Factors Affecting the Antibacterial Effects of Nd:YAG Laser In Vivo

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Background and Objectives: One of the main advantages of laser surgery is it's bactericidal effect which reduces the risk of postoperative infections. Several study designs have been set to investigate this effect. Aim of this study was to research if the bactericidal effect of laser tool was affected from several factors in vitro studies.
Study Design/Materials and Methods: To determinate and investigate the bactericidal effect of laser in an original model, α-hemolytic streptococcus, *Bacterioides fragilis*, *Neisseria*, *Streptococcus salivarius*, *Staphylococcus aureus*, and *Candida albicans* were prepared in 10^4, 10^6 and 10^8 inoculum and placed in Mueller-Hinton Broth which have five different proportions of sheep blood. Samples which exposed with various energy levels of Nd:YAG laser were spread on agar plates, and at the end of an incubation time the colonization counted comparatively. The lowest energy level without colonization was accepted as minimal bactericidal energy level.
Results: Highest minimum bactericidal energy level is used for α-hemolytic streptococcus and lowest values for *neisseria*. Bactericidal effect decreased on suspensions, of which population of microorganisms are high and hemoglobin concentration was high in the broth.
Conclusions: These findings suggest that the Nd:YAG laser has a higher bactericidal effect when sheep blood is added to the media. Factors like population and type of bacteria in the irradiated suspension affect minimum bactericidal energy level. Lasers Surg. Med. 32:197–202, 2003. © 2003 Wiley-Liss, Inc.

Key words: amount of microorganisms; broth concentration; minimal bactericidal dose

INTRODUCTION

Early in the 20th century Einstein described three processes known as absorption, spontaneous emission, and stimulated emission. In 1958, Schwalow and Townes used Einstein's theory of stimulated emission, and with the advent of Bohr's theory and optical resonators, they described the principles of maser (microwave amplification by stimulated emission of radiation). This laid the groundwork for others such as Maliman who first observed stimulated emission in the visible portion of the spectrum by using an excited ruby rod, thus generating the first laser beam. Following the development of laser (light amplification by stimulated emission of radiation) considerable experimentation has been done to identify applications to dentistry [1–4].

The lasers most commonly used for soft tissue surgery are the CO_2 and Nd:YAG, both of which produce radiation in the infrared range of the electromagnetic spectrum. Nd:YAG laser with fiberoptic delivery of a pulsed 1,060 nm laser beam to a small hand piece had been designed especially for intraoral use. Nd:YAG lasers with 1,060 nm wavelength is the direct opposite of the CO_2 lasers in relationship to its tissue effects in that it is minimally absorbed but with maximal penetration [5,6]. It presents a number of clinical advantages for its use in oral and maxillofacial surgery including hemostasis during and after surgery, reduction in postoperative pain, decreased swelling and scarring, no suturing, reduced surgical time, reduced mechanical trauma to the tissues and bactericidal effect [7–9].

Laser irradiation, apart from conventional methods, had been shown to have the potential to eliminate bacteria. Saks and Roth had reported the eliminating effects of ruby laser on various protozoa [10]. Klein et al. using greater than 250 J had found that it inhibited growth of *Pseudomonas aeroginosa* but did not had any effect on *Staphylococcus aureus* [11]. However, when McGuff and Bell used ruby, Nd:YAG and helium-neon lasers on the same microorganisms found no inhibitor effect at all [12].

A black pigment, having a broad absorption spectrum, will absorb the electromagnetic energy produced by a Nd:YAG laser. It has been shown that a black dye acts as an initiator, increasing localized effects of the laser and it is possible to produce effective bacterial elimination without risk of damaging side effects. Rooney and Midda demonstrates that the pulsed Nd:YAG laser was effective in destroying heat resistant bacteria in laboratory model and in the presence of black Suomi ink bactericidal effect was produced in lower energy levels [13]. Schultz et al. showed enhancement of bactericidal effects of various lasers

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including Nd:YAG when an artificial stain like methylene blue was added to the environment [14].

The aim of this study was to investigate the effect of pulsed Nd:YAG laser irradiation to six different oral flora organisms in a specific laboratory model and to assess the minimum bactericidal energy level required to kill these bacteria. Secondarily, efficiency of use of erythrocyte suspensions as dye material to enhance bactericidal effect of Nd:YAG laser was investigated.

MATERIALS AND METHODS

Model Designed

Instead of various other studies that used inorganic dyes, since hemoglobin is a natural pigment, we used both whole sheep blood and peptic digest of sheep blood (BBL) to compare the laser's bactericidal effect to have similar in vivo environment. Sheep blood was collected to a blood-collecting bag (Kansuk laboratories, Sefaköy, Istanbul) that is used at our blood bank on the day of the experiments and was used on the same day. Each time blood of the same sheep was used. Both peptic digest and whole blood were diluted with Mueller-Hinton broth (Difco laboratories) (Table 1).

Organisms Studied

An overnight culture of α-hemolytic streptococcus, *Bactericides fragilis*, *Neisseria*, *Streptococcus salivarius*, *Staphylococcus aureus*, and *Candida albicans* strains was inoculated to Mueller-Hinton broth and incubated to a log phase for 4–6 hours. Using 0.5 McFarland equivalence turbidity standard (Remel), turbidimetric comparison was made to give approximately 1×10^8 bacteria/ml as initial inoculation. From this inoculation serial dilutions of 10^6 and 10^4 colony-forming unit per milliliter (cfu/ml) were also prepared.

One hundred microliter of different (10^8, 10^6, and 10^4 cfu/ml) standardized bacterial suspensions for six microorganisms were placed in each of the 96 wells of pre-sterilized microtiter plates. In order to avoid secondary irradiation of a suspension from neighbor well, each well was isolated externally. Before inoculation, the outer surfaces of the wells were painted with a non-transparent-black dye, which absorbs the laser irradiation, to shield the scattered laser energy from adjacent well.

Laser

Pulsed Nd:YAG laser (Dornier Medilas fibertom, Dornier Med. Tech. Berlin, Germany) was used for this study. Delivery system of laser beam was 600 μm bare fiber, which is commonly used in clinical practice. Distance between the fiber tip and bacterial suspension was 10 mm constantly.

TABLE 1. Blood Concentrations in Muller-Hinton Broth

1	%100 Mueller-Hinton broth
2	%75 Mueller-Hinton broth + %25 sheep blood
3	%50 Mueller-Hinton broth + %50 sheep blood
4	%25 Mueller-Hinton broth + 75 sheep blood
5	%100 Sheep blood (undiluted)

To irradiate one sample with different energy levels, same sample was placed into 32 different wells (100 μl per well). This inoculation was fulfilled for each sample. Each well of the same sample was irradiated once. The first well exposed at 5 J/cm^2 and the following wells exposed at increasing energy levels (with 5 J/cm^2 intervals) up to 160 J/cm^2. After irradiation, 10 μm of each irradiated samples were inoculated for 18 hours at 35°C. After the incubation colony count was performed. Minimal bactericidal energy levels were determined for each solution. The total energy delivered was the product of power level and period of irradiation $J = w \times t$. A control group was also prepared from non-irradiated bacteria suspension to observe normal bacterial growth.

RESULTS

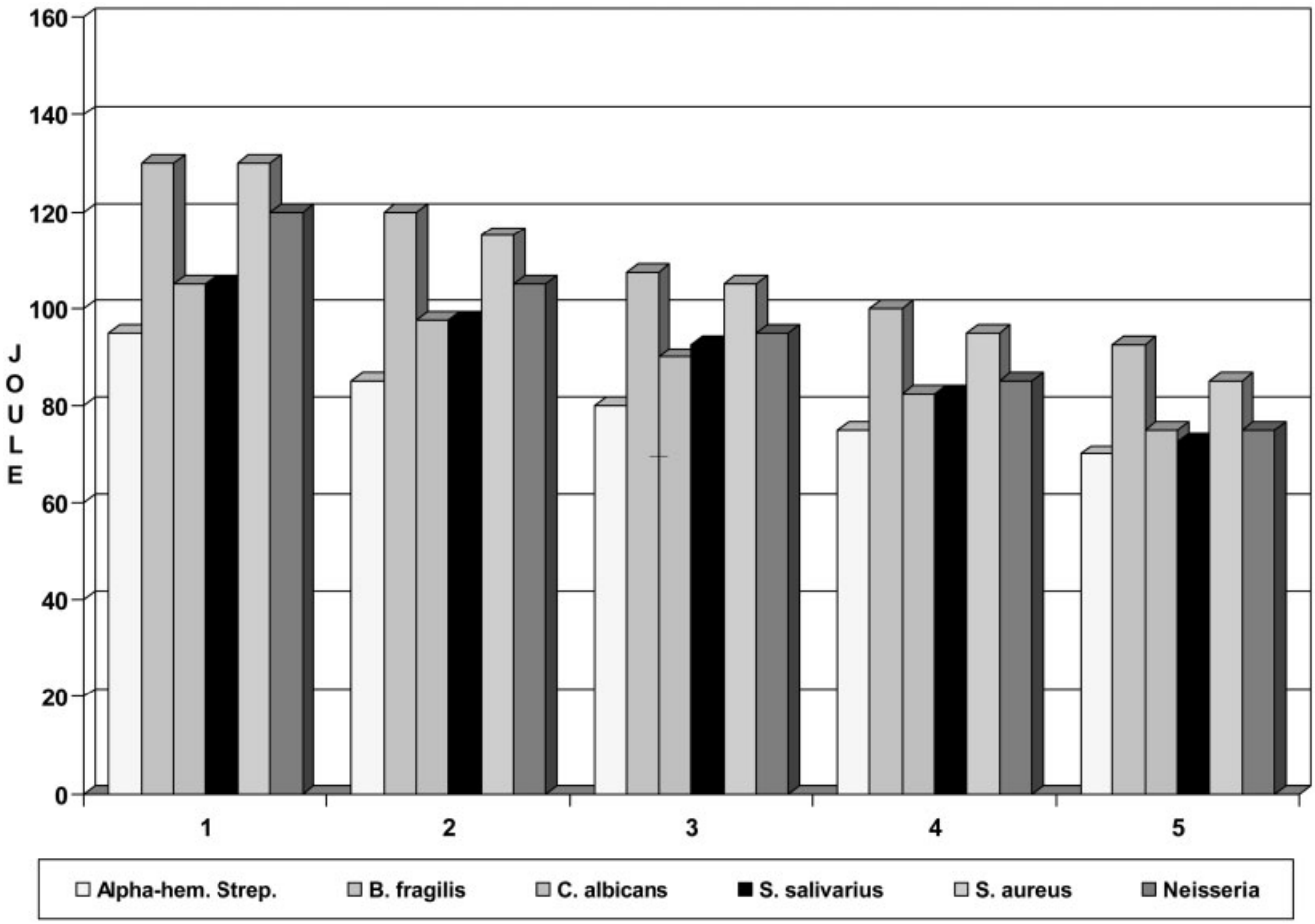
Table 2 summarizes the results and compares the effect of Nd:YAG laser irradiation on six different microorganisms with 10^4 count in the presence and absence of blood in the media. For all study organisms (α-hemolytic streptococcus, *Bactericides fragilis*, *Neisseria*, *Streptococcus salivarius*, *Staphylococcus aureus*, *Candida albicans*) the effect of the broth concentration on minimal bactericidal energy level is found statistically significant.

Table 3 shows the bactericidal effects of Nd:YAG laser on 10^6 number of microorganisms. A linear relationship between media sheep blood concentrations and minimal bactericidal energy level was observed and this relationship was also significant for microorganism numbers.

The bactericidal effects of applications with Nd:YAG laser for all microorganisms in the same joule power level at 4 and 5 levels of blood concentrations were statistically higher from other concentrations.

The minimal bactericidal energy level difference between 10^4 and 10^8 is found statistically when the data relating to the number of microorganisms were investigated. The higher population of microorganism needed the higher minimal bactericidal energy level of Nd:YAG laser. The effect of broth concentration on the applied energy level is statistically significant and in high hemoglobin concentrations bactericidal effect could be obtained with lower energy levels. Effects of these two parameters (concentration of bacteria and hemoglobin) are independent of each other. Similar results were found statistically significant for 10^8 bacterial inoculum (Table 4). Based on our results, it appears that the Nd:YAG laser has the potential to kill these six oral flora microorganisms and this effect is directly related to the amount of power, number of microorganisms, and concentrations of sheep blood in the media. Among these bacteria types *B. fragilis* required highest minimum bactericidal energy levels, while α-hemolytic streptococcus, *C. albicans*, *S. salivarius*, *S. aureus*, and *neisseria* could be eliminated with lower energy levels, respectively.

TABLE 2. Minimal Bactericidal Energy Level for Six Study Microorganisms in Five Different Broth Concentrations With 10^4 Bacterial Inoculum



DISCUSSION

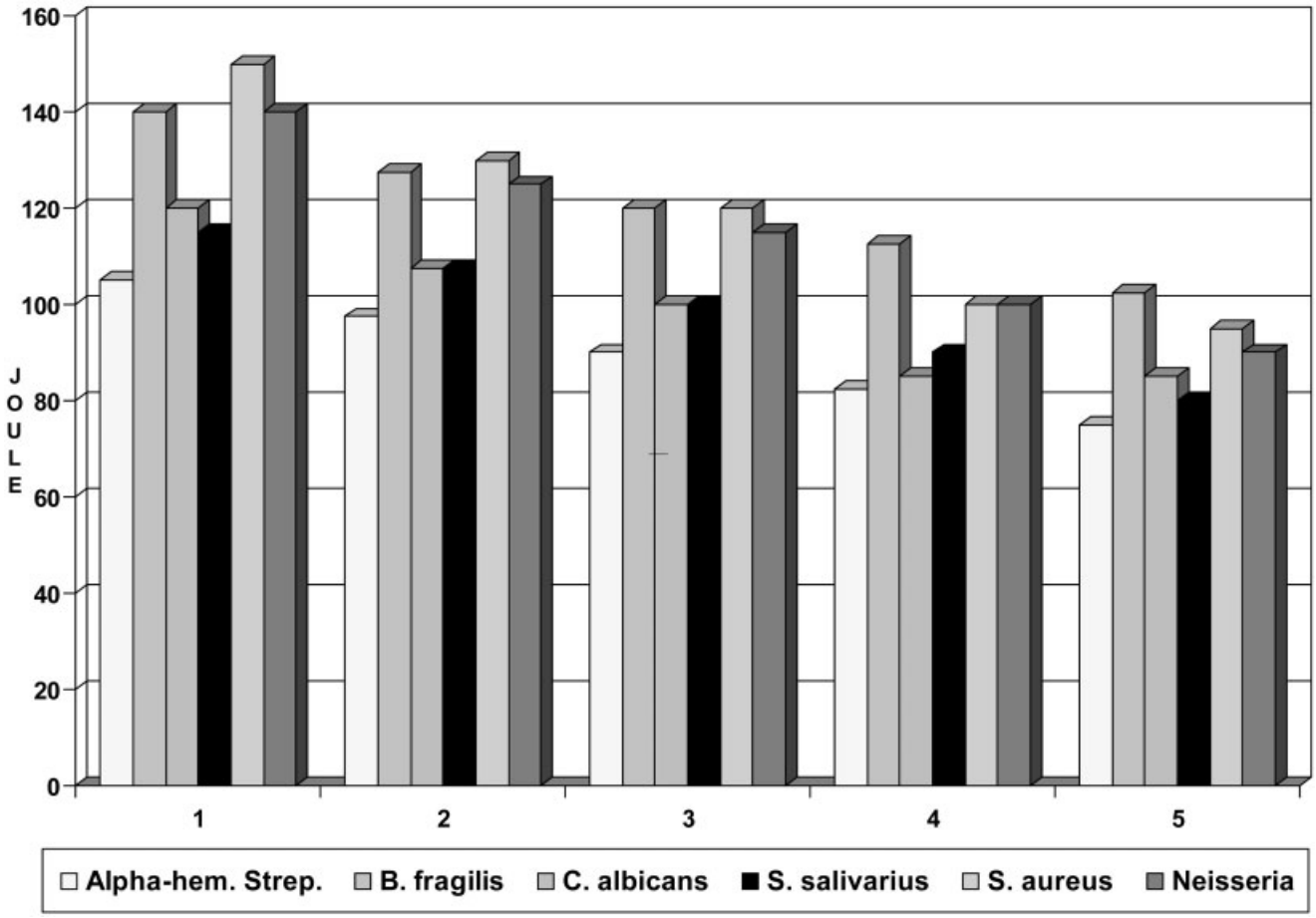
The most frequently used method to eliminate microorganisms from the wound surface is use of antibiotics and antiseptics. However, there is a need for alternative antimicrobial strategies, which could circumvent the problems associated with the use of these agents like development of resistance in the target organism or permitting the colonization of opportunistic pathogens. More recently, it has been suggested that high-power lasers, such as the Nd:YAG laser, which emit light in the infrared region may be useful for destroying such organisms, presumably by a thermal effect [2,15].

The ability of lasers to sterilize surfaces is well known. Adrian and Groos showed that the CO_2 laser could sterilize a scalpel blade that had been contaminated with spores [16]. The CO_2 laser has also been shown to be capable of sterilizing endodontic reamers [17]. Also, Powell and Whisenant showed the argon laser capable of sterilizing endodontic reamers at 120 J [18].

Although most species of oral bacteria do not absorb visible light and so are largely unaffected by such radiation, assimilation or absorption of a colored compound by these organisms can sensitize them to the visible light. When they are then exposed to light of an appropriate wavelength, absorption of photons by the sensitization results in its conversion to an excited triplet state. Photosensitizers such as haematoporhyrin, haematoporphyrin ester, and phthalocyanine, which are currently used in the photodynamictheraphy of tumors in man, gave disappointing results with the concentrations and exposure times employed. In the case of porphyrins, these results were not surprising, as most studies have shown that these compounds are not effective against gram (−) bacteria [19,20]. However, Wilson has shown that lethal photosensitization of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Actinomyces actinomycethemcommitans* was possible with haematoporhyrin ester [15].

Energy transfer from this to neighboring molecules can then result in the formation of reactive species, such as

TABLE 3. Minimal Bactericidal Energy Level Results for Six Microorganisms in Five Different Broth Concentrations With 10^6 Bacterial Inoculum



single oxygen, superoxide ions and hydroxyl and other radicals, which can damage and ultimately kill the cell [19,21].

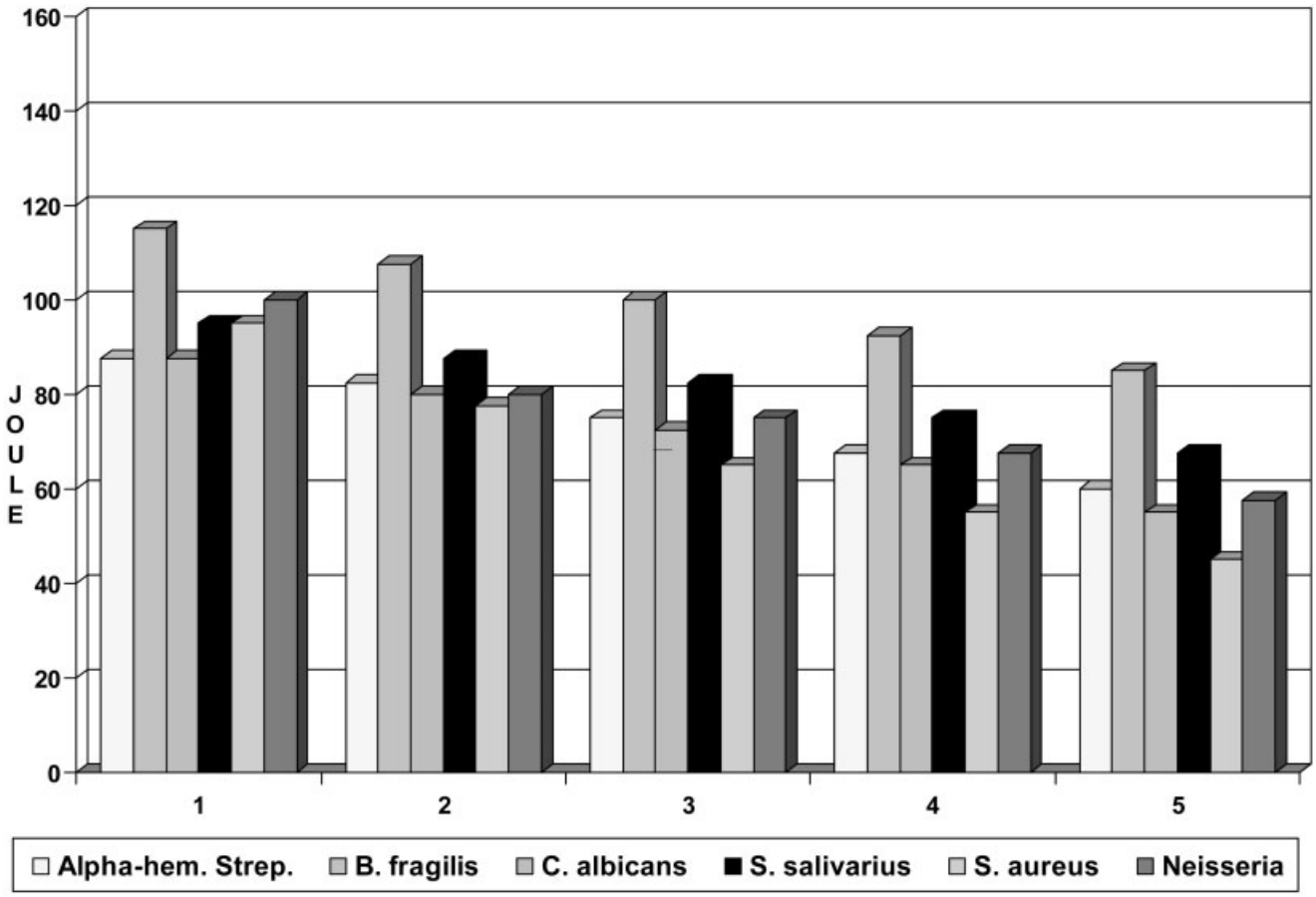
Mac Millan, Maxwell and Chichester, Dobson and Wilson have shown that dyes such as crystal violet, methylene blue, haemotoporphyrin HCI and toluidine blue, indigocarmine, arianor steel blue, aluminum disulphonade phthalocyanine can sensitize bacteria to killing by monochromatic light from a laser [21,22]. Dobson and Wilson used these agents as a photosensitizer to kill *Streptococcus sanguis*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Actinomyces actinomycethemcommitans* with He/Ne laser light [21]. They reported that bactericidal effect of He/Ne laser could be achieved at lower energy levels in the presence of dyes. However, these above mentioned dyes are artificial chemical agents and most of them are highly cytotoxic. Therefore, their clinical use to enhance the bactericidal effect of laser light is quite limited and can be hazardous for the patient. Thus results of in vivo studies that subject these artificial dyes have restricted clinical importance.

Instead of other cytotoxic artificial dyes, in our study both whole sheep blood and peptic digest of sheep blood, which mimic human erythrocytes, were used to compare the bactericidal effect of Nd:YAG laser light. By the way we established a laboratory setting, of which results are more meaningful for clinical use.

Schultz et al. performed Nd:YAG laser irradiation to a 0.5 ml bacterial suspension (1.0×10^8 cells/ml) in the wells. They reported that *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* exhibited decreased viability when exposed to energy density greater than 1,667 J/cm during a 10 seconds exposure [14]. However, in our study results showed that minimum bactericidal energy level for *Staphylococcus aureus* at same bacterial population (1.0×10^8 cells/ml) with previous study was 150 J/cm^2, which is approximately 11 times lower than Schultz's results. This is probably due to the difference between volumes of bacteria suspensions of two studies.

It is difficult to compare the results of our study with previous laser studies, because the laboratory settings and irradiation constants are quite different among papers. The

TABLE 4. Minimal Bactericidal Energy Level Measurements for Six Microorganisms in Five Different Broth Concentrations With 10^8 Microorganism Count



differences in the spot diameter, exposure time and distance between laser tip and target, and operating mode in a continuous or a pulse and the condition of the target organism in a bacterial colony or a suspension make it possible to compare the lethal effect of the laser by only their total delivered energy. However, comparing the results of our study with literature, the present study indicated that the Nd:YAG laser had a bactericidal effect and it was capable of killing both pigmented (sheep blood added Mueller-Hinton broth media) and non-pigmented (Mueller-Hinton broth) bacteria at the different energy levels. This antibacterial effect level is totally depended on environmental factors like microorganism count, type and pigmentation of tissue. High energy level is definitely bacterial but should be taken in to the consideration that in the same time it caused thermal damage. Antiseptic procedure would be expected with the use of Nd:YAG laser.

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