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Scanning electron microscopy evaluation of the root canal morphology after Er:YAG laser irradiation

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Abstract

The current limits of the endodontic disinfection strategies are not only a result of bacterial biofilm growth mode inside the root canals, they are equally due to the anatomical complexity of the root canal system, of its structure, of the dentin composition and of the factors associated with chemical disinfectants. One of the major problems is the fact that a great part of the endodontic anatomy remains uninstrumented after conventional treatment and even the accessible parts of the root canals are covered in smear layer, which results as a by-product of the instrumentation and acts as a barrier for irrigants, medication and even influencing the quality of the endodontic filling. Therefore, strategies in advanced disinfection in endodontics are developed and tested in order to meet these challenges. The present study aims to assess the possibility of improving the debridement of the root canals by using erbium-doped yttrium-aluminum-garnet (Er:YAG) laser radiation. We used extracted teeth, which were subjected to the conventional treatment protocol and then divided into three study groups: the negative control group and two other groups, which were exposed to laser radiation using two energy levels. Scanning electron microscopy (SEM) revealed the efficiency of the laser aided treatment *versus* the conventional methods of cleaning and disinfection of root canals.

Keywords: debridement, Er:YAG, laser irradiation, SEM.

Introduction

The conventional root canal treatment involves cleaning by using mechanical preparation and irrigation with anti-microbial solutions and solvents. During the preparation of the root canal system, it is cleaned of all debris of inorganic or organic nature and of microorganisms in order to perform the three-dimensional filling. The removal of necrotic pulp tissue, or in an advanced state of deterioration, as well as the removal of microorganisms, can be achieved with the aid of irrigation solutions and the mechanical action of instruments [1]. It is vital to understand that the current limits of the endodontic disinfection strategies are not only a result of bacterial biofilm growth mode inside the root canals, they are equally due to the anatomical complexity of the root canal system, of its structure, of the dentin composition and of the factors associated with chemical disinfectants. Therefore, strategies in advanced disinfection in endodontics are developed and tested in order to meet these challenges.

Studies have demonstrated that conventional chemo-mechanical preparation is limited regarding the decontamination of the endodontic space. Moreover, root canal instrumentation produces smear layer. This may limit optimal penetration of the irrigating agents and it can act as a barrier between the filling material and the root canal walls, altering the filling's quality and promoting further infiltration. Organisms at this level can be considered responsible for persistent endodontic infections, which is why removing the smear layer has its role and importance in the modern endodontic treatment [2]. Debris and smear layer removal lead to a better seal of canal filling substance and reduction of microleakage [3]. Many lasers, such as carbon dioxide (CO_2), neodymium-doped yttrium-aluminum-garnet (Nd:YAG), erbium (Er):YAG and erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) have been used in order to improve the removal of debris and smear layer from infected canals. Due to the specificity of the 2940 nm wavelength, the Er:YAG laser has shown his greater ability to remove debris and smear layer from the root canal walls following biomechanical instrumentation [4]. Many studies were conducted on this topic, having different protocols, different parameters, and tip design, different irrigants and it is nowadays a consensus that the association of laser radiation and irrigants offers a superior cleaning of the root canals by increasing the permeability of dentinal tissue, opening the dentinal tubules and reaching the collateral canals, which are not possible by means of conventional debridement using ethylenediaminetetraacetic acid (EDTA) and irrigants. One of the most valuable means of evaluation regarding the results in cleaning and disinfecting the root canals is by scanning electron microscopy (SEM), which has demonstrated to be a tool of great precision in offering information at higher level of magnification.

This study was designed in order to evaluate the effectiveness of Er:YAG laser therapy during the endodontic treatment. The aim was to investigate the possibility of improving the endodontic treatment without producing changes in the morphology of the root dentinal walls, by means of introducing the laser-assisted irrigation (LAI) into the conventional treatment protocol.

Materials and Methods

The study included 26 extracted monoradicular teeth, which corresponded to the inclusion criteria from among 57 initial analyzed teeth: teeth with a straight single canal, without any obstacles, cracks or other abnormalities, which could influence the results of our study.

It was performed a cone-beam computed tomography (CBCT) analysis for each tooth included in the study, in order to visualize the endodontic anatomy. The access cavity was made with a cylindrical diamond bur, followed by removal of pulp tissue and establishing the canal working length. All samples were prepared using rotating mechanical preparation (ProTaper system Dentsply, using a crown-down sequential technique) to an apical size of 0.3 mm and alternative irrigation with 5% sodium hypochlorite (NaOCl), distilled water and 17% EDTA solution. The files were replaced after instrumentation of five canals. All root canals were then dried with 0.06 tapered paper points (Roeko GmbH, Langenau, Germany). All root canals treatments were carried out by the same operator.

Subsequently, they were divided into three study groups: first group, the control group, containing two teeth, has not been treated with laser; the second group of 12 teeth was exposed to laser radiation, the Er:YAG laser, using the photon-initiated photoacoustic streaming (PIPS) fiber tip 300/14, at the following parameters: 50 ms pulse, 10 mJ, 10 Hz, variable square pulse (VSP) mode, 10 s, under continuous cooling with 2.5% NaOCl, followed by a resting time of 30 s; the protocol was repeated three times per each tooth (Figures 1 and 2). The third group (12 teeth) was treated with the same fiber tip, but at the following parameters: 50 ms pulse, 20 mJ, 15 Hz, super-short-pulse (SSP) 10–30 s duration, under 2.5% NaOCl irrigation; 30 s "resting time" between one cycle and the other (two cycle); at the end, 20 s irrigation with distilled water for neutralization was applied. The laser tip was introduced no further than 4 mm in the canal and was repeated four times per each tooth (Figure 3).

In order to irradiate the root canals after the conventional preparation, an Er:YAG laser with a wavelength of 2940 nm (Fidelis Plus III, Ljubljana, Slovenia) was used, with a cylindrical-conical shape PIPS, 400 μm in diameter at its apical end. During laser irradiation cycles, the root canals were irrigated continuously with distilled water under aspiration in order to maintain optimum hydration and cleaning.

All samples were then dried, sectioned and analyzed in scanning optical microscopy (FEI Inspect S in high-pressure mode; Department of Materials Science and Welding, Faculty of Mechanical Engineering, Politehnica University, Timişoara, Romania) in all three areas of the root canal: coronal third, middle third and apical third, monitoring the quality of dentinal walls debridement.

Results

In order to have a clearer evidence of the root canal changes in the debridement and morphology, these were analyzed in all three areas of interest, taking into account that, regardless of the technique used, it has limitations because of the complex anatomy of the endodontic space (Figure 4). The conventional technique has been shown to have an optimal decontamination and cleansing at the cervical level of the root canal, where the size and access are favorable, the effectiveness of this technique decreasing towards the apical third.

At the dentinal tubules level, at a magnification of 5000×, the differences between the non-smear tubules and those in which the smear layer is thick, covering the dentinal walls is evident (Figure 5). At 1000× magnification, with cross-section and lateral view, even the depth of debridement in the tubules can be approximated (Figure 6). The same magnification helps us to visualize areas of 50 μm, alternating between clear areas and thick smear layer (Figure 7).

By reducing the magnification to 800×, the electronic microscopy helps us to visualize the morphology of the secondary and lateral root canals (Figure 8), highlighting the differentially cleared areas: on a wall of the lateral canal, we observe areas of clean tubules, whilst the opposing wall is loaded with a thick smear layer, which suggests the uneven action of the technique used.

Figure 1 – ***Samples are fixed in a stable position using a special holder, simulating the intraoral position.***

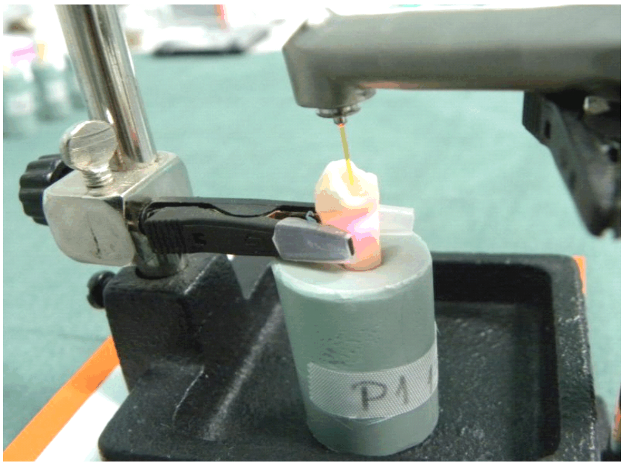

Figure 2 – ***Laser root canal irradiation of the samples.***

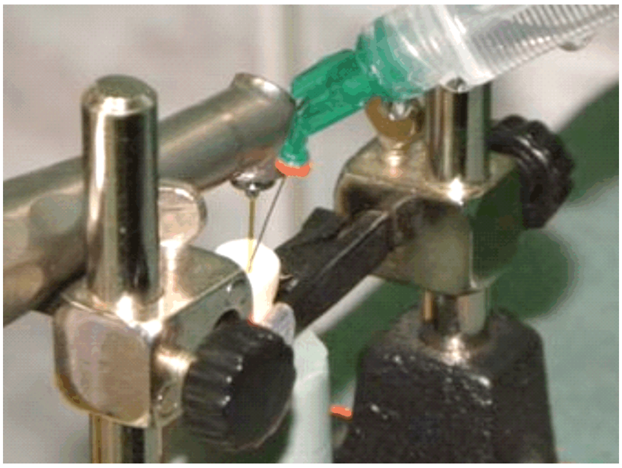

Figure 3 – ***The laser irradiation occurs under continuous cooling with 2.5% NaOCl, simultaneously with proper aspiration.***

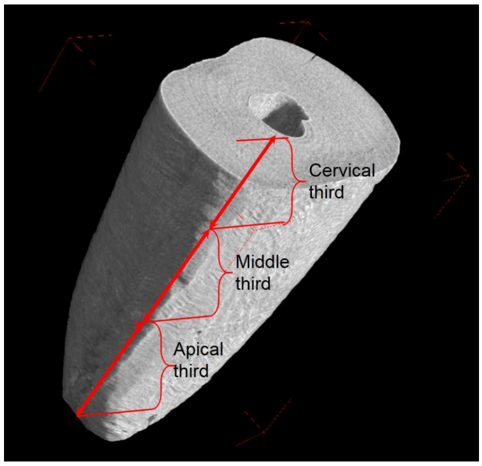

Figure 4 – ***Analysis of the root canal regarding all three parts: cervical third, middle third, apical third.***

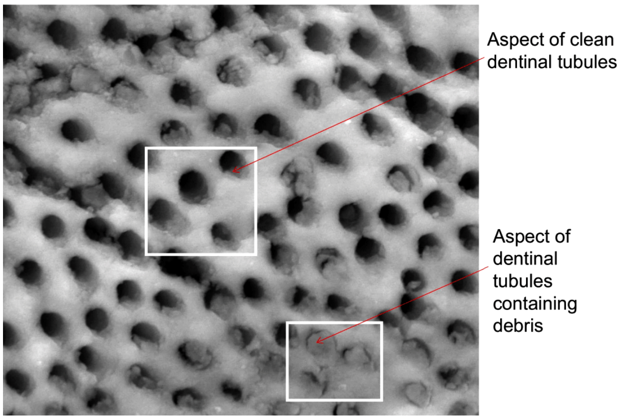

Figure 5 – ***Modification on morphological aspect of dentinal tubules in relation with the removal of debris (5000×).***

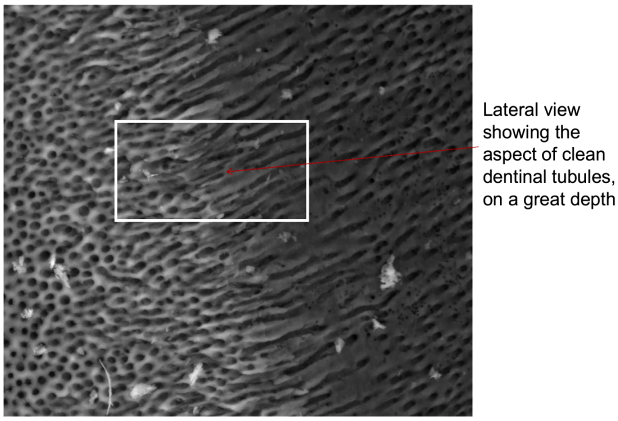

Figure 6 – ***Lateral view on dentinal tubules showing clean and open tubules on the surface of dentinal wall but also in the deeper areas (1000×).***

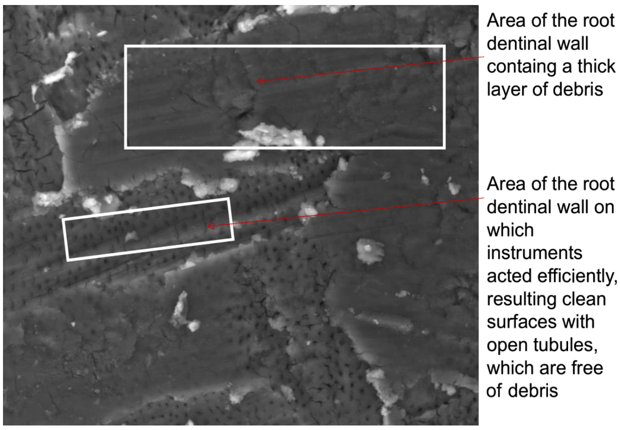

Figure 7 – ***Aspect of the dentinal tubules and smear layer (1000×).***

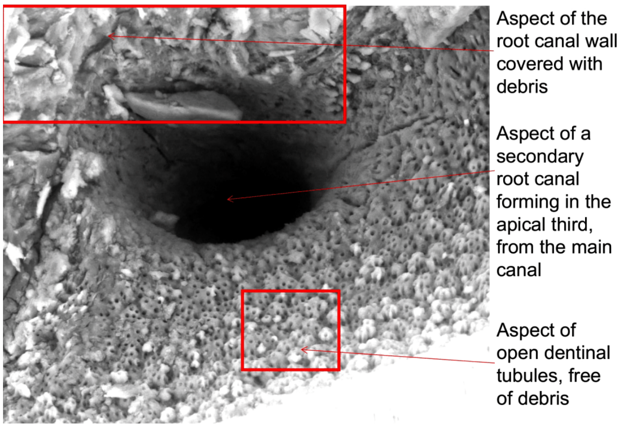

Figure 8 – ***Secondary canal and dentinal tubules view with identification of the debris inside (800×).***

Magnifications of 1000× and 2000× can also show areas where the action of the endodontic preparation techniques affects root canal morphology due to the use of incorrect tools, parameters, or techniques that can cause, for example, surface melting, narrowing dentinal tubules and determining a glassy surface (Figures 9 and 10).

In group 1, the control group (Figure 11) in which conventional preparation was used (without laser irradiation), it can be observed the presence of the smear layer in the apical third (Figure 11a) and the dentinal tubules partially opened in the middle area (Figure 11b) of the root canals. In the cervical third (Figure 11c), the debridement is improved, but there still is smear layer in the dentinal tubes.

In group 2 (Figure 12), which was irradiated with Er:YAG laser, using PIPS 300/14 at 10 mJ, 10 Hz, VSP mode, 10 s, in the apical third (Figure 12a) few dentinal tubules are still obstructed by the smear layer but in the middle third (Figure 12b) and the cervical third (Figure 12c) they can be observed clean dentinal areas with opened dentinal tubules.

In group 3, which was irradiated with Er:YAG laser, using PIPS 300/14 at 20 mJ, 15 Hz, VSP mode, 10 s, the SEM images highlight the improvement of root canal debridement in all three regions: in the apical area (Figure 13a) it can be observed that the majority of the dentinal tubules are without smear layer, while in the middle (Figure 13b) and cervical area (Figure 13c) the smear layer is absent (Figure 13). When assessing the morphology of the dentinal walls in the cervical part of the root canal, we have to take into consideration the position of the laser tip, which was inserted 4 μm into the root canal, thus its efficiency and the changes induced in the morphology will be mostly obvious at this level.

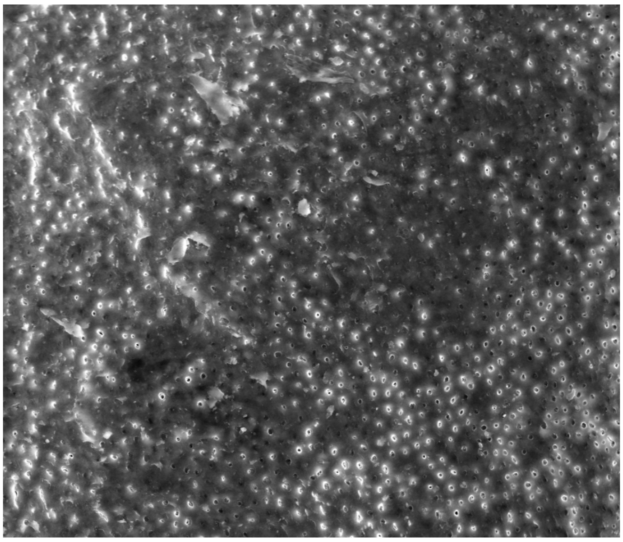

Figure 9 – ***Melted surface of the dentinal tubules after improper biomechanical treatment assisted by laser (1000×).***

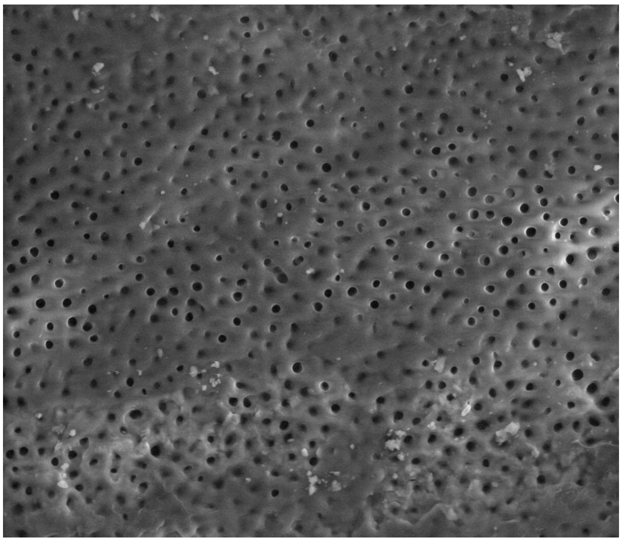

Figure 10 – ***Melted surface of the dentinal wall but most of dentin tubules are open and almost no debris are highlighted (2000×).***

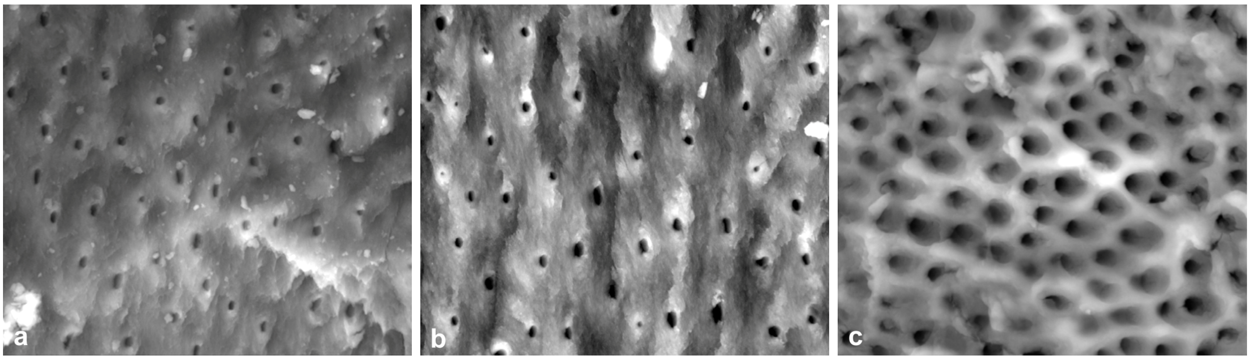

Figure 11 – ***SEM (×5000) of root canal dentin walls for group 1 (control group): (a) Apical third – thick layer of debris covering the tubules; (b) Medium third – narrow tubules with smear layer; (c) Cervical third – the majority of the tubules are clean, the smear layer is less represented. SEM: Scanning electron microscopy.***

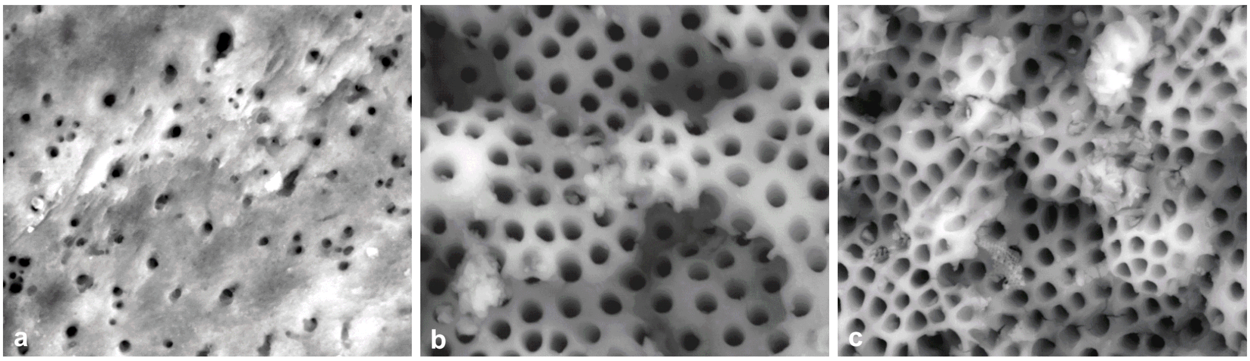

Figure 12 – ***SEM (×5000) of root canal dentin walls for group 2 (PIPS 300/14: 50 ms pulse, 10 mJ, 10 Hz, VSP mode, 10 s): (a) Some of the dentinal tubules are covered with debris – apical third; (b) The majority of the dentinal tubules are clean – medium third; (c) Clean surface and open dentinal tubules – cervical third. SEM: Scanning electron microscopy; PIPS: Photon-initiated photoacoustic streaming; VSP: Variable square pulse.***

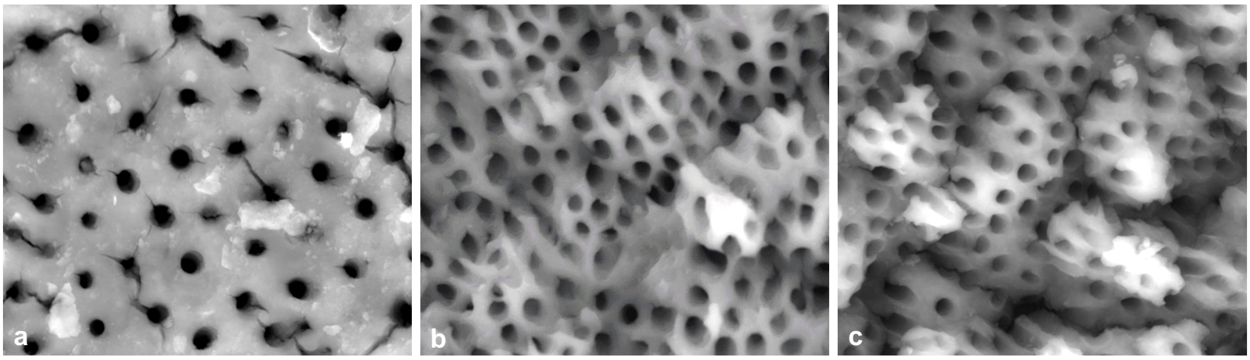

Figure 13 – ***SEM (×5000) of root canal dentin walls for group 3 (PIPS 300/14: 50 ms pulse 20 mJ, 15 Hz, VSP mode, 10 s) showing clean and open dentinal tubules in all three areas of interest: (a) Apical third; (b) Medium third – clean tubules; (c) Cervical third. SEM: Scanning electron microscopy; PIPS: Photon-initiated photoacoustic streaming; VSP: Variable square pulse.***

Discussions

Root canal therapy has to deal with some main issues, which are related to infection and microanatomy. These factors, which could influence the success of the endodontic treatment, are difficult to handle by means of conventional treatment, which is why scientists struggled to offer alternative methods and protocols in order to increase the prognosis of the treatment. One of the most important problems is the fact that a major part of the endodontic anatomy remains uninstrumented after conventional treatment and even the accessible parts of the root canals are covered in smear layer, which results as a by-product of the instrumentation and acts as a barrier for irrigants, medication and even jeopardizing the quality of the endodontic filling. A percentage of 35–53% of the root canal surface remains not treated by the instruments, thus the role of the irrigants is to compensate this issue [5–7].

These findings lead to the need for efficient irrigants, or association between different solutions having different effects. There is no irrigant that could fulfill all requirements, that is why the most common protocols use combination between EDTA and NaOCl. Another important aspect to be considered is that these irrigants need to reach all the endodontic anatomy in order to be effective and this purpose is difficult to be achieved through the conventional single needle irrigation. Research were conducted on different means to activate the movement of the irrigant inside the root canal anatomy by sonic, ultrasonic or laser activation.

Laser treatment has proven to be among the most effective tool in this respect, different wavelengths and different parameters being proposed to the clinicians: diode laser, Nd:YAG laser and Er:YAG laser are the three most common laser types which are being used in endodontics for activation of the irrigants in order to reach an increased area of the endodontic anatomy and also to be effective on smear layer debridement. Er:YAG has proven his superiority among different laser types in removing the smear layer [8, 9] and PIPS has shown better debridement than other geometries tips [10, 11].

Laser debridement necessitates a previous preparation of the root canals by using a conventional approach, because laser parameters used for disinfection do not have ablative effect on the dentine [12]. The antibacterial laser effect depends on the characteristics of the wavelength and on the energy used, and in most cases, also on the thermal effect, which produces some changes on the bacterial cellular wall, leading to osmotic changes and finally to cellular death. When laser irradiation penetrates the root canal dentine, the penetration depth depends on the parameters, such as wavelength and power density. Generally, the penetration depth decreases together with the increase of the absorption of the targeted tissue. The irradiation modes of the root canals, followed by the laser energy absorption of the dentinal surface represent important problems that need to be considered in laser-assisted debridement of the endodontic space. The fore mentioned aspects will influence the degree of tissue alteration in the dentine as well as the elimination of the bacterial biofilms inside the root canal system.

The more increased efficiency of Er:YAG laser is explained by its wavelength absorption in water and hydroxyapatite. In endodontics, Er:YAG laser energy is used in subablative mode. Thus, the debridement is superior to the one using Nd:YAG laser, the results being confirmed by studies of Takeda *et al.* [9]. Therefore, the observations of our study find their counterparts in earlier scientific researches, which in their majority have shown noticeable improvement debridement inside the root canals when using laser radiation. Moreover, the superiority of conical geometry and emission peaks side compared to previous models presenting cylindrical laser tips largely oriented toward the apical hole and to lesser extent the lateral walls of the root canals. We have considered this comparison in our previous studies, using two types of fiber tips – PIPS and Xpulse, showing different effects on the target tissue, as seen in the results section [2].

Even with all the clear advantages of the introduction of the laser technology in medicine, there are a number of precautions and side effects of laser radiation. One of these is related to the emission of laser energy to the tip of the optical fiber, which is directed essentially along the root canal and to a lesser degree to its sidewalls. Thus, it is difficult to obtain a uniform irradiation of the surface of the root canal by means of a laser [13, 14]. In addition, thermal effects on the periapical tissue may be possible, the safety of this procedure being however a limitation. Direct emission of laser radiation through an optical fiber tip near the apical foramen may lead to its transmission beyond the apical foramen, which could adversely affect periradicular-supporting tissues. This effect can be dangerous, especially if the foramens are located close to the mandibular foramen or canal [14]. These drawbacks being observed, a new tip was imagined and tested for Er:YAG laser. It allows laser emission to the sides, rather than direct emission through a single opening at the terminal end, as is the case with previous models. This new endodontic tip was designed to fit the shape and volume of root canals prepared by nickel–titanium rotary instruments. The purpose of this improvement is to enhance the antimicrobial effect of laser radiation in the dentinal tubules and lateral walls of the root canals.

Regarding the future of laser technology, a new area of interest for future research is the understanding of the interaction between irrigation and laser in root canal decontamination phase. This concept is the basis of LAI and the generation of currents initiated by PIPS in the disinfection of root canals [15–17]. The mechanism of interaction between Er,Cr:YSGG laser and liquid irrigants from the endodontic space has been attributed to efficient absorption of water by average wavelength radiation of the infrared spectrum. This leads to vaporization and bubble formation, which extend through the implosion and present side effects for the cavitation. This phenomenon is called cavitation and materializes itself through the outward movement of fluids in root canals. Thermal component in this interaction is considered to be moderate. The creation of the vapors is the same in both the sodium hypochlorite solution and water. If the fluid does not absorb the laser radiation, there is no vapor cavitation and no fluid movement. Using current knowledge in this respect, PIPS, one of the two tips used in this study, is based on direct shockwave generated by an Er:YAG laser in irrigating liquid. Laser system that develops this concept is equipped with a tip of 400 nm diameter, which at subablative parameters (average power 0.3 W, 20 mJ, at 15 Hz) has a photomechanical effect when light energy is pulsed at the level of the irrigant liquid. When activated in a limited volume of fluid due to absorption wavelength Er:YAG laser in water, with great power derived from the short duration of pulses (50 ms), photomechanical phenomenon results [15]. A recent study has investigated the bactericidal effect of Er:YAG laser combined with sodium hypochlorite irrigation against *Enterococcus faecalis* deep inside dentinal tubules in experimentally infected root canals and the conclusions was that all the Er:YAG laser irradiation protocols, combined with NaOCl irrigation, demonstrated effective bactericidal effects at all tested depths inside dentinal tubules [18].

These are future research directions that will bring additional scientific evidence in the large field of the use of laser technology in dentistry and in endodontics mostly.

Conclusions

Results emphasize the role of laser radiation to improve the quality of dentin smear layer debridement as a defining step in the endodontic treatment. We observed an increased efficiency in debridement of all interested areas when using the Er:YAG laser with PIPS tips. Using laser technology at the appropriate subablative parameters, the risk of side effects is minimized and debridement is both minimally invasive and superior to the conventional cleaning techniques. Laser radiation improves the endodontic treatment without any changes in the morphology of the root canals. Consequently, we obtain a dentin surface clean, disinfected and adequately prepared for the third stage of endodontic treatment, which is the three-dimensional sealing of the endodontic space.

Complete financial disclosure/Conflict of interest statement

The authors individually declare that they have no financial or other type of conflict of interest regarding the research or commercial products presented in this paper.

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