Original Article

Influence of Various Application Systems on the Amount of Extruded Irrigant in Simulated Immature Teeth With Regard to Gravity: An *ex-vivo* Study

E Namsoy, B Serefoglu[1], M Hülsmann[2], MK Çalışkan[1]

Department of Endodontics, Faculty of Dentistry, European University of Lefke, Lefke, Northern Cyprus, TR-10 Mersin, [1]Department of Endodontics, School of Dentistry, Ege University, Bornova, İzmir, Turkey, [2]Department of Preventive Dentistry, Periodontology and Cariology, University Medicine Göttingen, Göttingen, Germany

Abstract

Background: Gravity impact has been mainly evaluated in mature teeth related to debris extrusion, even though it may affect the amount of apically extruded irrigant. In the literature the influence of gravity on the amount of apically extruded irrigant in immature teeth has been studied by a 45o inclined plate to mimic the position of the maxillary teeth and 90o for the mandibular teeth. However, patients are positioned horizontally in the dental chair while treatment. There is no study in the literature testing the horizontal position to mimic the clinical settings realistically. **Aim:** The aim of this study was to evaluate the influence of various irrigation systems on the amount of extruded irrigant in simulated immature maxillary and mandibular teeth irrigated in vertical and horizontal positions. **Materials and Methods:** Twenty-five maxillary central incisors with an apical opening of 1.3 mm in diameter were included. Irrigation procedures were performed with EndoVac, closed-ended, and open-ended needles using a VATEA peristaltic pump. The amount of apically extruded irrigant was determined using a microbalance. Statistical analysis was performed using the Kruskal–Wallis test. **Results:** The EndoVac system caused almost no irrigant extrusion in all tested positions ($P > 0.05$); however, closed-ended and open-ended needles extruded more irrigant in a mandibular vertical position compared to maxillary vertical ($P < 0.05$) and maxillary horizontal positions ($P < 0.05$). Open-ended needles extruded the highest amount of irrigant. **Conclusions:** The EndoVac macrocannula is a more reliable and safer irrigation system as it prevents irrigant extrusion independent of the position of the tooth.

Keywords: *Apical irrigant extrusion, gravity, immature teeth, negative pressure irrigation, positive pressure irrigation*

Received: 17-Apr-2022; **Revision:** 13-Aug-2022; **Accepted:** 28-Sep-2022; **Published:** 18-Nov-2022

Introduction

Traumatic injuries in children mostly affect the maxillary permanent incisors resulting in pulp necrosis and the development of periapical lesions. Completion of root maturation is disturbed if the trauma occurs before the root development was accomplished.[1] Biomechanical preparation of these immature teeth with a necrotic pulp is a challenging procedure due to wider root canals, weak and divergent or parallel dentinal walls, and excessive removal of root dentine increases the risk of vertical root fractures.[2] Therefore, optimal disinfection of the root canal system should strive without mechanical preparation.

Sodium hypochlorite (NaOCl) is considered as the main endodontic irrigant due to its strong antimicrobial effect and tissue-dissolving ability.[3] However, when its use cannot be restricted to the confines of the root canal system, periapical tissues and stem cells might be damaged.[4,5] Moreover, preliminary data indicate that

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_270_22

Address for correspondence: Dr. E Namsoy,
European University of Lefke, Faculty of Dentistry,
Department of Endodontics, 99728, Lefke, Northern Cyprus,
TR-10 Mersin, Turkey.
E-mail: enamsoy-lau@eul.edu.tr

How to cite this article: Namsoy E, Serefoglu B, Hülsmann M, Çalışkan MK. Influence of various application systems on the amount of extruded irrigant in simulated immature teeth with regard to gravity: An ex-vivo study. Niger J Clin Pract 2022;25:1831-7.

0.1 mL or higher amounts of NaOCl can reduce cell viability by 40%.[6] Several factors should be considered to reduce the risk of potential extrusion of the irrigant, for instance, apical diameter and patency of the root canal. Also, mechanical and physical features such as insertion depth of the cannula, volume and flow rate of the irrigant, as well as size and design of the irrigant delivery needle or the irrigation system are important parameters regarding the safety and efficacy of the treatment.[7]

Even though syringe irrigation with different needle tip designs [open-ended needle (OEN) or close-ended needle (CEN)] has been frequently used,[8] the risk of apical extrusion is high with these systems due to the generation of apical positive pressure (APP).[9] To overcome this complication, the EndoVac™ (EV) system (Discus Dental, Smart Endodontics, Culver City, CA, USA) has been designed both to deliver irrigants to the apical part and to suck out debris and irritants from the root canals with the help of apical negative pressure (ANP).[10] Although this system has been compared with conventional needle irrigation systems especially in mature teeth at the mandibular position,[9,11] there are only three *ex vivo* studies that evaluated its effect in simulated immature teeth.[12-14]

Additionally, even though gravity may affect the amount of apically extruded irrigant, its impact has been mainly evaluated in mature teeth related to debris extrusion.[15,16] So far, only one study in the literature has evaluated the influence of gravity on the amount of apically extruded irrigant in immature teeth.[14] In the same study, a 45° inclined plate was utilized to mimic the position of the maxillary teeth and 90° was used for the mandibular teeth. However, endodontists do not treat patients in a standing position (vertical); rather, patients are positioned horizontally in the dental chair. Therefore, it is important to test the horizontal position to mimic the clinical settings more realistically to have more reliable data, particularly for maxillary anterior teeth. There is no other study in the literature comparing the amount of extrusion of irrigant in immature teeth evaluated in a maxillary horizontal position to imitate the lying position of patients for accurate simulation of the clinical settings.

Therefore, the aim of this *ex vivo* study was to evaluate the impact of gravity and the irrigation systems including EV, CEN, and OEN on the amount of apically extruded irrigants in simulated immature permanent maxillary central incisors, using standard irrigant volume and flow rates at mandibular and maxillary vertical and maxillary horizontal positions. The null hypotheses were that there would be no statistically significant differences in the amount of extruded irrigants between the irrigation systems, and that gravity would not affect the amount of extruded irrigants.

Materials and Methods

Selection and preparation of specimens

This study was approved by the Human Ethical Committee of Ege University, Turkey (No: 19-1/22, 08 January 2019). The minimum sample size for each group was obtained as 23 (f = 0.40, at 0.01 Type I error and 95% power) using 2 × 3 Factorial ANOVA method in the G*Power (version 3.1.9.2) package program. Twenty-five maxillary central incisor teeth without caries, cracks, or fractures which had been freshly extracted for periodontal and prosthodontic reasons were included. Each tooth was shortened coronally with a high-speed diamond bur to provide standardization of the tooth length of 18 mm [Figure 1a]. Simulation of the incomplete apical closure was performed using Peeso reamers (Dentsply Maillefer, Ballaigues, Switzerland) with sizes 1 to 4, respectively, under sterile saline irrigation to create an apical opening of 1.3 mm in diameter without destroying the structural integrity of the root [Figure 1a]. The root canal system was irrigated with 2.5% NaOCl and 17% EDTA to remove the smear layer and debris remnants before the experimental procedure. Since irrigation procedures were non-destructive, the same 25 samples were used in all experimental groups, thereby avoiding the impact of inherent variables such as canal anatomy and shape. These tooth preparation steps and the application of the same 25 samples for each group further improved the accuracy of the study.

Experimental model

The method of Myers and Montgomery[17] was utilized to collect the extruded irrigant using glass vials. Before the irrigation procedure, three consecutive measurements were obtained for each vial using a microbalance with a precision of 10^{-4} g (Sartorius, Göttingen, Germany) and the mean values were recorded. Rubber stoppers were used to fix and attach the teeth to the vials. The glass vials were connected to 20 mL dark glass flasks (Smart Kimya, Izmir, Türkiye). To equalize the air pressure inside and outside the flask, a 25-gauge needle was placed through the rubber stopper. For each tooth, the flask was covered using a rubber dam. The teeth were fixed at 90° to mimic the tooth position in the mandibular [Figure 1b] and maxillary [Figure 1c] arch when patients were considered to be treated in an upright position and the teeth were fixed at 180° to simulate treatment in a horizontal position [Figure 1d].

Irrigation procedures and irrigant collection

Depending on the irrigation procedure, three experimental groups each with three subgroups with regard to the position of the teeth were created [Figure 1b-d]. In each experimental group, the VATEA peristaltic pump (ReDent-Nova, Ra'anana, Israel) was used to standardize the flow rate of irrigant to 2 mL/min and irrigation was performed with 2.5% NaOCl for 4 min, delivering a total volume of 8 mL of irrigant. The aspiration of the suction unit (FedesaJarez Basic Dental Unit, Madrid, Spain) was set at -20 kPa. Using the VATEA peristaltic pump allowed an even distribution of the flow of volumes to each particular tooth.

Irrigation was performed as follows:

Group 1 – EV: The VATEA peristaltic pump was connected to the EV master delivery tip. While the tip was placed above the access cavity providing a constant flow of irrigant, the macrocannula was placed 2 mm short of the root tip [Figure 1b].

Group 2 – CEN: The VATEA peristaltic pump was connected to a 30-gauge CEN (KerrHawe SA, Bioggio, Switzerland). As in Group 1, the needle tip was placed 2 mm short of the root tip. During irrigation, an endodontic aspirator (Endo Tips 0.014" Angelus, Londrina, PR, Brazil) was attached to the suction of the dental unit and was placed at the coronal end of the root canal for suctioning the irrigant that flowed out of the access cavity [Figure 1c].

Group 3 – OEN: Irrigation was performed with a 30-gauge OEN (NaviTip; Ultradent Products Inc, South Jordan, UT, USA) in the same manner as described for Group 2 [Figure 1d].

For all experimental groups, irrigation was performed in mandibular and maxillary vertical and maxillary horizontal positions. After irrigation was finished, the glass vial with the collected extruded irrigant was immediately weighed again three times, and the mean values were recorded. Calculation of the amount of extruded irrigant was determined for each sample by subtracting the mean pre- and post-irrigation weight of the vials. This procedure was repeated for every sample in every group.

Statistical analyses

The effects of gravity and type of irrigation system on the amount of extruded irrigant were analyzed at an 0.05 level of significance with a nonparametric factorial design with a mixed procedure in the SAS Version 9.3 (SAS Institute, Cary, NC, USA). Since a significant interaction between the irrigation systems and tooth positions was found, the data were analyzed separately using nonparametric tests in IBM SPSS Statistics for Windows (IBM SPSS Inc., Chicago, IL, USA). Kruskal–Wallis tests were used to compare the effect of gravity on the amount of extruded irrigant among the irrigation systems in mandibular and maxillary vertical and maxillary horizontal positions. The Dunn Test (with Bonferroni correction) was used as the Post hoc method.

Results

The median, minimum, and maximum values of the amount of apically extruded irrigant related to each experimental group at the mandibular and maxillary vertical and maxillary horizontal positions are presented in Table 1. The EV system caused almost no irrigant extrusion in all tested positions (P =0.892). Although CEN and OEN extruded significantly more irrigant in the mandibular position compared to maxillary vertical ($P_{CEN} < 0.001$, $P_{OEN} < 0.001$) and maxillary horizontal positions ($P_{CEN} < 0.001$, $P_{OEN} <.003$), there were no differences between maxillary vertical and maxillary horizontal positions ($P_{CEN} = 1.000$, $P_{OEN} = 0.205$). In the mandibular position, the EV system caused significantly less irrigant extrusion than the needle groups ($P_{CEN} = 0.020$, $P_{OEN} < 0.001$) and CEN caused less extrusion than OEN ($P < 0.001$). However, in both the maxillary vertical and maxillary horizontal positions, there was no significant difference between EV and CEN ($P = 1.000$). On the other hand, significant differences between EV and OEN ($P < 0.001$) and also between OEN and CEN ($P < 0.001$) were observed [Table 2].

Discussion

The purpose of performing *ex vivo* studies is to

Table 1: Weight of apically extruded irrigant (g) related to three different irrigation systems in mandibular, maxillary vertical, and maxillary horizontal positions

System	Amount of extruded irrigant (g)									P
	Mandibular position			Maxillary vertical position			Maxillary horizontal position			
	Median	Min.	Max.	Median	Min.	Max.	Median	Min.	Max.	
EV	0.0001	0.0000	0.0020	0.0000	0.0000	0.0004	0.0001	0.0000	0.0004	0.892
CEN	0.0031	0.0000	0.1002	0.0000	0.0000	0.0020	0.0000	0.0000	0.0013	<0.001
OEN	0.7871	0.0172	1.5972	0.2390	0.0194	0.5399	0.3519	0.0515	0.6607	<0.001

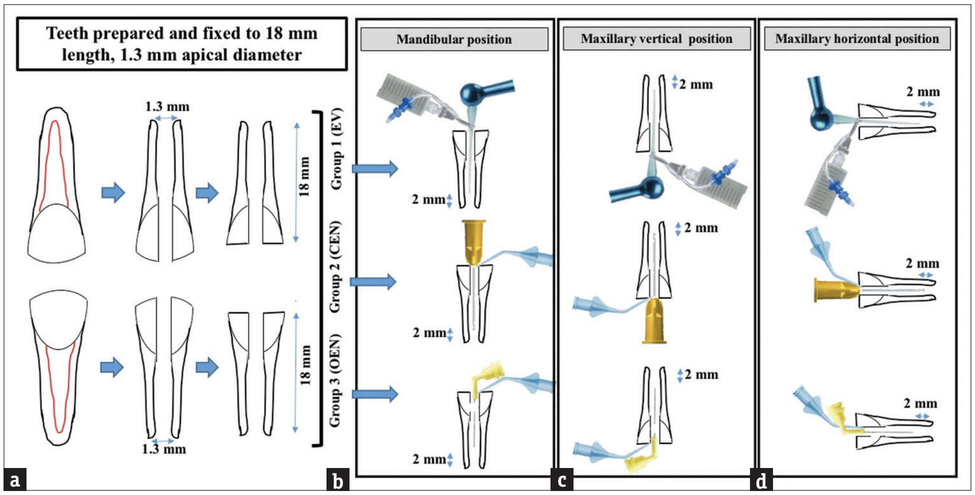

Figure 1: (a) Preparation of teeth; teeth are shortened to provide standardization of the tooth length to 18 mm and an apical opening of 1.3 mm in diameter. (b) Mandibular positioning of the teeth. (c) Maxillary vertical positioning of the teeth. (d) Maxillary horizontal positioning of the teeth. Each tooth was coronally shortened with a high-speed diamond bur to provide standardization of the tooth length to 18 mm (a). Simulation of the incomplete apical closure was performed using Peeso reamers (Dentsply Maillefer, Ballaigues, Switzerland) with sizes 1 to 4, respectively, under sterile saline irrigation to create an apical opening of 1.3 mm in diameter without destroying the structural integrity of the root

Table 2: *P* values of statistical intergroup comparisons according to teeth position

System	Tooth locations (*P*)		
	Mandibular position	Maxillary vertical position	Maxillary horizontal Position
EV - CEN	0.020*	1.000	0.744
EV - OEN	<0.001*	<0.001*	<0.001*
CEN - OEN	<0.001*	<0.001*	<0.001*

*Statistical significance

investigate materials and devices under simulated clinical conditions to provide more efficient materials and devices for clinical applications. In the literature on root canal irrigation, there are major limitations in *ex vivo* study models such as inadequate standardization and lack of presentation of the protocol for irrigation procedure including the insertion depth of the needle, the volume of delivered irrigant, the irrigant contact time, and the flow rate.[18] In this study, all these above-mentioned parameters were investigated with respect to gravity in simulated immature teeth in mandibular and maxillary vertical and maxillary horizontal treatment positions of the tooth. Also, in the CEN and OEN groups, considering that endodontic aspiration of the irrigant could influence the amount of apically extruded irrigant, the tip of an endodontic aspirator was placed at the coronal end of the root canal for suctioning the irrigant that flew out of the tooth coronally during the irrigation procedures.

The null hypotheses of the study were both rejected because there were statistically significant differences in the amount of extruded irrigant between irrigation procedures, and the gravitational force affected the amount of extruded irrigant, except in the EV group.

Preliminary *in vitro* studies in the literature used two different strategies to mimic the periapical tissue environment to detect and measure the amount of extruded irrigant; open root canal systems in which the teeth were exposed to atmospheric pressure[9,14,19] and closed root canal systems by using either saline agar or agarose gel.[11,20] In the literature, there is no realistic and precise *ex vivo* model described to simulate the actual periapical conditions, as both the photographic technique[11] and qualitative assessment[21] have limitations to measure the real volume of the extruded irrigants.[7,20] It is well known that in experimental set-ups using saline agar and agarose gel, extruded irrigant might be absorbed or expand.[22,23] Besides, the radiodensity values are different between saline agar/agarose gel setups in comparison to the periodontium and periapical lesions.[24] Because of all these reasons, the method of Myers and Montgomery[17] was chosen in the present study, as already been used in recent investigations.[12,14,19,25] This method also provided an advantage in terms of mimicry as it causes no material during the microbalance measurement of extrusion.[25] Additionally, in a previous study, it was revealed that the absence of the periapical tissue simulation resulted in an up to 60 times larger volume of irrigant extrusion, however, the tissue simulation in that study did not change the finding that the CEN caused significantly less extrusion compared with the OEN.[26] The limitation of the present study is the lack of stimulation of the periapical tissues because of the employment of an open canal system. Considering all these results, in the present study, an open root canal system without simulation of periapical pressure had been used to measure the amount of extruded irrigant.

In the literature, there are three studies that evaluated the effect of the EV system on immature teeth.[12-14] Velmurugan *et al*.[12] investigated the influence of EV microcannula, EV macrocannula, CEN, and OEN using 9 mL NaOCl per specimen in simulated immature teeth with a 1.3 mm diameter of the apical opening. The differences between the amount of extruded irrigant among four experimental groups in that study resulted in ranking order as follows: OEN (9 mL) = CEN (9 mL) > EV microcannula (7.53 mL) > EV macrocannula (0.23 mL). The significant difference between the EV macrocannula and microcannula was attributed to the large diameter (0.55 mm) of the macrocannula and the small diameter (0.32 mm) of the microcannula which also has only side vents.[12] However, in that study, NaOCl was delivered over a period of 60 s using a hand-controlled syringe, without utilizing the chair-side endodontic aspirator in the CEN and OEN groups. Thus, the manually controlled flow rate of the irrigant may not be adequately standardized.[27] In fact, the mandibular positions of the teeth and unemployment of an endodontic aspirator may increase the effect of gravity and cause extrusion of the irrigant in CEN and OEN groups. Our results for the EV macrocannula group were 0.0001 g for mandibular, 0.0000 g for maxillary vertical, and 0.0001 g for maxillary horizontal positions, and no statistically significant differences were observed with respect to tooth position. This variation between our results and the results reported by Velmurugan *et al*.[12] may be attributed to the above-mentioned factors.

In another *ex vivo* study by Jamleh *et al*.,[13] the irrigant volume and flow rate were standardized at 3.0 mL/min for the intracanal negative pressure (iNP) system and the EV system with microcannula or the OEN under APP. Simulated immature teeth with apical openings of ISO size 35 were used in a mandibular position, and saline agar was used apically to simulate a closed root canal system. These researchers prepared the teeth up to ISO size 35 to mimic immature teeth, but the apical opening of immature teeth clinically measures larger than a size ISO 80 file.[28] Thus, the study model may not adequately resemble *in vivo* conditions. The least extrusion was observed with the iNP system, but surprisingly, no difference was detected between the EV microcannula needle and the OEN. However, the authors only reported the number of teeth with extrusion and did not provide the volume of the extruded irrigant. In the present study, maxillary central incisors with a 1.3 mm diameter of the apical opening in stage IV of maturity according to Kling *et al*.[29] were selected as frequently seen in apexification treatment[30,31] and teeth in which regenerative endodontic treatment (RET) is performed.[32]

VandeVisse and Brilliant[33] demonstrated that instrumentation with irrigation generated significantly more apically extruded debris than instrumentation without irrigation. In addition, when a larger amount of irrigant was used, it was possible to collect larger amounts of extruded debris. Even though gravity is one of the factors that could influence the amount of extruded debris or irrigant, only a few studies evaluated the influence of gravity on the amount of extruded debris in mature teeth with respect to instrumentation systems regardless of the amount of extruded irrigant.[15,16] As for immature teeth, only one study assessed the effect of gravity on apically extruded irrigants.[14] In that study, the flow rate of the irrigant and irrigation time were not standardized. Moreover, the authors utilized a 45° inclined plane to mimic the position of the maxillary teeth and a 90° plane to simulate the position of the mandibular teeth. However, treatment is usually performed on patients in horizontal lying position and this setup could not represent the actual clinical conditions. In the present study, these factors were standardized, and in addition to mandibular and maxillary vertical positions, teeth were also evaluated in a maxillary horizontal position to imitate the lying position of patients in the dental chair for accurate simulation of clinical settings. The results revealed that OEN and CEN caused statistically significant more irrigant extrusion than the EV system in the mandibular position and OEN led to greater irrigant extrusion than CEN. These findings are consistent with the results from several irrigant extrusion studies in mature teeth that used an open canal system.[9,25] Additionally, in the CEN and OEN groups, although a statistically significant difference was not observed between maxillary vertical and maxillary horizontal positions, these devices caused more irrigant extrusion at the mandibular position. This finding might be explained by the negative influence of the gravitational force in APP groups.

In recent decades, RET, which allows for continued root development in immature teeth with apical periodontitis, has been recommended as an alternative treatment protocol to apexification with calcium hydroxide[30] and apical closure using mineral trioxide aggregate, especially in young patients.[31] Published protocols for RET commonly require the use of triple antibiotic paste as an intermediate medicament following limited or no root canal instrumentation with 1.5% to 3% NaOCl and 17% EDTA irrigation in a two-visit approach.[34] This procedure is associated with a high risk of apical extrusion resulting in the death of stem cells which are responsible for further root development. The results of this study revealed that the EV system is reliable and safe, as it enables to limit the circulation of irrigant solution to working length.

Conclusion

Within the limitations of this study, OEN extruded the most irrigant among the tested irrigation systems. The gravitational force has an impact on the amount of extruded irrigant when APP irrigation techniques are used. Utilizing the macrocannula of the EV system in immature teeth is a more reliable approach during irrigation as it prevents irrigant extrusion related to gravity.

Acknowledgments

The authors deny any conflicts of interest related to this study. The authors would like to thank Prof. Dr. Pelin Güneri for her valuable contributions and Assoc. Prof. Dr. Timur Köse for the statistical analysis of the study.

Author contributions

EN (enamsoy-lau@eul.edu.tr) carried out the experimental processes, analyzed the results, and wrote the manuscript, BS (burcuseref@yahoo.com) analyzed the results, reviewed the manuscript, MH (michael.huelsmann@med.uni-goettingen.de) reviewed results and manuscript, and MKC (kcaliskan1@yahoo.com) found the concept of the study, analyzed the results, and reviewed the manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Andreasen FM, Kahler B. Pulpal response after acute dental injury in the permanent dentition: Clinical implications- A review. J Endod 2015;41:299-308.
2. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: A comprehensive review. Int Endod J 2018;51:1367-88.
3. Zehnder M, Gold SI, Hasselgren G. Pathologic interactions in pulpal and periodontal tissues. J Clin Periodontol 2002;29:663-71.
4. Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, *et al.* Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod 2011;37:1109-15.
5. Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, *et al.* Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014;40:51-5.
6. Viola KS, Rodrigues EM, Tanomaru-Filho M. Cytotoxicity of peracetic acid: Evaluation of effects on metabolism, structure and cell death. Int Endod J 2018;51(Suppl 4):e264-7.
7. Boutsioukis C, Psimma Z, van der Sluis LWM. Factors affecting irrigant extrusion during root canal irrigation: A systematic review. Int Endod J 2013;46:599-618.
8. Dutner J, Mines P, Anderson A. Irrigation trends among American Association of Endodontists members: A web-based survey. J Endod 2012;38:37-40.
9. Desai P, Himel V. Comparative safety of various intracanal irrigation systems. J Endod 2009;35:545-9.
10. Nielsen BA, Baumgartner JC. Comparison of the EndoVac system to needle irrigation of root canals. J Endod 2007;33:611-5.
11. Mitchell RP, Yang SE, Baumgartner JC. Comparison of apical extrusion of NaOCl using the EndoVac or needle irrigation of root canals. J Endod 2010;36:338-41.
12. Velmurugan N, Sooriaprakas C, Jain P. Apical extrusion of irrigants in immature permanent teeth by using EndoVac and needle irrigation: An *in vitro* study. J Dent (Tehran) 2014;11:433-9.
13. Jamleh A, Fukumoto Y, Takatomo Y, Kobayashi C, Suda H, Adorno CG. A comparison between two negative pressure irrigation techniques in simulated immature tooth: An *ex vivo* study. Clin Oral Investig 2016;20:125-31.
14. Sharma R, Kumar V, Logani A, Chawla A, Sharma S, Koli B. Effect of gravity on periapical extrusion of irrigating solution with different irrigation protocols in immature anterior teeth. Eur Endod J 2020;5:150-4.
15. Uzunoglu E, Görduysus M. Apical extrusion of debris and irrigant using novel preparation systems. J Contemp Dent Prac 2014;15:423-7.
16. Kaşıkçı Bilgi I, Köseler I, Güneri P, Hülsmann M, Çalışkan MK. Efficiency and apical extrusion of debris: A comparative *ex vivo* study of four retreatment techniques in severely curved root canals. Int Endod J 2016;50:910-8.
17. Myers GL, Montgomery S. A comparison of weights of debris extruded apically by conventional filing and canal master techniques. J Endod 1991;17:275-9.
18. Konstantinidi E, Psimma Z, Chávez de Paz LE, Boutsioukis C. Apical negative pressure irrigation versus syringe irrigation: A systematic review of cleaning and disinfection of the root canal system. Int Endod J 2017;50:1034-54.
19. İnce Yusufoglu S, Keskin NB, Saricam E, Bozkurt DA. Comparison of apical debris extrusion using EDDY, passive ultrasonic activation and photon-initiated photoacoustic streaming irrigation activation devices. Aust Endod J 2020;46:400-4.
20. dos Reis S, Cruz VM, Hungaro Duarte MA, da Silveira Bueno CE, Vivan RR, Pelegrine RA, *et al.* Volumetric analysis of irrigant extrusion in immature teeth after different final agitation techniques. J Endod 2020;46:682-7.
21. Fukumoto Y, Kikuchi I, Yoshioka T, Kobayashi C, Suda H. An ex vivo evaluation of a new root canal irrigation technique with intracanal aspiration. Int Endod J 2006;39:93-9.
22. Altundasar E, Nagas E, Uyanik O, Serper A. Debris and irrigant extrusion potential of 2 rotary systems and irrigation needles. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e31-5.
23. Bürklein S, Benten S, Schafer E. Quantitative evaluation of apically extruded debris with different single-file systems: Reciproc, F360 and OneShape versus Mtwo. Int Endod J 2014;47:405-9.
24. Tanalp J, Gungor T. Apical extrusion of debris: A literature review of an inherent occurrence during root canal treatment. Int Endod J 2014;47:211-21.

25. Venumbaka NR, Baskaran P, Mungara J, Chenchugopal M, Elangovan A, Vijayakumar P. Comparative evaluation of EndoVac and conventional irrigating syringe on apical extrusion in primary molars. An *in vitro* study. J Clin Pediatr Dent 2018;42:135-40.
26. Psimma Z, Boutsioukis C, Vasiliadis L, Kastrinakis E. A new method for real-time quantification of irrigant extrusion during root canal irrigation *ex vivo*. Int Endod J 2013;46:619-31.
27. Boutsioukis C, Lambrianidis T, Kastrinakis E, Bekiaroglou P. Measurement of pressure and flow rates during irrigation of a root canal *ex vivo* with three endodontic needles. Int Endod J 2007;40:504-13.
28. Webber RT. Apexogenesis versus apexification. Dent Clin North Am 1984;28:669-97.
29. Kling M, Cvek M, Mejare I. Rate and predictability of pulp revascularization in therapeutically reimplanted permanent incisors. Endod Dent Traumatol 1986;2:83-9.
30. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Endod Dent Traumatol 1992;8:45-55.
31. Kandemir-Demirci G, Kaval ME, Güneri P, Çalışkan MK. Treatment of immature teeth with nonvital pulps in adults: A prospective comparative clinical study comparing MTA with $Ca(OH)_2$. Int Endod J 2020;53:5-18.
32. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: A data analysis of clinical protocols. J Endod 2015;41:146-54.
33. VandeVisse JE, Brilliant JD. Effect of irrigation on the production of extruded material at the root apex during instrumentation. J Endod 1975;1:243-6.
34. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, *et al.* European Society of Endodontology position statement: Revitalization procedures. Int Endod J 2016;49:717-23.