Endodontic files: To sterilize or to discard?

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Objective: The purpose of this study was to examine both qualitatively and quantitatively the presence of organic debris on endodontic files decontaminated and sterilized after use. Methods: Thirty K files #30 were used, 10 of which served as positive and negative control. Ten pig molars were instrumented using the Crown-Down technique by inserting one file in each root canal, totaling 25 files. The files in group 1 (n=10) were sterilized by autoclave. Files in group 2 (n=10) were placed in an ultrasonic bath with enzyme solution and then sterilized by autoclave. Those in group 3 (n=5) were used but not sterilized, and finally the files in group 4 (n=5) were neither used nor sterilized. The experimental and control files were subsequently stained with Van Gieson’s solution and observed by optical microscopy. A value representative of the amount of organic material still present on the file was then assigned in accordance with a previously established scale. The same measurement was carried out in the apical, middle and cervical thirds of each file body while tables were formulated comparing the different groups. Results: The results demonstrated that both experimental groups produced significantly inferior results compared to the files in the positive control group. Conclusions: In comparing the experimental groups, the files immersed in ultrasonic bath with enzyme solution exhibited values that were inferior to those of the files which had not been subjected to this procedure.


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Introduction

To be successful, endodontic treatment depends not only on a reliable diagnosis, but also on appropriate technical procedures and a chain of continuous asepsis in order to prevent cross infection. Cross infection control protocols are of paramount importance in all fields of medicine and particularly in Dentistry. These procedures are updated with newly acquired knowledge and original studies.1

Endodontic files are generally considered reusable instruments after sterilization. Recently it has been debated whether they should be sterilized or considered as single-use instruments since no tests have yet succeeded in completely removing the debris (organic and inorganic tissues) retained on the surface of the instrument after sterilization. In this context, the protocols for reusing, cleaning and sterilizing endodontic files must be cautiously reviewed.1 Manually cleaning endodontic files is a challenging procedure due to the anatomical complexity of these instruments.2 Walker et al2 found that 76% of endodontic files, after being subjected to manual cleaning, still displayed a considerable amount of organic debris prior to autoclaving. They concluded that manual cleaning is subjective and not reproducible.

Ultrasonic cleaning consists in immersing the instrument in a solution and subsequently subjecting it to high frequency pulses, which result in specific regions of alternating pressure. Steam bubbles are thus formed in the low pressure zones, which ultimately burst in the high-pressure zones, creating cavitations that aid in cleaning the file surface.2 Few studies have so far been conducted with the purpose of assessing the effectiveness of the sterilization protocols applied to endodontic files.3, 4, 5

Transmission of spongiform encephalitis, a.k.a. prion disease, is part of a group of rare fatal illnesses. This illness is characterized by the accumulation of an abnormal form of prion protein in the central nervous system. The risk of iatrogenic transmission of Creutzfeldt-Jakob disease (CJD), in its human variant, has aroused concern among health care professionals given their current reluctance to use conventional decontamination and sterilization thermal and chemical procedures.3, 4, 5 Sonntag and Peters7 suggested as decontamination options the use of a solution of sodium hydroxide 1 M (24 h) or 2 M (1 h), solution of sodium hypochlorite at 2.5% (24 h) or 5% (1 h), or a solution of guanidine thiocyanate 3, 4 or 6 M (24 h, 1 h and 15 min, respectively) followed by steam sterilization at 134°C for 18 minutes to 1 hour. They also suggested a fast decontamination protocol using chlorexidine.

As mentioned above, previous studies demonstrated that manual or ultrasonic cleaning are not effective in removing organic debris from endodontic files.1, 3 Smith et al5 used an optic microscope to examine endodontic files collected from dental offices and a hospital. Despite the fact that the files from the dental offices were cleaned through manual brushing, they still exhibited 76% of retained debris, whereas the files from the hospital, which had been placed in ultrasonic bath only displayed 14% of debris remnants. They also demonstrated that ultrasound accomplishes an efficient removal of the biological remnants from endodontic files (98.33%) when placed freely in the solution but not when inserted inside a container.

Conventional procedures such as autoclaving, exposure to ionic radiation, treatment with formaldehyde or ultrasound are ineffective.6 Sonntag and Peters7 suggested as decontamination options the use of a solution of sodium hydroxide 1 M (24 h) or 2 M (1 h), solution of sodium hypochlorite at 2.5% (24 h) or 5% (1 h), or a solution of guanidine thiocyanate 3, 4 or 6 M (24 h, 1 h and 15 min, respectively) followed by steam sterilization at 134°C for 18 minutes to 1 hour. They also suggested a fast decontamination protocol using chlorexidine.
and manual cleaning followed by immersion in a solution of sodium hypochlorite at 1% under ultrasound for 5 to 10 minutes prior to sterilization.

The World Health Organization (WHO) recommends immersion in a solution of sodium hypochlorite (20000 ppm) for 1 hour; boiling in a solution of sodium hydroxide (1 M) for 1 hour or sterilization in an autoclave at 121° C for 30 to 90 min in the presence of a sodium hydroxide solution (2 M) to ensure prion inactivation in the surgical material. However, these procedures are unsuitable for dental instruments due to corrosion of the metal surface, which can pose a risk for the operator and hamper the elimination of prions.2

Perakaki and Mellor3 found that endodontic files which had undergone a process of ultrasonic cleaning showed less debris than those cleaned with disinfectant solution. None of the files displayed an absolute absence of debris and they therefore suggested that these instruments be considered disposable.

Parashos et al10 recommended as a protocol for cleaning endodontic files that these be vigorously brushed 10 times with a dense sponge soaked in a chlorhexidine solution at 0.2%, leaving the files in an enzymatic solution for 30 minutes, followed by 15 minutes under ultrasound with enzymatic bath and rinsing in running water for 20 seconds. They performed this protocol on nickel-titanium files and found an absolute absence of biological debris stained with Van Gieson’s solution.11 This study was subsequently replicated by Azarpazhooh et al,9 who emphasized that the staining material was not specific for amyloid nor prion detection.

The purpose of this study was to evaluate the presence of remnants of organic substance in two groups of endodontic files using different sterilization protocols.

### Material and Methods

In the present study 30 hand K files #30 (Dentsply/Maillefer) were used, divided into four groups (Table 1).

To simulate clinical instrumentation pig mandibles were employed and the teeth underwent trepanation using round high-speed diamond burs #12 under constant irrigation. All files were handled by the same operator in order to ensure uniformity of procedures. The files were inserted twice into the root canal and rotated twice, each a half turn. After this stage, the files were randomly distributed in their respective groups and kept in individual sterile collectors.

The instruments were all subjected to Van Gieson’s staining technique in order to highlight organic matter by observing hues of red.12 The files were stained for 2 minutes and the excess dye was removed with bi-distilled water. The files were then dried at room temperature and placed on glass slides for microscopic observation.

### Disinfection and sterilization methods

#### Method A

The files in group 1 were manually cleaned with a wire brush and then with a swab soaked in ethylic alcohol at 70°. After packaging and sealing the files were subjected to a sterilization cycle in an autoclave at 121° C for 20 min and 1 atm.

<table>
<thead>
<tr>
<th>Group</th>
<th>Designation sterilization methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Method A (n=10) Files exposed to organic matter by instrumentation and subjected to cleaning and sterilization Method A. G2</td>
</tr>
<tr>
<td>2</td>
<td>Method B (n=10) Files exposed to organic matter by instrumentation and subjected to cleaning and sterilization Method A. G3</td>
</tr>
<tr>
<td>3</td>
<td>Positive Control (n=5) Files exposed to organic matter by instrumentation and subjected to manual cleaning and sterilization. G4</td>
</tr>
<tr>
<td>4</td>
<td>Negative Control (n=5) Files not exposed to organic matter by instrumentation and not sterilized</td>
</tr>
</tbody>
</table>
Method B
The files in group 2 were manually cleaned with a wire brush and then with a swab soaked in ethylic alcohol at 70°. They were subsequently placed in a commercial enzyme solution (Instrunet® EZ+T) for 20 min in an ultrasonic cleaner according to manufacturer’s directions. After packaging and sealing, files were subjected to a sterilization cycle in an autoclave at 121° C for 20 min and 1 atm.

Observation and quantification of results
The files were observed under an optical microscope (Nikon SMZ 1500) with external light source, lens HR Plan Apo 1x WD 54 by Nikon and photographed with an attached Nikon digital camera DXM 1200C with 150 X magnification of the apical, middle and cervical thirds. The images were assessed by two previously calibrated observers using Kappa test for inter-rater agreement.

The standards underpinning the classification were based on the following criteria (Fig 1).
» Absent (0) = No red color present.
» Small (1) = Presence of small red dots.
» Moderate (2) = Presence of red color in several areas.
» Abundant (3) = Presence of red color in many areas.

The results were statistically analyzed by SPSS software and the Chi-square test (p<0.05).

Results
Based on these results it was concluded that when method B was used an absence of organic debris was observed in only 20% of the cases, whereas with method A no file was free from debris.

With both methods (A and B) between 76% and 83% of the specimens exhibited a small amount of organic matter.

The presence of organic debris is not related to the file thirds as no significant differences were found between the results obtained (Tables 2-5).

Observations made regarding methods A and B combined with an analysis of the outcomes indicate a statistically significant difference between the two methods in the process of file disinfection and sterilization (p=0.009) (Fig 2).

Figure 1. Images of debris on the three thirds of files, according to the criteria used for classification.
Table 2. Group 4 (negative control).

<table>
<thead>
<tr>
<th></th>
<th>Absent (0)</th>
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<th>Moderate (2)</th>
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<tbody>
<tr>
<td>Apical 1/3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>Middle 1/3</td>
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<td>0</td>
</tr>
<tr>
<td>Cervical 1/3</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
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Table 3. Group 3 (positive control).

<table>
<thead>
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<tr>
<td>Apical 1/3</td>
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<td>3</td>
</tr>
<tr>
<td>Middle 1/3</td>
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<tr>
<td>Totals</td>
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<td>0</td>
<td>4</td>
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Table 4. Group 1 (Method A).

<table>
<thead>
<tr>
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<tr>
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<td>9</td>
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<td>0</td>
</tr>
<tr>
<td>Middle 1/3</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cervical 1/3</td>
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<td>0</td>
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<tr>
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Table 5. Group 2 (Method B).

<table>
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<th>Moderate (2)</th>
<th>Abundant (3)</th>
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<tr>
<td>Cervical 1/3</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>6</td>
<td>23</td>
<td>1</td>
<td>0</td>
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</table>

Method A  A total of 30 observations:
- 16.7% displayed a moderate amount.
- 83.3% showed a small amount.

Method B  In a total of 30 observations:
- 3.3% displayed a moderate amount.
- 76.7% showed a small amount.
- 20% showed no amount whatsoever.

Figure 2. Comparison between sterilization methods A and B.
Discussion

Although single-use endodontic files are available on the market, it is common practice for dentists to reuse these instruments after sterilization. Thus, this study — despite its limitations — has been deemed relevant as it addresses the debate over which sterilization method is the most suitable for endodontic instruments and whether such methods should be modified in order to improve the procedures employed in everyday practice.

Files #30 were used as this file size constitutes an average diameter amongst those most commonly used in delivering endodontic treatment to molars.

All instruments in the study exhibited organic debris, but when sterilized by means of method B, whereby an enzyme bath was introduced into an ultrasonic cleaner, there was a significant reduction in the amount of debris.

The literature has not reported cases of transmission of CJD through dental procedures, but since there is a potential risk of transmission of prion disease (albeit minor), as a preventive measure, in addition to the proper sterilization of endodontic instruments, professionals should obtain a medical history of the patients and their families.

Whereas none of the files in this study were found to be entirely free from organic debris after disinfection and sterilization, the recommendation that endodontic files be handled as single-use, disposable instruments should be underscored.

References