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Bactericidal Effect of Er,Cr:YSGG Laser on *Streptococcus mutans*

Murat TÜRKÜN[1], L. Şebnem TÜRKÜN[1], Esra Uzer ÇELİK[1] and Mustafa ATEŞ[2]
[1]Department of Restorative Dentistry and Endodontics, School of Dentistry, Ege University, Bornova, Izmir, Turkey
[2]Department of Basic and Industrial Microbiology, Faculty of Science, Ege University, Bornova, Izmir, Turkey
Corresponding author, Esra Uzer ÇELİK E-mail:esrauzer@yahoo.com

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The aim of this study was to compare the antibacterial activities of Er,Cr:YSGG laser with two different power outputs against a chlorhexidine gluconate-based cavity disinfectant. A cavity tooth model test was used to determine the antibacterial activity. Four cylindrical cavities were prepared on the dentin surface of 10 bovine incisors and left in contact with *Streptococcus mutans* for 72 hours to allow bacterial invasion. Following which, Er,Cr:YSGG laser with 0.75 W and 1 W power outputs and a chlorhexidine gluconate-based cavity disinfectant were applied separately on one of the three infected cavities, whereas the fourth was left untreated for control. Standardized amounts of dentin chips were obtained from the cavity walls, and the number of bacteria recovered was counted. Statistical analysis was carried out using one-way ANOVA and Dunnett's C test ($p=0.05$). No significant differences were observed among the data obtained from the chlorhexidine gluconate-based cavity disinfectant and the two Er,Cr:YSGG laser groups ($p>0.05$). However, when compared to the control group, both Er,Cr:YSGG laser groups and the chlorhexidine gluconate-based cavity disinfectant resulted in significantly less bacterial recovery ($p<0.05$). In conclusion, the antibacterial activity on *S. mutans* demonstrated by Er,Cr:YSGG laser with both energy outputs was similar to that of the tested chlorhexidine gluconate-based cavity disinfectant.

Key words: Er,Cr:YSGG laser, Chlorhexidine gluconate, *Streptococcus mutans*

INTRODUCTION

The principal objective of caries removal is to eliminate infected and necrotic tissues and microorganisms that may cause persistent inflammation and treatment failure. Thus, thorough removal of the infected dentin has a direct influence and impact on the clinical success of a restoration. However, the caries treatment procedures used presently neither always nor assuredly eliminate all of the microorganisms in residual tissues[1,2]. Bacterial sources which contribute to cavity infection are: 1) invasion from the tooth surface via marginal gap formation between a tooth and restorative material; 2) bacteria present in the smear layer; 3) bacteria present in dentinal tubules; 4) bacteria present at the dentinoenamel junction; and 5) bacterial re-contamination of the surface prior to restoration placement[3]. A number of studies have demonstrated that bacteria left in the dentin of a cavity − due to any of the aforementioned infection sources − could maintain their activity for a long time[4,5]. Brännström[6] indicated that residual bacteria in a cavity preparation can multiply from within the smear layer, even in the presence of a good seal within the oral cavity. As a result, this becomes a source of bacterial toxins − which can diffuse or even cause recurrence of the caries process[6].

To prevent the occurrence of residual caries, postoperative sensitivity, and pulp inflammation caused by bacteria, many studies have recommended the use of cavity disinfectants or restorative materials which have antibacterial activity[7,8].

Disinfecting the cavity preparation prior to its restoration eliminates the chance of having bacteria in the cavity[8]. Chlorhexidine gluconate-based solutions are the most popular cavity disinfectants used in daily dental procedures. The effectiveness of chlorhexidine lies in its chemical charge, as it is a compound which exhibits strong cationic properties. Since oral surfaces are mostly negatively charged, the positive charge of chlorhexidine accounts for its adherent ability and prolonged antimicrobial effect[9,10]. However, there are some concerns about the use of cavity disinfectants before the application of dentin bonding agents, since they may alter the ability of hydrophilic resins to seal dentin tubules[8,11,12]. These concerns are compounded by the many controversial results arising from various studies on the interaction between cavity disinfectants and dentin bonding agents[8,12−14].

In the perennial quest for better dental treatment methods, various kinds of laser have been investigated and the performances of lasers in the field of dentistry is indeed improving. When used in conjunction with a water spray, it has become safer and more efficient to remove dental hard tissue by laser − and thus this method is gaining wider acceptance in restorative procedures. In particular, erbium lasers are able to remove enamel, dentin, and carious tissue with minimal amount of thermal disruption to the residual tooth[15].

Recently, an erbium, chromium: yttrium, scandium, gallium, garnet (Er,Cr:YSGG) laser device that emits a 2.78-μm laser has been developed to cut

dental hard tissues[16]. The Er,Cr:YSGG laser employs a pulsed-beam system with a fiber delivery and sapphire tip that is bathed in a mixture of air and water vapor. This device has been shown to create precise hard tissue cuts by virtue of laser energy interaction with water at the tissue interface, and has therefore been termed as a hydrokinetic system. Laser energy has been shown to cause violent explosive forces, also known as hydrokinetic effect, on water droplets emitted from the handpiece[16,17]. In previous SEM studies, it has been shown that laser cuts led to less damage to prisms and tubules without smear layer and debris when compared with bur cuts[18,19]. This characteristic of the laser may be an advantage for the elimination of residual caries as the smear layer itself is a source of residual bacteria in a prepared cavity.

High-power laser light is known to be bactericidal, and investigations have shown that it is effective against caries organisms and inflammatory dental diseases. The antibacterial effect of a laser mainly depends on the effects produced by laser light in the target cell, tissue, or organism. These effects may be photochemical (due to the production of free radicals and other reactive species), photothermal, photoablative (due to the breaking of chemical bonds), or photomechanical (due to the shock waves produced by the dissipation of a plasma). In general, soft lasers induce only photochemical changes while hard lasers may produce any, or all, of the above-mentioned effects depending on the laser type and the conditions under which it is operating[20]. A number of studies demonstrated that different types of laser have antibacterial effects on different microorganizms[21-23]. However, the antibacterial effect of Er,Cr:YSGG laser on microorganisms associated with dental caries is still not well known. To eliminate residual caries thoroughly and efficiently, it is important to know the possible antibacterial effect of lasers on microorganisms related to dental caries.

The aim of this study, therefore, was to compare the antibacterial activities of Waterlase, an Er,Cr:YSGG laser with two different power outputs against Concepsis, a chlorhexidine gluconate-based cavity disinfectant.

MATERIALS AND METHODS

Specimen preparation and treatment

This part of the study was performed according to a method modified from that used by Özer *et al.*[24]. Ten extracted bovine incisors were stored at −80℃ until use. Enamel of teeth was cut horizontally with a water-cooled diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) to obtain flat dentinal surfaces. Four cylindrical cavities (1 mm in diameter, 2 mm in depth) were prepared on the flat surface of each tooth without causing pulp exposure.

Teeth were sterilized in an autoclave for 15 minutes at 121℃. To confirm sterility, the teeth were put into brain heart infusion (BHI) broth (CM225, Oxoid, England, UK) and incubated for 24 hours at 37℃. Each tooth was then transferred to 2 ml of sterile physiologic saline (SPS) in an individual tube, and stored for 24 hours at 37℃ to wash out the culture medium and to avoid dehydration. After drying with sterile paper points under a laminar flow cabinet, all teeth were placed in a bottle containing broth culture supplemented with 0.5% yeast extract of *Streptococcus mutans* CCUG 6519 and incubated at 37 ℃ for 72 hours with 5% CO_2 to create infected cavities. Following incubation, the teeth were taken out from the bottle, and the cavities were dried again with sterile paper points and a gentle stream of air.

Er,Cr:YSGG laser (Waterlase, Biolase, California, USA) with 0.75 W and 1 W power outputs and Concepsis (2% chlorhexidine gluconate, Ultradent Products Inc., South Jordan, USA) were applied on one of the three infected cavities separately, and the fourth cavity was left untreated for control. In each group, 10 cavities were treated. The laser groups were irradiated without water spray cooling, *i.e.*, 0% water level and 0% air cooling level, according to the manufacturer's instructions. Irradiation time was five seconds and was repeated five times with 15-second intervals. Concepsis was applied using a sterile brush, left undisturbed for 60 seconds and then dried gently with an air syringe. After application of Concepsis and laser beams, all the openings of cavities were sealed with a temporary restorative material (Cavit, Provit, Hamburg, Germany) until the drilling procedure to prevent infection of the cavities from environmental conditions. However, before the cavities were sealed, they were isolated with a piece of sterile cotton to prevent the temporary restorative material from adapting to the treated cavity walls.

Antibacterial activity determination

To evaluate the antibacterial effects of the tested material and device against the microorganisms penetrated to the deeper parts of the dentinal tubules during inoculation of the cavities, dentin chips from the cavity walls were collected. After removing the temporary restorative material, standardized amounts of dentin chips (25 ± 5 mg) were collected from the circumferential cavity walls (except the pulpal floor) by using a new carbide fissure crosscut bur (1.6 mm in diameter, H21 NTI Kahla, Germany) mounted to a low-speed contra-angle handpiece, and then put into sterile tubes. For each cavity, a new sterile bur was used to prevent overheating of dentinal walls during the cutting action. Although the size of the dentin chips collected varied from 30 to 60 μm in width and 125 to 300 μm in length, we did not grind them uniformly to prevent any possible damages to the microorganisms during this process.

Instead of the grinding procedure, suspensions with the dentin chips collected were obtained by adding 2 ml of SPS into the tubes and mixed using Vortex (Fisions Scientific Equipment, Leicestershire, UK) for 30 seconds. The purpose of which was to enable the microorganisms to pass through the solution and thereby produce a homogeneous suspension. Serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were achieved and the number of *Streptococcus mutans* recovered was determined by plate count using 5% sheep blood agar (CM854, Oxoid, England, UK).

Statistical analysis

Statistical analysis was carried out using one-way ANOVA and Dunnett's C test with SPSS 10.0 software package (SPSS, Inc., Chicago, IL, USA) as there was no homogeneity of variances. Level of significance was set at $p=0.05$.

RESULTS

Fig. 1 shows the number of bacteria recovered in each group. No significant differences were observed among the data obtained from Concepsis and the two Er,Cr:YSGG laser groups with different power outputs ($p>0.05$). Both Er,Cr:YSGG laser groups and Concepsis resulted in significantly less bacterial recovery than the control group ($p<0.05$).

DISCUSSION

In this study, the cavity tooth model test described by Özer *et al.*[24] was used to evaluate the antibacterial effects of Er,Cr:YSGG laser and Concepsis. However, this model was modified in terms of using bovine incisors instead of human molars. The intent of which was to obtain large dentinal surfaces so as to prepare four cavities on the same tooth. Other antibacterial activity test models such as the agar well and disc diffusion techniques were considered to be inappropriate for antibacterial activity comparison of different materials, since the diffusion rate of antibacterial solutions into the hydrophilic agar/material may vary significantly. To overcome these problems and to be able to compare materials using more precise clinical simulations, the cavity tooth model was developed[24,25].

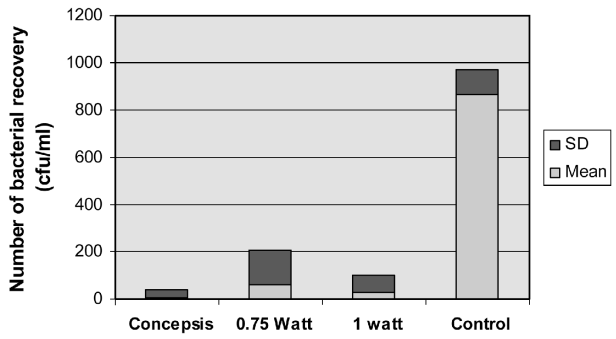

Fig. 1 Number of *Streptococcus mutans* recovered after tooth cavity test.

In restorative treatments, sterilization of the prepared cavity plays a critical role in ensuring a successful treatment. The most well-known odontopathogens associated with dental caries are *mutans streptococci*. These microorganisms can adhere to tooth surfaces and produce extracellular and intracellular polysaccharides that increase[26]. One way of preventing superinfections, especially by *S. mutans*, is to remove all infected tooth tissues and disinfect the area by using cavity disinfectants[27].

The Concepsis solution used in this study contained 2% chlorhexidine gluconate and is recommended for restorative purposes as a cavity disinfectant prior to cementation or luting procedure[8,28]. Chlorhexidine used as a mouth rinse or in dental gels applied by toothbrushing has been reported to yield two-fold results: a low or moderate reduction in *mutans streptococci* counts in plaque and saliva and a trend for less caries to develop in groups[29]. As a cavity disinfectant, Gultz *et al.*[30] — who had compared the antimicrobial activities of different cavity disinfectants — found that Concepsis solution was superior to the other products.

The antibacterial activity of different lasers has been investigated previously. Moris *et al.*[31] indicated that Nd:YAG, Ho:YAG, and Er:YAG lasers were strongly effective microbicides on *Escherichia coli* and *Enterecoccus faecalis* and could be considered as a valuable tool for root canal disinfection. Moreover, previous *in vitro* studies on the antibacterial effects of Er:YAG laser radiation clearly demonstrated bacterial killing for *Actinobacillus actinomycetemcomitans* and *Porphromonas gingivalis*[32]. Compared to the extensive researches into the antibacterial effects of different lasers on endodontic and periodontologic pathogens, much less attention has been given to pathogens associated with dental caries. In the present study, Er,Cr:YSGG laser radiation reduced the number of *S. mutans* — to a similar extent as Concepsis — on dentin surfaces. It could thus be assumed that Er,Cr:YSGG laser radiation also demonstrated antimicrobial activity on *Strep. mutans* while ablating the infected hard tissues.

In terms of bacterial killing rate, many factors play an influential role: laser energy, water content, volume, strength of the cellular wall, absorption properties, and the migration of bacteria into dentinal tubules[33]. In terms of depth of penetration, it depends on the morphological features of the bacteria (e.g., special characteristics of the cell surface such as capsules or pili)[33] and was reported to reach 150 μm *in vivo* and even more than 1000 μm under *in vitro* conditions[34]. Previous studies have reported

that although the intensity of laser irradiation decreased after penetrating dentin slices at 500-1000 μm depth, the bacterial mode of action was still effective at such depths[35-37]. On the other hand, chemical disinfectants penetrated no more than 130 μm into the dentin as indicated by Berutti *et al.*[38]. In laboratory researches, the inoculation period of samples also directly affects bacterial penetration depth. In the present study, the cavities were inoculated for only 72 hours. In clinical situations, this period is generally much longer and bacterial penetration depth may be deeper than that of our samples. On this note, the deeper penetration depth of laser beam is a favorable advantage in the elimination of microorganisms found in deeper layers of dentin during dental treatment.

The results of this study gave no information on whether the reduction of bacteria with Er,Cr:YSGG laser radiation was caused by either the removal of hard tissue or by the bactericidal effects of laser irradiation itself. The elimination of bacteria produced by ablation of infected hard tissue did not seem to be very probable, particularly considering the parameters of the Er,Cr:YSGG laser radiation used in this study. Instead, the antibacterial effect of the Er,Cr:YSGG laser was more likely to be due to the evaporation of cellular water — which was expanded quickly by the laser pulse, thus leading to abrupt disintegration of the bacterial cell wall. Another explanation for the bactericidal action stemmed from the thermal necrosis or dehydration of the germ, which was more affected by the repetition rate rather than the pulse energy. Then peradventure, it could be a combination of all the above cited mechanisms that effected the killing of bacteria[39].

Although there were no significant differences among the data obtained from Er,Cr:YSGG laser and Concepsis groups in this study, slight differences were found in the number of recovered bacteria among these groups. In particular, a high number of bacteria was recovered from some of the cavities disinfected by Er,Cr:YSGG laser. It was noted that these results depended on the shape of the cavities irradiated with Er,Cr:YSGG laser as well as the different properties of the tested device and material.

In our study, we used cylindrical cavities of 1-mm diameter and 2-mm depth instead of flat surfaces to simulate clinical conditions. The antibacterial effect of a laser beam is applicable only on irradiated surfaces. To get a homogenous laser application — to all parts of the cavity walls in a cylindrical cavity — with each radiation is almost an impossible feat. Therefore, we believe that during laser application, any unirradiated surface would cause a rise in the number of bacteria recovered due to the reproduction of microorganisms. As Concepsis is in a solution form, it can readily diffuse into cavity walls. Consequently, when Concepsis was applied to the cavity walls in this study, the probability of uncontacted surfaces was very low — thereby demonstrating antibacterial activity in all samples. On the other hand, when restorative treatments and cavity preparations are performed with laser energy, it is inevitable that there would be unirradiated cavity surfaces.

Parameters of the laser energy used in this study were quite different from the parameters generally employed in clinical applications. This was so largely due to the requirements of this microbiological study. For example, the cavities for bacterial inoculation were prepared by rotary instruments before irradiation. Hence, the power outputs used in this study to disinfect the cavities were lower than the ones used for cavity preparation in clinical applications.

In addition, water cooling — with the aid of a water spray — cannot be used in microbiological studies to preclude the risk of spreading the microorganisms to the other surfaces. Further, if high laser energy is used to irradiate the tooth surface, it will cause carbonization and cracks on the laser beam-applied surface[19]. In clinical applications, higher laser energy is used to prepare cavities since it is accompanied with water cooling. Thus, the antibacterial activity may appear to be more efficient.

In the light of this study, the use of Er,Cr:YSGG laser with a rubber dam in cavity preparation will produce cavities that are quite sterile. Indeed, this disinfection method will improve the prognosis of dental treatments, especially for pulp capping where microbial activity is an important consideration. Subsequently, residual caries can be prevented by virtue of the antibacterial activity of the laser energy. Another laudable advantage of this laser system is that it eliminates the need for an extra step of applying a cavity disinfectant.

Bacteria or bacterial products contained in the smear layer are potential sources from which residual caries can occur. Numerous studies have demonstrated that single microbes, entrapped within the smear layer, could multiply and replace most of the smear layer within four weeks[40-42]. The microbes in the smear layer get sufficient nutrients from the tissue fluid seeping outward from the pulp. When the superficial smear layer was removed with an antibacterial agent before restoration, bacteria were eliminated and no reaction was observed in the pulp[43]. It has also been documented in numerous studies that CO_2, Nd:YAG, Er:YAG, and Er,Cr:YSGG laser irradiation were able to remove the debris and smear layer efficiently[44]. In this way, removal of the smear layer serves to eliminate the microorganisms and thus prevent residual caries.

In this study, Waterlase was the Er,Cr:YSGG laser system employed for antibacterial activity comparison with Concepsis. Based on the results of this

study, it was shown that Waterlase — with two energy outputs at 0.75 W and 1 W — demonstrated similar antibacterial activity as Concepsis on *S. mutans*, whereby Concepsis has been proven with regard to antibacterial activity. Nonetheless, the antibacterial effects of this laser on other bacteria associated with dental caries should be evaluated in further studies.

To date, the traditional methods of treating caries and disinfecting teeth before restoration placement are still more economical when compared to the use of lasers. Until laser has been shown to produce scientifically sound results which are superior to those achieved with conventional methods, the use of lasers in dental practice will not gain wider acceptance. Despite the many advantageous properties, it is absolutely important for lasers to demonstrate antibacterial activity before dentists will consider using lasers in daily dental practice.

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