Comparison of the Antimicrobial Efficacy of the EndoVac System and Conventional Needle Irrigation in Primary Molar Root Canals

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Purpose: *The purpose of this study was to compare the antimicrobial efficacy of the EndoVac system and conventional needle irrigation to eliminate E faecalis in primary molar root canals.* ***Study Design:*** *60 extracted human primary second molar roots were instrumented up to an apical size .04/35 and randomly divided into two groups; Group 1: conventional needle (n=30) and Group 2: EndoVac (n=30), and four subgroups (two experimental subgroups; (a) 2.5% sodium hypochlorite (NaOCl) + ethylenediaminetetraacetic acid (EDTA) (n=20), (b) ozonated water (OW) + EDTA (n=20), and control groups (c) 5.25% NaOCl (n=10) and (d) saline (n=10). All roots were sterilized and then inoculated with E.faecalis. Before and after final irrigation procedures, root canals were sampled and the grown colony forming units (CFUs) were counted. Data were analyzed by Kruskall-Wallis and Mann-Whitney U tests using a 0.05 significance level.* ***Results:*** *The EndoVac reduced more bacteria than the conventional needle did but it was not statistically significant (p>0.05). NaOCl alone or followed by EDTA totally eliminated bacteria. OW + EDTA showed higher reduction of bacteria but could not totally eliminate bacterias.* ***Conclusions:*** *In the context of bacterial elimination, the EndoVac was not significantly better than the conventional needle. Although, there were fewer CFU/mg when using EndoVac, there was not any statistically significant superiority to conventional needle irrigation. An OW+EDTA regimen showed antibacterial effect in the primary molar root canals but it was significantly less effective than NaOCl+EDTA.*

Key Words: *antimicrobial, EndoVac, ozonated water, primary teeth, pulpectomy, root canal*

INTRODUCTION

Microorganisms are the main etiological factor of pulpal and periapical infections[1]. The main purpose of endodontic therapy is to eliminate all microorganisms from the root canal and prevent reinfection by optimally cleaning, shaping and filling the root canal hermetically[2].

Disinfection of the root canal in the endodontic infections is an important issue for the success of the primary teeh root canal treatments, especially during the root resorption or formation of the primary teeth[3]. Primary root canal infections are polymicrobial and dominated by anaerobic bacteria[3-5]. Enterococcus faecalis (*E.faecalis*) is the most frequently isolated bacteria in resistant infections and failed root canal treatments[1] with the prevalance ranging from 24% to 77% in such infections[6], and one of the bacteria isolated from necrotic pulps of primary teeth[3]. *E.faecalis* is found in 40% of primary endodontic infections[6] and has often been chosen as a test organism in primary molars root canal treatment[6, 7].

Mechanical instrumentation is not enough to entirely clean the root canal[8]. Irrigation solutions act as a bactericidal agent, tissue solvent, lubricant and debridement[9]. Although sodium hypochloride (NaOCl) is the most commonly used intracanal irrigant in endodontics, new alternative irrigation solutions have been investigated since NaOCl has cyotoxic effects on vital and periapical tissues[9]. Also, its extrusion can cause post-operative pain, swelling and higher bleeding[10].

Ozone (O_3) is a naturally found and powerful oxidizing agent having great antimicrobial effects on bacteria, fungi, protozoa and viruses[11]. Ozone has been investigated as a possible alternative antiseptic agent in dentistry because of its high antimicrobial effect without the development of drug resistance[12]. Ozone can be used either in gaseous, aqueous or oil forms in dentistry[13]. The ozonated water (OW) is an alternative irrigant to NaOCl with the advantage of higher biocompatibility on vital tissues and great antimicrobial efficacy[14].

The conventional needle irrigation is the most widely used technique in irrigation but positive pressure can cause extruding irrigants to periradicular tissues leading to tissue damage and post-operative pain[15]. Several irrigation devices and techniques have been introduced to entirely penetrate and contact irrigation solutions through the root canal system[16]. The EndoVac (Discus Dental, Culver City,

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CA) system is an apical negative pressure system designed to safely and entirely deliver irrigants to the root canal system[17]. Also, regarding smear layer removal, irrigant extrusion and antimicrobial efficacy, it has shown better results than conventional needle irrigation[18-20].

Endodontical techniques show differences from adult treatment since primary teeth roots have complex morphology. Widely divergent, curved and fragile primary molar roots, with incomplete access to root decreases the success of effective preparation[21-23]. Also, extruded irrigants and bacterias can have toxic effects on underlying tooth germ during endodontic treatment [24].

The EndoVac system and the OW have been investigated and found effective in permanent teeth but to date no study has been conducted on primary teeth. The purpose of this in vitro pilot study was to compare the antimicrobial efficacy of two final irrigation regimens (OW + EDTA and NaOCI + EDTA) to eliminate *E.faecalis* in primary molar root canals using two irrigation systems (EndoVac and conventional needle).. The null hypotheses of the present study were; (a): there is no difference between the EndoVac and conventional needle irrigation systems regarding the antimicrobial efficacy in infected human primary molar root canals, (b): there is no difference between OW + EDTA and NaOCl + EDTA final irrigation regimens regarding the antimicrobial efficacy in infected human primary molar root canals.

MATERIALS AND METHOD

Ethical approval was obtained from the Health Ethics Committee of the University of Cumhuriyet University, Sivas, Turkey (ID: 2012-04/04). This study was conducted on the totally 60 palatinal or distal roots of, respectively, primary maxillary or mandibular second molar teeth that were freshly extracted for orthodontic reasons. Each tooth was radiographed digitally (Novelix, Trophy) in order to determine root resorption degree; "res_i or $res_{1/4}$", described by Fanning[25] and root curvature less than 30°. The teeth with fractured, calcified or previous root canal treatment were excluded. Only one root of each tooth was used;- non-used roots were removed by a diamond blazer.

Specimen Preparation

Each tooth was decoronated and the root length was standardized to 11mm. After endodontic access, the working length (wl) was determined by inserting a no.10 K file (Dentsply/Maillefer, Ballaigues, Switzerland) into each root canal until visible apically under magnifying loupe x20 and then subtracting 1 mm from this point[26]. Thereafter, each root apical foramina was closed with soft modelling wax (Cera Reus, SA, Reus, Spain) in order to create a closed system and horizontal grooves were placed for mechanical retention in experimental setup [26]. Each root cementum was coated with two layer nail varnish to prevent bacterial retention[27]. Each tooth was inserted into a polyvinylsiloxane impression material[26] and adapted to the previously prepared experimental set up. Each root canal was instrumented crown down with nickel titanium rotary Profile.04 ISO (Dentsply/ Tulsa Dentall, Tulsa, Okla) up to an apical .04/35 file using 1 ml 2.5% NaOCl at each file change with a 27 gauge needle (Hayat, İstanbul, Turkey) in accordance with the manufacturer's recommendations[12]. All specimens were then sterilized in ethylene oxide gas for a 12-hour cycle using the Anprolone AN 74C Gas Sterilizer (Andersen Products Inc, Haw River, NC).

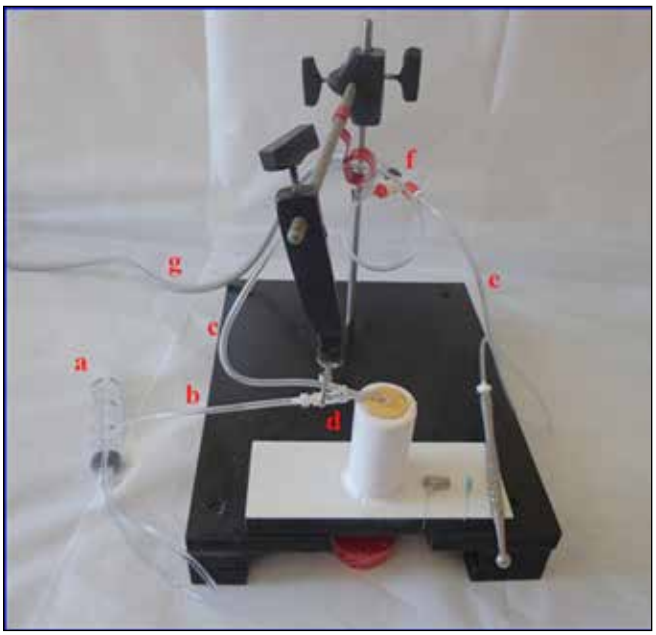
Contamination with E.faecalis

The microbiological procedure (contamination of the root canals, sampling and counting bacteria) was based on the previous studies by Siqueira et al. [28] and Kustarcı et al. [27] . A suspension was prepared by adding 1 ml of pure culture of *E.faecalis (*ATCC 29212), grown in brain heart infusion broth (BHI) (Difco, Detroit, MI) to 5 ml of BHI. The McFarland standard number 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5 X 10^8 colony forming units (CFU) ml^{-1}. Each root canal was filled with *E.faecalis* suspensions with a sterile automatic micropipette (Gilson, Villiers-le-Bel, France). A sterile K- type file was used to carry the bacterial suspension to the entire root canal length [27, 28]. Specimens were incubated at 37 °C for 24 hours[27, 28]. Bacterial colonization was checked by examining the three control specimens with a scanning electron microscope (Leo 440 CCD*,* Leica, Bensheim, *Germany).*

Experimental Design

An experimental set-up based on the fixture designed previously [26] was done in order to facilitate consistent irrigation protocols performed by one single operator (Figure 1). The ozonation of water was performed by bubbling ozone through sterile distilled water at 4mg/l with a custom-made ozone generator (TeknO_3zone, İzmir, Turkey). The concentration of ozone was controlled digitally by the automatic balancing system.

Figure 1 Experimental set-up to perform irrigation by a single operator. (a) 20-ml needle (b) Connector between 20 ml needle and the maser suction tip (MST) (c) Connector between the MST and high vacuum line (d) The MST (e) Connector between the high vacuum line and Endovac handpiece (f) High vacuum line.



Experimental Groups and Final Irrigation Procedure

60 extracted human primary second molar roots were randomly divided into two main groups regarding the irrigation activation system; Group 1: conventional needle (*n*=30) and Group 2: EndoVac (*n*=30), and four groups regarding final irrigation regimens (two

experimental subgroups; (a)= 2.5% NaOCl + 17% EDTA (*n*=20), (b)= 4 ppm OW + 17% EDTA (*n*=20), and control groups (c) 5.25% NaOCl (*n*=10) and (d) saline (*n*=10). Each tooth had the same total final irrigation time of 6 min and 5 ml/min irrigation average rate (total of 30 ml irrigation solution for each roots). The final irrigation procedure was carried out as;

Group 1: EndoVac

a. **NaOCl +EDTA:** The experimental group consisted of a 30 s period of irrigation with 2.5% NaOCl with the macrocanula, followed by leaving the canal full of irrigant for 30s. Three irrigation cycles were performed with the microcanula placed at wl for 6s, 2 mm shorter wl for 6s, and wl for 6s. The first cycle was 30s of 2.5% NaOCl, followed by 30s soaking; the second cycle was 1 min %17 EDTA, followed by 1 min of soaking and the third cycle was 1 min of 2.5% NaOCl, followed by 1 min of soaking.
b. **OW + EDTA group**: Same procedure as group NaOCl + EDTA, but 4 ppm OW was used instead of 2.5% NaOCl.
c. **5.25% NaOCl (negative control):** Same procedure as above but only 5.25% NaOCl was used as the irrigant.
d. **Saline (positive control):** 0.9% sterile saline was used as the only irrigant.

Group 2: Conventional needle

a. **NaOCl +EDTA:** A 27-gauge needle was inserted into the canal at 2 mm shorter wl and 2.5% NaOCl was delivered into the canal for 1 min active, followed by 1 min of soaking. 17% EDTA was delivered for 1 min active and soaked for 1 min. And finally, 2.5% NaOCl was delivered into the canal for 1 min active, followed by 1 min of soaking.
b. **OW +EDTA:** Same procedure as group NaOCl + EDTA, but 4 ppm OW was used instead of 2.5% NaOCl.
c. **5.25% NaOCl (negative control):** Same procedure as above but only 5.25% NaOCl was used as irrigant.
d. **Saline (positive control):** 0.9% sterile saline was used as the only irrigant.

Bacterial Evaluation

Before the final irrigation procedure, the root canals were filled with 0.9% saline and sterile paper points were placed at wl and maintained for 1 min inside the root canals. Paper points were transferred to tubes containing 5 ml of BHI broth. The tubes were vortexed for 5 min and 10 µl of the suspension was plated into one part of semi-divided blood agar plates and incubated at 37°C for 48 hours. The CFUs grown were counted and the initial mean number of viable microorganisms was determined 1×10^6 CFU ml^{-1} (6 $\log_{10}$ CFU ml^{-1}) for all teeth. After the final irrigation procedure, the same microbiological sample procedure was performed and suspension was plated into the other second part of semi-divided blood agar plates and incubated at 37°C for 48 hours. The colonies of remaining bacteria were counted and the results were given as $\log_{10}$ CFU ml^{-1}.

Statistical Analysis

All data were processed by SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). The mean numbers of the initial and the remaining bacteria after final irrigation were calculated. A $\log_{10}$ transformation was used in the statistical analysis because of the great CFU means. The data were analyzed by the Kruskall Wallis and Mann-Whitney U tests using a 0.05 significance level ($p< 0.05$).

RESULTS

Table 1 reveals the log CFU means of the remaining bacteria after final irrigation. The EndoVac reduced more bacteria than the conventional needle did but it was not statistically significant ($p>0.05$). NaOCl, alone or followed by EDTA, totally eliminated bacteria. OW + EDTA regimen showed higher reduction amounts of bacteria but could not totally eliminate bacteria.

Table 1 LogCFU mean numbers of remaining bacteria.

Groups	n	Mean CFU (*10⁶)	SD (*10⁶)
Irrigation System			
Conventional	30	1.61	1.85
EndoVac	30	1.45	1.65
Final Irrigation Regimen			
NaOCl+ EDTA [a-b,c SS]	20	0.00	0.00
OW+EDTA [b- a,c,d SS]	20	2.32	0.29
%5.25 NaOCl [c-b,d SS]	10	0.00	0.00
Saline [d- a,c,d SS]	10	4.58	0.36

Note. CFU: Colony forming units, SD: Standard deviation, SS and bold: Statistically significant, $p < 0.05$, [a]: NaOCl+ EDTA group, [b]: OW+EDTA group, [c]: %5.25 NaOCl group, [d]: Saline group.

$\chi^2 = 58{,}711$; $P = .00$ ($P < .05$).

Mann-Whitney *U* test was used for comparing the differences between all groups: [a-b] ($P = .00$, $P < .05$, SS), [a-c] ($P = 1.00$, $P > .05$), [a-d] ($P = .00$, $P < .05$, SS), [b-c] ($P = .00$, $P < .05$, SS), [b-d] ($P = .00$, $P < .05$, SS), [c-d] ($P = .00$, $P < .05$, SS).

DISCUSSION

A closed system model was used in our experimental set up to imitate the clinical environment, since the root is clinically enclosed with bone and acts as a closed end system. This makes gas entrapment, called a "vapor lock effect", which limits irrigation solutions to reach working length[29].

The root canal preparation with rotary instruments on primary molars reduces preparation time significantly more than manual instrumentation. Also, preparation with the Profile ISO instruments on primary molars reduces the zip formation and loss of working length[30]. In the present study, each root canal was instrumented crown down with nickel titanium rotary Profile.04 to an apical .04/35 size with the basis on the EndoVac manufacturer's recommendations regarding the minimal apical size up to the microcanulas.

There is no optimal concentration of NaOCl used in endodontic infections in primary teeth, with clinical ranges from 0.5% to 5.25%. In the primary root canal treatment, 2.5% NaOCl with larger volumes and continuous exchange provides totally elimination of viable bacteria in the root canal system[28]. EDTA is an demineralizing agent to remove the smear layer and a 17% concentration is widely used[9]. In the present study, the NaOCl and EDTA final

irrigation regimen was used since it is the most commonly used method clinically and the NaOCl and EDTA combination is more efficient at eliminating bacteria than NaOCl alone[9]. We should note that although there is no contraindication to use EDTA in primary teeth root canal treatment, apical extrusion of its can damage the permanent tooth germ.

The contact time of the irrigants, the delivery volume, the average rate and the different methodology can affect antimicrobial effectiveness. Some authors suggests different concentrations based on the belief that higher concentrations bring higher bactericide[31]. 4 ppm OW was used in the present study, based on a previous study[14]. Nagayoshi et al.[14] compared ex vivo the antimicrobial efficacy of 4 ppm OW with 2.5% NaOCl in bovine teeth and reported that OW had nearly the same antimicrobial activity compared with 2.5% NaOCl, also with lower cytotoxicity of OW on fibroblasts.

Similar to the previous studies, our study showed that OW greatly reduced amount of *E.faecalis* but could not totally eliminate it[31, 32]. In an in vitro study[32], 25 ppm OW with ultrasonication and without for 4 min was used in the primary molar root canals and OW was found less effective in eliminating *E.faecalis* than 2.5% NaOCl.

The antimicrobial ability of the EndoVac system while working on the root canal safely without extrusion beyond apical construction and removing smear layer has been shown in permanant teeth[17, 18]. But to date no study investigated using an EndoVac system in primary root canal treatment.

The antimicrobial effect of the EndoVac system was examined in permament teeth by many studies[18, 33-35], yet no study was conducted on primary teeth. Miller and Baumgartner[18] compared the antimicrobial efficacy of the EndoVac to the endodontic needle irrigation in the apical 5 mm of the root canals infected with *E.faecalis.* They found that although there is no statistical difference between the groups and there were fewer remaining bacteria after irrigation using the EndoVac. These findings are very similiar to our study. Miranda *et al*.[36] evaluated the ex vivo antimicrobial efficacy of the EndoVac irrigation system compared with PDT associated with chemomechanical debridement plus intracanal CaOH and found both systems to be effective.

There are many limitations in our study. One of these may be the bacteriological model used in the study, which mainly evaluates the suspension bacteria. Actually, the suspension model used in our study has often been previously chosen and acceptable as a laboratory bacteriological model in in vitro studies[27, 28], but to date, several reports have shown the existence of bacterial biofilms in infected root canal system[22, 24]. Therefore, the main goal of the endodontic treatment is to either remove biofilms from the root canal or eliminate all microorganism in the biofilms[24]. It is known that the elimination of bacterial biofilms is a major step for the success of endodontic treatment, especially in the root canal systems consisting of apical ramification, lateral canals and isthmus such as primary teeth[23]. Further, in vitro and in vivo studies must be carried out using biofilm formation to assess the antimicrobial efficacy in primary root canal treatment.

CONCLUSIONS

The present study shows that in the context of bacterial elimination, the EndoVac system was not significantly better than the conventional needle. Although there was no statistically significant benefit of the EndoVac system and OW in primary teeth endodontic infections, they have both greatly reduced amount of bacteria in the root canal system with hopeful results. Within the limitations of the present study, this is the first study examining the antimicrobial efficacy of both the EndoVac system and OW in primary teeth. More research needs to be performed in the primary teeth root canals using the EndoVac system and OW in both in vitro and in vivo studies.

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REFERENCES

1. Siqueira, J.F., I.N. Rôças, A. Favieri, and K.C. Lima. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. Journal of Endodontics 26:331-334, 2000
2. Rodd, H.D., et al. Pulp therapy for primary molars. Int J Paediatr Dent 16 Suppl 1:15-23, 2006
3. Sato, T., E. Hoshino, H. Uematsu, and T. Noda. Predominant obligate anaerobes in necrotic pulps of human deciduous teeth. Microbial ecology in health and disease 6:269-275, 1993
4. Topcuoglu, N., E. Bozdogan, and G. Kulekci. Presence of oral bacterial species in primary endodontic infections of primary teeth. J Clin Pediatr Dent 38:155-160, 2013
5. Sundqvist, G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 78:522-530, 1994
6. Bhangdia, M.B., B. Nandlal, G.S. Vijaykumar, P.K. Kulkarni, and R. Shanbhog. Clinical evaluation of sustained-release metronidazole gel versus metronidazole solution as an intracanal medicament in abscessed primary molars. Eur Arch Paediatr Dent 15:19-26, 2014
7. Önçağ, Ö., D. Gogulu, and A. Uzel. Efficacy of various intracanal medicaments against Enterococcus faecalis in primary teeth: an in vivo study. Journal of Clinical Pediatric Dentistry 30:233-237, 2006
8. Gondim, J.O., et al. Effect of a calcium hydroxide/chlorhexidine paste as intracanal dressing in human primary teeth with necrotic pulp against Porphyromonas gingivalis and Enterococcus faecalis. Int J Paediatr Dent 22:116-124, 2012
9. Zehnder, M. Root canal irrigants. J Endod 32:389-398, 2006
10. Becking, A.G. Complications in the use of sodium hypochlorite during endodontic treatment. Report of three cases. Oral Surg Oral Med Oral Pathol 71:346-348, 1991
11. Nogales, C.G., P.H. Ferrari, E.O. Kantorovich, and J.L. Lage-Marques. Ozone therapy in medicine and dentistry. J Contemp Dent Pract 9:75-84, 2008
12. de Gregorio, C., R. Estevez, R. Cisneros, A. Paranjpe, and N. Cohenca. Efficacy of different irrigation and activation systems on the penetration of sodium hypochlorite into simulated lateral canals and up to working length: an in vitro study. Journal of endodontics 36:1216-1221, 2010
13. Bocci, V.A. Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res 37:425-435, 2006
14. Nagayoshi, M., C. Kitamura, T. Fukuizumi, T. Nishihara, and M. Terashita. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod 30:778-781, 2004
15. Desai, P. and V. Himel. Comparative safety of various intracanal irrigation systems. J Endod 35:545-549, 2009
16. Boutsioukis, C., et al. Evaluation of irrigant flow in the root canal using different needle types by an unsteady computational fluid dynamics model. J Endod 36:875-879, 2010
17. Schoeffel, G.J. The EndoVac method of endodontic irrigation: safety first. Dent Today 26:92, 94, 96 passim, 2007
18. Miller, T.A. and J.C. Baumgartner. Comparison of the antimicrobial efficacy of irrigation using the EndoVac to endodontic needle delivery. J Endod 36:509-511, 2010
19. Iriboz, E., K. Bayraktar, D. Turkaydin, and B. Tarcin. Comparison of apical extrusion of sodium hypochlorite using 4 different root canal irrigation techniques. J Endod 41:380-384, 2015
20. Yucel, A.C., M. Gurel, E. Guler, and B. Karabucak. Comparison of final irrigation techniques in removal of calcium hydroxide. Aust Endod J 39:116-121, 2013
21. Ahmed, H.M. Anatomical challenges, electronic working length determination and current developments in root canal preparation of primary molar teeth. Int Endod J 46:1011-1022, 2013
22. Liu, H., X. Wei, J. Ling, W. Wang, and X. Huang. Biofilm formation capability of Enterococcus faecalis cells in starvation phase and its susceptibility to sodium hypochlorite. J Endod 36:630-635, 2010
23. Lin, J., Y. Shen, and M. Haapasalo. A comparative study of biofilm removal with hand, rotary nickel-titanium, and self-adjusting file instrumentation using a novel in vitro biofilm model. J Endod 39:658-663, 2013
24. Ricucci, D. and J.F. Siqueira, Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 36:1277-1288, 2010
25. Fanning, E.A. Most cited: number 1. A longitudinal study of tooth formation and root resorption. N Z Dent J 104:60-61, 2008
26. Parente, J.M., et al. Root canal debridement using manual dynamic agitation or the EndoVac for final irrigation in a closed system and an open system. Int Endod J 43:1001-1012, 2010
27. Kustarci, A., Z. Sumer, D. Altunbas, and S. Kosum. Bactericidal effect of KTP laser irradiation against Enterococcus faecalis compared with gaseous ozone: an ex vivo study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 107:e73-79, 2009
28. Siqueira, J.F., Jr., I.N. Rocas, A. Favieri, and K.C. Lima. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. J Endod 26:331-334, 2000
29. Tay, F.R., et al. Effect of vapor lock on root canal debridement by using a side-vented needle for positive-pressure irrigant delivery. J Endod 36:745-750, 2010
30. Canoglu, H., M.U. Tekcicek, and Z.C. Cehreli. Comparison of conventional, rotary, and ultrasonic preparation, different final irrigation regimens, and 2 sealers in primary molar root canal therapy. Pediatr Dent 28:518-523, 2006
31. Estrela, C., C.R. Estrela, D.A. Decurcio, A.C. Hollanda, and J.A. Silva. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. Int Endod J 40:85-93, 2007
32. Goztas, Z., H. Onat, G. Tosun, Y. Sener, and H.H. Hadimli. Antimicrobial effect of ozonated water, sodium hypochlorite and chlorhexidine gluconate in primary molar root canals. Eur J Dent 8:469-474, 2014
33. Brito, P.R., et al. Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: an in vitro study. J Endod 35:1422-1427, 2009
34. Nakornchai, S., P. Banditsing, and N. Visetratana. Clinical evaluation of 3Mix and Vitapex as treatment options for pulpally involved primary molars. Int J Paediatr Dent 20:214-221, 2010
35. Barcelos, R., P.N. Tannure, R. Gleiser, R.R. Luiz, and L.G. Primo. The influence of smear layer removal on primary tooth pulpectomy outcome: a 24-month, double-blind, randomized, and controlled clinical trial evaluation. Int J Paediatr Dent 22:369-381, 2012
36. Miranda, R.G., E.B. Santos, R.M. Souto, H. Gusman, and A.P. Colombo. Ex vivo antimicrobial efficacy of the EndoVac system plus photodynamic therapy associated with calcium hydroxide against intracanal Enterococcus faecalis. Int Endod J 46:499-505, 2013