European Journal of Research in Dentistry 2020; 4(2): 45–51
DOI: 10.35333/ERD.2020.265

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

An *in-Vitro* Study on the Effectiveness of Different Irrigation Solutions Against *Enterococcus Faecalis* with/without Erbium Laser Activation

Erbiyum Lazer Aktivasyonu olan/olmayan Farklı İrrigasyon Solüsyonlarının *Enterococcus Faecalis*'e Karşı Etkinliği Üzerine in vitro Bir Çalışma

Gülşah BALAN, Sadık KALAYCI, Ayşen YARAT, Serap AKYÜZ, Fikrettin ŞAHİN

Abstract

Objective: Questions have been raised on the safety of prolonged use of sodium hypochlorite. There is a demand on developing alternative agents and activation methods for effective irrigation in a short time. Our study compares sodium hypochlorite to alternative soltions with and without laser activation to evaluate antibacterial efficiency against *Enterococcus faecali*s.

Methods: Bacteria was inoculated into 54 human teeth and incubated for 28 days to build up biofilm. The teeth then were randomly divided according to the irrigation method into two; (1) syringe (S) and (2) Erbium laser activation (LA) groups. In each group, teeth were divided into 4 experimental subgroups to evaluate the antibacterial properties of four different solutions; saline (NaCl), sodium hypochlorite (NaOCl), sodium pentaborate pentahydrate (NaB) and deuterium depleted water (DDW). The amount of irrigant solution was 10 mL for each. Samples were taken from canal wall and colony forming units were determined by plate counting after 24 hours. The survival rates of the bacteria were calculated and recorded as data.

Results: As expected, NaOCl-LA was found to be more effective than Saline-LA ($p<0.05$) but not superior than NaOCl-S ($p>0.05$). NaB showed similar antibacterial efficiency as NaOCl ($p>0.05$). Both techniques reduced the bacterial survivals, despite, statistically there was no difference between S and LA ($p>0.05$).

Conclusion: NaOCl, which has the highest antibacterial efficacy, was followed by NaB, DDW and Salin, respectively. Laser activation activation did not make any difference on the antibacterial efficiency of the solutions.

Keywords: deuterium depleted water, *Enterococcus faecalis*, Erbium laser activation, sodium pentaborate pentahyrate, sodium hypochlorite

Amaç: Sodyum hipokloritin(NaOCl) uzun süreli kullanımı güvenlikle ile ilgili soruları gündeme getirmiştir. Kısa sürede ve etkili irrigasyon için alternatif ajanların ve aktivasyon yöntemlerinin geliştirilmesi gerekir. Çalışmamız, *Enterococcus faecalis*'e karşı antibakteriyel etkinliği değerlendirmek için lazer aktivasyonu olan ve olmayan farklı solüsyonlarla sodyum hipokloriti karşılaştırmaktadır.

Yöntem: Bakteriler 54 insan dişine ekildi ve biyofilm oluşturmak için 28 gün inkübe edildi. Dişler daha sonra irrigasyon metoduna göre rastgele ikiye ayrıldı; (1) şırınga (S) ve (2) Erbium lazer aktivasyon (LA) grupları. Her grup, antibakteriyel özellikleri değerlendirilecek solüsyonlara göre 4 deney alt grubuna ayrıldı; salin (NaCl), sodyum hipoklorit (NaOCl), sodyum pentaborat pentahidrat (NaB) ve döteryumu azaltılmış su (DDW). İrrigant miktarı, her biri için 10 mL idi. Kanal duvarından örnekler alındı ve koloni oluşturan birimler 24 saat sonra plaka sayımı ile belirlendi. Bakterilerin sağ kalım oranları hesaplandı ve kaydedildi.

Bulgular: Beklendiği gibi, NaOCl-LA'nın Salin-LA'dan daha etkili olduğu ($p<0.05$), ancak NaOCl-S'den daha üstün olmadığı bulundu ($p>0.05$). NaB, NaOCl ile benzer antibakteriyel etkinlik gösterdi ($p>0.05$). Her iki teknik de bakteri sağ kalımları azalttı, ancak S ve LA arasında istatistiksel fark bulunmadı ($p>0.05$).

Sonuç: Antibakteriyel etkinliği en yüksek bulunan NaOCl'i sırasıyla NaB, DDW ve Salin takip etti. Lazer aktivasyon, solüsyonların antibakteriyel etkinlikleri üzerinde fark yaratmadı.

Anahtar Kelimeler: döteryumu azaltılmış su, *Enterococcus faecalis*, Erbiyum lazer aktivasyonu, sodyum pentaborat pentahidrat, sodyum hipoklorit

Gülşah Balan (✉)
Marmara University, Faculty of Dentistry, Department of Pedodontics, Basibuyuk Yolu 9/3 34854 Basibuyuk, Maltepe, Istanbul, Turkey, e-mail: gulsahbalan@marun.edu.tr, gulsahbalan@hotmail.com

Serap Akyüz, Ayşen Yarat
Departments of Pediatric Dentistry and Basic Medical Sciences-Biochemistry, Faculty of Dentistry, Marmara University, Istanbul, Turkey

Sadık Kalaycı, Fikrettin Şahin
Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, Istanbul, Turkey

This work is supported in part by the grant Marmara University Scientific Research Projects Unit (SAG-C-DRP-241018-0573). I would like to thank all co-authors and the staff of Yeditepe University Biocidal and AR-GE Laboratory unit.

Submitted / Gönderilme: 20.04.2020 **Accepted/Kabul:** 10.09.2020

Introduction

Bacterial reduction within the root canal system is essential to remove residues from the dentin walls and also to avoid complications of periradicular structures (1), in which *Enterococcus faecalis* (*E. faecalis*) is often isolated from the root canals with chronic periapical pathology

(2). Besides mechanical preparation, final irrigation is an important chemomechanical part that facilitates bacterial elimination while removing necrotic tissue and smear layer. In this manner, irrigation solutions should propose high antibacterial efficiency and tissue solving abilities. Sodium hypochlorite (NaOCl) is a widely used broad spectrum potent antimicrobial (3). It is used in concentrations ranging from 0.5% to 6% (4). However, it is non-specifically cytotoxic and accidentally causes damage if it is in contact with the periapical area or adjacent structures such as the maxillary sinus (5). Therefore, research is ongoing to find alternative irrigation solutions that can replace NaOCl.

There is an attention to boron-containing compounds in order to develop novel therapeutics and dental materials (6) as boron has no adverse effect on vital organs and has some facilities to overcome healing complications (7, 8). Moreover, it expresses antifungal, antiviral and antibacterial action (9, 10). Boric acid solution was tested to consider capability of smear layer removal in root canal (11, 12) and disinfection action in periodontal pocket (13). Sodium pentaborate pentahydrate (NaB; $NaB_5O_8.5H_2O$) is one of the basic refined boron compound containing 5 moles of water (7). NaB has been found to be successful in a research for odontogenic and osteogenic differentiation capacity (14). NaB added dental composite has been evaluated on *S. mutans* in an aim of avoiding secondary caries and the physical changes of composite material (15). Also, previous research has shown that sodium perborate has similar antibacterial activity compared to 5% NaOCl against *E. faecalis* (16). Very little was found in the literature on the question of antibacterial properties of NaB, besides its osteogenic influences.

Deuterium depleted water (DDW), called as light water, has less deuterium than natural water. It has been reported that using water with low deuterium concentration does not have harmful effects on living organisms (17-19), however it causes necrosis or apoptosis on unhealthy cells (20). Naturally occurring deuterium is essential for maintaining normal cell growth (21) and in the presence of low deuterium, such as 30 ppm, the vital activity of cells slows to a halt. Studies have indicated that some metabolic enzymes play a role in the fractionation of deuterium localized in the mitochondria, so the functioning of electron transport in the mitochondria varies according to concentration of deuterium (20, 22). Thus, the H/D kinetic isotope effect appears as the major limiting factor of cellular functions under deuteration (23). In contrast, low organisms such as algae, yeast and mold and some other eukaryotic unicellular organisms can survive in the environment above 100% D_2O due to their cellular components (24-26). Although, single-cell organisms can often grow in conditions of full deuteration (24), it has not been tested before low deuterium (25 ppm) environment in a root canal system.

There are various ways that enhance efficiency of solutions such as using different concentrations, combination of agents, altered vehicles, and physical agitation to eliminate drawbacks of conventional syringe irrigation (S) (27). Erbium lasers (Er:YAG and Er,Cr:YSGG) have been investigated on solution activation as an alternative agitation technique in endodontics (28, 29). Laser activation (LA) is based on generation of laser-induced bubble collapses by high powered lasers, which creates shockwaves and high fluid dynamics in a liquid (30-32). This strong photoacoustic shockwaves triger physical removal of debris or biofilm in untouched areas in root canal system (33). It was found to be more effective in bacterial elimination than syringe or passive ultrasonic irrigation (34). Er,Cr:YSGG LA has been included in our study to enhance actions of the experimental solutions which have no tissue dissolving capacity.

Our study compares four different irrigants with and without Er,Cr;YSGG laser activation to evaluate antibacterial efficiency against *E. faecali*s.

Material and Method

Sample Preparation

The study protocol was approved by the Clinical Research and Ethics Committee of the University of Marmara (No. 2018–209). A total of 54 single-rooted human teeth were extracted by using therapeutic indication. The specimens were subjected to 0.5% NaOCl for 24 hours and cleaning on the external part of the root by a Gracey 7/8 curette (Hu-Friedy, Chicago, IL) to remove remnants of periodontal ligament and root surface calculus. Until the endodontic shaping step, the teeth were kept in a 0.1% thymol solution at +4 ° C.

A cylindrical diamond bur was used to create the root canal entrance and gates gladden burs were used to enlarge the coronal entrance. The apical permeability and single canal confirmation were verified with #10 K-file (Dentsply Maillefer). The working length was determined to be 1mm less because the #10 K-File was visible through the apical

foramen. Final instrument size was ISO 30 .06 taper 1 mm beyond the apical foramen. Each instrument was irrigated in between treatments with 2.5% NaOCl using a syringe and a 30-G side-vented needle (Becton Dickinson, Madrid, Spain). To remove the smear layer, the root canals were rinsed with 10 mL 17% ethylene-diamine-tetraacetic acid (EDTA) (Denta Flux, Madrid, Spain) for 3min followed by 10 mL of saline solution. The apical aperture was closed with a composite filler and the outer surface of the teeth was covered with at least two layers of nail polish to prevent extrusion of the irrigant and to simulate clinical conditions. The teeth were stored in eppendorf tubes and autoclaved at 121°C for 15 min, then stored at +4 ° C.

Cultivation and inoculation of bacteria

E. faecalis, ATCC 29212 (American Type Culture Collection), maintained by subculturing on tryptic soy agar (TSA) plates weekly. For experiments, single colonies were inoculated into 40 mL of tryptic soy broth (TSB) medium and incubated at 37°C for 24h. The *E. faecalis* culture was diluted 100 times in fresh TSB and then McFarland standard number 0.5 was used to evaluate the broth to ensure that the number of bacteria was 1.5×10^8 CFU/mL. Each specimen was inoculated with 10 µL of *E. faecalis* suspension using a sterile 100 µL pipette tip and was incubated in aerobic condition at 37 °C for 4 weeks to allow biofilm formation and maturation in the closed canal system, with the broth refreshed every 3-day.

Grouping and Treatment

After four weeks of incubation, the infected 54 teeth were randomly divided according to the irrigation method into two; (*1*) syringe (S) and (*2*) laser activation (LA) groups. In each group the teeth were randomly divided into 4 experimental subgroups to evaluate the antibacterial properties of four different solutions. Before experiment, paper points samples were taken from canal walls to ensure the biofilm formation on canal walls.

The teeth of S method were divided into four subgroups as follows: 10 mL of sterile phosphate buffered saline (PBS) solution for the positive control group (Saline-S; n=5), 10 mL of 5% sodium hypochlorite for the negative control group (NaOCl-S; n=8), 10 mL 3% sodium pentaborate pentahydrate for the first experimental group (NaB-S; n=8), 10 mL 25 ppm DDW for the second experimental group (DDW-S; n=7). In this method, 30G endodontic side-vented needles (NaviTip 31G/27 mm, Ultradent, New York, USA) were placed passively in the canal, 2 mm away from the apex. 10 mL of test solution was used for each tooth sample. Irrigation of the canals took approximately 5 min for each specimen.

The teeth of LA were divided into four as follows: as the positive control group 10 mL sterile PBS solution was used (Saline-L; n=5); as the negative control group 10 mL 5% sodium hypochlorite (NaOCl-L; n=7); as the first experimental group 10 mL of 3% sodium pentaborate pentahydrate (NaB-L; n=7); and as the second experimental group 10 mL of 25 ppm deuterium depleted water (DDW-L; n=7) were used. LA was performed using an Er,Cr:YSGG-pulsed laser (Waterlase iPlus; BIOLASE Technology, Irvine, CA) at 2780 nm wavelength, equipped with a glass RFT5 tip (BIOLASE Technology, Inc.; 500 µm in diameter, length 14mm, calibration factor >0.80). The treatment was at 0.75W average power at 10Hz (140sec/pulse). Irradiance was an energy density of 18.75 W/cm2 and activated with short movement (2–3 mm) up and down. During the laser activation the coaxial water spray from the gold hand piece (BIOLASE Technology) was switched off and tip positioned only in the coronal reservoir. 10-sec activation was set for 6 times, each followed by 3 sec rest phase. LA performed within an irrigant filled canal and solution was refreshed continuously.

As a final step, 10 mL of sterile distilled water was used to avoid residual test solution. After all irrigation and drying, dentine shavings from the canal walls were collected by scraping using sterile ISO 25 Henstrom files. Samples were incubated at 37 °C for 24 hours by inoculating 0.1 mL suspension to tryptic soy agar. After 24 hours the colony forming units (CFU) were recorded as CFU/mL and the survival fractions were counted by using the following formula:

Surviving bacteria (%) = $(CFU/mL_{\text{after experiment}} \text{ x}100)/CFU/mL_{\text{before experiment}}$.

Statistical Analysis

Data of survival ratio (%) were investigated statistically with one-way ANOVA. PostHoc Bonferroni tests were used for multiple comparisons by using PSPP 1.2.0 (GNU Public License, Free Software Foundation Inc.). The significance level for all analysis was set at $p<0.05$.

RESULTS

Survival ratio of E faecalis calculated from each group are summarized in Table 1. No viable bacteria (0%) was used as the main parameter to define effectiveness. In the S or LA groups, the survival ratios for irrigants have been found significantly different respectively ($p<0.0001$, $p<0.05$) (Table 1). According to the comparison of each irrigant between S and LA groups, both methods were found capable to reduce bacteria and there was no statistically difference between main groups (Table 1).

Table 1. Survival ratio (%) of *E. faecalis* by the effects of different irrigants in S and LA groups.

	Saline	NaOCl	NaB	DDW	P_{ANOVA}
S	7.03±1.10	0.00±0.00	0.85±0.83	2.41±0.93	*<0.0001*
LA	5.05±0.50	0.002±0.001	0.96±0.68	7.40±8.53	*<0.05*
$P_{PostHoc\ Bonferroni}$	*>0.5*	*>0.5*	*>0.5*	*>0.5*	

Values were given as mean± standart deviations
S: Syringe irrigation (5min); LA: laser activation (1 min); NaOCl: Sodium hypochloride; NaB: Sodium pentaborate pentahydrate; DDW: Deuterium depleted water

Table 2. Comparison of subgroups, and their p values

	Saline-S	NaOCl-S	NaB-S	DDW-S	Saline-LA	NaOCl-LA	NaB-LA	DDW-LA
Saline-S		**0.008***	**0.034***	0.440	1.000	**0.011***	0.053	1.000
NaOCl-S			1.000	1.000	0.200	1.000	1.000	**0.001***
NaB-S				1.000	0.658	1.000	1.000	**0.006***
DDW-S					1.000	1.000	1.000	0.134
Saline-LA						0.244	0.880	1.000
NaOCl-LA							1.000	**0.002***
NaB-LA								**0.011***
DDW-LA								

*p< 0.05

Furthermore, the PostHoc Bonferroni test for multiple comparisons showed that there was a difference between the subgroups ($p<0.05$) (Table 2); as expected NaOCl-LA found more effective than Saline-S ($p<0.05$) but not superior than NaOCl-S ($p>0.05$). It should be highlighted that DDW-LA failed to completely eliminate bacteria compared to NaOCl-LA ($p=0.002$) and NaB-LA ($p=0.011$), and also to 5 min syringe irrigation of NaOCl-S ($p<0.0001$) and NaB-S ($p=0.006$). 5% NaOCl has decreased the viable bacteria dramatically and found as the most effective solution in the study.

Discussion

E. faecalis was chosen for our study because it can penetrate into the dentinal tubules, which is the main cause of the secondary infection in endodontic cases (35, 36). It is a suitable bacterium that can be used for *in-vitro* research,

highly resistant to antimicrobial compounds, and can be easily produced in the laboratory. The bacteria broth was inoculated for 28 days to create a mature biofilm conditions within the root canal system of a necrotic tooth according to Latham et al. (35). However, it is also recognized that it cannot fully represent the variable intracanal flora in *in-vivo* conditions.

Saline is not a routine contemporary endodontic irrigant, not an effective antibacterial agent with no tissue dissolution properties (37). As it is expected, saline has showed high survival ratio after both syringe irrigation (7%) and LA method (5%). As far as compared to saline, DDW has been found inactive against Enterococcus species with 2.4 % survival ratio in S and 7% survival in LA. Ineffective outcome of the result is estimated that 25 ppm DDW has no antibacterial effect nor tissue solvent action on organic or inorganic matter. It could be suggested as an assist solution in endodontic irrigation such as distilled water. Moreover, DDW has the ability to overcome oxidative stress of the affected periradicular tissues when there is an apical extrusion (19).

The present findings seem to be consistent with the literature which suggest an increased antibiofilm effect when the concentration of NaOCl solution increases. Concentration is an important factor that 1 % and 2.5 % NaOCl solution was almost as effective after 30 min as a 5.25 % NaOCl solution after 5 min (38, 39). Christo et al reported that 4% concentration was more efficient than 1% NaOCl after 2 min laser activation, but it was not superior than 4% NaOCl syringe irrigation (40). In accordance with the present results, we have found that no bacteria survived after 5% NaOCl irrigation in both 5 min syringe irrigation and l min laser activation groups. On the contrary, previous studies have reported 2 min in the presence of 5.25% NaOCl was enough to achieve disinfection (41). It is accepted that higher concentrations of NaOCl are more cytotoxic and the risk of apical extrusion of the irrigant needs to be compared with the cytotoxicity of the irrigant (42).

Susceptibilities of NaB on *E. faecalis* have not been previously tested as an endodontic irrigant. Sodium perborate was compared to 5% NaOCl and the result showed similar antibacterial activity against *E. faecalis* (16). In line with the previous study, 3% NaB and 5% NaOCl showed similar antibacterial effect in root canals, whereas less bacteria were killed by NaB than by NaOCl. Also, there was no alteration in the efficiency when it is activated for 1 min LA (0.9%) compared to 5 min S irrigation (0.8%). These findings further support the idea of trying to find agitation techniques increasing the antibacterial effect would be a mistaken assumption, in which any solution can reduce bacterial count (43). However, these results were encouraging for further research on increased concentration of NaB. The present study also confirms previous findings and contributes additional evidence that suggests NaB as an irrigation solution due to the osteoinductive properties of NaB (14).

Our results indicated that LA was not superior to S irrigation on empowering the solutions against *E. faecalis* ($p>0.05$). This is in line with some researchers who did not find a significant bacterial reduction between S irrigation of 5% NaOCl or activated by Er,Cr:YSGG laser at low power (40, 44). Since laser induced cavitations enhance the fluid dynamics of irrigants (30, 31), there are expectations such as increased bactericidal efficiency and improved cleaning action of intracanal solutions. Yao et al reported that LA is 2.6 times more effective than syringe to eliminate dentin debris from complex canal areas (32). It was also found more effective in removing the artificially placed dentine debris from the root canal than syringe irrigation or passive ultrasonic irrigation when the irrigant was activated for 20 sec (45, 46). These results therefore need to be interpreted with caution. Laser cavitation can remove the bacterial biofilm but does not provide a bactericidal impact unless the solution has strong antibacterial properties.

The present study was designed to determine that NaB or DDW could be served as endodontic irrigants and also to evaluate the antibacterial impact of laser activation on these solutions against *E. faecali*s. The results of this investigation show that antibacterial properties of an irrigation solution have an important impact on reducing viable bacteria. Taken together, these findings support strong recommendations to use irrigants within a prolonged contact. Overall, the effectiveness of a solution depends on contact, the results of the 5 min syringe irrigation in the main channel should be questioned for the apical region or dentinal tubules. A greater focus on NaB could produce interesting findings that account more for its antimicrobial and osteoinductive properties. Another possible area of future research could be the investigation of DDW as a potential assist solution or a mixing material in dentistry.

Acknowlegdment This study was supported by Marmara University Scientific Research Projects Unit-BAPKO (SAG-C-DRP-241.018.0573).

References

1. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;85(1):86-93.
2. Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. International endodontic journal. 1998;31(1):1-7.
3. Pashley DH, Michelich V, Kehl T. Dentin permeability: effects of smear layer removal. J Prosthet Dent. 1981;46(5):531-7.
4. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic topics. 2005;10(1):77-102.
5. Hülsmann M, Hahn W. Complications during root canal irrigation–literature review and case reports. International Endodontic Journal. 2000;33(3):186-93.
6. Yang F, Zhu M, Zhang J, Zhou H. Synthesis of biologically active boron-containing compounds. Medchemcomm. 2018;9(2):201-11.
7. Dogan A, Demirci S, Caglayan AB, Kilic E, Gunal MY, Uslu U, et al. Sodium pentaborate pentahydrate and pluronic containing hydrogel increases cutaneous wound healing in vitro and in vivo. Biol Trace Elem Res. 2014;162(1-3):72-9.
8. Kuru R, Yarat A. Bor ve sağlığımıza olan etkilerine güncel bir bakış. Clinical and Experimental Health Sciences. 2017;7(3):107-14.
9. Bailey PJ, Cousins G, Snow GA, White AJ. Boron-containing antibacterial agents: effects on growth and morphology of bacteria under various culture conditions. Antimicrobial agents and chemotherapy. 1980;17(4):549-53.
10. Iyigundogdu ZU, Demir O, Asutay AB, Sahin F. Developing novel antimicrobial and antiviral textile products. Applied biochemistry and biotechnology. 2017;181(3):1155-66.
11. Turk T, Kaval ME, Sen BH. Evaluation of the smear layer removal and erosive capacity of EDTA, boric acid, citric acid and desy clean solutions: an in vitro study. BMC Oral Health. 2015;15:104.
12. Culhaoglu AK, Özcan E, Kilicarslan MA, Seker E. Effect of Boric Acid Versus Conventional Irrigation Solutions on the Bond Strength Between Fiber Post and Root Dentin. Journal of Adhesive Dentistry. 2017;19(2).
13. Luan Q, Desta T, Chehab L, Sanders V, Plattner J, Graves D. Inhibition of experimental periodontitis by a topical boron-based antimicrobial. Journal of dental research. 2008;87(2):148-52.
14. Taşlı PN, Doğan A, Demirci S, Şahin F. Boron Enhances Odontogenic and Osteogenic Differentiation of Human Tooth Germ Stem Cells (hTGSCs) In Vitro. Biological Trace Element Research. 2013;153(1):419-27.
15. Demirci S, Kaya MS, Dogan A, Kalay Ş, Altin NÖ, Yarat A, et al. Antibacterial and cytotoxic properties of boron-containing dental composite. Turkish Journal of Biology. 2015;39:417-26.
16. Shakouie S, Milani AS, Eskandarnejad M, Rahimi S, Froughreyhani M, Galedar S, et al. Antimicrobial activity of tetraacetylethylenediamine-sodium perborate versus sodium hypochlorite against Enterococcus faecalis. Journal of dental research, dental clinics, dental prospects. 2016;10(1):43.
17. Harbeson SL, Tung RD. Deuterium in Drug Discovery and Development. In: Macor JE, editor. Annual Reports in Medicinal Chemistry. Annual Reports in Medicinal Chemistry. 46: Academic Press; 2011. p. 403-17.
18. Mosin O, Ignatov I. Studying of Isotopic Effects of Deuterium in Biological Objects. European Reviews of Chemical Research. 2015;3(1):25-42.
19. Rasooli A, Fatemi F, Akbarzadeh K, Dini S, Bahremand S. Synergistic protective activity of deuterium depleted water (DDW) and Satureja rechingeri essential oil on hepatic oxidative injuries induced by acetaminophen in rats. Journal of Essential Oil Bearing Plants. 2016;19(5):1086-101.
20. Basov A, Fedulova L, Baryshev M, Dzhimak S. Deuterium-Depleted Water Influence on the Isotope (2)H/(1)H Regulation in Body and Individual Adaptation. Nutrients. 2019;11(8):1903.
21. Somlyai G, Jancsó G, Jákli G, Vass K, Barna B, Lakics V, et al. Naturally occurring deuterium is essential for the normal growth rate of cells. FEBS Letters. 1993;317(1-2):1-4.
22. Fisher-Wellman KH, Lin C-T, Ryan TE, Reese LR, Gilliam LA, Cathey BL, et al. Pyruvate dehydrogenase complex and nicotinamide nucleotide transhydrogenase constitute an energy consuming redox circuit. The Biochemical journal. 2015;467(2):271.
23. Opitz C, Ahrné E, Goldie KN, Schmidt A, Grzesiek S. Deuterium induces a distinctive Escherichia coli proteome that correlates with the reduction in growth rate. Journal of Biological Chemistry. 2019;294(7):2279-92.
24. Flaumenhaft E, Bose S, Crespi H, Katz J. Deuterium isotope effects in cytology. International review of cytology. 18: Elsevier; 1965. p. 313-61.
25. Katz JJ, Crespi HL. Deuterated organisms: cultivation and uses. Science. 1966;151(3715):1187-94.
26. Unno k, Busujima h, Shimba s, Narita k, Okada s. Characteristics of growth and deuterium incorporation in Chlorella ellipsoidea grown in deuterium oxide. Chemical and pharmaceutical bulletin. 1988;36(5):1828-33.
27. Wright PP, Walsh LJ. Optimizing Antimicrobial Agents in Endodontics. In: Kumavath RN, editor. Antibacterial Agents. Rijeka: InTech; 2017. p. Ch. 05.
28. Peeters HH, Gutknecht N. Efficacy of laser-driven irrigation versus ultrasonic in removing an airlock from the apical third of a narrow root canal. Aust Endod J. 2014;40(2):47-53.
29. Peeters HH, De Moor RJ, Suharto D. Visualization of removal of trapped air from the apical region in simulated root canals by laser-activated irrigation using an Er,Cr:YSGG laser. Lasers Med Sci. 2015;30(6):1683-8.
30. Blanken JW, Verdaasdonk RM. Cavitation as a Working Mechanism of the Er, Cr: YSGG Laser in Endodontics: A Visualization Study. Journal of Oral Laser Applications. 2007;7(2).
31. Matsumoto H, Yoshimine Y, Akamine A. Visualization of Irrigant Flow and Cavitation Induced by Er:YAG Laser within a Root Canal Model. Journal of Endodontics. 2011;37(6):839-43.

32. Yao K, Satake K, Watanabe S, Ebihara A, Kobayashi C, Okiji T. Effect of Laser Energy and Tip Insertion Depth on the Pressure Generated Outside the Apical Foramen During Er:YAG Laser-Activated Root Canal Irrigation. Photomed Laser Surg. 2017;35(12):682-7.
33. De Moor RJ, Blanken J, Meire M, Verdaasdonk R. Laser induced explosive vapor and cavitation resulting in effective irrigation of the root canal. Part 2: evaluation of the efficacy. Lasers Surg Med. 2009;41(7):520-3.
34. De Moor RJ, Meire M, Goharkhay K, Moritz A, Vanobbergen J. Efficacy of ultrasonic versus laser-activated irrigation to remove artificially placed dentin debris plugs. J Endod. 2010;36(9):1580-3.
35. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res. 1987;66(8):1375-9.
36. Love RM. Enterococcus faecalis– a mechanism for its role in endodontic failure. International Endodontic Journal. 2001;34(5):399-405.
37. Vatkar NA, Hegde V, Sathe S. Vitality of Enterococcus faecalis inside dentinal tubules after five root canal disinfection methods. J Conserv Dent. 2016;19(5):445-9.
38. del Carpio-Perochena AE, Bramante CM, Duarte MAH, Cavenago BC, Villas-Boas MH, Graeff MS, et al. Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. Journal of endodontics. 2011;37(8):1134-8.
39. Retamozo B, Shabahang S, Johnson N, Aprecio RM, Torabinejad M. Minimum contact time and concentration of sodium hypochlorite required to eliminate Enterococcus faecalis. Journal of Endodontics. 2010;36(3):520-3.
40. Christo JE, Zilm PS, Sullivan T, Cathro PR. Efficacy of low concentrations of sodium hypochlorite and low-powered Er,Cr:YSGG laser activated irrigation against an Enterococcus faecalis biofilm. Int Endod J. 2016;49(3):279-86.
41. Radcliffe C, 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. International endodontic journal. 2004;37(7):438-46.
42. Spangberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg Oral Med Oral Pathol. 1973;36(6):856-71.
43. Sippus J, Gutknecht N. Deep disinfection and tubular smear layer removal with Er:YAG using photon-induced photoacoustic streaming (PIPS) contra laser-activated irrigation (LAI) technics. Lasers in Dental Science. 2019;3(1):37-42.
44. Pedulla E, Genovese C, Campagna E, Tempera G, Rapisarda E. Decontamination efficacy of photon-initiated photoacoustic streaming (PIPS) of irrigants using low-energy laser settings: an ex vivo study. Int Endod J. 2012;45(9):865-70.
45. Ordinola-Zapata R, Bramante CM, Aprecio RM, Handysides R, Jaramillo DE. Biofilm removal by 6% sodium hypochlorite activated by different irrigation techniques. International Endodontic Journal. 2014;47(7):659-66.
46. Betancourt P, Merlos A, Sierra JM, Camps-Font O, Arnabat-Dominguez J, Vinas M. Effectiveness of low concentration of sodium hypochlorite activated by Er,Cr:YSGG laser against Enterococcus faecalis biofilm. Lasers Med Sci. 2019;34(2):247-54.