Efficacy of Different Techniques for Removing Debris from Endodontic Files Prior to Sterilization

Saeid Nosouhian¹, Farshad Bajoghli², Mahmoud Sabouhi³, Masoud Barati⁴, Amin Davoudi⁵, Maryam Sharifipour⁶

Contributors:
¹Assistant Professor, Department of Prosthodontics, Dental Implant Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran; ²Associate Professor, Department of Prosthodontics, Dental Implant Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran; ³Assistant Professor, Department of Endodontic, Private Practice, Isfahan, Iran; ⁴Dentistry Student, Dental Students Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran; ⁵Dentist, Torabinejad Dental Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Correspondence:
Dr. Sabouhi M. Department of Prosthodontics, Dental Implant Research Center, Isfahan University of Medical Sciences, Hezarjarib Street, Isfahan, Iran. Phone:+ 983137922838. Email: Sabouhi@dnt.mui.ac.ir

Introduction

Removing contaminations and irritate factors from the root canal are one of the basic goals in endodontic therapies. Using instruments, conveying remaining debris from other patients, not only does not help clinicians to eliminate the canals from contaminations, but also imposes new infections to patients.¹,² Prion proteins and their way of transmission have been focused in many studies previously.³,⁴ They are resistant to autoclaving, so dental pulps could be a source for contamination due to their nerves.⁵,⁷ As dental instruments such as matrix bands, burs, endodontic files and are hardly disinfected,⁶ trying to introduce a method to reach the optimum level of decontamination have been challenging recently.¹¹-¹⁵

Vadrat and Darbord conducted a study to evaluate the efficacy of different sterilizations and disinfections. Their results indicated that using autoclave and 1 N sodium hydroxide for 15 min reduced the rate of prion transmission.¹⁶

Linsuwanont et al. compared the effects of different cleaning methods in Ni-Ti rotary files. They used 180 endodontic files in four methods of removing debris (brushing, immersion in NaOCl 1% for 10 min after brushing, immersion in NaOCl 10% for 10 min, and ultrasonic for 5 min, combination of all previous techniques). Their result showed that combination of all techniques was more effective.¹⁷

In another study, Parashos et al. used 36 endodontic files and observed the efficacy of debris removing in three ways (chemical, mechanical, and ultrasonic). They recommended using scouring sponge soaked by chlorhexidine 0.2% and empower enzyme for removing debris.¹⁸

In 2009, Aasim et al. assessed the effect of pre-soaking files in an enzymatic cleaner prior to ultrasonic cleaning. They claimed that there were no benefit in pre-soaking files and the optimum time for ultrasonic cleaning was between 5 and 10 min.¹⁹

As removing debris prior to sterilization seems important and using enzyme and ultrasonic technique are recommended by many authors,¹⁸,²⁰-²² the aim of this study was to evaluate the efficacy of mechanical, chemical, and ultrasonic (in combinations or separately and step by step) method for removing debris from endodontic files prior to sterilization.

Materials and Methods:
Totally, 90 new endodontic files with used 36 endodontic files of 1 files of each one kept as negative control (NC) group. The rest of files (29 files of each size) were divided into five groups one files of each one as AC (Chemical), and BC (chemical and ultrasonic). Using Micro 10 enzyme with ultrasonic. Finally, the samples were observed in an enzymatic cleaner prior to ultrasonic cleaning. They claimed that there were no benefit in pre-soaking files and the optimum time for ultrasonic cleaning was between 5 and 10 min.¹⁹

Results:
Significant differences were observed in heads of the files among groups C and PC (P = 0.02), and high amount of debris were seen in the shafts of groups A and D (P < 0.001). The amount of remaining debris were significant in the shafts of sizes 15 (P < 0.001) and 25 (P = 0.01).

Conclusion:
Using Micro 10 in both ultrasonic and conventional methods were acceptable for removing debris from the files. Furthermore, higher amounts of debris were found in the shafts and heads of files with lower sizes (15 and 25).

Key Words: Contamination, debris, endodontic files, ultrasonic
Materials and Methods
In this observational-analytical study, 90 new endodontic files with size of 15, 25, and 40 (30 files of each one) (Dentsply Maillefer, Ballaigues, Switzerland) were sterilized and one files of each size assumed as negative control group (NC). The rest of the files (29 files of each size) were distributed among 29 dentistry students (Isfahan University of Medical Sciences, Dental School) randomly in the way that each student had one file of each size. After accomplishments of endodontic therapy by students, the files were collected and divided into following five groups:

Positive control (PC): One file of each size (3 files totally) were stored in autoclave machine for sterilization without any interventions.

Group A: Totally, 7 files of each size (meaning 21 files totally) were administered to scouring sponge, which were soaked in chlorhexidine 0.2% (Shahr Daru, Tehran, Iran), before subjecting in autoclave machine. The files were cleaned with the sponge by rotational movement in the way that all the flouts were involved.

Group B: Totally, 7 files of each size (21 files in total) were decontaminated like group A plus storing in Micro 10 enzyme (1/133) (Unident, Chêne-Bourg, Switzerland) in conventional way for 15 min before autoclaving.

Group C: Totally, 7 files of each size (meaning 21 files in total) were decontaminated like group A, but they were subjected to Micro 10 enzyme (1/133) in both conventional and ultrasonic (Mini Piezon, EMS Co., Domat, Switzerland) techniques for 15 min in each one before autoclaving.

Group D: Totally, 7 files of each size (21 files in total) were decontaminated like group A, but they were subjected to Micro 10 enzyme (1/133) only in ultrasonic way for 15 min before autoclaving.

After autoclaving, the samples were observed under a metallographic microscope (×75) (Metallographic Laboratory of Sanati Isfahan University). The length of each file presumed as two parts of head and shaft equally from the middle. The files rotated 90° clock-wisely for four times in which all the circumferential of the files surface were observed under the microscope. At the end, scoring was done from 0 to 9 based on remaining residual debris and each file got 8 numbers (scores) (Figures 1 and 2).

The collected data were analyzed by Tuckey, paired t-test, two-way, and one-way ANOVA tests using SPSS software version 15 at a significant level of 0.05.

Results
The results of two-way ANOVA test showed no significant difference in heads of the files among groups A, B, C, and D (P = 0.1). Furthermore, the differences among file sizes were not significant (P = 0.5).

A significant difference was found among different file sizes (P < 0.001). Furthermore, Tuckey test clarified significances between groups A and C (P = 0.03); and size of 15 with 25 (P = 0.02) and 40 (P < 0.001). Furthermore, the results showed a significant difference in shafts among groups A, B, C, and D (P = 0.02).

The results of observing entire length of the files revealed significant difference among groups A, B, C, and D (P = 0.02) and different file sizes (P < 0.001). Tuckey test showed significant difference between groups A and C (P = 0.001) and size of 15 with 25 (P = 0.03) and 40 (P = 0.001).

Comparing groups with NC and PC groups
The results of two-way ANOVA and Tukey test showed a significant difference in heads of the files among groups C and

Figure 1: The scores (0-4) based on a percentage of remaining debris under observation of metallographic microscope (×75).
Removing debris from endodontic files ... Barati M et al

The results showed a significant difference in shafts between groups PC with A, B, C, D, and NC ($P < 0.001$). In contrast, the differences among file sizes were not significant ($P > 0.05$).

The results of observing entire length of the files revealed a significant difference between groups PC with A, B, C, D, and NC ($P < 0.001$). The differences between groups A and C was significant too ($P = 0.04$). However, the differences among file sizes were not significant ($P > 0.05$).

The result of remaining debris by paired $t$-test (Table 1) showed a high amount of debris in the shafts, especially in groups A ($P < 0.001$) and D ($P < 0.001$). Furthermore, the amount of remaining debris (Table 2) were significant in the shafts of sizes 15 ($P < 0.001$) and 25 ($P = 0.01$).

Discussion

The present study tried out to evaluate both mechanical and chemical methods of removing debris from the endodontic file step by step and individually.

Group A: As the results showed, the shafts and the entire length of files represented significant differences in comparison with PC group, which indicates the positive efficacy of this method.

In Parashos et al.’s study, mechanical techniques for removing debris were compared. Scouring sponge, which were soaked in chlorhexidine 0.2%, showed the best results. In that study, the efficacy of each stage did not observe separately, and the results were reported in a combination of chemical and ultrasonic methods, but the present study tried to evaluate the efficacy, separately.18

In another study, Linsuwanont et al. compared both mechanical and chemical techniques. Their result showed that the files, which were cleaned by a nylon brush showed higher contamination than those cleaned ones with a combination of mechanical and chemical methods.17 The result of the present study confirmed insufficient mechanical removing of debris too.

Group B: In this group, both mechanical and chemical removing were used, and lower amount of remaining debris were observed in comparison with group A, but no significant difference was established. Furthermore, a lower amount of debris were found in larger sizes.

Table 1: The mean score of remaining debris between groups in heads and shafts.

| Groups | Heads | Shafts | Difference between heads and shafts | $P$-value |
|--------|-------|--------|-----------------------------------|-----------|
| A      | 1.83  | 2.87   | 1.03                              | 0.00      |
| B      | 1.88  | 2.14   | 0.26                              | 0.36      |
| C      | 1.44  | 1.76   | 0.32                              | 0.24      |
| D      | 1.70  | 2.67   | 0.97                              | 0.00      |
| PC     | 2.67  | 6.75   | 4.08                              | 0.05      |
| NC     | 1.41  | 2.66   | 1.25                              | 0.01      |

PC: Positive control, NC: Negative control

Table 2: The mean score of remaining debris between different file size in the heads and shafts.

| File size | Means in the heads | Means in the shafts | Mean difference between heads and shafts | $P$-value |
|-----------|--------------------|---------------------|-----------------------------------------|-----------|
| 15        | 1.85               | 3.13                | 1.28                                    | 0.00      |
| 25        | 1.65               | 2.30                | 0.65                                    | 0.01      |
| 40        | 1.71               | 2.12                | 0.41                                    | 0.81      |

PC: Positive control, NC: Negative control
Linsuwanont et al. stated higher impacts of debris removing with both mechanical and chemical materials were reported, too.

Parashos et al. claimed that empower enzyme is useful for removing debris. Another difference with that study is in the type of enzymes. In the present study, Micro 10 was used instead of Empower as it contains surfactant and anti-corrosion substances besides four enzymes for removing organic debris.

Group C: In this group ultrasonic was used besides chemical and mechanical techniques. The shafts and the entire length of files showed a significant difference compared to group A, which indicates the positive efficacy of this method.

Linsuwanont et al. reported a decrease of remaining debris in ultrasonic and immersion methods (together) in comparison to only immersion technique. In that study, brushing with immersion and ultrasonic showed 100% debris removal, but in the present study, the results did not reach to 100% success. That might be due to different observing methods with different accuracy (using metallography vs. staining).

Group D: In this group, ultrasonic with Micro 10 showed lower removing of debris than group C.

Two other studies compared ultrasonic with thermal disinfector and wash disinfector. Their results reflected the higher capability of ultrasonic than other methods. In the present study, PC group was defined to make more decisive comparisons. A significant difference was recognized between group C and PC in the files’ heads, which showed high efficacy method in group C.

In all the groups as the size increased the remaining debris decreased, which would be due to larger floats and easier removing of debris. Also, more amount of debris in the shafts might be due to encountering with tight canal space in the apical.

Conclusion
With limitations of in vitro studies, it can be concluded that using Micro 10, in both ultrasonic and conventional, was acceptable for removing debris from the floats of the endodontic files. Furthermore, the reaming debris was more in the shafts than head especially in files with lower sizes (15 and 25).

References
1. Morrison A, Conrod S. Dental burrs and endodontic files: Are routine sterilization procedures effective? Tex Dent J 2010;127(3):295-300.
2. Venkatasubramanian R, Jayanthi, Das UM, Bhatnagar S. Comparison of the effectiveness of sterilizing endodontic files by 4 different methods: An in vitro study. J Indian Soc Pedod Prev Dent 2010;28(1):2-5.
3. Walker JT, Dickinson J, Sutton JM, Raven ND, Marsh PD. Cleanability of dental instruments – Implications of residual protein and risks from Creutzfeldt-Jakob disease. Br Dent J 2007;203(7):395-401.
4. Smith A, Dickson M, Aitken J, Bagg J. Contaminated dental instruments. J Hosp Infect 2002;51(3):233-5.
5. Prusiner SB, McCarty M. Discovering DNA encodes heredity and prions are infectious proteins. Annu Rev Genet 2006;40:25-45.
6. Blanquet-Grossard F, Szadovitch V, Jean A, Deslys JP, Dormont D, Hauw JJ, et al. Prion protein is not detectable in dental pulp from patients with Creutzfeldt-Jakob disease. J Dent Res 2000;79(2):700.
7. Smith AJ, Bagg J, Ironside JW, Will RG, Scully C. Prions and the oral cavity. J Dent Res 2003;82(10):769-75.
8. Lowe AH, Bagg J, Burke FJ, MacKenzie D, McHugh S. A study of blood contamination of S iqveland matrix bands. Br Dent J 2002;192(1):43-5.
9. Reuber M, Al-Din AS, Baborie A, Chakraborty A. New variant Creutzfeldt-Jakob disease presenting with loss of taste and smell. J Neurol Neurosurg Psychiatry 2001;71(3):412-3.
10. Whitworth CL, Davies K, Palmer NO, Martin MV. An investigation of the decontamination of S iqveland matrix bands. Br Dent J 2007;202(4):E12.
11. Johnson MA, Primack PD, Loushine RJ, Craft DW. Cleaning of endodontic files, Part I: The effect of bioburden on the sterilization of endodontic files. J Endod 1997;23(1):32-4.
12. Popovic J, Gasic J, Zivkovic S, Petrovic A, Radicevic G. Evaluation of biological debris on endodontic instruments after cleaning and sterilization procedures. Int Endod J 2010;43(4):336-41.
13. Saghiri MA, Karamifar K, Mehrvazfar P, Asgar K, Gutmann JL, Lotfi M, et al. The efficacy of foam cleaners in removing debris from two endodontic instruments. Quintessence Int 2012;43(9):811-7.
14. Van Eldik DA, Zilm PS, Rogers AH, Marin PD. Microbiological evaluation of endodontic files after cleaning and steam sterilization procedures. Aust Dent J 2004;49(3):122-7.
15. Alexandrou G, Chrissafis K, Vasiliadis L, Pavlidou E, Polychroniadis EK. Effect of heat sterilization on surface characteristics and microstructure of Mani NRT rotary nickel-titanium instruments. Int Endod J 2006;39(10):770-8.
16. Vadrot C, Darbord JC. Quantitative evaluation of prion inactivation comparing steam sterilization and chemical sterlants: Proposed method for test standardization. J Hosp Infect 2006;64(2):143-8.
17. Linsuwanont P, Parashos P, Messer HH. Cleaning of rotary nickel-titanium endodontic instruments. Int Endod J 2004;37(1):19-28.
18. Parashos P, Linsuwanont P, Messer HH. A cleaning protocol for rotary nickel-titanium endodontic instruments. Aust Dent J 2004;49(1):20-7.
19. Aasim SA, Mellor AC, Qualtrough AJ. The effect of
pre-soaking and time in the ultrasonic cleaner on the cleanliness of sterilized endodontic files. Int Endod J 2006;39(2):143-9.

20. Filho MT, Leonardo MR, Bonifacio KC, Dametto FR, Silva AB. The use of ultrasound for cleaning the surface of stainless steel and nickel-titanium endodontic instruments. Int Endod J 2001;34(8):581-5.

21. Popovic J, Gasic J, Radicevic G. The investigation of ultrasound efficacy in cleaning the surface of new endodontic instruments. Srp Arh Celok Lek 2009;137(7-8):357-62.

22. Whitworth CL, Davies K, Palmer NO. Can protein contamination be removed from hand endodontic instruments? Prim Dent Care 2009;16(1):7-12.

23. Van Eldik DA, Zilm PS, Rogers AH, Marin PD. A SEM evaluation of debris removal from endodontic files after cleaning and steam sterilization procedures. Aust Dent J 2004;49(3):128-35.

24. Perakaki K, Mellor AC, Qualtrough AJ. Comparison of an ultrasonic cleaner and a washer disinfector in the cleaning of endodontic files. J Hosp Infect 2007;67(4):355-9.