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Evaluation of Cleaning Protocols on Endodontic Instruments

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Abstract: To evaluate an effective cleaning protocol for endodontic instruments. Total 180 new endodontic k-files (sizes 15, 25 and 40) were contaminated by preparing canals of extracted teeth. The practices used different cleaning protocols for the instruments. These protocols involved four components the use of sponges soaked with chlorhexidine, brushing, pre-soaking in MICRO 10 ENZYME and ultrasonication. After cleaning, the files were immersed in Van Gieson's solution and examined under magnification for stained debris. The effectiveness of cleaning sequences was tested on the instruments. The mean percentage of highest contamination score was 35% in sponge soaked with chlorhexidine gluconate group, 15% in the brushing only group, 5% in MICRO 10 ENZYME soak plus brushing group, 0% in MICRO 10 ENZYME soak and brushing and ultrasonication group and 4% in ultrasonicatin only group. There were no clean instruments in these groups except group 4. There was a statistically significant difference between the full cleaning method (group 4) and other groups ($p<0.05$). Statistically, size of instruments did not significance influence on debris removal. The best method for the efficient debris removal was the one that included mechanical, chemical and ultrasonic cleaning of instruments.

Key words: Cleaning protocol, endodontic instruments, biological debris, sponge, chemical

INTRODUCTION

Cleaning of instruments to remove organic residue is an important step before sterility of instruments (Miller and Sheldrake, 1991; Parker and Johnson, 1995). Endodontic instruments may directly contact with saliva blood and infected pulp tissue and these fluids should be considered as potentially infectious. Root canals are considered as the critical sites and instruments used in root canal treatment should be cleaned and sterilized at the time of used, even before the first use (USPHS, 1988; Martins *et al*., 2002). Presence of debris on the surface of instruments can influence the effectiveness of the sterilization process (Muscarella, 1998). Organic debris may prevent a disinfectant or sterilant from contacting the instrument being processed and may also bind and inactivate chemical disinfectants (Muscarella, 1998). The cleaning of instruments to remove micro-organisms and biological debris effectively eliminates the majority of micro-organisms (Rutala *et al*., 1998; Alvarado, 1999; Chu *et al*., 1999). Instruments must be pre-cleaned to remove organic debris before sterilization in order to prevent continuing viability of pathogens (Miller and Sheldrake, 1991; Parker and Johnson, 1995).

Debris adherence prevents complete autoclave steam penetrations into the instruments decreasing antibacterial efficacy of the chemical solutions and inactivating their antibacterial molecules. They also increase bacteria and spores resistance against the heat due to the dried protein layer of the organic material (Alexandrou *et al*., 2006; Morrison and Conrod, 2010). Endodontic files have no internal surfaces, therefore it would be expected that a cleaning protocol could be developed that results in files free of organic debris.

Different methods have been developed to clean endodontic instruments. Cleaning by wiping endodontic instruments with gauze during use is recommended but a large amount of debris still remained on the instruments after cleaning (Segall *et al*., 1977). Brushing is generally used but because of some disadvantages, the use of sponge embedded into a disinfectant or a detergent has been recommended (Linsuwanont *et al*., 2004). Effectiveness of various mechanical cleaning methods, such as gauze soaked with alcohol, a sponge soaked with alcohol an ultrasonic bath were investigated and reported none of these methods was able to clean the instruments totally (Murgel *et al*., 1990). Other investigations reported that ultrasonic cleaning was an ineffective method to totally remove debris from rotary NiTi instruments (Marending *et al*., 1998; Eggert *et al*., 1999). To date, combination of mechanical and chemical cleaning procedure has been developed (Parashos *et al*., 2004). The purpose of the present study was to evaluate cleaning efficacy of different debris removal techniques from the endodontic hand files before sterilization.

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MATERIALS AND METHODS

Total 210 new endodontic k-files (sizes 15, 25, 40 and 25 mm, Mani, Tochigi, Japan) were used for this experimental research. Before the cleaning procedures, instruments were used under simulated clinical conditions to prepare canals of extracted human molars and premolars teeth until debris was easily visible on the files with the naked eye in order to produce a build-up of organic material on the instruments.

The debris-laden files were used in experiments to determine feasible cleaning protocols. The protocols involved different methods of mechanical and chemical removal of the root canal debris.

Experimental groups

Positive control group: Total 10 new instruments of each size (15, 25 and 40) were contaminated by using them to prepare canals of extracted teeth and then stained without any cleaning procedure.

Negative control group: Total 10 new instruments of each size that were not contaminated. Total 150 new instruments were used to prepare canals of extracted teeth.

After visual debris was noted, all instruments were inserted into a sponge soaked in 0.2% chlorhexidine gluconate aqueous solution (Shahre Daru Co. Ltd., Tehran, Iran) for 30 min. The instruments were randomly assigned into five equal groups representing five different cleaning procedures.

Group 1: Total 30 files of total instruments that described above (inserted into a sponge soaked in 0.2% chlorhexidine gluconate aqueous solution for 30 min) were placed in this group.

Group 2: Total 30 files were brushed for 20 strokes per row with a tube nylon bristle brush (Abzarantools, Tehran, Iran) under running distilled water.

Group 3: Total 30 files were placed in a soaking container at 2% MICRO 10 ENZYME concentration (Unident, Chene Bourg, Geneve, Switzerland), leave for 15 min and brushed as in group 2 and rinsed under running distilled water.

Group 4: The instruments were immersed in a soaking container at 2% micro 10 enzyme concentration for 15 min and brushing the files then, placed into ultrasonic bath in the same solution for 15 min and rinsed.

Group 5: Without soaking in micro 10 enzyme and brushing, the files were only placed into ultrasonic bath in 2% micro 10 enzyme concentration for 15 min and rinsed.

Scoring system: After the cleaning procedure, instruments were air-dried and immersed in Van Gieson's solution (Electron Microscopy Sciences, Hatfield, Pennsylvania, United States) for 3 min, rinsed in running tap water for 30 sec and allowed to air dry on the endodontic stand. Instruments were then scored for debris at ×75 magnification using a metallographic microscope (Kemet International Ltd., Maidstone, Kent, UK).

To resist movement of the instruments during examination, a specially designed holder was used. The files were placed into a hollow rectangular block, square in cross section with an insert of rubber impression material to accept the instrument handle. Scoring of the files involved recording the presence of red or orange stained material of unstained material or of totally clean files. Each instrument was examined for debris at two levels: Apical and coronal. At each level, the instruments were examined on four sides by sequentially rotating the block through 90°. The entire flute surface of each file was scored. Any stained material anywhere on the file led to a rating of dirty. Both totally-clean files and files with slight non-stained debris were considered clean. The category and extent of stained debris were recorded using the criteria is shown in Table 1. Only one category of debris was assigned to each site examined.

Each file was assessed at 8 sites. A rank score was given to each site depending on the extent of biological contamination (Table 1). The scores from all sites on each file were summed. The minimum score for each file was 0 (no stained material present) and the maximum score was 24 (all surfaces heavily contaminated with particulate debris). The mean score for each file was calculated and then converted into the mean percentage to compare with the highest biological debris score.

Table 1: Scoring system for debris on instruments

Category of debris	Instruments
Dirty	Stained particulate debris: Particulate matter stained red or orange
Clean	Unstained particulate debris: Fine particles that did not exhibit any red/orange coloration (without debris)
Extent of stained debris	
0	None
1	Slight: Scattered particles spaced widely apart on the flute surface
2	Moderate: Numerous particles with areas of continuous coverage of surfaces
3	Heavy: Areas where the flutes were packed with debris to their entire depth

Statistical analysis was carried out using the one way ANOVA and student's unpaired t-test (SPSS 21.0 statistical software; SPSS Inc, Chicago, IL, USA); to comparing different groups and instrument sizes (15, 25 and 40) in each group. A p-value of 0.05 was considered to be statistically significant.

RESULTS

All 30 new files of negative control group were clean except 1 that was slightly contaminated with organic material. In positive control group, instrumentation of root canals of extracted teeth resulted in considerable accumulation of debris on the instruments and all instruments contained organic material. The mean percentage of highest contamination score for each group was summarizing in Table 2.

Comparison of different cleaning procedures indicates that all methods in cleaning were partially effective in removing debris.

In group 1, inserting instruments into a sponge soaked with 0.2% chlorhexidine gluconate resulted in a significantly lower debris scores (mean percentage = 35%; $p< 0.05$; Table 2).

Mechanical removal by brushing alone reduced the level of debris remaining on the instruments. Mean percentage of highest contamination score was lower in the brushing only group compared to sponge of C.H.X group (15% compared to 35%). Brushing alone did not eliminate all of organic materials.

By soaking in micro 10 enzyme and brushing (group 3) or placing into ultrasonic bath in 2% micro 10 enzyme concentration (group 5) organic materials was successfully removed (5% compared to 4%). With the sequence of combined mechanical and chemical removal (soaking at 2% micro 10 enzyme and brushing then placed into ultrasonic bath in the same solution) all organic materials was totally removed (0.0%). There was a statistically significant difference between the full cleaning method (group 4) and other groups ($p<0.05$).

Statistically, size of instruments did not significance influence on debris removal ($p>0.05$; one way ANOVA).

Table 2: Effectiveness of biological debris removal and extent of stained debris

Groups	n	No. clean	Mean % of HCS[a]	SE[b]
Positive control	30	0	60.0	0.5
Negative control	30	29	0.4	0.3
1 (G_1)	30	0	35.0	0.4
2 (G_2)	30	0	15.0	0.3
3 (G_3)	30	22	5.0	0.3
4 (G_4)	30	30	0.0	0.0
5 (G_5)	30	23	4.0	0.4

[a]HCS = Highest contamination score; [b]SE = Standard Error of the mean; F-tes = $p<0.05$; Pair comparison: G_1 vs. G_2, $p<0.05$; G_2 versus G_3 $p<0.05$; G_2 versus G_4, $p<0.05$; G_3 versus G_5; $p>0.05$

DISCUSSION

Muscarella (1998) reported that heat sterilization is completely effective in killing bacteria and viruses on dental instruments even in the presence of organic debris. Unlike heat, low temperature sterilization requires direct contact between the sterilant and instrument for effective sterilization. Ideally, all organic materials should be removed by cleaning before sterilization to minimize risk (Linsuwanont *et al*., 2004). Due to safety of multiple used files, it is extremely important to consider that highly specific cross-infection control measures in dentistry are required for patients with or at notable risk of prion diseases (Porter *et al*., 2000; Porter, 2002).

This study attempted to detect the presence of biological material anywhere on the flutes of three sizes of instruments as well as evaluating the effectiveness of different techniques to produce endodontic files that were microscopically free of stained (biological) debris.

For identification of debris, Van Gieson's stain was used in this study. It is an easy single-step technique to perform and is able to stain strongly a wide range of organic materials which are considered to be potential biological risk factors.

Murgel *et al*. (1990) reported that files could not be totally cleaned with sponge, gauze or ultrasonics. However, these researchers used only two thrusts into the sponge and the ultrasonic time was for just 5 min. Other studies demonstrate that efficacy of ultrasonic and washing disinfectors were 98.33 and 88.57%, respectively (Van Eldik *et al*., 2004a, b).

The present study clearly indicates that both the mechanical and chemical aspects of the cleaning protocol must be applied in order to proper cleaning to occur. It has been previously established that debris and micro-organisms can be reduced by a cleaning protocol that includes a mechanical action (Hubbard *et al*., 1975; Murgel *et al*., 1990; Marsicovetere *et al*., 1996) and ultrasonic agitation of the instruments in a solvent solution (Cafruny *et al*., 1995; Zmener and Speilberg, 1995; Burkhart and Crawford, 1997). The present study has confirmed these previous findings. The use of a sponge implies that all sides of the instrument are likely to be contacted by the sponge simultaneously. A sponge soaked with 0.2% chlorhexidine gluconate reduced the mean percentage of highest contamination score from 60-35%. The purpose of the sponge is not only the physical cleaning action but also to keep the files and remaining debris moist which is an important aspect of instrument cleaning (Miller, 2002).

The use of nylon bristle brushes and metal bur brushes to clean endodontic instruments is a common and

long-used method. However in one study shown that brushing was not a very successful procedure (Parashos *et al*., 2004). In this study, brushing alone removed a significant amount of debris but could not clean the instruments. Brushing reduced the mean percentage of highest contamination score from 35-15%. The procedure of brushing in the large files (40) resulted in better cleanliness than small files (15, 25) whereas it was insignificant ($p>0.05$). Pre-soaking before ultrasonication has been shown to be an important step in the cleaning process (Sanchez and Macdonald, 1995; Miller, 2002).

Hypochlorite effectively dissolves pulp tissue (Hand *et al*., 1978) but the possibility of corrosion damage to the instruments is a concern. The present investigation was used micro 10 enzyme that is composed of a complex triple enzyme combination which is non corrosive (Unident, 2012).

Filho *et al*. (2001) recommended the ultrasonic bath as the most effective method to remove foreign particles from the surface of the instruments. The present study showed that ultrasonic use is an important step in instrument cleaning and this is consistent with other studies (Cafruny *et al*., 1995; Burkhart and Crawford, 1997; Miller, 2002).

The literatures recommends 6-10 min in special solutions (24-26) but as suggested by Parashos *et al.* (2004), the 15 min of ultrasonication was suitable. However, neither study could demonstrate any totally clean instrument after ultrasonic cleaning with a few sparsely distributed particles remaining on the flutes (19, 20, 24-26). The result from the present study confirm that the use of an ultrasonic bath combined with soaking at 2% micro 10 enzyme and brushing was needed for removal all organic materials and combined use of 2% micro 10 enzyme and brushing or ultrasonic bath in 2% micro 10 enzyme concentration for 15 min could not completely remove organic material from the instruments.

CONCLUSION

These results emphasize the necessity of all steps (chemical, mechanical and mechanical agitation) for removal of organic materials.

The method for decontaminating instruments that are routinely applied in dental practices are ganarally ineffective in removing biological debris. The initial cleaning with a scouring sponge is important and it is simple and quick to perform for the clinician and the assistan. Also, the subsequent pre-soaking, brushing and ultrasonication are very important stages. Hence, it is recommended that this technique shold be used in routine dental practice.

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