Science Arena Publications
Specialty Journal of Medical Research and Health Science
ISSN: 2521-3172
Available online at www.sciarena.com
2019, Vol, 4 (3): 43-50

The Effect of Intracanal Medication with Calcium Hydroxide on Recovering E. Faecalis from Root Canals after Obturation of Root Canal System

Jalil Modaresi[1, 2], Fatemeh Alahkasi[1], Narjes Hoshyari[3], Hengameh Zandi[3], Mehri Esfandyar[5]*

[1] Associate Professor, Department of Endodontics, Faculty of Dentistry, Sahid Sadoughi University of Medical Sciences, Yazd, Iran.
[2] Social determinants of oral health research center, Sadoughi University of Medical Sciences, Yazd, Iran.
[3] Associate Professor, Department of Endodontics, Sari dental school, Mazandaran University of Medical Sciences, sari, Iran.
[4] Postgraduate Student of dental and maxillofacial radiology, Department of dental and maxillofacial radiology, Faculty of Dentistry, Sahid Sadoughi University of Medical Sciences, Yazd, Iran.
[5] Postgraduate Student of endodontics, Department of Endodontics, Faculty of Dentistry, Sahid Sadoughi University of Medical Sciences, Yazd, Iran.

***Corresponding Author**

Abstract: *Aim: E.feacalis is one of the most common species isolated from the canals after instrumentation and medication. Therefore, a variety of intracanal medicaments such as Ca(OH)2 have been recommended for interappointment periods with the aim of complete disinfection of the root canal. It has been shown that this bacteria may remain in the canal even after obturation of root canal system. There is controversy regarding the effectiveness of interappointment medicaments and the aim of this study was assessing the effectiveness of medication with calcium hydroxide after obturation of the root canal system. Materials and methods: Access preparations were done on 66 extracted human teeth which were consequently infected with E.faecalis for 3 weeks. The canals were prepared by NiTi rotary instruments and irrigated with saline solution. The specimens were divided into two groups. In the first group, Calcium hydroxide was used as intracanal medication for 7 days followed by obturation of root canal system with gutta percha and sealer. The specimens of group 2 were obturated with gutta percha and sealer immediately. In the next stage, all teeth were incubated for 60 day at 37°c. Each specimen was transversally cut with diamond disk in middle of the root. Dentine chips were removed from two different depths of the middle intra walls with two sequential sterile round burs (iso 10,16) using a low speed handpiece. The Samples obtained by each bur were immediately collected in separate test tubes containing BHI broth medium, and then cultured in agar plates. Colony forming units were counted after 24 hours at 37°c. Data were ranked and analyzed using the Mann-Whitney U test and chi-square test. Results: E. Faecalis was recovered from 87.9% of superficial dentin and 78.3% of deep layer dentin in Group1 and 39.4% of super facial dentine and 24.2% of deep layer dentine in group 2. Based on colony counting, which were 41.92 and 42.4 colony count/mL($\times 10^{-2}$) for superficial and deep dentin respectively in group 1 and 25.08 and 24.8 colony count/mL($\times 10^{-2}$) for superficial and deep dentine respectively in group 2; Growth rate of E.feacalis was higher in superficial and deep layer dentine when calcium hydroxide was used. Conclusion: Even after 60 days, E.faecalis remained viable inside dentinal tubules. The result supports the findings which indicate that interappointment calcium hydroxide may not add to the antibacterial effectiveness of the treatment.*

Keywords: *E.faecalis, Ca(OH)2, root canal, obturation*

INTRODUCTION

The role of bacterias and their byproducts in the initiation and development of pulpal and periapical diseases is well known (Gutmann and Manjarrés, 2018); and one of the primary goals of the treatment is to kill or remove the microbes from the root canal system (Zhang et al., 2019). Biochemical instrumentation of root canal system has been suggested to achieve this goal (Junior et al., 2014). However, due to complexity of the root canal system, it has been shown that complete elimination of debris and achievement of a sterile root canal system is very difficult (Atila-Pektaş et al., 2013). Moreover, there is a lot of evidence in the literature indicating that many, if not most, root canals contain viable microorganisms even after complete chemomechanical preparation by the end of first appointment (Singh and Kasat, 2015; Kheirieh et al., 2014). Therefore, a variety of intracanal medicaments such as $Ca(OH)_2$ have been recommended to be used between appointments to complete disinfection of the root canal (Dixit, Dixit and Kumar, 2014). Calcium hydroxide is a strong antimicrobial agent with a PH of 12.5 (Iqbal, 2012). On the other hand, one of the most common species isolated from the canals after instrumentation and medication is E.faecalis. It has been considered one of the most resistant species in the oral cavity and one possible cause of post treatment diseases after root canal therapy (Jhajharia et al., 2015; Wang et al., 2012).

Until now, Calcium hydroxide medicament pastes are used widely in endodontics (Teoh, Athanassiadis and Walsh, 2018); but there has been a lot of debate about the effect of calcium hydroxide on E.faecalis among various studies. Mehvarzfar, Siqueira and some others have shown that Calcium hydroxide, as an interappointment medicament, can eliminate E.faecalis biofilm (Mehrvarzfar et al., 2011; Siqueira et al., 2003; Zehnder et al., 2003). In contrast, Shojaee, Mattigatti and some others have shown that it doesn't affect E.faecalis (Badr, Omar and Badria, 2011; Sapna et al., 2012; Mattigatti et al., 2012; Adl, Shojaee and Motamedifar, 2012). Antimicrobial effect of Calcium hydroxide is due to its high alkaline PH, which results in hydroxyl ions and also affects cell membrane (Kim and Kim, 2014). Compared to other bacterias, E.faecalis is more persistent to Calcium hydroxide (Portenier et al., 2005). This is due to the fact that E. faecalis has different amounts of sensitivity in various phases of its development cycle and also because the cell wall changes under stressful conditions in the canal (Portenier et al., 2005). Thus, other interappointment medicaments for eliminating E.faecalis biofilm, such as chlorhexidine gel and the combination of Calcium hydroxide and chlorhexidine have been suggested (Ghabraei et al., 2018; Lakhani et al., 2017).

However, up to now, we've only found few studies comparing the bacteriological post obturation status of the root canals, when the canals have been obturated with gutta-percha and sealer immediately or medicated with an antibacterial dressing before obturation (Vivacqua-Gomes et al., 2005). Therefore, the aim of this study is to evaluate E. faecalis recovery after obturation of root canal system.

Materials and Methods:

The methodology used in this study was a modification of the one previously described by Haapasalo & Qrstavik by adapting it to extracted human teeth.

The steps of this study are as follow:

1. **Sample Selection and Initial Preparation**

Sixty-six caries-free mandibular premolars without any cracks, freshly extracted, with complete apex formation and a straight root canals were used (Haapasalo and Ørstavik, 1987). The teeth were cleaned with curettes to remove periodontal tissue and bone. Then, the specimens were kept in 0.5% hypo in less than 7 days period. The crowns were removed in order to an enhanced accessing with diamond disk (DiaDent, Maribor, Slovenia)to the level of CEJ resulting in roots 15mm in length. The teeth were instrumented to the apex using #20 k file (Mani Inc., Tochigi, Japan) to create a patency and facilitate the intra canal contamination procedures. The roots were stored in water at all times to avoid dehydration.

Teeth were placed in 5.25% NaOCl solution (NaOCl, Yekta, PakNam Co., Tehran, Iran) for 10 minutes and then they were put in ultrasonic bath for another 10 minutes. After these steps, they were placed in 17% EDTA (Ariadent, Asia Chemi Teb Co., Tehran, Iran) and then again for 10 minutes in ultrasonic bath.
In order to remove the residuals of Sodium NaOCl and EDTA, each of the samples were washed with water for 10 minutes.

2. Inoculation of E. faecalis

Then, each of the roots were placed separately in bottles containing Brain–Heart Infusion Medium (BHI; Oxoid, Basingstoke, UK) and autoclaved at 121°C, 15psi, for 15 min to be sterilized. Isolated colonies (24h) of pure culture of E.faecalis(ATCC 29212) grown on 5% defibrinated sheep blood agar plates were suspended in 5.0 ml of BHI. The cell suspension was adjusted sepectrophotometrically to match the turbidity of 1.5×10^8 colony forming unit (CFU) ml^{-1} (equivalent to ± 0.5 Mac farland standard). Specimens were placed separately in bottles containing microbial suspension for bacterial penetration through the root canals and dentinal tubules. Finally, they were kept in incubator at 37°C for 3 weeks.

3. Final Preparation and Obturation

Following the contamination period each specimen was removed from its bottle under aseptic condition and the canal was irrigated with 3.0 ml of normal saline.
All the samples were instrumented using NiTi rotary instruments (protaper dentsply. Co) up to F2
followed by manual apical preparation up to #35 K file (Mani Inc., Tochigi, Japan). The specimens were divided randomly into 2 groups, each containing 33 specimens. In the first group, a creamy mixture of $Ca(OH)_2$ powder (Golchai Co., Tehran, Iran) and 0.9% normal saline was placed in root canals using files, as intracanal medication for 7 days. By the end of the 7th day, the canals were irrigated with normal saline in order to remove Ca(OH)2 and dried with sterile paper point (Ariadent Co., Tehran, Iran). Then, the canals were obturated with gutta-percha (Ariadent Co, Tehran, Iran) and ZOE based sealer (Pishro dandan Co,Tehran,Iran) using lateral condensation method and under sterile conditions.
The specimens of group 2 were obturated with gutta-percha and sealer identical to group 1, immediately after final preparation but no medications were used.

4. Incubation period

Then, the teeth from both groups were coronally sealed with temporary restoration, soaked in 50 mL BHI and were incubated for 2 months at 37°C. BHI Solution was replaced every 72 hours.

5. Counting of Colony

Each specimen was transversally cut with diamond disk (DiaDent, Maribor, Slovenia) in middle of the root under sterilized conditions. Dentine chips for detection E. Faecalis were removed from two different depths of the intra walls with two sequential sterile round burs (Iso 10, 16, Teeskavan Co., Tehran, Iran) with a low speed handpeice. In order to standardize the volume of dentin chips, equal dentinal volumes were removed from samples. Samples obtained by each bur were immediately collected in separate test tubes containing 1mL BHI medium and 0/01mL of this suspension was cultured on BHI medium enriched by 0/05 mL sheep blood agar plates and and were kept at $37C^o$ for 24 hours to allow count of colony.
In order to be certain of development of E. faecalis colonies, samples were checked using gram staining test, catalase test and Bile esculin medium(March Company 6.0% NaCl) test, and then the colonies were counted.

6. Statistical Analysis

Data were ranked and analyzed using the Mann- Whitney and chi-square tests.

Results

The results of this study show that placing Calcium Hydroxide in the canal before obturation the canal compared to immeadiate obturation of the root canal system, not only didn't have any decreasing effect on the

colonies, but also provided more possibility for growth of E. faecalis. .group 1 had more colonies compared to group 2 and the difference between group 1 and group 2 was significant regarding both superficial and deep dentine (P-Value<0.001). In both groups, after 2 months, E. faecalis bacterias were present in both superficial and deep dentine; which indicates that appropriate conditions for surviving and multiplication of the colonies of E. faecalis in dentinal tubules of treated root canal systems will still be available.

Table 1: Comparison of mean number of E. faecalis colony count/mL($\times 10^{-2}$)

Colony count/mL($\times 10^{-2}$)	Group I (with $Ca(OH)_2$)		Group II (without $Ca(OH)_2$)	
	Superficial dentin	Deep dentin	Superficial dentin	Deep dentin
Mean	41.92	42.4	25.08	24.8
P-Value	P<0.001		P<0.001	

Table 2: Analysis of data with Chi- square test (based on detection of E.F visually)

	Antimicrobial effect	Group I (with $Ca(OH)_2$)	Group II (without $Ca(OH)_2$)	Total	P-value
superfacial dentin	Viability of E.F	87.8%	39.4%	63.6%	<0.0001
	Elimination of E.F	12.1%	60.6%	36.4%	<0.0001
In Deep dentin	Viability of E.F	78.8%	24.2%	51.5%	<0.0001
	Elimination of E.F	21.2%	75.8%	48.5%	<0.0001

Discussion

In this study, we evaluated the decreasing effect of intracanal calcium hydroxide in infected dental canals by E. faecalis compared with immediate obturation of the tooth. It was concluded that using intracanal calcium hydroxide for 7 days not only doesn't decrease the count of E. faecalis, but also leads to increased colony counts of E. faecalis compared to immediate obturation of the root canal system with gutta percha.

Several models have been proposed in the literature for the study of dentin infection and most of them use E.feacalis as the microorganism of choice (Ørstavik and Haapasalo, 1990; Komorowski et al., 2000; Ferraz et al., 2001). E.feacalis is a facultative gram-positive anaerobic cocci, and is related to persistent root canal infections (Gomes et al., 1996; Peciuliene et al., 2000). It is more resistant to at least some local endodontic medicament than most other microbes (Stuart et al., 2006).

Calcium hydroxide is the material of choice for intracanal medicament in endodontics (Manohar and Sharma, 2018). In this investigation, calcium hydroxide mixed with normal saline was used as an intracanal medicament due to better diffusion capacity through dentin and higher PH values compared to others (Pacios et al., 2004; Mori et al., 2009).

The methodology used was a modification of the one previously described by Haapasalo & Qrstavik (Haapasalo and Ørstavik, 1987). It has been also used in other studies regarding effect of antibacterial materials on the amount of E.faecalis in dentin tubules (Spangberg, Engström and Langeland, 1973).

E.feacalis has been detected within dentinal tubules up to 500-700 μm deep after 60 days of incubation (Gomes et al., 2003). Saleh et al used 3 weeks of incubation and found microorganisms up to 300-400 μm deep within the tubules (Saleh et al., 2004). So, in this study, the samples were incubated 3weeks for bacterial penetration.

Nerwich et al demonstrated that hydroxyl ions derived from a calcium hydroxide dressing, diffuse through root dentin faster and reach higher levels in the cervical part of the root than in the apical region (Nerwich, Figdor and Messer, 1993). for this study, the samples were taken from mid portion of the root canals.

Various studies have evaluated the effect of intaracanal antibacterial materials on elimination of E.faecalis (Ghabraei et al., 2018; Lakhani et al., 2017); but only in a few of these studies the comparison of immediate root filling in a single visit and filling after use of a calcium hydroxide dressing in multiple visits were done.

Vivacqua-Gomes et al. studied the amount of E. faecalis after intracanal calcium hydroxide for 14days compared to immediate obturation of the root canal system. It should be noted that they used CHX irrigation for canal debridement. They concluded that no statistical differences in E. faecalis counts were found between single-visit and multiple-visits root canal treatments. Although there was an increase, no significant difference was seen (Gomes et al., 1996). the reason for this contradiction between our results can be due to the different irrigation materials used in our studies.

The principal known benefit of calcium hydroxide lies in its bactericidal effect conferred by its PH. calcium hydroxide kills the bacteria producing an alkaline environment by diffusion of hydroxyl ions through the dentinal tubules (Tronstad et al., 1981). The result of this study indicated that interappointment calcium hydroxide did not add to the antibacterial effectiveness of the treatment and this result is compatible with other studies that have shown calcium hydroxide is ineffective in complete elimination of E.feacalis in root canal even after an extended time of incubation (Vivacqua-Gomes et al., 2005; Haapasalo and Ørstavik, 1987; Safavi, Spngberg and Langeland, 1990).

Although Estrela et al (2001) showed that E.faecalis was eradicated within 1 hr in direct exposure to calcium hydroxide in saline and Evans et al (2002) found that a functioning intracellular proton pump was the primary factor of alkaline resistance of E.faecalis. The protein pump of E.faecalis appears to function until it is overwhelmed at PH values of 11.5 and higher (Tronstad et al., 1981). Tronstad et al assessed the PH of various areas of dentin after the placement of calcium hydroxide paste with PH of 12.2 into the root canal. The PH within the canal ranged from 10 to 12.2, whereas in adjacent dentin, PH was 8 to 11.1 and peripheral dentin had PH range of 7.4 to 9.6 (Tronstad et al., 1981).

It's been shown after application of Calcium Hydroxide, its PH won't reach a level to affect E. faecalis in different depths of dentinal tubules (Tronstad et al., 1981; Nerwich, Figdor and Messer, 1993). Thus, bacterias will be exposed to PH levels below 11, in which E. faecalis is able to survive and continue to grow due to protein pump function.

Haapasalo et al. stated that causes of increasing of E. faecalis colonies can be inhibitory⁄neutralizing effect of dentin on calcium hydroxide and poor diffusion of hydroxyl ions into infected dentin (Haapasalo et al., 2000).

In this investigation, the canals were obturated with ZOE based sealer. Zinc oxide is a valuable component of this sealer due to its antimicrobial function. It creates a low level but long lasting antimicrobial effect (Saleh et al., 2004). Furthermore, Siqueira et al demonstrated that a new preparation of ZOE sealer had large zones of inhibition against all microorganisms tested (Siqueira et al., 2000).

Also, according to Pizzo and Cobankara studies ZOE sealer was one of the three most potent bacterial – growth inhibitors of E.feacalis (Pizzo et al., 2006; Çobankara et al., 2004).

In Vivacqua-Gomes et al's study it was shown that the most important factor in decreasing E. faecalis bacterias is using sealer; because, in absence of sealer, all the samples were contaminated by E. facelis 2 months after the obturation of root canal system (Gomes et al., 1996).

The result of this investigation illustrated no aggregatory effect of calcium hydroxide with ZOE sealer in eliminating E.feacalis. vice versa, group 1(with calcium hydroxide) demonstrated higher recovery of E.feacalis two months after obturation. This might be because of the interaction between calcium hydroxide and ZOE sealer. Margelos J in his study on the effect of calcium hydroxide on ZOE sealer has concluded that Calcium hydroxide preferably interacts with eugenol inhibiting the ZnO-eugenol chelate formation (Margelos et al., 1997). The $Ca(OH)_2$-eugenol interaction is rapid, and leading to residual eugenol in the set product. The ZnOE sealers in contact with calcium hydroxide are brittle in consistency and granular in structure (Margelos et al., 1997; Metzger, Basrani and Goodis, 2011). This composition may have not ideal antibacterial efficacy.

One of the other reasons for increasing of E.faecalis in two session obturation with interappointment placement of Calcium Hydroxide can be remaining of Calcium Hydroxide in the walls of root canal and inhibiting the permeation of sealer into the dentinal tubules. Therefore interaction with the antibacterial effect of the sealer would occur.

Conclusion

Even after 60 days E.feacalis remained viable inside dentinal tubules. The result supports the findings indicating that interappointment calcium hydroxide may not add to the antibacterial effectiveness of the treatment.

References

1. Adl, A., Shojaee, N. S., & Motamedifar, M. (2012). A comparison between the antimicrobial effects of triple antibiotic paste and calcium hydroxide against Entrococcus faecalis. Iranian endodontic journal, 7(3), 149.
2. Atila-Pektaş, B., Yurdakul, P., Gülmez, D., & Görduysus, Ö. (2013). Antimicrobial effects of root canal medicaments against E nterococcus faecalis and S treptococcus mutans. International endodontic journal, 46(5), 413-418.
3. Badr, A. E., Omar, N., & Badria, F. A. (2011). A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. International endodontic journal, 44(1), 51-58.
4. Çobankara, F. K., Altinöz, H. C., Erganiş, O., Kav, K., & Belli, S. (2004). In vitro antibacterial activities of root-canal sealers by using two different methods. Journal of endodontics, 30(1), 57-60.
5. Dixit, S., Dixit, A., & Kumar, P. (2014). Nonsurgical treatment of two periapical lesions with calcium hydroxide using two different vehicles. Case reports in dentistry, 2014.
6. Estrela, C., de Araújo Estrela, C. R., Bammann, L. L., & Pecora, J. D. (2001). Two methods to evaluate the antimicrobial action of calcium hydroxide paste. Journal of endodontics, 27(12), 720-723.
7. Evans, M., Davies, J. K., Sundqvist, G., & Figdor, D. (2002). Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. International Endodontic Journal, 35(3), 221-228.
8. Ferraz, C. C. R., de Almeida Gomes, B. P. F., Zaia, A. A., Teixeira, F. B., & de Souza-Filho, F. J. (2001). In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. Journal of Endodontics, 27(7), 452-455.
9. Ghabraei, S., Bolhari, B., Sabbagh, M. M., & Afshar, M. S. (2018). Comparison of Antimicrobial Effects of Triple Antibiotic Paste and Calcium Hydroxide Mixed with 2% Chlorhexidine as Intracanal Medicaments Against Enterococcus faecalis Biofilm. Journal of Dentistry (Tehran, Iran), 15(3), 151.
10. Gomes, B. P. F. A., Lilley, J. D., & Drucker, D. B. (1996). Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. International endodontic journal, 29(4), 235-241.
11. Gomes, B. P. F. A., Souza, S. F. C., Ferraz, C. C. R., Teixeira, F. B., Zaia, A. A., Valdrighi, L., & Souza-Filho, F. J. (2003). Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. International Endodontic Journal, 36(4), 267-275.
12. Gutmann, J., & Manjarrés, V. (2018). Historical and Contemporary Perspectives on the Microbiological Aspects of Endodontics. Dentistry journal, 6(4), 49..
13. Haapasalo, H. K., Siren, E. K., Waltimo, T. M. T., Orstavik, D., & Haapasalo, M. P. P. (2000). Inactivation of local root canal medicaments by dentine: an in vitro study. International endodontic journal, 33(2), 126-131.
14. Haapasalo, M., & Ørstavik, D. (1987). In vitro infection and of dentinal tubules. Journal of dental research, 66(8), 1375-1379.
15. Iqbal, A. (2012). Antimicrobial irrigants in the endodontic therapy. International journal of health sciences, 6(2), 186.
16. Jhajharia, K., Parolia, A., Shetty, K. V., & Mehta, L. K. (2015). Biofilm in endodontics: a review. Journal of International Society of Preventive & Community Dentistry, 5(1), 1.

17. Junior, E. C. S., da Fonseca, T. S., da Frota, M. F., de Carvalho, F. M. A., Marques, A. A. F., & Garcia, L. D. F. R. (2014). Cleaning capacity of hybrid instrumentation technique using reamer with alternating cutting edges system files: Histological analysis. Contemporary clinical dentistry, 5(2), 203.
18. Kheirieh, S., Fazlyab, M., Torabzadeh, H., & Eghbal, M. J. (2014). Extraoral retrograde root canal filling of an orthodontic-induced external root resorption using CEM cement. Iranian endodontic journal, 9(2), 149.
19. Kim, D., & Kim, E. (2014). Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review-Part I. In vitro studies. Restorative dentistry & endodontics, 39(4), 241-252.
20. Komorowski, R., Grad, H., Wu, X. Y., & Friedman, S. (2000). Antimicrobial substantivity of chlorhexidine-treated bovine root dentin. Journal of endodontics, 26(6), 315-317.
21. Lakhani, A. A., Sekhar, K. S., Gupta, P., Tejolatha, B., Gupta, A., Kashyap, S., ... & Farista, S. (2017). Efficacy of triple antibiotic paste, moxifloxacin, calcium hydroxide and 2% chlorhexidine gel in elimination of E. Faecalis: an in vitro study. Journal of clinical and diagnostic research: JCDR, 11(1), ZC06.
22. Manohar, M. P., & Sharma, S. (2018). A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. Indian Journal of Dental Research, 29(6), 716.
23. Margelos, J., Eliades, G., Verdelis, C., & Palaghias, G. (1997). Interaction of calcium hydroxide with zinc oxide-eugenol type sealers: a potential clinical problem. Journal of Endodontics, 23(1), 43-48.
24. Mattigatti, S., Ratnakar, P., Moturi, S., Varma, S., & Rairam, S. (2012). Antimicrobial effect of conventional root canal medicaments vs propolis against Enterococcus faecalis, Staphylococcus aureus and Candida albicans. J Contemp Dent Pract, 13(3), 305-9.
25. Mehrvarzfar, P., Akhavan, H., Rastgarian, H., Akhlagi, N. M., Soleymanpour, R., & Ahmadi, A. (2011). An in vitro comparative study on the antimicrobial effects of bioglass 45S5 vs. calcium hydroxide on Enterococcus faecalis. Iranian endodontic journal, 6(1), 29.
26. Metzger ZVI, Basrani B, Goodis e. (2011). Instruments, Materilas, and devices. In: Cohen S, Hargreaves KM. Pathway of the pulp. 10th ed. St. Louis: Mosby, 264.
27. Mori, G. G., Ferreira, F. C., Batista, F. R. D. S., Godoy, A. M. D. S., & Nunes, D. C. (2009). Evaluation of the diffusion capacity of calcium hydroxide pastes through the dentinal tubules. Brazilian oral research, 23(2), 113-118.
28. Nerwich, A., Figdor, D., & Messer, H. H. (1993). pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. Journal of endodontics, 19(6), 302-306.
29. Nerwich, A., Figdor, D., & Messer, H. H. (1993). pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. Journal of endodontics, 19(6), 302-306.
30. Ørstavik, D., & Haapasalo, M. (1990). Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Dental Traumatology, 6(4), 142-149.
31. Pacios, M. G., de la Casa, M. L., de los Ángeles Bulacio, M., & López, M. E. (2004). Influence of different vehicles on the pH of calcium hydroxide pastes. Journal of oral science, 46(2), 107-111.
32. Peciuliene, V., Balciuniene, I., Eriksen, H. M., & Haapasalo, M. (2000). Isolation of Enterococcus faecalis in previously root-filled canals in a Lithuanian population. Journal of endodontics, 26(10), 593-595.
33. Pizzo, G., Giammanco, G. M., Cumbo, E., Nicolosi, G., & Gallina, G. (2006). In vitro antibacterial activity of endodontic sealers. Journal of dentistry, 34(1), 35-40.

34. Portenier, I., Waltimo, T., Ørstavik, D., & Haapasalo, M. (2005). The susceptibility of starved, stationary phase, and growing cells of Enterococcus faecalis to endodontic medicaments. Journal of Endodontics, 31(5), 380-386.
35. Safavi, K. E., Spngberg, L. S., & Langeland, K. (1990). Root canal dentinal tubule disinfection. Journal of endodontics, 16(5), 207-210.
36. Saleh, I. M., Ruyter, I. E., Haapasalo, M., & Ørstavik, D. (2004). Survival of Enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. International Endodontic Journal, 37(3), 193-198.
37. Sapna, H., Priti Kamlesh, L., Dinesh Rao, B., & Shubha, A. B. (2012). An in vitro evaluation of antimicrobial efficacy of primary root canal filling materials. Journal of Clinical Pediatric Dentistry, 37(1), 59-64.
38. Singh, M., & Kasat, V. O. (2015). Efficacy of various combinations of irrigants and medicaments on Candida albican: An in vitro study. Journal of International Society of Preventive & Community Dentistry, 5(3), 157.
39. Siqueira Jr, J. F., Favieri, A., Gahyva, S. M., Moraes, S. R., Lima, K. C., & Lopes, H. P. (2000). Antimicrobial activity and flow rate of newer and established root canal sealers. Journal of endodontics, 26(5), 274-277.
40. Siqueira Jr, J. F., Rôças, I. N., Lopes, H. P., Magalhães, F. A., & de Uzeda, M. (2003). Elimination of Candida albicans infection of the radicular dentin by intracanal medications. Journal of Endodontics, 29(8), 501-504.
41. Spangberg, L., Engström, B., & Langeland, K. (1973). Biologic effects of dental materials: 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surgery, Oral Medicine, Oral Pathology, 36(6), 856-871.
42. Stuart, C. H., Schwartz, S. A., Beeson, T. J., & Owatz, C. B. (2006). Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics, 32(2), 93-98.
43. Teoh, Y. Y., Athanassiadis, B., & Walsh, L. (2018). Comparison of commercial calcium hydroxide pastes for prolonged antibacterial effect using a colourimetric assessment. Materials, 11(3), 348.
44. Tronstad, L., Andreasen, J. O., Hasselgren, G., Kristerson, L., & Riis, I. (1981). pH changes in dental tissues after root canal filling with calcium hydroxide. Journal of endodontics, 7(1), 17-21.
45. Tronstad, L., Andreasen, J. O., Hasselgren, G., Kristerson, L., & Riis, I. (1981). pH changes in dental tissues after root canal filling with calcium hydroxide. Journal of endodontics, 7(1), 17-21.
46. Vivacqua-Gomes, N., Gurgel-Filho, E. D., Gomes, B. P. F. A., Ferraz, C. C. R., Zaia, A. A., & Souza-Filho, F. J. (2005). Recovery of Enterococcus faecalis after single-or multiple-visit root canal treatments carried out in infected teeth ex vivo. International Endodontic Journal, 38(10), 697-704.
47. Wang, Q. Q., Zhang, C. F., Chu, C. H., & Zhu, X. F. (2012). Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis. International journal of oral science, 4(1), 19.
48. Zehnder, M., Grawehr, M., Hasselgren, G., & Waltimo, T. (2003). Tissue-dissolution capacity and dentin-disinfecting potential of calcium hydroxide mixed with irrigating solutions. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 96(5), 608-613.
49. Zhang, D., Shen, Y., de la Fuente-Núñez, C., & Haapasalo, M. (2019). In vitro evaluation by quantitative real-time PCR and culturing of the effectiveness of disinfection of multispecies biofilms in root canals by two irrigation systems. Clinical oral investigations, 23(2), 913-920..