

COMBINATION EFFECT OF NITROGEN LASER AND CHLOROHEXIDINE ON THE VIABILITY OF *Pseudomonas aeruginosa*

Ayad G. Anwer

Institute of laser for postgraduate studies, University of Baghdad. Baghdad-Iraq.

Abstract

To evaluate the Combination effect of Nitrogen laser and chlorohexidine on the viability of *Pseudomonas aeruginosa* that isolated from burns, the samples of bacteria were irradiated with 337.1 nm Nitrogen laser using 5 and 10 pulses/second repetition rates , at 1 , 5, 10 ,15, 25 minutes exposure times . The irradiation was done with and without treatment with chlorohexidine respectively. The results showed that there is a noticeable effect of Laser on the viability of bacteria with the presence of chlorohexidine. The viability of bacteria decreased with increasing the exposure time and pulse repetition rate.

التأثير الجمعي لليزر النتروجين و الكلورهيكسدين في حيائية بكتريا *Pseudomonas aeruginosa*

الخلاصة

لتقييم التأثير الجمعي لأشعة ليزر النتروجين و الكلورهيكسدين في حيائية بكتريا الزوائف الزنجارية المعزولة من الحروق. تم تشيع عينات البكتريا بليزر النتروجين ذي الطول الموجي 337.1 نانومتر و بتكرارية نبضات : 5 و 10 نبضة / ثانية و لأوقات التشيع 1, 5, 10, 15, 25 دقيقة. وقد اجريت تجارب التشيع بوجود و عدم وجود الكلورهيكسدين . اظهرت النتائج بأن هناك تأثيراً ملحوظاً لأشعة الليزر في حيائية البكتريا بوجود الكلورهيكسدين و نقصان حيوية البكتريا عند زيادة زمن التعرض و زيادة معدل تكرارية النبضة

Introduction

Since the first report on laser radiation by maiman in 1960, many potential fields for its application have been investigated. Among these, laser application in biology certainly belongs to the most significant advances of our present century. Actually, various kinds of lasers have been used in many fields of biology and microbiology [1].

Several studies proved the significant effect of laser radiation with different parameters in reducing the viability of some bacteria with the presence or absence of dyes or photosensetizers. *Pseudomonas aeruginosa* is one of the most common causes of burns and wound infection . It can even infect clean surgical wounds, especially in people with impaired humoral immunity, and is especially troublesome in those with extensive burns several of its attributes

have been linked with virulence. Its over all effect is to produce tissue damage ,prevent healing ,and increase the risk of septic shock [2]. Many chemicals kill or prevent growth of microorganisms. Such chemicals have been called antimicrobial agents, and they have various applications [3].

critical factors that determine the effectiveness of antimicrobial agent against a particular organism. Microorganisms vary in their sensitivity to particular microbial agents.

The aim of this research is to evaluate the combination effect of nitrogen laser and Chlorohexidine on the viability of *Pseudomonas aeruginosa*

Materials and methods

A sample of bacteria was obtained from a patient with severe burn. Sterile swab was used for taking the sample from the burned skin. The isolate of bacteria were identified according to the microscopic examination (Gram-Negative-cocobacilli) and biochemical tests; *P. aerognoisa* give positive results for oxidase , catalase . Alkaline protease. and motile in the semisolid medium. The bacteria have the ability to ferment glucose and production of H₂S. the isolates give negative results to the indole, Voges-Proskauer and methyl red tests, while positive to citrate utilization test Another diagnostic test includes production of pyocyanine haemolysin and growth at 42C [4]. A colony of pure culture of the isolated bacteria was inoculated into tubes containing 5 ml of brain heart infusion broth. After incubation at 37C for 24 hours, serial dilution were prepared to get 90x10⁶ cell/ml. Pulsed nitrogen laser (Molectron UV24) was used in the irradiation experiments, this type of laser emits light In the ultraviolet region of the electromagnetic spectrum at 337.1nm wavelength, the system is operated with repetition rates between (1-50)pulse/second ,with 1milijoule pulse energy and 10 nanoseconds pulse width . The spot diameter of laser beam was 8mm.The samples of bacterial dilutions were divided into these groups : First group (control group) , bacteria without irradiation or treatment with Chlorohexidin (2%).Second group, bacteria treated with Chlorohexidin(2%). Third group, bacteria irradiated with laser beam with repetition rates 5, 10 pulses/sec for exposure periods:1, 5, 10, 15 and 25 minutes for each repetition rate without treatment with Chlorohexidin. Fourth group, bacteria irradiated with laser beam with the same parameters of the third group with treatment with Chlorohexidin.The irradiation was carried out with glass box (50cm*30cm*40cm)with a sterile cavity. After irradiation, the pour plate technique was used for enumeration the living bacteria. 0.1ml of bacterial suspension of each irradiated dilution and control group were mixed with melted agar in plates and cooled to approximately 42 to 45C.After incubation for 16-24 hours at 37C, the number of colony forming unit per mL (CFU) was obtained by the formula:

CFU/ml =Number of Colonies x dilution factor [5]

Results and discussion

The results of the effect of nitrogen laser with 5 pulses / second and chlorohexidine on the viability of *Pseudomonas aerugenoisa* are illustrated in figure (1) and data in table (1).The viability of bacteria decreased with the increasing of exposure time, that is when the bacteria were irradiated with laser only. It is clear that the value of Colony Forming Units/ml (CFU), decreased from 90x10⁶ cfu/ml in control to 70x10⁶ cfu/ml at 1 minute exposure time . 75x10⁶ cfu/ml at 5minutes exposure time . 63x10⁶ cfu/ml and 60x10⁶ cfu/ml at 10 ,15 minutes exposure times respectively and 55x10⁶ cfu/ml at 25 minutes exposure time.

Treatment of the bacterial solution with 2% chlorohexidene lead to decrease the viability of bacteria from 90x10⁶ cfu/ml in control to 40x10⁶ cfu/ml. The noticeable decreasing of the viability of bacteria was observed when the bacteria were treated with chlorohexidene and irradiated with laser. The value of cfu decreased to 30x10⁶ cfu/ml at 1minutes exposure time . 21x10⁶ cfu/ml and 18x10⁶ cfu/ml at 5,10 minutes exposure times respectively. No growth was seen at 15 and 25 minutes exposure times. Using 10 pulses/second pulse repetition rate, more noticeable effect on decreasing the viability of bacteria was seen. After irradiation the bacteria with laser only, the viability of bacteria decreased from 90x10⁶ cfu/ml in control to 60x10⁶ cfu/ml at 1 minute exposure time . 62x10⁶ cfu/ml at 5minutes exposure time . 54x10⁶ cfu/ml and 48x10⁶ cfu/ml at 10 ,15 minutes exposure times respectively and 40x10⁶ cfu/ml at 25 minutes exposure time . When the bacteria were treated with chlorohexidene and irradiated with laser, growth was seen only at 1 minute exposure time with 22x10⁶ cfu/ml No growth was observed at 5 , 10 , 15 , 25 minutes exposure times.

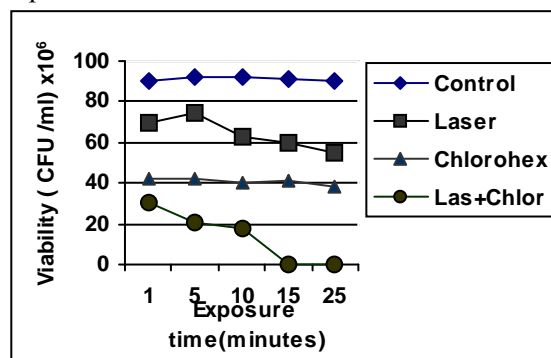


Figure (1): Effect of Nitrogen laser (5 pulses / sec) repetition rate and Chlorohexidine on Viability of *Pseudomonas aeruginosa*.

Table (1): Data of the Effect of Nitrogen laser (5 pulses / sec) repetition rate and Chlorohexidine on Viability of *Pseudomonas aeruginosa*

Exposure time	1	5	10	15	25
Group	minute	minutes	minutes	minutes	minutes
Control	90	92	92	91	90
Laser	70	75	63	60	55
Chlorohexidine	42	42	40	41	38
Laser + Chlorohexidine	30	21	18	0	0

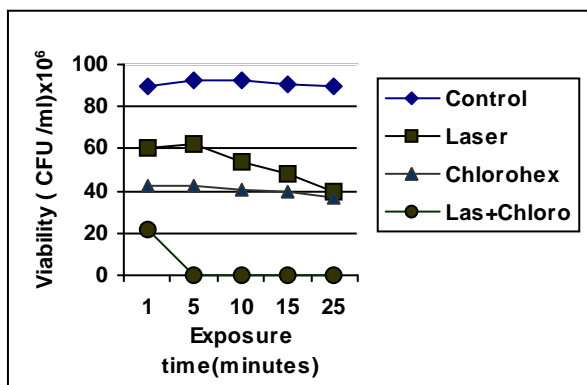


Figure (2): Effect of Nitrogen laser (10 pulses /sec) repetition rate and Chlorohexidine on Viability of *Pseudomonas aeruginosa*

Table (2): Data of the Effect of Nitrogen laser (10 pulses / sec) repetition rate and Chlorohexidine on Viability of *Pseudomonas aeruginosa*

Exposure time	1	5	10	15	25
Group	minute	minutes	minutes	minutes	minutes
Control	90	92	92	91	90
Laser	60	62	54	48	40
Chlorohexidine	42	42	41	40	37
Laser + Chlorohexidine	22	0	0	0	0

The most probable mechanism of the effect of nitrogen laser on the viability of bacteria is photochemical, taking into account the term of absorbing chromophore or Photoacceptor that have high absorbance at the wavelength of the applied laser light, the suggested chromophore is the reduced form of Nicotinamide Adenine Dinucleotide Phosphate NADP (H) that exhibits high absorbance at the wavelength of nitrogen laser at (337.1) nm..

Laser light at high doses (high repetition rates and long exposure times) increases the probability of bonds breaking between the atoms

of the chromophore due to absorption of high photon energy of nitrogen laser light that equals to 3.6 eV , so ,the bond with dissociation energy equal or less than this value undergo breaking.

Damaging of chromophore which is important in metabolic processes will lead to interrupting of cell activity and even death of the cell.

As it is clear, the effect increased with increasing the pulse repetition rate and exposure time, since the dose equals to energy density at certain exposure time at certain pulse repetition rate. Chlorohexidine is one of the phenolic compounds that destroy cell membrane and cause denaturation of protein. The combination effect of laser and chlorohexidine that lead to decrease the viability of bacteria to zero at 5 minutes exposure time with 10 pulses/second repetition rate , may be due to the accumulative effect of laser and chemical agent.

Studies have demonstrated this bactericidal effect of lasers with output powers of ≥ 6 mW directed toward pathogenic or opportunistic bacteria previously treated with a photosensitizing agent. It was found in a study that the irradiation of Bacterial suspension of *Pseudomonas aeruginosa* containing 50 μ g/ml of a photosensitizer, with He-Ne laser (632.8nm), resulted in a gradual reduction of the total viable count of the bacterial cells in laser doses (2.4, 4.8, and 7.2) J/cm², and no growth was found for 9.6 J/cm² when TBO (Toluidin Blue O), and MB (Methylen Blue) were used. Meanwhile the reduction in the viable counts for CV (Crystal Violet), and TH (Thionine) were fewer for the same laser doses[6].

Lasers operating in the UV region were used in the field of photoinactivation of microorganisms especially bacteria and viruses; for example, Gurzadyan et al. used a picosecond Nd: YAG laser 4W (266 nm) to inactivate viruses and bacterial plasmid. The 4W of Nd: YAG laser was used also to inactivate the yeast (*Candida utilis*) [7].

XeCl laser (308nm) was used to inactivate cells of *Escherichia coli* and to study the mutagenesis of excimer laser on bacterial cells [8].

In 2003 Nussbaum, et al shows that *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* when irradiated using a wavelength of 810 nm at irradiances of 0.015 W/cm² (0-50 J/cm²) and 0.03 W/cm² (0-80 J/cm²). This show exposure to 810 nm irradiation (0.03 W/cm²) could potentially benefit wounds infected with *P. aeruginosa*[9]. In 2004 Al- Nu'aimi found that the irradiation of

He-Ne laser (with output power= 3mW, and wavelength= 632.8 nm) to determine Laser Death Time (LDT) of *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* with three photosensitizers (Toluidin Blue O, Methylene Blue, and Crystal Violate) that the laser death time (LDT) of the most susceptible isolate of each bacterial species in presence of the most efficient photosensitizing dye (TBO) was performed. Survival percentages decreased with increasing the exposure time till 100% of killing was achieved. [10]

Institute of laser for post graduate studies,
University of Baghdad.

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