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RESEARCH ARTICLE

INFLUENCE OF PRE-SOAKING AND DIFFERENT TIME INTERVALS IN ULTRASONIC CLEANER ON STERILIZATION OF ENDODONTIC FILES

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ABSTRACT

Endodontic treatment may directly involve contact with saliva, blood and infected pulp tissue. So, cross infection is the major issue in endodontic treatment. All instruments should be cleaned prior to sterilization using methods like pre-soaking and ultrasonic cleaning. Contaminated instruments are pre-soaked prior to ultrasonic cleaning. This study investigated the effect of pre-soaking of instruments prior to ultrasonic cleaning on sterilization. Endodontic files were divided into 10 groups. Group 1 to Group 4 includes pre-soaking and ultrasonic cleaning with different time intervals. Group 5 to Group 8 includes no pre-soaking but ultrasonic cleaning with same time intervals as above groups. Group 9 and 10 were control groups. Group 1-4 instruments were pre-soaked before ultrasonic cleaning. Then all instruments from Group 1-8 were subjected to ultrasonic cleaning. Then all instruments were sterilized using autoclave. Visualization and scoring of debris is done at tip and blade of the file. There was no significant difference with pre-soaking of instruments before ultrasonic cleaning but ultrasonic cleaning has significant effect on cleanliness of files with time intervals of 5-10 mins. There were no further improvement upto 1 hr. This was concluded that there is no improvement in cleanliness of instruments with pre-soaking and optimum ultrasonic cleaning time was 5-10 mins.

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INTRODUCTION

Infection control is a major issue in dentistry because of concern over communicable diseases being transmitted in health care settings. So, aseptic technique is especially important in endodontics because microorganisms are the major cause of endodontic infections.

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Although, root canal disinfection can be achieved through chemomechanical debridement and the use of intracanal medicaments, but use of sterilized endodontic files for mechanical debridement is must. Therefore, endodontic file sterilization is important for two reasons: Elimination of patient cross-contamination and for increased endodontic success. Furthermore, there is evidence that some instruments used in endodontics, e.g. files and reamers, are particularly difficult to clean, and may carry significant material residues after washing (Gill *et al.,* 2001). 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. All instruments should be mechanically cleaned prior to sterilization to remove adherent materials on the external surface. Ultrasonic cleaning is recommended because it reduces direct handling of contaminated instruments, decreases the chance of puncture injuries and has superior cleaning ability compared with other cleaning techniques (Murgel et al. 1990, Palenik 1993, Cafruny et al. 1995). Pre-soaking of instruments in a cleaner prior to ultrasonic cleaning has been shown to increase the effectiveness of cleaning (Sanchez & Macdonald 1995). There has been various recommendations regarding time for which dental instruments should remain in an ultrasonic bath for optimum cleaning (Miller & Hardwick 1988, Miller 1993, Palenik 1993).

The aim of the present study was

- To assess whether pre-soaking of the files in an cleaner prior to ultrasonic cleaning had any effect on cleanliness.
- To assess the effect of the time that endodontic files spend in an ultrasonic bath prior to sterilization on the overall cleanliness of the instruments.

MATERIALS AND METHODS

The present In - vitro study was undertaken in the Department of Conservative Dentistry and Endodontics, Genesis Institute of Dental Sciences and Research, Ferozepur, Punjab (INDIA). Teeth were selected which required root canal treatment based on the diagnosis criteria of AAE(American Association of Endodontics).Twenty root canals were treated by postgraduate endodontic students using sets of files. A fresh sterilized set of six K-Flex stainless steel files (size 15–40) was used for each root canal treated. A standard endodontic access cavity was prepared using a diamond bur in a high-speed handpiece. Conventional cleaning and shaping of the root canals was carried out using the crown-down technique. During instrumentation, the canals were copiously irrigated with 3 % sodium hypochlorite solution (Prevest DenPro Hyposol). All the used files were kept in a file holder. These file holders were kept in airtight plastic containers until they were subjected to the cleaning procedures.

Sample Groups

The files were divided into the following groups, with each group having twelve files (two sets of six files – No. 15–40):

Group 1: pre-soaking + 5 min of ultrasonic cleaning.
Group 2: pre-soaking + 10 min of ultrasonic cleaning.
Group 3: pre-soaking + 30 min of ultrasonic cleaning.
Group 4: pre-soaking + 60 min of ultrasonic cleaning.
Group 5: no pre-soaking + 5 min of ultrasonic cleaning.
Group 6: no pre-soaking + 10 min of ultrasonic cleaning.
Group 7: no pre-soaking + 30 min of ultrasonic cleaning.
Group 8: no pre-soaking + 60 min of ultrasonic cleaning.
Group 9: control; no pre-soaking + no ultrasonic cleaning.
Group 10: control; pre-soaking + no ultrasonic cleaning.

Pre-soaking of the files

Files in groups (1-4) were immediately pre-soaked in Cidex solution(Korsolex Rapid Instrument Sterilizer) for 5 min ; rinsed under tap water for 5 mins and then kept in air-tight container prior to ultrasonic cleaning on next day. Files in group 10 were also pre-soaked but then passed directly for autoclave sterilization. Group (5 -9) files were not pre-soaked.

Cidex solution

Endodontic files pre-soaked in solution

Ultrasonic cleaning of files

Each set of files of groups(1-8) were placed in ultrasonic cleaner for appropriate time (5,10,30,60 min).The solution used in ultrasonic cleaner is Chlor X (2%) (Pervest Denpro Chlor X) containing chlorhexidine digluconate, ethyl alcohol and water. The files were then rinsed under tap water for 10-15 secs and then placed in air-tight container prior to autoclaving next day.

Endodontic files pre-soaked in solution

Autoclave sterilization

The file stands were packed using porous autoclave paper that permits steam penetration to the instruments and the files were then subjected to a standard autoclaving procedure (121 °C for 15 min at 15 lb of pressure).

Visualization of debris

The sterilized instruments were visualized for any debris, blood or contaminants using stereomicroscope (64x). The examination was carried out in a clean and dust free environment to try to prevent contamination from dust particles in the air. Each file was rotated 360 degree before scoring. A computer was attached to microscope in order to save the pictures in the system. The whole length of the file was not visible under the stereomicroscope under 64x magnification. Therefore the cutting element of the file, which was 17 mm in length, was divided into two equal halves, the tip and the blade. Each half was photographed and scored for debris separately.

Debris scoring

The scale used to measure the amount of debris on the surface of the file was a modification of the scale used by Smith et al. (2002). Using the Smith et al. (2002) scale, a file that had only one or two specks of dentine debris present scored the same as a file that had 25% coverage of debris.

A scale of 0 to ++++ was therefore used, where:

0 = No debris on the surface of the file.
+ = 0–5% of the file contaminated with visible debris.
++= 6–15% of the file contaminated with visible debris.
+++ = 16–25% of the file contaminated with visible debris.
++++ = >25% of the file contaminated with visible debris.

The scoring was blinded by a colleague handing the files to the scorer (SAA) in a random manner without revealing the identity of the group to which each file belonged. The computer attached to the microscope was used to cross check the scores recorded by the microscope. A random sample of files was re-examined a second time to check intra-examiner reliability.

Statistical analysis: The debris data was analysed using one-way analysis of variance.

RESULTS

The debris scores were normally calculated at both the tip and the blade of the file.

Significance of pre-soaking phase

An analysis of variance was performed using a univariate test for debris scores at the tip and the blade of the files to discover any significant difference between pre-soaking and no pre-soaking of the files before ultrasonic cleaning. The results showed that there was no statistically significant difference between the debris scores on files that had been pre-soaked or not pre-soaked before ultrasonic cleaning as P values at the tip and blade both was not significant(P=0.92 at the tip and 0.95 at the shaft). As there was no significant difference with presoaking of the instruments before ultrasonic cleaning, the data for the pre-soaked and the non pre-soaked instruments were combined together for the purpose of further statistical tests. The new groups were recoded as below:

Group A: group 1 (pre-soak + ultrasonic cleaning for 5 min) and group 5 (no pre-soak + ultrasonic cleaning for 5 min), 24 files
Group B: group 2 (pre-soak + ultrasonic cleaning for 10 min) and group 6 (no pre-soak + ultrasonic cleaning for 10 min), 24 files.
Group C: group 3 (pre-soak + ultrasonic cleaning for 30 min) and group 7 (no pre-soak + ultrasonic cleaning for 30 min), 24 files.
Group D: group 4 (pre-soak + ultrasonic cleaning for 60 min) and group 8 (no pre-soak + ultrasonic cleaning for 60 min), 24 files.
Group E: group 9 (no pre-soak + no ultrasonic cleaning) and group 10 (pre-soak + no ultrasonic cleaning), 24 files.

Significance of ultrasonic cleaning

The debris scores for the files that had been ultrasonically cleaned (groups A–D) were compared with those files that had not been ultrasonically cleaned (group E). The results are shown in (Table 1) and show a highly significant statistical difference (P = 0.000 at the tip and at the blade) with ultrasonically cleaned files having low debris. These results demonstrate the benefit of using an ultrasonic cleaner to remove debris.

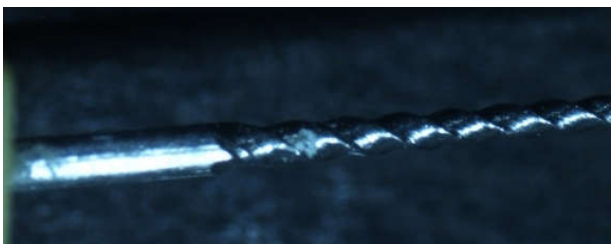
Table 1.

Ultrasonic cleaning	df	Mean Square	F	Significance
Tip	1	607.500	186.376	0.000
Blade	1	589.633	77.972	0.000

Comparison of debris scores at the tip and the blade

The mean and standard deviation of debris scores at the tip and the blade of the files were calculated (Table 2). Mean debris score at tip was 2.375 ± 2.887 and at blade it was 4.8167 ± 3.529 (for all groups combined) which shows that blade had significantly higher mean debris score than tip ($p < 0.001$).

Debris at tip



Debris at blade

Significance of different exposure time in ultrasonic cleaner

The data was subjected to one-way analysis of variance to compare the debris scores in the different groups for the different times in the ultrasonic cleaner (Table 3).The results showed a highly significant difference ($P < 0.000$).

For each group the mean debris score was calculated to detect the most effective method of cleaning. Using a post hoc Bonferroni multiple-comparison test applied to the Anova table, it was possible to identify which pairs of means differed. This is illustrated in Table 4 for the tip of the files and Table 5 for the blade. One-way analysis of variance was performed to compare the effect of the different ultrasonic cleaning times on the different file sizes but no significant difference was found ($P > 0.05$).

Table 2.

Group (cleaning time)	Tip			Shaft		
	N	Mean	SD	N	Mean	SD
5 min	24	1.7083	1.19707	24	4.0000	2.46718
10 min	24	1.3333	1.16718	24	4.4583	2.81269
30 min	24	0.7083	0.75060	24	1.9167	1.21285
60 min	24	1.2500	1.29380	24	4.4583	1.97768
No cleaning	24	6.8750	3.34030	24	9.2500	3.84764

Table 3. One-Way Anova

		Sum of Squares	df	Mean Square	F	Sig.
Tip	Between Groups	619.750	4	154.938	47.849	0.000
	Within Groups	372.375	115	3.238		
	Total	992.125	119			
Shaft	Between Groups	695.717	4	173.929	25.440	0.000
	Within Groups	786.250	115	6.837		
	Total	1481.967	119			

Table 4. Post HOC test at Tip

		Mean Difference	Standard Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
5 min	10 min	0.37500	0.51946	1.000	-1.1118	1.8618
	30 min	1.00000	0.51946	0.567	-.4868	2.4868
	60 min	0.45833	0.51946	1.000	-1.0285	1.9451
	No cleaning	-5.16667*	0.51946	0.000	-6.6535	-3.6799
10 min	5 min	-.37500	0.51946	1.000	-1.8618	1.1118
	30 min	0.62500	0.51946	1.000	-.8618	2.1118
	60 min	0.08333	0.51946	1.000	-1.4035	1.5701
	No cleaning	-5.54167*	0.51946	0.000	-7.0285	-4.0549
30 min	5 min	-1.00000	0.51946	0.567	-2.4868	.4868
	10 min	-0.62500	0.51946	1.000	-2.1118	.8618
	60 min	-0.54167	0.51946	1.000	-2.0285	.9451
	No cleaning	-6.16667*	0.51946	0.000	-7.6535	-4.6799
60 min	5 min	-0.45833	0.51946	1.000	-1.9451	1.0285
	10 min	-0.08333	0.51946	1.000	-1.5701	1.4035
	30 min	0.54167	0.51946	1.000	-.9451	2.0285
	No cleaning	-5.62500*	0.51946	0.000	-7.1118	-4.1382
No cleaning	5 min	5.16667*	0.51946	0.000	3.6799	6.6535
	10 min	5.54167*	0.51946	0.000	4.0549	7.0285
	30 min	6.16667*	0.51946	0.000	4.6799	7.6535
	60 min	5.62500*	0.51946	0.000	4.1382	7.1118

Table 5. Post hoc test at blade

		Mean Difference	Standard Error	Significance	95% Confidence Interval	
					Lower Bound	Upper Bound
5 min	10 min	-0.45833	0.75482	1.000	-2.6188	1.7021
	30 min	2.08333	0.75482	0.067	-0.0771	4.2438
	60 min	-0.45833	0.75482	1.000	-2.6188	1.7021
	No cleaning	-5.25000	0.75482	0.000	-7.4104	-3.0896
10 min	5 min	0.45833	0.75482	1.000	-1.7021	2.6188
	30 min	2.54167	0.75482	0.010	0.3812	4.7021
	60 min	0.00000	0.75482	1.000	-2.1604	2.1604
	No cleaning	-4.79167	0.75482	0.000	-6.9521	-2.6312
30 min	5 min	-2.08333	0.75482	0.067	-4.2438	0.0771
	10 min	-2.54167	0.75482	0.010	-4.7021	-0.3812
	60 min	-2.54167	0.75482	0.010	-4.7021	-0.3812
	No cleaning	-7.33333	0.75482	0.000	-9.4938	-5.1729
60 min	5 min	0.45833	0.75482	1.000	-1.7021	2.6188
	10 min	0.00000	0.75482	1.000	-2.1604	2.1604
	30 min	2.54167	0.75482	0.010	0.3812	4.7021
	No cleaning	-4.79167	0.75482	0.000	-6.9521	-2.6312
No cleaning	5 min	5.25000	0.75482	0.000	3.0896	7.4104
	10 min	4.79167	0.75482	0.000	2.6312	6.9521
	30 min	7.33333	0.75482	0.000	5.1729	9.4938
	60 min	4.79167	0.75482	0.000	2.6312	6.9521

DISCUSSION

Numerous studies have recommended cleaning instruments before sterilization, to minimize the risk of cross-infection. The current concern over the risk of iatrogenic transmission of prion diseases has contributed to the view that consideration should be given to treating endodontic instruments as single use, but because of cost implications this has not been routinely followed. A recent study has shown that complete removal of organic debris from rotary nickel-titanium endodontic files is possible using a combination of cleaning procedures (moist storage, brushing followed by immersion in 1% sodium hypochlorite, ultrasonic cleaning) but this requires a meticulous technique (Linsuwanont *et al.,* 2004). This study has shown that the pre-soaking of files prior to ultrasonic cleaning does not produce any beneficial effect. This may be because of the cleaners used in ultrasonic cleaning has effects of both pre-soaking agent and cleaner. According to this study,time spent by instruments in ultrasonic cleaner is not proportional to degree of cleanliness. Significant difference is seen only in first 5-10 minutes, further there was no improvement upto 60 mins. So, preferable time for ultrasonic cleaning is 10 mins maximum. Tip and the blade of file were visualized separately because magnification was used and it was not possible to visualize in one view. The control files of group 9 and 10 has almost same number of debris on tip and blade but other files in all groups show more debris on blade than tip.This may be due to ultrasonic cavitation effect with greater movement of tip in ultrasonic cleaning. The cleaned instruments were sterilized in an autoclave with a standard sterilization procedure of 121° C for 15 min. This has shown to kill most microbes, including spores, and is most common method of instrument sterilization (Van Eldik *et al.,* 2004).

Conclusion

Pre-soaking prior to sterilization has no effect on cleanliness of files**.** Cleaners used in ultrasonic cleaning appears to play dual role as cleaner and detergent. The optimum ultrasonic cleaning time is between 5 to 10 min. Presence of debris even after 1 hour of ultrasonic cleaning supports the case of single use only.

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