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Bactericidal Effects of Diode Laser on *Streptococcus mutans* After Irradiation Through Different Thickness of Dentin

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Background and Objectives: A reliable method to eradicate the bacteria of residual carious dentin has not yet been developed. The aim of this study was to evaluate the antibacterial effect of a diode laser on *Streptococcus mutans* through different thickness (500, 1,000, and 2,000 μm) of human dentin. The thermal effect of laser irradiation was also investigated.

Study Design/Materials and Methods: Dentin specimens were inoculated with 2 μl of *S. mutans* on one side and irradiated by a diode laser on the other side with a power output ranging from 0.5 to 7 W. The laser tip was swept with the whole irradiation area of 7 mm×3 mm at a speed of about 10 mm/second with a total irradiation time of 30 seconds. Cooling with distilled water (30 ml/minute) was applied simultaneously during laser irradiation. After laser irradiation, the bacteria was removed from the dentin surfaces and cultured for 48 hours at 37°C anaerobically to assess the colony forming units (CFU) per ml. The morphology of the lased bacteria and the temperature rise during laser irradiation were observed by scanning electron microscope (SEM) and measured by thermocouple, respectively.

Results: The results revealed that 7 W of laser power could kill 97.7% of CFU through 500 μm thickness of dentin. However, the bactericidal efficiency was significantly reduced as the dentin thickness was increased. The morphological changes of lased bacteria ranged from less affected such as loss of their wall bands and existence of minicells to more severely degenerated, such as disintegration and fusion of cells with pores on the cell wall. Only the dentin specimens with a thickness of 500 μm exhibited a temperature rise greater than 5.5°C after receiving 5 or 7 W of laser irradiation.

Conclusions: A diode laser can eliminate the *Streptococcus mutans* of the residual carious dentin without inducing high pulpal temperature rise when the remaining dentin thickness is greater than 1 mm. Lasers Surg. Med. 38:62–69, 2006. © 2006 Wiley-Liss, Inc.

Key words: bactericidal effects; diode laser; *streptococcus mutans*

INTRODUCTION

The minimal intervention dentistry is a prevailing concept in operative dentistry which addresses that the amount of enamel and dentin should be maximally conserved through the sterilization of cariogenic bacteria, and the stimulation of remineralization [1]. Clinically, the bulk of carious lesion was usually removed by hand instruments or rotary burs, however, the quantity of residual carious dentin to be removed, exhibits great differences among practitioners. A general judgment of residual carious dentin is based on the color by visual inspection and the hardness detected by a sharp excavator [2]. However, this diagnostic criterion is rather subjective and cannot be applied to every dentist. Caries-disclosing dyes were recommended as an objective method to discriminate the healthy dentin from infected dentin, but the results were not always reliable [3]. In addition, either the hand instruments or rotary burs can not guarantee thorough a cleaning of the infected dentin, and residual bacteria often present before the placement of restorations. Mertz-Fairhurst et al. [4] have reported that numerous bacteria might remain in dentinal tubules after cariostatic-sealed restorations. Therefore, many researchers spend much efforts in developing an optimal method to treat the residual carious dentin.

One of the proposed ways was to use the fluoride release and anticariogenic materials such as glass ionomer cement (GIC) [5]. However, cariogenic bacteria that remained viable beneath GIC for 2 years has been reported [6] and secondary caries often led to the failure of GIC restorations [7]. Montanaro et al. [8] also demonstrated that *Streptococcus mutans* could be adhered and colonized on GIC surfaces in a period as short as 4 hours, and the fluoride

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release capability of GIC did not inhibit the early bacterial adhesion. Another method suggested the addition of antibacterial agents to GIC, but the physical properties of GIC were compromised after incorporation of these agents [9]. Other researchers claimed that the use of a cetrimide containing GIC dentin conditioner [10] or 3 aged dental cements [11] had prominent antimicrobial activity by agar diffusion test and direct-contact test. However, most of the bacteria of residual infected dentin existed in the dentinal tubules and there was no direct contact with the materials. Those two tests could not reflect the actual clinical situations.

On the contrary, the transmission characteristics of laser light exhibit the potential to sterilize the residual infected dentin through certain dentin thickness. Previous studies mainly focused on the antibacterial effect of the diode laser in the root canals [12–15], the bactericidal effect of the diode laser on residual infected dentin through different dentin thickness has seldom been evaluated. The primary aim of this study was to investigate the influence of the diode laser irradiation on *Streptococcus mutans* through different thickness (500, 1,000, and 2,000 μm) of human dentin. In addition, the lased bacteria and the thermal effect of laser irradiation were also investigated.

MATERIALS AND METHODS

Specimen Preparation

Extracted human third permanent molars were used in this study and they were obtained with informed consent of the donors. Crowns with caries, restorations, or fractures were discarded. Any remaining soft tissues were thoroughly removed from the tooth surfaces with a dental scaler (Sonicflex 2000, KaVo Co., Biberbach, Germany) under running water. All teeth were then stored in 4°C distilled water containing 0.2% thymol to inhibit microbial growth until use.

While fully hydrated, each third molar was first cut just below the occlusal pit and fissure, perpendicular to the long axis of the tooth by means of a low-speed diamond wafering blade (Isomet; 10.2 cm×0.3 mm, arbor size 1/2 inch, series 15HC diamond; Buehler Ltd., Lake Bluff, IL). Second, the crown dentin disks with thickness of 500, 1,000, and 2,000 μm were obtained by a second cut horizontal to the first one. The enamel of each specimen was removed with a plain-cut tungsten carbide fissure bur at high speed under continuous water spray. Each dentin disk was subsequently cut to an orthorhombic shape with the dimensions of length×width = 7 mm×3 mm. Then the dentin specimens were wet-polished with a 600 grit silica paper to create uniform flat surfaces and to finely adjust the specimen thickness. The specimen thickness was precisely determined by an electronic vernier (CD-10CX, Mitutoyo Co., Ltd., Tokyo, Japan). To ensure complete removal of the smear layer, the specimens were immersed in an ultrasonic cleaner (Delta, Mandarin Scientific Co., Ltd., Taipei, Taiwan) filled with 10% ethylenediaminetetraacetic acid (EDTA)(Wako, Pure Chemical Industries, Ltd., Osaka, Japan) for 2 minutes and 2.5% sodium hypochlorite (NaOCl) for 1 minute, followed by three washes with physiological saline solution for a period of 2 minutes.

Laser Irradiation

A diode laser (LaserSmile™, Biolase, CA) that provided a constant beam of coherent, continuous monochromatic light with an emission wavelength of 810 nm was used in this study. A light-emitting diode (LED) (630 nm, 5 mW) was used as an aiming device and the laser beam was delivered through a flexible 400-μm optic fiber with a straight handpiece in continuous mode. Before the laser irradiation, the laser energy was carefully calibrated with a power meter (Coherent; Morita Mfg. Corp., Tokyo, Japan) to control the output energy from the fiber tip within the desired irradiation condition. The calibration of laser energy with a power meter after laser irradiation was also performed. The results showed that the laser energy output was the same as our desired irradiation parameters. The laser tip was held perpendicular to the irradiated surface in a light contact motion and was cut 1 mm after irradiating each specimen to prevent energy loss. It was swept with the whole irradiation area of 7 mm×3 mm at a speed of about 10 mm/second with a total irradiation time of 30 seconds to simulate clinical manipulation. In order to reduce the thermal effect of diode laser, distilled water cooling (30 ml/minute) was applied simultaneously during laser irradiation.

Eighteen specimens with 2,000 μm in thickness were used for observing morphological changes of lased dentin by scanning electron microscopy (SEM). The specimens were randomly divided into six groups (A–F) with three specimens in each group. From Group A to F, the received power outputs were 0.5, 1, 3, 5, 7, and 9 W, respectively. Average energy densities of the target surfaces of Group A–F were 71.4, 142.9, 428.6, 714.3, 1,000, and 1285.7 J/cm^2, respectively.

Scanning Electron Microscopy (SEM) Examination of Laser Irradiated-Dentin

The morphology and microstructures of irradiated-dentin were examined by SEM. All specimens were immersed in 2.5% cold glutaraldehyde in 0.1 mol/L cacodylate buffer at a pH of 7.4 for 8 hours. They were then serially dehydrated in graded ethanol solutions (50, 60, 70, 80, 90, 95, and 100% ethanol) at 45-minute intervals, critical point-dried by CO_2, mounted on aluminum stubs, sputter-coated with ~20 nm of gold/palladium, and finally examined by a Hitachi SEM (Model S-800, Tokyo, Japan) at an accelerating voltage of 15 kV.

Bacterial Inoculation and Bactericidal Evaluation

Streptococcus mutans were used in this study because they were commonly isolated from carious lesions and were closely associated with dental caries [16]. Moreover, *S. mutans* were extensively used to test the antibacterial effect of restorative materials [17]. In this experiment, *S. mutans* (MT 8148) were cultured in the brain heart infusion (BHI, Difco, Kansas City, Missouri) broth in an anaerobic glove box (Modular Atmosphere Controlled

System, Forma Scientific, New York, NY) containing an atmosphere of 80% N_2, 10% H_2, and 10% CO_2 overnight. The bacteria were harvested to a concentration of 10^8–10^9 organisms/ml determined by the optical density using a microplate reader (ELx 800, BIOTEK).

Since the specimens of Group F that received 9 W power output demonstrated detrimental appearance such as crater and crack under SEM examination, this group was not included in the evaluation. Seventy-eight dentin specimens (500 μm in thickness) were randomly allocated to Groups A–E with 13 specimens in each group. The remaining 13 specimens did not receive laser treatment and served as the control group. Ten specimens in each group were used to evaluate the bactericidal effect and three specimens were used to perform morphological examination of lased bacteria by SEM. Additionally, 10 specimens (1,000 μm in thickness) and 10 specimens (2,000 μm in thickness) were used to test the bactericidal activity of laser with 7 W power output.

All specimens were autoclaved (Melatronic 23, Melag, Berlin, Germany) for 20 minutes at 121°C under 1.2 psi pressure to achieve sterility. They were then inoculated with 2 μl of *S. mutans* (10^5–10^6 CFU/ml) on one side by means of a micropipette and then irradiated with laser on the other side immediately. The control group was only inoculated with 2 μl of *S. mutans* but without receiving laser treatment. The specimens of experimental groups were irradiated in combination with water cooling on the bacteria-free side in a motion mentioned previously. Immediately upon irradiation, the specimens were placed into sterile Eppendorf tubes containing physiological saline solution with 0.1% Tween (Sigma, Munich, Germany). Each tube was then vortexed for 1 minute to remove the bacteria from the dentin surfaces. Upon vortexing, 100 μl of the extracted fluid were diluted in log 10 steps. One hundred micro liters portions of the dilutions (10^{-1} to 10^{-5}) were applied to the BHI culture plates and incubated for 48 hours at 37°C anaerobically. The colonies were then counted and the total number of bacteria (colony forming units (CFU) per ml) was assessed.

The results were quoted in "percentage of killing" as:

$$\text{Percentage of killing} = [100\% - (\text{CFU of experiment group}/\text{CFU of control group}) \times 100\%]$$

Statistical analysis was performed using the Kruskal–Wallis test (significant level of 5%, for non-parametric data of percentage of killing CFU and log of killing CFU) followed by Mann–Whitney *U* test (significant level of 5%, for multiple comparison). The dentin specimens with lased bacteria were prepared for SEM investigations in the same manner described previously.

Temperature Elevation Measurement

The temperature rise of specimens with different thickness (500, 1,000, 2,000 μm) during different laser irradiation powers (0.5, 1, 3, 5, 7 W) was investigated. The non-irradiation surfaces of each specimen were applied with a silicone heat transfer compound (Unick, Unick Chemical Co., Taipei, Taiwan) to promote heat conduction in the thermocouple (Philips, type K025, diameter 0.25 mm, Tokyo, Japan). Paraffin wax was used to isolate the thermocouple and prevent the influence of environment temperature. A thermocouple was connected to a digital oscilloscope (LeCroy 9310M, Dual 300 MHz, oscilloscopes; LeCroy Corp., Geneva, Switzerland), plotter (X-Y plotter, DXY-880; Roland Digital Group Co., Tokyo, Japan), and digital thermometer (YF-160, type K; Yu Hong Co., Taipei, Taiwan) to record the mean temperature elevation and standard deviation (Fig. 1).

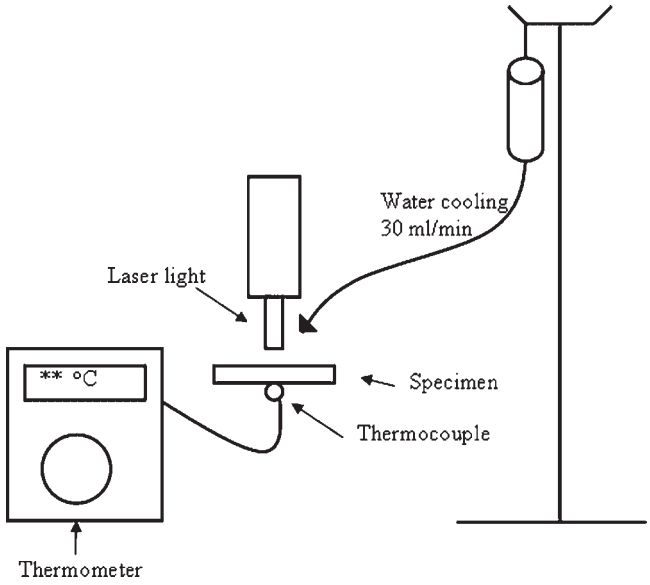

Fig. 1. The thermocouple connected with a digital thermometer was used to measure the temperature elevation during laser irradiation with water cooling.

RESULTS

SEM Examination of Laser Irradiated-Dentin

All dentin specimens after 10% EDTA and 2.5% NaOCl treatments, but without laser irradiation (control group) demonstrated even and clean surfaces (Fig. 2a). No smear layer coverings and exposed dentinal tubules orifices were noted. After receiving 7 W of diode laser irradiation for 30 seconds (Group E), every examined specimen showed many occluded dentinal tubules orifices (Fig. 2b). No detrimental structure changes were found. The specimens received laser irradiation below 7 W (Groups A–D) all demonstrated similar microstructures illustrated in Figure 2b. However, when the power output reached 9 W (Group F), crater and melted substances at the periphery of the crater were observed in every specimen (Fig. 2c). Most of the dentinal tubules orifices could be clearly found.

Bactericidal Efficiency of Diode Laser Through Different Dentin Thickness

When the dentin thickness was 500 μm, the percentage of killing CFU (%) of diode laser at 0.5, 1, 3, 5, and 7 W was

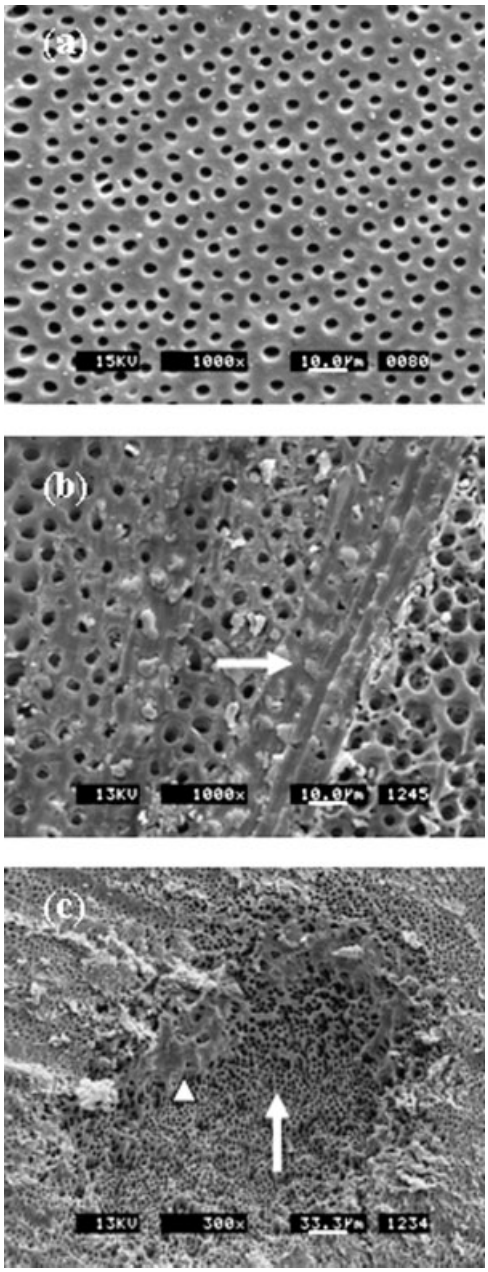

Fig. 2. **a**: An even and clean surface free of smear layer coverings was found at the dentin specimen without laser irradiation. **b**: After receiving 7 W of diode laser power for 30 seconds, many dentinal tubules orifices were occluded (arrow). No detrimental structure changes were found. **c**: The specimen surfaces exhibited a crater (arrow) and melted substances (arrowhead) were observed at the periphery of the crater when the energy output reached 9 W.

19.4, 32.5, 56.8, 90.8, and 97.7, respectively (Fig. 3). No significant differences were found between the two groups receiving 0.5 and 1 W, and the two groups receiving 5 and 7 W using the Kruskal–Wallis test followed by Mann–Whitney *U* test. However, the percentage of killing CFU (%) with diode laser at 7 W reduced to 50.9 and 20.1 when the

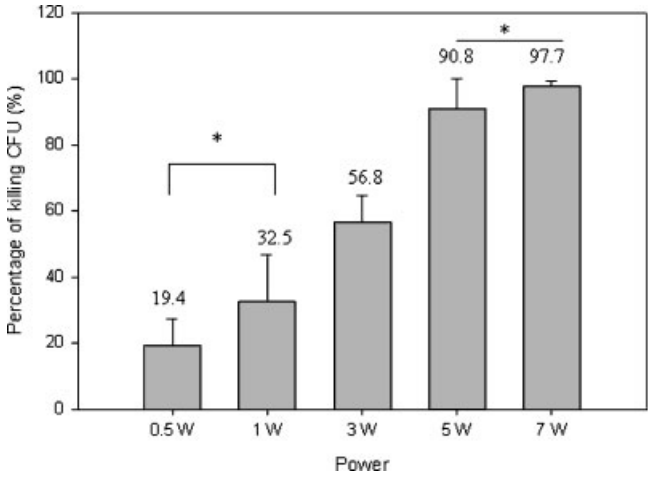

Fig. 3. The percentage killing of CFU (%) with diode laser through the 500 μm thick dentin specimens was increased as the laser power was elevated from 0.5 to 7 W. No significant differences (*) were found between the two groups receiving 0.5 and 1 W, and the two groups receiving 5 and 7 W.

dentin thickness was increased to 1,000 and 2,000 μm, respectively (Fig. 4). There were statistically significant differences between any two groups.

Microbiological Observation of Lased Bacteria

S. mutans was formed by the linkage of diplococci which was composed of a couple of cocci connected by a wall band. The normal diplococci appeared as a short-rod in shape and exhibited wall band on the surface before laser irradiation (Fig. 5a). After receiving 3 W of diode laser irradiation through the 500 μm thickness of dentin, some cells lost their wall bands and became uncoupled, indicating this power setting was sufficient to inflict cell wall damage on this microorganism (Fig. 5b). Moreover, the morphology of some

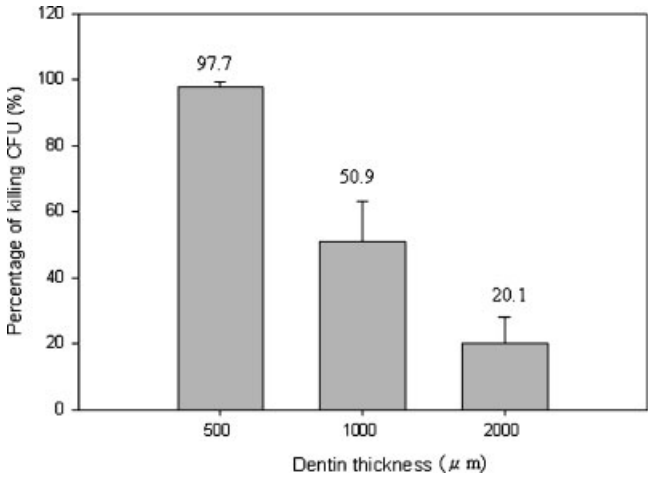

Fig. 4. The percentage killing of CFU (%) with diode laser at 7 W reduced to 50.9 and 20.1 when the dentin thickness was increased to 1,000 and 2,000 μm, respectively. There were statistically significant differences between any two groups.

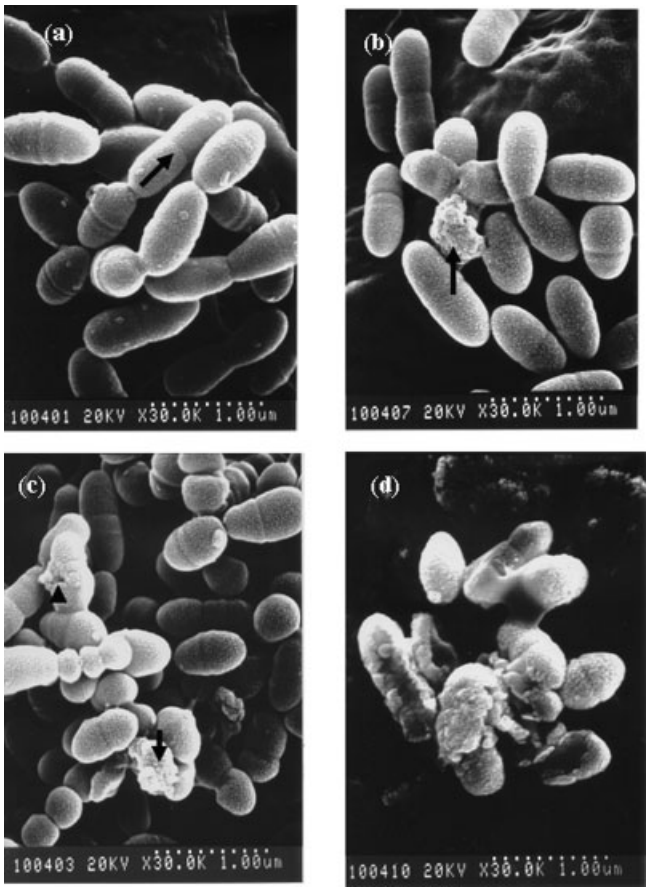

Fig. 5. **a**: The normal *S. mutans* appeared an even short-rod in shape and exhibited wall band on the surface (arrow) before laser irradiation. **b**: After receiving 3 W of laser power through 500 μm thickness of dentin, some cells lost their wall bands and became uncoupled (arrow). **c**: As the laser power was increased to 5 W, the *S. mutans* displayed various sizes either elongated or shrank. Disintegration (arrow) or fusion (arrowhead) of cells with pores on the cell wall was found. **d**: When the laser power was elevated to 7 W, large amount of cells appeared shriveled and displayed perforations on their cell envelopes. The cell bodies seemed to be totally fused together and no morphologically intact bacteria were discernible.

microorganisms was severely damaged, leaving depression and many pores on the surfaces. As the laser power was increased to 5 W, the microorganisms demonstrated prominent morphological changes (Fig. 5c). The *S. mutans* displayed various sizes either elongated or shrank. Disintegration or fusion of cells with pores on the cell wall was found. When the laser power was elevated to 7 W, completely disrupted cells could be seen (Fig. 5d). Large amount of cells appeared shriveled and displayed perforations on their cell envelopes. Furthermore, coagulated and amorphous masses in which the shape of the bacteria merged with one another were found. The cell bodies seemed to be totally fused together and no morphologically intact bacteria were discernible.

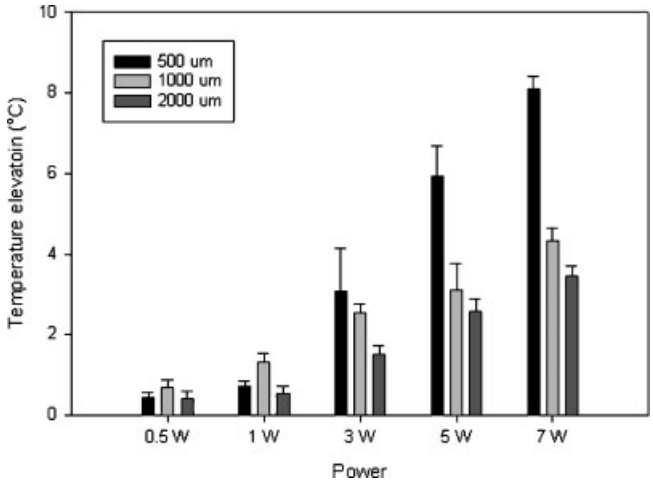

Fig. 6. Generally, the temperature rise during diode laser irradiation in the presence of water cooling (30 ml/minutes) was decreased as the dentin thickness was increased. The dentin specimens with a thickness of 500 μm exhibited a temperature rise of 5.9 and 8.1°C after receiving 5 and 7 W of laser irradiation, respectively. The temperature rise was less than 5.5°C for other conditions.

Temperature Elevation Measurement

The temperature rise during diode laser irradiation in combination with water cooling (30 ml/minute) is shown in Figure 6. Generally, the temperature rise was decreased as the dentin thickness was increased. Only the dentin specimens with a thickness of 500 μm exhibited a temperature elevation greater than 5.5°C after receiving 5 or 7 W of laser irradiation.

DISCUSSION

The interaction of laser light and dental hard tissues is determined by the irradiation parameters such as wavelength, repetition rate, pulse energy, duration of exposure, and optical properties of the tissue. The operator can avoid any thermal damage on dentin by using water cooling or selecting the suitable power output. In this study, 9 W of diode laser power could create a crater and melt the dentin substance (Fig. 2c), representing this power output would cause harmful effect on dentin. When the laser power was reduced below 9 W, some dentinal tubules orifices were occluded and no signs of thermal side effects (charring or crater) were found (Fig. 2b). Therefore, the 7 W was selected as the highest power to proceed the evaluation of bactericidal effect.

In this experiment, *S. mutans* were subcultured to a concentration of 10^8–10^9 CFU/ml. Each dentin specimen was inoculated with 2 μl of *S. mutans* which corresponded to 10^5–10^6 CFU/ml, and immediately followed by laser irradiation. Upon laser irradiation, the bacteria adhered to the specimens were immersed in physiological saline solution containing 0.1% Tween and was then vortexed for 1 minute. The quantity of collected bacteria in the control group was also 10^5–10^6 CFU/ml, indicating that

almost all of the inoculated bacteria could be collected via this method. Moreover, the bacteria were collected immediately upon laser irradiation because the primary purpose of this study was to examine the influence of human dentin thickness (500, 1,000, and 2,000 μm) on the antimicrobial efficiency of the diode laser irradiation. Longer period of incubation would allow for the penetration of bacteria into the dentinal tubules, and thereby compromise the thickness factor we intended to investigate. Unlike some previous studies whose aims were to evaluate the bactericidal effect of the diode laser in root canals [12,15], therefore, we shortened the incubation period instead of several hours of incubation.

The percentage of killing CFU (%) with diode laser through 500 μm thickness of dentin specimens at 5 and 7 W was 90.8 and 97.7, respectively (Fig. 3). Compared with chlorhexidine whose antibacterial activity was reduced to 54% using the same thickness of dentin disks [18], the efficiency of diode laser is prominently higher. In addition, another antibacterial monomer, 12-methacryloyloxydodecylpyridinium bromide (MDPB) was claimed to have effective antimicrobial activity and inhibition of root caries progression [19]. However, MDPB could only penetrate 140 μm into the demineralized lesion [20] that was less than the effective depth of diode laser.

Nevertheless, the antibacterial effect of diode laser at 7 W was reduced to 50.9% and 20.1% when the dentin thickness was increased to 1,000 and 2,000 μm, respectively (Fig. 4). Although the study examining the maximal penetration depth of bacteria in residual infected dentin has not been reported, the depth of *S. mutans* in the dentinal tubules of open infected root canals was found to reach 1,100 μm [21]. Therefore, the thorough removal of soft dentin is encouraged to decrease the thickness effect on laser irradiation.

It has been reported that 2.5 W of diode laser irradiation could result in partial carbonizations of the root surfaces [22], but could achieve a bacterial reduction in periodontal pockets up to three log steps [23]. In another study, more than 50% of bacteria in the infected root canals could be killed by diode laser at a single irradiation with 2 W of laser power [13]. The powers selected in this study were higher than those in the previous studies, but did not contribute thermal side effects to dentin specimens (Fig. 2). The major reasons could be attributed to the continuous movement of fiber tip with a speed of about 10 mm/second in combination with distilled water cooling (30 ml/minute), which could reduce the thermal effect and simultaneously reach high bactericidal efficiency.

S. mutans is a gram-positive, non-motile, short-rod facultative anaerobe (Fig. 5a). Compared with gram-negative bacteria, it is relatively resistant to laser irradiation because of their tough cell wall, which is composed, of highly cross-linked murein [24]. In this study, *S. mutans* began to notably lose their normal cell morphology after receiving 3 W of diode laser irradiation through 500 μm thick dentin (Fig. 5b). The morphological changes of lased bacteria ranged from less affected such as loss of their wall bands and existence of minicells to more severely degenerated such as disintegration and fusion of cells with pores on the cell wall (Fig. 5c,d). Furthermore, the severity of cell damage in morphology was positively correlated with the antimicrobial activity of laser power. The higher the laser power was used, the greater bactericidal effect and cell damage could be achieved.

The significance of morphological changes of lased *S. mutans* was explained as follows. The cell division in gram-positive cocci begins with centripetal penetration of the wall followed by the appearance of wall bands [25]. Subsequently, the new wall bands are separated by a nascent cross-wall. Therefore, loss of wall bands indicated that the cell division was interfered. The existence of small, round cells, namely minicells, resulted from the division of bacteria at the inappropriate junction region when normal division in the center of the cell was blocked under external harmful stimuli [26]. For the disintegration or fusion of cells with pores on the cell wall, the turnover of gram-positive cell walls during cell growth will be under precise control. When the microorganism receives heat stress, strong fabrication of cell walls will be hampered because older peptidoglycan of cell walls will be vulnerably stretched to its breaking point [27]. The decomposed peptidoglycan will be rapidly solubilized by peptidoglycan hydrolases, causing pores on the cell walls. All of the above-mentioned phenomenon represented that the laser light could hamper the normal cell division of *S. mutans*.

The possible mechanisms regarding the antibacterial effect of diode laser are summarized in the following. Thermal and photodisruptive effects were considered the principal reasons for the laser to eliminate the bacteria [28]. Immediate cell death might not occur during laser irradiation, but sublethal damage inhibited the cell growth after exposure to laser irradiation [29]. The sublethal damage included destruction of cell wall integrity (Fig. 5) and possibly the accumulation of denatured protein. Integrity of cell wall is intimately related to the mechanical stability of gram-positive bacteria. The damage of cell wall will cease the cell growth and successive cell lysis [30]. On the other hand, the cellular protein is highly sensitive to thermal changes. The laser irradiation might produce denatured protein and induce the cell to create new proteins to compensate the denaturation [31]. Some proteins such as IDG-60 immunodominant glycoprotein is indispensable for maintaining the integrity of the cell wall and the structure uniformity of cell shape [32]. The stress on the cells to prevent the accumulation of denatured protein debris could also cause cell death [29].

The temperature rise during laser irradiation was measured to evaluate the thermal effect on the pulp. According to the studies of Zach and Cohen [33] regarding pulp response to externally applied heat, 15% of teeth failed to recover from an intrapulpal temperature increase of 5.5°C. If the temperature increase was 11°C, 60% of teeth could not recover. In this study, only the dentin specimens with a thickness of 500 μm exhibited a temperature rise of 5.9 and 8.1°C after receiving 5 and 7 W of laser irradiation, respectively (Fig. 6). Other lased conditions gave rise to temperature elevation no more than 5.5°C. However, the clinicians should be cautious if the utilized parameters are

put into clinical practice. The reason is that the 810 nm diode laser is easily absorbed by the pigmented pulp tissue and the temperature rise lower than 5.5°C does not exclude possible damage to the pulp. On the contrary, the temperature rise above 5.5°C does not necessary indicate the detrimental effect to the pulp in vivo because blood flow, blood perfusion, and conduction through gingiva and bone in the clinical situation can act as a heat reservoir of teeth [34]. The temperature rise of teeth placed in a 37°C water bath after laser irradiation was reported to be 5°C lower than the temperature rise of isolated teeth [34]. Nonetheless, the measurement of temperature rise is widely used as a convenient and fundamental method in evaluating the thermal effect of laser on pulp tissue [35–37], as the precisely controlled laboratory conditions can hardly be conducted in vivo.

Streptococcus mutans were selected in this study because they were the predominant bacteria in carious lesions [16] and were broadly used to evaluate the bactericidal effect of restorative materials [17]. However, the efficacy of the diode laser in disinfecting residual carious dentin needs to be further investigated as the causative bacteria in carious lesions are not limited to one species and these different species of bacteria might have different susceptibility to the laser irradiation.

In conclusion, the diode laser irradiation at 7 W output power in association with distilled water cooling could reach 97.7% killing of CFU through 500 μm thickness of dentin. It can be an adjunct tool to disinfect the residual carious dentin with minimal risk of thermal damage to the pulp when the remaining dentin thickness is greater than 1 mm.

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