EFFECT OF DIODE LASER 805NM ON THE VIABILITY OF SOME TYPES OF GRAM NEGATIVE AND GRAM POSITIVE PATHOGENIC BACTERIA

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Abstract

The effect of diode laser radiation 805nm on the viability of some types of Gram negative and Gram positive pathogenic bacteria (*Escherichia coli, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus, Salmonella enteritidis* and *Bacillus cereus*) was studied. These types of bacteria were irradiated at 0.94, 2.01 and 2.76 Wat for 1 minute as an exposure time of laser radiation. The results showed that this type of laser radiation had an inhibition action on *E. coli, S. pyogene, B. cereus, and S. aureus* and a stimulation effect on *S. enteritidis and K. pneumonia*. In both cases, the effect was dose-dependent.

تاثير الليزر الثنائي الصمام 805 نانوميتر على حيوية بعض انواع البكتريا المرضية السالبة والموجبة لصبغة كرام

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الخلاصه

درس تأثير اشعة الليزر الثنائية الصمام على حيوية بعض انواع البكتريا المرضية الموجبة والسالبة لصبغة كرام (المسبحيات والمكورات العنقودية والعصويات وبكتريا القولون والسالمونيلا والكلبسيلا). حيث شععت هذه الانواع البكتيرية بـ(0.94 و2.01 و2.76) واط لمدة دقيقة واحدة كفترة تعرض للاشعاع. وقد بينت النتائج إن هذا النوع من اشعة الليزر يملك تأثير تثبيطي على المسبحيات, وبكتريا القولون والعصويات والمكورات العنقودية يزداد بزيادة الجرعة, وتأثير تحفيزي على السالمونيلا والكلبسيلا و يزداد أيضا هذا التأثير التحفيزي بزيادة الكثافة الضوئية.

Introduction

Laser, an acronym for **l**ight **a**mplification by **s**timulated **e**mission of **r**adiation, has several therapeutically physical properties, these are: **monochromaticity** (the ability of selectivity target chromophores with an appropriately single wavelength), **coherence** (the proper alignment of light wave, allowing high intensity to be focused over a small area), **compressibility** (the use of ultrashort pluses that delivers localized energy) and **collimation** (the transmission of parallel rays of light without divergence or loss of intensity as a distance increases, thus creating a spot size that is maintained over a wide distance) (1).

Since the first appearance in 1960, lasers have been used as a potentially useful light source for medical and biological application because they have many characteristics, which distinguish

them from conventional light sources as those properties mentioned before (2).

Diodes laser emitting in 780 – 905 nm of spectrum have a number of advantages over other lasers (compact size, electrical safety, simplicity of use), which makes them very promising for medical and biological applications (3).

Since there are many types of bacteria that have an important medical role in our life such as *Escherchia coli*, which is considered as the head of large bacterial family, Enterobacteraceae, the enteric bacteria, which are the facultative anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. The enterobacteriaceae are among the most important bacteria medically. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella, Shigella,* and *Yersinia*). Several other are normal colonists of human gastrointestinal tract (e.g. *E.coli, Enterobacter* and *klebsiella*) but these bacteria, as well may occasionally be associated with diseases of human (4). In the present study, some of them and others were subjected to the effect of low level laser radiation (805nm) from diode laser on their viability in vitro.

Materials and Methods

Biological model system

Isolates of pathogenic bacteria (*E. coli, Klebsiella pneumonia, Streptococcus pyogenes, Staphylo-coccus aureus)* were isolated from urine of urinary tract infected patients (UTI) (5,6,7). *Salmonella enteritidis* was isolated from stool of salmonellosis patients and *Bacillus cereus* was *i*solated from stool of patients with enterocolitis. After isolation and identification by using microscopic examination and biochemical testes was done by catalase test, manitol salt agar test, coagulate test and hemolysis test for Gram positive bacteria and indole test, Simmons citrate agar, urease test and motility test) for Gram negative bacteria (5,6,7,8,9) A single colony from each isolate was transferred from MacConky agar, blood agar, Staph 110 agar and nutrient agar culture respectively to brain heart infusion broth BHIB (Difco, England), which was centrifuged at 6000 r.p.m. for 10 min. Cell pellets were washed twice with physiological saline then mixed by vortex and resuspended in 5 ml of normal saline ([10).

Method of irradiation

After incubation of bacterial suspension for 16-24 hours at 37˚C, total viable count was carried out for the plates that contain 30-300 bacterial colonies.

Number of bacteria per milliliter was calculated as follows

CFU (colony forming unit) = Number of colonies × Dilution factor (10)

One ml of the suitable dilution of each bacterial suspension was put in an Eppendroff tubes and exposed to different power densities of 805nm of diode laser in dark, then these irradiated tubes poured again on the selective media for each type of bacteria and incubated for 24 hours at 37˚C for calculating the number of colonies and obtaining the CFU and compare it with control (Not irradiated) (10).

Laser model system

The CW diode laser (Eltech S.R., Italy) emitting laser light at 805 nm, IR light (light in the invisible spectrum below red from 700-2000nm) (11) and a thin flexible glass fiber with a diameter of 8mm (12), the output power of **(**0.94, 2.01 and 2.76) W and the exposure time was 1 minute. So the power densities according to the following equation:

Power Density = P / A

P = power

A = Area in cm^2

The power densities were 1.87, 4.0 and 5.49 W/cm^2 for 0.94, 2.01 and 2.76 W, respectively.

Results and Discussion

This *in vitro* study showed that the laser light at 805 nm have an inhibition action on the viability of some types of bacteria such as *E. coli, Streptococcus pyogenes, Staphylococcus aureus and Bacillus cereus*, where the viability of these bacteria decreased as the power density increases as shown in the figure below, especially for *Streptococcus pyogenes* and *E. coli*. While there was a full decrease or full inhibition action of laser light on the *Staphylococcus aureus and Bacillus cereus* from the first dose of laser radiation. This may be due to the damage of photoacceptors (cytochrom *bd*), where the photo-biological reaction involves the absorption of a specific wavelength by a functional photoreceptor molecule (13). The photoacceptor (cytochrom *bd*) take part in a metabolic reaction in a cell, after absorbing the light of the wavelength used for irradiation, this molecule assumes an electronically excited state

from which primary molecular processes can lead to a measurable biological effect in certain circumstances where one of the physical and / or chemical changes can occur as a result of photoexcitation of their electronic state:

1-Alteration of redox properties and an acceleration of electron transfer, this can cause folding of protein which influence the activity of enzyme.

2-A fraction of excitation energy may be converted to heat, which causes a local transient increase in temperature of the absorbing chromophores this may cause structural changes and biochemical activity such as activation or inhibition of enzymes.

3-The superoxide anion (O^{-}_{2}) production increase by activating electron flow in the respiratory chain and subsequently an increase in the concentration of the product of its dismutation,H_2O_2, in a cell results in multiple secondary responses.

4-Some respiratory chain components such as porphyrins and flavoprotiens (NADH dehydrogenase) can be reversibly converted to photosensitizers and the absorption of light quanta by these molecules is responsible for generation of singlet oxygen $^1O_2$ which is toxic to cell. These processes can cause the death of the cell and the death increase as the dose increase. The results of this study show agreement with (10) where the laser light at 632.8 nm caused an inhibition action on *Staphylococcus aureus* and *E .coli.*

From the other hand, the photo-control of cell metabolism from the view point of positive (stimulating) effect take place (15) as the results shown when *Salmonella enteritidis* and *Klebsiella pneumonia* irradiated with low level laser light of diode 805nm as it is illustrated in figure below where the effect of stimulation (i.e. the ratio of number of viable cells in irradiated and control samples) is increasing with increase of the dose. As mentioned previously the photoacceptor molecule absorbed light by its chromophores in different redox state causing physical or /and chemical changes when the IR light absorbed by oxidased CUA(one of the component of cytochrom *bd*) resulting in an increase in nitric oxide (NO) which regulates the activity of cytochrom *bd* where (NO) is an electron acceptor is competitor with oxygen for binding to reduced form of cytochrom (*bd)* oxidase.(NO) binding to the binuclear center requires the presence of an intermediate, so the laser light and activation of electron flow can reverse the partial inhibition of catalase center by (NO) and in this way the binding of O_2 increase and respiration rate increases .this is cause an increase of the oxidased form of CUB and subsequently the superoxide anion O^{-}_{2} production increased as well as its dimutation H_2O_2. These O^{-}_{2} and H_2O_2. Participate in photosignal transduction chain leading to increase of proliferation (14).

An analysis of the data "respiration stimulation - culture growth rate stimulation" and "respiration inhibition - growth rate inhibition" makes it possible to content that in both cases the same molecules can play the role of photoacceptors while the dose and the intensity of the light dictate the sign of the end macroeffect. (16). From this study we can say that a further of the dose cause destruction of photoreceptors which is accompanied by growth inhibition and cell lethality. The fact that irradiation of cells with laser light by the same molecules has both a positive effect (acceleration of cell division) and a negative effect (damage to intracellular systems and even the death of cells) can be explained as following: there are two processes involving one and the same primary photoprocess _ electronic excitation. One of them is the acceleration of the electron transfer in the redox pairs in some sections of the respiratory chain, and the other, the transfer of the excitation energy to oxygen to form $1O_2$ (as will be recalled, flavins and cytochromes are photosensitizers).

The results of this study agree with (17) where there was a stimulation effect of diode laser light 890 nm on *streptococcus mutans.*

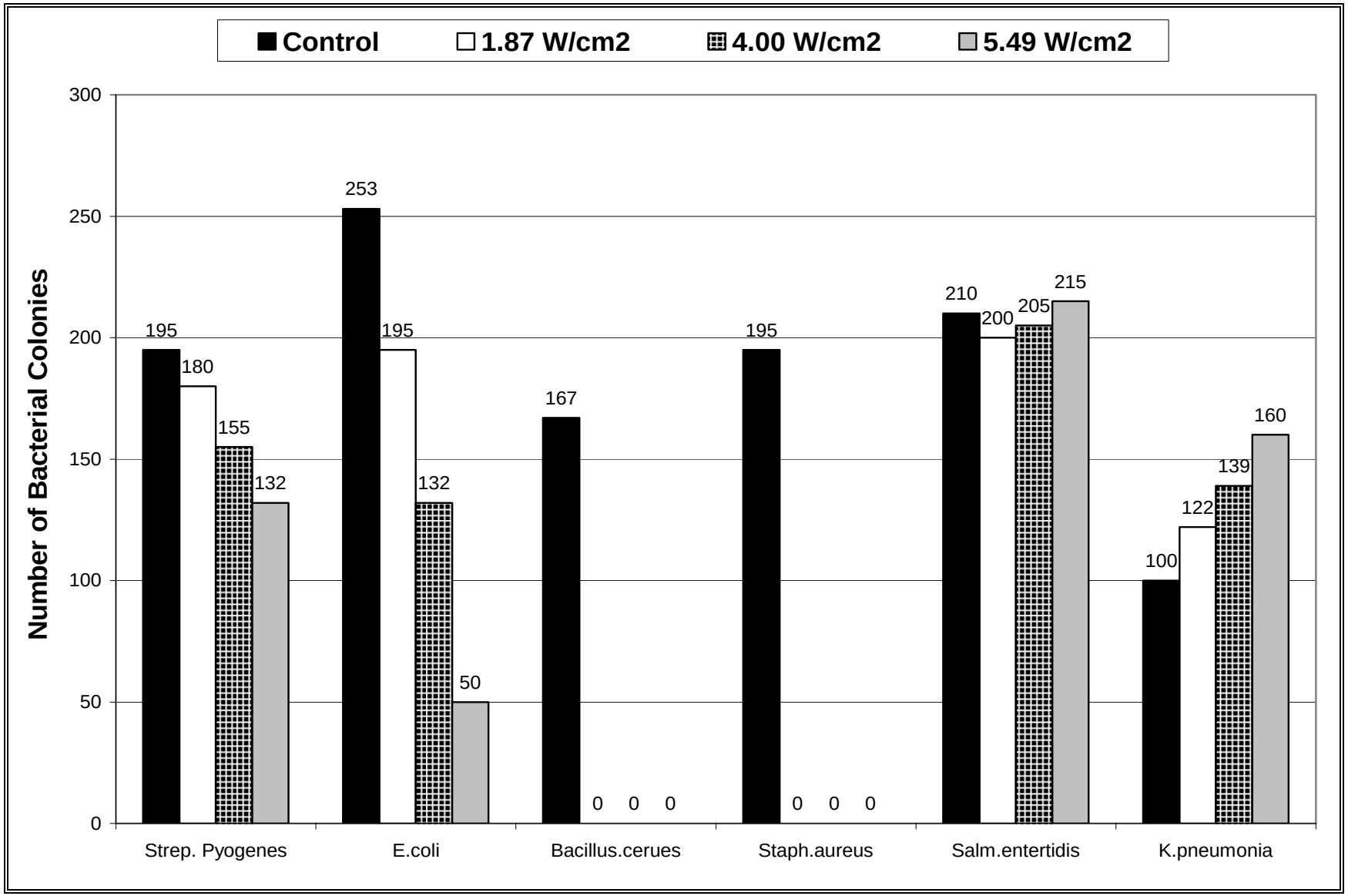

Figure: Effect of diode laser 805nm on different types of bacteria at different power densities.

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