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EFFECT OF CO_2 LASER ON PERI-IMPLANT INFECTIONS

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ABSTRACT

The purpose of this study was to assess the bactericidal effect of CO_2 laser 10,600 nm on (*S. oralis, S. aureus* and *S. epidermis*) causing peri-mucositis and peri-implantitis. Five pure bacterial isolates including, isolate for *Streptococcus oralis*, two isolates for *Staphylococcus aureus* and two for *Staphylococcus epidermis* were chosen out of 25 samples according to antibiotic sensitivity test to perform this study. Bacterial suspension (10^{-6} CFU/ml) was prepared by serial dilution method of each isolate then 100µl of each suspension was spread over agar plates before irradiation for the control groups and 100µl of each bacteria suspension was spread over agar plates after being irradiated with 10,600 nm CO_2 laser, CW mode emission using different power densities 500 -3000W/cm^2 (500 W/cm^2 increment) with different exposure times 10-60sec. (10sec. increment for isolating of *Streptococcus oralis*) and 5-30sec. (5 sec. increment for isolates of *Staphylococcus aureus* and *Staphylococcus epidermis*). All the plates were incubated at 37ºC for 24-48 hours. The colony forming units (CFUs) was counted and compared with the control group then the bactericidal effect of CO_2 laser was assessed in relation to the colony forming units of the control group.The maximum bactericidal effect of CO_2 laser on *S. oralis* was 100% at 2500W/cm^2 with exposure times 50 and 60sec. , while the CO_2 laser killed 100% of resistant *S. aureus*

and *S. epidermis* to antibiotic test of 3000W/cm^2 at 25 sec. for *S. aureus* whereas 2500W/cm^2 at 30 sec. for *S. epidermis*. However, with regard to the sensitive isolates the maximum effect of CO_2 laser on *S. aureus* was found at 2000W/cm^2 with all exposure times (5-25 sec.) while *S. epidermis* was appeared at 2000W/cm^2 when exposure time 20 second. The results revealed that irradiation by CO_2 laser CW mode emission may be useful in reducing the bacterial counts almost completely at low and completely at high power densities.

KEYWORDS: CO_2 laser irradiation, bactericidal effect, Peri-implant infection**.**

1. INTRODUCTION

The inflammatory lesions that appear in the tissues around implants are collectively defined as peri-implant diseases. [1] These conditions are classified into peri-implant mucositis where the infection is confined to the peri-implant soft tissue and peri-implantitis where the infection involved the alveolar bone support.[2] These lesions take place at a previously stabilized and integrated implants causing late biological complications .An adequate per mucosal seal of the soft tissue to the implant surface conserves the base of the sulcus against the penetration of chemical and bacterial substances. Although it has been found that the bacterial profile in the sulci around healthy implants resembles the microbiota associated with healthy periodontal tissues,[3] that are predominantly Gram-positive cocci such as *Streptococcus, Staphylococci* species and rods microorganisms.[4] and in smaller proportions Gram-negative bacteria for example *Prevotella intermedia* and *Porphyromonas gingivalis*.[5] but with the loss of this initial seal, differences in bacterial numbers and morphotypes can be found and the peri-implant mucosa may transmit from health to peri-implant mucositis and possibly to peri-implantitis.[6]

Dental implants, like natural teeth, are susceptible to inflammatory diseases that are predominantly driven by the accumulation of dental plaque.[2] The biofilm formation (biofilm) on oral implants may be identical to that seen on natural teeth, with the formation of pellicle and subsequent microbial colonization.[7] Biofilm is a very thin layer of micro-organisms that acts as an interphase between the surface of the implant and the initial microorganisms, for example *Streptococcus mitis, Streptococcus sanguis, Streptococcus oralis* and *Streptococcus gordonii*. [8,9] These bacteria create a series of prior conditions for the adhesion of periodontal pathogens likes *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, being able to induce the development of peri-implant infection.[10] The most bacteria associated with biofilm are *Streptococcus* species, which is the early

colonizer for the plaque formation and *Staphylococci* species, which is appeared within the oral cavity and their isolation from peri-implant infection, is evident. Both *Staphylococcus aureus* and coagulase-negative *Staphylococci* are frequently isolated and are responsible for infections associated with metallic biomaterials and indwelling medical infections.[11] More recently, *Staphylococcus aureus* has been demonstrated to have the ability to adhere to titanium surfaces. This may be significant in the colonization of dental implants and subsequent infections. [12]

The use of lasers for therapy has become very common to the medical field. In view of the recent advances and development of different delivery systems with a wide range of laser wavelengths, lasers can also be applied for dental treatments.[13]

Laser applications for the field of oral implantology had a considerable scientific interest throughout the recent years.[14,15] Lasers are expected to be one of the most promising new technical modalities for the treatment of dental implant diseases because they can perform excellent tissue ablation with high bactericidal and detoxification effects.[16]

Surgical lasers can be used in a variety of ways, starting from insertion, second stage recovery and gingival management to the treatment of peri-implantitis.[14,15] Lasers were proposed to the treatment of peri-implant infection, based on their successful application with positive results as an adjunctive or alternative treatment for periodontal diseases,[13] and it has been introduced as a potential alternative in reducing pathogens on implant surfaces.[17,18]

Now a day, Lasers have been expected to resolve the difficulties and problems of conventional mechanical treatment concerning periodontal problems. [13] Recently, the results from the published studies indicate that among all laser used in the field of dentistry only 10,600 nm CO_2 (carbon-dioxide) laser, 810, 980 nm diode laser and 2940 nm Er:YAG (erbium-doped: yttrium, aluminum and garnet) may be useful for the decontamination of implant surfaces because of their bactericidal effects. In addition, their specific wavelength is poorly absorbed by titanium and the implant body temperature do not increase significantly after laser irradiation, [19,16,20,21,23] due to their hemostatic properties and selective calculus ablation. [22]

The CO_2 laser has been used for decades in surgical procedures because of their speed and efficiency in cutting soft tissue.[31] The CO_2 laser also has minimal depth of penetration, thus the lateral thermal damage is less.[25.26]

2. MATERIALS AND METHODS

2.1 Bacterial samples

Twenty five samples were taken from the oral cavity of 20 volunteering human subjects complaining from peri-implant infections attended Al-Alweyia, the college of dentistry- Al-Mustansiriya and Al-Karkh dental implant centers in Baghdad, their age ranged from (22–52 year). The patients were not suffering from any systemic diseases like hypertension, diabetes mellitus and heart diseases.

All patients were subjected to clinical examination to detect the type of infection with signs like, redness of gum, swelling, suppuration, slight mobility of dental implant and symptoms like pain.

Collection of the samples were done by using a dental curette to collect the supragingival plaque, containing *Streptococci* species, which is the early colonizer for the plaque formation in the case of mucositis while in the case of peri-implantitis, each sample was taken using a paper point, inserted inside the space between the soft tissue and dental implant and left for 15 seconds to absorb the gingival crevicular fluid.[27] The samples were then transported to the laboratory in a transport medium (Brain Heart Infusion Broth), which helps to maintain the viability of the organisms. [28]

The purification of the primary bacterial isolates was done by culturing the collected samples on blood and brain heart infusion agar medium at 37 ºC for 24 – 48 hrs.in aerobic and anaerobic conditions according to the nature of the bacterial growth then the pure isolates of *S. aureus* and *S. epidermis* were incubated on selective medium (mannitol salt agar) aerobically for 24 hr.at 37℃ while the isolate of *Streptococcus oralis* was incubated anaerobically for 48 hr. at 37℃ in a candle jar (CO_2 5%) on brain heart infusion agar medium after that the plates of the medium were identified by microscopic examination and biochemical tests including catalase, coagulase tests and API kit strips (Analytical Profile Index) to confirm the identification of pure bacterial isolates.

The selection of microorganisms were according to the antibiotic sensitivity test, four pure bacterial isolates were selected from eleven isolates of *Staphylococci* species and one isolate was chosen from five *Streptococci* species which were used in the present work. The pure isolates were preserved in the refrigerator at -4 ºC until required for further study. A standardized suspension of bacterial growth with dilution of (10^{-6} viable cells/ml) was chosen from the other serial dilutions (stepwise dilution) for each isolate of *S. oralis*, *S. aureus* and *S. epidermis*. then 100µl of each suspension was spread over the surface of brain heart infusion agar plates (Merck, United Kingdom) before irradiation for control group then plates incubated aerobically or anaerobically at 37 °C for 24-48hrs.according to the natural growth of bacteria. Until the growth was visible, three replicates were used for each bacterial isolate.

2.2 Laser irradiation experiment

One isolate of *S. oralis*, two isolates of *S. aureus* and two isolates of *S. epidermis* were selected according to antibiotic sensitivity test. The laser system used in this experiment was CO_2 laser System, DS-40U, Daeshin Enterprise Co., Ltd., Korea) emitting at 10,600 nm.

2.3 Bacterial irradiation

Four hundered µl of each bacterial suspension was placed in the sterile eppendroof tube. The hand piece of CO_2 laser was perpendicular to the opening of eppendroof tube. The end of opening of the laser hand piece was placed in intimate contact (direct contact) with opening of eppendroof tube (Himedia, India). The sample was subjected to laser irradiation experiment using different power densities at different exposure times. In this experiment temperature of suspension was measured with thermocouple device. The irradiation experiments were done in a sterilized hood (Marubeni, Japan). Irradiated isolates were subjected to six power densities 500, 1000, 1500, 2000, 2500 and 3000 W/cm^2 with exposure times 10-60 sec. (10 sec. increment for isolate of *Streptococcus oralis*) and 5-30 sec. (5 sec. increment for isolates of *S. aureus* and *S. epidermis*). Then 100 µl of each suspension was spread over the surface of brain heart infusion agar plates (In the current study after irradiation there is no need to use the selective media for culturing because the bacterial isolates were already used from a pure culture). Then plates incubated aerobically or anaerobically at 37°C for 24-48hrs.according to the natural growth of bacteria. Until the growth was visible, three replicates were used for each bacterial isolate.

2.4 STATISTICAL ANALYSIS

The mean of colony forming units per milliliter CFU/ml were obtained from three replicates of each isolate was analyzed by using the SPSS statistical software package. The results were analyzed using one-way analysis of variance (ANOVA) test. The P values less than 0.05 were considered statistically significant. The data were presented as mean and standard deviation (S. D).

3. RESULTS

The statistical analysis was shown that there were statistical significant differences between the control group and the test group for all bacterial isolates.

3.1 Effect of CO_2 laser irradiation on the viability of *S. oralis*

The results have shown a reduction in the bacterial counts of the test group after CO_2 laser irradiation by using different power densities (500,1000,1500,2000 and 2500 W/cm^2) with different exposure times 10-60s (10 sec. increment) for *S. oralis* as compared to the control group (135 CFU/ ml). In the present study, the complete killing of *S. oralis* was noticed 100% when the exposure times increased to 50 and 60 seconds at the power density 2500W/cm^2 tab(1).

Table1. Effect CO_2 laser irradiation on the mean values of *S. oralis* and killing percentage at different exposure times

Control Group CFU/ml	Test Group CFU/ml						
	Power density	Time (sec.)					
		10	20	30	40	50	60
135	500	60.77 ± 4.38 a	59.23 ±3.62 b	71.53 ± 3.88 b	67.69 ± 1.54 b	56.15 ± 4.41 c	70.00 ± 1.42 c
135	1000	79.23 ± 4.12 a	100.00 ±0.0 a	98.46 ± 0.74 b	74.61 ± 5.40 b	83.85 ± 3.93 c	79.23 ± 2.62 c
135	1500	76.15 ± 3.42 a	68.46 ±4.75 b	79.23 ± 3.52 b	70.77 ± 4.50 b	81.54 ± 1.74 c	94.62 ± 1.09 c
135	2000	96.15 ± 0.87 a	64.62 ±4.52 b	90.00 ± 0.71 b	84.62 ± 2.84 b	96.15 ± 3.73 c	92.31 ± 2.42 c
135	2500	73.07 ± 4.65 a	78.46 ±1.47 a	95.38 ± 0.92 b	98.46 ± 0.83 b	100.00 ±0.00 c	100.00 ±0.00 c

a < 0.05 , b <0.01 , c< 0.001

3.2 Effect of CO_2 laser irradiation on the viability of *S. aureus* (resistant isolate to antibiotic test)

The present study displayed statistically significant differences between the test group and the control group. There was highly statistical significant differences ($P < 0.05$) observed among different parameters in the test group**.** A reduction in the mean value of *S. aureus* after irradiation with CO_2 laser was revealed in this study when compared with the mean value of the control group (86 CFU/ ml) before laser irradiation. High percentage of bacterial eradication was recorded 100% when the exposure time reached to 30 sec.at power density 3000 W/cm^2 tab(2).

Table2. Effect CO_2 laser irradiation on the mean values of *S. aureus* (resistance isolate) and killing percentage at different exposure times.

Control Group CFU/ml	Test Group CFU/ml						
	Power density	Time (sec.)					
		5	10	15	20	25	**30**
86	500	28.30 ± 2.18 a	43.03 ± 7.79 a	60.47 ± 1.74 b	48.07 ± 3.19 b	46.13 ± 1.99 b	23.65 ± 3.81 a
86	1000	64.95 ± 2.42 b	58.92 ± 1.18 b	56.59 ± 1.87 b	56.59 ± 2.14 b	73.65 ± 1.98 b	44.19 ± 6.14 a
86	1500	30.95 ± 9.18 a	68.99 ± 7.24 b	71.32 ± 3.44 b	63.57 ± 4.95 b	60.08 ± 1.17 b	39.15 ± 4.46 a
86	2000	45.35 ± 6.94 b	54.27 ± 1.76 b	57.76 ± 2.21 b	74.03 ± 9.78 b	84.89 ± 9.93 b	70.16 ± 3.79 b
86	2500	37.99 ± 8.09 a	53.11 ± 2.12 b	51.17 ± 4.69 b	73.26 ± 1.77 b	96.13 ± 2.05 c	98.86 ± 0.67 c
86	3000	43.80 ± 8.93 b	32.56 ± 3.95 a	68.99 ± 8.50 b	85.47 ± 3.37 b	99.22 ± 0.35 c	100.00 ± 0.0 c
a < 0.05 , b <0.01 , c< 0.001.							

3.3 The effect of CO_2 laser irradiation on the viability of *S. aureus* (sensitive isolate to antibiotic test)

The results of the present work demonstrated that there were remarkable statistical significant differences ($P < 0.05$) between different power densities and different exposure times after CO_2 laser irradiation when compared with control group (130 CFU/ml) for *S. aureus*. This behavior was observed to be dependent on various doses regarding the time and the power density. The killing 100% has been observed with increasing the exposure times tab(3).

Table3. Effect CO_2 laser irradiation on the mean values of *S. aureus* (sensitive isolate) and killing percentage at different exposure times

Control Group CFU/ml	Test Group CFU/ml					
	Power density	Time (sec.)				
		5	10	15	20	25
130	500	64.85 ± 8.67 a	63.85 ± 8.23 a	50.00 ± 5.07 a	96.92 ± 1.55 c	53.08 ± 3.28 b
130	1000	53.85 ± 4.66 a	43.08 ± 6.57 b	56.15 ± 2.97 a	64.08 ± 9.20 b	100.00 ±0.00 c
130	1500	50.76 ± 4.36 a	53.85 ± 2.82 a	70.00 ± 4.24 a	100.00 ± 0.00 c	100.00 ±0.00 c
130	2000	100.00 ± 0.0 c	100.00 ± 0.0 c	100.00 ± 0.0 c	100.00 ± 0.00 c	100.00 ± 0.0 c
130	2500	100.00 ±0.00 c	82.31 ± 0.44 a	100.00 ± 0.00 c	100.00 ± 0.00 c	100.00 ±0.00 c
130	3000	60.00 ± 5.74 a	60.00 ± 7.24 a	66.92 ± 5.75 a	94.62 ± 2.47 c	100.00 ±0.00 c
a < 0.05 , b <0.01 , c< 0.001						

3.4 The effect of CO_2 laser irradiation on the viability of *S. epidermis* (resistant isolate to antibiotic test)

The statistical differences between the mean values of *S. epidermis* (resistant isolate to antibiotic test) by using different power densities with different exposure time after irradiation by CO_2 laser when compared with the control group (53 CFU/ml) was found. There was high significant statistical difference ($p<0.05$) appeared between exposure times when the power density was considered a constant.

The highest killing percentage of CO_2 laser on *S. epidermis* was 100% when the exposure time increased to 30 sec.by using power densities 2500 and 3000 W/cm^2 tab (4).

Table4. Effect CO_2 laser irradiation on the mean values of *S. epidermis* (resistant isolate) and killing percentage at different exposure times.

Control Group CFU/ml	Test Group CFU/ml						
	Power density	Time (sec.)					
		5	10	15	20	25	30
53	500	47.17 ± 6.21 a	81.14 ± 6.30 b	62.26 ± 4.32 b	37.74 ± 4.75 c	54.72 ± 7.02 b	26.42 ± 3.59 b
53	1000	37.73 ± 4.38 a	73.58 ± 6.64 b	56.60 ± 8.24 b	41.51 ± 3.07 c	64.15 ± 1.69 b	43.40 ± 4.15 b
53	1500	15.09 ± 6.32 b	62.26 ± 8.19 b	64.15 ± 5.28 b	79.24 ± 5.81 b	83.02 ± 4.17 b	35.85 ± 7.03 b
53	2000	55.71 ± 3.58	62.26 ± 7.35	49.05 ± 4.75	66.04 ± 5.28	90.57 ± 5.13	81.13 ± 7.85

		a	b	b	b	c	c
53	2500	26.42 ± 4.28 b	18.87 ± 3.65 c	47.17 ± 4.30 b	88.68 ± 3.77 c	98.11 ± 1.09 c	100.00 ± 0.00 c
53	3000	41.51 ± 4.10 a	41.51 ± 4.57 b	77.36 ± 3.62 b	96.23 ± 2.8 c	96.22 ± 1.28 c	100.00 ± 0.00 c
a < 0.05, b < 0.01 , c < 0.001							

3.5 The effect CO_2 laser irradiation on the viability of *S. epidermis* (sensitive isolate to antibiotic test)

It is very clear from statistical analysis, that there were highly remarkable significant differences ($p<0.05$) in mean values of *S.epidermis* after irradiation with highest reduction in bacterial count in comparison with control group (166 CFU/ml). The killing percentage of *S. epidermis* reached into 100% at exposure times 20 and 25 seconds by using power densities 2000, 2500 and 3000 W/cm^2 tab(5).

Table5. Effect CO_2 laser irradiation on the mean values of *S. epidermis* (sensitive isolate) and killing percentage at different exposure times

Control Group CFU/ml	Test Group CFU/ml					
	Power density	Time (sec.)				
		5	10	15	20	25
166	500	87.95 ± 5.45 b	86.75 ± 5.13 b	88.55 ± 5.59 b	91.57 ± 2.44 b	91.57 ± 3.27 c
166	1000	89.16 ± 5.69 b	87.35 ± 6.63 b	92.17 ± 3.69 b	85.54 ± 7.43 b	92.77 ± 3.42 c
166	1500	86.75 ± 5.12 b	90.96 ± 6.03 c	90.36 ± 4.67 b	92.77 ± 3.75 b	89.16 ± 5.53 c
166	2000	94.57 ± 2.86 c	96.39 ± 1.59 c	95.78 ± 2.52 b	100.00 ± 0.0 c	94.58 ± 2.13 c
166	2500	98.19 ± 0.44 c	96.99 ± 1.57 c	96.99 ± 1.64 c	100.00 ± 0.0 c	100.00 ± 0.0 c
166	3000	96.39 ± 2.08 c	96.99 ± 1.59 c	99.40 ± 0.34 c	100.00 ± 0.0 c	100.00 ± 0.0 c
a < 0.05, b < 0.01 , c < 0.001						

3.6 Antibiotics susceptibility for all pure bacterial isolates

The antibiotics susceptibility of *S. oralis* showed that the bacterial isolate was sensitive to five drugs, which are: Tetracycline (TE), Penicillin (P), Ampicillin (AM), Clindamycin (DA) and Amoxicillin (AX) while being resistant to Metronidazole (MET) only.

On other hand the antibiotics susceptibility of *S. aureus* (resistant to antibiotics) has shown resistance to three antibiotics those were: metronidazole (MET), penicillin (P) and ampicillin

(AM) while *S. epidermis* has shown resistance to four antibiotics the same previously antibiotics with adding lincomycin (L). Whereas S. *aureus* and *S. epidermis* (sensitive to antibiotics) have revealed sensitive to six disks those were: lincomycin (L), Tetracycline (TE), penicillin (P), neomycin (N), ampicillin (AM) and amikacin (AK).

DISCUSSION

Generally, in the present work, there were variable percentages of killing for all bacterial isolates either resistant or sensitive to antibiotic test this may be explained due to virulence factors for each bacterial isolate.

Regarding to *Streptococcus oralis* the maximum percentage of killing reached 100% of power density 2500 W/cm^2 when exposure times increased in 50 and 60 seconds. On the other hand, the results revealed that the maximum percentage of killing by using CO_2 laser reached 100% for resistant isolates (*S. aureus* and *S. epidermis*) when the exposure time reached 30s. for *S. aureus* at power density 3000 W/cm^2 while for *S. epidermis* the maximum percentage of bacterial eradication was occurring when the exposure time increased into 30 sec. at power densities 2500 and 3000 W/cm^2. Whereas for sensitive isolates of *S. aureus* and *S. epidermis* the results have shown that there were variables maximum percentages of killing after irradiation by CO_2 laser. All these results were at a recorded temperature ranging from 45 -75 ºC. These results may be explained to the photo thermal interaction mechanism of a CO_2 laser. When the laser light is absorbed by bacterial cell wall, the photon energy of laser light is absorbed by bacterial cell structure (in which the water is main component affected) it will be converted into heat energy and the latter will lead to changes in the permeability of the cell wall or may affect enzymes, resulting in reduction of energy transfer within the bacterial cell and lead to cell immobility or may lead to denaturation of protein as a result the bacteria is killed.[29] So the heat resulting from the absorption of photon energy of CO_2 depending on the composition of tissue thus, temperature increases causing the bacterial cell to change in structure and composition.[30]

Generally, the results of present work revealed that the bacterial isolates which were more sensitive to antibiotics are more liable and showed higher response to the effect of CO_2 laser than the isolating which were resistant to antibiotics as a result of the weak virulence factor of bacteria and or may be due to a reduction in activity of bacteria.

High power densities with increasing the exposure times were needed for bacterial isolating of *S. aureus* and *S. epidermis*, which are resistant to antibiotic this may be due to the capsule

of bacteria which may prevent the penetration of CO_2 laser through the cell wall as capsule enhance microbial virulence.

There was no study found to be identical or approaches to the present work, except one related to isolate *S. aureus* used parameters different such as exposure time from those used in the current study. That study [31] revealed that the CO_2 laser could eliminate 100% of the *S. aureus* at 6W, 20 Hz and at 10 ms exposure time/pulse while 97% the bacteria was killed at power 4W, 20 Hz and at 10 ms exposure time/pulse and as well as at 2 W, 20 Hz and at 20 ms exposure time/pulse for the decontamination process. Whereas the present study revealed that the CO_2 laser could eradicate 100% of the *S. aureus* at power density 3000 W/cm^{2}and at at exposure time 30 second.

The results of this study showed that irradiation by CO_2 laser had maximum killing effect on the all bacterial pure isolates. The maximum effect of CO_2 laser on all bacterial isolates was reached when the duration of the exposure time was increased during irradiation.

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