Laser irradiation during root canal treatment is efficient but in order to prevent irreversible thermal damage to dentine, cementum and other periapical structures continuous water cooling is required.

The aim of this study was to ultrastructurally analyze the surface of intracanal dentine and cementum at the root apex after Er:YAG laser irradiation with and without sterile saline solution irrigation.

The study involved 70 intact maxillary and mandibular single-rooted and two-rooted human teeth, divided into two experimental groups and one control group. In all teeth access cavity was created.

For root canal irradiation, Er:YAG laser with the wavelength of 2940 nm, energy output of 140 mJ per pulse, pulse duration of 125-160 ms, pulse repetition rate of 10 Hz and power of 15 W was used. Total irradiation time for each root canal was 10 seconds.

The ultrastructural analysis of intracanal dentine and the cementum of the root apex was done by using scanning electron microscope (JEOL JSM-5300 Tokyo Japan).

Intracanal Er:YAG laser irradiation without water cooling showed melted circum-pulpal dentine with large cracks in it, whereas cementum at the root apex was thermally modified in the form of plates and cracks.

Intracanal Er:YAG laser irradiation with continuous water cooling resulted in clean intracanal dentine surface without morphological changes of the cementum.

The canal system irradiation is acceptable and possible only when using constant water cooling; otherwise, irreversible thermal damage to intracanal dentine and cementum at the root apex occur. Acta Medica Medianae 2016;55(1):26-32.

Key words: laser, cementum, apex, ultrastructure, intracanal dentin
phosphatase. Instant water evaporation and a significant volume increase occur with the absorption of laser energy, resulting in the cracking of the dentine structure. External root resorption is also possible. The bone is more sensitive to thermal stress than periodontium because of its reduced vascularisation (13).

Still, there hasn’t been much data on possible thermal effects of laser beams on dentine tissue and the cementum of the root apex.

A successful root canal irradiation without consecutive changes on periodontal tissues implies an appropriate use of laser equipment. An inappropriate use can lead to thermal damage of all the tissues of endoperiodontal system and the soft tissues of the oral cavity as well.

The aim of this in vitro study was to examine the effects of Er:YAG laser irradiation on intracanal dentine and the cementum of the root apex, with and without sterile saline solution irrigation.

**Material and method**

The control group (C) consisted of 10 teeth. After removal of the root canal content, the samples were treated using a step back technique with #15-30 K-files and irrigated with sterile saline solution.

The first/A (I/A) experimental group of samples included 20 teeth, Er:YAG laser-irradiated without irrigation.

The first/B (I/B) experimental group of samples consisted of 20 teeth, Er:YAG laser-irradiated with constant sterile saline solution irrigation.

Er:YAG laser working protocol included intracanal Er:YAG laser irradiation with the wavelength of 2940 nm, energy output per pulse 140 mJ, pulse duration of 125-160 ms, pulse repetition rate of 10 Hz and power of 15W. Total irradiation time for each root canal was 10 seconds.

After intracanal laser irradiation, the crowns from the control, the I/A and the I/B group were cut off at the cemento-enamel junction by using diamond disc and continuous distilled water irrigation in order to remove superficial debris that occurred during cutting. Tooth roots were cut longitudinally into two equal segments - mesial and distal. Then, the samples were prepared for SEM evaluation. The coronal, middle and apical thirds of the root canal samples were examined. The quantitative evaluation of the smear layer after laser irradiation was done in accordance with the Hülsmann criteria (15).

The second/A (II/A) experimental group of samples consisted of 10 teeth, Er:YAG laser-irradiated without using sterile saline solution.

The second/B (II/B) experimental group of samples consisted of 10 teeth, Er:YAG laser-irradiated with continuous sterile saline solution irrigation.

Four-millimetre-long samples of the apical thirds from the II/A and the II/B group were transversely cut off with diamond disc and prepared for SEM evaluation in order to analyse the cementum at the root apex.

The SEM preparation included air-drying of the samples, their fixing to aluminium stubs with a fixing agent (Dotite paint xc 12 Carbon JEOL, Tokyo, Japan) and sputter-coating with gold/palladium (in the unit JFC 1100E Ion Sputter JEOL). The samples were then examined by scanning electron microscopy (SEM) (JEOL-JSM-5300).

**Results**

The obtained results are shown in figures 1-5 and tables 1 and 2.

Control group (C)

In the control group, after removal of the root canal content, the samples were treated using a step back technique with #15-30 K-files and irrigated with sterile saline solution.

In the coronal and the middle third of intracanal dentine, smaller particles of superficial debris adhered to the smear layer surface were present, whereas in the apical third of intracanal dentine larger debris particles were dominant. Dentine tubules were completely closed. In the I/A group of samples, after Er:YAG laser irradiation without irrigation, a glass-like and irregular surface of intracanal dentine was observed on all thirds of the root canals. Circum-pulpal dentine was melted and large cracks were observed. Irregular dentine structure was observed with the occurrence of micro-openings in the coronal third. The use of Er:YAG laser without irrigation also
showed specific, melted, lava-like areas in the middle third, and the apical third of intracanal dentine showed that intertubular dentine suffered more ablation than the peritubular dentine, leaving protruded, clearly exposed and completely closed dentinal tubules (Figure 2).

In the I/B group of samples, after Er:YAG laser irradiation with continuous irrigation using sterile saline solution, clean dentine surfaces with preserved structure were obtained. The openings of dentinal tubules were of regular shape and almost equal diameter. There were no signs of demineralisation (Figure 3).

The results of the II/A group refer to the analysis of the ultrastructural appearance of cementum at the root apex after Er:YAG laser irradiation without irrigation. The obtained results showed that cellular cementum was thermally modified with significant morphological changes. Cellular cementum was in the form of plates and cracks (Figure 4).

The results obtained in the II/B group showed that the apex structure and the morphology of cementum were preserved after Er:YAG laser irradiation with continuous irrigation using sterile saline solution. Numerous preserved cementocytes were observed within lacunae (Figure 5).

The values of the quantitative evaluation of dentinal debris and smear layer according to the Hülsmann criteria on the samples of the I/A group showed the presence of a high amount of non-homogenous smear layer covering the root canal of the quantitative evaluation of dentinal debris and smear layer according to the Hülsmann criteria on the samples of the I/B group that were Er:YAG laser-irradiated with continuous sterile saline solution irrigation showed a small amount of smear layer or the complete absence of it. Thirteen samples in total were scored 1 and seven samples were scored 2 (Table 1).

| Group  | N   | Irrigant                      | 5 | 4 | 3 | 2 | 1 |
|--------|-----|-------------------------------|---|---|---|---|---|
| I/A    | 20  | Er:YAG laser without irrigation| 20| 0 | 0 | 0 | 0 |
| I/B    | 20  | Er:YAG laser with sterile saline solution irrigation| 0 | 0 | 0 | 7 | 13 |
Comparing the values of the quantitative evaluation of dentinal debris and smear layer between the samples in the I/A and the I/B group by using Mann-Whitney U test, a statistically significant higher value in the I/A group in relation to the I/B group was determined (3.20 vs 1.50; p<0.001) (Table 2).

Table 2. The comparison of the quantitative analysis values of the dentinal debris and smear layer in the I/A and the I/B group of samples: Mann-Whitney U test

|     | N  | SD | Min | Max | Z     | p     |
|-----|----|----|-----|-----|-------|-------|
| I/A | 20 | 3.20 | 1.88 | 5.00 | -4.119 | <0.001 |
| I/B | 20 | 1.50 | 0.51 | 2.00 |       |       |

Discussion

The first attempts at removing the smear layer from the canal system using Excimer laser were made by Liesenhoff et al. (16), whereas Gordon et al. (17) compared the effects of pulse and continuous wave of 1.06 µm Nd:YAG laser wavelength.

Marchida et al. (18) succeeded in completely removing the smear layer and debris on intracanal dentine walls of the extracted teeth by using KTP:YAG laser.

Blum and Abadie et al. (19) evaluated the cleanliness of intracanal dentine obtained by five different preparation techniques and they discovered that the combined use of ultrasonic preparation and laser showed the cleanest structure of intracanal dentine with open dentinal tubules and very little debris.

Er:YAG laser efficiency in removing the smear layer was also reported by Kesler et al. (20), who explained the elimination of smear layer by the high coefficient of water absorption and minimal thermal energy release, especially when using continuous water cooling.

Harashima et al. and Takeda et al. (21) demonstrated the relation between the smear layer removal and energy emitted by a laser. They reported the melting of dentine when using high laser energy. In another study, Takeda et al. (12) evaluated the effects of three different endodontic irrigants (17% EDTA, 6% phosphoric acid i 6% citric acid) and two types of laser (carbon dioxide and Er:YAG) on the smear layer in the middle and the apical third of the root canal in vitro. The highest efficiency in removing the smear layer was reported after Er:YAG laser irradiation had been applied. Lower efficiency was shown by a CO2 laser, whereas the use of the three irrigants did not result in a complete smear layer removal, even the erosive demineralisation of intertubular dentine occurred. Intracanal Er: YAG laser irrigation with water cooling efficiently removed the smear layer without destructive effects and den-tine recrystallisation. The CO2 laser removed the smear layer by melting but Er: YAG laser was by far the most efficient (22, 23).

The bactericidal effect of Er:YAG laser is proportional to the applied input laser power but it does not provide the complete sterilisation of the canal system (13).

The main problem when using lasers for hard dental tissue cutting is heat production. Lasers applied in endodontics lead to the evaporation of organic tissue in the root canal (20). Thermal energy release during laser endodontic treatment depends on the laser type, power level, device calibration, duration of a laser treatment, fiberoptic system of laser beam transfer, the presence of water cooling and the type of tissue that is being irradiated (23, 24).

Apart from the thermal effect, the anatomical complexity of the root canal systems and the apical curvatures that reduce the laser efficiency should also be considered, and that requires technical improvement of the laser. The complete cleaning of all the surfaces of the intracanal system with a laser is made difficult in curved and narrow root canals since a laser beam is emitted in a straight line (12, 20, 25-27). Thermal side effects of modern laser devices are significantly reduced but not completely eliminated.

An increase in thermal energy during endodontic treatment can have destructive effects on the dentine structure and the tissues of periodontal system. An increase of 10 degrees per minute is considered harmless for periodontal tissue. The bone is more sensitive to thermal lesions than periodontium. Operators should be careful during laser procedure since the temperature rises, and in some cases is higher than 100 C. For this reason, the use of lower energy parameters and shorter periods of laser procedures in clinical conditions are suggested in order to preserve periodontal tissue (28-30).

Thermal effects of laser irradiation on cementum are less known. Due to possible thermal lesions in the cementum and other periodontal structures, temperature of the root surface before the experimental treatment of the root canal should be determined (28).

Laser irradiation, due to an increase in thermal energy during endodontic treatment can have destructive effects on the dentine structure, cementum and the tissues of periodontal system as well. It has been considered that the temperature transferred through dentine and cementum causes enzyme denaturation, especially alkaline phosphatase with the occurrence of external root resorption (28).

The results of the ultrastructural appearance of the cementum structure at the root apex after Er:YAG laser irradiation without water cooling showed thermally modified cellular cementum without its recognisable morphological characteristics. The cellular cementum was in the form of plates and cracks. These findings confirmed that inadequately applied laser treatment in the root canal resulted in consecutive changes on cementum at the root apex.
On the other hand, the results of the ultrastructural examination of the appearance of the cementum structure at the root apex after Er:YAG laser irradiation with continuous sterile saline solution irrigation showed preserved cementum morphology and structure. Lacunae with numerous preserved cementocytes were observed.

These results showed that the use of lasers in the sterilisation of the canal system is desirable and possible but under strictly determined conditions and with continuous water cooling.

Lasers with lower energy parameters and shorter periods of laser procedures with constant water cooling should be used in clinical conditions in order to preserve the tissues of endoperiodontal system.

Blum et al. (19) evaluated the cleanliness of intracanal dentine obtained by five different preparation techniques and they discovered that the combined use of ultrasonic preparation and laser irradiation showed the cleanest structure of intracanal dentine with open dentinal tubules and very little debris, which is in correlation with the results of this study.

The results of this research showed that laser irradiation without water cooling thermally modified the structure of intracanal dentine and cementum at the root apex in endodontic procedure. In all the thirds of the root canal, the glass-like and irregular surface of intracanal dentine was observed, whereas circumpulpal dentine was melted and with large cracks. The use of Er:YAG laser irradiation without water cooling also showed specific melted, lava-like areas (middle third), and the apical third showed that intertubular dentine suffered more ablation than peritubular dentine, leaving protruded, clearly exposed and completely closed dentinal tubules, which was in accordance with literature data (8-10, 26, 27).

Intracanal Er:YAG laser irradiation with continuous sterile saline solution irrigation resulted in clean dentine surface with preserved structure. The openings of dental tubules were of regular shape and almost equal diameter. There were no signs of demineralisation.

Conclusion

Er:YAG laser irradiation without water cooling results in carbonisation, lava-like melting, the occurrence of large cracks in intracanal dentine and the thermal modification of cellular cementum in the form of plates and cracks.

Intracanal Er:YAG laser irradiation with water cooling efficiently removes smear layer without damage and dentine recrystallisation, while the morphology and structure of cementum remain unchanged.

Using laser irradiation in the sterilisation of the canal system is desirable and possible but under strictly determined conditions and with constant water cooling.

References

1. Matuse T, Shirakami T, Ozaki K, Nakanishi T, Yumoto H, Ebisu S. An immunohistological study of the localization of bacteria invading root pulpal walls of teeth with periapical lesions. J Endod 2003; 29: 194-202. [CrossRef][PubMed]
2. Zivkovic S, Brkanic T, Dacic D, Opacic V, Pavlovic V. Smear layer in endodontics. Serb Dent J 2005; 52: 7-19. [CrossRef]
3. Zivkovic S, Kolar M, Blazic L, Vucetic M, Toscic T. Smear layer on dentin in Restorative Dentistry. Serb Dent J 2004; 51: 169-76. [CrossRef]
4. Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. J Endod 1990; 16: 566-9. [CrossRef]
5. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of different concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endod 2003; 29: 233-9. [CrossRef][PubMed]
6. Mitic A, Mitić N, Muratovska I, Stojanovska V, Popovska L, Mitic V. Ultrastructural investigation of root canal dentine surface after application of active ultrasonic method. Serb Arch Med 2008; 5-6: 226-31. [CrossRef]
7. Gutknecht N, Raoufi P, Franzen R, Lampert F. Reduction of specific microorganisms in periodontal pockets with the aid of an Nd:YAG laser-an in vivo study. J Oral Laser Applications 2002; 2: 175-479.
8. Mitic A. Ultrastructural research of the dentine surface of the tooth root canal after the appliance of chemomechanical, ultrasound and laser technique [dissertation]. Nis (Serbia): Faculty of Medicine, University of Nis; 2009.
9. Mitic A, Mitic N, Zivkovic S, Toscic G, Savic V, Dacic S, Stojanovic M. Efficiency of final irrigation of root canal in removal of smear layer. Serb Arch Med 2009; 137: 482-9. [CrossRef]
10. Stevanovic M, Petrovska M, Stevanovic M, Mirceva M. Bactericidal effects of Er:YAG laser irradiation on root canals. J Oral Laser Applications 2004; 4: 43-6.
11. Mehl A, Folwaczny M, Haffner C, Hickel R. Bactericidal effects of 2.94 microns Er:YAG –laser radiation in dental root canals. J Endod 1999; 25: 490-3. [CrossRef]
12. Takeda FH, Harashima T, Kimura Y, Matsumoto K. A comparative study of the removal of the smear layer by three endodontic irrigants and two types of laser. Int Endod J 1999; 32: 32-9. [CrossRef][PubMed]
13. Jukic S, Miletic I, Bozic Z, Anic J, Smoljak D, Kalenic S. Antibacterial effects of Nd:YAG laser in root canal samples: in vitro study. Acta Clin Croat 2004; 43: 3-7.
14. Kimura Y, Wilder-Smith P, Matsumoto K. Lasers in endodontics: a review. Int Endod J 2000; 33: 173-85. [CrossRef][PubMed]
for endodontic disinfection. J Endod 1999; 25: 105-8. [CrossRef]

24. Fegan SE, Steiman HR. Comparative evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation: an in vitro study. J Endod 1995; 21: 415-7. [CrossRef]

25. Moshonov J, Ørstavik D, Yamauchi S, Pettiette M, Trope M. Nd:YAG laser irradiation in root canal disinfection. Endod Dent Traumatol 1995; 11: 220-4. [CrossRef][PubMed]

26. Schoop U, Moritz A, Kluger W, et al. The Er:YAG laser in endodontics: results of an in vitro study. Laser Surg Med 2002; 30: 360-4. [CrossRef][PubMed]

27. Matsumoto K, Hossain M, Tsuzuki N, Hessain M, Tsuzuki N, Yamada Y. Morphological and compositional changes of human dentin after Er:YAG laser irradiation. J Oral Laser Applications 2003; 3: 15-20.

28. Khabbaz MG, Makropoulou MI, Serafetines AA, Papadopoulos D, Papagiakoumou E. Q-switched versus free running Er:YAG laser efficacy on the root canal walls of human teeth: a SEM study. J Endod 2004; 30: 585-8. [CrossRef][PubMed]

29. Matuset, Shikrakami T, Ozaki K, Nakanishi T, Yumoto H, Ebisu S. An immunohistological study of the localization of bacteria invading root pulpal walls of teeth with periapical lesions. J Endod 2003; 29: 194-202. [CrossRef][PubMed]

30. Berkiten M, Berkiten R, Okar I. Comparative evaluation of antibacterial effects of Nd:YAG laser irradiation in root canals and dentin tubules. J Endod 2000; 5: 268-70. [CrossRef][PubMed]
EFEKTI LASERO-TERAPIJE NA INTRAKANALNI DENTIN I CEMENTNO TKIVOAPEKSA KORENA ZUBA

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Obrada kanala korena zuba laserom je efikasna, ali zahteva obavezno hlađenje vodom kako bi se sprečila ireverzibilna termička oštećenja dentina, cementa i ostalih peri-apeksnih struktura.
Cilj ovog rada bio je da se ultrastrukturno analizira površina intrakanalnog dentina i cementnog tkiva na apeksu korena zuba posle obrade kanala Er:YAG laserom bez irigacije i sa irigacijom sterilnim fiziološkim rastvorom.

Korišćeno je 70 intaktnih maksilarnih i mandibularnih jednokorenih i dvokorenih humanih zuba, podeljenih u dve eksperimentalne i jednu kontrolnu grupu. Kod svih zuba je formiran pristupni kavitet.
Za obradu kanala korišćen je Er:YAG laser talasne dužine 2940 nm, izlazne energije za impuls 140 mJ; u pulsnom režimu 125-160ms, stopom pulsne repeticije od 10 Hz i energijom od 15 W. Vreme obrade svakog kanala laserom iznosilo je 10 sekundi.

Ultrastrukturna analiza intrakanalnog dentina i cementnog tkiva na apeksnom delu korena zuba urađena je na skening elektronskom mikroskopu (JEOL JSM -5300 Tokyo Japan).

Intrakanalna obrada Er:YAG laserom bez vodenog hlađenja pokazuje stopljen cirkumpulparni dentin sa velikim pukotinama u dentinu, a cementno tkivo na apeksu je termički modifikovano u vidu ploča i pukotina.

Intrakanalna obrada Er:YAG laserom uz kontinuirano vodeno hlađenje daje čistu intrakanalnu dentinsku površinu bez morfoloških promena cementnog tkiva.

Obrada kanalnog sistema zuba je opravdana i moguća samo u uslovima stalnog hlađenja vodom, u protivnom nastaju ireverzibilna termička oštećenja intrakanalnog dentina i cementa na apeksu korena zuba. Acta Medica Medianae 2016;55(1):26-32.

Ključne reči: laser, cement, apeks, ultrastruktura, intrakanalni dentin