Research Article

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The Influence Of Combining Different Irrigation Techniques On The Reduction Of Endodontic Microflora.

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ABSTRACT

This study evaluated the effectiveness of various irrigation agitation systems in reducing endodontic microflora. The methods examined included EndoActivator, EndoVac, a combination of EndoActivator and EndoVac, and passive syringe irrigation.

Keywords : *EndoVac, EndoActivator*.

INTRODUCTION

Successful endodontic treatment relies on the thorough elimination of microorganisms from the root canal system and the prevention of reinfection. Consequently, chemo-mechanical preparation is a crucial step in root canal therapy. The primary objective of instrumentation is to facilitate effective irrigation, disinfection, and filling, thereby meeting both mechanical and biological goals.

However, achieving complete debridement within the root canal can be challenging, as instruments primarily address the central canal area. Areas such as canal fins and isthmuses often remain untouched after preparation, potentially harboring debris, microorganisms, and their by-products, which can lead to periradicular inflammation.

Additionally, the vapor lock effect occurs when sodium hypochlorite (NaOCl) interacts with organic material in the root canal, forming micro-gas bubbles at the apical termination. These bubbles can coalesce into an apical vapor lock, hindering the flow of irrigants to the apex. Therefore, agitation of irrigants within the root canal is essential.

Various agitation techniques exist, one of which is sonic agitation. This method operates through two mechanisms—cavitation and acoustic streaming—both of which enhance debridement and aid in the removal of endodontic biofilm. The EndoActivator system is designed to improve disinfection by generating mechanical oscillations primarily at the activator tip, with frequencies ranging from 1 to 10 kHz.

Another technique involves using apical negative pressure to draw fluids apically, which provides effective cleaning and reduces the risk of irrigation accidents. Research indicates that apical negative pressure systems are particularly effective in reducing bacterial counts, especially in the apical onethird of the root canal.

Based on these insights, we hypothesize that the combined method of sonic activation followed by apical negative pressure as a final irrigation protocol will yield superior results in canal debridement and bacterial reduction compared to passive syringe irrigation. The null hypothesis posits that there is no significant difference between the combination method and passive syringe irrigation.

MATERIALS AND METHODS

Samples selection

Forty-four permanent maxillary human anterior teeth featuring a single canal, an apical foramen, canal curvature between 0 and 10 degrees, and no signs of apical resorption were selected. The teeth were digitally radiographed to verify the presence of a single patent root canal, free from complex anatomical features. They were then autoclaved for 40 minutes for sterilization and stored in distilled water until needed to prevent dehydration.

Preparation of Samples

The teeth were decoronated, and the root length was

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standardized to 15 mm using a diamond disc operated at low speed with ample coolant. After decoronation, a #10 K-file was inserted and measured until its tip was just visible at the apical foramen, establishing the working length by subtracting 1.0 mm from this measurement. Protaper Universal files were then used to prepare the canals up to the F4 file. Each time a file was exchanged, the canals were irrigated with 5 ml of 2.5% NaOCl using a 30 G needle. At the end of the instrumentation, the canals were flushed with 5 ml of NaOCl, followed by a 1-minute irrigation with 17% EDTA, and finally rinsed with 3 ml of saline. The canals were dried using #40 paper points. To ensure complete sterilization of the root canals, the teeth were autoclaved at 121°C for 20 minutes.

Canal Inoculation with Enterococcus faecalis

A standard suspension of E. faecalis was prepared. Each canal was filled to the orifice level with this suspension using sterile 1-mL insulin syringes fitted with a 30-gauge needle. The roots were then placed in 15-mL tubes containing 10 mL of Brain Heart Infusion broth and incubated at 37°C for 21 days in 100% humidity, allowing the bacteria to colonize the canal walls and penetrate the dentinal tubules. Every three days, 5.0 mL of the culture medium was replaced with fresh broth. After 21 days, the specimens were removed from the inoculation tubes, and the root apices were sealed with composite resin in a clean environment to create a closed system.

Sample Classification

The samples were randomly divided into four groups, along with one positive control and one negative control group.

Group 1: EndoActivator (n=10)

The red EndoActivator tip (25/04) was manually fitted loosely within 1 mm of the working length and activated at a speed of 10 kHz in an up-and-down motion for 1 minute using NaOCl, followed by 1 minute with EDTA.

Group 2: EndoVac (n=10)

Following the method described by Nielsen & Craig Baumgartner, a delivery tip was attached to a syringe connected to the dental chair's high-speed suction. A small tube connected a macro- or microcannula to the suction. The delivery/evacuation tip introduced the irrigant into the chamber while removing excess fluid. Negative pressure pulled the irrigant apically toward the cannula. After reaching the working length with the F4 file, macro-irrigation with NaOCl was performed over 30 seconds, moving the macrocannula up and down in the canal. The canal was then left undisturbed, filled with irrigant for 60 seconds. Three cycles of microirrigation followed: the first with 5.25% NaOCl, the second with 15% EDTA, and the third again with 5.25% NaOCl.

Group 3: EndoActivator and EndoVac (n=10)

This group first used the EndoActivator with the red tip (25/04), followed by the EndoVac system using the same technique, but with the irrigation duration halved.

Group 4: Passive Syringe Irrigation (n=10)

A 30-gauge side-vented needle was placed 1 mm from the working length to deliver 3 mL of 2.5% NaOCl. During the irrigation, the needle was moved up and down to enhance agitation.

Positive Control: This group did not receive any treatment, ensuring the presence of *E. faecalis*.

Negative Control: These specimens were mechanically prepared, autoclaved, and did not receive *E. faecalis*, ensuring they remained sterile.

Method of evaluation

Confocal laser scanning microscopy

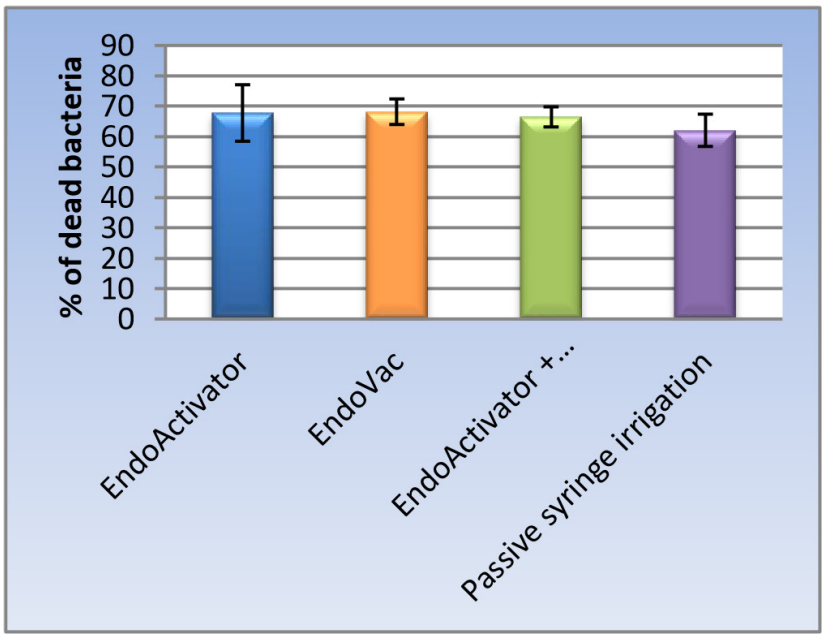
To assess the effectiveness of each disinfection technique in eliminating bacteria from the dentinal tubules, confocal laser scanning microscopy (CLSM) was utilized to directly visualize live and dead bacteria. Specimens were stained with 10 microliters of the LIVE/DEAD BacLight Bacterial Viability kit for 15 minutes, following the manufacturer's instructions. After staining, the specimens were washed with distilled water and mounted on a glass slide, which was then covered with a cover slip. A Zeiss Confocal Laser Scanning Microscope 710 Axio Observer was configured with an excitation wavelength of 543 nm and emission wavelengths of 561 nm, operating at a 16-bit depth to examine the tooth samples. CLSM images were captured using the Zen Lite 2012 software (Carl Zeiss) at a resolution of 1024 × 1024 pixels, and the resulting image stacks were analyzed with the LSM browser. For CLSM analysis, images were taken from three random locations within the root (coronal, middle, and apical) at various depths. At each depth, the software quantified the intensities of red fluorescence (indicating dead bacteria) and green fluorescence (indicating live bacteria).

RESULTS

Statistical analysis was conducted using IBM® SPSS® Statistics Version 20 for Windows. The results indicated that both the irrigation technique and the root level, as well as the interaction between these two variables, had a statistically significant impact on the mean percentage of dead bacteria. Given the statistical significance of the interaction, it suggests that these variables are dependent on one another.

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Figure 1.



Effect of irrigation technique regardless of root level

Regardless of root level; there was no statistically significant difference between EndoActivator, EndoVac and EndoActivator+ EndoVac techniques; all showed statistically significantly higher mean percentage of dead bacteria than passive syringe irrigation.

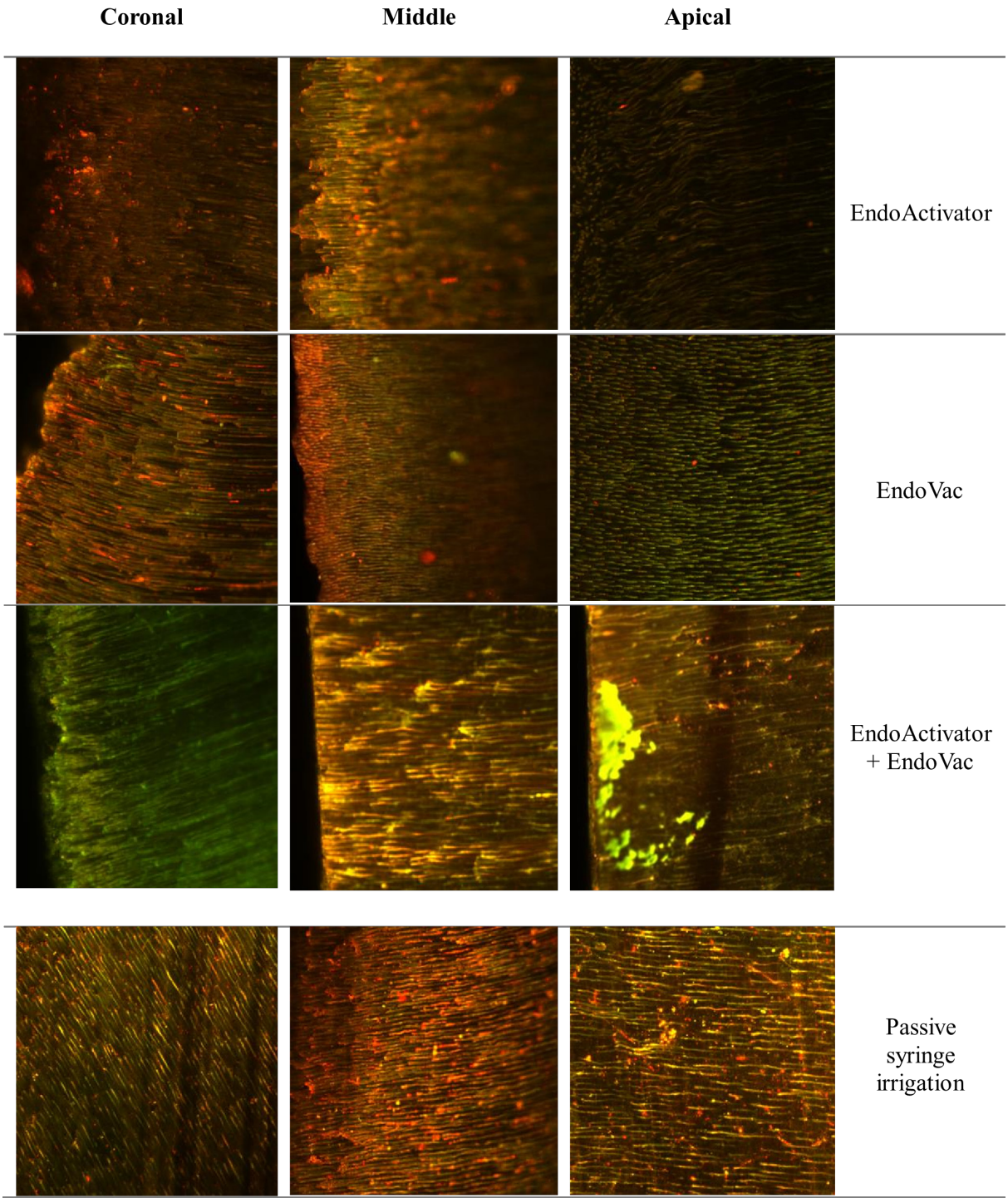
Table (1). The mean, standard deviation (SD) values and results of repeated measures ANOVA test for comparison between percentages of dead bacteria with different irrigation techniques regardless of root level.

Table 1 : * Significant at P ≤ 0.05, Different superscripts are statistically significantly different.

EndoActivator		**EndoVac**		**EndoActivator + EndoVac**		**Passive syringe irrigation**		**Pvalue**
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
67.8 ^{A}	9.3	68.2 ^{A}	4.2	66.5 ^{A}	3.3	62.1 ^{B}	5.3	0.034*

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Figure 2: shows live and dead bacteria in coronal, middle and apical third of EndoActivator, EndoVac, combination between both and passive syringe irrigation.



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DISCUSSION

The primary objectives of endodontic therapy are to thoroughly clean and shape the root canal and to achieve complete three-dimensional obturation of the root canal system, thereby facilitating apical healing. [3]

Research has demonstrated that 35% or more of the root canal surface area remains unaltered following instrumentation[4], Thus, adequate irrigation is crucial for cleaning these untouched areas. Various methods have been used to irrigate the root canal and eliminate microorganisms; however, none of these techniques can completely eradicate bacteria from the apical 1 mm of the root canal[5, 6].

Passive syringe irrigation is a widely used technique for root canal irrigation; however, it is often ineffective in the apical third of the root canal. The smaller diameter of this region compared to other areas limits the circulation and effectiveness of the irrigating solutions[7]. Previous studies have indicated that current irrigation methods, such as passive syringe irrigation, are effective in cleaning the coronal portions of root canals but are significantly less effective in the apical sections[5,8]. as mechanical flushing action of conventional passive syringe irrigation is very weak. Another problem that encountered during irrigation with needle which is presence of vapor lock that hinder the penetration of the irrigation into apical one third which has a negative effect on root canal debridement [9]. So the aim of the study was to assess the effectiveness of different agitation techniques in bacterial reduction in the root canal space. Human teeth were selected to simulate the clinical condition that might face any practitioner during root canal treatment, so upper central incisors were used because of the straight and single root canal configuration. All samples were autoclaved to allow safe handling. Teeth were decoronated to a length 15 mm to represent root portion only. Preparation was done by using protaper Universal as protaper Universal was considered a gold standard files. Preparation was done up to F4 file to allow standardization of the preparation, ensure presence of progressive taper, increase the efficacy of the irrigation [10] and to allow 30-gauge needle to reach 1 mm of the working length so the apical preparation (0.40 mm tip size) must be larger than the needle diameter. During preparation, NaOCl was used as an irrigant of choice as it is the most widely used irrigant in the chemomechanical preparation of root canal system because it has a strong antimicrobial activity and has ability to dissolve organic materials [11], however NaOCl alone cannot effectively remove the smear layer, so the association of EDTA and NaOCl solutions has proved to be effective in removing smear layer [8]. E. faecalis was chosen for our study as it has been the most commonly isolated bacteria from the root canal system especially in failing endodontic cases [12, 13, 14] In addition, E. faecalis can penetrate into the dentinal tubules and form biofilms [15, 16], which are more resistant to canal disinfection [17, 18]. Samples were left for 21 days because it was a sufficient time to ensure proper inoculation of bacteria inside dentinal tubules and formation of well matured biofilm. After 21 days of incubation period, root apices were sealed by composite resin to prevent bacterial leakage and to simulate in vivo condition (closed system) [19].

EndoActivator which represent the sonic agitation device, as found in literature, EndoActivator has increased the antibacterial activity of NaOCl on E. faecalis[1]. Because it generates subsonic micro acoustic streaming in an irrigant and cavitation. When cavitation bubbles are produced by acoustic waves, they eventually collapse and the energy released is transferred to the root canal, providing effective biofilm dislodgement which could be the reason for the reduction of bacteria after using EndoActivator in this study.

EndoVac system which uses the idea of apical negative-pressure irrigation can effectively safely irrigate the root canal system up to the working length without extrusion of the solution beyond the apical constriction of the canal [20]. This can be explained by the design of the microcannula, which eliminates vapor lock, allowing the apical exchange of irrigants. The volume of irrigant delivered to the canal apically by the EndoVac system was significantly higher than the volume delivered by conventional syringe needle irrigation during the same time period.

The combination between the EndoActivator and EndoVac might show better results on bacterial reduction as it combines the benefit of using sonic energy and negative apical pressure.

Passive syringe irrigation which was the control group as it is the most widely used technique of irrigation.

Following treatment, samples were stored in clean sterile Eppendorf containing distilled water until splitting was done. During sectioning copious amount of coolant was necessary to avoid thermal elevation and bacteria killing.

There are many tools used to detect bacteria like wide field microscope, scanning electron microscope and confocal laser scanning microscope. Confocal Laser Scanning Microscope was a valuable tool to detect live and dead bacteria. It provides detailed information about the presence and distribution of bacteria inside dentinal tubules in the total circumference of the root canal walls at relative low magnification through the use of fluorescent stains.

The main reason for using the confocal laser scanning approach is its spatial filtering capabilities, which effectively eliminate out-of-focus light and glare in specimens that are thicker than the immediate plane of focus. Confocal technology is indeed one of the most significant advancements in optical microscopy.

Our findings indicated that complete eradication of *E. faecalis* from the root canal system is unattainable, as bacteria can

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infiltrate the dentinal tubules beyond the reach of NaOCl. Previous studies have also highlighted that no irrigation technique is capable of completely removing bacteria from the root canal system, a result consistent with those reported by Mancin.

Regardless root level, EndoActivator, EndoVac and the combination between both showed statistically significant higher mean percentage of dead bacteria than passive syringe irrigation and this was return to acoustic streaming and cavitation that produced during using EndoActivator and the large amount of irrigation that delivered into apical one third during using the EndoVac, this result was similar to Kadhom et al.

Brito et al. compared the effectiveness of three irrigation techniques on the reduction of intracanal Enterococcus Faecalis and found that there was no significant difference among conventional irrigation, EndoActivator and EndoVac irrigation technique. Regardless irrigation technique, the coronal level showed the highest mean percentage of dead bacteria as this third was received the highest amount of irrigation and there is no vapor lock effect, while middle and apical showed the lowest mean percentage of dead bacteria and there was no statistically significant difference between middle and apical.

The null hypothesis tested was rejected.

CONCLUSION

There is no significance difference between using EndoActivator or EndoVac separately or using them in combination.

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