Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i4.5388

COMPARATIVE EVALUATION OF DIFFERENT IRRIGATION AGITATION METHODS IN BACTERIAL REDUCTION OF OVAL SHAPED CANALS: AN IN VITRO STUDY

Santosh Hugar[1]*, Ruhi Sankpal[2], Girish Umashetty[3], Suvarna Patil[4]

[1]*M.D.S, HOD , Department of Conservative Dentistry and Endodontics, Vasantdada Patil Dental College and Hospital , Kavalapur.
[2]M.D.S, Student III year, Department of Conservative Dentistry and Endodontics, Vasantdada Patil Dental College and Hospital, Kavalapur.
[3]M.D.S , HOD , Department of Conservative Dentistry and Endodontics, Vasantdada Patil Dental College and Hospital, Kavalapur.
[4] M.D.S, Professor, Department of Conservative Dentistry and Endodontics, Vasantdada Patil Dental College and Hospital , Kavalapur.

***Corresponding Author :** Santosh Hugar
*M.D.S, HOD , Department of Conservative Dentistry and Endodontics, Vasantdada Patil Dental College and Hospital , Kavalapur.

ABSTRACT

INTRODUCTION: Thorough cleaning of the pulp space is a challenging task. The mechanical instrumentation alone is usually not sufficient to completely debride the canals, and therefore, it requires the chemical action of irrigants also to disinfect the difficult to reach areas. Sodium hypochlorite is the most used irrigating solution in endodontics, because its mechanism of action causes biosynthetic alterations in cellular metabolism and phospholipid destruction, formation of chloramines that interfere in cellular metabolism, oxidative action with irreversible enzymatic inactivation in bacteria, and lipid and fatty acid degradation.

MATERIAL AND METHOD: : Fourty extracted human teeth with similar dimensions were selected. Access cavities were prepared and the root canals were instrumented using Heroshaper Gold (25/4
%). The root canals were then contaminated with an E. faecalis suspension following incubation for thirty days. The contaminated roots were divided into three experimental groups (n=10) according to the irrigant agitation protocol (Passive ultrasonic irrigation XP ENDO Finisher Group and sonic irrigation), in addition to a positive control group (n=5) and a negative control group (n=5). Microbiological samples were taken from the root canals before instrumentation (S1), after instrumentation (S2) and after the final irrigation protocol (S3). The samples were assayed and incubated for 48 hours in order to obtain the residual titer of E. faecalis cells. Viable cells were quantified by colony-forming units (CFU) measurement. The collected data was submitted to statistical analysis descriptive quantitative data was expressed in mean and standard deviation respectively. Data normality was checked by using Shapiro – Wilk test. Confidence interval is set at 95% and probability of alpha error (level of significance) set at 5%. Power of the study set at 80%.

RESULTS: Result demonstrated that all final agitation protocols were effective in reducing the bacteria with more number of reduction in XP endo finisher 0.1 (0.31) and passive ultrasonic irrigation 0.1 (0.31) followed by Endoactivator 0.4 (0.51).

CONCLUSION: All additional cleaning techniques improved the disinfection, regardless of the region under study . XPEFR was more effective than PUI and EAS. However, none of the techniques was capable to render the root canals completely free of microorganism

INTRODUCTION

- The mechanochemical cleaning of the pulp chamber and canal space contributes greatly to the success of root canal therapy.
- Not only the shaping of the canal but irrigation also plays a prime role in eliminating any remaining pulp tissues and debris.
- Sodium hypochlorite (NaOCl) is the most frequently used irrigant in endodontic therapy, but its periapical extrusion can cause adverse effects such as severe pain, edema, profuse hemorrhage, and ecchymosis. Hence, it is essential to achieve a balance between safety and effectiveness in this area
- The conventional needle (CN) irrigation is the most commonly used technique because of its easy availability.
- However, its cleaning efficacy greatly relies upon the depth to which the needle is placed in the canal, affecting the canal debridement.
- Thus, newer irrigation techniques have been introduced in recent times.
- Pécora et al. reported that sodium hypochlorite exhibits a dynamic balance as is shown by the reaction:

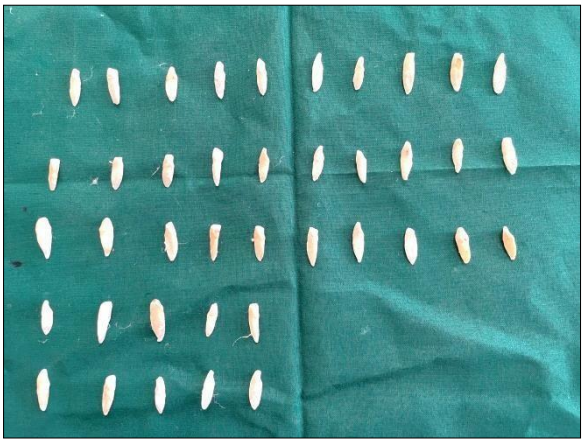
NaOCl + H2O « NaOH + HOCl « Na+ + OH- + H+ + Ocl

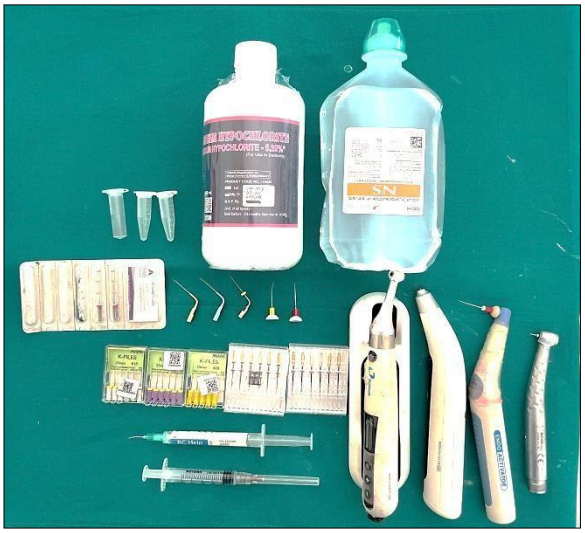
- Interpreting these chemical reactions, it can be observed that sodium hypochlorite acts as an organic and fat solvent degrading fatty acids, transforming them into fatty acid salts (soap) and glycerol (alcohol), that reduces the surface tension of the remaining solution (scheme 1).
- Sodium hypochlorite neutralizes amino acids forming water and salt (scheme 2 - neutralization reaction).

- Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as a solvent, releases chlorine that, combined with the protein amino group, forms chloramines (scheme 3 - chloramination reaction).
- Several studies have demonstrated that proportionally large areas of the main root-canal wall remains untouched by the instruments. Chemomechanical debridement is a crucial step in root canal therapy. Several investigations have revealed that the substantial areas of the root canal, especially at the apical third, are left undisturbed by the instruments
- Emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal.

MATERIALS AND METHODS

- Fourty extracted teeth were selected according to the following inclusion criteria: single-rooted mandibular human incisors, complete root formation, no evidence of root resorptions, no fracture or cracks, 20- to 22-mm in length, oval-shaped root canals (long/short cross section diameter ratio of ≥2.5 at 5mm from the apex) based in preoperative radiographs that were taken in the buccolingual and mesiodistal directions. The specimens were stored in 0.9% NaCl solution prior to their use . Traditional endodontic accesses were executed by means of round . Dental crowns were maintained so a reservoir for E. faecalis suspension could be obtained. A size10 K-file was chosen to determine apical patency observing its tip in the apical foramen and the working length (WL) was delimited 1 mm shorter.

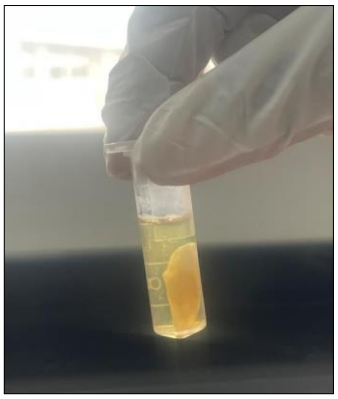




- The apical constriction was instrumented up to a size 20 K-file to standardize the apical constriction size. The smear layer was removed by irrigating with 3 mL of 17% EDTA for 3 minutes, in combination with 5 mL of 2.5% NaOCl. The bactericidal effect of NaOCl was inactivated by rinsing 5 mL 5% sodium thiosulfate. The teeth received two layers of clear nail polish on the entire external surface, including the apical foramen. This procedure was performed to prevent microleakage of bacteria through lateral canals. Next, a sterile size 15 K-file was placed 0.5 mm beyond the apical foramen to remove any blockage from the nail polish layers

TEST APPARATUS.

- Each tooth was inserted vertically in a Eppendorf tube up to the cervical region. The entire model was sterilized in an autoclave for 20 minutes at 127°C before infection procedures. Afterwards, 2 mL of sterile saline was added into the Eppendorf tubes to eliminate the entrapped air and to guarantee the vapor lock effect.



SPECIMEN INFECTION WITH E. faecalis

The infection of the study samples and sampling procedures were done according to de Brito et al. (15) with modifications. A pure culture of E. faecalis (ATCC 29212) (American Type Culture Collection, Virginia, EUA) was incubated overnight in brain-heart infusion broth and used to prepare an inoculum suspension. The bacterial suspension was adjusted spectrophotometrically to match the turbidity of 1.5x108 colony-forming units (CFU mL) equivalent to ±0.5 McFarland standard. Sterile pipettes were used, under Class I laminar flow to inoculate each specimen with 2 µL of the bacterial suspension. Thirty specimens were contaminated. A sterile size 10 K-file (Dentsply) was used to spread the bacterial suspension along the entire root canal length. Subsequently, the infected specimens were incubated at 37°C for 30 days and every 2 days received new sterile BHI medium to maintain the

biofilm growth. Subsequently, the root canals were irrigated with 1 ml of sterile NaCl and the first bacterial sample (S1) was taken by means of three sterile paper points size 15 introduced sequentially into the canals for 1 min. The paper points were then transferred to a sterile Eppendorf tube containing 1 ml of sterile saline solution. The CFU counts were performed as described in the quantification of the bacterial load.

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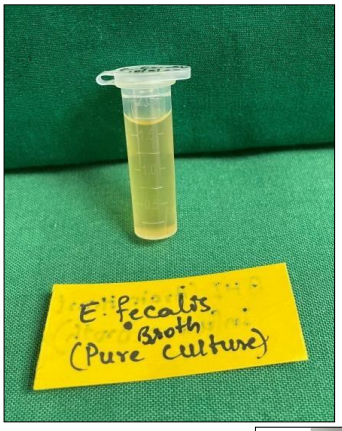

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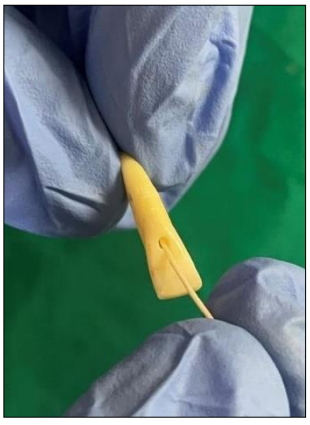

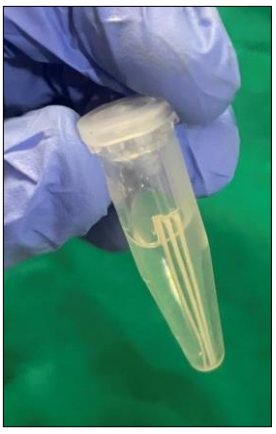

ROOT CANAL PREPARATION

- Root canal preparation One expert operator, using aseptic techniques, carried out canal preparation and sampling procedures on each specimen under a class I laminar flow hood, to prevent airborne contamination
- The apical preparation was done till 25/4 with Heroshaper Gold.
- Samples were irrigated intermittently with 5.25% sodium hypochlorite with 30-G side vented needle
- Then, the canals were filled with 2 mL of sterile saline and the second bacterial sample (S2) was collected

FINAL IRRIGATING PROTOCOL

- Teeth were randomly divided into three groups n=10
- Positive control n=5 – In this group, five root canals were contaminated but not instrumented.
- Negative control n=5 – To assure the absence of cross contamination during the experiment, five samples that were sterilized but not contaminated

All specimens from all groups were irrigated with 2 mL of 2.5% NaOCl (16) and the different final irrigant agitation protocols were performed, as follows:

- Passive Ultrasonic Group-Irrigating tip gently moved up and down till 1mm short of working length for 1 min
- XP ENDO Finisher Group-Rotated at 850 RPM with 1 NCM torque for 1 min till 1mm short of working length
- Sonic Irrigation Group-Irrigating tip gently moved up and down till 1mm short of working length for 1 min
- Then, the canals were filled with 2 mL of sterile saline and the second bacterial sample (S3) was collected with paper points.
- The Eppendorf tubes containing the collected samples (S1, S2 and S3) were taken and Each colony was counted as one Colony-Forming Unit (CFU).

STATISTICAL ANALYSIS

- Statistical analysis was performed using Statistical Package for Social science (SPSS) version 21 for Windows (SPSSInc).
- Descriptive quantitative data was expressed in mean and standard deviation respectively. Data normality will be checked by using Shapiro – Wilk test.
- Confidence interval is set at 95% and probability of alpha error (level of significance) set at 5%. Power of the study set at 80%.

- Overall intergroup comparison among three groups will be done using One-way Anova 'F' test followed by Tukey's post hoc test for pairwise intergroup comparison between each groups

	Before Treatment (S1) Mean (SD)	After Instrumentation (S2) Mean (SD)	After Irrigation & Agitation (S3) Mean (SD)
Group 1 (PUI Group)	472.0 (69.08)	1.5 (0.84)	0.1 (0.31)
Group 2 (XP Group)	483.4 (66.69)	2.3 (3.09)	0.1 (0.31)
Group 3 (EC group)	470.0 (88.47)	1.4 (0.51)	0.4 (0.51)

Table 1: Mean values (log 10) of the bacterial content found before (S1) and after the root canal instrumentation (S2) as well as after the final irrigation and agitation protocols (S3), with their respective percentage of bacterial reduction

Reduction of bacterial load	S1 -S2 Mean (SD)	S1-S3 Mean (SD)
Group 1 (PUI Group)	470.6 (68.95)	471.6 (69.4)
Group 2 (XP Group)	481.1 (67.08)	483.3 (66.7)
Group 3 (EC group)	387.5 (88.62)	388.9 (88.39)
One way Anova F test value	F = 4.610	F = 4.651
P value, Significance	p =0.019*	p =0.018*

*p<0.05 – Statistical significant difference

Table 2: Overall comparison of reduction of bacterial content from S1 to S2 stage and S1 to S3 Stage respectively using One way Anova F test

DISCUSSION

- XPEF file was introduced by FKG in 2015.It is suggested to be used at 800 rpm and 1.0 Newton Torque with irrigating solution after root canal preparation size #25 or larger
- In Passive ultrasonic irrigation the technique is based on the passive insertion of a metal tip/file coupled to an ultrasonic device oscillating at a frequency of 30 kHz into a root canal filled with an irrigating or chelating solution.
- The EndoActivator system (EAS) is a sonically driven irrigant activation tool which was developed to produce vigorous fluid agitation within the root canal.
- A significant bacterial reduction was observed in all groups after instrumentation . However, remaining bacteria inside the specimens was detected in all groups, confirming the need of supplementary disinfection protocol.
- Result demonstrated that all final agitation protocols were effective in reducing the bacteria with more number of reduction in XP endo finisher and passive ultrasonic irrigation followed by Endoactivator
- This favorable result of XPEFR might be explained by the innovative alloy (MaxWire; FKG) used in manufacturing the XPEFR, which allows the material to expand at body temperature, leading to compression of its elliptical part, caused by the resistance settled by the root canal anatomy . In turn, the resistance presses the semiactive tip of the file against the root canal walls.
- This means that after the file expands inside the root canal space, it is able to scrape the dentinal walls with its semiactive tip.
- PUI does not touch the root canal walls. Its mechanism of action is based on activation of irrigating solutions

- EAS is less effective than XPEFR in reducing the amount of residual filling materials, owing to the lower hydrodynamic phenomenon promoted by the blunt and flexible polymer tip.

CONCLUSION

- All additional cleaning techniques improved the disinfection, regardless of the region under study. XPEFR was more effective than PUI and EAS.
- However, none of the techniques was capable to render the root canals completely free of microorganisms

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