ORIGINAL RESEARCH

DOI: 10.62838/ASMJ.2024.1.03

Comparison of the effectiveness of sodium hypochlorite, citric acid, and diode laser in disinfection of the root canal system.

Andrea-Csinszka Kovács-Ivácson[1], Alexandra-Mihaela Stoica[1], Örs Bodó[2], Timea Dakó[1], Monica Monea[1]

[1] George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Târgu-Mureș, Romania

[2] Oral and Maxillofacial Surgery Clinic, County Emergency Clinical Hospital of Târgu Mureș, Romania

Abstract

Introduction: To achieve a proper disinfection of the root canal system, besides many irrigant solutions, laser has become increasingly popular in recent years. Bacteria that penetrate deep in the dentine can be destroyed by laser up to 1150 µm. The aim of the study is to investigate and to compare the efficiency of conventional chemical disinfection using cleaning solutions - sodium hypochlorite (2%), citric acid (20%) - and the physical disinfection using a diode laser (940 nm, 1 W) in vitro using extracted teeth. Material and Methods: 23 intact, single rooted teeth were prepared and inoculated with Enterococcus Faecalis. Afterwards samples were taken from each group and placed on solid mediums. The following were applied to members of each group prior to sampling: 2% sodium hypochlorite, 20% citric acid and diode laser (940 nm, 1W). To evaluate the results, so that the bacterial strains on the medium could be counted, quenches were performed from 1/10 and 1/100 dilutions. Statistical analysis was performed using Kruskall-Wallis and unpaired T-test with a value of $p>0.05$. Results: Statistical analysis on the 3 groups (NaOCl, citric acid, and diode laser) showed significant differences between the counted remaining colonies after disinfection. Conclusions: The diode laser used for disinfection under the used settings is not effective enough, but as an adjuvant, associated with conventional irrigation effective disinfection can be obtained.

Keywords: root canal, E. Faecalis, sodium hypochlorite, citric acid, diode laser.

Introduction

To perform a correct and successful root canal treatment, proper mechanical cleaning and shaping of the canals is essential prior to the final obturation. Cleaning and shaping are important to minimize the number of remnant bacteria and prevent subsequent complications that would require retreatment of the tooth [1, 2].

Many studies have focused on the effectiveness of conventional irrigating solutions, but according to the obtained results, no agent meets all expectations [3, 4].

To achieve proper disinfection, the use of laser as an adjuvant in root canals has become increasingly popular in recent years [5]. One of the main reasons for this is that laser light is able to kill bacteria that penetrate deeper into the dentin than the solutions used. Bacteria that have penetrated deeper than 100 µm can be destroyed by the laser up to 1150 µm [6].

The most often used laser types in decontamination of the root canal are Er: YAG, Nd: YAG and diode lasers. Wavelength of the Er: YAG laser is well-absorbed in tissues containing water and hydroxyapatite, therefore it is effective in removing the smear layer. Its energy is absorbed at the first 400 µm, therefore it is not able to kill bacteria located deeper. Nd: YAG laser is mainly used for disinfection due to its deep penetrating light (1000 µm), but is not able to remove the smear layer [7, 8].

The use of a diode laser in dentistry is common in everyday practice due to its low price, small size, ease of operation, and versatility. Its wavelength is in the infrared range (800-1064 nm), but this is still visible as a perceptible red light. The performance of the laser varies from 0.5 up to 7 W [9]. Studies are still underway to determine the appropriate wavelength, power, and application time, with no agreement according to which the optimal parameters that provide the most effective treatment are [10].

The aim of this study is to investigate and to compare the efficiency of conventional chemical disinfection using cleaning solutions – sodium hypochlorite (2%), citric acid (20%) – and the physical disinfection using a diode

laser (940 nm, 1 W) in vitro using extracted teeth.

Material and methods

This study was performed at the University of Medicine, Pharmacy, Science, and Technology of Târgu Mureş in collaboration with two departments – the department of Odontology and Oral Pathology, Faculty of Dentistry, and the department of Microbiology, Virusology and Parasitology, Faculty of Medicine.

For this research 23 intact single-rooted teeth were used. Teeth were extracted for periodontal reasons. The roots were separated at the enamel-cement border with a diamond bur, because only the roots were needed for the experiment. The roots were cleaned on the external surface using an ultrasonic depurator. The teeth were stored in sterile physiological saline solution during collection.

After removing the dental pulp, root canals were prepared using hand endodontic instruments-Kerr files (VDW)-up to ISO size 40 (black) using standardization technique (Figure 1). There was no need to use a step-back technique because the root canals were straight, without any curvature [11].

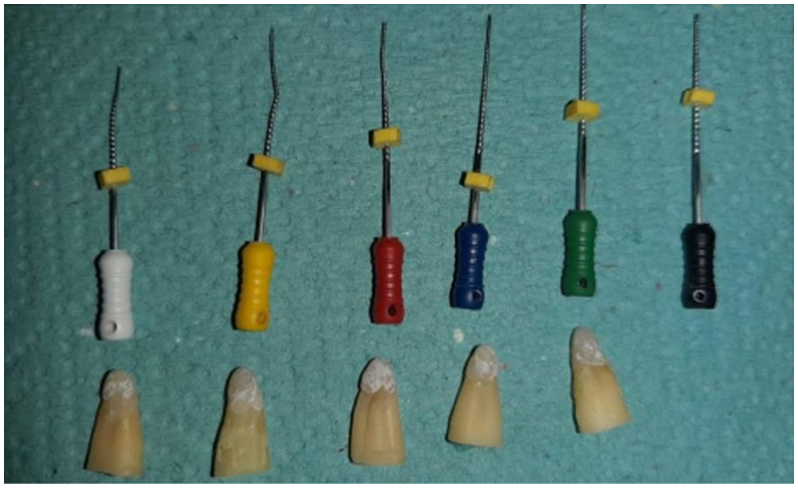


Figure 1. The prepared roots and the used K-files.

Sterile physiological saline solution was used for lubrication. After enlargement, each root was irrigated with 17% EDTA, applying 1 ml in each for 1 min to remove the smear layer. This was important to help the bacteria penetrate the dentin [12, 13].

After that, the root tips were closed with a composite material on the outside to prevent any subsequent leakage. The teeth were sterilized in an autoclave at 121°C for 20 min. According to the literature, autoclaving of teeth does not significantly change the hardness and other properties of dentin and can therefore be used in experiments conducted in vitro [14].

Afterwards the teeth were placed in a sterile container and kept in this during the experiment. Sterile distilled water was placed in the empty areas of the tank to maintain moisture and thus prevent the roots from drying out and the bacteria from dying.

The 23 teeth were divided into negative, positive control, and three experimental groups (NaOCl, citric acid, and laser group). The control groups contained four teeth each and the experimental groups contained five teeth each.

A negative control group was required to be possible to check for the presence or absence of contamination. In case of contamination, the experiment needed to be performed again. In the present case, there was no detectable bacterial growth in the negative control group, so there was no contamination.

In addition to the negative control group, all teeth were inoculated with Enterococcus Faecalis (Figure 2).

Figure 2. The solid medium inoculated with Enterococcus Faecalis.

10 µl of Tryptic Soy Broth (TSB) liquid medium was added to the roots, which contained the bacteria. Bacteria-free TSB was included in the members of the negative control group. This group was needed to rule out contamination of the teeth with other bacteria.

After inoculation, the teeth were placed in an incubator where they were kept for one week at 37°C. Meanwhile, the TSB was refreshed every day by using a pipette. In each case, disposable, sterile ends were placed on the pipette, which were replaced after each use.

Each root received 10 µl of TSB (as during the inoculation) every day, but it no longer contained bacteria, as the only goal here was to maintain the growth of bacteria in the teeth and prevent dehydration. When the sterile distilled water evaporated, the free spaces were refilled in the tank (Figure 3).

Figure 3. Refreshing of the TSB

After refreshing the TSB and distilled water, the teeth were placed back in the incubator and work continued for a week.

After one-week, samples were taken from each group and placed on solid media. This process consisted of several steps.

During sampling, 10 µl of sterile saline solution was placed in each root canal, and then sterile paper tips were used to absorb the solution from the canals. Paper points were manipulated using sterile tweezers. The paper tips were then placed in plastic Eppendorf tubes containing 1 ml of sterile physiological

saline solution. Using vortexing for a minute, the bacteria on the paper tips were mixed with the saline solution in the Eppendorf tubes. 100 µl of the obtained suspension was added dropping to solid medium and spread with a rod.

To evaluate the results, so that the bacterial strains on the medium could be counted, quenches were performed from 1/10 and 1/100 dilutions (Figure 4).

Figure 4. The Eppendorf tubes contain the 1/10 and 1/100 dilutions.

For the positive and negative control groups, sampling and quenching were performed as detailed above, without affecting the contents of the root canals. In the three experimental groups sodium hypochlorite, citric acid, and diode laser were applied to members of each group prior to sampling and quenching.

Sodium hypochlorite of 2% concentration and citric acid of 20% concentration were introduced in each root canal for 3 min, after which the root canals were dried with sterile paper points and then sampled and quenched as mentioned above. On the samples of the laser experimental group, a diode laser (Biolase) with a wavelength of 940 nm and a power of 1 W was used with 200 µm diameter and front emitting end only (Figure 5).

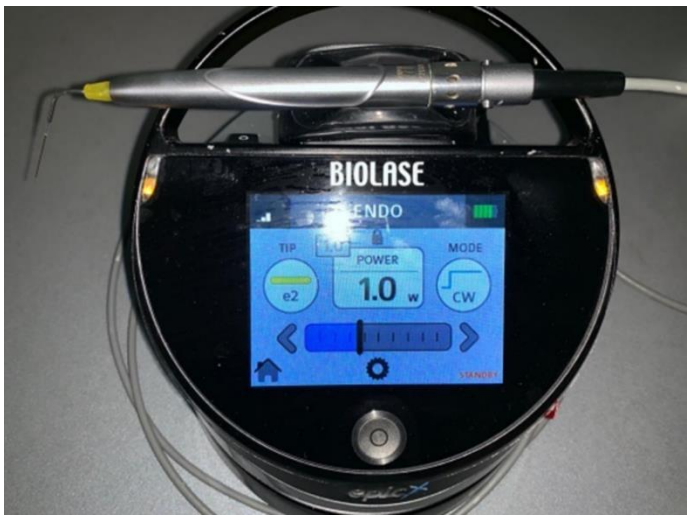


Figure 5. The used diode laser.

The laser travelled at a speed of 1 mm/ 2 seconds using circular (helical) movements from the top of each root to the top of the canal. This sequence of movements was repeated four times for each tooth (Figure 6).

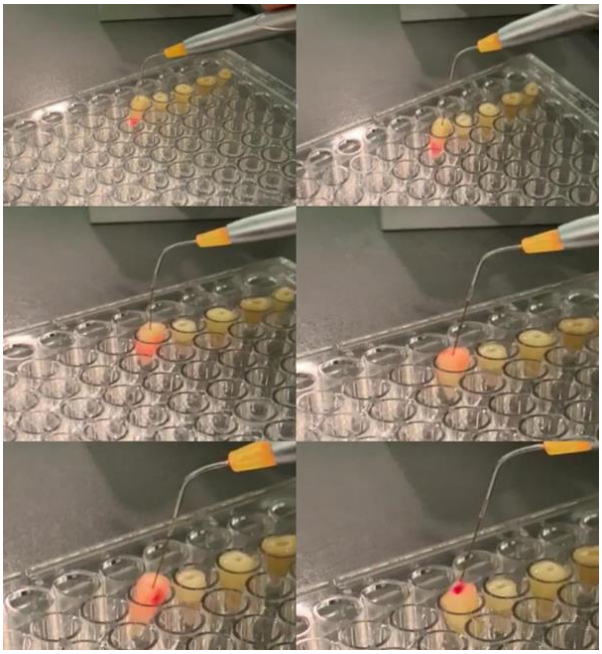


Figure 6. The usage of diode laser on the samples.

After the laser was applied, sampling and quenching were performed. After quenching the samples from the two control groups and the three experimental groups into solid media, these were placed in an incubator for 24 hours, after which the results were read, and the germ count for each tooth and group was determined.

All data were collected in Microsoft Excel work sheets (Microsoft Corporation, Washington, DC, USA, 2018). The statistical analysis was carried out in GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, CA, USA). To evaluate the results, the Kruskal-Wallis and the unpaired T-test were used. The results were considered significant at a value of $p<0.05$.

Results

The obtained results after counting bacterial culture on each group substrate are shown in Figure 7.

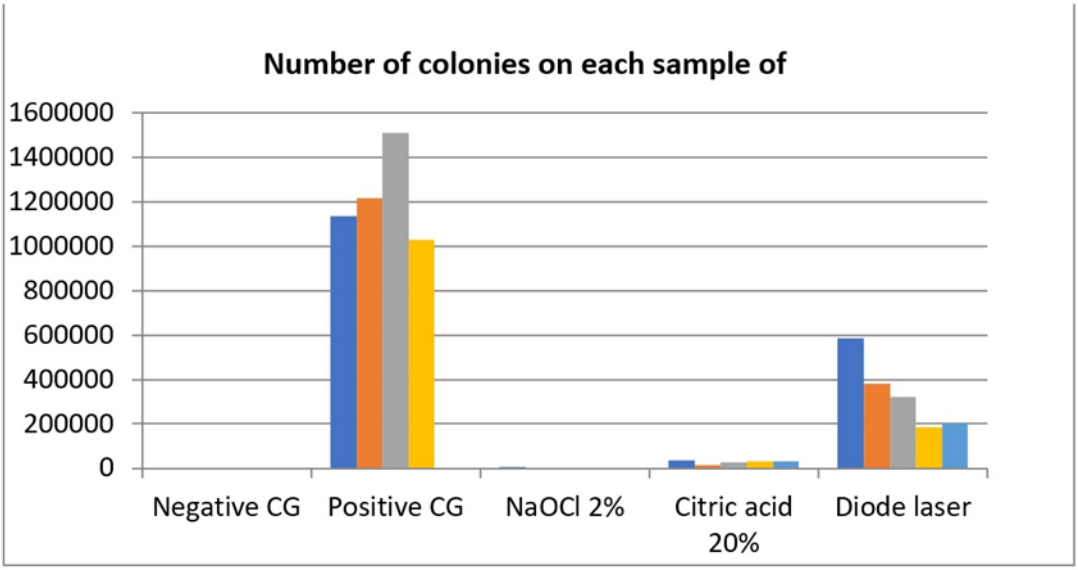


Figure 7. Number of colonies on each sample of each group

Statistical analysis of the 3 groups (NaOCl, citric acid, and diode laser) showed significant differences between the counted number of remaining colonies after disinfection performed with the mentioned solutions and method. (Figure 8)

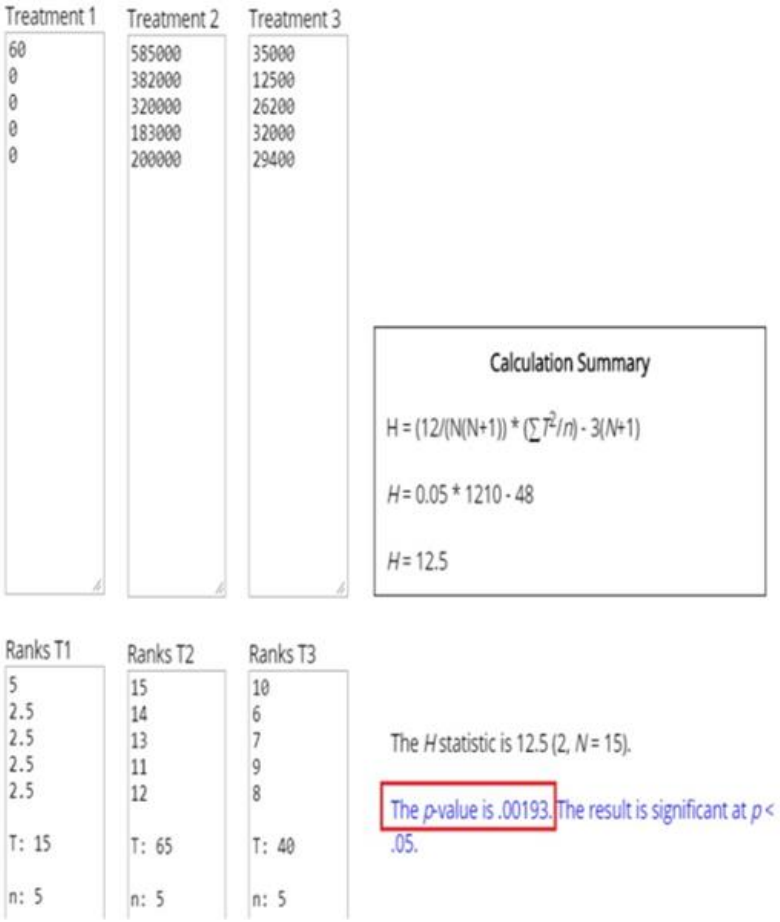
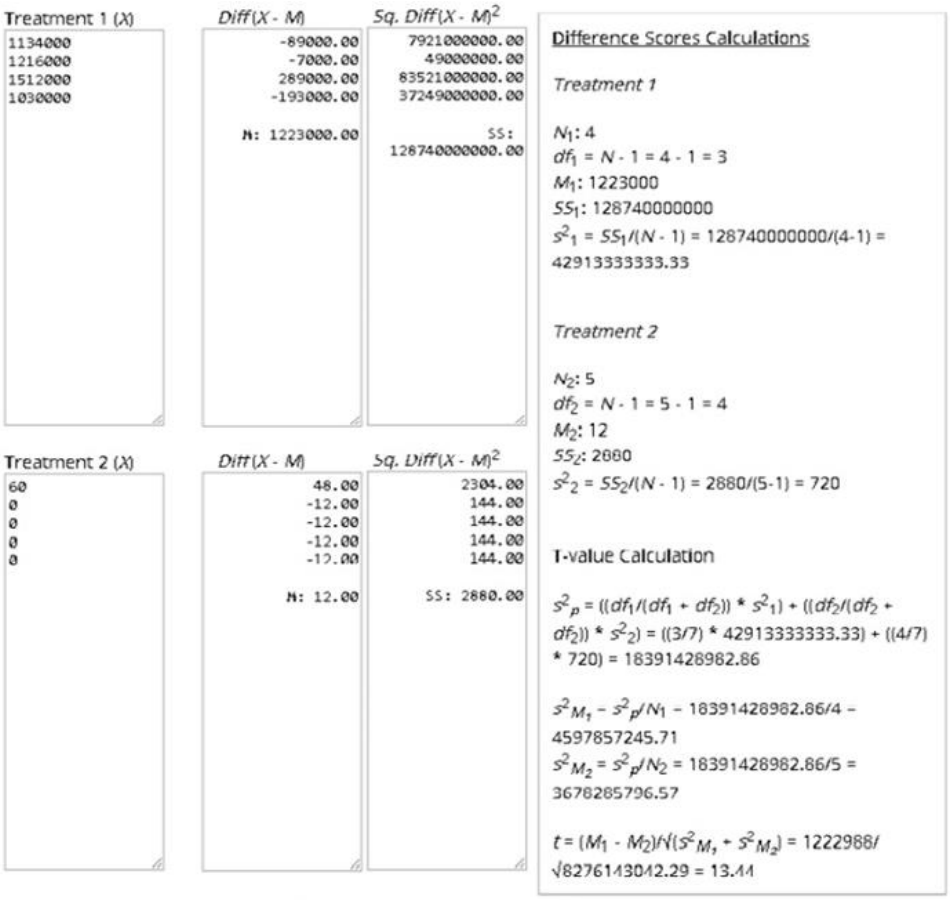


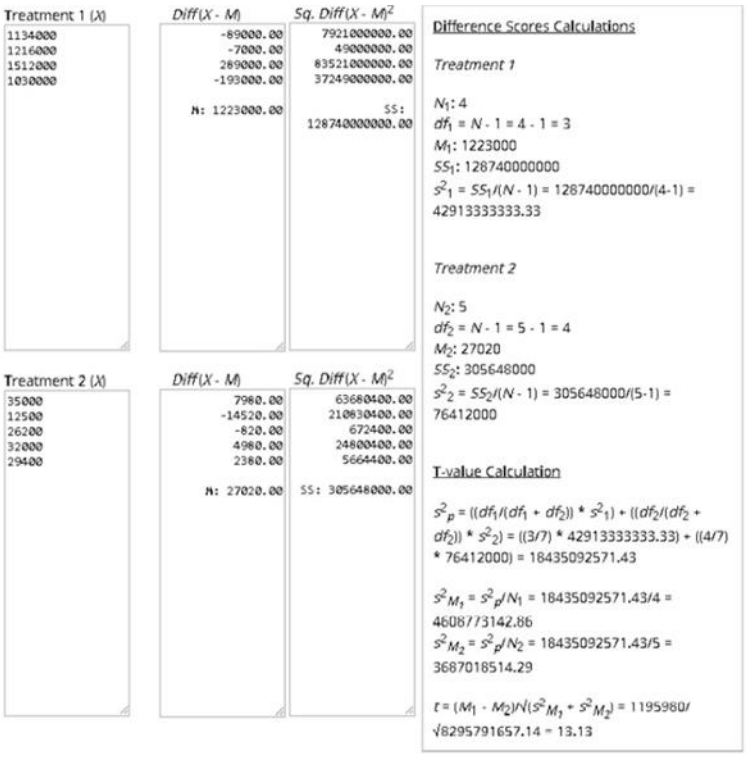
Figure 8. The performed Kruskal-Wallis test on NaOCl, citric acid, and diode laser groups

Comparing the number of colonies on the positive control group to NaOCl, citric acid, and diode laser group statistically significant results were found in every case. These results are shown in Figures 9-11.



The *p*-value is < .00001. The result is significant at *p* < .05.

Figure 9. Unpaired T-test comparing positive control group and NaOCl group.



The *p*-value is < .00001. The result is significant at *p* < .05.

Figure 10. Unpaired T-test on positive control and citric acid group

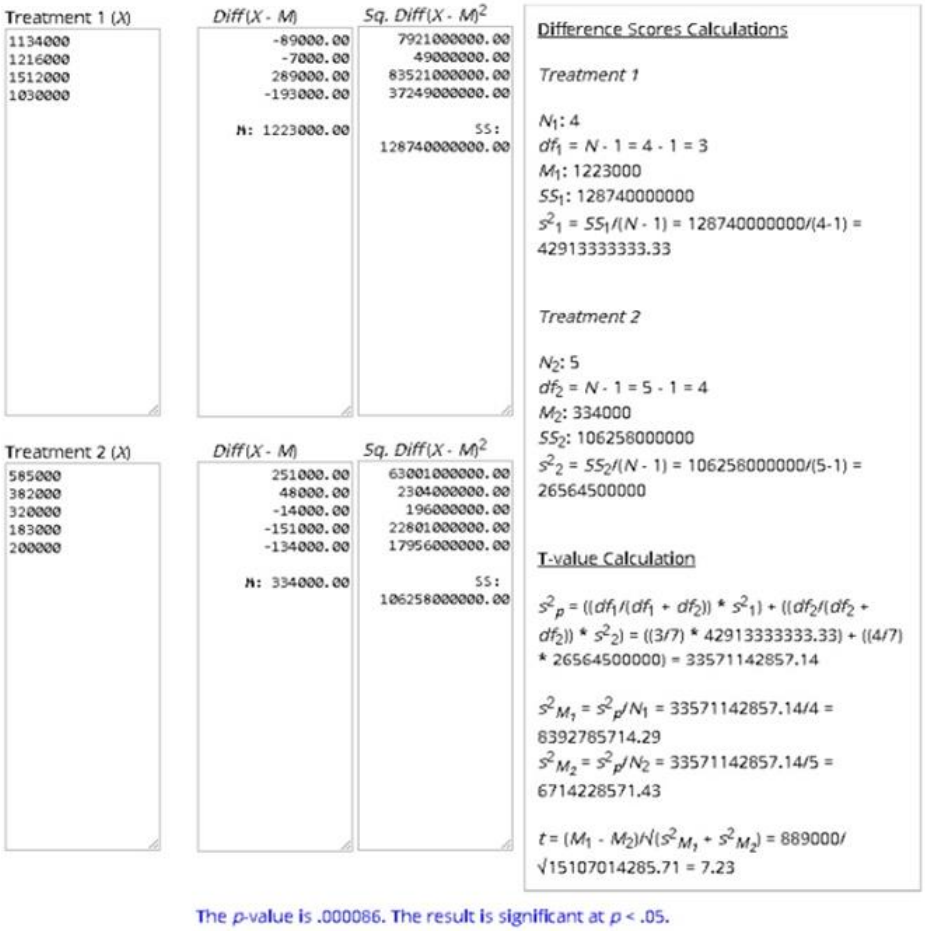


Figure 11. Unpaired T-test for positive control and diode laser group

Figure 12 shows solid media without bacterial growth on the negative control group.

Figure 13 shows bacterial growth on the solid media in case of the positive control group.

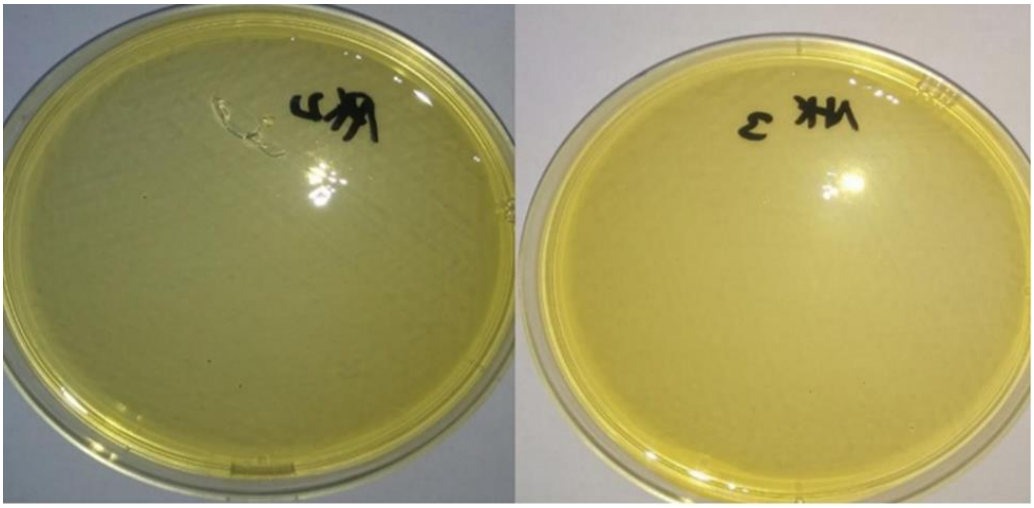


Figure 12. No bacterial growth on negative control group medium.

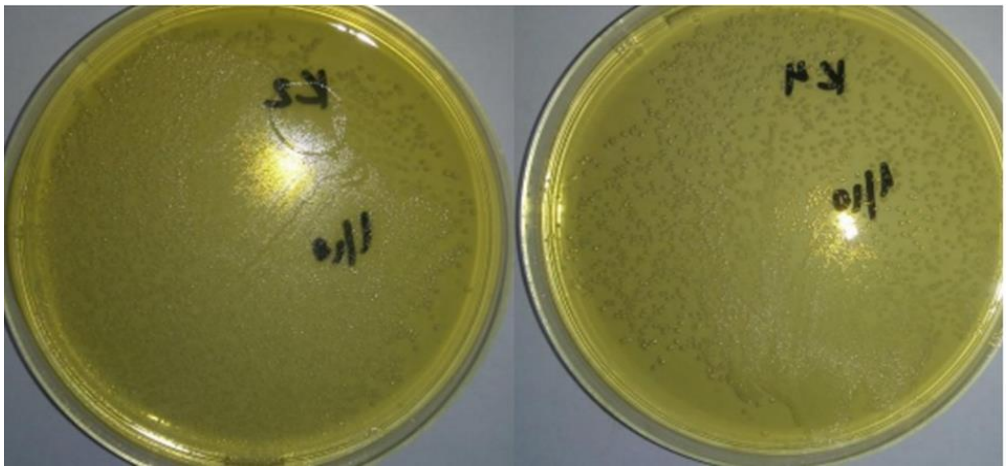


Figure 13. Bacterial growth on positive control group medium.

The number of grown bacteria on the solid media in case of NaOCl, citric acid, and diode laser group are shown in Figure 14-16.

Figure 14. Bacterial growth on NaOCl 2% medium.

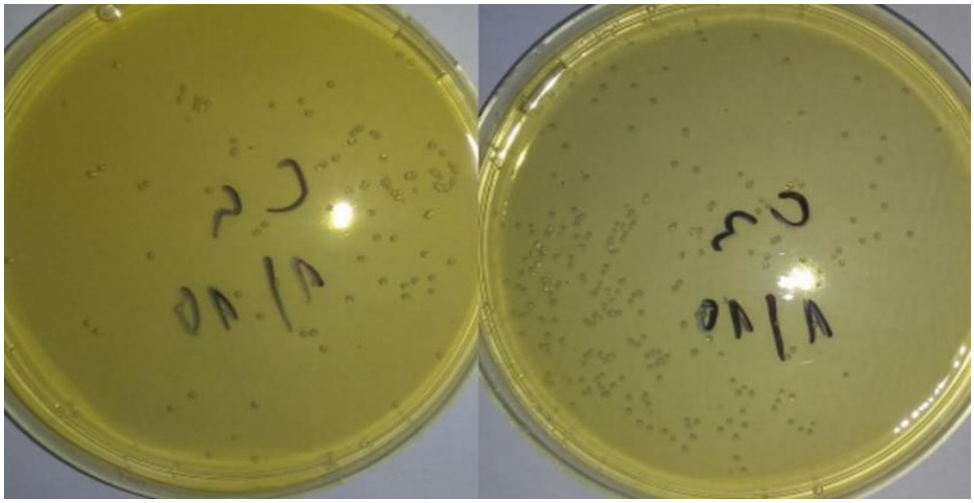


Figure 15. Bacterial growth on 20% citric acid medium.

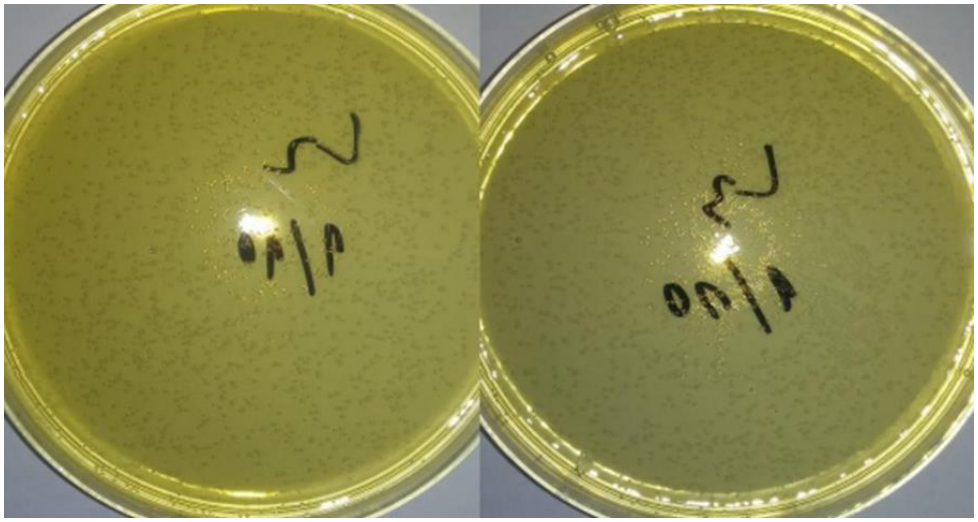


Figure 16. Bacterial growth on diode laser medium.

Discussion

During root canal treatments, the main goal is to treat or preserve the health of the periapical tissues. This can be obtained by cleaning the endodontic system. Literature data, besides the successful root canal treatments, also mention a 2-14% failure rate. In case of failure, clinical symptoms and persistent periapical or periradicular radiolucency are present [1, 2].

A common reason for failure is insufficient disinfection and proper cleaning of the root canals, due to which the remaining bacteria in the endodontic space subsequently generate inflammation. Among the pathogens that induce secondary infection, the largest number of Enterococcus Faecalis, Gram-positive, facultative anaerobic bacteria have been detected [15, 16].

To perform a successful root canal treatment, mechanical and chemical cleaning is essential. Removal of tissue debris by mechanical cleaning, a space for irrigants is created so that these can develop their effect. Therefore, various microorganisms and their endotoxins are removed from the endodontic space. For this reason, cleaning and shaping are inseparable from each other [17].

In practice, it is impossible to implement 100% cleaning of the root canal system exclusively by using irrigation, which is why many doctors use adjuvants together with the irrigants to achieve even greater disinfection. One of these methods is laser disinfection, which can be done with several types of lasers. This is a simple and effective method due to its application, so many studies recommend diode laser treatments in combination with conventional irrigation [18, 19].

According to our results found in the three experimental groups, members of the sodium hypochlorite (2%) had the highest rate of bacterial mortality, 4 out of 5 media were germ free. Thus, on one medium 6 colonies grew after treatment. The presence of these few colonies suggests that it is likely that the other 4 teeth are also not considered sterile. If it had been possible to do without dilution, to extinguish the sample taken from the teeth more concentrated, then also on the other 4 media a low number of bacteria could have been detected. Though, at such a small amount of saline solution used, this was not possible. However, despite this, NaOCl was very effective in bacterial destruction, presenting more than 99% efficiency.

The antibacterial effect of citric acid (20%) - less studied in the literature - is not proved to be quite effective compared to NaOCl, but still destroyed the bacteria in 97.8%. Higher concentrations of citric acid are likely to be even more effective from an antibacterial point of view [11].

The diode laser used in only one session destroyed 72.7% of the bacteria inside the roots. This value is similar to the results of other studies. Other studies also mention that the combined use of laser and NaOCl can also show 100% disinfection of root canals [20-22]. However, when applying only laser treatment,

it is recommended to repeat it at least 2-3 times to achieve an adequate reduction in the number of bacteria [23-25].

In other similar studies, where one-off treatment was also used, the effectiveness of the laser turned out to be much better, occasionally even producing the complete elimination of the bacterial flora. In these cases, the lasers were set to higher power (2.5 W, 4 W) and the wavelength was also different [26].

Setting the right parameters is difficult and dangerous, because the surrounding soft and hard tissues can also be damaged by the laser if it is not properly calibrated. Currently, there is still no agreement on the optimal parameters.

Conclusions

1. The diode laser used for disinfection under the mentioned settings is not effective enough, but as an adjuvant, associated with conventional irrigation effective disinfection can be obtained.

2. Citric acid irrigation is recommended for smear layer removal, but its antibacterial effect prevails also during treatment.

3. When used alone, sodium hypochlorite irrigation still has the highest antibacterial effect.

Conflict of interest: None declared.

References

1. Tabassum S, Khan FR. Failure of endodontic treatment: The usual suspects. Eur J Dent 2016;10(1):144-147.
2. Raedel M, Hartmann A, Bohm S, Walter MH. Three-year outcomes of root canal treatment: Mining an insurance database. Journal of Dentistry. 2015;43:412-417.
3. Dioguardi M, Di Gioia G, Illuzzi G, Laneve E, Cocco A, Troiano, G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent. 2018;12(3):459-466.
4. Spencer HR, Ike V, Brennan PA. Review: the use of sodium hypochlorite in endodontics - potential complications and their management. British Dent Jour. 2007;202:555-559.
5. Faras F, Abo-Alhassan F, Sadeq A, Burezq H. Complication of improper management of sodium hypochlorite accident during root canal treatment. J Int Soc Prev Community Dent. 2016;6(5):493-496.
6. S Najeeb S, Khurshid Z, Zafar MS, Ajlal S. Applications of Light Amplification by Stimulated Emission of Radiation (Lasers) for Restorative Dentistry. Med Princ Pract. 2016;25(3):201-211.
7. Verma SK, Maheshwari S, Singh RK, Chaudhari PB. Laser in dentistry: An innovative tool in modern dental practice, Natl J Maxillofac Surg. 2012;3(2):124-132.
8. Asnaashari M, Safavi N. Application of Low-level Lasers in Dentistry (Endodontic). J Lasers Med Sci. 2013;4(2):57-66.
9. Davoudi A, Sanei M Badrian H. Application of Laser Irradiation for Restorative Treatments. Open Dent J. 2016;10:636-642.
10. Juric I.B, Anic I. The Use of Lasers in Disinfection and Cleanliness of Root Canals: a Review. Acta Stomatol Croat. 2014;48(1):6-15.
11. Arslan H, Barutcigil C, Karatas E, Topcouglu HS. Effect of citric acid irrigation on the fracture resistance of endodontically treated roots. Eur J Dent. 2014;8(1):74-78.
12. Attur K, Joy MtT, Karim R, Anil Kumar VJ, Deepika C, Ahmed H. Comparative analysis of endodontic smear layer removal efficacy of 17% ethylenediaminetetraacetic acid, 7% maleic acid, and 2% chlorhexidine using scanning electron microscope: An in vitro study. J Int Soc Prev Community Dent. 2016;6(Suppl 2):160–165.
13. Babaji P, Jagtap K, Lau H, Bansal N, Thajuraj S, Sondhi P. Comparative evaluation of antimicrobial effect of herbal root canal irrigants (Morinda citrifolia, Azadirachta indica, Aloe vera) with sodium hypochlorite: An in vitro study. J Int Soc Prev Community Dent. 2016;6(3):196-199.
14. Josic U, Mazzitelli C, Maravic T, Fidler A, Breschi L, Mazzoni A. Biofilm in Endodontics: In Vitro Cultivation Possibilities, Sonic-, Ultrasonic- and Laser-Assisted Removal Techniques and Evaluation of the Cleaning Efficacy. Polymers (Basel). 2022;14(7):1334.
15. Asnaashari M, Tahmasebi Ebad L, Shojaeian S. Comparison of Antibacterial Effects of 810 and 980- nanometer Diode Lasers on Enterococcus Faecalis in the Root Canal System - An in vitro study. Laser Ther. 2016;25(3):209-214.
16. 16.Van Tyne D, Martin MJ, Gilmore MS. Structure, Function, and Biology of the Enterococcus Faecalis Cytolysin. Toxins (Basel). 2013;5(5):895- 911.
17. Dioguardi M, Di Gioia G, Illuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent. 2018;12(3):459-466.
18. Higuchi N, Hayasi JI, Fujita M, Iwamura Y, Sasaki Y, Goto R et al. Photodynamic Inactivation of an Endodontc Bacteria Using Diode Laser and Indocyanine Green-Loaded Nanosphere. Int. J. Mol. Sci. 2021;22(16):8384.

19. Ohsugi Y, Niimi H, Shimohira T, Hatasa M, Katagiri S, Aoki et al. In Vitro Cytological Responses against Laser Photobiomodulation for Periodontal Regeneration. Int. J. Mol. Sci. 2020;21(23):9002.
20. Derikvand N, Chinipardaz Z, Ghasemi S, Chiniforush N. The Versatility of 980 nm Diode Laser in Dentistry: A Case Series. J Lasers Med Sci. 2016;7(3):205-208.
21. Asnaashari M, Safavi N. Disinfection of Contaminated Canals by Different Laser Wavelengths, while Performing Root Canal Therapy. J Lasers Med Sci. 2013;4(1):8-16.
22. Hegde MN, Bhat R, Shetty P. Efficiency of a semiconductor diode laser in disinfection of the root canal system in endodontics: An in vitro study. Journal Of ICDRO. 2015;7(1):35-38.
23. Bordea IR, Hanna R, Chiniforush N, Grădinaru E, Câmpian RS, Sîrbu A, Amaroli et al. Evaluation of the outcome of various laser therapy applications in root canal disinfection: A systematic review. Photodiagnosis Photodyn Ther. 2020;28:101611.
24. Sarda RA, Shetty RM, Tamrakar A, Shetty, SY. Antimicrobial efficacy of photodynamic therapy, diode laser, and sodium hypochlorite and their combinations on endodontic pathogens. Photodiagnosis Photodyn Ther. 2019;28:265-272.
25. Katalinić I, Budimir A, Bošnjak Z, Jakovljević S, Anić I. The photo-activated and photo-thermal effect of the 445/970 nm diode laser on the mixed biofilm inside root canals of human teeth in vitro: A pilot study. Photodiagnosis Photodyn Ther. 2019;26:277-283.
26. Kasić S, Knezović M, Beader N, Gabrić D, Ivanišević Malčić A, Baraba A. Efficacy of Three Different Lasers on Eradication of Enterococcus faecalis and Candida albicans Biofilms in Root Canal System. Photomed Laser Surg. 2017;35(7):372-377.

Corresponding author:
Timea Dako
George Emil Palade University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, 38 Gheorghe Marinescu street, Târgu Mureș, 540139, Romania
Email: timea.dako@umfst.ro

Received: December 12, 2023 / Accepted: February 2, 2024