Original Article

Antibacterial Effect of Surface Pretreatment Techniques against *Streptococcus Mutans*

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ABSTRACT

Objective: The aim of this study was to evaluate the antibacterial surface pretreatment methods against *Streptococcus mutans* within the infected dentin surface using a tooth cavity model. **Material and Methods:** Seventy-two cavities were prepared on caries-free third molars ($n = 8$). After sterilization, teeth were inoculated with *S. mutans* for 48 h. One cavity of each tooth was used to evaluate the infection. Following inoculation, infected cavity surfaces were treated either with (1) Er:YAG Laser (1W; 5x5s, Smart 2940D Plus, Deka Laser), (2) Ozone (80s; HealOzone, Kavo), (3) ErYAG-Ozone combination, (4) Er:YAG-Ozone-CHX combination, (5) Chlorhexidine (CHX), (6) Clearfil Protect Bond (PB), (7) potassium-titanyl-phosphate (KTP) Laser (1W; 60 s, SMARTLITE D, Deka Laser), (8) KTP-Ozone combination, and (9) KTP-Ozone-CHX. Standardized amounts of dentin chips were obtained from the cavity walls, and the number of bacteria recovered was counted. Kruskal–Wallis test was used for statistical analyzes. **Results:** Both sole antibacterial materials, CHX or Protect Bond application, exhibited the most effective antibacterial activity with 125 and 156 CFU is an acronym of "colony forming unit" usullay mentioned by acronym. (CFU/ml), respectively, among the groups evaluated ($P < 0.05$). Er:YAG laser irradiation and its combinations with other antibacterial surface pretreatment applications also inhibited the bacterial growth with, respectively, 1444, 406, and 294 CFU/ml bacterial recovery being more efficient than KTP laser irradiation and ozone combinations. **Conclusions:** As an alternative device with photodynamic effects, Er:YAG and KTP laser irradiations and their further combinations during the cavity pretreatment procedure with chlorhexidine and ozone treatments exerted antibacterial effect against *S. mutans*, whereas chlorhexidine and antibacterial dentin bonding application solely have the highest antibacterial effects.

KEYWORDS: *Antibacterial effect, cavity surface pretreatment techniques, cavity preparation, dental, dental infection control, laser antibacterial*

INTRODUCTION

The minimal intervention dentistry addresses maximum conservation of enamel and dentin in addition to elimination of cariogenic bacteria to maintain the stimulation of remineralization through the caries removal process.[1] Thus, cavity preparation should include removal of infected carious dentin and further obtain a sound and disinfected cavity surface. But criteria for judging the complete removal of the infected dental tissue still remains as a confusion.[2]

Conventional caries removing instruments cannot maintain a surface purified from bacteria resulting a raised residual bacteria left after the preparation of the lesion within the smear layer.[3,4] This assumption can induce secondary caries, postoperative sensitivity, and

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even lead to pulp damage afterward.[3–5] To overcome the bacterial consequences leading, cavity disinfecting plays an effective role to eliminate the residual bacteria left in the cavity. Remaining dental tissue disinfection is numerously recommended to be complemented by chemical or bonding agents with antibacterial properties or pretreating with antibacterial photodynamic devices.[6]

Mechanical removal of infected carious dentin should be complemented by chemical agents with antibacterial properties, which should provide a more aseptic environment prior to the use of adhesive restorative materials to maintain ideal conditions for dentin–pulp complex healing and bond durability. Antibacterial cavity cleansers are recommended to disinfect dentin by removing the residual bacteria. Chlorhexidine is frequently used as cavity disinfectant through its effective antibacterial properties exerted by binding to the dentin amino acids and continuous killing bacteria for several hours.[7] Furthermore adhesive materials with antibacterial effects were developed to overcome the bacterial consequences and secondary caries to extend the survival of the restorations. An adhesive resin containing antibacterial monomer (methacryl-dodecyleaminepyridinium bromide, MDPB) has been launched due to its outstanding antibacterial activity through the contact but without releasing and furthermore compromising the bonding efficiency in order to disinfect the carious bacteria remained.[8]

Ozone has been introduced in the dental practice due to its antimicrobial activity and also has been investigated as a cavity disinfectant prior to bonding procedures.[9,10] Ozone is an energy-rich and highly unstable form of oxygen. It is a strong and fast oxidizer of cell walls and cytoplasmatic membranes of bacteria and is considered to be one of the best bactericidal, antiviral, and antifungal agents.[11] The antibacterial effect of ozone was also shown by reducing the *mutans streptococci* in carious lesions.[9,12-14] Ozone has an advantage of exerting low toxicity because it rapidly degrades after contact with organic components.[15]

Antibacterial photodynamic therapy is a promising method to treat the carious dentin lesions by enabling a conservative approach through its antibacterial effect for residual cariogenic pathogens in the smear layer. In addition to the various use of lasers with different wavelengths for removing oral soft and dental hard tissues without pain relief, irrigating root canals, scaling and for tooth bleaching, antibacterial use is also recommended for an advanced tissue pretreatment technique. Er:YAG laser devices with 2.94 μm can be used as an alternative cavity preparation technique to reduce patient's discomfort and pain caused by tactile

stimulation of the rotary instruments, for caries removal and dentin pretreatment prior to the adhesive procedures in addition to eliminating the residual bacteria mainly causing the secondary caries in the smear layer.[16,17] KTP lasers emitting at a wavelength of 532 nm are also introduced to be used for its disinfecting effect to reduce the number of bacteria when used to disinfect the infected root canals, and proposed to be an effective way for disinfecting pretreatment of dentin surface.[18]

The aim of the present in vitro study is to evaluate and compare the antibacterial surface pretreatment methods and their combinations against *Streptococcus mutans* within the infected dentin surface using a tooth cavity model. The hypothesis is that the alternative surface pretreatment techniques will demonstrate antibacterial effects when used alone but deepen and enhance the antibacterial effect in synergy by their combined use.

MATERIALS AND METHODS

Eighteen freshly extracted human caries-free third molars were cleaned from debris and soft tissue remnants and stored in physiological saline solution at + 4°C within no more than 6 weeks until used. The occlusal third part (±3 mm) of the crowns were removed with water cooled, slow-speed (4000 rpm) diamond saw to obtain flat dentinal surfaces (Isomet 1000; Buehler, Lake Buff, Illinois, USA). The apices of the teeth were sealed with glass ionomer cement.

Seventy-two cavities ($n = 8$) distributed as four cylindrical cavities for each tooth were prepared (diameter 2 mm, depth 2 mm) on occlusal surface without causing pulp exposure. The teeth were then autoclave-sterilized for 15 min at 121°C and taken into BHI (Brain Heart Infusion) broth and incubated for 24 h at 37°C to confirm the sterility. Respectively, the teeth were washed out the culture medium by taking into individual tubes filled with 2 ml of SPS (Sterile Physiologic Saline) and kept for 24 h at 37°C.

After drying with sterile paper-points, the cavities were filled with 10 μl of *S. mutans* suspension (106 CFU/ml) and left for 3 min to invade and further placed in individual sterile tubes containing 5 ml of 106 CFU/ml *S. mutans* suspension for 48 hours to obtain bacterial colonization into dentin to simulate infected dentin surfaces. The teeth were dried with sterile paper-points following the incubation process. Following inoculation, one of the infected cavity surfaces were treated with (1) Er:YAG laser (1W; five times for 5 s with a frequency of 15 s at noncontact mode (Smart 2940D Plus; Deka Laser, Florence, Italy), (2) Ozone (80 s (HealOzone, KaVo, Germany) by narrow ended vacuum applicator tips), (3) ErYAG–Ozone combination, (4) ErYAG–

Ozone–CHX combination, (5) Chlorhexidine [CHX; 2% chlorhexidine gluconate solution (Cavity Cleanser; BISCO)], (6) Antibacterial adhesive system; PB (Clearfil Protect Bond; Kuraray), (7) KTP laser 60 s at 1W (6.7 J/cm²; SMARTLITE D; Deka, Calenzano Firenze, Italy), (8) KTP–Ozone combination, and (9) KTP–Ozone–CHX combination. Er:YAG laser, KTP laser and Ozone were applied as guided by the manufacturer's instructions for disinfection. CHX was applied to the cavity surfaces for 40 s and left to air dry. Clearfil Protect Bond was applied according to the manufacturer's instructions, primer was applied for 20 s, air blowed gently, and then bond was applied and light cured for 10 s. In combination groups of different applications used, cavity surfaces were irradiated, ozonated, and pretreated with CHX, respectively. After pretreating the inoculated surfaces, each cavity was covered with a piece of a sterile sponge and a fluoride/eugenol-free temporary filling material. The teeth were separately kept in SPS at 37°C for 72 h. Sponges were removed using sterile excavators and tweezers without coming into contact with the cavity dentin walls. Dentin chips were collected with cooled round-shaped tungsten carbide burs from the surrounding walls of each cavity and collected into sterile tubes. The dentin chips were weighed and respectively diluted 1:100 in PYB medium. The solution was stirred for 30 s and a series of 10-fold dilutions was prepared. The numbers of *S. mutans* (CFU/ml) were determined by viable plate counting on sheep Blood Agar.

Mann–Whitney *U* and Kruskal–Wallis tests were used for comparison of the groups evaluated with a statistical level of 0.05.

RESULTS

Tooth cavity model was used to simulate the clinical model to assess the antimicrobial activity. The weights of the dentin chips collected were standardized and diluted 1:100 in PYB medium. The numbers of *S. mutans* collected from the dentin chips were determined by counting viable bacteria recovered.

Table 1 showed mean values and the standard deviations (SD) of the numbers of *S. mutans* recovered (CFU/ml) after cavity surfaces were pretreated with antibacterial applications by tooth cavity model. The number of viable cells was significantly decreased when the cavity surfaces were treated with CHX (125 CFU/ml) and Protect Bond (156 CFU/ml) among the groups evaluated ($P < 0.05$) as shown in Figure 1. Er:YAG laser irradiation (1444 CFU/ml) and its combinations with either Ozone or Ozone plus CHX also resulted in low bacterial recovery; 294 and 406 CFU/ml, respectively. The other combined group of KTP laser irradiation, ozone and CHX application resulted in 144 CFU/ml, which is significantly has less
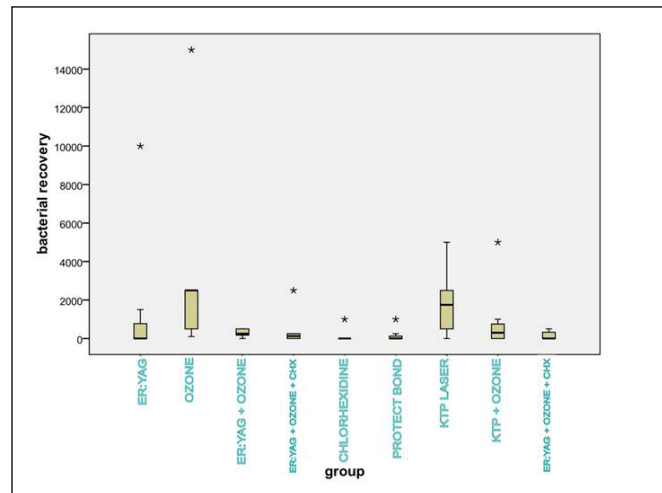

Figure 1: Viable bacteria recovered (CFU/ml) after application in tooth cavity model

Table 1: Number of viable bacteria recovered (CFU/ml) after application in tooth cavity model (mean value and standard deviations)

Surface pretreatment technique	Mean (CFU/ml)	SD
Er:YAG laser	1444AB	3469
Ozone	3262ACDEF	4860
Chlorhexidine (CHX)	125DH	354
Er:YAG and Ozone	294	192
Er:YAG and Ozone and CHX	406CG	855
KTP Laser	1812BGHIJ	1646
KTP+Ozone	888	1699
KTP and Ozone and CHX	144FJ	209
Clearfil Protect Bond	156EI	352

Same superscripts in each group of the every given line show statistically significant differences among the groups displayed

bacterial recovery than the control group ($P < 0.05$). All the combined groups of KTP, Er:YAG laser irradiation and ozone application had the potential to exert higher antibacterial effects than used alone but not as high as the CHX pretreatment and Protect Bond used.

DISCUSSION

Through the minimal tissue removal trend, the cavity surface residually contaminated by the cariogenic microbiota should be disinfected by cavity cleansers or pretreated with photodynamic devices to overcome the secondary caries occurrence, and furthermore to maintain a sterile substrate to bond and a desirable sealing with an adhesive system exerting some antibacterial properties.[8,19] The microbiota reasoning dental caries is mainly composed of *streptococci*, *lactobacilli*, and *actinomyces*, whereas the mutans group streptococci such as *S. mutans* and *S. sobrinus* are considered as the pioneer

etiological agents in coronal and root carious lesions.[20-22] Thus, in the present study the cavity surfaces were inoculated with the most susceptible and broadly used *S. mutans* to simulate the remaining infected or affected dentin to evaluate the antibacterial effect of the surface pretreatment techniques.[20,22] *In vitro* results of this microbiologic study revealed that the antibacterial agents and photodynamic techniques used alone or in combination exerted antibacterial effects against *S. mutans* at different levels. Thus, the hypothesis that the alternative surface pretreatment techniques would demonstrate some antibacterial effects against was partly accepted.

Dental lasers used at low energy levels reduce the bacterial load, remove the surface dentin layer and easily provide an access to mechanically unreachable areas of dentinal tubular network and can exert additional antibacterial effects.[23] In previous studies several authors demonstrated significant bacterial reductions occurred following laser irradiation in challenging surfaces such as root canal system.[24,25] Residual infected dentin can be potentially sterilized by laser light through its transmission characteristics. Depending on the energy output and pulse duration of laser irradiation, bactericidal effect through the photothermal interaction exerts on bacteria.[26] Er:YAG laser with 2.94 μm is shown to have bactericidal effects on *S. mutans* photochemically rather than photodynamically.[27] However, results of the present study does not highlight the laser irradiations as effective as CHX solution and PB adhesive system. Dentin surfaces were irradiated by 1 W energy output for both Er:YAG and KTP laser devices to disinfect the cavity surface and resulted in 1444 and 1812 CFU/ml bacterial recovery, respectively, which are rather high when compared with the combined techniques. Contrary to our results, Er,Cr:YSGG irradiated dentin surfaces resulted in reduced *S. mutans* recovery even in low energy outputs an in vitro study of Turkun *et al.*[28] Difference between the energy settings and type of Erbium lasers might be one of the reasons why the antibacterial effect was low among the groups evaluated. As mentioned above principal reasons for the laser to eliminate the bacteria are the thermal and photodisruptive effects.[28] The low energy output was also selected regarding the possible harmful effects of temperature rise in dentin, and moreover the reduction of the dentin hardness. However, high bacterial recovery levels in the study may further be explained as *S. mutans* being resistant to laser irradiation because of their strong cell wall, which is a composition of highly cross-linked murein and was reported to lose its cell composition after 3 W energy output, which is higher than the energy output used in the present study. This finding evidently supported that the severity of

cell damage in morphology was positively correlated with the antimicrobial activity of laser power, which is lower in the present study. In respect of the approach the higher laser power used, the greater bactericidal effect and cell damage could have been achieved, low bactericidal effects demonstrated by the laser groups seems reasonable.[29] Combinations of the lasers with ozone or CHX exerted higher antibacterial effects might also be as result of the synergistic effect of CHX, which directly hamper the cell wall of the bacteria. We can also assume that the antibacterial effect of CHX might have been induced and easily damage the cell wall because the dentin surface was already affected by the priorly irradiated laser.

In the present study cylindrical cavities were prepared on a flattened dentin surface as defined in the tooth cavity model.[30] To simulate the clinical conditions thus the geometric variability and the difficulty of access angle in the narrow dowels might be responsible for the lower antibacterial effects demonstrated by the laser irradiation groups where a homogeneous irradiation or access to deeper dentin surface was not so possible for Ozone and laser application tip for an optimum access whereas CHX solution and adhesive system used can easily diffuse.[30] In another respect, long incubation period could have been resulted in a deeper bacterial penetration compromising the antibacterial effectiveness of laser irradiation through the dentin tubules. In the present study design, combined groups were enrolled to evaluate whether the laser irradiation or ozone would deepen and enhance the antibacterial effect regarding the photodynamic characteristics as stated in the literature.[31] The higher bacterial recovery levels of the combination techniques also contradict to the synergy of the combination techniques. This contradiction may arise from the inhibition of the infiltration of the antibacterial solutions by the recrystallization of the surface dentin tissue. However, the degree of light penetration was asserted to be limited and result in a shallower penetration into dentin tubules which seems to support the present results.[32] Further methodologies might be designed to evaluate the subsurface activity of the antibacterial agents.

Gaseous or aqueous ozone is reported to have a strong oxidizing power with a reliable microbiocidal effect by mediating oxidation that destroys the cell walls and cytoplasmic membranes of bacteria beside its low cytotoxicity with a rapid degrading just after contact with organic compounds.[33,34] Previous studies showed that it is significantly effective in reducing the numbers of *S. mutans* and *S. sobrinus* in dental samples via a mechanism involving the rupture of their membranes.[9,35,36] The results of the present study showed that ozone

application revealed low antibacterial effect when used alone but showed synergist effect when used with both kind of lasers regarding their higher bactericidal effectiveness in accordance with data published.[31] Previous studies revealed that time required to achieve a total bacterial inactivation was 10–30 min.[10] It is also stated that not only the application time of ozone but also the dose used plays an important role on the antibacterial effect of ozone. An application of a higher ozone dose would probably need a lower application time in order to achieve higher disinfecting effect.[37] Furthermore, 80s ozone application time applied in the present study seems to be insufficient to exert an optimal antibacterial effect on the infected dentin surface and vacuum applicator of the ozone device might have been inadequate for reaching the cylindrical cavity surfaces.

Chlorhexidine still remains as the most effective and frequently used antibacterial solution for controlling bacterial plaque and caries.[38] Potential of residual caries and incidence of postoperative sensitivity decreases when CHX is used to disinfect the cavity.[6] Also many in vitro studies have demonstrated that the application of chlorhexidine did not have a negative effect on the bond strength of adhesive systems even more increasing the durability of resin–dentin bonds through its anti-MMP activity.[39-41] The high antibacterial effect exerted by CHX in the current study contributes its widespread use in the operative dentistry.

Self-etching adhesive systems with acidic primers demonstrates only limited antibacterial activities because of the buffering effect of dentin tubular fluid and the existence of aciduric bacteria.[42] Moreover, dentin bonding systems possessing antibacterial activity even after being cured are beneficial for eliminating the harmful effect caused by bacterial microleakage and would decrease the occurrence of secondary caries.[43] The incorporation of MDPB in the Protect Bond adhesive system possesses its antibacterial activity before and after curing, both *in vivo* and *in vitro*.[44-47] The present results also proved that Clearfil Protect Bond is antibacterial through its MDPB content even when compared application without a surface pretreatment. These data enable Protect Bond to be considered as a positive control material as CHX. Significantly lower bacterial recovery counts achieved in the present study supports its effectiveness as a "therapeutic adhesive system" and assuming that cured antibacterial adhesive could inhibit the growth of invading bacteria at the interface of dental restoration and dentine even if incomplete sealing or failure of the restoration occurs.[41,48]

Clinician's demands on recently developed devices especially for removing the dental tissue address the requirement of multifunctional properties. Innovative advanced technological dental devices such as laser and ozone devices enable for such multipurpose use in the contemporary dentistry. The results of the study concludes that the Er:YAG, KTP laser irradiation and ozone application help to disinfect the infected dentin surface beside evidently proven antibacterial chlorhexidine or antibacterial monomer MDPB use against *S. mutans*. Further in vitro microbiologic investigations with different methodologies should better be performed to evaluate their combined antibacterial effect activation and dynamics through infected dentin.

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Conflicts of interest

There are no conflicts of interest.

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