Evaluation of the effect of two types of laser on the growth of *streptococcus mutans*

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**Background and aims:** This study was done to compare the antibacterial effect of Photodynamic therapy (PDT) on *streptococcus mutans* (*S. mutans*) using two different light sources and photosensitizers (PS).

**Materials and methods:** Five groups were studied in this research: no light and no toluidine blue ortho (TBO) as PS for control group, irradiation only (CO2 laser or Nd:YAG laser), and irradiation with PS (CO2 laser and TBO or Nd:YAG laser and TBO). Standard suspensions of *S. mutans*, based on the type of group, were used in different PDTs. Bacterial suspension from each treatment was subcultured onto the surface of Mueller-Hinton agar plates, and bacterial growth was assessed. The results were analyzed by analysis of variance (ANOVA).

**Results:** There was a statistically significant reduction in the viability of *S. mutans* in TBO with CO2 laser and TBO with Nd:YAG laser groups (p value < 0.05). However, there was no significant difference between control and groups treated with lasers only. The highest number of the colonies of *S. mutans* in treated groups was observed in CO2 laser irradiation only and the lowest number was seen in CO2 laser with TBO. In the groups irradiated alone (without TBO), no significant reduction of colonies was observed. There was no significant difference between the experimental groups.

**Conclusions:** The colonies of *S. mutans* were susceptible to either CO2 laser or Nd:YAG laser in the presence of TBO with no significant difference. So these lasers with this photosensitizer may be useful in prevention of dental caries and antimicrobial treatment protocols.

**Key words:** *Streptococcus mutans* • Lasers • Photodynamic Therapy

**Introduction**

Orthodontic appliances are able to change the microbial flora of the mouth. Because it is difficult to maintain enough oral hygiene in orthodontic patients and keep the appliances clean, carious lesions are possible to increase 1). In fact, these lesions are caused by the activity of oral bacteria, including *streptococcus mutans* (*S. mutans*). These bacteria use carbohydrates in the mouth and produce organic acid and cause dental caries 2, 3).

In these conditions, the bacterial population, including the mutant bacteria increases. Altering the mineralization-deminerlization balance by decreasing oral PH and pushing it towards demineralization, which results in the formation of white spot lesions and subsequently causes cavitation in the teeth 4-6).

Anti-caries strategies are divided into two stages: prevention and treatment. The prevention stage includes restrictions on the consumption of sugar substances while at the same time observing the health by mechanical and chemical antibacterial agents 7, 8).

The treatment stage is also possible through traditional treatments, but due to the high limitations of these treatments such as mechanical damage to oral mucosa, alternative methods such as photodynamic therapy are suggested instead 9).

PDT is a therapeutic process that combines light and light-sensitive materials called photosensitizers (PS).

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When the light-sensitive agents are exposed to a certain wavelength of light, they are activated. As a result, the target cells exposed to PS and irradiation are destroyed by the formation of free oxygen radicals, which can alter or damage the vital element of the cells and ultimately lead to cell death \(^{10,11}\).

Antimicrobial PDT (a-PDT) is a local antibacterial method used to reduce bacterial contamination in oral infections. It is also a non-thermal and non-invasive method \(^{12}\).

PDT has many applications in dentistry, including treatment of oral cancer and bacterial, viral and fungal infections \(^{13}\).

Rolim et al (2012) investigated the antibacterial activity of various photosensitizers against \(S. mutans\) by analyzing the amount of oxygen produced. They studied PS such as methylene blue, toluidine blue ortho (with the same concentration of toluidine blue ortho (TBO) (0.1 mg/mL in this study), erythrosine, malachite green, rose Bengal and eosin. They reported TBO to be the most effective method in reducing microorganisms \(^{9}\). Zanin et al (2006) used TBO 0.1 mg/mL combined with light-emitting diode (LED) in order to achieve the load of \(S. mutans\). They also stated that the greatest effect on \(S. mutans\) was obtained with TBO at 0.1 mg/mL in combination with LED \(^{14}\). There would be other methods for PDT activation other than LED, like lasers which have been studied in a limited number of studies. Common lasers used in dentistry are Neodymium-doped yttrium aluminium garnet (Nd:YAG) laser and CO\(_2\) laser, whose antimicrobial effect on \(S. mutans\) was evaluated in the present study. The purpose of this study was to compare the antibacterial effect of PDT on \(S. mutans\) using two different light sources and photosensitizers.

### Materials and Methods

#### Testing microorganism and growth conditions

To prepare fresh bacterial cultures, \(S. mutans\) (PTCC 1683, Tehran, Iran) were inoculated in brain heart infusion (BHI) broth (Merck KGaA, Darmstadt, Germany) and incubated in an aerobic atmosphere at 37°C for 48 h. Then, a few colonies of fresh cultures were introduced into a tube containing 5 mL of sterile physiological serum, and an equivalent suspension of 0.5 McFarland was obtained. To ensure the opacity of \(1.5\times10^8\) bacteria per mL, we used spectrophotometer.

#### Light sources and light-sensitizers

In the present study, two different light sources and one photosensitizer were used, as follows:

- Toluidine blue O (TBO) powder (BIOCHEM Chemopharma, ZA Cosne sur loire, France) was dissolved in distilled water to obtain a clear and homogenous solution with 0.1 mg/mL concentration.

- The light sources for activation were as follows:
  1. CO\(_2\) laser (Daeshin Enterprise, Seoul, Korea;DS-40UB, 10.6 μm wave length, 20 Hz repetition rate) with output power of 2 W, within 20 seconds: 10-ms pulse duration, 10-ms of time off.
  2. Nd:YAG laser (FotoSan Stegnet, 1210 Ljubljana, Slovenia) with output power of 2 W, within 20 seconds: 10-ms pulse duration, 10-ms of time off.

#### Table 1: Laser Characteristics and Parameters

|                       | CO\(_2\) laser                              | Nd:YAG laser                               |
|-----------------------|--------------------------------------------|--------------------------------------------|
| Laser type            | Gas mixture                                | Solid state                               |
| Wave length           | 10600 nm                                   | 1064 nm                                   |
| Peak power            | 2 w                                        | 2 w                                        |
| Working mode          | Normal pulse                               | Short pulse                               |
| Pulse duration        | 10 ms                                      | 10 ms                                     |
| Repeat time           | 10 ms                                      | -                                          |
| Pulse repetition rate | -                                          | 20 HZ                                      |
| Beam delivery unit    | 7 joints articulated arm balanced with spring | Fiber optic delivery                      |
| Fiber delivery        | -                                          | 320 μm                                     |
| Hand piece            | Needle type                                | R21-A                                      |
| Tip diameter          | 0.6 mm                                     | -                                          |
| Area                  | 5.3066 cm\(^2\)                           | 5.3066 cm\(^2\)                           |
| Power density         | 0.3768 W/cm\(^2\)                         | 0.3768 W/cm\(^2\)                         |
S. mutans solution was prepared for five 24-well (6 mm diameters) flat bottom plates with black lids separately, as below:

**Group 1**: Control (no light, no photosensitizer)

**Group 2**: Laser CO2 + TBO (L CO2 + TB+)

**Group 3**: Laser Nd:YAG + TBO (L Nd:YAG + TB+)

**Group 4**: Laser CO2 (L CO2 + TB -)

**Group 5**: Laser Nd:YAG (L Nd:YAG + TB -)

In groups 2 (L CO2 + TB+) and 3 (L Nd:YAG + TB+), the tubes contained 200 μL S. mutans suspension and 200 μL TBO. In groups 1 (control), 4 (L CO2 + TB -) and 5 (L Nd:YAG + TB -), the tubes contained sterile phosphate-buffered saline (PBS) and 200 μL S. mutans suspension in order to equalize the level of all the wells. Then, specimens were kept in a dark environment for 5 minutes and were then exposed to irradiation. Laser therapy was done by one of the researchers and the radiation conditions were the same in both types of lasers. The laser devices were placed vertically against and level with wells. In order to prevent light transmission from one sample to another, the wells, as spaced apart with two empty wells and plates, were covered with a black shield with the same diameter of the wells. After irradiation, the plates were incubated overnight. The samples were diluted in PBS. In order to evaluate bacterial viability, Hinton Agar was incubated for 24 hours at 37°C in a partial atmosphere of 5% CO2. After incubation, the number of colony forming units per milliliter (CFU/mL) was determined [15].

**Statistical analysis**

The results were transformed into logarithm (Log 10) and analyzed by analysis of variance (ANOVA) test using SPSS statistical software (version 20). Statistical significance was defined as *p* < 0.05.

**Results**

The results of this study showed a statistically significant decrease in the life of S. mutans in TBO with CO2 laser and TBO with Nd:YAG laser groups (*p* value < 0.05). There was no significant difference between control (group 1) and CO2 laser only (group 4) or Nd:YAG laser only (group 5). The highest number of colonies of S. mutans in the treated groups was found in CO2 laser irradiation only (group 4) and the lowest number was seen in CO2 laser with TBO (group 2). In the groups irradiated alone (without TBO), no significant reduction of colonies was observed (Table 2 and Fig. 1).

As shown in Table 3, there is no significant differ-

| Groups                  | Mean ± Std | P value |
|-------------------------|------------|---------|
| 1: (control)            | 3.91 ± 3.60| -       |
| 2: (laser CO2 + TBO)    | 1.60 ± 1.90| 0.003 * |
| 3: (laser Nd:YAG + TBO) | 1.84 ± 2.12| 0.007 * |
| 4: (laser CO2)         | 2.84 ± 2.62| 1.000   |
| 5: (laser Nd:YAG)      | 2.34 ± 2.25| 0.268   |

*significant difference between study groups and control (*p* < 0.05)
ence between the experimental groups.

**Discussion**

PDT is a combination of visible light and PS. The light source is usually a laser or light-emitting diodes (LEDs) in PDT and toluidine blue, radachlorin, curcumin, etc. in PS. PS is a material that absorbs a certain wavelength of light and is able to convert it to useful energy. CO\(_2\) laser is high laser absorption, TBO was used as PS in this study.

PS is selectively absorbed by bacteria and cancer cells and is activated by light radiation with appropriate wavelengths. During this process, free radicals are fatal to microorganisms. Generated products can cause cell death through damage to essential cell components, or by causing irreversible changes in cellular metabolism.

TBO which was used in this study as Photosensitizer has the ability to absorb the maximum wavelength of 630 nm in the red light spectrum and to disable many bacteria. Due to its physical, chemical and hydrophilic properties, it can freely pass through bacterial membrane and can directly affect mitochondria.

Usacheva et al (2001) compared the efficacy of toluidine blue and methylene blue photobactericidal against gram-positive and gram-negative microorganisms. They found that TBO could bind to the polyphosphates in the outer membrane of the cells and damage the fats and proteins.

A lot of studies have been done to confirm the effectiveness of TBO as a PS in PDT. Owing to the antimicrobial effect of TBO and its high laser absorption, TBO was used as PS in this study.

The CO\(_2\) laser morphologically and chemically modifies the surface of the enamel and makes it resistant to demineralization. It can also lead to changes in the surface energy and disrupt the formation of biofilm. These changes interfere with the adhesion of bacteria to the tooth surface, thereby preventing the formation of primary caries.

Considering these properties, we used CO\(_2\) laser in this study. The main component of CO\(_2\) laser is carbo-dioxide. CO\(_2\) laser wavelength is 10.6 micrometers. The advantage of radiation with a long wavelength is that it is greatly absorbed by water and objects which contain water such as connective tissue. Because of its high absorption rate in water and organic matter, the CO\(_2\) laser beam has a shallow depth of penetration; 99% is absorbed within 0.2 mm. It can be used in dentistry in gingivectomy, frenectomy, root canal therapy and caries removal.

Nd:YAG is a crystal (Chemical formula Nd:Y\(_{3}\)Al\(_{5}\)O\(_{12}\)) that is commonly used as a lasing medium for solid-state lasers. The output of Nd:YAG laser is variable between 0 and 15 Watts. The output wavelength is then the typical 1064 nm. The advantage of this laser is its smaller size. Nd:YAG lasers can be used for teeth anesthesia, treatment of caries and periodontal disease.

In the present study, 10.6 \(\mu\)m CO\(_2\) laser and Nd:YAG laser with and without TBO were used to assess their antimicrobial effects on the growth of \(S.\) mutans. According to explanation above, CO\(_2\) laser and Nd:YAG laser were used as light sources in PDT due to the common use in dentistry and their effectiveness in preventing caries and TBO used as PS in PDT due to its antimicrobial effect.

TBO as a PS in present study, actually makes the target cell (\(S.\) mutans) more sensitive to light sources of radiation (CO\(_2\) laser and Nd:YAG laser) and increases their effectiveness.

The findings of the present study showed that the mean count of mutans bacterial colony in all groups of laser exposure (with and without PS) was reduced, but this reduction was significant only when lasers were used with TBO simultaneously.

Vahabi et al (2011) used TBO 0.1% as PS and diode laser with 633nm wavelength and 3 J/cm\(^2\) as light source for PDT. They reported that PDT with this PS and light source was effective in reduction of \(S.\) mutans. Although the types of lasers used in the present study were different, the results confirmed the effectiveness of light sources only in the presence of TBO.

In the present study, we found that Laser alone could not cause significant reduction in bacterial colony count, which is confirmed by similar studies.

**Conclusions**

The results of this study demonstrated that the colonies of \(S.\) mutans were susceptible to either 10.6 \(\mu\)m CO\(_2\) laser or Nd:YAG laser in the presence of TBO with no significant difference. On the other hand, these lasers with this photosensitizer may be useful in prevention of caries and other antimicrobial treatment protocols.

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