Effect of 405 nm Laser Light on the Cariogenic Bacteria Streptococcus Mutans

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Abstract
In this study, Streptococcus Mutans bacteria causing the decay of the teeth was isolate and diagnosed. The results showed that the most casualties were in men. The results showed that the highest absorbance of the isolated bacteria was in the ultra violet (UV) wavelengths. This result make us to use Diode-Pumped Solid State Laser (DPSSL) with a wavelength of (405nm), power of its laser was 20 mw. The result showed that the time required to kill the bacteria was about (22 min). The increase of the exposure time of the laser gradually reduces the survival of the bacteria. The energy density increased with increasing the exposure time and it was sufficient to cause damage inside the bacterial cell and kill the bacteria. The study proved that the laser used is able to kill bacteria that cause tooth decay (Streptococcus Mutans) and can be used in the sterilization of dental equipment or materials used in dentistry to eliminate such bacteria.

Keywords: Streptococcus Mutans Bacteria, Laser Irradiation, Diode-Pumped Solid State Laser.

Introduction
The current worldwide increase in resistant bacteria and the simultaneous downward trend in the discovery of novel antibacterial agents to combat resistant strains is a serious threat to the treatment of life-threatening infections. The existence of bacteria resistant to topical and systemic antibiotics dramatically reduces the possibilities of treating infected wounds effectively and results in delayed wound healing and complications such as sepsis that may lead to death (Vazquez, 2006). Therefore there is a drive to develop non-invasive and non-toxic novel antimicrobial strategies that act more efficiently and faster than the current antibiotics, to which pathogens will not easily develop resistance (Taylor et al., 2002). During the last year's low intensity visible laser radiation has been successfully used in some areas of medicine and biology (karu et al., 1990). The most interesting application of laser in biology is the photo- inactivation of microorganism, especially bacteria and viruses. For example, (Gurzadyan et al., 1981) used picoseconds Nd-YAG laser 4w (λ=266 nm) to inactivate viruses and bacterial plasmid. The (4 w) of Nd-YAG laser was used also to inactivate the yeast (Candida utilis) (Rubin et al., 1983), while XeCl laser (λ=308 nm) was used to inactivate cells of E.coli (Tipliova et al., 1988).

Photodynamic inactivation of bacteria has been studied widely by Wilson et al, where low-power laser (He-Ne or diode laser) is used to kill the bacteria in the
presence of a suitable photosensitizer (toluidine blue or aluminum disulphonated phthalocyanin) (Wilson et al., 1995). Dental caries is one of the most common infectious diseases in the human oral cavity. The enamel and dentin are demineralized by acids, such as lactic acid, which are produced as a by-product of the carbohydrate metabolism by cariogenic bacteria in dental plaque. Among the oral bacteria, mutans streptococci have been implicated as major cariogenic bacteria (So Young Yoo et al., 2007). S. mutans is a significant pathogen of oral cavity and initiates dental caries. Many factors contribute to the cariogenicity of this organism such as rapid generation time, fermentation of wide range of carbohydrates, ability to withstand low pH, presence of enzyme glucosyl transferase that converts sucrose to glucose to enhance adhesion. S. mutans is reported to cause systemic infections like endocarditis and intravascular infections (Beena Antony et al., 2010). For investigating the bactericidal effect laser, Deferent types of laser have been used with different wavelengths e.g. far IR ,NIR ,red ,blue ,green and UV . The present study was carried out to investigate and characterize the bactericidal effect of visible light in the violet region (405 nm) on S. mutans.

Materials and methods

Isolation and characterization of cariogenic bacteria: A total of 100 swaps from human mouth were collected. The samples were directly inoculated in a brain heart broth and then incubated at 37°C for 24 hour. Then it was cultured onto plates of mannitol salt agar, Mac Conkey agar, ascolin agar, MRS agar and Blood agar then incubated at 37°C for 24 hour. After incubation period, bacteria were characterized and identified microscopically and biochemically. Then it was incubated in the brain heart broth over night before exposing to laser light.

Spectroscopic Study : The solution of brain heart broth containing the S.mutans bacteria put in the quartz cell of digital spectrophotometer and the result shows that the maxima absorption was in the UV region, Although there was a significant absorption exist in the violet region (Beena Antony et al.,2010).

Laser Irradiation Experiments: The laser used in this study was diode-pumped solid-state laser (DPSSL), with a wavelength of 405 nm, 20 mW product of Instek, see Fig.(1). Bacterial solution where subjected to laser light at different exposure times. Irradiation times ranges from 2.5 min. to 40 min. energy density ranges from 5.3 to 84.9 J/cm². The viability of bacterial cells was examined depending on the variation in the sample absorption between before and after irradiation and incubation for one hour. for CW Laser the Fluence equation is given by : the parameter that summaries the effects of laser radiation on biological parts is fluence and is given by

\[
Fluence = \frac{P * t}{A} \quad (1)
\]

where \( P \) is the power density, \( t \) is the exposure time and the \( A \) is the spot area.
Fig. (1) The laser device working on the bacteria

Results and discussion

The results of bacterial examination are shown in table (1).

Table (1): Illustrates the human infection with bacteria

<table>
<thead>
<tr>
<th>No. of patient Examined</th>
<th>Haemophilus</th>
<th>Lactobacillus</th>
<th>Streptococcus</th>
<th>Staphylococcus</th>
<th>Bacillus</th>
<th>Actinomycetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>52</td>
<td>48</td>
<td>/</td>
<td>8</td>
<td>/</td>
<td>3</td>
<td>35</td>
</tr>
</tbody>
</table>

These results indicate that $S.mutans$ was the most common type of oral flora and thus it can be regarded as the main cause of dental caries. Spectroscopic examination are shown below, it shows that the absorption of the bacterial cells was increases toward the UV region, it reaches its maximum near 380nm.
Results of laser treatment of bacterial suspension are illustrated in fig. (3). Control group shows the maximum absorption after incubation period due to the huge number of bacterial cells that were grown in the absence of the bactericidal effect of laser light. Other groups that are treated with laser light show inhibition in their growth ability gradually with increasing the irradiation time. This can be explained by the decreases in the bacterial number after irradiation due to bacterial death, and the inhibition of cell division, both led to decreased cell number or even the absence of bacteria after certain irradiation time.
Fig. (3): Shows the decrease in the survival of the bacteria as a function of exposure time.

Fig. (4): Show the relation between the energy density and the absorption of the solution.

Fig. (4) show the decreases in absorbance after irradiation due to bacterial death, and the inhibition of cell division, both led to decreased the absorbance of bacteria after certain irradiation time.

Because we used low power laser, with relatively short wavelength, we can expect no thermal effect. The most common type of interaction associated with such laser wavelengths is the photochemical interaction. Photochemical interactions take place at very low power densities (typically 1W/cm²) and long exposure times ranging from seconds to continuous wave. Photochemical reactions generally do not result in
a significant rise in temperature. Its effects involve either a change in the course of biochemical reaction due to the presence of an electromagnetic field or photodecomposition due to high energy photons that rupture molecular bonds (Chopra and Chawla, 1992). In biological tissue, absorption is mainly due to the presence of free water molecules, proteins, pigments, and other macromolecules. Except water, these molecules had a high absorption around 400 nm. (Niemz, 1996). The energy density used in this study was high enough to damage the intra-cellular systems and even death of cell due to different quantum yields of the respective reactions involved after electronic excitation of the photo acceptor molecules (Karu, 1999). The various photochemical processes in tissue components, initiated by absorption of light these processes are dependent on fluence (irradiance) rather than intensity (Paras, 2003). This effect was increased with increasing the energy density of laser light that was increased with increasing the irradiation time.

Conclusions

The results of laser irradiation of the bacterial samples show that:

- The survival rate of the bacteria decreased with increasing laser exposure time.
- The laser wavelength (405nm, 10mW) can kill the Streptococcus mutans.
- The energy density used in this study was high enough to damage the intra-cellular systems and it was increased with increasing the irradiation time.
- The last energy densities have a more significant effect more than that at the first two energy densities and this indicates that the bactericidal effect depend on the energy density (dose) and period (exposure time) according to equation (1).
- It is clear that this type of laser light with this high intensity has both photochemical and Photo thermal effects on the bacterial components which are play together a significant role in the lethality of bacterial cells.
- The most infection in the streptococcus mutans in the male.

References


