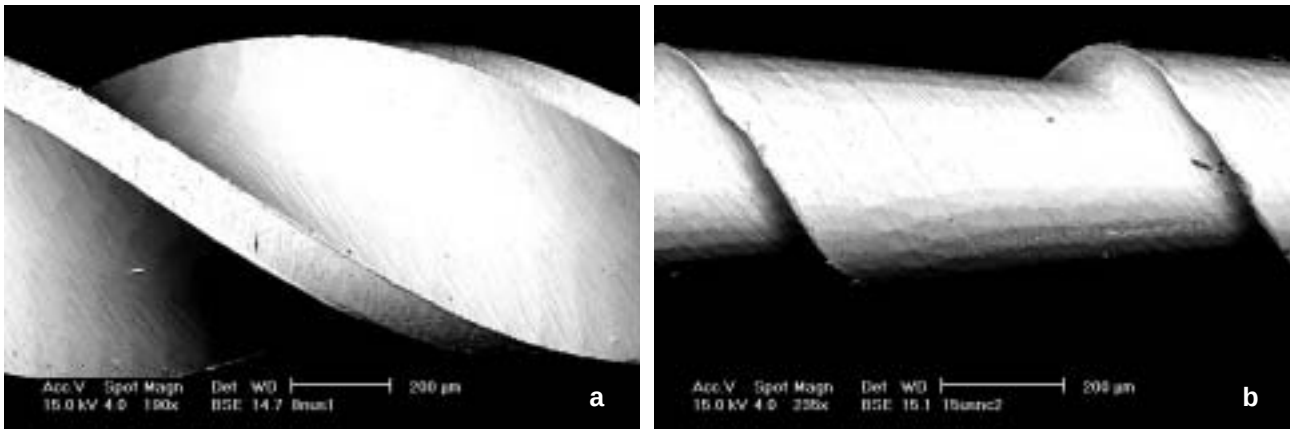


Fig 5a. Scanning electron micrograph of effective bioburden removal from a GT 0.08 file after cleaning by ultrasonication without a container. **5b.** Scanning electron micrograph of a Hedström ISO size 15 file after cleaning by ultrasonication without a container.

procedure and ultrasonication with a container demonstrated the lowest average proportion of clean surface area (80.67 per cent).

DISCUSSION

There has been limited investigation of the efficacy of current cleaning procedures for endodontic files. Previous studies have examined either inadequate numbers of files[9] or evaluated cleaning methods that are inappropriate for modern infection control practices.[2,10]

Several investigations have used scanning electron microscopy to quantify the surface debris on endodontic files. However, the quantification method has commonly involved a numerical rating based on a subjective assessment by the examiners.[2,4,11,12] Objective quantification of biological debris using a computerized analysis of the SEM image similar to that of Murgel *et al*.[3] has been performed in the present study. In contrast to Murgel *et al*.,[3] a greater length of each file was examined. This provided a more accurate representation of the proportion of biological debris for the entire length of the fluted sections.

The larger tapers of the unused rotary files had significantly greater amounts of manufacturing debris when compared to the unused ProFile 0.04 taper files but this may have resulted from variations in manufacturing or post-manufacturing processes for each file manufacturer. The efficacy of cleaning Hedström files appeared to be influenced by varying the size of the files. When compared with the smaller files, Hedström ISO size 35 files demonstrated significantly greater surface areas of retained biological debris after intentional contamination (Procedure II) and ultrasonication with a container (Procedure III). The relatively larger cutting flutes of the Hedström ISO size 35 files may have contributed to a more aggressive cutting nature, which could have resulted in a greater accumulation of contaminated biological debris. However, cleaning the Hedström files with either a thermal disinfector or ultrasonication without a container demonstrated effective removal of biological debris, irrespective of the size of the files. These results suggest that variation of the size of Hedström files will only affect the efficacy of biological debris removal when a less effective cleaning procedure is employed.

Rotary files retained significantly less biological debris after cleaning in an ultrasonic with a container. However, Hedström files retained less biological debris after cleaning in the thermal disinfector. This variation in the cleaning efficacy may depend on the interaction of the mechanical action of each cleaning process with the flute design of the different file type. However, a definite reason for this discrepancy is uncertain. An important facet of an effective cleaning procedure should be consistent cleaning efficacy, despite variations in the size and shape of the instruments to be cleaned. Ultrasonication without a container was the only cleaning procedure examined in this study that fulfilled this requirement.

For rotary files, ultrasonication without a container produced significantly greater proportions of clean surface area compared to both of the other cleaning methods. There was no significant difference in the clean surface area of Hedström files when they were cleaned by either ultrasonication without a container or the thermal disinfector. When considering the results for both rotary and Hedström files, ultrasonication without a container demonstrated the greatest efficacy of cleaning, with an average of 98.33 per cent of the surfaces of the files being free of biological debris. Cleaning with the thermal disinfector was less effective, producing an average clean surface area of 88.57 per cent, while ultrasonication with a container demonstrated the least effective removal of biological debris (80.67 per cent).

The effective removal of debris from endodontic files in this study differs with previously reported retention of debris after ultrasonic cleaning.[4] This variation may be due to differences in the cleaning procedures such as whether the files were placed in a container, the number of other instruments placed in the ultrasonic bath, or differing types of ultrasonic cleaners.

A significant difference was demonstrated between the two methods of ultrasonic cleaning for both file

types. The use of the container enabled easier and safer handling of the files. However, it appeared to shield the files from the propagation of the ultrasonic energy. Therefore, on the results of this study, the use of these containers in ultrasonic cleaners cannot be recommended. Other designs of containers or holding devices may not inhibit ultrasonic cleaning to the same degree as the one used in the present study. Further investigation should be undertaken to evaluate the effect of different types of holding devices on the efficacy of cleaning procedures.

At present, Australian Standards[13] recommend that endodontic files should demonstrate a 'macroscopic cleanliness' after a pre-sterilization cleaning procedure. 'Macroscopic cleanliness' of endodontic files can be difficult to assess due to their small size and fluted design. In addition, the accuracy of this assessment will vary with each individual. Adequate infection control protocols require a cleaning procedure that produces consistent and effective cleaning of endodontic files so that there is less reliance on subjective and inaccurate methods of assessment.

The results of the present study demonstrate that cleaning methods may vary in the efficacy of biological debris removal but effective removal of biological debris was demonstrated by a cleaning procedure that is readily available in Australia. However, trace amounts of debris may be retained in microscopic crevices or grooves that are created during the manufacturing process. The importance of this debris is unknown. The transmission of bacterial and viral diseases *via* endodontic files can be reduced to negligible levels by careful handling and standard infection control procedures. Even in the presence of biological debris, the viability of most microorganisms will not be protected when the files are subjected to a steam sterilization cycle.[6,7]

There has been concern that retained biological debris on dental instruments may pose a risk of CJD transmission.[4] At present, the risk of transmitting CJD *via* oral tissues is theoretical. However, the disease demands consideration in infection control protocols due to its mortality rate and the resistance of the aetiological agent to routine sterilization methods. Despite the suggestions that oral tissues may harbour infective prions,[14-16] routine dental treatment has no proven association with the transmission of CJD.[8] The potential to transmit CJD, especially vCJD, requires reliable and relevant investigation to reassess the current infection control protocols.[8] In the absence of this investigation, strict infection control procedures are enforced when treating high-risk or known CJD-affected patients. However, when effective cleaning and sterilization procedures are used, it remains uncertain whether these rigid protocols need to be implemented for endodontic treatment while the incidence of CJD is very low ($1:10^6$). Recently, the Department of Health in the United Kingdom has altered its guidelines to recommend that confirmed or suspected CJD cases may be treated in general dental practice provided that optimal standards of infection control are maintained.[17]

CONCLUSION

Inadequate removal of biological debris from both Hedström and rotary files was demonstrated when the endodontic files were placed in a perforated container and then cleaned in an ultrasonic bath. Despite the effective removal of biological debris from Hedström files, the thermal disinfector did not achieve favourable results for the rotary files. Ultrasonic cleaning of endodontic files provides effective removal of biological debris (98.33 per cent) when the files are loosely placed in the cleaning solution rather than in a container. When this method of cleaning is used, variation in the type of file or the size or taper of the files did not have a significant effect on the efficacy of biological debris removal.

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