Original Article

In Vitro Comparative Evaluation of Effectiveness of Sodium Hypochlorite with Conventional Irrigation Method versus EndoVac and Ultrasonic Irrigation in the Elimination of Enterococcus faecalis from Root Canals

Silpa Thulaseedharan¹, Pradeep Kabbinale², Anand Kumar Vallabhdas², Vijay Kumar Chittenahalli Ninge Gowda³, Kusuma Shikaripura Chandrashekar２, Rekha Marulappa³

¹Department of Conservative Dentistry & Endodontics, A J Institute of Dental Sciences, Kuntikana, Mangalore, Karnataka, and India, ²Department of Conservative Dentistry & Endodontics, ³Department of Oral pathology and Microbiology, Subbaiah Institute of Dental Sciences, Shivamogga, Karnataka, India

Aim: The aim of this study was to compare the decrease in bacterial population in the root canals with 2.5% sodium hypochlorite (NaOCl) and three irrigation systems: Conventional Needle Irrigation, Passive Ultrasonic Irrigation, and EndoVac Apical Negative Pressure Irrigation system

Materials and Methods: Access open was carried out in 48 extracted single-rooted maxillary and mandibular human premolar teeth. The canals were instrumented 1 mm short of apical foramen up to No. 50 size file; irrigated and apical foramen was sealed with cyanoacrylate. The teeth were mounted in plaster and autoclaved for 20 min at 121°C. The plaster blocks containing the teeth were inoculated with Enterococcus faecalis and incubated at 37°C for 24 h. The specimens divided randomly into four groups (n = 12) depending on the type of irrigation system used: Group 1: Conventional Irrigation Needle, Group 2: Ultrasonic system, Group 3: EndoVac Negative Pressure Irrigation system used with 2.5% NaOCl, and Group 4: Control group, normal saline irrigation. The normal saline is used as control. The samples were collected using 45 size sterile paper points and quantitative bacterial assessment was carried out by seeding each dilution on plates containing brain heart infusion broth. Then, incubated at 37°C for 7 days and colony-forming units were counted. Data obtained were statistically analyzed.

Results: Statistically, EndoVac irrigation systems were more effective in reducing the number of bacteria than the other irrigation systems.

Conclusion: EndoVac irrigation system was more effective in reducing bacterial numbers in infected root canals than the other two irrigation systems.

Keywords: EndoVac negative pressure irrigation system, Enterococcus faecalis, sodium hypochlorite, ultrasonic irrigation

Received : 24-01-2020.
Revised : 13-03-2020.
Accepted : 09-03-2020.
Published : 28-08-2020.

INTRODUCTION

Long-term success of endodontically treated tooth is not due to a single factor but relates to three aspects of treatment, what you may call an “endodontic triad.” Due to the complex anatomy of the root canal system such as irregularities of canal wall, oval extensions isthmus, and apical deltas, it is very difficult to clean the canal completely. In fact, within oval canals only 40% of the apical root canal wall area can be contacted by instruments when a rotating technique is used. Therefore, irrigation is an essential part of

Address for correspondence: Dr. Pradeep K, Department of Conservative Dentistry & Endodontics, Subbaiah Institute of Dental Sciences, Shivamogga 577222, Karnataka, India
E-mail: endopradeep@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Thulaseedharan S, Kabbinale P, Vallabhdas AK, Ninge Gowda VC, Chandrashekar KS, Marulappa R. In vitro comparative evaluation of effectiveness of sodium hypochlorite with conventional irrigation method versus EndoVac and ultrasonic irrigation in the elimination of Enterococcus faecalis from root canals. J Pharm Bioall Sci 2020;12:S105-8.
root canal treatment as it allows for cleaning beyond the root canal instruments.\textsuperscript{[5]}

*Enterococcus faecalis*, a Gram-positive facultative anaerobe, was selected because it is commonly found in the root canals of failing endodontically treated cases.\textsuperscript{[6]} It is difficult to clean the apical portion of the root canal with conventional irrigation methods, because of the complex anatomy in most cases of root canals.\textsuperscript{[7]} Ultrasonically activated files have the ability to prepare and clean the root canals mechanically.\textsuperscript{[8]} Ultrasonic activation has been shown to be less effective in removing simulated pulp tissue from the root canal system or smear layer from the root canal wall than Passive Ultrasonic Irrigation (PUI).\textsuperscript{[9,10]}

**MATERIALS AND METHODS**

Description of the materials used in the study is explained in Table 1. Forty-eight freshly extracted human premolar teeth. The selected specimens were stored and surfaced adhering to CDC (Centers for Disease Control and Prevention) infection control protocols global. Conventional access cavity preparation was carried out on all 48 samples using Endo Access Bur connected to a torque limited reduction gear handpiece. Step back canal preparation technique was adopted for cleaning and shaping of all 48 samples starting from initial apical binding file increasing the size up to #50. The tip of each root was blocked with two layers of cyanoacrylate. The teeth were then mounted vertically in plaster blocks and sterilized in an autoclave for 20 min at 121°C. A pure culture of *E. faecalis* (ATCC 29212) in brain heart infusion broth was used to obtain a suspension. A 10 μL of the suspension was placed in each root canal, and sterile cotton was placed in the canal entrance. The blocks were then placed inside stainless steel boxes and incubated at 37°C for 24 h. The incubated specimens are randomly divided into three groups and one control group (12 teeth in each group, $n = 12$).

Group 1: 2.5% NaOCl and Conventional Needle Irrigation were performed with a 30-gauge side-vented needle (Nipro India Corporation) and a syringe.

Group 2: 2.5% NaOCl + PUI. The ultrasonic irrigation cannula was a 25-gauge ultrasonic irrigation needle (Satelec, A-dec).

Group 3: 2.5% NaOCl + EndoVac irrigation system.

Group 4: Control group, normal saline irrigation.

**Bacterial sample collection from irrigated groups**

The paper point was placed inside the root canal space of each sample for 1 min and then kept on to petri plates containing brain heart infusion broth, which were incubated for 37°C with. Colonies were counted, and the number of colony-forming units per milliliter (CFU/mL$^{-1}$) was calculated. Comparison of microbial

![Graph 1: Microbial load after irrigation (10^5 CFU)](image)

![Graph 2: Comparison of microbial load before and after irrigation (10^5 CFU)](image)
load in the root canal after irrigation among the three study groups was carried out using analysis of variance (ANOVA).

RESULTS
The data were entered in the excel sheet and analyzed using Statistical Package for the Social Sciences (SPSS) software program, version 16.0 (SPSS, Chicago, IL, USA). The data were presented in the form of mean and standard deviation (SD). The study found that EndoVac Negative Pressure Irrigation group (mean ± SD, 0.512 × 10^5 ± 0.021 × 10^5 CFU/mL) has the least microbial load in the root canal after irrigation, whereas root canals in Conventional Needle Irrigation Group have the highest microbial load (mean ± SD, 1.320 × 10^5 ± 0.079 × 10^5 CFU/mL) [Graph 1]. When the difference in the microbial load in the root canals before and after irrigation was compared, it was observed that there was a statistically significant (P < 0.001) reduction in the microbial load in all the study groups [Graph 2]. When the data were compared using one-way ANOVA, the difference in the microbial load among the three groups was observed to be statistically significant (P < 0.001).

DISCUSSION
Most of the microbes in the root canal are taken care of with routine instrumentation of the pulp space and standard regimen of irrigant solutions. In vitro tests such as antimicrobial susceptibility allow experimental conditions to be controlled; however, this is often difficult to do when performing an experiment in vivo. This study is an in vitro evaluation of effectiveness of three irrigation systems with the use of 2.5% NaOCl as the irrigant in the experimental groups (Groups 1–3) and normal saline in the control group (Group 4). It performs bactericidal cytotoxicity, dissolution of organic material, and minor lubrication, the reason why NaOCl was used in this study. Ultrasonic devices were first introduced in Endodontics by Richman. The acoustic streaming that occurs in the root canal during ultrasonic irrigation has been described as acoustic micro streaming. The presence of apical vapor lock created by the organic decomposition of NaOCl into a bubble of carbon dioxide and ammonium adversely affects debridement efficacy when using a positive pressure system. Palazzi et al. conducted a study evaluating cleaning efficacy of two apical negative pressure irrigation techniques compared to needle irrigation. Ahmed Alkahtani et al. found that the EndoVac irrigation system extruded significantly less debris than needle irrigation system. Nielsen and Craig Baumgartner compared the cleanliness efficacy between the EndoVac irrigation system and a 30-gauge ProRinse irrigation; they found that EndoVac cleaned better at apical portion of root canal.

CONCLUSION
Within the conditions of this in vitro study, it can be concluded that EndoVac Negative pressure irrigation (Group 3) showed maximum reduction of E. faecalis from the pulp space preparation. Statistically significant differences were observed compared to Conventional Needle Irrigation (Group 1) and PUI system (Group 2).

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Ricucci D, Bergenholtz G. Bacterial status in root-filled teeth exposed to the oral environment by loss of restoration and fracture or caries: a histobacteriological study of treated cases. Int Endod J 2003;36:787-802.
2. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after “one-visit” endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:231-52.
3. Wu MK, Wesselink PR. A primary observation on the preparation and obturation of oval canals. Int Endod J 2001;34:137-41.
4. Wu MK, van der Sluis LW, Wesselink PR. The capability of two hand instrumentation techniques to remove the inner layer of dentine in oval canals. Int Endod J 2003;36:218-24.
5. Berber VB, Gomes BP, Sena NT, Vianna CC, Zaia AA, et al. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. Int Endod J 2006;39:10-7.
6. Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J 2004;37:438-46.
7. Garberoglio R, Becce C. Smear layer removal by root canal irrigants: a comparative scanning electron microscopic study. Oral Surg Oral Med Oral Pathol 1994;78:359-67.
8. de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. J Endod 2003;29:891-5.
9. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod 1980;6:740-3.
10. Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J Endod 1987;13:490-9.
11. van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007;40:415-26.
12. Alani AH, Al-Hashimi WN. A study to compare the cleaning efficiency of three different irrigation devices at different root canal levels (an in vitro study). Restor Dent 2011;23:10-15.

13. Garg G, Talwar S. Comparison of the efficacy of “F-File” with sonic and ultrasonic debridement to remove artificially placed dentine debris from human root canals: an in vitro study. J Endod 2010;22:39-47.

14. Koçani F, Kamberi B, Dragusha E, Mrasori S, Haliti F. The cleaning efficiency of the root canal after different instrumentation technique and irrigation protocol: a SEM analysis. Open J Stomatol 2012;2:69-76.

15. Palazzi F, Giardino L, Mohammadi Z, Rengo S, Riccitiello F. Debridement effectiveness of two different techniques using negative pressure irrigation system. G Ital Endod 2012;1-10.

16. Alkahtani A, Al Khudhairi TD, Anil S. A comparative study of the debridement efficacy and apical extrusion of dynamic and passive root canal irrigation systems. BMC Oral Health 2014;14:1-7.

17. Nielsen BA, Craig Baumgartner J. Comparison of the EndoVac system to needle irrigation of root canals. J Endod 2007;33:611-5.