

SEM and microbiological analysis of dirt of endodontic files after clinical use and your influence on sterilization process

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ABSTRACT

Objective: The aim of this study was to assess the level of cleaning of endodontic files after its use in root canals preparation and their influence on the sterilization process. **Methods:** Fifty files were divided into two groups: one group of 25 files for analysis in scanning electron microscopy (SEM) for verification of cleaning and another group of 25 files for microbiological analysis in thioglycolate and BHI after sterilization. **Results:** The results

showed that endodontic files had different degrees of dirt on his active part through evaluation by scanning electron microscopy. The bacterial growth wasn't detected through microbiological test after sterilization. **Conclusion:** It was concluded that despite the significant presence of dirt on endodontic files in their active part, this dirt don't interfere in the sterilization process.

Keywords: Dirt. Endodontic files. Microbiological test. Scanning electron microscopy.

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Introduction

The success of endodontic therapy is grounded not only in the correct diagnosis, but also the proper planning and technical implementation, and especially in caring for the maintenance of the aseptic chain during the patients care.

The endodontic instruments are used to remove the remnants of pulp tissue during the procedures of cleaning and shaping of the root canal system. These instruments can be recycled for reuse after its first use. In a large study was reported that 88% of general practitioners dentists in the UK re-process the endodontic files after use¹.

The mandatory conduct of biosecurity recognizes that the endodontic instruments, to be reused, they must go through a cleaning process before sterilization², since the presence of organic matter and/or debris on the instruments may interfere with the sterilization process. These organic compounds creates barriers to protect the microorganisms, which may prevent the penetration of the sterilizing agent³.

The procedures of pre-cleaning and autoclave can be used to sterilize endodontic instruments^{4,5}. However, the complex architecture of endodontic files could difficult these procedures⁵. Dental structures and organic debris have been observed on the surface of rotary instruments, especially in the cracks⁶. According to a previous study, 66% of endodontic files retrieved dental general practitioners remained visibly contaminated⁷. Thus, there is the possibility of cross contamination associated with the inability to properly clean and sterilize them, and suggested that these instruments should be single-use devices.

Therefore, the aim of this study was to determine the presence of debris left on the surface of endodontic files after performing a cleaning process and analyze its influence on the sterilization process.

Materials and methods

Fifty endodontic files K #25 were selected for this study, regardless of their trademark. The samples were divided into two groups (n = 25) according to the method of analysis, prior use and performance of disinfection protocol, according to Table 1.

The endodontic instruments were obtained directly from students of the School of Dentistry of Pontifical Catholic University of Rio Grande do Sul

Table 1. Distribution of samples in groups.

Group	Method	N	Prior use	Clean	Sterilization
G1	SEM	25	Yes	Yes	Yes
G2	Culture	25	Yes	Yes	Yes

(PUC-RS, Porto Alegre, Brazil). The cleaning protocol used consisted of brushing with chlorhexidine gluconate 2% (Globomedia, Sacomã, SP, Brazil), washing in water and drying. Prior to analysis, samples were placed in plastic Eppendorf tubes (Eppendorf AG, São Paulo, Brazil) and sterilized by autoclaving (Dabi Atlante, Ribeirão Preto, Brazil), for 30 minutes at a temperature of 120°C.

Analysis in Scanning Electron Microscopy

The first group of endodontic files was removed from the Eppendorf plastic tubes with clinical tweezers and manipulated only by the cable, avoiding any contact of the active part of the instrument. The cables of instruments were removed through a wire cutter and its metal rods, made by the blade and the intermediate portion, were fixed in stubs for further observation.

After this process, samples were taken to a scanning electron microscope. The initial portion of the active blade of each instrument was evaluated under magnification of 150 X and 15 kV, recording the images for each instrument.

The images were evaluated by four examiners previous calibrated by Kappa test for inter-examiner agreement. A numeric score was assigned for each image, representing its degree of dirt for each instrument: 1 = no residues in the file, 2 = file almost clean surface, i.e., with low residue, 3 = surface file with an average amount of waste, and 4 = the surface of the file with a large amount of waste.

The data were submitted to Kruskal-Wallis test, using the mode to qualitative assessment on a significance level of 1%.

Microbiological Analysis of Contamination of Files

All procedures were performed under strict aseptic conditions inside a laminar flow camera. Each endodontic file was removed from the Eppendorf plastic tube with a sterile tweezers and then introduced into

a glass tube containing BHI (Brain Heart Infusion, Himedia, Curitiba, PR, Brazil). Then it was removed and placed in a test tube containing thioglycolate broth (Himedia, Curitiba, PR, Brazil). As a negative control, two tubes of BHI liquid and thioglycolate were used. These tubes didn't receive samples. The positive control was performed by inoculating strains of periodontal pathogens from clinical specimens and isolates of *Enterococcus* spp. The tubes were incubated in a microbiological stove, in the presence of oxygen at 37°C for 72 hours. The presence of microorganisms was confirmed by observing turbidity of the liquid culture medium after 24, 48 and 72 hours. The negative samples were those which do not lead to change in the culture medium, whereas the positive samples were those that caused the turbidity of it.

To prove the sterility of files, after observing the presence or absence of turbidity in liquid media, was made the inoculation in solid medium. A 10µl aliquot of BHI was inoculated on the surface of the culture medium (agar plain), allowed to dry and incubated aerobically at 37°C. The same procedure was performed with sodium thioglycolate, but the plates were incubated in microaerophilic by the method of the candle flame.

Results

The results showed that in group 1 the endodontic files showed different degrees of dirt after performing the same cleaning protocol (Fig 1) providing, in most cases, a surface with large quantities of waste, represented by score 4 (Graph 1).

Moreover, the results didn't show presence of bacterial growth on the surface of endodontic files for 24, 48 and 72 hours after incubation, both in BHI and in thioglycolate medium, except the positive control where there was the presence of growth bacteria in all periods of compliance and in both culture media (Graphs 2 and 3).

Discussion

The endodontic instruments are used to remove the remnants of pulp tissue during the procedures of cleaning and shaping of the root canal system. These instruments are submitted by a cleaning process before sterilization to be reused, with the aim of removal of organic matter and waste tissue in the instruments.

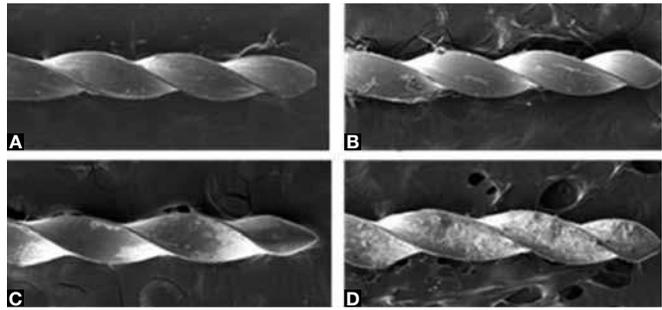
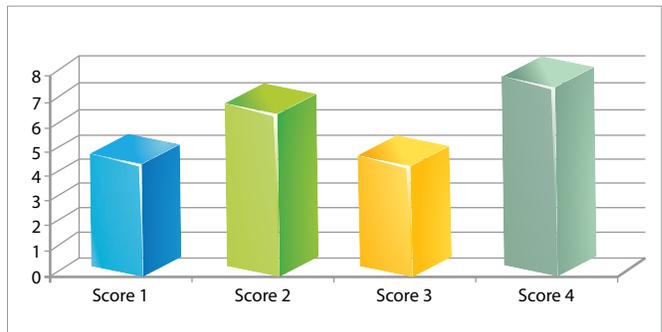
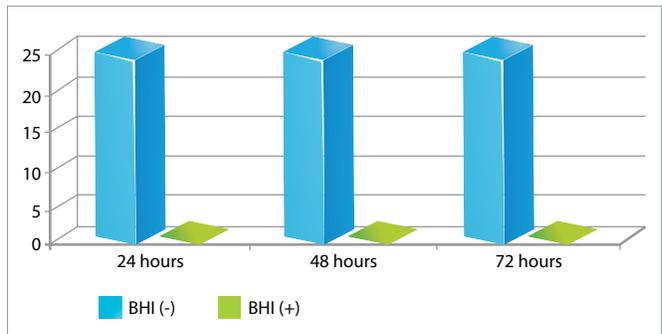


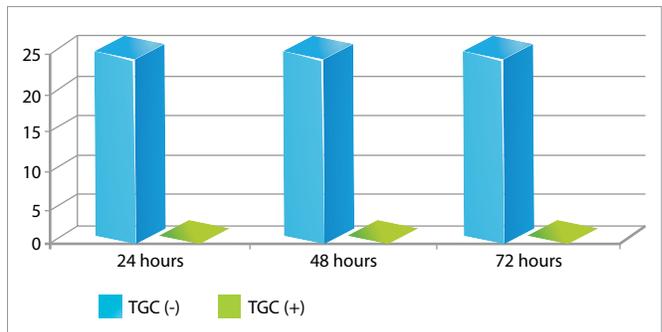
Figure 1. Scores for determining the amount of dirt on the surface of endodontic files: **A)** Score 1 - no residues in the file; **B)** Score 2 - file almost clean surface, i.e., presenting a small quantity of waste; **C)** Score 3 - surface of the file with an average amount of waste and, **D)** Score 4 - surface of the file with a large amount of waste.



Graph 1. Assessment of the degree of contamination of endodontic files.



Graph 2. Presence / absence of bacterial growth on the surface of endodontic files in culture media BHI/Time.



Graph 3. Presence / absence of bacterial growth on the surface of endodontic files in culture media Thioglycolate TGC/Time.

Several studies approach the cleaning techniques of endodontic files, including brushing, enzymatic cleaners and ultrasonic aid. However, these methods aren't able to clean completely the instrument, leaving it free of any residue, although the best results have been obtained by combining the resources of brushing and ultrasonic.^{2,8,9,10}

The ultrasonic cleaning has some advantages over the manual, such as higher cleaning efficiency; reduces the aerosolization of infectious particles released during the brushing; instruments with reduced incidence, increased cleaning, including removal of oxidation, better use of time and reduction of manual work.^{3,11,12,13}

The files collected for this study were subjected to cleaning by brushing performed by students of the School of Dentistry of Pontifical Catholic University of Rio Grande do Sul. SEM analysis demonstrated that 20% of files were included on the score 1, 28% in score 2, 20% in score 3 and 32% in score 4. This may be related to the fact that the feature was not used to perform ultrasonic cleaning of endodontic files, showing them a significant degree of dirt on their surfaces.

Previous study states that the presence of organic

matter and/or debris on the instruments may interfere with the sterilization process, because it creates barriers to protect the microorganisms, which may prevent the penetration of the sterilizing agent.³ However, these findings aren't in agreement with the findings in our study, where was shown that, despite the presence of dirt and organic matter on the surface of endodontic files, no bacterial growth was detected after the sterilization process of them. This can be explained by the efficient sterilization process that is able to reduce and eliminate all forms of microbial content present on the surfaces of endodontic instruments.

Results similar to our study were found by previous study which compared the microbiological conditions of files used by undergraduate students in six Schools of Dentistry of Rio Grande do Sul.¹⁴ The results showed that 53 samples were sterile of a total of 60 samples examined, whereas 7 were contaminated. The collected endodontic files obtained 100% of negative cultures only in two schools.

According to the limitations of this study, was concluded that despite a significant presence of dirt on the surface of endodontic files after cleaning, this factor doesn't influence the process of sterilizing them.

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