Acta Odontologica Scandinavica, 2013; 71: 1261–1266

informa
healthcare

ORIGINAL ARTICLE

A comparison of residual smear layer and erosion following different endodontic irrigation protocols tested under clinical and laboratory conditions

ZAFER C. CEHRELI[1], M. OZGUR UYANIK[2], EMRE NAGAS[2], BEHRAM TUNCEL[2], NURAY ER[3] & FUGEN DAGLI COMERT[2]

[1]*Department of Pediatric Dentistry,* [2]*Department of Endodontics, and* [3]*Department of Oral Sugery, Faculty of Dentistry, Hacettepe University, Ankara, Turkey*

Abstract

Objective. To compare the smear layer removal efficacy and erosive effects of different irrigation protocols under clinical and laboratory conditions. ***Materials and methods.*** Mandibular third molars (n = 32) of 30–45 year-old patients were instrumented with rotary files and were randomly assigned to one of the following groups for final irrigation: (1) 5.25% NaOCl; (2) 17% EDTA; and (3) BioPure MTAD. Thereafter, the teeth were immediately extracted and processed for micromorphological investigation. *In vitro* specimen pairs were prepared by repeating the clinical experiments on freshly-extracted mandibular third molars. To compare open and closed systems, laboratory experiments were repeated on 32 additional teeth with enlarged apical foramen. The cleanliness of the root canals and the extent of erosion were assessed by environmental scanning electron microscopy. ***Results.*** Specimens prepared under clinical and laboratory conditions had similar cleanliness and erosion scores ($p > 0.05$). Under both conditions, the tested solutions were more effective in removing the smear layer in the coronal and middle regions than in the apical one. Comparison of closed and open systems showed similar levels of cleanliness and erosion in all regions ($p > 0.05$), with the exception of 17% EDTA showing significantly higher levels of cleanliness and erosion in the apical third of open-end specimens. ***Conclusions.*** Based on clinical correlates of *in vitro* root canal cleanliness and erosion, laboratory testing of root canal irrigants on extracted teeth with closed apices can serve as a reliable method to simulate the clinical condition. EDTA was the most effective final irrigation solution in removing the smear layer at the expense of yielding the greatest erosive effect.

Key Words: *human experimentation, root canal irrigants, scanning electron microscopy, tooth apex, vapor pressure*

Introduction

The success of root canal treatment depends largely on the root canal system being thoroughly cleansed and disinfected, followed by complete obturation of the prepared canal space with a biocompatible and inert material. Since the first description of the smear layer in instrumented root canals [1], accumulating evidence has demonstrated the importance of smear layer removal, which results in a more thorough disinfection of the root canal system and the dentinal tubules that would ensure a better adaptation between the obturation materials and the root canal dentin [2].

Chemomechanical preparation has been described as the removal of micro-organisms, tissue remnants and dentin chips from the root canal systems [3]. Current concepts of chemomechanical preparation imply that chemicals should be applied on instrumented root canal surfaces so as to remove the smear layer [4,5]. Complete removal of the smear layer requires the use of irrigation solutions that can dissolve both the organic and inorganic components of the smear layer. Since no single solution is known to provide both effects alone [6,7], the use of chelating agents and/or acids followed by tissue solvents has been advocated. Consequently, the alternating use of ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) solutions has gained wide acceptance as an effective irrigation regimen [5,7–9]. BioPure MTAD (Dentsply Tulsa, Tulsa, OK), an

Correspondence: Zafer C. Cehreli, DDS, PhD, Department of Pediatric Dentistry, Faculty of Dentistry, Hacettepe University, Ankara, Turkey. Fax: +0090 312 3243190. E-mail: zcehreli@gmail.com

(Received 5 July 2012; accepted 1 November 2012)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2012.757647

aqueous solution of doxycycline, citric acid and polysorbate 80 detergent, has been recommended as a final irrigation solution with antibacterial properties [10].

To date, endodontic irrigants have been tested on intact or sectioned root canals of extracted human teeth [11–13]. While *in vitro* studies are relatively simple and may provide faster results and closer control of the variables involved in the irrigation protocols than clinical studies, the question as to whether these laboratory outcomes are somehow related or can be predictive of clinical performance remains dubious. No previous study has validated the efficacy of root canal irrigants by comparing them under both clinical and laboratory conditions. Consequently, the aim of this study was to investigate and compare the smear layer removal ability and erosive effects of different endodontic irrigation regimens under *in situ* and *in vitro* conditions. The null hypothesis tested was that clinical and laboratory test conditions have no significant effect on the cleaning efficiency and erosive potential of different irrigation protocols.

Materials and methods

A parallel clinical and laboratory design was used to facilitate comparisons. The clinical research protocol, the laboratory study protocol including the use of extracted human teeth and the consent form were approved by the Ethics Committee for Research on Human Subjects.

Clinical procedures

Healthy, 30–45 year-old volunteer patients participated in the clinical part of this study. The inclusion criteria for the selection of patients were: (1) The presence of one fully-erupted, intact, non-functional mandibular third molar scheduled for extraction; (2) Each selected molar exhibiting roots with a degree of curvature less than 20° as determined radiographically by the method described by Schneider [14]; and (3) Informed consent from the patients for endodontic procedures, followed by retrieval of extracted teeth for research. Thirty-two patients were selected in accordance with the inclusion criteria.

Following administration of local anesthesia, endodontic access cavities were prepared under rubber dam isolation. The working length was established using an apex locator (Apex pointer, MicroMega, Besançon, France) and confirmed by radiograph. New ProTaper rotary files (Dentsply Tulsa Dental Products, Tulsa, OK) were used for root canal preparation, utilizing ProTaper F3 as the final apical file. The root canals were irrigated with 2 ml 5.25% NaOCl between each file size. Thereafter, the teeth were randomly assigned to one of the following groups (n = 10/group) with respect to the solution used as a final irrigant: (1) 5.25% NaOCl; (2) 17% EDTA; and (3) BioPure MTAD. The test solutions (5 ml each) were delivered via 27-gauge needles which penetrated to within 2 mm of the working length [15]. In all groups, the root canals received a final rinse with 5 ml sterile distilled water and were dried with sterile paper points. The remaining two teeth were utilized as controls in which sterile distilled water was used as an irrigant. Following irrigation procedures, a sterile cotton pellet was placed in the pulp chamber and the access was sealed with Cavit (3M ESPE, St Paul, MN). The rubber dam was removed and the teeth were immediately extracted and processed for scanning electron microscopic investigation.

Laboratory procedures

In vitro specimen pairs (n = 32) were prepared by repeating the clinical procedures on freshly-extracted human mandibular third molars of similar patient age and tooth morphology. In an attempt to simulate the periradicular environment, the root segments were inserted into moistened floral foams [16] and were kept therein during chemomechanical preparation and irrigation. Drying of the root canals and sealing of the access cavity were performed as with the *in situ* specimens.

To investigate whether vapor lock has an impact on the results, additional extracted teeth (n = 32) were selected to serve as 'open system' specimens. The apical foramens were enlarged by establishing apical patency to a #30 file [17], after which a small plastic tube was attached to the external root surface with epoxy resin to permit unrestricted fluid extrusion during canal preparation and irrigation [18]. The remaining experimental procedures were performed as with *in situ* specimens.

Scanning electron microsopy (SEM)

Longitudinal grooves were prepared on the mesial and distal external root surfaces using a slow-speed diamond disk, without penetrating into the root canal space. The roots were then split in two halves in the mesiodistal plane with a small chisel. The root halves were coded and investigated under an EVO 50 EP environmental scanning electron microscope (Carl Zeiss NTS GmbH, Oberkochen, Germany) at extended variable pressure (XVP) mode without surface coating.

Each root canal was first examined at low (100×) magnification. Then, the coronal, middle and apical portions of root canals were investigated at 5000× [17]. Digital micrographs were obtained at both magnifications and were recorded as TIFF files at 1280 × 1024 resolution. The intra-examiner and inter-examiner agreement for the SEM evaluation was

Table I. The cleanliness scores of the coronal, middle and apical sections of the root canals. The values are expressed as mean ± SD.

	Coronal		Middle		Apical		
Irrigant	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro* (closed system)	*In vitro* (open system)
NaOCl	2.58 ± 0.51	2.20 ± 1	2.25 ± 0.62	3	3	2.75 ± 0.50	2.83 ± 0.39
EDTA	2.23 ± 0.83	2.16 ± 0.71	2.40 ± 0.63	2.37 ± 0.50	2.66 ± 0.49	2.61 ± 0.50	2.27 ± 0.75
MTAD	2.44 ± 0.88	2.20 ± 0.77	2.25 ± 0.86	2.27 ± 0.89	2.81 ± 0.60	2.93 ± 0.25	2.54 ± 0.66
Control	3	3	3	3	3	3	3

verified by the Kappa test, following blind evaluation of 20 specimens by two independent observers [19]. The cleanliness of the root canals was evaluated using a 3-point scoring system developed by Torabinejad et al. [17], which measures the presence, quantity and distribution of the smear layer as follows:

- Score 1 = No smear layer (no smear layer on the surface of the root canals with all tubules clean and open).
- Score 2 = Moderate smear layer (no smear layer on the surface of root canals but tubules contain debris).
- Score 3 = Heavy smear layer (smear layer covers the root canal surface and the tubules).

The same observers scored the degree of erosion of dentinal tubules, using the following scoring system coded by Torabinejad et al. [17]:

- Score 1 = No erosion (all tubules look normal in appearance and size).
- Score 2 = Moderate erosion (the peritubular dentin was eroded).
- Score 3 = Severe erosion (the intertubular dentin was destroyed and tubules were connected with each other).

Statistical analysis

The Mann-Whitney U-test and Kruskal Wallis analysis of variance were used to determine the differences among cleanliness and erosion scores, with statistical significance at $p = 0.05$. When the p-values from the Kruskal Wallis test were significant, the Kruskal Wallis multiple comparison test was used to determine the group(s) that differed significantly from others. Bonferroni correction was applied for all possible multiple comparisons controlling Type I error.

Results

The Kappa values for root canal cleanliness and the degree of erosion indicated high agreement between observers (both ≥ 0.9). The cleanliness and erosion scores are presented in Tables I and II, respectively.

There was no significant difference between (closed-end) specimens prepared under clinical and laboratory conditions with respect to the cleanliness and erosion scores (Mann-Whitney U-test, $p = 0.526$ and $p = 0.925$, respectively). All irrigation solutions were more effective in removing the smear layer in the coronal and middle thirds than in the apical third ($p < 0.001$), with the former two portions demonstrating similar cleanliness scores ($p = 0.876$). Under both test conditions, EDTA was the most effective irrigation solution in removing the smear layer, followed by MTAD and NaOCl (Kruskal Wallis test, $p < 0.05$). Pairwise comparisons showed that significant differences existed between the EDTA and NaOCl groups under both clinical and laboratory conditions ($p = 0.003$ and $p = 0.022$, respectively).

Compared with MTAD and NaOCl, specimens treated with 17% EDTA showed significantly higher scores for erosion under both clinical and laboratory conditions (Kruskal Wallis test, $p < 0.001$ and $p = 0.004$, respectively). Although final irrigation with MTAD yielded higher erosion scores compared with that of NaOCl, there was no significant difference between the two groups ($p > 0.05$).

In vitro comparison of closed and open systems showed that final irrigation with 17% EDTA caused significantly higher levels of cleanliness and erosion in

Table II. The erosion scores of coronal, middle and apical thirds of the root canals. The values are expressed as mean ± SD.

	Coronal		Middle		Apical		
Irrigant	*In vivo*	*In vitro*	*In vivo*	*In vitro*	*In vivo*	*In vitro* (closed system)	*In vitro* (open system)
NaOCl	1	1	1	1	1	1	1
EDTA	1.66 ± 0.97	1.83 ± 1	1.69 ± 0.94	1.75 ± 0.93	1.08 ± 0.28	1.07 ± 0.27	2.15 ± 0.90
MTAD	1.58 ± 0.90	1.20 ± 0.4	1.33 ± 0.70	1.11 ± 0.32	1.09 ± 0.30	1	1.07 ± 0.27
Control	1	1	1	1	1	1	1

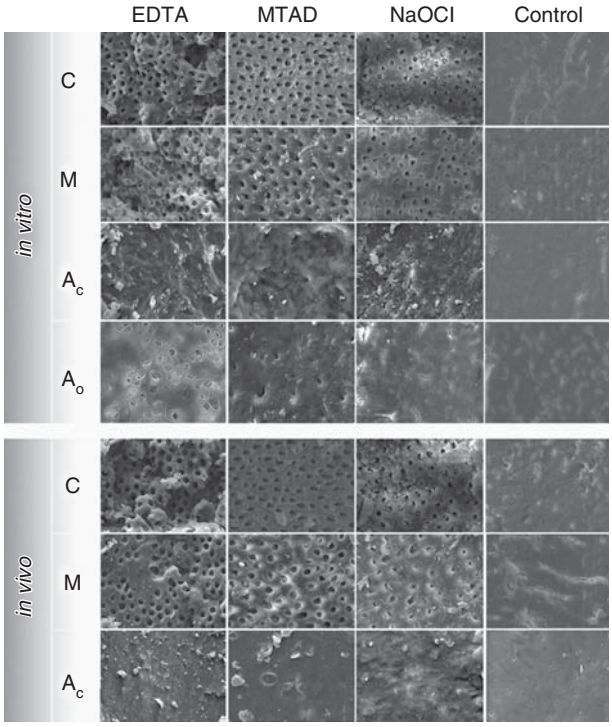

Figure 1. Representative scanning electron micrographs of the test groups. C, Coronal; M, Middle; A_c, Apical (Closed system); A_o, Apical (Open system).

the apical third of open system specimens ($p < 0.05$). For the coronal and middle regions, however, the levels of cleanliness and erosion were similar in the open and closed systems ($p > 0.05$). For the MTAD and NaOCl groups, both the open and closed systems showed similar levels of cleanliness and erosion in all regions ($p > 0.05$).

Representative SEM images of the test groups are presented in Figure 1. In teeth with a closed apex, a heavy smear layer was observed in the apical thirds of all *in situ* and *in vitro* specimens. The erosive destruction caused by EDTA was evident at the coronal and middle portions of root canals. For the open system specimens, final irrigation with EDTA resulted in a 'cleaner' apical third, compared with those of the MTAD and NaOCl groups.

Discussion

In vitro irrigation studies allow for controlling several experimental parameters including proper access cavity, obtaining accurate working lengths by visualizing files at the apex and shaping/irrigating the root canals without difficulty in access or clinical time limitations. Further, environmental factors which can influence the results (e.g. higher relative humidity and intra-oral temperature) can be controlled and multiple investigations can be performed at the same time. Because clinical conditions cannot offer those advantages, recommendations based on *in vitro* irrigation studies are merely deductive and need to be interpreted with care [7]. These observations justify our attempt to compare the effects of endodontic irrigation protocols under clinical and laboratory conditions. Based on the results, the null hypothesis was accepted, since *in situ* and *in vitro* test conditions had no significant effect on the cleaning efficiency and erosive potential of different irrigation protocols tested.

Currently, there is a lack of consensus on an ideal experimental method to assess smear layer removal [20]. While different types of microscopes are available for research, SEM is still the most common method for obtaining information about dentin surfaces, for both pratical reasons and to facilitate comparisons with previous studies that utilize the SEM. However, the damage caused in biological samples by the loss of water, which occurs in traditional high-vacuum SEMs, is a major drawback. To overcome this problem, De-Deus et al. [20] recommended the use of non-conventional microscopy techniques (e.g. environmental scanning electron microscope) in which the observation can be performed in low-vacuum conditions without the need for metal coating of non-conducting samples [20,21]. Here, the dentin specimens were investigated under an environmental scanning electron microscope using the extended variable pressure mode, a low-vacuum environment for investigation of non-coated, non-conducting biological specimens.

Irrespective of the present testing conditions, both the root canal cleanliness and the degree of erosion were differentially affected by the final irrigation regimens. In the NaOCl group, the relatively better scores of cleanliness in the coronal and middle thirds may indicate that treatment of prepared radicular dentin with NaOCl may not only remove the organic matrix, but also some of the inorganic content that ultimately renders dentin much cleaner than normal [22,23]. NaOCl was not as effective in the apical region as it was in the coronal and middle ones, probably because it has been shown to be less effective in reducing the surface tension at the apical region than in the middle and coronal thirds [24]. Compared with the NaOCl-only group (group 1), the combined use of NaOCl with BioPure MTAD and EDTA resulted in higher levels of root canal cleanliness in the middle and coronal regions. It has been shown that the combined use of NaOCl and EDTA results in a markedly higher rate of decalcification obtained with EDTA alone [23], indicating the significant contribution of NaOCl in the overall decalcifying effect. This was substantiated in the present study through the observation of the higher rate of dentin erosion in the EDTA group. Regarding group 2 (MTAD), our findings are in line with those of Torabinejad et al. [17], who showed that MTAD is an effective solution for the removal of the smear layer and that it causes an insignificant level of erosion when root canals were irrigated

with sodium hypochlorite and followed with a final rinse of MTAD. In contrast to the findings of Mancini et al. [19], there was no significant difference between the MTAD and NaOCl groups. A possible explanation could be that the present study utilized more than double the volume of NaOCl used in the [25]. Finally, the amount of smear remaining in the root canal may be related to the internal canal morphology and the type of instrumentation used [11]. In the present study, the root canals were prepared with rotary nickel-titanium instruments and the apical portion of each canal was enlarged to a # 30 file [17,19]. Torabinejad et al. [17] described this technique as an effective method to prepare root canals, explaining that it both creates a significant amount of smear layer [26] and allows for adequate cleaning and penetration of the solution to the apical third of root canals. On the other hand, none of the present irrigation protocols were effective in removing the smear layer in the apical region of teeth with closed apex. Our results corroborate with those of previous laboratory studies [19,27,28], showing that irrigation solutions are less effective or ineffective in the apical third. This could be attributed to the comparatively smaller apical canal dimensions, which hinders the penetration of irrigants, resulting in limited contact between the root canal walls and the irrigants [29]. Morever, since the roots were kept in moistened floral foam during canal preparation and irrigation procedures, it is highly possible that the foam prevented fluid extrusion from the apical foramen, thus allowing the root canal to behave as a closed-end channel. Consequently, this would produce a vapor lock effect which jeopardizes the efficacy of apical debridement during the delivery of irrigation solutions [18]. While it is also possible that the foam material could have failed to provide sufficient seal to the apical termination, thus preventing formation of the apical vapor lock and allowing irrigations solutions to flow through the apex, the consistent observation of apical smear in all test groups still support the present finding that the tested irrigation protocols were ineffective in removing smear in the apical portion.

In the present study, the comparison of open- and closed-end systems were only made under laboratory conditions, necessitating careful interpretation of the results with respect to the clinical conditions. According to the present results, unrestricted apical fluid movement did not necessarily enhance the cleansing efficacy of the tested final irrigation regimens. In fact, the only significant difference was observed with 17% EDTA at the apical region.

Owing to the limited number of clinical participants herein, the effects of the tested irrigation solutions could only be investigated using conventional needle irrigation. Future clinical-laboatory comparisons should include ultrasonically activated instruments, since they have been shown to enhance the cleaning efficiency of irrigation, particularly in the apical third [30]. Other limitations of the present study include the lack of different exposure times and concentrations of the irrigation solutions, whose effects remain to be substantiated in future comparative studies.

Conclusions

Based on the micromophological correlates of *in situ*/*in vitro* root canal cleanliness and erosion, it can be concluded that, for roots with a closed apex, laboratory testing of root canal irrigants on extracted teeth can serve as a reliable method to simulate the clinical condition. Both clinical and laboratory testing of 5.25% NaOCl, 17% EDTA and BioPure MTAD as final irrigation solutions showed that apical cleanliness remains to be a major problem when conventional needle irrigation is employed. Finally, irrigation with 17% EDTA yielded the best cleansing effect at the expense of causing intraradicular dentin erosion. In teeth with an open apex, final irrigation with EDTA appears to provide the best cleansing effect.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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