Effects of Erbium:Yttrium–Aluminum–Garnet Laser Irradiation on Bovine Dentin Contaminated by Cariogenic Bacteria

Qing Du, PhD,[1] Lihong Ge, PhD,[1] Sun Zhang, MD,[1] and Qian Zhang, MD[2]

Abstract

Objective: This study was performed to determine the bactericidal effects of erbium:yttrium–aluminum–garnet (Er:YAG) laser irradiation and the morphological and chemical composition changes in bovine dentin.

Methods: Dentin slabs were prepared from bovine incisors, and then cultured with *Streptococcus mutans* to produce bacteria-infected dentin samples. The samples were randomly divided into five groups with Er:YAG laser irradiation energy densities of 0, 6.37, 12.73, 19.11, and 25.47 J/cm^2. After irradiation, samples were stained and observed by confocal laser scanning microscopy. The bactericidal abilities were measured using live/dead staining. The morphology and chemical components were investigated by scanning electron microscopy and energy-dispersive spectrometry.

Results: After irradiation, the elimination of bacteria and the smear layer were significantly better in the high energy density groups (19.11, 25.47 J/cm^2) than in the low energy density groups (6.37, 12.73 J/cm^2; $p < 0.001$). On morphological examination, the group with minimum energy density (6.37 J/cm^2) showed superficial melting. In the high energy density groups (12.73, 19.11, and 25.47 J/cm^2), laser-irradiated dentin showed a clean surface with open orifices. Significant increases were observed in the weight percentages of calcium (from 19.75 ± 0.69 to 34.47 ± 2.91, $p < 0.001$) and phosphate (from 8.58 ± 0.43 to 15.10 ± 1.81, $p < 0.001$), whereas significant decreases were observed for oxygen (from 49.84 ± 0.69 to 36.39 ± 2.86, $p < 0.001$) and carbon (from 26.06 ± 3.58 to 12.80 ± 2.26, $p < 0.01$) with increasing energy density.

Conclusions: This study confirmed that Er:YAG laser irradiation has bactericidal and dentin conditioning effects.

Keywords: laser, caries, dentin, bactericidal effect

Introduction

DENTAL CARIES IS a widespread chronic disease that results in progressive damage to the teeth caused by bacteria.[1] According to the results of the Fourth National Epidemiological Survey of Oral Health in China, the prevalence rates of caries in children aged 5 and 12 years have increased by 5.8–70.9% and by 7.8–34.5%, respectively, compared with 10 years ago.[2] Worldwide, dental caries constitutes a huge disease burden on human health.[3]

In clinical practice, the conventional method of caries removal involves use of a high-speed handpiece. However, this does not guarantee cleaning of the infected dentin and removal of residual bacteria.[4] Novel minimally invasive dentistry techniques have been investigated.[5] In 1997, the erbium:yttrium–aluminum–garnet (Er:YAG) laser was the first dental laser to be approved by the US Food and Drug Administration for treating dental hard tissue ablation.[6] This method has become a feasible substitute for mechanical caries removal using a handpiece in pediatric dentistry,[7] as it produces less noise and no vibration thus making it more acceptable for use in young children.[8]

The Er:YAG laser has an emission wavelength of 2940 nm, which corresponds to the absorption peak of water in process of the wavelength' interaction with hard tissue.[9] Dentin contains 70% inorganic hydroxyapatite crystals, 20% organic matter, and 10% water by weight.[10,11] Caries of the dentin involves demineralization followed by bacterial invasion.[12] Bacterial acidification not only induces demineralization and exposure of the organic matrix in the dentin

[1]Department of Pediatric Dentistry, Peking University School and Hospital of Stomatology, Beijing, China.
[2]Central Laboratory, Peking University School and Hospital of Stomatology, Beijing, China.

surface, but also results in activation of dentin-embedded and salivary matrix metalloproteinases and cathepsins.[13] Because carious tissue has a higher water content than healthy tissue, it shows higher Er:YAG laser energy absorption.[14] The Er:YAG laser has potential for the selective removal of carious tissue, and can be used in minimally invasive treatment of dental caries.[15] In addition, Er:YAG laser irradiation has bactericidal effects during cavity preparation,[16] and is strongly absorbed by water inside bacteria, causing physical breakdown of the bacterial cell structure.[17]

Most studies of the bactericidal effects of the Er:YAG laser have focused on the root canal, and tested lasers against *Enterococcus faecalis* and *Escherichia coli*.[18,19] Previous studies have shown that the Er:YAG laser is the most appropriate laser for intracanal debris and smear removal.[20–22] Nevertheless, there have been few studies on the bactericidal effects of Er:YAG laser irradiation on cariogenic *Streptococcus mutans*.

Er:YAG laser irradiation can be used to modify the chemical compositions of enamel and dentin, which has been investigated as a means of preventing or reducing the tooth demineralization that occurs in dental caries.[23,24] Er:YAG laser irradiation ($1.2 J/cm^2$, $10 Hz$) significantly prevents demineralization by decreasing solubility of the enamel.[25,26] The level of carbonate influences the susceptibility of the enamel to demineralization.[27] However, most studies have focused on the enamel, and the clinical impact and mechanism of action on dentin remain largely unknown.[28] Considering the differences in composition between enamel and dentin, it is necessary to determine the effects of Er:YAG laser irradiation on dentin.

This study examined whether Er:YAG laser irradiation can kill the bacteria within dentin, and lead to changes in the morphology and chemical composition of contaminated bovine dentin.

Materials and Methods

Sample preparation

Incisors from freshly slaughtered cattle without any previous lesions were obtained from a local abattoir. After washing with distilled water, the crown was cut and midcoronal dentin disks $\sim 1 mm$ thick (average area, $10 \times 10 mm^2$) were obtained. Twenty-seven dentin specimens were prepared and stored in physiological saline at $4°C$ until use.

Microbial culture

Before the experiment, dentin disks were ultrasonicated for 30 min and autoclaved at $121°C$ for 20 min in distilled water. *S. mutans* (UA 159) was chosen as a standard cariogenic bacterial species.[29] The bacteria were cultivated in brain heart infusion (BHI) broth (Difco, Detroit, MI) at $37°C$ for 24 h. Then the culture was adjusted to 10^8 colony-forming unit/mL with an optical density of 0.3 based on preliminary experiments. Dentin slabs were incubated in 1 mL bacterial suspension for 2 days ($37°C$, $5\% CO_2$). After coculture, the dentin specimens were transferred to fresh medium and immersed in liquid BHI broth for 5 days. The medium was changed daily.

Laser application

After a 7-day experimental period, the biofilm formed over the slabs was removed with a sterile cotton rod to expose the carious dentin. A commercially available pulsed Er:YAG laser (LiteTouch, Syneron, Israel) was used. The irradiation pattern was designed to simulate clinical conditions with manual operation. The specimens were irradiated from the surface in close contact mode under constant scanning movement (1 mm/sec), with a water spray level of 8. A straight sapphire tip, 1 mm in diameter, was placed at an angle of 15° to the vertical axis of the slab. The laser tip was maintained at a distance of 1 mm from the surface and the irradiation area was $10 mm.^2$ Table 1 shows the laser parameters used in each group. Each group consisted of five samples. Group 1 had no laser energy output as a control, but was handled with water and air at the same levels. After irradiation, the samples were immediately placed in phosphate-buffered saline (PBS; Solarbio, Beijing, China) to keep the bacteria alive. To confirm that the slab was not contaminated by other bacteria, two slabs were only exposed to noninoculated BHI broth and saline separately. As expected, no bacterial growth was observed.

Bacteriological evaluation

After irradiation, the dentin slabs were stained with LIVE/DEAD® BacLight Bacterial Viability Kits (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's instructions. This kit includes component A (SYTO 9® dye), which stains live bacteria with a green pigment, and component B (propidium iodine dye), which stains dead bacteria with red pigment, thus enabling easy identification of viable bacteria. In this study, $0.5 \mu L$ component A and $0.5 \mu L$ component B were mixed in 1 mL PBS. The sample surface was dried with sterilized cotton, and then samples were placed in 12-well plates (Corning, Corning, NY), with each well containing $100 \mu L$ mixed dye. After incubation at room temperature in the dark for 15 min, the slabs were eluted for 30 min. Images obtained by confocal laser scanning microscopy (CLSM, LSM 5 EXCITER; Carl Zeiss, Oberkochen, Germany). Five random images were taken of each sample at the center of the irradiation area at a $20 \times$ magnification. In total, 25 images were taken for each group. Standard images were obtained using 30 sections with a step size of $1 \mu m$, in a format of $1024 \times \times 1024$ pixels. The images were fragmented into many layers and converted into TIFF format. These images were exported to ZEN Blue software (ver.1.1.1; Carl Zeiss) to quantify the amounts of live (green) and dead (red) bacteria. Each layer was evaluated

TABLE 1. ER:YAG LASER PARAMETERS USED IN EACH GROUP

	Output power	Energy density (J/cm^2)
Group 1	0	0
Group 2	0.5	6.37
Group 3	1.0	12.73
Group 4	1.5	19.11
Group 5	2.0	25.47

Each group consisted of five samples. Group 1: with no laser energy output, but handled with water and air at the same level.

separately and the bacterial viability data were obtained to evaluate the bactericidal effects by measuring the red to green fluorescence area ratio in each slab.

Scanning electron microscopy and energy-dispersive spectrometry observations

After CLSM observation, slabs were preserved in distilled water and sent to the University Analytical Instrumentation Center, Peking University (Beijing, China), for morphological and structural analyses. The protocol for sample preparation was as previously described.[30] The specimens were dehydrated in the molecule pump inside the scanning electron microscope (Bruker Nano, Bremen, Germany). However, the samples were not sputter coated with gold to preserve their innate characteristics. The morphology of the dentin surfaces was observed by scanning electron microscopy (SEM) at an acceleration voltage of 10 kV, under $500\times$, $2000\times$, and $5000\times$ magnifications. Five random images were taken of each sample at the center of the irradiation area. Energy-dispersive spectrometry (EDS; EDS QUAN-TAX; Bruker, Billerica, MA) analyses were performed in the same area using an X-ray detector system attached to the scanning electron microscope. The carbon, oxygen, calcium, and phosphate contents, as well as the Ca (mol)/P (mol) ratio, and C (mol)/O (mol) ratio were calculated. The stoichiometric Ca/P ratios and C/O ratios were calculated using the following formula, considering the respective atomic masses: Ca (mol)/P (mol) = [Ca (wt%)/40.08 (g/mol)]/[P (wt%)/30.97 (g/mol)]; C (mol)/O (mol) = [C (wt%)/12.00 (g/mol)]/[O (wt%)/15.99 (g/mol)]. A total of 25 images and corresponding data were obtained for each group.

Statistical analyses

Based on CLSM images, the quantities of carbon, oxygen, calcium, and phosphate, as well as the Ca (mol)/P (mol) ratio and the C (mol)/O (mol) ratio according to EDS, were calculated and analyzed. The Kolmogorov–Smirnov test confirmed the normality of the data. One-way analysis of variance was performed to detect significant differences among the groups. Statistical analyses were performed using SPSS software (ver. 20.0; SPSS, Inc., Chicago, IL). In all of the analyses, $p < 0.05$ was taken to indicate statistical significance.

Results

Bacteriological evaluation

CLSM observation and live/dead staining allowed us to distinguish live and dead bacteria in dentinal tubules. Green (vital) and red (nonvital) fluorescence were visible within the tubules of infected dentin slabs (Fig. 1). The red to green fluorescence area ratio can be used to measure the bactericidal effect after irradiation. The means and standard deviations of the red to green fluorescence area ratios were determined for each slab and are shown in Table 2. The area ratios significantly increased with increasing energy density (from 0.33 ± 0.08 to 123.48 ± 11.71, $p < 0.001$). In Group 1 without laser energy output, most bacteria were alive and showed green fluorescence (Fig. 1a). In Groups 2 and 3 (6.37 and 12.73 J/cm², respectively), the red to green fluorescence area ratios were increased (from 0.58 ± 0.17 to
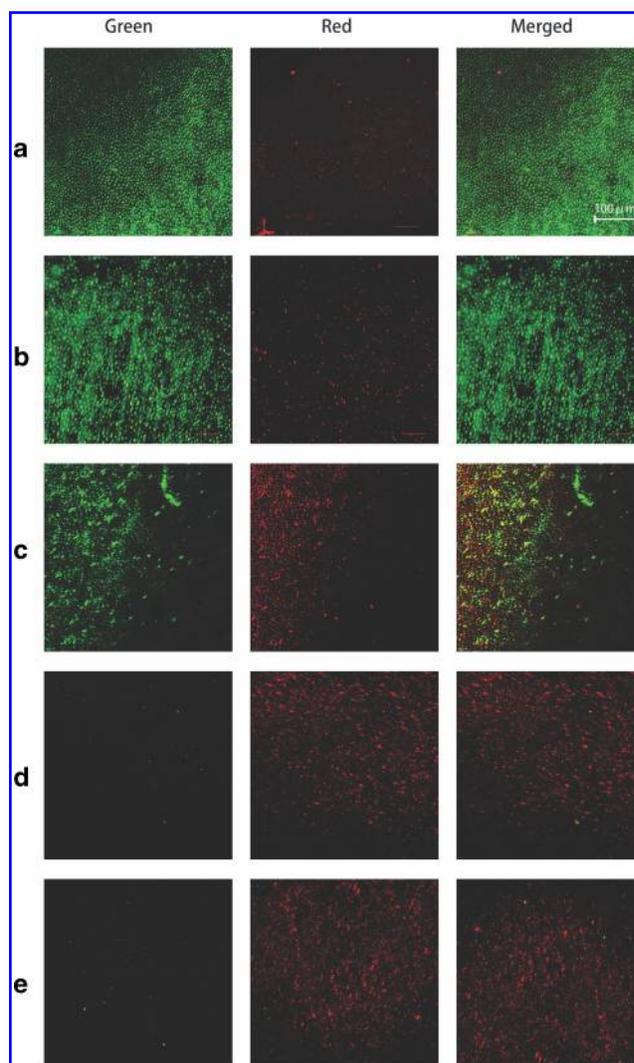

FIG. 1. Confocal laser microscopy analysis of the distributions of vital and nonvital bacteria. **(a)** Dentin contaminated by *Streptococcus mutans* after treatment with Er:YAG laser irradiation at energy density 0 J/cm². **(b)** 6.37 J/cm². **(c)** 12.73 J/cm². **(d)** 19.11 J/cm². **(e)** 25.47 J/cm². Columns (red) and (green) show the same sections, visualizing live and dead bacteria. Column (merged) shows composite images superimposing red and green fluorescence.

1.12 ± 0.31) as red fluorescence area increased (Fig. 1b, c), indicating the increasing bactericidal capability. Groups 4 and 5 (19.11 and 25.47 J/cm², respectively) showed mostly red fluorescence with little green fluorescence (Fig. 1d, e); the red to green fluorescence area ratio was >10, and live bacteria were rarely observed with the extent of bacterial reduction reaching almost 100%.

Morphological analyses

Under SEM observation, normal bovine dentin slabs had a smooth surface with a little smear layer (Fig. 2a). In Group 1, however, the dentin surface was rougher with a heavy smear layer on the slab surface after contamination (Fig. 2b), while the orifice could still be distinguished. In Group 2, superficial melting occurred and partial closure of

TABLE 2. THE RED-TO-GREEN FLUORESCENCE AREA RATIO (MEAN ± STANDARD DEVIATION) OF DENTINE

	Group 1	Group 2	Group 3	Group 4	Group 5
Ratio	0.33 ± 0.08^a	0.58 ± 0.17^a	1.12 ± 0.31^a	16.78 ± 3.88^b	123.48 ± 11.71^c

[a–c]Data with different lowercase superscript letters indicate a significant difference between randomly two groups ($p < 0.001$).

dentin tubules was observed (Fig. 2c). Laser-irradiated dentin in Groups 3–5 showed a clean surface and the absence of a smear layer or debris with open orifices. The orifices were widened and peritubular dentin protruded (Fig. 2d–f). No cracks or destructive appearance was observed in any of the groups.

Chemical composition analyses

EDS is a semiquantitative method for analyzing elements. Thus, the results are the relative contents of elements. The weight percentages of carbon, oxygen, calcium, and phosphate are presented in Fig. 3. Compared with Group 1, significant increases were observed in the weight percentages of calcium (from 19.75 ± 0.69 to 34.47 ± 2.91, $p < 0.001$) and phosphate (from 8.58 ± 0.43 to 15.10 ± 1.81, $p < 0.001$), whereas significant decreases were observed in

oxygen (from 49.84 ± 0.69 to 36.39 ± 2.86, $p < 0.001$) and carbon (from 26.06 ± 3.58 to 12.80 ± 2.26, $p < 0.01$) in Groups 2–5 with increasing energy density. In Groups 2–5, the C (mol)/O (mol) ratio significantly decreased (from 0.70 ± 0.10 to 0.47 ± 0.06, $p < 0.05$) compared with Group 1, whereas there were no differences in the Ca (mol)/P (mol) ratio after irradiation (95% confidence interval: 1.72–1.87, $p = 0.324$).

Discussion

The interaction of laser light with dental hard tissues is determined by the parameters of irradiation such as the wavelength, repetition rate, pulse energy, duration of exposure, and optical properties of the tissue.[27] The appropriate Er:YAG laser irradiation parameters for achieving antimicrobial effects in carious dentin have not been
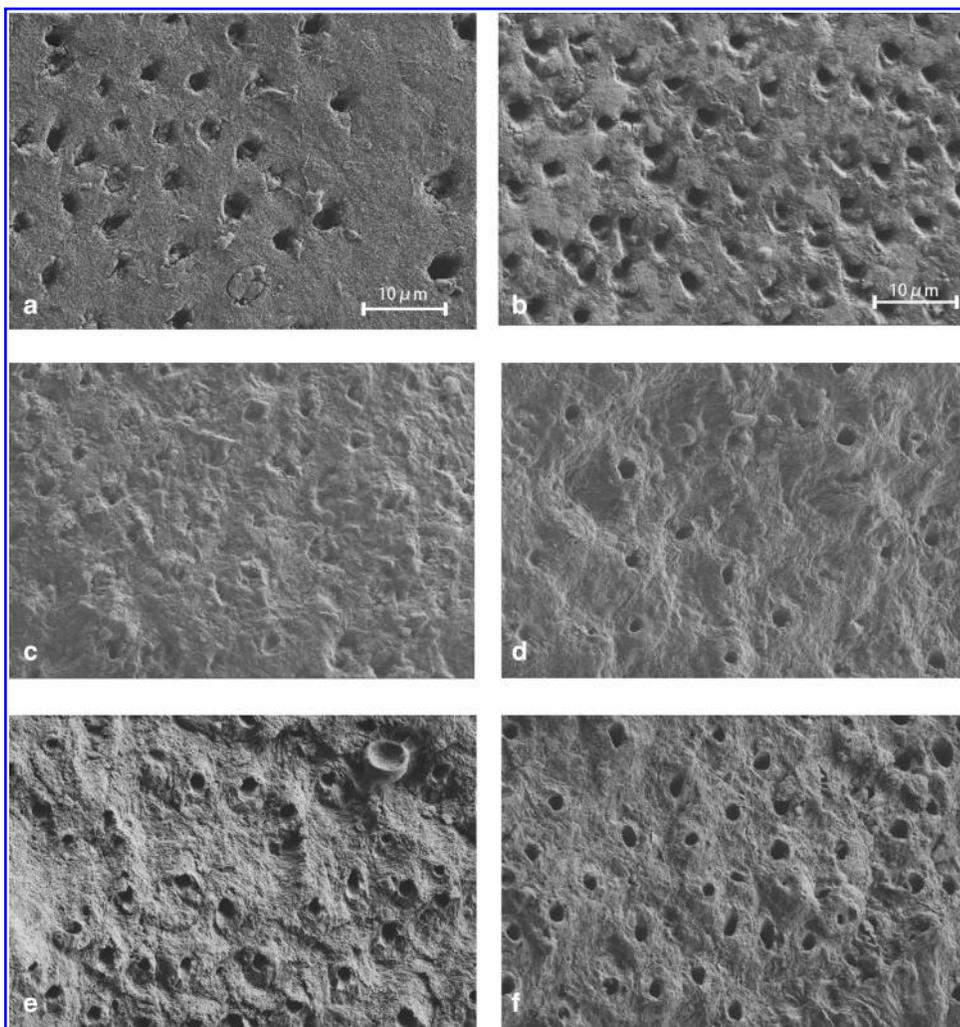

FIG. 2. SEM images of dentine surface after Er:YAG laser irradiation (magnification ×2000). **(a)** The normal bovine dentin slab without bacteria contamination and Er:YAG laser irradiation had a little smear layers. **(b)** Er:YAG laser irradiation at energy density $0 J/cm^2$: a heavy smear layer on the slab surface. **(c)** Er:YAG laser irradiation at energy density $6.37 J/cm^2$: surface melting occurred. **(d–f)** Er:YAG laser irradiation at energy density: 12.73, 19.11, 25.47 J/cm^2: a clean surface and the absence of a smear layer or debris with open orifices. SEM, scanning electron microscopy.
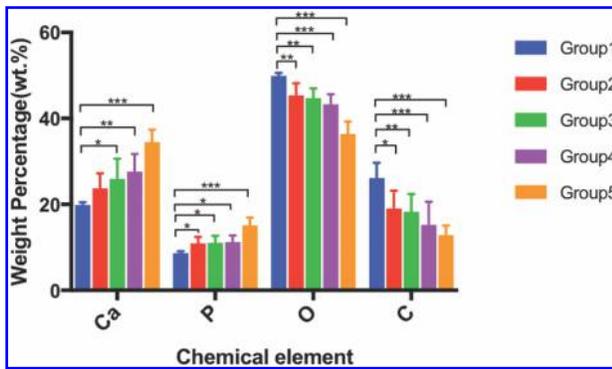
FIG. 3. Weight percentages of calcium, phosphorus, oxygen, and carbon (mean ± SD) of dentin according to Groups 1–5 ($n=5$). Groups 1–5 are dentin after treatment with Er:YAG laser irradiation at energy density: 0, 6.37, 12.73, 19.11, 25.47 J/cm^2 separately. $*p<0.05$, $**p<0.01$, $***p<0.001$.

clarified. This is because few studies have focused on carious dentin. Kukidome et al.[28] investigated the effects of laser irradiation with a wavelength of 2940 nm on cell suspensions of *S. mutans* with an energy density of 3.1 J/cm^2 (0.8 W, 40 Hz for 30 sec). Under these conditions, the numbers of viable bacteria were significantly decreased. However, they placed the bacterial cell suspension on a dentin plate rather than building a carious model. In addition, there have been few studies regarding the bactericidal effects of Er:YAG laser irradiation on cariogenic bacteria. The effects of Er:YAG laser irradiation have been investigated in root canal therapy, but these studies focused mainly on *E. coli*, *E. faecalis*, and *Staphylococcus aureus*.[31,32]

In this study, the irradiation parameters were chosen according to the results of a pilot study conducted in our laboratory and were designed based on a literature review.[28,32] At lower energy levels (from 6.37 to 12.73 J/cm^2), the number of live bacteria was reduced after irradiation, indicating that Er:YAG laser irradiation had bactericidal ability, although it was relatively weak. With higher energy densities (from 19.11 to 25.47 J/cm^2), Er:YAG laser irradiation showed superior bactericidal effects with nearly 100% bacterial reduction after irradiation. This energy density was higher than the energy previously reported in cell suspensions of *S. mutans*.[28] In this study, bovine dentin contaminated with cariogenic bacteria was used to simulate clinical dentin caries. Bacteria had penetrated into the dentin tubules after coculture for 7 days.[33] We assumed that the energy required to kill bacteria inside the dentin would likely be higher than that for sterilization on the surface, as the laser energy would be absorbed while passing into the dentin.

The Er:YAG laser showed bactericidal effects against cariogenic bacteria, which were mainly mediated through thermal effects and shockwave of the mid-infrared laser.[21] The Er:YAG laser emission wavelength is 2940 nm. Therefore, the fundamental chromophores that absorb erbium wavelengths are water and hydroxyapatite, the former of which is present throughout bacterial cells. Therefore, the laser energy damages the cell wall, leading to cell swelling and death.[17,34] Immediate cell death may not occur, but

sublethal damage inhibits cell growth after exposure to laser irradiation.

The morphological changes after Er:YAG laser irradiation depend on the irradiation parameters.[34] Under different energy levels, the Er:YAG laser can be used for the treatment of deep dentin caries for primary teeth and young permanent teeth[21] to preserve pulp vitality. To achieve this goal, the Er:YAG laser irradiation procedure consists of two steps. The first step involves decontamination of the surface with higher energy density. The second step involves melting dentin to decrease postoperative pain. In this study, surface melting occurred with partial bacterial reduction at the minimum energy level (6.37 J/cm^2). This suggestion was also supported by the findings of Moosavi et al.[36] Surface melting with tubular blockage can decrease postoperative pain to some degree.[21]

Previous studies have confirmed that laser irradiation reduces the degree of dentin dissolution. The studies tested the weight percentages of calcium and phosphate and the Ca/P and C/O ratios to determine the changes in dentine apatite crystals.[37] It has been suggested that changes in the chemical composition after Er:YAG laser irradiation have mechanistic effects.[38] In this study, we found significant increases in the weight percentages of calcium and phosphate, and significant decreases in those of oxygen and carbon. These observations were consistent with the study of Mei et al.,[39] who reported that laser irradiation of dentin led to improvements in mechanical properties. The Ca/P ratio of amorphous calcium phosphate is variable and can contribute to the lack of significance of the measured Ca/P ratio. However, it is not clear whether laser irradiation increases the acid resistance of dentin. Manesh et al. reported that laser irradiation was not particularly effective for increasing or decreasing the rate of dentin demineralization,[40,41] whereas Ceballos et al.[42] reported positive effects on both of these parameters. This discrepancy may have been due to the lower energy in the former study (8 J/cm^2), and to differences in the test instruments used between the studies. Therefore, we speculated that the rate of dentin demineralization may be decreased after Er:YAG laser irradiation. For clinical application, this may be useful for management of deep caries. However, additional studies are required as the causative bacteria in carious lesions are not limited to one species, and the different species may have different susceptibilities to laser irradiation.[43,44] In addition, future studies should be performed with larger sample sizes and with use of human teeth.

Conclusions

In conclusion, the results showed that Er:YAG laser irradiation has bactericidal effects and can cause both morphological and chemical changes in bovine dentin. A relatively weak bactericidal effect and melting of the dentin surface were observed with irradiation at lower energy densities. Superior antimicrobial capacity was observed with higher energy densities, and the dentin surface was clean with open orifices. Significant increases were observed in the weight percentages of both calcium and phosphate with increasing irradiation energy density, indicating that Er:YAG laser irradiation facilitated an asymptomatic postoperative course and prevented recurrence of caries.

References

1. Aas JA, Griffen AL, Dardis SR, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol 2008;46:1407–1417.

2. Bai J. The results of the Fourth National Epidemiological Survey of Oral Health in China [EB/OL]. 2017. Available at: http://www.gov.cn/xinwen/2017-09/20/content_5226224 .htm

3. Petersen PE. World health organization global policy for improvement of oral health—world health assembly 2007. Int Dent J] 2011;58:115–121.

4. Burrow M, Ricketts D, Kidd E, Innes N, Clarkson J. Complete or ultraconservative removal of decayed tissue in unfilled teeth. Cochrane Database Syst Rev 2009;54:274–276.

5. De ANA, Coutinho E, Cardoso MV, Lambrechts P, Van MB. Current concepts and techniques for caries excavation and adhesion to residual dentin. J Adhes Dent 2011;13:7.

6. Fried D, Featherstone JDB, Visuri SR, Seka WD. Caries inhibition potential of Er:YAG and Er:YSGG laser radiation. Proc SPIE Int Soc Opt Eng 1996;2672:73–78.

7. Ramazani N, Ahmadi R, Daryaeian M. Oral and dental laser treatments for children: applications, advantages and considerations. J Lasers Med Sci 2012;3:44–49.

8. Sarmadi R, Hedman E, Gabre P. Laser in caries treatment—patients' experiences and opinions. Int J Dent Hyg 2014;12:67–73.

9. Hibst R, Keller U. Experimental studies of the application of the Er:YAG laser on dental hard substances: I. measurement of the ablation rate. Lasers Surg Med 2010;9:338–344.

10. Caruso S, Bernardi S, Pasini M, et al. The process of mineralisation in the development of human tooth. Eur J Paediatr Dent 2016;17:322–326.

11. Berkovitz BKB, Moxham B, Linden RWA, Sloan AJ. Master Dentistry Volume Three Oral Biology. London: Elsevier, 2011.

12. Nyvad B, Crielaard W, Mira A, Takahashi N, Beighton D. Dental caries from a molecular microbiological perspective. Caries Res 2013;47:89–102.

13. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res 2011;90:294–303.

14. Borsatto MC, Torres CP, Chinelatti MA, Pécora JD, Corona SA, Palma-Dibb RG. Effect of Er:YAG laser parameters on ablation capacity and morphology of primary enamel. Photomed Laser Surg 2009;27:253–260.

15. Galia Z, Maya R, Jean-Paul R. Minimally invasive treatment of dental caries in primary teeth using an Er:YAG laser. Laser Ther 2014;23:249–254.

16. 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;35:111–116.

17. Lee BS, Lin YW, Chia JS, et al. Bactericidal effects of diode laser on Streptococcus mutans after irradiation through different thickness of dentin. Lasers Surg Med 2010;38:62–69.

18. Arnabat J, Escribano C, Fenosa A, et al. Bactericidal activity of erbium, chromium:yttrium-scandium-gallium-garnet laser in root canals. Lasers Med Sci 2010;25:805–810.

19. Wang Q, Zhang CX. Evaluation of the bactericidal effect of Er,Cr:YSGG, and Nd:YAG lasers in experimentally infected root canals. J Endod 2007;33:830–832.

20. Guidotti R, Merigo E, Fornaini C, Rocca JP, Medioni E, Vescovi P. Er:YAG 2,940-nm laser fiber in endodontic treatment: a help in removing smear layer. Lasers Med Sci 2014;29:69–75.

21. Olivi G, Margolis FS, Genovese MD. Pediatric Laser Dentistry: A User's Guide. China: Quintessence Publishing Co., Inc., 2011.

22. Perin FM, França SC, Silva-Sousa YTC, Alfredo E, Saquy PC, Estrela C, Sousa-Neto MD. Evaluation of the antimicrobial effect of Er:YAG laser irradiation versus 1% sodium hypochlorite irrigation for root canal disinfection. Aust Endod J 2010;30:20–22.

23. Manesh SK, Darling CL, Fried D. Imaging natural and artificial demineralization on dentin surfaces with polarization sensitive optical coherence tomography. Proc SPIE Int Soc Opt Eng 2008;6843:1004–1020.

24. De Andrade LEH, Pelino JEP, Lizarelli RFZ, Bagnato VS, De Oliveira OB. Caries resistance of lased human enamel with Er:YAG laser—morphological and ratio Ca/P analysis. Laser Phys Lett 2010;4:157–162.

25. Altinok B, Tanboga I, Peker S, Eren F, Bakkal M, Peker F. The effect of laser-activated acidulated phosphate fluoride on enamel submitted to erosive solution only: an in vitro preliminary evaluation. Eur J Paediatr Dent 2011;12:13–19.

26. Chow LC. Next generation calcium phosphate-based biomaterials. Dent Mater J 2009;28:1–10.

27. Ramalho KM, Hsu CY, de Freitas PM, et al. Erbium lasers for the prevention of enamel and dentin demineralization: a literature review. Photomed Laser Surg 2015;33:301–319.

28. Kukidome N, Amagai, T., Osuka, K., et al. Bactericidal effects of 2.94 μm and 1.67 μm laser. Bull Tokyo Dent Coll 2010;51:185–192.

29. Park SN, Lim YK, Choi MH, et al. Antimicrobial mechanism of oleanolic and ursolic acids on Streptococcus mutans ua159. Curr Microbiol 2017;75:1–9.

30. D'Ercole Simonetta DT, Giuseppe M, Sara B, et al. Microleakage of bacteria in different implant-abutment assemblies: an in vitro study. J Appl Biomater Funct Mater 2015;13:e174–e180.

31. Saharhelft S, Stabholtz A, Moshonov J, Gutkin V, Redenski I, Steinberg D. Effect of Er:YAG laser-activated irrigation solution on Enterococcus faecalis biofilm in an ex-vivo root canal model. Photomed Laser Surg 2013;31:334.

32. Asnaashari M, Safavi N. Disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy. J Lasers Med Sci 2013;4:8–16.

33. Melo MAS, De-Paula DM, Lima JPM, et al. In vitro photodynamic antimicrobial chemotherapy in dentine contaminated by cariogenic bacteria. Laser Phys Lett 2010;20:1504–1513.

34. Dos Santos Antonio MP, Moura-Netto C, Camargo SE, Davidowicz H, Marques MM, Maranhão de Moura AA. Bactericidal effects of two parameters of Er:YAG laser intracanal irradiation: ex-vivo study. Lasers Med Sci 2012;27:1165–1168.

35. Featherstone JD. Caries detection and prevention with laser energy. Dent Clin North Am 2000;44:955–969.

36. Moosavi H, Ghorbanzadeh S, Ahrari F. Structural and morphological changes in human dentin after ablative and subablative Er:YAG laser irradiation. J Lasers Med Sci 2016;7:86–91.

37. Celik EU, Ergücü Z, Türkün LS, Türkün M. Effect of different laser devices on the composition and microhardness of dentin. Oper Dent 2008;33:496–501.

38. Hossain M, Nakamura Y, Tamaki Y, Yamada Y, Murakami Y, Matsumoto K. Atomic analysis and knoop hardness measurement of the cavity floor prepared by Er,Cr:YSGG laser irradiation in vitro. J Oral Rehabil 2010;30:515–521.

39. Mei ML, Ito L, Chu CH, Lo EC, Zhang CF. Prevention of dentine caries using silver diamine fluoride application followed by Er:YAG laser irradiation: an in vitro study. Lasers Med Sci 2014;29:1785–1791.

40. Manesh SK, Darling CL, Fried D. Imaging natural and artificial demineralization on dentin surfaces with polarization sensitive optical coherence tomography. Proc SPIE Int Soc Opt Eng 2008;6843:1004–1020.

41. Manesh SK, Darling CL, Fried D. Nondestructive assessment of dentin demineralization using polarization-sensitive optical coherence tomography after exposure to fluoride and laser irradiation. J Biomed Mater Res B Appl Biomater 2009;90B:802–812.

42. Ceballos L, Toledano M, Osorio R, Garcíagodoy F, Flaitz C, Hicks J. Er-YAG laser pretreatment effect on in vitro secondary caries formation around composite restorations. Am J Dent 2001;14:46–49.

43. Chalmers NI, Oh K, Hughes CV, et al. Pulp and plaque microbiotas of children with severe early childhood caries. J Oral Microbiol 2015;7:25951.

44. Simón-Soro A, Guillen-Navarro M, Mira A. Metatranscriptomics reveals overall active bacterial composition in caries lesions. J Oral Microbiol 2014;6:25443.

Address correspondence to:
Sun Zhang, MD
Department of Pediatric Dentistry
Peking University School and Hospital of Stomatology
22 Zhongguancun Nandajie, Haidian District
Beijing 100081
China

E-mail: zhangsun_1@sina.com

Qian Zhang, MD
Central Laboratory
Peking University School and Hospital of Stomatology
22 Zhongguancun Nandajie, Haidian District
Beijing 100081
China

E-mail: zhangqian860203@163.com