ENDODONTIC BACTERIAL REDUCTION USING DIODE LASER RADIATION – SHORT LITERATURE REVIEW

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ABSTRACT:

The rapid evolution of laser technology and the better understanding of laser-tissue interaction, allowed the growth of possible laser treatments, including laser assisted endodontics. Diode laser was introduced in endodontic procedures in late 1980’s and today is used for decontamination of the root canals. Lasers are used in association with various endodontic techniques, either to irradiate directly the dentinal walls, or to irradiate/activate photoactive substances/irrigants, having an indirect clinical effect on the endodontic system. Side effects resulting from irradiation with laser light do not appear if the correct parameters are used. Further clinical studies are necessary in order to evaluate the healing of endodontic diseases from laser assisted therapy.

Key words: diode laser, endodontics, decontamination, bactericidal effect.

1. INTRODUCTION

According to National Academy of Sciences, laser development is one of the greatest achievements after one century of innovations in engineering (1).The word “laser” stands for Light Amplification by Stimulated Emission of Radiation (2).

When the first dental laser became available, at the end of the 1980’s, practitioners were thrilled. Unfortunately, the wave length chosen for this first device, was the only one available, not the best option in order to satisfy the purpose. The use of dental lasers has evolved greatly since then, following scientific research of the laser radiation interaction with soft and hard tissues (2).

The laser light is different from the one emitted by the majority of luminous common sources, such as incandescent light bulbs, fluorescent lamps and high power arc lamps. Laser light has specific properties, useful especially in science and technology: monochromaticity, coherence and directionality (1, 3).

Depending on the optical properties of the tissue, the energy of the laser light can have 4 different interactions with the target: reflection, absorption, transmission and scattering (1-3).

2. LASER IN ENDODONTICS

Studies regarding the use of laser in endodontics were first done in the 1970's by Weichman, Johnson and Lenz (4-6), and were later developed, mainly in the early 1990's.

The rapid evolution of laser technology along with the rise in the understanding of laser-tissue interaction, allowed the growth of possible laser treatments, including laser assisted endodontics. The first intent was to use the laser light in all the endodontic treatment procedures, but after research was done and the devices were used in clinical practice, the indication was limited to cleaning/debridement and decontamination of the endodontic system. In recent years, all the available wave lengths were studied, and in vivo and in vitro studies confirmed the efficiency of several laser wave lengths in reducing the bacterial population in infected canals (3).

Diode laser was introduced in endodontic procedures in late 1980's, first for treating dentin hypersensitivity (7, 8) and for bio-modulation of the inflammatory response during vital pulp therapy (pulp capping and pulpectomy) and other inflammatory conditions of the maxillary bone (9, 10). Today, diode lasers are an alternative for Nd:YAG lasers for incision, vaporization and coagulation of soft tissues (gingiva, mucosa, dental pulp), for treating periodontal pockets (curettage, decontamination) and for decontamination of the radicular canals (3).

3. DENTAL LASERS FOR ENDODONTIC DECONTAMINATION

Bacterial contamination of the endodontic system is considered to be the primary etiologic factor in the development of pulpal and periapical lesions (11-13). For the root canal treatment to be successful it is necessary the complete removal of intracanalicular bacterial population and its metabolism products (1). A more reasonable and easily achievable goal is to reduce the number of microorganisms to a level that permits the body to heal itself (3).

In endodontics the golden standard is today considered to be the use of chemical irrigants, especially sodium hypochlorite (14). However, conventional manual irrigation technique showed a limited capacity of penetration in dentinal tubules, and Berutti et al (1997) reported that the use of sodium hypochlorite is effective for decontamination up to 130 microns in the dentinal wall (15), while bacterial colonies were found even at 1.15 mm from the main canal in dentinal tubules (16).

Lasers are used in association with various endodontic techniques. These devices can be used to irradiate directly the dentinal walls or to irradiate/activate photoactive substances/irrigants, having an indirect clinical effect on the endodontic system. Laser irradiation produces a direct thermal effect on dentinal walls and bacteria, but also generates unwanted side effects, depending on the wave length and the interaction with the dentinal walls (3).

3.1. CONVENTIONAL LASER ENDODONTICS

Since the beginning of laser assisted endodontics, the technique used involved end firing tips or fibers, placed inside the canal, usually at 1 mm away from the apex. This technique is appropriate for the majority of visible wave lengths (445 and 532 nm), near-infrared (810 – 1,340 nm) and medium-infrared (2,780 and 2,940 nm) of the electromagnetic spectrum. The laser energy is transmitted through fibers or tips inserted at the end of the hand piece. Endodontic tips have a specific diameter of 200-300 µm and a length appropriate for their clinical use, allowing their insertion inside the root canal. These tips are flexible and resistant for use in anatomical curvature (keeping or reducing them) of dental roots with a low risk of fracture. When a thin fiber (200 µm) is used inside the root canal, the decontamination of the endodontic system is done up to 500-750 µm from the main canal. The laser activation begins when a movement of withdrawal is performed with the fiber/tips, in a circular or helical motion in a certain amount of time (usually 2 mm/s) (3).

3.2. LASER-ACTIVATED IRRIGATION (LAI)

One of the most recent techniques available for agitation or automatic activation of the irrigant in the root canal is the laser-activated irrigation. This technique involves the use of laser energy to agitate or activate the irrigant. For this specific technique the practitioner can use erbium lasers such as ErCrYSGG and ErYAG. These lasers have wavelengths in the medium-infrared region (2,780-2,940 nm) which is absorbed in water and NaOCl (17).

In order to be effective on the whole working length of the root canal, LAI technique has had different protocols for applying the laser tips in different segments of the root canal. In one scenario, the tip was placed near the opening orifice of the root canal, in the pulp chamber filled with irrigant (18, 19). In doing so, the agitation/activation and flow of the irrigant in the whole radicular system was done. This specific LAI technique known as photon-induced photoacoustic streaming (PIPS)uses special laser tips, tapered and stripped, with low energy (20 m J) and short duration of pulsation (50 microseconds). These fibers allow for the lateral emission of light to occur (19).

3.3. ANTIMICROBIAL PHOTODYNAMIC THERAPY (A PDT)

This technique is also known as photodynamic inactivation (PDI), photoactivated disinfection (PAD)or photodynamic antimicrobial chemotherapy (PACT) and involves applying a photoactive or photosensitive dye (photosensitizer PS) which is capable of producing species of reactive oxygen when irradiated with light at the right wavelength for it to be absorbed by PS (1, 3).

Photosensitive substances, such as methylene blue (MB), toluidine blue (TB), tolonium chloride (TC) and green indocyanine (IG), make the bacteria more sensible to laser light (3). In order to accomplish the aPDT, PS must be capable to absorb efficiently light and then to transform it in one of the species of reactive oxygen: “singlet oxygen”, anion superoxide and hydroxyl radical. Also, it has to present a certain degree of affinity for the microorganism membranes and to penetrate the microbian biofilm (20). Some bacteria produce their own PS and can be destroyed just by applying light. The PS produced is usually a porphyrin and can be excited efficiently with red or blue light.

Unfortunately, the majority of organisms responsible for endodontic infections do not produce PS and thus the technique requires using external PS (1). External factors can interfere with the photoreaction, such as the interaction between phenothiazine dyes and blood or saliva from the oral cavity can reduce the efficiency in spite of correct illumination and PS concentration (21).

Studies regarding the use of PDT in treating oral and dental infections are becoming popular. Particularities of this noninvasive method and bacterial resistance to antibiotics transformed PDT in an alternative to the treatment with chemical agents for eliminating bacteria in oral infections such as periodontitis and peri-implantitis (1).

The use of PDT for treating endodontic infections caused by bacterial biofilm was analyzed in ex vivo- studies with extracted teeth. Enterococcus faecalis is the pathogen frequently associated with current endodontic infections (22) and some studies evaluated toluidine blue effect (23, 24) and methylene blue (24-26) with PDT in endodontic treatment. Studies (27-32) showed that endodontic therapy done with PDT is effective in bacterial reduction from root canal, both in ex vivo samples and in patients, although the success rate varies and comparint them is difficult (different PS, light parameters, ways of applying the light) (1).

4. BACTERICIDAL EFFECT

Nowadays it is accepted that laser irradiation has the potential to kill mirorganisms. In most cases, this effect is linked to the energy level and the exposure (2). A precise and clear determination of the bactericidal effect of different laser systems has not been accomplished, many results were contradictory. Olivi reported that laser irradiation of root canals using conventional techniques (introducing a fiber/tip at the working length and activating while withdrawal), does not satisfy modern endodontics principles (3).

The first mechanism proposed for explaining the bactericidal effect of laser irradiation was that the laser light is absorbed by bacteria and thus is responsible for direct destruction of the bacterial cell. It was considered that this phenomenon was due to the presence of black pigments in some bacteria (protoporphyrin IX), which can absorb a limited portion of wavelengths, especially those in the near infrared portion of the spectrum (33). Deterioration is produced at the membrane of the cell and induces osmotic alteration which translates into cell death (34, 35).

The second mechanism proposed suggested that laser light is absorbed strongly in dentinal substrate on which bacteria adhere, the resulting heat causes a local rise of temperature, that leads to the death of the microorganisms attached (33, 36). However, some studies reported the existence of insignificant relationship between the temperature rise and the bactericidal effect (37). In this case, the bactericidal effect was linked to an unidentified photodamage effect (38, 39), which contradicts with most studies that reported a bactericidal effect according to the power applied and the thermal effect of lasers. It is unclear the real mechanism that stands for the laser decontamination of root canals. Thermographic and morphologic studies represented a step forward to the identification of safe parameters for laser decontamination of root canals (3).

Diode lasers 810 nm, 940 nm and 980 nm, and Nd:YAG laser 1064 nm have been studied in association with various irrigants as a final step in cleaning and decontaminating at the end of the endodontic therapy. Several studies (40-44) reported that when the root canal is irradiated after or at the same time with the irrigation procedure (distilled water, EDTAC, chlorhexidine, sodium hypochlorite) the morphologic pattern at the dentinal surface is better or similar to the one seen after irrigation.

The ability of diode lasers with 2 wavelengths (810 nm and 940 nm) has been compared by Beer (45) in vitro. Diode lasers have accomplished a bacterial reduction of E. coli of 76.06% (810 nm) and 68.15 % (940 nm). This study concluded that an additional irradiation in the access cavity produces a better result concerning the bactericidal effect: average reduction of E. coli of 97.84% (810 nm) and 98.83% (940 nm) and average reduction of E. faecalis of 98.8% (810 nm) and 98.66% (940 nm) (3).

In 2018 Masilionyte conducted a study in which he gathered all the clinical cases of apical periodontitis from a private practice in order to evaluate results of endodontic (re)treatment assisted by diode laser 940 nm and EDTA versus endodontic conventional therapy. His study concluded that laser assisted endodontic therapy using diode laser 940 nm is an alternative to conventional endodontic therapy, allowing the reduction of chemical irrigants, intracanal medicamentation and systemic antibiotics. This treatment protocol allowed a quicker healing of periapical lesions, but the author emphasized the need of extensive clinical studies (46).

Dai et al (47) conducted a study on temporary teeth inoculated with Enterococcus faecalis for the evaluation of the bactericidal effect of diode laser irradiation (810 nm) and of the association of laser irradiation with sodium hypochlorite 5.25% irrigation. The bactericidal effect was superior when diode laser was used along with sodium hypochlorite 5.25% irrigation. The authors stress that this protocol could be an ideal one to ensure the treatment success.

5. SMEAR LAYER REMOVAL FROM THE ROOT CANAL WALLS

Cleaning the radicular system from pathogens is a major goal of the endodontic therapy, conventionally fulfilled by biomechanical instrumentation. Given the complexity of the radicular system, the complete removal of debris and the gain of a sterile radicular system if difficult to obtain (48, 49). Peters et al (50) showed that over 35% of the root canal surface has no alteration after preparation using 4 different techniques. Pashely (51) reported that smear layer with its bacteria and bacterial products plays an important role as a reservoir for pathogens. Thus, its complete removal can signify the removal of pathogens from the radicular system (2, 52).

Practitioners must consider the development of modern treatment strategies for the removal of microorganisms from the radicular system given the fact that most substances used inside the root canal have a limited bacterial spectrum and a limited ability to diffuse in dentinal tubules (2, 53).

Many laser systems have been reported as being useful in the removal of the smear layer from root canal walls, including argon fluoride (ArF) laser and excimer laser, ion argon laser, KTP laser (532 nm), diode laser, Nd:YAG, HoYAG, Er:YAG, Er,Cr:YSGG lasers and CO_2 laser (54).

Near-infrared lasers kill bacteria, but do not remove smear layer. The heat stored by diffusing thermal energy determines partial closure of dentinal tubules in a process named fusion of organic and inorganic dental structure. Furthermore, when the terminal portion of the tip or fiber contacts the dental wall during laser light emission, it can produce thermal alteration named hot spots”, bubles and fissures. When the near-infrared laser is used in a wet root canal, filled with water or irrigants, water based fluids limit the direct interaction of the laser radiation on the dentinal walls, reducing the unwanted side effects, but also limiting the decontamination effect (3).

In this case, near-infrared lasers activate the irrigants through heating and this rises the irrigation efficiency. Diode laser irradiation (2.5 W, gated mode) and Nd:YAG laser (1.5 W, pulsed mode at 15 Hz), performed in a root canal filled with fluids, produces a dentinal pattern similar to the one obtained when the irradiation is performed in a dry environment. When the irradiation follows EDTA irrigation, cleaner surfaces are obtained, with less smear layer, open dentinal tubules and less thermal alteration, than when laser irradiation is performed in a dry environment (3)

Wang (55) used a diode laser with 980 nm wavelength at 5 W for 7 s for the removal of smear layer. However, there is concern regarding the heat transmission in the tooth surrounding tissues when high power is used (54, 56)

Matsumoto (57) emphasized the possible limited use of laser in the radicular system, sugesting that “smear layer removal and debris with laser is possible, but it is difficult to clean all root canal walls, because the laser light is emitted in a straight direction, making it almost impossible to irradiate lateral canal walls”. The author recommends the improvement of endodontic tips as to allow irradiation of all the areas of the root canal walls (2).

6. LIMITATIONS WHEN USING LASERS IN ROOT CANALS

6.1. INSERTING AND ACTIVATION OF ENDODONTIC TIPS

Despite the flexibility of modern endodontic fibers/tips, reaching the whole working length in curved or narrow root canals, or the entire contaminated areas, is still difficult to obtain given the complex anatomy of the radicular system (3).

In order to facilitate the insertion and removal of the fiber/tip, the root canal must be enlarged and properly prepared, at a diameter bigger than of the fiber/tip (200-300 microns) and up to ISO 30-35, .06. This manner of preparing the root canal leads to the reduction of radicular dentine which can be unsafe in difficult canals (3).

Furthermore, laser energy emission through the tip of the optic fiber is directed along the root canal and not necessarily in its lateral areas, make it almost impossible to obtain a uniform coverage of 360 degrees of the internal aspect of the radicular system (58, 59).

The helical movement proposed for the withdrawal of the fiber from the apical portion of the root canal does not produce an efficient rise of the lateral diffusion of energy. The increase of the angle of irradiation obtained is minimal and the movement can force the fiber/tip into a tight contact with the dentine, producing most probably a morphological alteration (hot spots) at the surface. Alterations can vary from a minimal superficial thermal effect, with a partial closure of the dentinal tubules, to producing areas of carbonization (near-infrared lasers) of ablative effects (medium-infrared lasers) that can lead to the perforation of the apical portion or ledges in dentinal walls (3).

Anatomical and operative problems related to the insertion of the fiber in narrow and curved root canals remain unresolved, with the impossibility or probable inefficiency of the procedure when being performed in teeth with complicated root canal anatomy (3).

Stabholz et al (59, 60) described a new endodontic tip which can be used with erbium lasers. This new endodontic tip named "side-firing spiral" was designed to adapt to root canal walls through its spiral slots located along the tip. The tip is sealed at its end, preventing the transmission of radiation beyond the apical foramen of the tooth.

6.2. THERMAL EFFECT

The practitioner who uses dental laser systems must be aware of the thermal effects that appear in biological tissues when a device of such high power is being activated. The temperature rise in a tissue can lead to the development of structural changes, such as protein denaturation, water evaporation, coagulation etc. In hard dental tissues, the temperature rise can lead to the alteration of crystallographic characteristics of the mineral matrix, such as crystalline skeleton, hydroxyapatite crystals volume and formation of new compounds (61-64).

When techniques of photothermal disinfection with dental lasers are used, it is important to apply pulsed modes and to respect the rest period to allow the cooling of the radicular structures, without collateral alteration of the periodontal ligament through thermal stress. Safety evaluation in laboratory environment showed that the accepted degree of temperature rise at the radicular surface is 5.5-7^{o}C (54, 65, 66).

The apical portion of the root canal is more predisposed to thermal alteration because the thickness of the dentine is the lowest at this level (67) and the end portion of the fiber will pass close to the root canal walls. To minimize the thermal effects during laser assisted endodontic treatment with plain fibers, the end of the tip must be constantly moved in a circular motion during activation and withdrawal (68).

Parameters such as wavelength, energy density, power, peak power, average power, pulse length and rate of repetition are very important to determine the degree of heat generation from irradiating tissues. The amount of heat generated inside a tissue is highly dependent on the optical properties of that tissue (absorption coefficient and scattering coefficient). Also, the heat transfer is dependent on certain properties of the tissues (thermal diffusion and thermal conductivity) (1, 69).

Direct emission of laser radiation through tips near the apical foramen of a tooth can result in the transmission of energy beyond that foramen. This phenomenon can affect supporting tissues and has great importance in the case of teeth located near the mental foramen or mandibular nerve (2, 59, 60).

Many studies have evaluated the rise of temperature while using near-infrared lasers and the morphological alterations that appear on the dentinal walls. Diode laser 980 nm action has been studied in vitro by Wang (2005) when irradiating monoradicular teeth for 7 s using fibers of various diameters (550 and 365 microns) at power of 5 W. The maximum rise in temperature was 8.1^{o}C. Laser irradiation led to an improved removal of smear layer, with a superior cleaning than the one noticed in the control group which had no irradiation (55).

De Moura-Netto (2005) compared in a in vitro study the effects of Nd:YAG laser (1.5 W, 100 m J, 15 Hz) with the effects of diode laser 810 nm (2.5 W in continuous wave) using a speed while withdrawing the fiber of

2 mm/s for 20 s. Both lasers proved partial removal of debris and smear layer, with the presence of morphological alterations (recrystallization and liquefaction), more prominent in Nd:YAG laser (70).

Da Costa Ribeiro (2007) studied the rise of temperature when irradiation is performed with diode laser 810 nm. At 2.5 W, the thermal rise in the apical third varied from 1.6 to 8.6^{o}C, while at 1.25 W, 10 Hz, the rise was from 1.2^{o}C to 3.3^{o}C(56).

Parirokh (2007) concluded in an in vitro study that irradiation with diode laser 808 nm led to closure of dentinal tubules in that area in all scenarios, especially in the apical third of the root canal (71).

Alfredo (2008) evaluated temperature variations using thermocouples located at the radicular walls after irradiation with diode laser 980 nm at: 1.5, 3.0 and 5.0 W in continuous and gated wave mode at 100 and 1000 Hz, in dry canals and in ones irrigated with distilled water. The cervical portion of the root canal showed the highest temperature rise (+9.68±5.80 ^{o}C), followed by the medium third (+7.66±4.87 ^{o}C) and the apical third (+6.70±4.23^{o}C), seeing a statistically significant difference between them (72).

Hmud (2010) conducted a study in which the temperature changes were evaluated inside the root canal and on its walls when irradiation was performed with diode laser 940 nm and 980 nm at 4 W/10 Hz and 2.5 W/25 Hz with fibers/tips of 2000 µm. Both diode lasers irradiation led to modest thermal changes at the external root surface (68).

Da Fonseca Alvarez (2012) used thermocouples to register and analyze the temperature variation at the external root surface of lower incisors in order to evaluate possible periodontal damage. Samples were irradiated while the fiber/tip had a circular retrograde motion using various parameters (1.5, 2.0, 2.5, 3.0 and 3.5 W). Temperature was measured at the vestibular and lingual aspects of the roots, in the apical and middle third. The temperature variations recorded were considered to be acceptable and safe, lower than 7^{o}C. When using 3.5 W the temperature rise was beyond 7^{o}C (73).

Haidary (2016) evaluated in a study the root surface temperature while irradiating the root canal with Er,Cr:YSGG laser 2780 nm anddiode laser 940 nmin an alternating sequence. Authors concluded that the alternative irradiation with these lasers is a safe method for decontaminating the root canal (74).

Similar studies were performed in 2015 by Al-Karadaghi (75) and in 2017 byTrišić (76),which concluded that the use of diode lasers at the mentioned parameters does not lead to unwanted thermal effects.

7.CONCLUSIONS

1. The use of dental lasers for decontamination of the endodontic system is an alternative to conventional endodontic therapy.

2. Side effects resulting from irradiation with laser light do not appear if the correct parameters are used.

3. The mechanism for the bactericidal effect or laser irradiation is still unclear.

4. Further clinical studies are necessary in order to evaluate the healing of endodontic diseases from laser assisted therapy.

5. Researchers must build new endodontic tips with an improved design that makes them efficient in complex root canals.

REFERENCES

1. Patrícia M. de Freitas. Lasers in Dentistry Guide for Clinical Practice. Wiley Blackwell, 2015
2. Robert A. Convissar. Principles And Practice Of Laser Dentistry, Second Edition,Elsevier, 2016.
3. Giovanni Olivi, Roeland De Moor, Enrico Divito. Lasers In Endodontics - Scientific Background And Clinical Applications. 2016.
4. Weichman JA, Johnson FM. Laser use in endodontics. A preliminary investigation. Oral Surg Oral Med Oral Pathol. 1971;31(3):416–20.
5. Weichman JA, Johnson FM, Nitta LK. Laser use in endodontics. II. Oral Surg Oral Med Oral Pathol. 1972;34(5):828–30.
6. Lenz P. The use of laser beams in dentistry. Zahnarztl Mitt. 1974;64(3):112–7. German.
7. Furuoka M, Yokoi T, Fukuda S, Usuki M, Matsuo S, Taniguchi K, Kitamura K. Effects of GaAlAs laser diode in treatment of hypersensitive dentine. Fukuoka Shika Daigaku Gakkai Zasshi. 1988;15(1):42–8. Japanese.
8. Yamaguchi M, Ito M, Miwata T, Horiba N, Matsumoto T, Nakamura H, Fukaya M. Clinical study on the treatment of hypersensitive dentin by GaAlAs laser diode using the double blind test. Aichi Gakuin Daigaku Shigakkai Shi. 1990;28(2):703–7. Japanese.
9. Kireev AK, Evstigneev AR, Voroshnin PA, Aleksandrov MT. The use of the Uzor laser apparatus for treating infl ammatory diseases of the maxillofacial area. Stomatologiia (Mosk). 1989;68(5):42–5. Russian.
10. Kurumada F. A study on the application of Ga-As semiconductor laser to endodontics. The effects of laser irradiation on the activation of inflammatory cells and the vital pulpotomy. Ou Daigaku Shigakushi. 1990;17(3):233–44. Japanese.
11. Kakehashi S, Stanley HR, Fitzgerald RJ: The effect of surgical exposures of dental pulps in germ-free and conventional laboratory rats, Oral Surg Oral Med Oral Pathol 20:340–349, 1965.
12. Bergenholz G: Microorganisms from necrotic pulps of traumatized teeth, Odontologisk Revy 25:347–358, 1974.
13. Moller AJ, Fabricius L, Dahlen G, et al.: Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys, Scand J Dent Res 89:475–484, 1981.
14. Mohammadi Z, Shalavi S. Antimicrobial activity of sodium hypochlorite in endodontics. J Mass Dent Soc. 2013;62(1):28–31.
15. Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. J Endod. 1997;23(12):725–7.
16. Kouchi Y, Ninomiya J, Yasuda H, Fukui K, Moriyama T, Okamoto H. Location of Streptococcus mutans in the dentinal tubules of open infected root canals. J Dent Res. 1980;59(12):2038–46.
17. Meire MA, Poelman D, De Moor RJ. Optical properties of root canal irrigants in the 300–3,000-nmwavelength region. Lasers Med Sci. 2014;29(5):1557–62.
18. Peeters HH, Suardita K. Efficacy of smear layerremoval at the root tip by using ethylenediaminetetraaceticacid and erbium, chromium: yttrium, scandium, gallium garnet laser. J Endod. 2011;37:1585–9.
19. Peters OA, Bardsley S, Fong J, Pandher G, Divito E.Disinfection of root canals with photon-initiated photoacousticstreaming. J Endod. 2011;37:1008–12.
20. Dai T, Huang YY, Hamblin MR. Photodynamic therapy for localizedinfections--state of the art. Photodiagn Photodyn Ther 2009; 6(3‐4): 170‐188.
21. Nunez SC, Garcez AS, Gomes L, Baptista MS, Ribeiro MS. Methylene blueaggregation in the presence of human saliva. Proc SPIE. 2008; 9: 684608-1‐7.
22. Rocas IN, Siqueira JF, Jr., Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004; 30: 315–320.
23. Fonseca MB, Junior PO, Pallota RC, et al. Photodynamic therapy for root canals infected with Enterococcus faecalis. Photomed Laser Surg 2008; 26: 209–213.

24. Souza LC, Brito PR, de Oliveira JC, et al. Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of Enterococcus faecalis. J Endod 2010; 36: 292–296.
25. Soukos NS, Chen PS, Morris JT, et al. Photodynamic therapy for endodontic disinfection. J Endod 2006; 32: 979–984.
26. Ng R, Singh F, Papamanou DA, et al. Endodontic photodynamic therapy ex vivo. J Endod 2011; 37: 217–222.
27. Foschi F, Fontana CR, Ruggiero K, et al. Photodynamic inactivation ofEnterococcus faecalis in dental root canals in vitro. Lasers Surg Med 2007;39(10): 782–787.
28. Garcez AS, Ribeiro MS, Tegos GP, Nunez SC, Jorge AO, Hamblin MR. Antimicrobial photodynamic therapy combined with conventional endodontic treatment to eliminate root canal biofilm infection. Lasers Surg Med 2007; 39: 59–66.
29. Fimple JL, Fontana CR, Foschi F, et al. Photodynamic treatment ofendodontic polymicrobial infection in vitro. J Endod 2008; 34(6): 728–734.
30. Garcez AS, Nunez SC, Hamblin MR, Ribeiro MS. Antimicrobial effects ofphotodynamictherapy on patients with necrotic pulps and periapical lesion. JEndod 2008; 34(2): 138–142.
31. Kishen A, Upadya M, Tegos GP, Hamblin MR. Efflux pump inhibitor potentiates antimicrobial photodynamic inactivation of Enterococcus faecalis biofilm. Photochem Photobiol 2010; 86(6): 1343–1349.
32. Garcez AS, Nunez SC, Hamblim MR, Suzuki H, Ribeiro MS. Photodynamic therapy associated with conventional endodontic treatment in patients with antibiotic-resistant microflora: a preliminary report. J Endod 2010; 36(9): 1463–1466.
33. Pirnat S, Lukac M, Ihan A. Study of the direct bactericidal effect of Nd:YAG and diode laser parameters used in endodontics on pigmented and nonpigmented bacteria. Lasers Med Sci. 2011;26(6):755–61. Epub 2010 Jun 27.
34. Moritz A, Jakolitsch S, Goharkhay K, Schoop U, Kluger W, Mallinger R, Sperr W, Georgopoulos A. Morphologic changes correlating to different sensitivities of Escherichia coli and enterococcus faecalis to Nd:YAG laser irradiation through dentin. Lasers Surg Med. 2000;26(3):250–61.
35. Moritz A. Oral laser application. Berlin: Quintessence Verlags-GmbH; 2006. p. 258–77.
36. Murray BE. The life and times of the Enterococcus. Clin Microbiol Rev. 1990;3:46–65.
37. Schoop U, Kluger W, Moritz A, Nedjelik N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med. 2004;35:111–6.
38. Neuman KC, Chadd EH, Liou GF, Bergman K, Block SM. Characterization of photodamage to Escherichia coli in optical traps. Biophys J. 1999; 77:2856–63.
39. Mirsaidov U, Timp W, Timp K, Mir M, Matsudaira P, Timp G. Optimal optical trap for bacterial viability. Phys Rev E Stat Nonlin Soft Matter Phys. 2008;78(2 Pt 1):021910.
40. Santos C, Sousa-Neto MD, Alfredo E, Guerisoli DM, Pecora JD, Comelli Lia RF. Morphologic evaluation of the radicular dentine irradiated with Nd:YAG laser under different parameters and angles of incidence. Photomed Laser Surg. 2005;23(6): 590–5.
41. Gurbuz T, Ozdemir Y, Kara N, Zehir C, Kurudirek M. Evaluation of root canal dentin after Nd:YAG laser irradiation and treatment with five different irrigation solutions: a preliminary study. J Endod. 2008;34(3):318–21.
42. Faria MI, Souza-Gabriel AE, Marchesan MA, Sousa-Neto MD, Silva-Sousa YT. Ultrastructural evaluation of radicular dentin after Nd:YAG laser irradiation combined with different chemical substances. Gen Dent. 2008;56(7):641–6.
43. Marchesan MA, Brugnera-Junior A, Ozorio JE, Pécora JD, Sousa-Neto MD. Effect of 980-nanometer diode laser on root canal permeability after dentin treatment with different chemical solutions. J Endod. 2008;34(6):721–4.
44. Alfredo E, Souza-Gabriel AE, Silva SR, Sousa-Neto MD, Brugnera-Junior A, Silva-Sousa YT. Morphological alterations of radicular dentine pretreated with different irrigating solutions and

irradiated with 980-nm diode laser. Microsc Res Tech. 2009;72(1):22–7.
45. Beer F, Buchmair A, Wernisch J, Georgopoulos A, Moritz A. Comparison of two diode lasers on bactericidity in root canals-an in vitro study. Lasers Med Sci. 2012;27(2):361–4. Epub 2011 Feb 2.
46. Masilionyte, M., Gutknecht, N. Outcome of 940-nm diode laser-assisted endodontictreatment of teeth with apical periodontitis: a retrospective study of clinical cases. Laser Dent Sci 2, 169–179 (2018). https://doi.org/10.1007/s41547-018-0035-2
47. Dai S, Xiao G, Dong N, Liu F, He S, Guo Q. Bactericidal effect of a diode laser on Enterococcus faecalis in human primary teeth-an in vitro study. BMC Oral Health. 2018;18(1):154. Published 2018 Aug 31. doi:10.1186/s12903-018-0611-6
48. Bystrom A, Sundquist G: Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy, Scand J Dent Res 89:321–328, 1981.
49. Sjogren U, Hagglund B, Sundquist G, et al.: Factors affecting the long-term results of endodontic treatment, J Endod 16:498–504, 1990.
50. Peters OA, Schonenberger K, Laib A: Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography, Int Endod J 34:221–230, 2001.
51. Pashley DH: Smear layer: physiological considerations, Oper Dent Suppl 3:13–29, 1984.
52. Drake DR, Wiemann AH, Rivera EM, et al.: Bacterial retention in canal walls in vitro: effect of smear layer, J Endod 20:78–82, 1994.
53. Oguntebi BR: Dentin tubule infection and endodontic therapy implications, Int Endod J 27:218–222, 1994.
54. Coluzzi DJ, Parker SPA. Lasers in Dentistry- CurrentConcepts. New York: Springer, 2017.
55. Wang X, Sun Y, Kimura Y, Kinoshita J, Ishizaki NT, Matsumoto K. Effects of diode laser irradiation on smear layer removal from root canal walls and apical leakage after obturation. Lasers Med Sci. 2005; 20(2):99–103.
56. da Costa Ribeiro A, et al. Effects of diode laser (810 nm) irradiation on root canal walls: thermographic and morphological studies. J Endod. 2007;33(3):252–5.
57. Matsumoto K: Lasers in endodontics, Dent Clin North Am 44:889–906, 2000.
58. Goodis HE, Pashley D, Stabholz A: Pulpal effects of thermal and mechanical irritants. In Hargreaves KM, Goodis HE, editors: Seltzer and Bender’s dental pulp, Chicago, 2002, Quintessence, pp 371–410.
59. Stabholz A, Zeltzser R, Sela M, et al.: The use of lasers in dentistry: principles of operation and clinical applications, Compendium 24:811–824, 2003.
60. Stabholz A: The role of laser technology in modern endodontics. In Ishikawa I, Frame JW, Aoki A, editors: Lasers in dentistry: revolution of dental treatment in the new millennium, Elsevier Sci BV Int Congr Series 1248:21–27, 2003.
61. Bachmann L, Craievich AF, Zezell DM. Crystalline structure of dental enamel after Ho:YLF laser irradiation. Arch Oral Biol 2004; 49: 923–929.
62. Ana PA, Bachmann L, Zezell DM. Lasers effects on enamel for caries prevention. Laser Phys 2006; 16: 865–875.
63. Bachmann L, Rosa K, Ana PA, et al. Crystalline structure of human enamel irradiated with Er,Cr:YSGG laser. Laser Phys Let 2009; 6: 159–162.
64. Botta SB, Ana PA, de Sa Teixeira F, et al. Relationship between surface topography and energy density distribution of Er,Cr:YSGG beam on irradiated dentin: an atomic force microscopy study. Photomed Laser Surg 2011; 29: 261–269.
65. Zach L, Cohen G. Pulp response to externally applied heat. Oral Surg Oral Med Oral Pathol. 1965;19:515–30.
66. Nammour S, et al. External temperature during KTP-Nd:YAG laser irradiation in root canals: an in vitro study. Lasers Med Sci. 2004;19(1):27–32.
67. Gutknecht N, Franzen R, Meister J, Vanweersch L, Mir M. Temperature evolution on human teeth root surface after diode laser assisted endodontic treatment. Lasers Med Sci 2005;20:99–03.

68. Hmud R, Kahler WA, Walsh LJ. Temperature changes accompanying near infrared diode laser endodontic treatment of wet canals. J Endod. 2010;36(5):908–11.
69. Seka W, Featherstone JDB, Fried D, et al. Laser ablation of dental hard tissue: from explosive ablation to plasma-mediated ablation. SPIE 1996; 2672:144–158.
70. de Moura-Netto C, de Moura AA, Davidowicz H, Aun CE, Antonio MP. Morphologic changes and removal of debris on apical dentin surfaces after Nd:YAG laser and diode laser irradiation. Photomed Laser Surg. 2008;26(3):263–6.
71. Parirokh M, Eghbal MJ, Asgary S, Ghoddusi J, Stowe S, Forghani F, Shahravan A. Effect of 808 nm diode laser irradiation on root canal walls after smear layer removal: a scanning electron microscope study. Iran Endod J. 2007;2(2):37–42. Epub 2007 Jul 5.
72. Alfredo E, Marchesan MA, Sousa-Neto MD, Brugnera-Júnior A, Silva-Sousa YT. Temperature variation at the external root surface during 980-nm diode laser irradiation in the root canal. J Dent. 2008;36(7):529–34. Epub 2008 May 6.
73. da Fonseca Alvarez A, Moura-Netto C, Daliberto Frugoli A, Fernando C, Correa Aranha AC, Davidowicz H. Temperature changes on the root surfaces of mandibular incisors after an 810-nm high-intensity intracanal diode laser irradiation. J Biomed Opt. 2012;17(1):015006.
74. Haidary D, Franzen R, Gutknecht N. Root Surface Temperature Changes During Root Canal Laser Irradiation with Dual Wavelength Laser (940 and 2780 nm): A Preliminary Study. Photomed Laser Surg. 2016;34(8):336-344. doi:10.1089/pho.2015.4007
75. Al-Karadaghi TS, Gutknecht N, Jawad HA, Vanweersch L, Franzen R. Evaluation of Temperature Elevation During Root Canal Treatment with Dual Wavelength Laser: 2780 nm Er,Cr:YSGG and 940 nm Diode. Photomed Laser Surg. 2015;33(9):460-466. doi:10.1089/pho.2015.3907
76. Dijana Trišić, Bojana Ćetenović, Igor Jovanović, Elizabeta Gjorgievska, Branka Popović, Dejan Marković. Diode Laser Irradiation in Endodontic Therapy through Cycles - in vitro Study. Balk J Dent Med, 2017;108-111.