Antibacterial Effects of Diode Laser and Chlorhexidine gluconate on Streptococcus mutans in Coronal Cavity

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Abstract

Background: The principal objective of caries removal is to eliminate the infected and necrotic tissues and microorganisms that may cause a persistent inflammation and treatment failure. The aim of this study was to compare the antibacterial activities of diode laser, commercially available chlorhexidine gluconate (CHX) and the prepared one as a cavity disinfectant.

Methodology: 70 extracted sound human premolar teeth used. Crown of teeth was cut horizontally to obtain flat dentinal surfaces. One cylindrical cavity (2 mm in diameter, 1 mm in depth) prepared on the flat surface. Samples were divided into 7 groups, each consisted of 10 prepared teeth. The first twenty samples disinfected with (commercially available) Chlorhexidine (2% and 0.2%), the second twenty samples (prepared) chlorhexidine gluconate (2% and 0.2%), and the last twenty diode laser (1w and 1.30w). Dentin chips from the cavity walls collected immediately after treatment and put into sterile tubes containing 0.5mm of sterile normal saline. A 200µm from this saline was dispensed over Petri-dish contain Mitis-Salivarios agar.

Results: The results showed that the CHX solution, CHX powder and diode laser have a significant difference from the control group. Highest antibacterial effect on S. mutans achieved by CHX solution (2%) followed by CHX solution (0.2%). In the second order group, CHX powder (2%) followed by CHX powder (0.2%) showed comparably higher mean values of bacterial colonies than the first two groups mentioned above, and there is a difference between them but not significant.

Conclusion: The antibacterial effect of CHX (solution, powder) at all concentrations (2% and 0.2%) and diode laser at all powers (1w and 1.30w) in the infected coronal cavities with S. mutans was significantly different from untreated control group.

Introduction

The principal objective of caries removal is to eliminate the infected and necrotic tissues and microorganisms that may cause a 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 not always assuredly eliminates all of the microorganisms in residual tissues. Bacterial sources which contribute to cavity infection come from: Invasion from the tooth surface via marginal gap formation between a tooth and the restorative material, bacteria present in the smear layer, bacteria present in the dentinal tubules, bacteria present at the dentinoenamel junction and bacterial recontamination of the surface prior to restoration placement. A number of studies have demonstrated that the bacteria left in the dentin of a cavity due to any of the above mentioned infection sources could maintain their activities for a long time [1].

Chlorhexidine gluconate-based solutions are the most popular cavity disinfectants. The chlorhexidine application reported a significant decrease in the number of bacteria in the dentinal tubules. The effectiveness of chlorhexidine lies in its chemical charge, as it is a compound which exhibits strong cationic properties. The positive charge of chlorhexidine accounts for its adherent ability and prolonged antimicrobial effect [1].

Using the antibacterial effect of laser depends on the effects produced by laser light in the target cell, tissue, or organism. These effects may be photochemical (the production of free radicals and other reactive species), photothermal, photoablative (the breaking of chemical bonds), or photomechanical (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 [2].

A number of studies demonstrated that different types
of lasers have antibacterial effects on different microorganisms. In particular, diode and erbium lasers are able to produce an antibacterial effect on enamel, dentin, and carious tissue with a minimal amount of thermal disruption to the residual tooth [3].

To eliminate the residual caries thoroughly and efficiently, it is important to know the possible antibacterial effect of diode lasers on the microorganisms related to dental caries [4].

Materials and Methods

Sample Preparation
A total of 70 extracted sound human premolars were cleaned and scaled to remove the debris and calculus. The teeth were examined under a stereomicroscope (20x) to exclude the external root resorption, immature apex and vertical root fracture. Crowns of the teeth were cut horizontally with a water-cooled diamond disk to obtain flat dentinal surfaces under water coolant to expose the mid-coronal dentin. The teeth were embedded in a cylindrical polyvinyl tube, poured with autopolymerizing acrylic resin, then the specimens were attached to the survoyer and each specimen received a cylindrical cavity (2 mm in diameter, 1 mm in depth) and any specimen with pulp exposure was excluded. The specimens of all groups adapted in the stainless steel boxes which were covered with aluminum foil and placed in autoclave for 15 minutes at 121°C [5].

Sample Groups
The total number of samples was 70, divided into 7 groups (n= 10). One served as control and the others were divided into 3 subgroups of 20 specimens according to the disinfective treatment (prepared chlorhexidine solution, commercially chlorhexidine solution and diode laser), each subgroup again was divided into two groups according to the CHX concentrations (2% and 0.2%) [6] and diode laser powers (1w and 1.30w)

Preparation of Bacterial Suspension
A single colony of S. mutans inoculated in 5ml Brain heart infusion broth (BHI-broth) in the screw capped vial and incubated at 37°C for 24 hrs. After that 0.5ml of bacterial suspension was added to 0.5ml BHI-broth in the screw capped vial giving a final concentration of 4x10^7 CFU /ml [7].

Inoculation of coronal cavities with S. mutans
Before inoculation, the coronal cavities were dried by sterile absorbent endodontic paper points. Then each cavity for each group inoculated with 10µl of a bacterial suspension containing 4x10^5 CFU by micropipette and incubated at 37°C for 24 hrs.

Control groups divided into control positive (cavities infected with bacteria but not treated) and control negative (cavities not infected and not treated).

Group 1
(n=5): Control group +ve.
(n=5): Control group -ve.

Groups Disinfected with CHX
Each infected cavity was treated with 10 µl of CHX solution via micropipette for the selected time, then each cavity was dried with a sterilized absorbed endodontic paper point.

Group 2 (n=10): 2% CHX (commercially available), 40 sec.
Group 3 (n=10): 0.2% CHX (commercially available), 40 sec.
Same procedure used for CHX (Freshly prepared from powder)
Group 4 (n=10): 2% CHX (Freshly prepared), 40 sec
Group 5 (n=10): 0.2% CHX (Freshly prepared), 40 sec

Groups Disinfected with Laser
Each cavity was irradiated in contact mode with continuous wave of radiation. The laser light was transferred through a 600µm flexible fiber optic tip by a special hand piece. The fiber optic was disinfected for each use by 70% ethyl alcohol and inserted inside the cavity to 1mm with a spiral continuous movement clockwise from the top to the floor and anti-clockwise in the reverse direction. This procedure improves the distribution of the laser light inside the cavity and to avoid excessive heat generation and carbonization in the internal cavity surface. Irradiation time was 15 seconds and repeated 5 times with 15 second intervals with contact, according to manufacture instructions. During this study the output power was adjusted at 1W and 1.30W.

Group 6 (n=10): 1W, 15 sec, 5 cycles.
Group 7 (n=10): 1.30W, 15 sec, 5 cycles.

Antibacterial activity determination
To evaluate the antibacterial effects of the CHX and Diode laser against S. mutans, a standardized amounts of dentin chips?25±5 mg ) was collected from the circumferential cavity walls using a new, sterile
carbide round bur mounted to a low-speed contra-angle headpiece. Collected dentine chips with bur was transferred into sterile tubes containing a 0.5ml normal saline. A 200µl from this saline was dispensed onto the separate Petri dishes of MSA and incubated at 37°C for 24hrs [7].

Colony Counting

After 24hrs of incubation, the number of bacterial colonies was counted.

Results

The comparison of antibacterial activity between CHX and those disinfected with diode laser is summarized in Table (1) and Figure (1). The results showed that the highest antibacterial effect against S. mutans was achieved by CHX solution (2%) followed by CHX solution (0.2%). In the second order group, CHX powder (2%) followed by CHX powder (0.2%) showed comparably higher mean values of bacterial colonies than the CHX solution, and there was a difference between them but not significant.

Also the results showed that the antibacterial effect of CHX solution and powder both (2% and 0.2%) was significantly different from that of diode laser both powers (1.30W and 1W).

Discussion

Among various oral microorganisms, S. mutans has been identified as a plaque-forming bacterium capable of producing dental caries in experimental animals and in humans [8].

The principal objective of caries removal is to eliminate infected and necrotic tissues and microorganisms that may cause a 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. The caries treatment procedures used presently not always eliminate all of the microorganisms in the residual tissues [9]. But it has been demonstrated that bacteria are capable of invading the dentinal tubules up to a depth of 1mm, and unfortunately the chemical disinfectants penetrate up to 130µm inside the dentine [10]. This difference in depth of penetration between the invading bacteria and the disinfectant solution is often responsible for the recurrent caries in many of the cases which can be observed in the conventional dental procedures.

A culture dependant approach was used in this study. It is considered to be one of the most reliable methods of detecting viable bacteria, particularly when samples are taken immediately after the antimicrobial treatment [6].

A study showed that CHX produced the largest mean inhibition zones and was effective against all microorganisms [10]. Also, it adsorbs onto the dental tissues and mucous membranes resulting in its prolonged gradual release at therapeutic levels [11].

The bactericidal effect of CHX against S. mutans was detected in the present experiment, which varied with different concentrations. The results of the current study showed no significant difference between concentrations (2% and 0.2%) at 40 seconds contact time, and the results showed that CHX solution at (2%) was the most efficient concentration which eliminated a large amount of S. mutans after 40 sec. while CHX solution at (0.2%) showed a difference from (2%) but not significant. Also the current study showed that CHX powder at (2%) was statistically not different from CHX powder at (0.2%) in the elimination of S. mutans from coronal cavity after 40 sec. of application. These results are in agreement with results obtained by Mohammadi and Abbott [6] who showed that both concentrations (2% and 0.2%) of CHX solution reduced S. mutans in the coronal cavity. Other study did not demonstrate any significant antibacterial difference between different concentrations (0.1% and 0.2%) of CHX in reduction of S. mutans in the prepared cavities [12] and such a difference may be due to the difference in time of application.

The disinfectant efficiency of CHX depends on the mechanism by which the adsorption of CHX occurs onto the cell wall of microorganisms causing the leakage of intracellular components [13]. At low concentration, CHX has a bacteriostatic effect causing the leaking of a small molecular weight substance from microorganisms. At a higher concentration, CHX has a bactericidal effect due to the cytoplasmic precipitation and/or coagulation that probably was caused by protein cross-linking [13]. Also the cationic properties of CHX enable their adsorption by dentine surface, making CHX molecules penetrate deeper inside dentinal tubes and elongating its residual antimicrobial activity [14].

Different mechanisms regarding the antibacterial effect of diode laser include: Thermal and photodiseruptive
effects that are considered the principal reasons for the laser to eliminate the bacteria [15]. Lethal damage includes destruction of the cell wall integrity and possibly the denature of protein. The damage of the cell wall will cease the cell growth and successive cell lysis. At the same time, the cellular protein is highly sensitive to the thermal changes [14]. Another possible explanation could be due to the possibility of occluding the dentinal tubules which results from melting of dentin that leads to the entrapping of the invading microorganisms and reduction of the dentinal fluid as a source of nutrition [16]. A further explanation of the antibacterial effect of laser is believed due to the fact that with laser it is possible to achieve expansion of intratubular water and collapse of water vapor as deep as possible, which is capable of producing an acoustic wave strong enough to disrepute the intratubular bacteria [17].

The present study showed a significant antibacterial difference between the output powers. When the power of laser increased, the effect against S. mutans also increased. The results of this study showed that 15 sec. (5 cycles) with (1.30W) output power was more effective than 15 sec. (5 cycles) with (1W) output power. These results indicated that the power played an important role in the cavity disinfection. The results of this investigation are in accordance with others, [18] who found that the damage of bacteria increased with the amount of energy applied and there was a significant antibacterial difference between the powers output after the laser irradiation using (Nd:YAG) 1064nm and diode 980nm laser. Castro et al., [19] showed that 0.75 W and 1 W power outputs significantly reduced the numbers of S. mutans in the infected cavities using diode laser 810nm.

In this study the irradiation technique was as cycles, each cycle consisted of 15 sec. Five cycles of irradiation inside the infected cavities were used in this study. This technique was applied because bacterial growth had been formed inside the infected cavities which required more than one cycle of laser irradiation to reach the deeper layer and disrupt it. Using one cycle may probably result in partial disruption of the bacterial layer and the extensive bacterial reduction was achieved in all cases by repeating laser treatment with a high power diode laser [20].

Using laser as a cavity disinfectant may have advantages over the chemical solutions by reducing the tissue toxicity which results from using disinfectant solutions as a cavity disinfectant. The penetration of small amount of the disinfectant into the periradicular tissue has toxic and allergic reactions which lead to the inflammation. In addition, the higher concentrations of these solutions may also have an effect on the properties of dentin [21].

Conclusions

The antibacterial effect of CHX (solution, powder) at all concentrations (2% and 0.2%) and diode laser at all powers (1w and 1.30W) in the infected coronal cavities with S. mutans was significantly different from untreated control group.

Acknowledgements

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References

Illustrations

Illustration 1

Figure (1): A Duncan histogram showing the antibacterial activity of diode laser and CHX treated groups.
Illustration 2

Table (1) Comparison between antibacterial activity among all treated groups at different powers and concentrations

<table>
<thead>
<tr>
<th>Watt-Conc.</th>
<th>Descriptive statistic</th>
<th>F-value</th>
<th>P-value</th>
<th>Duncan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Max</td>
<td>Min</td>
</tr>
<tr>
<td>CHX S. (2%)</td>
<td>3.63</td>
<td>3.815</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>CHX S. (0.2%)</td>
<td>35.13</td>
<td>11.420</td>
<td>53</td>
<td>22</td>
</tr>
<tr>
<td>CHX P. (2%)</td>
<td>71.00</td>
<td>17.936</td>
<td>96</td>
<td>44</td>
</tr>
<tr>
<td>CHX P. (0.2%)</td>
<td>132.38</td>
<td>31.973</td>
<td>181</td>
<td>87</td>
</tr>
<tr>
<td>Diode laser (1.30w)</td>
<td>279.25</td>
<td>144.645</td>
<td>425</td>
<td>114</td>
</tr>
<tr>
<td>Diode laser (1w)</td>
<td>670.38</td>
<td>147.160</td>
<td>863</td>
<td>470</td>
</tr>
<tr>
<td>Control +ve</td>
<td>1123.25</td>
<td>182.065</td>
<td>1308</td>
<td>907</td>
</tr>
</tbody>
</table>
According to the ANOVA, means with different letters vertically have a significant difference at p<0.05 according to the Duncan test.

SD = Standard deviation.
Max = Maximum.
Min = Minimum.

Mean with different letters are significantly differed at P <0.5%.
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