



Comparative Effects of Er:YAG Laser, Sodium Hypochlorite, and QMix on Root Canals Infected With *Enterococcus faecalis* and *Candida albicans*

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Abstract

Introduction: During endodontic treatment, the smear layer can reduce disinfectant efficacy. The aim of this study was to evaluate the antimicrobial efficacy of Er:YAG laser radiation and its combination with NaOCl and QMix against *Enterococcus faecalis* and *Candida albicans* and their comparison with conventional irrigation with only NaOCl and QMix.

Methods: Two hundred extracted single-rooted teeth after root canal preparation were divided into two groups and inoculated with *E. faecalis* and *C. albicans*. According to the treatment method, all samples were divided into five treatment groups: (1) Er:YAG laser, (2) NaOCl, (3) QMix, (4) Er:YAG laser plus NaOCl, and (5) Er:YAG laser plus QMix. After 24 hours of agar plate cultivation, cell viability was recorded with a flow cytometer.

Results: All treatment modalities showed efficiency in the reduction of microbial cells. For laser treatment alone after exposure for 90 seconds, significantly fewer non-death cells were seen, compared to 30-second treatment. For both timings (30 and 90 seconds), irrigation with NaOCl or QMix after laser application resulted in significantly fewer vital cells of *E. faecalis* and *C. albicans*, compared with laser treatment alone ($P < 0.001$). The samples treated with only NaOCl showed a significantly higher percentage of vital *E. faecalis* and *Candida albicans* cells, compared to the samples treated with only QMix ($P < 0.001$).

Conclusion: Higher Er: YAG laser exposure time (90 seconds) after its combination with QMix and NaOCl improves the efficacy in the reduction of *E. faecalis* and *C. albicans* from the root canal, compared to 30-second laser exposure time and conventional irrigation methods with NaOCl and QMix.

Keywords: Er:YAG laser; QMix; Root canal; *Candida albicans*; *Enterococcus faecalis*.



Introduction

Complete mechanical-chemical irrigation and shaping of the root canal, together with three-dimensional hermetic root canal system obturation and coronal seal, are the key factors that ensure successful endodontic therapy.¹ However, after mechanical preparation, a smear layer is formed, and it consists of organic and inorganic substances that can reduce disinfectant efficacy.² This can then lead to root canal infection even after irrigation with chlorhexidine and sodium hypochlorite (NaOCl).^{3,4}

The microorganisms that are commonly isolated in infected root canals from failed endodontic therapy and those with persistent infections are *Enterococcus faecalis* and *Candida albicans*.^{5,6} These microorganisms are often hidden in anatomical regions that cannot be reached by mechanical procedures, such as accessory and lateral root canals or isthmuses.⁷ Furthermore, these microorganisms may be resistant to antimicrobial agents.⁸

The other reason is that during mechanical cleaning, a smear layer can cover root canal walls that were prepared with endodontic instruments.⁹ This inorganic

and organic layer may contain microorganisms and by-products,¹⁰ which presents a potential reservoir of irritants. Chelators are intended to eliminate the smear layer and improve disinfectant activity.^{11,12} In addition to removing the smear layer and debris, EDTA and QMix play a role in improving root canal sealer permeability.^{13,14} However, such procedures are not sufficient to eradicate all microorganisms because of the difficulty of accessing root canals and dentin tubules.¹⁵

Newer treatment strategies are designed to enable agent penetration of the entire endodontic space together with lateral and accessory canals, isthmuses, and dentin tubules. For example, laser irradiation can be effective in cleaning the root canal system after mechanical preparation because of its potential antimicrobial effects.¹⁶ Although various laser systems have been used in endodontic therapy, Er:YAG laser treatment in combination with sodium hypochlorite has been reported to be particularly useful in disinfecting the dentin tubules, according to the time and dose.^{17,18}

However, this in vitro study aimed to determine the

antimicrobial efficacy of Er:YAG laser radiation alone and in combination with NaOCl and QMix against *E. faecalis* and *C. albicans*, and to compare it with conventional irrigation with NaOCl and QMix.

Materials and Methods

Sample Preparation

We used 200 extracted single-rooted human teeth that were extracted for periodontal reasons. The crowns of the tested teeth were cut at the cement enamel junction using a water-cooled diamond blade in a low-speed saw (Isomet 1000; Buehler GmbH, Germany) to obtain 15-mm root specimens (Figure 1).

A manual #10 Kerr file (Maillefer, Dentsply, Switzerland) was used to determine the working length of the specimen. Apical enlargement of the root canal to #35 was obtained using a ProTaper F3 rotary file (Maillefer, Dentsply, Switzerland). Between each file, a 2.5% NaOCl (Chloraxid, Cerkamed, Stalowa Wola, Poland) solution was used for root canal irrigation. After root canal preparation, the teeth were irrigated for one minute with 17% EDTA (Calasept EDTA, Nordiska Dental, Ängelholm, Sweden) and then sterilized. After drying the root canals with paper points, root apices were closed with composite materials. Sterilization was confirmed microbiologically on blood agar plates for 24 hours at 37 °C. The teeth were then divided into two experimental groups, depending on which microbial strain was inoculated: *E. faecalis* or *C. albicans*.

The samples were then divided into five treatment groups: (1) Er:YAG laser, (2) NaOCl, (3) QMix, (4) Er:YAG laser plus NaOCl, and (5) Er:YAG laser plus QMix.

Microorganism Preparation

Enterococcus faecalis (ATCC 29212, Thermo Fisher Scientific) and *C. albicans* (ATCC 10231, Liofilchem, Roseto degli Abruzzi, Italy) microbial strains were prepared by inoculation from frozen vials on blood agar plates, which were incubated for 24 hours in an aerobic atmosphere at 37 °C. The microbial strains were then inoculated at a density of 5 McFarland (1.5×10^9 cells/mL) in thioglycolate broth measured by a spectrophotometer and subsequently incubated for seven days at 37 °C in an anaerobic atmosphere generated by an Anoxomat Mark II Jar system (Mart Microbiology B. V, the Netherlands). We added 30 µL of fresh bacterial or yeast suspension every two days. After seven days, the samples were rinsed with 3 mL of 5.2% NaOCl for 20 seconds, flushed with NaOCl, and then washed with 2 mL of 10× phosphate buffer. Finally, the microorganisms were suspended in 5 mL of suspension and 500 µL was taken to measure cell viability by flow cytometry.

Treatment

Er:YAG laser: The samples in the first group were

treated with Er:YAG laser (Fotona, TwinLight, Ljubljana, Slovenia) pulse irradiation (2940 nm, power output 15 W, 20 Hz, pulsing rate 50 µs, 1500 mJ, conical fiber tip) (Figure 2). The laser was transmitted into the root canal with a 200-µm optical fiber for 30 or 90 seconds. After laser treatment, the root canals were flushed with 5 mL of phosphate buffer (pH=8.3). After washing, the cell suspensions were inoculated on blood agar plates and incubated for 24 hours at 37 °C. The next day, the growth of bacteria/yeasts was recorded if more than 300 bacterial/yeast colonies were obtained.



Figure 1. Preparation of Samples for Further Procedures



Figure 2. Er:YAG laser TwinLight® Endodontic Treatment (TET) (Fotona, Ljubljana, Slovenia)

NaOCl: The samples from the second group were first irrigated with 3 mL of 5.2% of NaOCl and washed with 2 mL of 10× phosphate buffer with fetal bovine serum (FBS) to neutralize the toxic effects of persistent microbes. After washing, 500 µL from the suspension was taken to measure cell viability with a flow cytometer (BD FACSCanto II) (Figure 3).

QMix: Root canals from the third group were irrigated with 3 mL of QMix (Dentsply Tulsa, Tulsa, OK, USA) and then washed with 2 mL of 10× phosphate buffer with FBS. Afterwards, 500 µL of the suspension was used to determine cell viability as described above.

Er:YAG laser plus NaOCl: The samples from the fourth group were treated with an Er:YAG laser in combination with NaOCl. After a photoacoustic streaming method using an Er:YAG laser for 10 seconds to create non-thermal photo acoustic shockwaves, the root canals were disinfected with 3 mL of 5.2% NaOCl. After irrigation, the samples were washed with 2 mL of 10× phosphate buffer with FBS to neutralize the toxic effects of persistent microbes. After washing, 500 µL from the suspension was taken to measure cell viability with a flow cytometer.

Er:YAG laser plus QMix: The samples of the fifth treatment group were treated similarly to those of the fourth group, but irrigation was performed with 3 mL of QMix rather than NaOCl.

Cell Viability

After 24 hours of agar plate cultivation, we determined cell viability by flow cytometry using thiazole orange and propidium iodide (Figures 4 and 5). A cell viability kit with liquid containing beads (BD, Becton Dickinson, Biosciences, USA) was used to assess cell viability.

Statistical Analysis

Statistical analysis was performed with SPSS 20 (IBM; New York, USA). A two-way ANOVA with a Tukey post hoc test was used to evaluate any significant variance in vital microbial cells among groups, as well as between the types of microorganisms and disinfecting methods. Statistical significance was set at $P < 0.05$.

Results

Table 1 reveals the percentage of vital *E. faecalis* cells after treatment with an Er:YAG laser for 30 or 90 seconds and after the combination of the Er:YAG laser with either NaOCl or QMIX. For laser treatment alone, 90 seconds resulted in significantly fewer non-death cells compared with those seen after a 30-second treatment (19.6% vs. 25.5%; $P < 0.001$). In groups treated with NaOCl or QMix after laser application, significantly fewer vital cells were observed, compared with those seen with laser treatment alone (Er:YAG: 25.52% and 19.6%, respectively; Er:YAG + NaOCl: 18.84% and 9.64%; ER:YAG + QMix: 9.5% and 4.79%; $P < 0.001$).



Figure 3. BD FACSCanto II Flow Cytometer

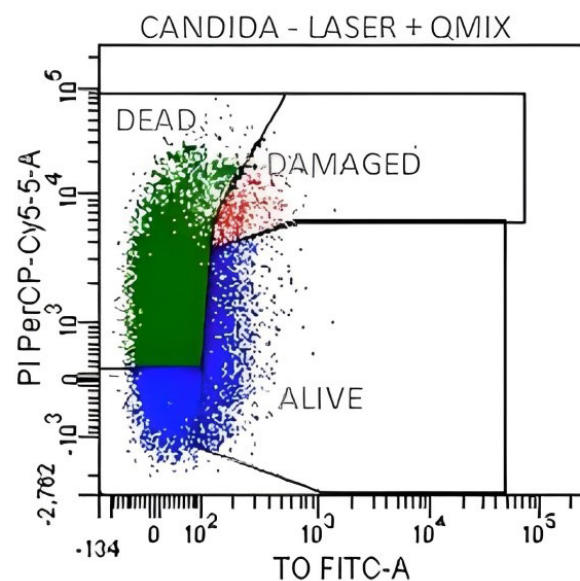


Figure 4. Example of Flow Cytometry Analysis of Dead *Candida albicans* Cells After Combined Er:YAG Laser Radiation and Qmix Irrigation Methodology

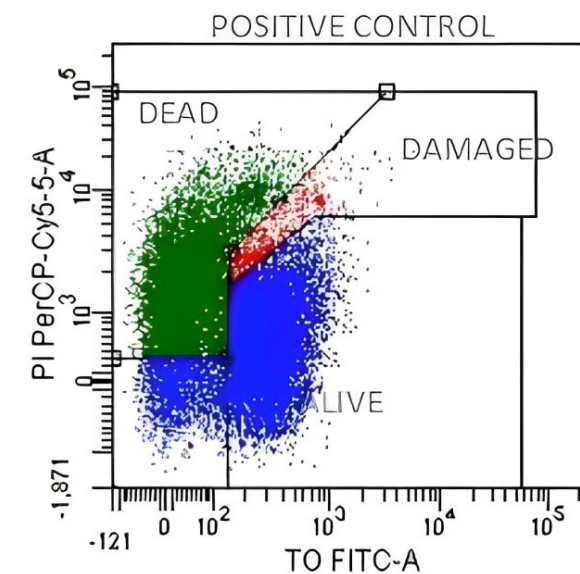


Figure 5. Example of *Candida albicans* Positive Control on Flow Cytometry Analysis

Table 1. Percent of Vital Microbial Cells Measured by Flow Cytometry

	<i>Enterococcus faecalis</i> (n=100)	<i>Candida albicans</i> (n=100)	P Value
Er:YAG			
30 s	25.52	20.98	0,049*
90 s	19.53	10.91	0,034*
5.2% NaOCl	40.46	33.43	0,022*
Qmix	24.49	20.55	0,052
Er:YAG + NaOCl			
30 s	18.84	15.37	0.041*
90 s	9.64	7.58	0.152
Er:YAG + Qmix			
30 s	9.5	9.12	0.112
90 s	4.79	3.51	0.687
P value	<0,001*	<0,001*	
CFU/mL			
Positive control	>300	>300	0.741
Negative control	0	0	0.001

In the group treated with only NaOCl, the percentage of vital *E. faecalis* cells significantly resulted in more cells (40.76%), compared to treatment with only QMix (24.49%) ($P < 0.001$).

Also, in the table are shown the percentages of vital *C. albicans* cells after treatment with only the Er:YAG laser for 30 or 90 seconds and after its combination with either NaOCl or QMix irrigation. Similar to what was seen with *E. faecalis*, irrigating the root canals with NaOCl or QMix resulted in significantly fewer vital cells than Er:YAG laser treatment alone for both time intervals (Er:YAG: 20.98% and 10.96%, respectively; Er:YAG + NaOCl: 15.37% and 7.58%; ER:YAG + QMix: 9.12% and 3.51%; $P < 0.001$).

In the group treated with only NaOCl, the percentage of vital *C. Albicans* cells significantly resulted in more cells (33.43%), compared to the group treated with only QMix (20.55%) ($P < 0.001$).

Discussion

The strains used in this study were chosen because they are most commonly present in root canal persistent infections. The complex root canal system anatomy and the penetration of microorganisms into dentine tubules can affect the occurrence of persistent infections.¹⁹ Additionally, both strains are often found to be resistant to pharmacological treatment.²⁰

One of the current endodontic irrigants is QMix which contains EDTA, chlorhexidine and detergent. Although its pH is slightly alkaline, we used it in our study because it has the antimicrobial properties of chlorhexidine along with the smear-layer removal properties of EDTA.^{21, 22} Importantly for our study, Al-Sheik Abidal et al²³ showed that QMix had bactericidal properties toward *E. faecalis*. Furthermore, Kalyoncuoglu et al²⁴ found that QMix was

shown to be effective against *C. albicans* when used as final irrigation. Regarding smear layer removal, Eliot et al¹⁷ in their study proved that irrigation with QMix was more effective than EDTA in the removal of the smear layer and dentinal tubules exposure. Smear layer removal helps to prevent the reduction of the root canal and pharmacological penetration into dentinal tubules and to improve root canal filling adaptation.^{25, 26}

In recent years, root canal irrigation activated with a laser (LAI) has presented a powerful method as a treatment technique.²⁷ The mechanism action of the Er:YAG laser is based on the highest absorption in water and a high affinity for hydroxyapatite and thus ensures the effective removal of the debris and smear layer from the root canal system.²⁸ This property is a result of the collapsed bubbles produced by the laser, leading to a secondary cavitation by the pulsed energy transferred to the solution.²⁹ Koçak et al,³⁰ after diode laser treatment with solutions, reported decreased amount of smear layer.

In our study, we compared the effectiveness of root canal disinfection with NaOCl and QMix activated by the Er:YAG laser with conventional irrigation using NaOCl and QMix alone. We found that combinatorial treatment with either NaOCl or QMix was more effective than any treatment alone. Perin et al³¹ also reported a stronger antimicrobial effect of the Er:YAG laser when used in combination with 1% NaOCl against five types of microorganisms, including *E. faecalis* and *C. albicans*. However, they noted that if the laser treatment and irrigation solution were used 3 mm below the apex, 70% of the strains remained. This demonstrates the importance of treatment positioning to maximize the antimicrobial effect.

In our study, laser irradiation combined with QMix was more effective in eliminating microorganisms than that observed when combined with NaOCl. This can be attributed to the fact that the combination of laser irradiation and QMix irrigation ensures the closest total microorganism elimination.

In order to emit laser irradiation laterally to the root canal wall, we used the laser tip with the side-firing spiral tip in our study. For the prevention of irradiation transmission beyond the apical foramen, the tip was sealed at the end.

Comparing the individual treatments, we reported that Er:YAG was superior to either NaOCl or QMix in promoting microbial death. This is consistent with previous studies illustrating that treatment with the Er:YAG laser showed a better effect on *E. faecalis* biofilm removal than conventional syringe irrigation with NaOCl or EDTA.²⁹ This is likely because of the fact that the energy of the Er:YAG laser can be absorbed and removed in dental hard structures biofilms very quickly due to the water content of biofilms and high laser light absorption by water.³¹

Exposure time also influenced the effectiveness of the combinatorial treatments in our study as a 90-second treatment showed greater microorganism eradication than 30-second exposure. Previous studies have also demonstrated a time-dependent effect of Er:YAG laser irradiation on microbial elimination.^{32,33} Longer exposure may have greater efficacy because laser energy kills bacteria directly and also activates the disinfection agents to enhance its bactericidal action.³⁴

For irrigation techniques, we also showed that QMix yielded better results than NaOCl, similar to Gründling et al.³⁵ This is likely because QMix not only penetrates through and removes debris that forms on the root canal walls after instrumentation but also kills microorganisms within the dentine tubules.¹⁷

However, because these effects were seen only in vitro, we recommend evaluating these treatments in patients in future studies to demonstrate their effectiveness in a clinically relevant population to improve dental health.

Conclusion

We demonstrate that combinatorial treatment with the Er:YAG laser and conventional irrigation techniques with NaOCl and QMix are more effective than individual treatments in removing microorganisms from root canals. We showed that increasing the laser exposure time (90 seconds) improves the efficacy of the treatment.

The combination of the Er:YAG laser with QMix was more effective in *E. faecalis* and *C. albicans* elimination from the root canal than its combination with NaOCl.

Author's Contribution

Conceptualization: Agime Dragidella.

Data curation: Agime Dragidella, Ariana Kameri.

Formal analysis: Agime Dragidella.

Investigation: Agime Dragidella.

Methodology: Agime Dragidella, Ariana Kameri.

Writing—original draft: Agime Dragidella, Ariana Kameri.

Writing—review & editing: Ariana Kameri.

Competing Interests

The authors deny any conflict of interest related to this study.

Ethical Approval

This research was approved by the Ethics Committee of the University Dentistry Clinical Center of Kosova and performed with the principles of medical ethics according to the Helsinki Declaration on human research.

References

- Greco K, Cantatore G. A critical approach to the root canal obturation techniques. *G Ital Endod.* 2014;28(2):48-78. doi: [10.1016/j.gien.2014.09.002](https://doi.org/10.1016/j.gien.2014.09.002).
- Jena A, Sahoo SK, Govind S. Root canal irrigants: a review of their interactions, benefits, and limitations. *Compend Contin Educ Dent.* 2015;36(4):256-61.
- Berber VB, Gomes BP, Sena NT, Vianna ME, Ferraz CC, Zaia AA, et al. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. *Int Endod J.* 2006;39(1):10-7. doi: [10.1111/j.1365-2591.2005.01038.x](https://doi.org/10.1111/j.1365-2591.2005.01038.x).
- Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*. *J Endod.* 2009;35(1):95-7. doi: [10.1016/j.joen.2008.09.004](https://doi.org/10.1016/j.joen.2008.09.004).
- Siqueira JF Jr, Rôças IN. PCR methodology as a valuable tool for identification of endodontic pathogens. *J Dent.* 2003;31(5):333-9. doi: [10.1016/s0300-5712\(03\)00051-4](https://doi.org/10.1016/s0300-5712(03)00051-4).
- Siqueira JF Jr, Sen BH. Fungi in endodontic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2004;97(5):632-41. doi: [10.1016/s1079210404000046](https://doi.org/10.1016/s1079210404000046).
- Alfirdous RA, Garcia IM, Balhaddad AA, Collares FM, Martinho FC, Melo MA. Advancing photodynamic therapy for endodontic disinfection with nanoparticles: present evidence and upcoming approaches. *Appl Sci.* 2021;11(11):4759. doi: [10.3390/app11114759](https://doi.org/10.3390/app11114759).
- Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *Int Endod J.* 2001;34(6):429-34. doi: [10.1046/j.1365-2591.2001.00411.x](https://doi.org/10.1046/j.1365-2591.2001.00411.x).
- Munoz HR, Camacho-Cuadra K. In vivo efficacy of three different endodontic irrigation systems for irrigant delivery to working length of mesial canals of mandibular molars. *J Endod.* 2012;38(4):445-8. doi: [10.1016/j.joen.2011.12.007](https://doi.org/10.1016/j.joen.2011.12.007).
- Adorno CG, Fretes VR, Ortiz CP, Mereles R, Sosa V, Yubero MF, et al. Comparison of two negative pressure systems and syringe irrigation for root canal irrigation: an ex vivo study. *Int Endod J.* 2016;49(2):174-83. doi: [10.1111/iej.12431](https://doi.org/10.1111/iej.12431).
- Kolosowski KP, Sodhi RN, Kishen A, Basrani BR. Qualitative time-of-flight secondary ion mass spectrometry analysis of root dentin irrigated with sodium hypochlorite, EDTA, or chlorhexidine. *J Endod.* 2015;41(10):1672-7. doi: [10.1016/j.joen.2015.06.010](https://doi.org/10.1016/j.joen.2015.06.010).
- Kolosowski KP, Sodhi RN, Kishen A, Basrani BR. Qualitative analysis of precipitate formation on the surface and in the tubules of dentin irrigated with sodium hypochlorite and a final rinse of chlorhexidine or QMix. *J Endod.* 2014;40(12):2036-40. doi: [10.1016/j.joen.2014.08.017](https://doi.org/10.1016/j.joen.2014.08.017).
- Aksel H, Serper A, Kalayci S, Somer G, Eriskan C. Effects of QMix and ethylenediaminetetraacetic acid on decalcification and erosion of root canal dentin. *Microsc Res Tech.* 2016;79(11):1056-61. doi: [10.1002/jemt.22745](https://doi.org/10.1002/jemt.22745).
- Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMix. *Int Endod J.* 2012;45(4):363-71. doi: [10.1111/j.1365-2591.2011.01985.x](https://doi.org/10.1111/j.1365-2591.2011.01985.x).
- Lin LM, Pascon EA, Skribner J, Gängler P, Langeland K. Clinical, radiographic, and histologic study of endodontic treatment failures. *Oral Surg Oral Med Oral Pathol.* 1991;71(5):603-11. doi: [10.1016/0030-4220\(91\)90371-i](https://doi.org/10.1016/0030-4220(91)90371-i).
- Do QL, Gaudin A. The efficiency of the Er:YAG laser and photoninduced photoacoustic streaming (PIPS) as an activation method in endodontic irrigation: a literature review. *J Lasers Med Sci.* 2020;11(3):316-34. doi: [10.34172/jlms.2020.53](https://doi.org/10.34172/jlms.2020.53).
- Eliot C, Hatton JF, Stewart GP, Hildebolt CF, Jane Gillespie M, Gutmann JL. The effect of the irrigant QMix on removal of canal wall smear layer: an ex vivo study. *Odontology.* 2014;102(2):232-40. doi: [10.1007/s10266-012-0102-1](https://doi.org/10.1007/s10266-012-0102-1).
- Al Omari T, El-Farraj H, Alzenate HM, Al Charabi N, Al Khatib R, Ateş AA. The usage of lasers in cleaning, shaping, and disinfection of root canal system. *Saudi Endod J.* 2022;12(3):253-60. doi: [10.4103/sej.sej_179_21](https://doi.org/10.4103/sej.sej_179_21).
- Pereira TC, Dijkstra RJB, Petridis X, Sharma PK, van de Meer WJ, van der Sluis LWM, et al. Chemical and mechanical influence of root canal irrigation on biofilm removal from lateral

- morphological features of simulated root canals, dentine discs and dentinal tubules. *Int Endod J*. 2021;54(1):112-29. doi: [10.1111/iej.13399](https://doi.org/10.1111/iej.13399).
20. Alshanta OA, Albashaireh K, McKlound E, Delaney C, Kean R, McLean W, et al. *Candida albicans* and *Enterococcus faecalis* biofilm frenemies: when the relationship sours. *Biofilm*. 2022;4:100072. doi: [10.1016/j.biofilm.2022.100072](https://doi.org/10.1016/j.biofilm.2022.100072).
 21. Elnaghy AM. Effect of QMix irrigant on bond strength of glass fibre posts to root dentine. *Int Endod J*. 2014;47(3):280-9. doi: [10.1111/iej.12145](https://doi.org/10.1111/iej.12145).
 22. Rahmati A, Karkehabadi H, Rostami G, Karami M, Najafi R, Rezaei-Soufi L. Comparative effects of Er:YAG laser, and EDTA, MTAD, and QMix irrigants on adhesion of stem cells from the apical papilla to dentin: a scanning electron microscopic study. *J Clin Exp Dent*. 2022;14(4):e310-e5. doi: [10.4317/jced.59129](https://doi.org/10.4317/jced.59129).
 23. Al-Sheik Abidal AK, Abdul-Rahman GY, Tawfeeq AW. The antibacterial effect of QMix, a novel root canal irrigant (ex vivo study). *Rafidain Dent J*. 2020;13(3):537-46. doi: [10.33899/rden.2020.165415](https://doi.org/10.33899/rden.2020.165415).
 24. Kalyoncuoglu E, Tunc ES, Ozer S, Keskin C, Bilgin K, Birinci A. Evaluation of antifungal efficacy of QMix 2in1 as a final irrigant: an in vitro study. *Niger J Clin Pract*. 2016;19(6):807-10. doi: [10.4103/1119-3077.164344](https://doi.org/10.4103/1119-3077.164344).
 25. Machado R, da Fonseca Roberti Garcia L, da Silva Neto UX, de Miranda da Cruz Filho A, Silva RG, Vansan LP. Evaluation of 17% EDTA and 10% citric acid in smear layer removal and tubular dentin sealer penetration. *Microsc Res Tech*. 2018;81(3):275-82. doi: [10.1002/jemt.22976](https://doi.org/10.1002/jemt.22976).
 26. Plotino G, Cortese T, Grande NM, Leonardi DP, Di Giorgio G, Testarelli L, et al. New technologies to improve root canal disinfection. *Braz Dent J*. 2016;27(1):3-8. doi: [10.1590/0103-6440201600726](https://doi.org/10.1590/0103-6440201600726).
 27. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. *J Endod*. 1975;1(7):238-42. doi: [10.1016/s0099-2399\(75\)80226-3](https://doi.org/10.1016/s0099-2399(75)80226-3).
 28. DiVito E, Peters OA, Olivi G. Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation. *Lasers Med Sci*. 2012;27(2):273-80. doi: [10.1007/s10103-010-0858-x](https://doi.org/10.1007/s10103-010-0858-x).
 29. Kourtí E, Pantelidou-Papadopoulou O, Tolidis K, Strakas D. Evaluation of smear layer after Er:YAG laser irradiation in middle and apical third of mesial root canals: a comparative SEM investigation. *Int J Exp Dent Sci*. 2021;10(1):14-8.
 30. Koçak S, Çiçek E, Sağlam BC, Koçak MM, Türker SA. Influence of diode laser application on the efficiency of QMix and EDTA solutions in removing smear layer. *Photomed Laser Surg*. 2015;33(11):564-7. doi: [10.1089/pho.2015.3910](https://doi.org/10.1089/pho.2015.3910).
 31. Perin FM, França SC, Silva-Sousa YT, Alfredo E, Saquy PC, Estrela C, et al. Evaluation of the antimicrobial effect of Er:YAG laser irradiation versus 1% sodium hypochlorite irrigation for root canal disinfection. *Aust Endod J*. 2004;30(1):20-2. doi: [10.1111/j.1747-4477.2004.tb00162.x](https://doi.org/10.1111/j.1747-4477.2004.tb00162.x).
 32. Eick S, Meier I, Spoerlé F, Bender P, Aoki A, Izumi Y, et al. In vitro-activity of Er:yag laser in comparison with other treatment modalities on biofilm ablation from implant and tooth surfaces. *PLoS One*. 2017;12(1):e0171086. doi: [10.1371/journal.pone.0171086](https://doi.org/10.1371/journal.pone.0171086).
 33. Cheng X, Chen B, Qiu J, He W, Lv H, Qu T, et al. Bactericidal effect of Er:YAG laser combined with sodium hypochlorite irrigation against *Enterococcus faecalis* deep inside dentinal tubules in experimentally infected root canals. *J Med Microbiol*. 2016;65(2):176-87. doi: [10.1099/jmm.0.000205](https://doi.org/10.1099/jmm.0.000205).
 34. Tabassum S, Khan FR. Failure of endodontic treatment: the usual suspects. *Eur J Dent*. 2016;10(1):144-7. doi: [10.4103/1305-7456.175682](https://doi.org/10.4103/1305-7456.175682).
 35. Gründling GL, Melo TA, Montagner F, Scarparo RK, Vier-Pelisser FV. QMix® irrigant reduces lipopolysaccharide (LPS) levels in an in vitro model. *J Appl Oral Sci*. 2015;23(4):431-5. doi: [10.1590/1678-775720140488](https://doi.org/10.1590/1678-775720140488).