



Residual protein levels on reprocessed dental instruments

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Summary Reduction of the initial bioburden on instruments, prior to sterilization, is believed to reduce transmission risks of iatrogenic Creutzfeldt-Jakob disease. Endodontic files are used in the preparation of root canals and are likely to have close contact and become contaminated with neural material from branches of the maxillary and mandibular cranial nerves. This study examined methods used by 22 dental practices to clean endodontic files, and scored visible debris and residual protein levels adhering to 220 dental endodontic files that had been used, cleaned, autoclaved and were deemed ready for re-use. Visible debris was scored after examination under a dissecting light microscope. Residual protein was quantified using a fluorescent assay based on reaction of proteins with *o*-phthaldialdehyde/*N*-acetyl cysteine. There was wide variation in the methods used by practices to clean endodontic files. The cleaning process varied from a wipe with an alcohol-impregnated cloth to hand scrubbing and/or use of an ultrasonic bath. Surface debris was visually detected on 98% of files. Residual protein was detected on all the files examined (median amount: 5.4 µg; range: 0.5-63.2 µg). These results demonstrate that the cleaning of some instruments reprocessed routinely in primary care is incomplete, and such instruments cannot be excluded as a potential source of cross-infection.

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Introduction

Iatrogenic transmission of variant Creutzfeldt-Jakob disease (vCJD) has been highlighted by recent concerns over the possibility of transmission by blood transfusion.¹ There is also potential for onward transmission of vCJD via re-usable medical devices, and the large volume of instruments used in dental surgery has raised the theoretical possibility of transmission in that setting.² The disease-associated form of prion protein has been demonstrated in trigeminal ganglion, pulpal tissue and gingival tissue in animal models.³ In vCJD, the abnormal prion form has been observed using immunohistochemistry in the trigeminal ganglion but not in the salivary gland, alveolar nerve, dental pulp or gingival tissue.⁴ Previous risk assessments for the potential transmission of vCJD via surgical procedures have stressed the importance of cleaning instruments prior to sterilization.⁵

Endodontic files are used to debride and shape the root canal system following removal of the dental pulp. These instruments pose an infection control problem due to their small size and complex surface topography, which make them difficult to clean effectively. Debris may accumulate between the flutes of the instruments.⁶ These instruments are decontaminated and re-used routinely in UK dental practice.⁷ In view of the ongoing debate over the presence of abnormal prion protein in tissues out with the central nervous system and lymphoid tissues,⁸⁻¹⁰ this is a particular concern. The purpose of this study is to extend previous work by quantifying residual protein levels on endodontic files after use and reprocessing in general dental practice, and to determine whether these could be related to the cleaning method.

Materials and methods

Sample collection

A random sample of 22 general dental practices was identified from a group of general dental practitioners interested in clinical research [the GRID (Glasgow Research in Dental Practice) group]. A coding system was used for each practice to maintain anonymity. A questionnaire was administered to each dental practitioner during a practice visit and completed via a face-to-face interview. The questionnaire was used to collect data on methods used to reprocess endodontic files. The visits were carried out by one investigator (SL). Ten endodontic files were collected from each of 22

practices. The 220 files had each been used to treat at least one patient, had been subjected to the routine cleaning and sterilization procedures for that practice and were ready for re-use. No attempt was made to identify the number of times each file had been used and reprocessed. Each file was handled aseptically and placed in a sterile Universal container for transportation and storage prior to analysis.

Visual score of debris

Each file was examined visually for contamination using a dissecting light microscope at $\times 40$ magnification. The full length of the file was examined and visual debris was scored between 0 (no visible debris) and 3 (extensive amounts of debris visible) (Table I).

Protein analysis

For each file, only the metal working section of the device was assayed for residual debris. Protein was desorbed from each file by submersion in a measured volume of 1 v/v% Decon[®] in water followed by sonication for 30 min. The protein desorbed was quantified using a variant of a sensitive protein assay¹¹ based on using *o*-phthalaldehyde/*N*-acetyl cysteine as the fluorescent reagent with reference to a standard curve derived using bovine serum albumin.⁶ Controls included uninoculated solutions and unused files.

Data analysis

The method of decontamination and results of both the visual examination and the protein assay were entered into Minitab (v12.0) and StatXact (v6.0), and subsequently analysed. The data were not normally distributed and hence scores were compared using the Mann-Whitney test (for two groups) and the Kruskal Wallis test (when considering more than two groups).

Results

Decontamination of endodontic files

All practices in this survey re-used endodontic files routinely. Files were discarded when visibly deformed or broken. There was wide variation in the methods used by practices to clean endodontic files. The cleaning process varied from a wipe with an alcohol-impregnated cloth (Azowipe[®]) to hand

Table I Number of visually contaminated files

Visual score		Number of files (%)
0	No contamination visible through the microscope	4 (2)
0.25	Very few and very small particles only	44 (20)
0.5	Few and very small particles only at one place on the thread	65 (30)
0.75	Small particles spread over about 50% of the thread	21 (10)
1.0	Small parts (~20%) of the file covered with particles	39 (18)
1.5	Parts of the file (~40%) covered with particles, visible without microscope	9 (4)
2	More than half of the thread covered with particles, visible without microscope	23 (10)
2.5	Some part already thickly covered with particles, visible without microscope	7 (3)
3	Totally covered with particles, ~50% thickly covered, visible without microscope	8 (4)

scrubbing or use of an ultrasonic bath. The most common method of cleaning was manual cleaning, and the most common method of sterilization was in a benchtop steam sterilizer (Table II). Eight of the 22 practices decontaminated the files by hand scrubbing with a variety of proprietary cleaning agents and steam sterilization (method 1). A further seven practices employed hand scrubbing with a variety of proprietary detergents, ultrasonic bath and steam sterilization (method 2). Other practices used a number of different decontamination procedures but these were too small for meaningful statistical analysis (Table II). For the purposes of statistical analysis, these two methods (methods 1 and 2) of decontamination were examined more closely.

Visual contamination

The results of the examination for visual contamination are shown in Table I. This demonstrated that 216 of 220 (98%) files showed some degree of visual contamination.

Protein contamination

All of the 220 files demonstrated residual protein contamination. The median amount was 5.4 µg with a range from 0.5 µg to 63.2 µg (Table III).

Effect of decontamination method

The median visual score for those practices using decontamination method 1 was 1 and for method 2 was 0.5, with a corresponding *P* value from the Mann-Whitney test of 0.001. This suggests that method 2 is marginally more effective than method 1.

The median amount of protein found on files decontaminated using method 1 was 5.0 µg, compared with 5.8 µg for method 2. A Mann-Whitney test for equal medians resulted in a *P* value of

0.151, suggesting insufficient evidence to support a difference between methods in residual protein contamination.

Between practice variation within decontamination methods

The median value for visual contamination for method 1 ranged from 0.5 to 1.5 between practices. A test for equal medians gave a *P* value of 0.013 showing a significant difference between practices using the same method. The median value for visual contamination for method 2 ranged from 0.25 to 2 between practices. A test of equal medians again indicated a significant difference between practices (*P* < 0.001).

Median values for the amount of residual protein for practices using method 1 ranged from 3.5 µg to 10.8 µg. A test for equal medians gave a *P* value of 0.001, indicating a statistically significant difference between practices. Median values for practices using method 2 ranged from 4.8 µg to 7.6 µg. A *P* value of 0.513 was observed in a test for equal medians, and so differences between practices using method 2 were not statistically significant.

Discussion

This analysis has furthered earlier work⁷ by confirming and extending the information on residual debris remaining on instruments reprocessed in primary care facilities. These findings highlight that endodontic files are consistently contaminated following reprocessing methods commonly used in primary dental care facilities. The maximum amount of protein detected on a single file was 63 µg, although multiple files may be used on a patient during a single course of treatment. Similar levels of residual protein contamination can be achieved in laboratory studies on protein removal. Levels of up to 50 mg of protein have been found on

Table II Method of decontamination of endodontic files

Method of cleaning and sterilization	Number of practices
Hand scrub with cleaning agent and steam sterilization	8
Hand scrub with cleaning agent, ultrasonic bath and steam sterilization	7
Presoak, ultrasonic bath and steam sterilization	2
Presoak, hand scrub with cleaning agent and steam sterilization	1
Ultrasonic bath and steam sterilization	1
Presoak, ultrasonic bath and cold sterilization	1
Hand scrub with cleaning agent, autoclave and cold sterilization	1
Impregnated wipe and steam sterilization	1

surgical instruments immediately prior to use in operating theatres (DP, unpublished observations). Further work is required to analyse the nature of the residual contamination, e.g. type and origin of protein adhering to instruments.

The Department of Health's risk assessment for vCJD and dentistry (2003)² categorizes dentistry as 'low risk' for potential transmission of vCJD. However, the report recognizes that the possibility of infectivity in dental pulpal tissue cannot be ruled out and endodontic files, the delicate cutting instruments coming in direct contact with the pulp chamber, are particularly difficult to clean. Re-use of endodontic files was reported by 100% of

practitioners in this study. A variety of decontamination methods was employed, none of which have been validated. Even among practices using the same decontamination method, there was significant variation in the amount of visible debris and residual protein, indicating the lack of reproducibility of the cleaning methods in the practices surveyed. Furthermore, there may be a gradual accumulation of contaminants on files following multiple episodes of decontamination and re-use.

Previous workers using decontamination techniques readily available in general dental practice have demonstrated that manual debridement techniques are time consuming, have considerable

Table III Residual protein contamination on endodontic files (μg) following cleaning and sterilization procedures

Practice number	Method of cleaning and sterilization ^a	Median amount of protein (μg) per file (range)
1	1	10.8 (3.7-55.9)
2	1	8.0 (2.9-35.1)
3	1	6.1 (3.3-11.8)
4	1	5.0 (0.5-6.5)
5	1	4.8 (1.2-60.9)
6	1	3.9 (1.7-6.0)
7	1	3.7 (0.9-7.2)
8	1	3.5 (1.5-10.2)
9	2	7.6 (5.1-15.1)
10	2	6.9 (3.8-11.6)
11	2	5.8 (0.7-15.1)
12	2	5.6 (2.2-25.6)
13	2	5.3 (2.0-18.2)
14	2	5.0 (1.3-15.3)
15	2	4.8 (1.1-24.3)
16	3	13.5 (2.9-32.9)
17	3	10.1 (2.4-28.2)
18	3	5.0 (1.4-12.7)
19	3	4.9 (1.0-63.2)
20	3	4.8 (2.4-16.2)
21	3	3.1 (0.7-34.3)
22	3	2.2 (0.7-6.5)

^a Method of cleaning and sterilization: 1, hand scrub with cleaning agent and steam sterilization (autoclave); 2, hand scrub with cleaning agent, ultrasonic bath and steam sterilization (autoclave); 3, other.

operator error and risk additional contamination.^{12,13} None of the techniques for cleaning files in these studies consistently produced files that were visibly free of debris. Furthermore, other workers¹⁴ have found the cutting efficiency of endodontic files decreased to 77% depending on the type of file design and decontamination procedure performed.

These results demonstrate that endodontic files are routinely contaminated with tissue debris after reprocessing, and cannot be excluded as a potential risk for transmission of infectious agents. It is recommended that these instruments should be viewed as single-use devices, unless significantly more efficient cleaning processes can be developed and validated for use in general dental practice.

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