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# Effect of a Modified Irrigation Protocol on the Cleanliness of Moderately Curved Canals

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## Abstract

**Objectives** This study tested the hypothesis that modifying the sequence of sodium hypochlorite (NaOCl)/ethylene diamine tetra-acetic acid (EDTA) irrigation during root canal shaping would improve apical cleanliness in moderately curved canals.

**Materials and Methods** Forty-five root canals were prepared using Protaper Gold with three irrigation protocols. Standard irrigation (SI) used 0.5 mL 3% NaOCl between each instrument, followed by 5 mL 17% EDTA manually agitated for 30 seconds. Reverse irrigation (RI) used 0.5 mL of 17% EDTA between each instrument, then 5 mL of 3% NaOCl, manually agitated for 30 seconds. Reverse irrigation plus (RI+) was similar to RI, except NaOCl (5 mL), used as a final rinse, (5 mL), used as a final rinse, was allowed to interact for 3 minutes with dentin before manual agitation (30 seconds). Root canal cleanliness was evaluated under the scanning electron microscope (SEM) (Hulsmann score); the chemical composition of dentin after irrigation was analyzed by energy dispersive X-ray (EDX).

**Statistical Analysis** Results were compared using Kruskal–Wallis ANOVA by ranks and Wilcoxon matched paired posthoc tests. A Chi-square test assessed whether the best cleanliness would demonstrate a significant association with one irrigation protocol; odds ratio calculation was performed using score "1" versus score "2 or more" (2+) ($p < 0.05$).

**Results** In the apical region, cleanliness was better in RI+ than SI and both significantly better than RI. Odd ratios indicate that the cleanliness in RI+ was significantly better than RI and SI groups ($p < 0.000$ and 0.003, respectively). Independently of the irrigation protocol, EDX analyses showed no chemical alteration of root dentin.

**Conclusions** Using 17% EDTA during shaping, followed by 3% NaOCl rinse for 3 minutes, improved apical cleanliness without inducing erosion of dentin.

**Keywords**
- EDTA
- endodontic
- irrigation
- NaOCl
- smear layer

## Introduction

The success of endodontic therapy strongly depends on proper shaping and cleaning of the root canal system before three-dimensional (3D) filling. Whereas shaping is primarily aimed at cutting infected dentin and enlarging root canals, cleaning mainly consists of eliminating tissue debris and bacteria

Sodium hypochlorite (NaOCl, 0.5–5.25%) is a well-established irrigant for cleaning root canals because of both its antimicrobial activity and organic tissue dissolution capability.[1,2] High concentrations (5% and higher) faster

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dissolve organic tissue but there are also concerns about their toxicity when inadvertently injected into periapical tissue.[3] Other studies showed that less concentrated solutions (1–3%) maintain excellent antibacterial properties and that larger volumes and longer duration of irrigation have more impact on canal debridement.[2] Thus, concentrations ranging between 2.5 and 3% are claimed to offer a good balance between efficiency and toxicity. Unfortunately, sodium hypochlorite has no effect on the inorganic fraction of dentin extracted during shaping, which contributes to the formation of the endodontic smear layer.[4] This 0.5 to 2 µm-thick layer of organic and inorganic debris, packed onto canal walls, was shown to harbor bacteria and occlude 6% of the volume of mesial roots in mandibular molars.[5,6] Thus, it is advocated to remove the smear layer in order to enhance canal disinfection.[7-10]

Current clinical procedures rely on the use of sodium hypochlorite during shaping, followed by calcium complexing agents such as 17% ethylene diamine tetra-acetic acid (EDTA), to chelate calcium ions extracted from dentin.[4,11,12] The efficiency of this combination of chemicals has long been demonstrated, but evidence also suggests that the cleanliness at the apical level of curved canals may be improved.[13,14] Cutting debris accumulating along the inner curvature of root canals or occluding accessory canals are less likely to be removed or dissolved once they have formed.[15] One may argue that the chelating action of EDTA would be more useful during shaping to prevent the formation and accumulation of the smear layer onto canal walls, whereas the use of NaOCl would be more appropriate as a final disinfection step when shaping is completed.

In the current study, it is hypothesized that reversing the sequence of irrigants during shaping would lead to cleaner root dentin surfaces without altering its chemical composition. Specifically, scanning electron microscopy (SEM) was used to evaluate the cleanliness of root canals after different irrigation protocols, and energy-dispersive X-ray spectroscopy (EDX) was used to analyze chemical changes in dentin after treatment.

## Materials and Methods

In accordance with the regulations of the Ethic Commission on Human Research of Geneva (CCER-Geneva), which authorizes the use of anonymous extracted teeth for *in vitro* research without written consent, 45 human necrotic single-rooted teeth were collected just after extraction. Teeth were radiographed from both buccolingual and proximal directions to determine canal curvature, according to the Schneider method.[16] Only moderately curved canals (10–30°) were selected for the study and teeth exhibiting wide opened apices or straight roots were excluded. The Schneider method was used to measure canal curvature because it is widely in used in research and clinics to evaluate case difficulty.[17]

The access cavity was prepared using a cavity access Z set (Dentsply Sirona Endodontics; Ballaigues, Switzerland), and root canal length was established using a #10 K-file (Micro-Mega; Besancon, France), pointing out the apical foramen, which were observed under the operatory microscope (Extaro 300 Carl Zeiss; Oberkochen, Germany); working length was defined 0.5 mm shorter. The apical foramen was sealed with cyanoacrylate glue (Zapit; Dental Ventures of America, Corona, California, USA) to reproduce a closed system.[18] Canals were manually enlarged with #15 K-file (Micro-Mega; Besancon, France) before using ProTaper Gold (Dentsply Sirona Endodontics; Baillaigues, Switzerland). Briefly, S1-S2-F1 and F2 progressively reached the working length, whereas the F3 was stopped 0.5 mm shorter. This final preparation (0.3 mm diameter, 9°taper) allowed the placement of the needle tip 3 mm shorter than the root canal terminus.[19] The apical patency was verified between each rotary instrument using a #10 K-file, which was allowed pass through the apical seal.

For all groups, a 30G open-ended needle (Miraject Endo Luer; Hager & Werken, Duisburg, Germany) was used for canal irrigation; final solutions were manually activated (MDA: manual dynamic agitation) with a matching gutta-percha point (F3). Specimens were randomly divided into three groups ($n$ = 15) that followed different irrigation protocols (see below).

For the standard irrigation group (SI), 0.5 mL of 3% NaOCl was delivered between each instrument. This volume of irrigant was selected because a higher volume is unlikely to penetrate deep inside the root canal before shaping is completed.[20] A final rinse of 1 minute was made using 5 mL of 17% EDTA, which was manually agitated for 30 seconds.

For the reverse irrigation group (RI), 0.5 mL of 17% EDTA solution was used during shaping and renewed between each instrument. A final irrigation of 1 minute was made using 5 mL of 3% NaOCl and manually agitated for 30 seconds.

Specimens of the reverse irrigation plus group (RI+) followed the same protocol as the reverse irrigation group, but the final irrigation using 5 mL NaOCl was extended to 3 minutes and the solution manually agitated during the last 30 seconds. The rationale for using a prolonged application time was to increase the proteolytic action of sodium hypochlorite against canals debris that may contain a higher organic content.

All specimens were rinsed with 2 mL of distilled water to neutralize the final solution and stored at 4°C.

### Preparation of the Samples for the Scanning Electron Microscopy (SEM)

The specimens were embedded into polyvinylsiloxane material to produce individual molds designed to maintain the root during the splitting process necessary for SEM observation. A F3 gutta-percha point was fitted into the root canal to prevent the penetration of external debris.[21] Longitudinal grooves (buccal and lingual) and transversal grooves were prepared to allow the placement of a scalpel blade (10; KLS Martin Group, Tuttlingen, Germany) that received a brief load in order to force the root to fracture. The samples were labeled, dehydrated (ascending concentrations of ethanol: 70%, 80%, 90%, 100%), glued onto aluminum stubs, and gold sputtered.

### Field Emission Scanning Electron Microscope (FESEM) Evaluation

The samples were observed under a field emission scanning electron microscope (Zeiss Sigma 300 VP-FESEM; Oberkochen, Germany). A low-magnification (x50) was used to delineate two regions of interest, extending from 0 to 5 mm from the apex (apical) and from 5 to 10 mm (middle). The coronal portion of the root was excluded from evaluation because, independently of the irrigation protocol used, this region is easily cleaned.[14,22] For both regions, two microphotographs were taken (x350 and x1000) and evaluated by two calibrated observers, who blindly assessed the cleanliness of the canal using the Hulsmann score.[13] In case of disagreement, the worst score was selected for calculations.

### EDX

An Oxford X max 50 EDX spectroscopy system with dual silicon drift detectors, each with an area of 50 $mm^2$, and a resolution of 125eV (Zeiss Sigma 300 VP-FESEM, Oberkochen, Germany) were used to measure the relative amounts of calcium, phosphorus, carbon, oxygen, sodium, and magnesium on dentin surfaces after treatment. EDX analysis was performed on each sample at the middle and apical regions. A mean value of the atomic percentage of each of the six elements that were measured across the root canal was calculated for the middle and apical regions.

### Statistical Analysis

Mean scores and standard deviations were calculated for the apical and middle regions of each group and statistically compared using Kruskal–Wallis ANOVA by ranks and Wilcoxon matched pairs posthoc tests. A Chi-square test was used to assess whether the best cleanliness (score 1) would demonstrate a significant association with one of the three irrigation groups; odds ratio calculation was performed using score "1" versus score "2 or more" (2+).

## Results

Results indicate that the cleanliness of the middle and the apical regions were similar ($p > 0.05$), except for the RI group. As shown in ►**Fig. 1**, mean scores observed in the middle region of the SI group (1.87 ± 0.74) and RI+ group (1.47 ± 0.74) were statistically equivalent ($p > 0.05$), whereas the mean score for the RI+ group was significantly better ($p = 0.014$) than the RI group (2.67 ± 1.29). In the apical region, mean scores of the SI group (2.20± 1.01) and RI+ group (1.60 ± 0.74) were statistically equivalent but both significantly better than the mean score reported for the RI group (3.53 ± 1.19). Results of the odd ratios test indicate that the occurrence of score 1 at the apical region of RI+ group was significantly higher than RI and SI groups (respectively $p < 0.000$ and 0.003).

SEM evaluation of the middle and the apical regions showed small amounts of smear layer, partially occluding dentinal tubules in SI group (►**Fig. 2A** and **2B**), whereas an amorphous surface layer was observed covering the root dentin of RI samples (►**Fig. 2C** and **2D**). RI+ samples showed no smear layer and opened dentinal tubules in both regions (►**Fig. 2E** and **2F**). Independently of the irrigation protocol used, results from the EDX analysis showed that the relative percentages of the six elements measured on the middle and apical surfaces of the canal walls remained comparable. ►**Fig. 3A, 3B, 3C** specifically shows the proportions of mineral (43.1–45.3%) and organic elements (54.7–56.9%) measured in the apical region after irrigation.

## Discussion

The use of NaOCl followed by EDTA during biomechanical canal preparation is the gold standard.[4,11] This sequence of irrigation was used as a control in the SI group. Although this protocol was shown to allow clinicians to obtain clean canal surfaces in most instances, other reports suggest that

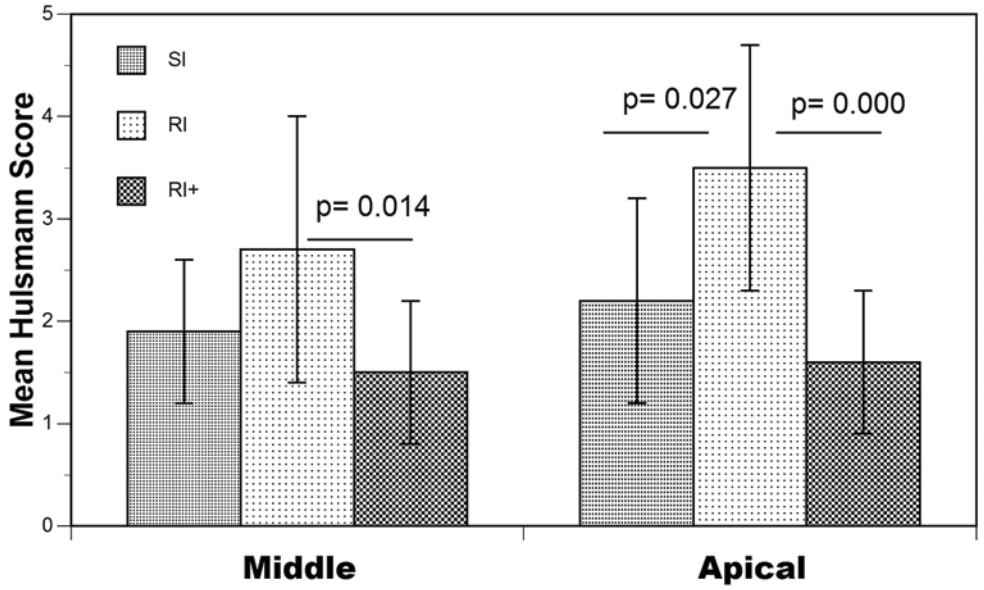


**Fig. 1** Mean Hulsmann scores (± standard deviation [SD]) in the middle and apical regions after standard irrigation (SI), reverse irrigation (RI) and reverse irrigation plus (RI+).

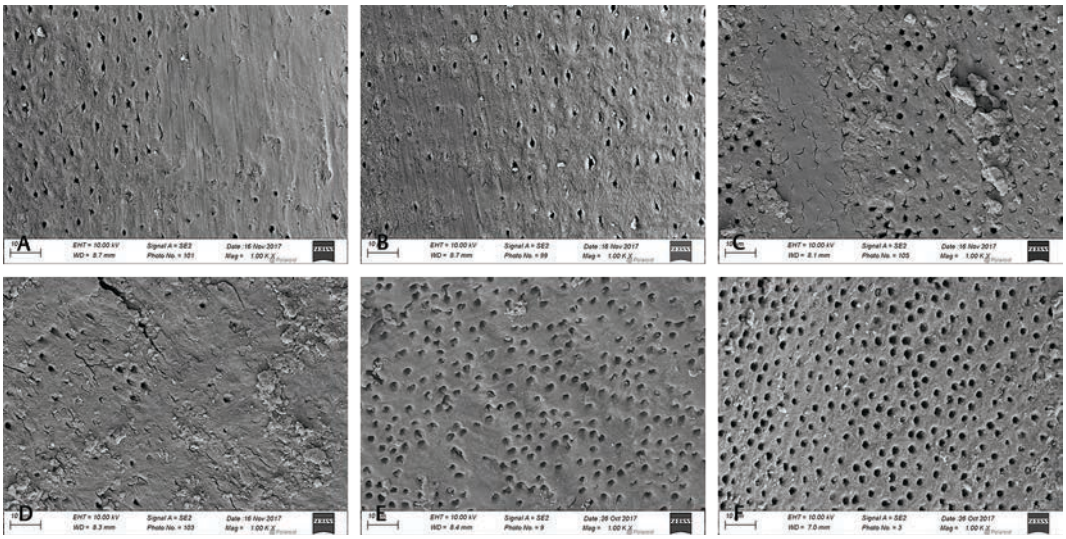

**Fig. 2** SEM micrographs of: (**A**) Standard irrigation (SI) group, middle region x1000. (**B**) SI group, apical region x1000: the orifices of the dentin tubuli appear through a thin smear layer. (**C**) Reverse irrigation (RI) group, middle region x1000. (**D**) RI group, apical region x1000: Large areas covered by an amorphous layer are visible. (**E)** RI+ group, middle region x1000. (**F**) RI+ group, apical region x1000: opened dentin tubuli and the absence of smear layer are observed.

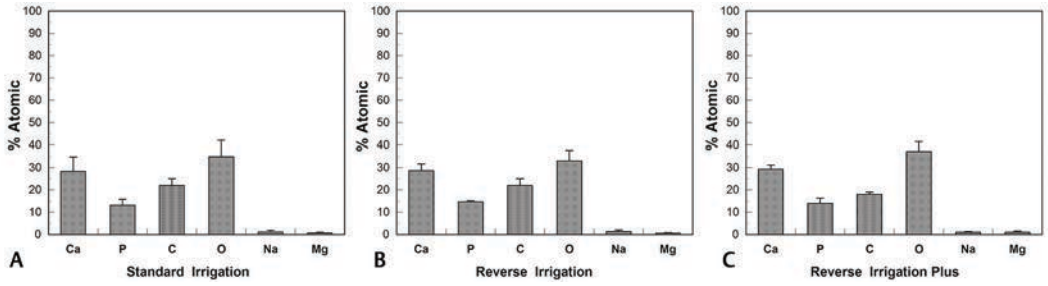


**Fig. 3** Atomic percentage of calcium, phosphorous, carbon, oxygen, sodium, magnesium measured at: (**A**) apical region SI specimens, (**B**) apical region reverse irrigation (RI) specimens, and (**C**) apical region RI+ specimens.

cleaning the apical third of curved canals remains critical and advocate the use of supplementary procedures based on mechanic, sonic, ultrasonic, or laser devices. However, a recent meta-analysis that compared manual dynamic activation (MDA), passive ultrasonic irrigation (PUI), sonic irrigation (SI) or apical negative pressure (ANP) failed to identify any superiority among these different activation techniques.[23] In the current study, we suggest reversing the sequence of irrigation to improve canal cleanliness without using supplemental instruments for activating irrigants.

Teeth used in this study had an intact or restored crown to provide a four-wall reservoir, promoting fluid exchange and renewal throughout shaping and irrigation. Selected root canals were moderately curved canals (17°± 6) because they are harder to clean apically.[14,24] No statistical difference in canal length or canal curvature was found among the three experimental groups. The apical permeability was kept minimal to prevent the risk of excessive fluid communication between the root canal and the external environment that was shown to modify fluid dynamics during irrigation.[18] The use of the Protaper Gold system, comprising five successive instruments (S1 to F3), allowed the placement of a total volume of 2.5 mL of solution within five successive events. Boutsioukis et al reported that the 9% tapered apical preparation of Protaper F3 allows the deep placement of the irrigation needle and a higher shear stress to develop along canal walls during irrigation.[25-27] Finally, a F3 gutta-percha point was used to manually agitate the solutions (MDA), in order to help the dispersion and mixing of irrigants in the stagnation zone and ensure that air inside the apical third (vapor-lock) was displaced.[28]

Results for the SI group are in agreement with previously published reports that used the same scoring method. Whereas Caron et al reported a mean apical score of 2.21 in curved-canals, Ali-Ali et al obtained similar results after evaluating smear layer and debris removal effectiveness of several irrigation methods by combining the use of SEM and histological cross-sections.[21,29] Recently, several authors have raised concerns about the use of qualitative scores to evaluate root canal cleanliness, mostly because the selection of the observed area is operator-dependent. It must be pointed out that in the current study, both examiners were blinded before scoring specimens and interexaminer reproducibility was verified using Kappa statistics ($K$ = 0.82). More recently, the observation of root canals using micro-CT has been proposed to increase the quality of the results because the assessment of the same specimen before and after the experimental procedure is made possible.

However, scanning and reconstruction procedures take a considerable amount of time and a perfect knowledge of dedicated software to provide accurate results. As reported by De Deus et al, the ideal experimental model to assess smear layer removal is not currently available.[30]

Interestingly, apical specimen exhibiting score 1 was 5.5 times more likely to be associated with RI+ than SI (OR 5% 95% confidence interval 3.12–9.68), supporting the superiority of this cleaning protocol. On the contrary, the RI tended to promote the accumulation of debris on canal walls (►**Fig. 2C** and **2D**) when compared with RI+ canal surfaces (►**Fig. 2E** and **2F**). It is assumed that insufficient amounts of chlorine have been released to dissolve this organic rich layer. When the solution was left in place for 3 minutes (RI+) without increasing the total volume applied before activation for 30 seconds, this organic layer was no longer observed.[31] In agreement with Zehnder et al in 2005, the prolonged use of sodium hypochlorite helps to wash out any residual EDTA before developing full proteolytic and antimicrobial activity.[8]

Although erosion of dentin consecutive to the use of EDTA followed by NaOCl has been reported by others, this phenomenon was not observed in the samples of the RI+ group. This could be explained by differences in EDTA/NaOCl concentration ratios, pH and volume of solutions, duration of the reaction, and geometry of the specimens.[32-34] As reported by Gu et al in 2017, dentin erosion mostly occurs when EDTA is applied onto NaOCl-treated dentin, as EDTA readily dissolves the collagen-depleted apatite crystallites at the surface of root dentin before propagating downward across the subsurface. Their results clearly indicated that high concentrations of hypochlorite (4% or higher) or extended irrigation times (30 minutes and more) are necessary to induce erosion of dentin.[35] Also, in agreement with Calt and Serper, the application of EDTA either during (RI and RI+ groups) or after shaping (SI group), never exceeded 1 minute.[36] Obviously, the risk that alterations in the chemical or structural composition of root dentin may have occur after a 3-minute period of application of 3% NaOCl solution is unlikely. A lack of erosion is further supported by EDX analysis showing comparable mineral-organic ratios among groups and percentages of elements similar to others.[33] Thus, a potential decline in the mechanical properties of NaOCl-treated dentin can be ruled out when using 3% sodium hypochlorite over 3 minutes. From a clinical point of view, it should be noted that the total volumes of irrigants applied in each group were strictly identical and that reversing the irrigation sequence would undoubtedly benefit the treatment of necrotic teeth where the tissue dissolving capability of NaOCl is less important compared with the antimicrobial activity.

Irrigation plays several roles during endodontic treatment, the most important being the elimination of endodontic pathogens causing infection; another role being the cleaning of the dentin surfaces covered by smear layer. As reported by Haapasalo et al in 2012, the smear layer has to be removed because it contains bacteria and bacterial antigens that were embedded into it during instrumentation of necrotic root canals.[37] The smear layer also occludes dentin tubules, which are potentially infected and restrict the penetration of disinfecting solutions into dentin tubules. Wang et al in 2013 examined the effect of the smear layer on the antibacterial effect of different disinfecting solutions in infected dentinal tubules.[38] They applied confocal laser scanning microscopy to detect live versus dead bacteria in the dentinal tubules after irrigation and concluded that the smear layer clearly decreases the antibacterial activity of NaOCl in dentin. More recently, Morago et al in 2016confirmed that that the presence of the smear layer significantly reduces the antimicrobial activity of 2.5% NaOCl. [39] Thus, the cleanliness of the root canal walls and the quality of the final disinfection are strongly linked. Finally, there is evidence that a complete removal of the smear layer increases the sealing ability of several endodontic sealers.[40]

## Conclusions

Using 17% EDTA during shaping followed by 3% NaOCl rinse for 3 minutes moderately improved apical cleanliness without inducing any sign of erosion on dentin canal walls. This irrigation protocol was significantly more likely to be associated with cleaner dentin than the SI protocol in the apical region. Further studies are needed to confirm this cleaning effect in teeth, exhibiting more complex anatomies such as transverse anastomosis, fins isthmus and lateral canals.

**Conflict of Interest**
None declared.

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