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ARTICOLO ORIGINALE

Debridement effectiveness of two different techniques using negative pressure irrigation system

Efficacia nella detersione di due differenti tecniche d'irrigazione a pressione negativa

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KEY WORDS

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PAROLE CHIAVE

Sistema canale chiuso;
EndoVac;
Irrigazione con siringa;
Irrigazione a pressione negativa;
Fango dentinale.

Summary

Objectives: To evaluate the cleaning efficacy of two apical negative pressure irrigation techniques compared to needle irrigation.

Materials and Methods: Eighty extracted human single canal teeth were shaped and assigned to 3 experimental groups (n = 20) according to the irrigation technique performed and two negative control groups (n = 10) as follows: 1) NI (Max-I-Probe side-vented needle irrigation); 2) EV (EndoVac system); 3) EVM (EndoVac-modified technique); 4) EV-C (EndoVac-negative control); 5) NI-C (needle irrigation-negative control). A scanning electron microscopic evaluation was performed. The presence of debris and smear layer at all levels (coronal, middle, apical) was evaluated.

Results and conclusions: A new irrigation protocol, using EndoVac System resulted in better removal of the smear layer at all levels.

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Riassunto

Obiettivi: Valutare l'efficacia di due tecniche d'irrigazione a pressione apicale negativa paragonandole a quella tradizionale con siringa.

Materiali e metodi: Ottanta denti umani monocalari, assegnati a 3 gruppi sperimentali (n = 20) in accordo alla tecnica d'irrigazione utilizzata e due gruppi controllo (n = 10). 1) NI

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(Irrigazione con siringa); 2) EV (EndoVac); 3) EVM (EndoVac-tecnica modificata); 4) EV-C (EndoVac-controllo negativo); 5) NI-C (Irrigazione con siringa-controllo negativo). Dopo detersione e sagomatura i campioni sono stati valutati attraverso la microscopia elettronica a scansione. I risultati sono stati analizzati statisticamente mediante analisi non parametrica della varianza ($p < 0.05$).

Risultati e conclusioni: Un nuovo protocollo d'irrigazione con il sistema endovac si è dimostrato significativamente più efficace nella rimozione del fango dentinale a tutti i livelli.

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Introduction

The ideal outcome of root canal treatment is the effective destruction and removal of bacterial biofilms and their by-products from the root canal system (RCS), or at least their significant reduction to levels compatible with periradicular tissue healing [1]. Due to its anatomical complexities, such as isthmi, fins, deltas, and accessory canals, complete debridement of the RCS is a laborious challenge [2].

Current instrumentation techniques are ineffective in shaping and cleaning all surfaces and irregularities of the root canal space [3–6]. Additionally, accumulation of debris and producing smear layer are potential side effects of mechanical instrumentation [7], which may impede disinfection of the RCS in cases with apical periodontitis [8,9], harbour microorganisms and disrupt the seal between the root filling material and canal walls, possibly leading to treatment failure [10,11]. Therefore, irrigation using antimicrobial and tissue-dissolving irrigants is complementary to instrumentation in facilitating the removal of bacteria and disinfection of the RCS, flushing debris and necrotic tissue, and removing smear layer [12,13], especially from areas that are routinely left uninstrumented following root canal preparation, e.g. isthmuses, oval extensions and apical deltas [4,14].

Because of its broad spectrum antimicrobial efficacy [15,16] as well as its unique ability to dissolve organic debris [17,18], sodium hypochlorite (NaOCl) is recommended as the main irrigant during endodontic therapy [19]. However, it is not able to dissolve the inorganic components of dentine debris [20]. Seventeen per cent ethylenediaminetetraacetic acid (17% EDTA) is a chelating agent that is often used as the active final rinse to remove the inorganic component of the smear layer.

It is best used in combination, but not coincident, with NaOCl [19,21]. It was stated that removal of debris relies mostly on the flushing action of the irrigant [22,23]. Therefore, a sufficient volume, a high flow rate and an unrestricted flow of the irrigant along the canal walls are crucial for thorough debridement of the RCS [24–26]. Conventional manual irrigation with a syringe and needle remains widely accepted [4], although its flushing action is not sufficient in removing debris from root canal irregularities [13,14,27,28]. The flushing action and the extent of irrigant replacement of syringe irrigation is dependent on many factors such as the insertion depth and diameter of the needle [25,26,29]. The optimal needle depth may be also influenced by the presence of a curvature and by the final size and taper of the prepared root canal [22,30,31]. Moreover, gas entrapment could prevent optimal irrigant delivery and flow 0-2 mm from

the end-point of canals [32]. Several studies have shown that current irrigation methods are effective in cleaning root canals coronally but less are effective apically [33–35]. Huang et al. [31] and Sedgley et al. [36] reported that a thorough cleaning was attainable with the tip of the syringe located apically.

Different techniques and devices have been proposed to improve the flow and distribution of irrigating solutions within the RCS [27]. EndoVac (Discus Dental, Culver City, CA) is a commercially available negative pressure irrigation system that is designed to deliver irrigating solution to the apical end of the canal system and remove debris via a negative pressure mechanism. This system combines a master delivery/suction tip (MDT) (fig. 1) that simultaneously delivers and evacuates irrigants to/from the access cavity while drawing irrigants into the canal space by using macro- (fig. 2) and micro-cannulas (fig. 3). The EndoVac has been shown to introduce a higher flow of irrigant and produce better debridement at 1 mm from the working length (WL) when compared with positive pressure needle irrigation [37]. Nonetheless, no differences were observed for the canal area 3 mm short of the WL, in agreement with the findings of Siu and Baumgartner [38]. However, their comparisons are biased. Syringe irrigation was performed at 2 mm away from WL or even more, while Endovac was inserted to full WL. To be effective, endodontic irrigants should ideally be delivered close to WL. Irrigant replacement reached the WL only when the side-vented needle was placed within 1 mm from the WL [39]. Using an *ex vivo* open-end canal model, Abarajithan et al. [40] showed that both Endovac and conventional irrigation were ineffective in complete removal of smear layer from the apical third of root canal instrumented up to a master apical file (MAF) ISO size 60. Additionally, Susin



Figure 1 Master delivery tip.

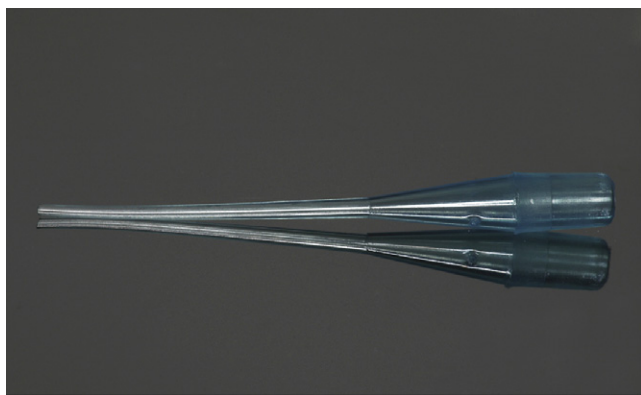


Figure 2 Macro-cannulus.

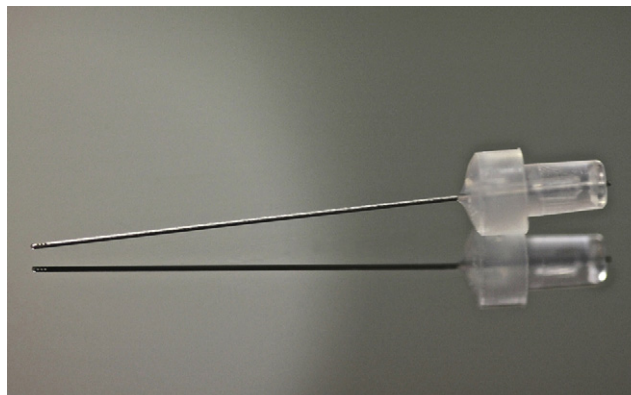


Figure 3 Micro-cannulus.

et al. [41] studied canal and isthmus debris debridement efficacies of apical negative pressure (ANP) technique in comparison with manual dynamic irrigation [42], using an *ex vivo* closed-end canal model. The ANP technique was unable to completely remove debris from the narrow isthmi present between the canals in the mesial root of mandibular molars, because of the difficulty in getting irrigating solutions to reach the isthmus and to create a strong enough current to flow through the isthmus.

Thus, the purpose of the present *ex vivo* study was to examine the canal debridement and smear layer removal efficacy of two irrigant delivery ANP techniques with EndoVac system versus positive pressure needle irrigation, using a closed canal design. The null hypotheses was that there is no difference between the canal debridement and smear removal by using two different ANP irrigant delivery techniques and needle irrigation, at different levels from the anatomical apex, in a simulated closed canal system.

Materials and Methods

Tooth selection and preparation

Eighty freshly extracted human permanent anterior single-rooted teeth with straight root canal (maxillary lateral incisors and mandibular incisors) were collected and stored in sterile saline before the investigation. The age of the patients from whom these teeth were extracted was less than (40%) and over (60%) 30. The inclusion criteria were small restorations, intact pulp chamber, and intact closed apices, whereas the exclusion criteria were previous root canal treatment, extensive restorations, root caries, root fractures, teeth with an irregular root canal anatomy, and root length less than 16 mm.

The study was approved by the Ethics Committee of Federico II Naples University. After preparation of the access cavity, root canal was negotiated using a size 10 stainless steel K-file, inserted into the root canal until the tip of the instrument was just visible at the apical foramen. The WL was determined by subtracting 1 mm from this length. Each tooth was radiographed in buccolingual and mesiodistal projections to detect any possible obstruction, to evaluate the shape of the root canal and to determine the degree of root canal curvature [43]. Teeth with no single canal system, canal

curvature angles of more than 20 degrees, calcified root canals, or root canals allowing introduction of an instrument exceeding ISO size 30 to the apical foramen were excluded. The incisal edge was adjusted, so that the length of each tooth was 21 mm from the apical foramen. To simulate *in vivo* conditions, each root had their apical foramina covered and sealed by hot flexible glue expressed from a hot glue gun. This set-up permitted recapitulation of canal patency but prevented fluid extrusion from the apical foramen during canal preparation. Then the cementum, totally from apex to the cemento-enamel junction and set glue were coated with tray adhesive (Dentsply Caulk, Milford, DE, USA). Access cavity finishing and pulp canal orifice expansion were performed with sizes 5 and 3 ultrasonic tips Start-X (Dentsply, Maillefer, Baillagues, Switzerland). The cervical bulge of dentine was removed by using X-Gates bur (Cavity Access Set; Dentsply, Maillefer, Baillagues, Switzerland). A glide path was established by mechanical instrumentation up to an apical diameter of 0.19 mm at the WL with sizes #13/.02, #16/.02, #19/.02 nickel-titanium (NiTi) rotary Pathfiles (Dentsply, Maillefer, Baillagues, Switzerland). Briefly, the coronal two thirds of the root canals were enlarged by using Protaper S1 NiTi rotary instrument (Dentsply, Maillefer, Baillagues, Switzerland) at the WL. Root canals were then instrumented to final size #40/.04 taper NiTi rotary GT Series X file (Dentsply, Maillefer, Baillagues, Switzerland) in a crown-down approach to a standardized WL of 21 mm. Apical patency was confirmed with a small file (stainless steel hand k-file size 10) throughout the procedures after each larger file size. Before instrumentation, the teeth were divided into three experimental groups, according to the irrigation technique performed of 20 teeth each and two negative control groups for negative and positive pressure irrigation, of 10 teeth each, balancing the 5 groups with regard to age of the teeth, root curvature and number of round-shaped and oval-shaped canals.

Experimental groups

For all experimental groups, irrigation with 5.25% NaOCl (Nicolor 5; Ognalaboratori Farmaceutici, Muggiò, Italy) at 37 °C and with 17% EDTA (Ognalaboratori Farmaceutici, Muggiò, Italy) began before the use of the X-Gates drill. The canals were kept flooded with 5.25% NaOCl throughout

the instrumentation procedure. Finally, the canal was flushed with 17% EDTA followed by 5.25% NaOCl [44]. Each tooth in each group received an equal amount of time for irrigation and the same volume of irrigants. Altogether, 35 mL 5.25% NaOCl was used: three milliliters during access cavity finishing and pulp canal orifice expansion, 16 mL during rotary instrumentation, 10 mL during macro-irrigation and 6 mL for micro-irrigation (the first cycle and the final flush after EDTA application). Furthermore, 3 mL 17% EDTA was used for each tooth.

a) EV group (n = 20): EndoVac system

For the EV group, irrigation began during the use of the X-Gates drill. The irrigant was delivered into the pulp chamber by using MDT connected to the NaOCl syringe and placed above the access opening.

Suction tubing attached to the syringe tip through an aluminum adapter (fig. 4) removed any excess irrigant. This allowed the canal and pulp chamber to be full of irrigant at all times. During all instrumentation, the chamber was flooded with 2 mL 5.25% NaOCl replenished with 2 mL after each instrument. Once instrumentation was complete, when the MAF (#40/.04) reached WL, the canal was macro-irrigated and micro-irrigated according to the manufacturer's instructions. Thirty seconds of macroirrigation (active irrigation) with 5.25% NaOCl were accomplished as follows: 10 mL were delivered coronally by the MDT while macro-cannulus inserted into the canal was constantly moved up and down, from a point up to apically binding point (4 mm short of

the WL) to a point just below the canal orifice. The NaOCl was suctioned through the tip of the macro-cannulus while the NaOCl was constantly being replenished via the syringe tip. The irrigant was then left undisturbed for 60 seconds in a totally filled canal to allow further chemical reactions between fresh NaOCl and residual organic debris. Then 3 cycles of micro-irrigation were accomplished. During each cycle, pulp chamber was maintained full of irrigant delivered by metal needle of MDT over 30 seconds while the micro-cannulus was placed in sequence, at WL for 6 seconds, 2 mm short of the WL (6 seconds) and back to the WL for 6 seconds: this alternating movement between these positions lasted 30 seconds, allowing 18 seconds of active irrigation directly at WL. The micro-cannulus was at last withdrawn from the canal in the presence of a full irrigant pulp chamber, ensuring a totally filled canal for a 60 seconds passive wait. This completed 1 cycle of micro-irrigation. NaOCl (5.25%) was used in the first cycle. EDTA (17%) was used in the second cycle and NaOCl (5.25%) was used once again in the third cycle. After 3 cycles sequence and 60 seconds passive wait, NaOCl was aspirated using the micro-cannulus at WL.

b) EVM group (n = 20): EndoVac system-modified technique

For the EVM group, macro-irrigation began during rotary instrumentation as follows: 2 mL of 5.25% NaOCl between each instrument change by using at the same time MDT on pulp chamber and macro-cannulus into the root canal placed up to the apically binding point without "up-down" motion. The NaOCl was suctioned through the tip of the macro-cannulus while the NaOCl was constantly being replenished via the syringe tip. Once the MAF reached WL, the micro-cannulus replaced macro-cannulus and a 3 cycles sequence of micro-irrigation started at WL. The micro-cannulus was constantly left at WL without "up-down" movements. NaOCl (5.25%) was used in the first cycle. EDTA (17%) was used in the second cycle. NaOCl (5.25%) was used once again in the third cycle. After each cycle of micro-irrigation (30 seconds) a 60 seconds passive wait (pulp chamber and root canal full of fresh irrigant) followed. After micro-irrigation completion NaOCl was aspirated using the micro-cannulus at WL.

c) NI-group (n = 20): Needle irrigation group

For the needle irrigation group, irrigation began during the use of the X-Gates drill. The pulp chamber and canal were irrigated by using a conventional syringe and 30-gauge Haws Max-i-Probe side-vented needle (Dentsply Rinn). A two milliliters flush of 5.25% NaOCl over 30 seconds was used after each instrument, leaving the canal filled with irrigant and undisturbed for 60 seconds before using the next file. During irrigation, the needle was inserted in the canal as deep apically as possible without binding and to full WL. During irrigation the needle was constantly moved up and down (simulating macro-cannulus) from apically binding point on dentinal wall to a point just below root canal orifice to properly improve apical irrigant replacement [44]. Once the MAF reached WL, the canal received irrigation with 10 mL of 5.25% NaOCl over 30 seconds. The irrigant was then left undisturbed for 60 seconds. After a 60 seconds passive wait, three additional cycles of irrigation were used



Figure 4 Multi-Port Adapter.

(simulating micro-irrigation cycles). Each cycle involved irrigation with the needle constantly moving in 2 mm amplitudes (simulating micro-cannulus): this alternating “up-down” movement every 6 seconds between full WL and 2 mm short of the WL lasted 30 seconds, allowing 18 seconds of active irrigation directly at WL followed by a 60 seconds passive wait during what irrigant was left undisturbed. NaOCl (5.25%) was used in the first cycle. EDTA (17%) was used in the second cycle. NaOCl (5.25%) was used in the third cycle. The irrigant was aspirated from the canal by using a 30-gauge open-ended needle (NaviTip Ultradent, South Jordan, UT) that was placed at WL.

Control groups

a) EV-C group (n = 10): EndoVac-negative control

In this group, the same protocol for irrigation was followed as in EV group, but only saline solution was used as an irrigant.

b) NI-C group (n = 10): Needle irrigation-negative control

In this group, the same protocol for irrigation was followed as in NI group, but only saline solution was used as an irrigant.

Root Sectioning and Scanning Electron Microscope Examination

Upon completion of the respective irrigation protocol, the root canals were rinsed with 2.5 mL of sterile saline solution per canal to dilute the NaOCl solution and dried with multiple paper points. Additionally, shallow horizontal grooves were placed at 5 mm intervals from the apical foramen marking the apical third, middle third and coronal third of each root. Subsequently, the roots were split longitudinally in a buccolingual direction, resulting in 20 and 40 samples per control and experimental groups respectively. Two longitudinal grooves, which did not penetrate into the canal, were prepared along the buccal and lingual external root surface, using a narrow, pointed, high-speed tungsten carbide bur under copious water cooling to facilitate longitudinal splitting of the root and to expose the instrumented canal. Gentle tapping of a new razor blade placed in one of the grooves, with the root secured with two fingers, caused the splitting of the root into two longitudinal halves.

Both halves were fixed in 2% glutaraldehyde, dehydrated by using a graded series of ethanol solutions, mounted in aluminium stubs, gold-sputtered and examined under scanning electron microscope (SEM) (Autoscan Siemens, Erlangen, Germany) operating at 15 kV.

SEM Evaluation

Micrographs for assessing the efficacy of debris and smear layer removal were taken at 200X and 1,000X magnifications respectively, in the coronal, middle and apical parts of the canal walls according to a scale developed by Hülsmann et al. [45]. This five stage scale included a detailed verbal description and a visual example (i.e. a SEM photograph) for each gradation (1-5). **Debris** was defined as dentine chips, remnants of necrotic pulp tissue, and particles lying loose on the canal wall. The five-level scoring system employed for

assessing the efficacy of debris removal was: 1, clean root canal wall, only very few debris particles; 2, few small conglomerations of debris; 3, many conglomerations of debris covering less than 50% of the root canal wall; 4, more than 50% of the canal wall covered with conglomerations of debris; 5, complete or nearly complete cover of the canal wall with conglomerations. The **smear layer** was defined as a surface film of debris retained on dentine or other surfaces after instrumentation with either rotary instruments or endodontic files. The five-level scoring system employed for assessing the efficacy of smear layer removal was: 1, smear layer is completely absent. Most tubules are patent and debris-free (coronal third and middle third) or occluded with sclerotic casts (apical third); 2, smear layer covering less than 25% of the canal wall. Dentinal tubule orifices, when identified, may be reduced in dimensions owing to partial or complete occlusion by debris; 3, homogenous smear layer covering the root canal wall and evident in 25%–50% of the canal surface. Only a few dentinal tubules open. Dentinal tubule orifices, when identified, may be reduced in dimensions owing to partial or complete occlusion by debris; 4, homogeneous smear layer evident in 50%–75% of the canal surface and tubules; no open dentinal tubules; 5, heavy, homogeneous smear layer covering 75%–100% of the canal surface and tubules. As the last 2 mm of the apical third of most canal walls was highly sclerotic and the tubules were occluded by sclerotic casts, scoring could not be conducted based on the presence or absence of patent dentinal tubule orifices only. In those regions, assessment was made based on whether the sclerotic dentine was covered by the smear layer. The former, even in the complete absence of dentinal tubules, still retained the anatomy of sclerotic dentine. The latter could always be discerned by the presence of a flat surface that contains evidence of instrumentation. For each root half, 10 images for debridement effectiveness and 10 images for smear layer retention were taken from the coronal third, the middle third and the apical third (i.e. experimental groups: 20 images at 200X magnification · three locations · 60 roots = 3600 images/ 20 images at 1000X magnification · three locations · 60 roots = 3600 images).

The images were selected by an independent blinded operator in a random walk manner through the defined sections. Thereafter, the selected images were photographed, coded and randomly mixed. Separate blind evaluations were undertaken by four trained observers who were blinded and well versed in the interpretation of SEM morphology.

Separate evaluations were undertaken at each canal level. When agreement independently occurred on a score among the four examiners, agreed score was recorded. When discrepancies exist during the course of evaluation, a “forced agreement” between the four examiners was used, so that all examiners agreed on the scores for each image taken from each canal level. Intra-examiner and inter-examiner reliability and reproducibility for SEM assessment was verified using *Kappa* statistics to data, with a significance of 0.5. Statistical analysis of differences between groups with respect to debris and smear scores was performed by using the Kruskal Wallis nonparametric analysis of variance, followed by Dunn’s rank sum test for pair-wise comparisons. The Friedman’s test was used to analyze the results from each third of the same group. The level of significance was set at $p < 0.05$.

Results

The Cohen’s *k* statistic was used to analyze intra-examiner and inter-examiner agreement among the evaluators: kappa test results, showed good to excellent reliability and reproducibility among the four observers, with all *k*-values ≥ 0.9 for the different groups and the difference between matched grades never exceeded 1 score. Debris and smear scores of the three experimental groups and the two control groups at coronal, middle and apical levels are expressed as percentage distribution in the *fig. 5* (*graphs 5a* and *5b* respectively). Examination of the surface of the root canal walls in the

EV-control and NI-control groups revealed its complete or nearly complete cover with conglomerations of debris and a heavy homogeneous smear layer (*fig. 6*).

Examination of the surface of the root canal walls in the EVM group revealed clean root canal wall with only very few debris particles and few small conglomerations of debris and there was no smear layer at all (*fig. 6*).

Comparative evaluation of debris and smear layer scores between groups at each level has been demonstrated in *Tables 1* and *2*. The Kruskal-Wallis statistic showed that the smear layer and the debris scores for all the experimental groups were significantly different from those for the two control groups ($p < 0.001$). The Dunn’s rank sum test showed no significant differences ($p > 0.05$) among all the experimental groups at the apical third with respect to the debris scores. Nonetheless, significantly better results were obtained for the coronal ($p < 0.001$) and middle ($p < 0.01$) areas in EVM group than in EV group. Furthermore, there was no statistically significant difference between NI and EVM groups ($p > 0.05$) at the coronal and middle levels, but significantly more debris were removed from the coronal ($p < 0.001$) and middle ($p < 0.001$) areas in NI group than in EV group. EVM technique performed significantly better than EV technique ($p < 0.001$) at all levels with respect to removal of the smear layer. Moreover, EVM protocol resulted in significantly more smear layer removal than needle irrigation at the coronal ($p < 0.05$), middle ($p < 0.001$) and apical ($p < 0.001$) levels. For the coronal region, significantly better results ($p < 0.001$) were detected in NI group than in EV group with respect to smear score; no significant difference ($p > 0.05$) between NI and EV groups was observed at the middle level.

Significantly more residual smear layer than in the EV group ($p < 0.001$), with scores of 3-5, was observed at the apical level in the NI group. The differences among all thirds of the same group, analyzed statistically by using the Friedman’s test are illustrated in *Table 3*.

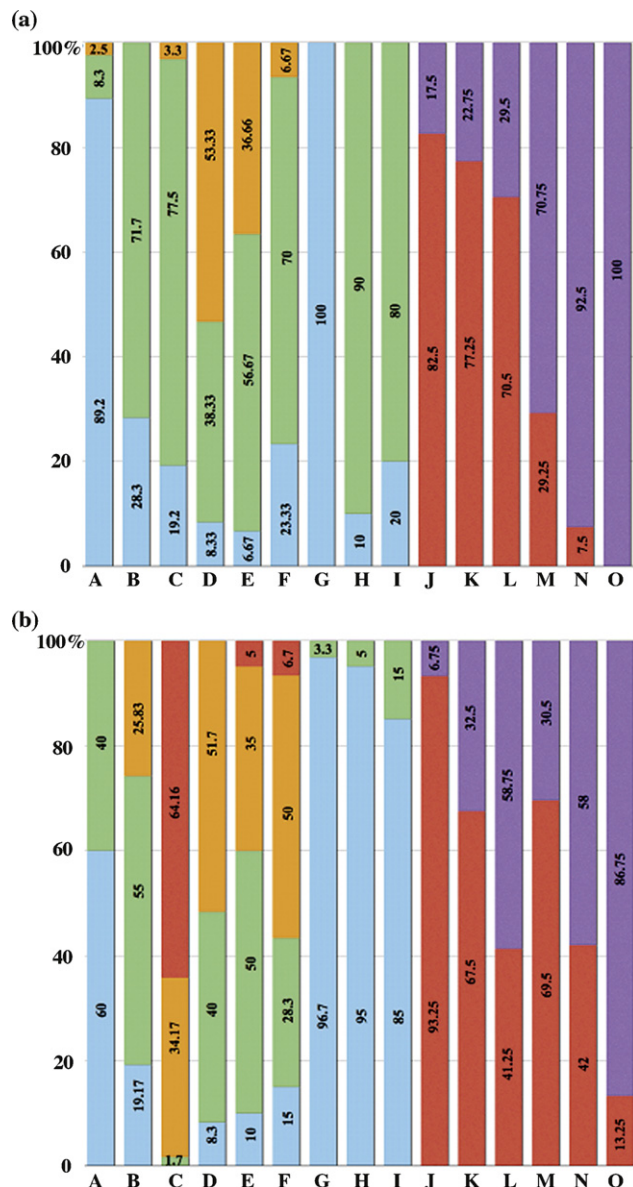


Figure 5 Percentage distribution of debris scores (a) and smear scores (b) at coronal (A,D,G,J,M), middle (B,E,H,K,N) and apical (C,F,I,L,O) levels in NI group (Needle irrigation / 30G-Max-I-Probe side-vented needle) (A-C), EV group (EndoVac System) (D-F), EVM group (EndoVac /modified technique) (G-I), EV-C group (EndoVac-negative control) (J-L), NI-C group (Needle irrigation-negative control) (M-O).

Discussion

In the present study cleansing and debridement efficacy of three different irrigation regimens, in the coronal, middle and apical thirds of root canal walls were investigated using SEM micrographs, separately for debris and smear layer, and the scoring system proposed [45]. The use of an *ex vivo* closed-end canal model more accurately simulates *in vivo* situations, in which the tooth’s foramen and outer surface are sealed by the periodontal ligament and further embedded in alveolar bone [5,34].

A new protocol for ANP irrigation (EVM) with EndoVac system (Discus Dental, Culver City, CA, USA) was evaluated in comparison with EndoVac according to manufacturer’s instructions and needle irrigation. For the EVM group macro-irrigation began during rotary instrumentation, between each instrument change by using at the same time MDT on pulp chamber and macro-cannulus into the root canal, placed up to the apically binding point without “up-down” motion; once the MAF, reached WL, the micro-cannulus replaced macro-cannulus and a 3 cycles sequence of micro-irrigation started at WL; the micro-cannulus was constantly left at WL without “up-down” movements.

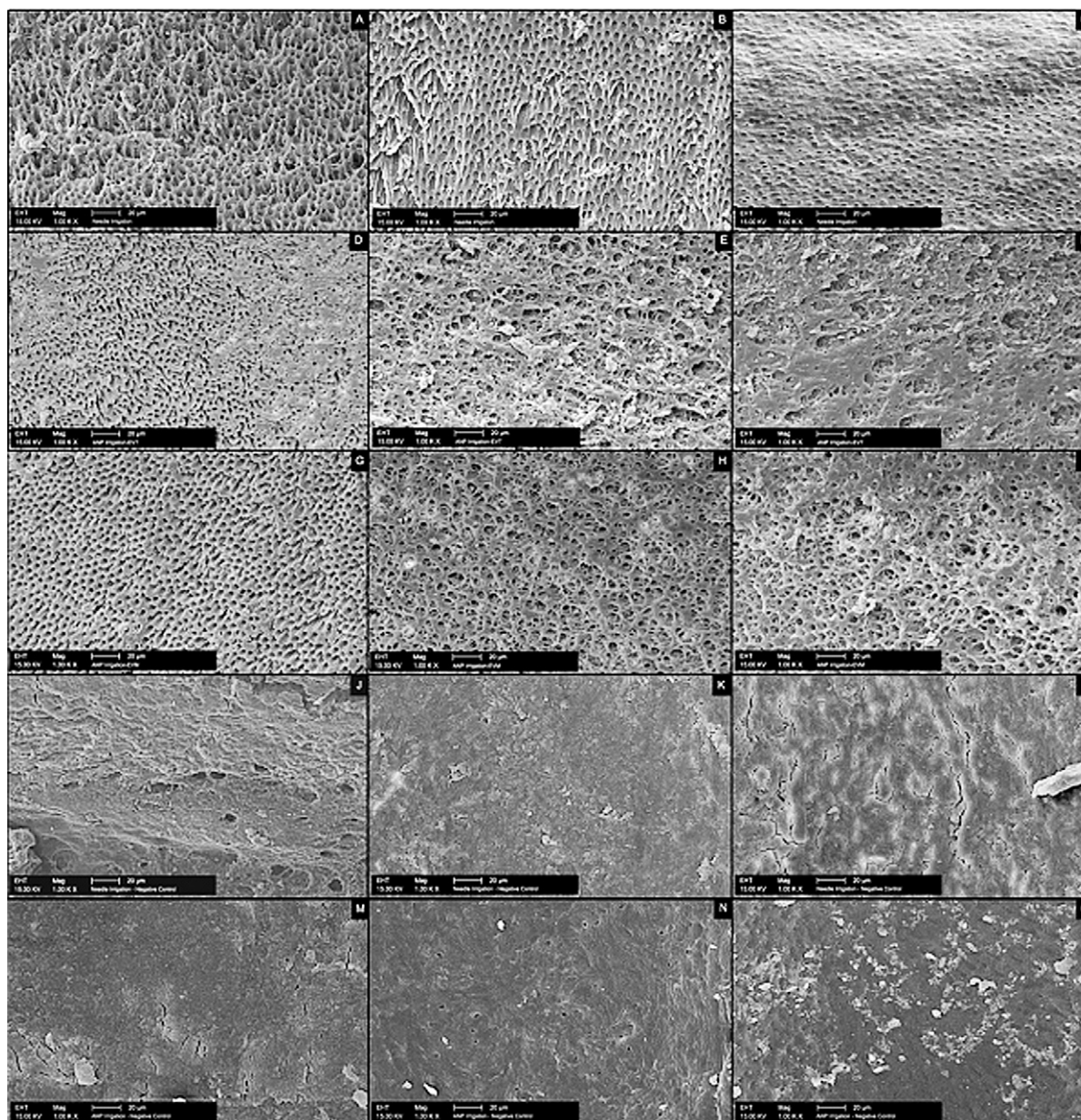


Figure 6 Scanning electron microscopy (SEM) images of the cleaned and shaped canal walls (Scale = 20 μm ;Mag = 1.00 K X; EHT = 15.00 kV) taken from coronal (A,D,G,J,M), middle (B,E,H,K,N) and apical (C,F,I,L,O) thirds for NI group (Needle irrigation) (A-C), EV group (EndoVac System) (D-F), EVM group (EndoVac-modified technique) (G-I), EV-C group (EndoVac-negative control) (J-L), NI-C group (Needle irrigation-negative control) (M-O).

An isolated observation of the coronal and middle thirds displayed that the EndoVac system (EV) was significantly less effective than needle irrigation in debris removal with only 8.33% and 38.33% of the coronal images and 6.67% and 56.67% of the middle micrographs showing scores 1 and 2 respectively. The introduction depth of cannulas tips and the distance to the dentinal wall seem to play an important role in the removal of debris, reinforcing the benefit of the physical flushing action [29]. Boutsoukis et al. [46] found that increasing the apical preparation size or taper of the root canal further than a certain value might in fact decrease the debridement efficacy of needle irrigation, because the average velocity and the wall shear stress decrease. A similar finding has been reported in a previous study simulating

irrigant flow in root canals with different apical sizes [47]. The larger distance of the cannulas and their tips to the dentinal walls in the coronal and middle thirds might have an effect on the mechanical debridement in these sections of the RCS. The closer distance of the cannulas and needle, and their tips to the dentine walls in the apical third might explain debris scores in this section of the RCS. The Friedman's statistic confirmed significantly better performance ($p < 0.001$) of the EndoVac system (EV) at the apical third than at the coronal and middle thirds with respect to debris score. When macro-irrigation began early during instrumentation (EVM protocol), it provided significantly more effective debris removal in the coronal and middle regions than in EV group, with 100% and 90% (respectively) of SEM

Table 1 Dunn's rank sum test for multiple pair-wise comparisons of debris scores for needle irrigation (NI) and ANP irrigation with EndoVac system according to manufacturer's instructions (EV) and to a new protocol (EVM).

Subgroups [*]		<i>p</i> value
(NI Coronal) ^a	<i>versus</i> (EV Coronal) ^b (EVM Coronal) ^a (EV-C Coronal) ^c (NI-C Coronal) ^c	<i>p</i> < 0.001
(NI Middle) ^d	<i>versus</i> (EV Middle) ^e (EVM Middle) ^d (EV-C Middle) ^f (NI-C Middle) ^f	
(NI Apical) ^g	<i>versus</i> (EV Apical) ^g (EVM Apical) ^g (EV-C Apical) ^h (NI-C Apical) ^h	

Significance level of $\alpha = 0.05$; *Subgroups with same superscript are not statistically significant ($p > 0.05$); EV-C: EndoVac-negative control; NI-C: Needle irrigation-negative control; ANP: apical negative pressure.

Table 2 Dunn's rank sum test for multiple pair-wise comparisons of smear scores for needle irrigation (NI) and ANP irrigation with EndoVac system according to manufacturer's instructions (EV) and to a new protocol (EVM).

Subgroups [*]		<i>p</i> value
(N Coronal) ^a	<i>versus</i> (EV Coronal) ^b (EVM Coronal) ^c (EV-C Coronal) ^d (NI-C Coronal) ^e	<i>p</i> < 0.001
(NI Middle) ^f	<i>versus</i> (EV Middle) ^f (EVM Middle) ^g (EV-C Middle) ^h (NI-C Middle) ^h	
(NI Apical) ^l	<i>versus</i> (EV Apical) ^j (EVM Apical) ^k (EV-C Apical) ^l (NI-C Apical) ^l	

Significance level of $\alpha = 0.05$; * Subgroups with same superscript are not statistically significant ($p > 0.05$); EV-C: EndoVac-negative control; NI-C: Needle irrigation-negative control; ANP: apical negative pressure.

micrographs showing score 1 and no significant difference between middle and apical thirds. Susin et al. [41] suggested that the difficulty in getting irrigating solutions to reach the isthmus and to create a strong enough current to flow through the isthmus between canals could explain why ANP irrigation did not completely remove debris from the isthmus regions in a closed canal system. It should be noted that debris removal is certainly much more difficult in the narrow isthmus regions

Table 3 Statistical analysis of differences among all thirds of the same group (Friedman's test).

Groups		C vs M	C vs A	M vs A
NI	debris scores	***	***	*
	smear scores	***	***	***
EV	debris scores	**	***	***
	smear scores	ns	ns	*
EVM	debris scores	***	***	ns
	smear scores	ns	*	ns
EV-C	debris scores	ns	ns	ns
	smear scores	***	***	***
NI-C	debris scores	***	***	US
	smear scores	***	***	***

C: coronal third; M: middle third; A: apical third; significance level of $\alpha = 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

than in the instrumented canals, since this complicated morphology renders it extremely difficult for the delivery of a large volume of irrigant with a high flow rate even with the use of the ANP technique. It is possible to assume that ANP irrigation performed following EVM protocol could improve debridement efficacy into uninstrumented areas of RCS.

No significant differences were detected in debris removal among NI apical, EV apical, and EVM apical subgroups with most of the SEM images showing score 1 (19.2%, 23.33%, 20% respectively) and 2 (77.5%, 70%, 80% respectively). It is possible to assume that apical size and taper of the root canal results in a sufficient increase in the cross-sectional area of the annulus between the needle or cannulas and the root canal wall to supply adequate irrigant flow rate to the WL without blocking the backflow. It must be emphasized that the disruption or detachment of debris cannot ensure their removal unless there is a favorable irrigant flow to carry them toward the canal orifice (reverse flow) [47]. Although no consensus exists regarding the minimum apical preparation size or taper, instrumentation to size ISO #35 or #40 results in clinically adequate irrigant volume amounts for both positive and negative pressure systems [48]. Following the manufacturer's recommendations, an ISO #35 was considered to be the smallest apical size to effectively allow irrigant to pass circumferentially around the 0.32 mm micro-cannulus. An increase in size from ISO #35 to ISO #40 resulted in a percentage gain of approximately 44% in mean irrigant volume [48]. As the apical size increases to a size of ISO #40, there is a decreased chance of suction holes, along the side of the last 0.70 mm of the micro-cannulus, contacting the root canal wall and becoming blocked. Also, the concomitant and more potent coronal aspiration with the MDT competes with the micro-cannulus for fluid evacuation.

For the EV group, the micro-cannulus was placed in sequence at WL for 6 seconds, 2 mm short of the WL and back to the WL and so on: this alternating movement allows for the removal of micro-bubbles of ammonium and carbon

dioxide resulting from the hydrolysis of organic tissue. In the apical third these micro-bubbles could isolate residual tissue from further contact with hypochlorite, adhering to the dentinal walls, the micro-cannulus and tissue remnants. As the apical size increases to a size of ISO #40, the larger area surrounding the micro-cannulus also allows for increased volume of irrigant to the micro-cannulus tip and increased wall shear stress [48]: “up-down” movements become unnecessary.

Concerning coronal level, the needle irrigation achieved significantly more smear layer free canal walls than EndoVac System (EV) with 60% and 40% (versus 8.3% and 40%) of micrographs showing smear score 1 and 2 respectively, with a trend toward better smear layer removal in the middle third too (19.17% and 55% versus 10% and 50% of the images showing score 1 and 2 respectively). It might be speculated that the coronal and middle thirds are flushed more often with NaOCl during the clinical procedure, resulting in better debridement and smear removal coronally. In the EV group, only after reaching WL with the MAF, macro-irrigation with the EndoVac was accomplished. Sodium hypochlorite was then used to replenish the irrigant only in the pulp chamber after each rotary NiTi instrument, injecting fresh NaOCl down the canal, since fresh NaOCl was dynamically exchanged throughout instrumentation. When the instrument is removed coronally, according to Archimedean principle of fluid displacement, the NaOCl from the pulp chamber should replace it [49]. This constant exchange would negate the need for injecting fresh NaOCl down the canal. It is possible to assume that upon completion of all rotary preparations, the root canals could be clean, but not enough to allow effective debris and smear removal at the end of root canal treatment. The micro-hurricane of NaOCl created inside the RCS by using the macro-cannulus, could create a pressure-washing effect along the dentinal walls not sufficient to ensure effective flushing action of irrigants and macro debris evacuation.

Abarajithan et al. [40] showed that ANP irrigation and conventional irrigation were equally effective in removing smear layer from the coronal and middle thirds of the root canals, while in the apical third the Endovac system performed significantly better than needle irrigation. Their results are questionable because apical size preparation was in their study standardized to ISO size #60 to improve the irrigant flow into open-end root canals. The difference in smear layer removal at all levels between EVM and EV protocols was statistically significant ($p < 0.001$), and both techniques performed significantly better than needle irrigation in the apical region ($p < 0.001$). A possible explanation of this finding is that positive pressure irrigation might fail to avoid vapor lock effect in a closed canal system, that more accurately simulate *in vivo* application of irrigants, with less effective contact time between irrigants and dentine in the apical third. Vapor lock that results in trapped air in the apical third of root canals might hinder the exchange of irrigants and affect their debridement efficacy [32].

Parente et al. [50] reported that ANP irrigation can overcome the fluid dynamics challenges inherent in closed canal systems, producing clean dentinal surfaces in closed-end root canals instrumented to size #40/.06 taper. The EndoVac system's effectiveness in producing clean dentinal surfaces may be attributed to its ANP approach. Placement of the

macro-cannulus at middle-apical third of the canal followed by the placement of the micro-cannulus directly at the apical end enables an irrigant to be suctioned in sufficient volume and flow to displace debris and remove smear layer. Additionally, the orifices of the micro-cannulus provide a portal of exit for canal debris in closed-end canal systems. In our study, root canal preparation to final size #40/.04 taper was estimated [48] to represent a good balance of tooth structure preservation and adequate volume of irrigation at the apical third when using the ANP irrigation system, since an increase in preparation taper from size #40/.04 taper to size #40/.06 taper resulted in volume percentage gain of only 5.4% [48]. The negative pressure irrigation according to EVM technique performed significantly better than EndoVac system (EV) also at the apical level ($p < 0.001$): this finding might be attributed to 30 seconds of active irrigation for “microcycle” with micro-cannulus constantly placed at full WL, providing a supportive effect on smear layer removal. The Friedman's test confirmed almost uniform high effectiveness for EVM protocol throughout root canals with respect to smear score, with no significant difference between middle and apical thirds ($p > 0.05$) and between coronal and middle thirds ($p > 0.05$). The results obtained from this study rejected the null hypothesis. Under laboratory conditions both negative and positive pressure irrigations with NaOCl and EDTA as irrigants, showed no statistically significant difference in antimicrobial efficacy against *E. faecalis* [51,52], confirming that deep disinfection depends on penetration ability of irrigants into dentinal tubules.

Negative pressure irrigation may improve irrigants volumes, intimacy and time of contact with root canal walls, especially into uninstrumented areas of the RCS, enhancing surface debridement and disinfection: it would be of interest to optimize exposure time and volume for root canal irrigants balancing debridement and disinfection effectiveness with respect of the structural and mechanical properties of dentine.

Conclusions

Under the conditions of the present study, ANP irrigation showed significantly better performance in removing smear layer compared with needle irrigation in the apical third of RCS. A new irrigation protocol by using EndoVac System resulted in better removal of the smear layer than EndoVac system used according to the manufacturer's instructions and needle irrigation, at all levels, in a simulated closed canal system. Further research is needed to confirm our results in curved canals and to determine whether this difference in remaining canal debris affects clinical success.

Clinical relevance: Although instruments remove most of the canal contents in the main root canal area, irrigation plays an indispensable role in all areas of the RCS inaccessible for instrumentation. One of the most favorable features of irrigants is their flushing action. A new irrigation protocol by using EndoVac System could ensure a pressure-washing effect along the dentinal walls sufficient to allow effective flushing action of irrigants at all levels. Moreover it could produce a strong enough current to flow through the isthmus between canals delivering large volume of irrigants with a high flow rate and allowing effective macro debris evacuation.

Conflict of interest

The authors deny any conflicts of interest related to this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.gien.2012.09.001>.

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