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Bactericidal Effect of CO_2 Laser on Bacteria Associated With Dental Implant Infection: An In Vitro Study

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Abstract: One of the most popular causes for implant infection is dental plaque bacteria. Previous studies have shown the bactericidal effect of CO_2 laser irradiation on bacteria associated with soft tissue surrounding the implant materials. No published studies have examined the effect of irradiation by CO_2 laser on *Streptococcus oralis* and *Staphylococcus aureus.*The aim of this study was to evaluate the bactericidal effect of CO_2 laser on bacteria that are causing dental implant infections. This study was carried out on two isolates of bacterial species out of 25 samples, isolated from patients having soft tissue infections around the dental implant. These two pure isolates including *Streptococcus oralis* and *Staphylococcus aureus* were identified by microscopic examination, culture characteristics ,biochemical tests and API system. Bacterial suspension (10^{-6} CFU/ml) was irradiated with 10600 nm CO_2 laser,CW mode emission using different power densities 500 -3000W/cm^2 (500 W/cm^2 increment)with different exposure times 10-60s (10 sec.increment for isolate of *Streptococcus oralis*) and 5-30s (5 sec. increment for isolate of *Staphylococcus aureus*).After the irradiation, 100µl of bacterial suspension was spread over agar plates and incubated at 37 ºC for 24-48 hrs. under aerobic and anaerobic conditions according to the nature growth of bacteria. Colony forming units (CFUs) were counted and compared with control group then the bactericidal effect of CO_2 laser was assessed in relation to the colony forming units of control group.In this study the maximum bactericidal effect of CO_2 laser on *S.oralis* was 100% at 2500W/cm^2 with exposure times 50 and 60s, whereas the CO_2 laser eliminated 100% of *S.aureus* at 3000W/cm^2 at 25 and 30 s exposure time.The results indicate that irradiation by CO_2 laser CW mode emission may be useful in reducing bacterial colony forming units at low (such as 1000 W/cm^2) and high power density. Also the results of this study reveal that complete or nearly complete reduction in the bacterial counts may be achieved.

Introduction

The inflammatory lesions that appear in the tissues around implant are collectively defined as peri-implant diseases (Zitzmann and Berglundh 2008) and it takes place at a previously stable integrated implant and hence constitute a late biological complication (Rohit and Suchetan 2012). Dental implants, like natural teeth, are susceptible to inflammatory diseases that are predominantly driven by the accumulation of dental plaque, major early colonizer bacteria of dental plaque biofilm is *streptococci*(Jakubovics and Kolenbrander 2010),which provide adhesion for *Actinomyces* and *Fusobacterium.* These bacteria create a series of prior conditions for the adhesion of periodontal pathogens, being able to induce the development of peri-implantitis (Heuer 2007). 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 (Harris 2006).

Laser applications in the field of oral implantology have been of considerable

scientific interest throughout the recent years (Parker 2007).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 (Kreisler 2002). Surgical lasers can be used in a variety of ways, ranging from insertion, second stage recovery and gingival management to the treatment of peri-implantitis (Marotti *et al.*, 2008). Lasers were proposed for the treatment of peri-implant infections, based on their successful application with positive results as an adjunctive or alternative treatment for periodontal diseases (Ishikawa *et al.*, 2009), and it has been introduced as a potential alternative in reducing pathogens on implant surfaces **(**Ma´ximo *et al.*,2009). Now a days, Lasers have been expected to resolve the difficulties and problems of conventional mechanical treatment concerning periodontal problems (Ishikawa.*et al.*, 2009). The results from recently published studies indicate that among all lasers used in the field of dentistry only the CO_2 (carbon-dioxide) laser, the diode laser and the Er:YAG (erbium-doped: yttrium, aluminium and garnet), may be useful for the decontamination of implant surfaces. This is because of their bactericidal effects and because their specific wavelength is poorly absorbed by titanium. Aslo the implant body temperature does not increase significantly after laser irradiation(Romanos *et al.*, 2002, Kreisler *et al.*, 2002 ,Kreisler *et al.*, 2002).The latter is due to their hemostatic properties and selective calculus ablation(Marotti *et al.*, 2010, Stubinger *et al.*, 2005 and Marotti *et al.*, 2011).

Materials and Methods

Bacterial samples

The bacteria used in this study were *S.oralis* and *S.aureus* taken from the oral cavity of patients, complaining from infection of soft tissues around dental implant materials. The samples were collected using dental curate for collecting the supraginigival plaque (in case of mucositiis) as well as paper point, which inserted inside the space between the soft tissue and dental implant for absorbing the gingival crevicular fluid left for 15 seconds (in case of peri-implantitis). The samples were then transported to the laboratory in a transport medium, which helps to maintain the viability of the organisms. Bacterial isolates were cultured on brain heart infusion agar medium at 37 ºC for 24 – 48 hrs. In aerobic and anaerobic conditions. These bacteria were identified using microscopic examination, culture characteristic, biochemical test and API system. The pure isolates were preserved in the refrigerator at (-4 ºC) until required for the study.

Laser irradiation experiment

One isolate of *S.oralis* and *S.aureus* were selected according to antibiotic test.
The laser system used in this experiment was CO_2 laser System, DS-40U, Daeshin Enterprise Co., Ltd., Korea) emitting at 10600 nm.

Bacterial irradiation

Standardized suspensions of bacterial growth with dilution of (10^{-6} viable cells/ml) was chosen from the other serial dilutions for *S.oralis* and *S.aureus*. 400 µl of this suspension was placed in sterile appendorof tube. The hand piece of CO_2 laser was perpendicular on the opening of appendorof tube. 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. After irradiation, 100µl of the irradiated suspension was spread over the surface of brain heart infusion agar plates for each isolate. Then plates incubated aerobically and anaerobically at 37 °C for 24-48 hrs. according to the nature growth of bacteria. Until the growth was visible, 3 replicates were used for each bacterial isolate. The irradiation experiments were done in sterilized hood. Irradiated isolates were subjected to six power densities 500,1000,1500,2000,2500 and 3000 W/cm^2 with exposure times 10-60s (10s. increment for isolate of *Streptococcus oralis*) and 5-30s (5s. increment for isolate of *Staphylococcus aureus*).
The data were analyzed by using the available software statistical packages of SPSS, Microsoft office excel and least significant difference-LSD test. The number of colony forming units per milliliter CFU/ml can calculate manually from the following equation:
CFU/ml = No. of colonies x 1/dilution factor x10

The effect of CO_2 laser irradiation on the viability of *S.oralis.*

The results have revealed that there was a reduction in mean value of CFU/ml for *S.oralis* compared with control group (135 CFU/ml) as shown in figure (1).

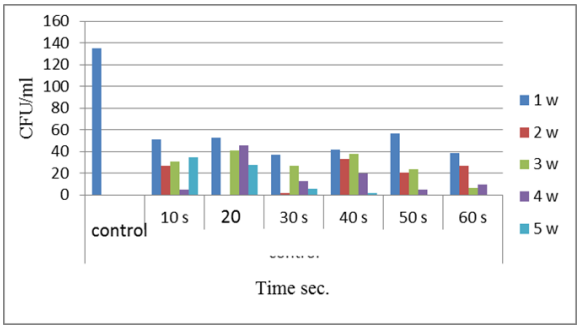

Fig. (1): The effect CO_2 laser on the viability of S. oralis using output power from 1-5 W (1W increment) with exposure times 10-60s (10s. increment) corresponding to power densities 500, 1000, 1500, 2000 and 2500 W/cm2.

According to the results of statistical analysis by using analysis variance of ANOVA and LSD test , it was found that there were statistical significant differences (P< 0.05) in the bacterial number (CFU/ml) between different power densities and different exposure times as shown in table (1).

Table (1): The effect CO_2 laser using different power densities at different exposure times compared with control group on CFUs of bacterial isolate *S.oralis*

Power	**Time (sec.)**						**P-Value**
	10	20	30	40	50	60	
1W	51.00 ± 6.08	53.33 ± 4.48	36.00 ± 5.29	41.67 ± 0.88	56.67 ± 10.86	39.33 ± 1.76	0.448 NS
2W	27.00 ± 5.51	0.00 ± 0.0	2.33 ± 1.12	33.33 ± 7.12	20.67 ± 5.20	27.33 ± 3.84	0.0025 **
3W	31.33 ± 4.05	41.33 ± 6.22	27.00 ± 4.35	37.67 ± 5.78	24.33 ± 1.20	7.33 ± 2.40	0.0042 **
4W	5.33 ± 1.33	46.00 ± 5.85	12.67 ± 0.88	20.33 ± 3.28	5.00 ± 2.21	10.00 ± 3.00	0.0031 **
5W	35.00 ± 5.68	28.33 ± 0.33	5.33 ± 1.20	2.00 ± 1.15	0.33 ± 0.14	0.00 ± 0.0	0.0049 **
P- Value	0.0036**	0.0026**	0.0041**	0.0062**	0.0014**	0.0013**	---

** (P<0.01) , NS: Non-significant.

In the present study, the CO_2 laser killed 100% of bacteria at 1000 W/cm^2 and 2500W/cm^2 for exposure times 20, 50 and 60s while it killed 94.62% of bacteria at 1500 W/cm^2 at 60s as well as 96.15% at 2000 W/cm^2 at exposure time of 50s, whereas the low percentage reached 71.53% at exposure time 30s when using power density 500 W/cm^2 as shown in table (2).

Table (2): Percentage killing of *S. oralis* after CO_2 laser irradiation

Power	**Time (sec.)**					
	10	**20**	**30**	**40**	**50**	**60**
1W	60.77 %	59.23 %	71.53 %	67.69 %	56.15 %	70.00 %
2W	79.23 %	100%	98%	74.61%	83.85 %	79.23 %
3W	76.15 %	68.46 %	79.23 %	70.77 %	81.54 %	94.62 %
4W	96.15 %	64.62 %	90%	84.64%	96.15%	92.31 %
5W	73.07 %	78.46 %	95.38 %	98.46 %	100%	100%

The minimum time that kills 99% of bacteria using 2500W/cm^2 was 49 sec. for *S.oralis* as shown in figure (2).

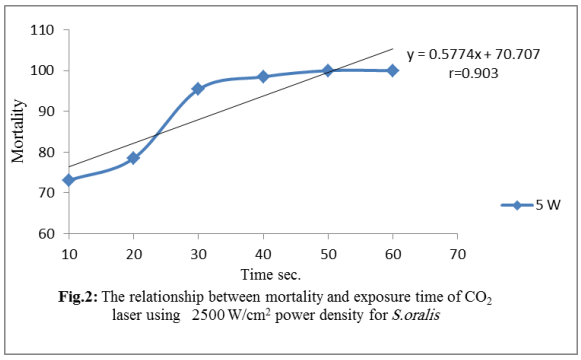

Fig.2: The relationship between mortality and exposure time of CO_2 laser using 2500 W/cm^2 power density for *S.oralis*

Fig. (2): The relation between mortality and exposure time of CO_2 laser using 2500 W/cm2 power density for S. oralis

Results the Effect CO_2 Laser Irradiation on the Viability of *S.Aureus*

The reduction in the mean value of CFU/ml for *S.aureus* after irradiation with CO_2 laser was observed when compared with mean value before laser irradiation (86CFU/ml). A reduction in viable number count was observed with increasing exposure times at different power densities as illustrated in figure (3).

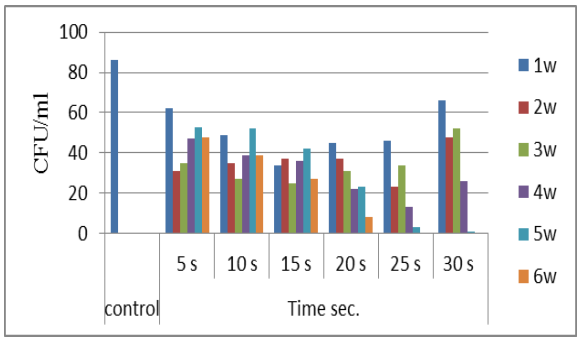

Fig. (3): The effect CO2 laser on the viability of S. aureus using output power from 1-6 W (1 W increment) with exposure times 5-30s (5s. increment) corresponding to power densities 500,1000,1500,2000,2500,3000 W/cm2 .

Significant differences (P< 0.05) were observed between different exposure times when power density is considered a constant (range of power density is considered a constant during the experiment work from 500 to 3000 w/cm^2 (500 increment), only the exposure time is a variable) as shown in table (3).

Table (3): The effect CO_2 laser irradiation at using different power densities with different exposure time compared with control group on CFUs of bacterial isolate *S.aureus*.

Power	Time (sec.)						P- Value
	5	10	15	20	25	30	
1W	61.67 ± 17.36	49.00 ± 15.30	34.00 ± 9.24	44.67 ± 11.34	46.33 ± 16.33	65.67 ± 3.28	0.035 *
2W	31.00 ± 2.08	35.33 ± 9.61	37.33 ± 9.35	37.33 ± 17.32	22.67 ± 17.18	48.00 ± 22.47	0.042 *
3W	52.50 ± 16.50	26.67 ± 6.23	24.67 ± 2.96	31.33 ± 12.86	34.33 ± 13.91	52.33 ± 3.84	0.0028 **
4W	47.00 ± 14.57	39.33 ± 9.26	36.33 ± 17.38	22.33 ± 9.41	13.00 ± 8.54	25.67 ± 11.86	0.0032 **
5W	53.33 ± 6.96	40.33 ± 18.17	42.00 ± 4.04	23.00 ± 1.52	3.33 ± 1.76	1.00 ± 0.57	0.0002 **
6W	48.33 ± 7.68	58.00 ± 12.00	26.67 ± 7.31	12.50 ± 11.50	0.67 ± 0.33	0.00 ± 0.0	0.0004 **
P- Value	0.049 *	0.027 *	0.043 *	0.002**	0.002**	0.0004**	----

* (P<0.05), ** (P<0.01).

The present study recorded high percentage of killing 100% at exposure time 25s at 3000 W/cm^2 while 98.84% at 2500 W/cm^2 for 30s, in addition to that 84.89% at 25s at 2000 W/cm ^{2} while 70.30% at 1500 W/cm^2 at 15s as well as 73.26% at 1000 W/cm^2 at 25s and 60.47% at 15s when using 500 W/cm^2 as shown in table(4).

Table (4): Percentage killing of *S.aureus* after irradiation with CO_2 laser

Power/area	Time (sec.)					
	5	10	15	20	25	30
1w	27.91%	43.02%	60.47%	47.68%	46.51%	23.26%
2w	63.95%	59.31%	56.98%	56.98%	73.26%	44.19%
3w	59.31%	68.61%	70.30%	63.96%	60.47%	39.54%
4w	45.35%	54.66%	58.14%	74.42%	84.89%	69.77%
5w	38.38%	39.54%	51.17%	74.26%	96.52%	98.84%
6w	54.19%	54.65%	68.61%	90.70%	100%	100%

The minimum time that kills 99% of *S.aureus* using 2500 and 3000 W/cm^{2}was 29.1 and 26.7 sec. respectively as shown in figure(4).

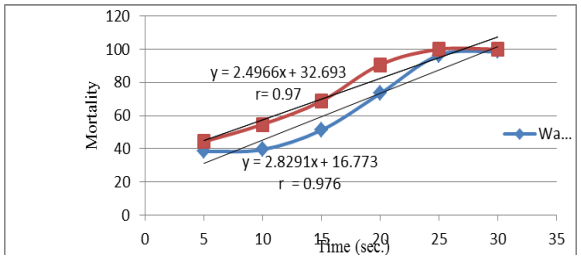

Fig. (4): The relation between mortality and exposure time of CO2 laser using (2500 and 3000 W/cm2) power densities for S.oralis

Conclusions

The results of this study showed that there are maximum effect of CO_2 laser on viability of pure isolates of bacteria .The percentage of killing reached 100% at 2500W/cm^2 at exposure times of 50 and 60s for *S.oralis* whereas 3000 W/cm^2 at exposure times of 25 and 30s for *S.aureus* as shown in tables (2 and 4*)*.The maximum effect of CO_2 laser on isolates revealed when increased the exposure times during irradiation. The temperature of suspension was 45 -75ºC as measured by the thermocouple device. The results may be explained as due to the photo-thermal interaction mechanism of CO_2 laser. The light is absorbed by the tissue the photon energy is converted to heat energy and hence the target tissue temperature increases. The energy is transferred to neighboring molecules, which in turn, quickly diffuse to an area much larger than initially irradiated one. The photon energy of laser light is absorbed by bacterial cell structure (main component water) then converted into heat energy and latter lead to change in the permeability of the cell wall or may be effected on enzyme, which resulting in reducing energy transfer within the cell and lead to cell immobility or may lead to denaturation of protein and the result bacteria is killed.

References

Harris LG, Mead L, Muller-Oberlander E, Harris L and Richards RG. (2006) .Bacteria and cell cytocompatibility studies on coated medical grade titanium surfaces.J Biomed MaterRes A. ,***78***:50-58.

HeuerW,Elter C, Demling A, Neumann A,Suerbaum S ,HannigM,et al.(2007) .Analysis of early biofilm formation on oral implants in man.J Oral Rehabil ,***34***:377-82.

Ishikawa I, Aoki A., AristeoA.Takasaki, MizutaniK. ,Katia M. Sasaki and Izumi Y. (2009). Application of lasers in periodontics: true innovation or myth, Periodontology 2000, ***50***, 90 126.

Jakubovics, N. S. & Kolenbrander, P. E. (2010).The road to ruin: the formation of disease-associated oral biofilms. Oral Dis ***16***, 729–739.

Kreisler M, Al Haj H, Gotz H, Duschner H, d'Hoedt B.(2002). Effect of simulated CO_2 and GaAlAs laser surface decontamination on temperature changes in Ti-plasma sprayed dental implants. Lasers Surg. Med , ***30***(3):233-9.

Kreisler M, Go¨ tz H, Duschner H.(2002).Effect of Nd:YAG, Ho:YAG, Er:YAG, CO_2 and GaAIAs laser irradiation on surface properties of endosseous dental implants. Int J Oral Maxillofacial Implants ***17***:202–11.

Kreisler M, Gotz H, Duschner H. (2002).Effect of Nd:YAG, Ho:YAG, Er:YAG, CO_2 and

GaAIAs laser irradiation on surface properties of endosseous dental implants.Int J Oral MaxillofacImplants ,***17***(2):202-11.

Ma´ximo MB, de Mendoncxa AC, Renata Santos V, Figueiredo LC, Feres M, Duarte PM. (2009). Short-term clinical and microbiological evaluations of peri-implant diseases before and after mechanical anti-infective therapies. Clin Oral Implants Res , ***20***:99-108.

Marotti J, Geraldo-Martins VR, Bello-Silva MS, de Paula EC, Apel C, Gutknecht N (2010). Influence of etching with erbium, chromium:yttrium-scandium-gallium-garnet laser on microleakage of class V restoration. Lasers Med. Sci. ***25***(3):325–329. doi:10.1007/s10103-008-0623-6 308 Lasers Med Sci , ***28***:303–309 .

Marotti J, Pigozzo MN, Nakamae EDM, Tortamano-Neto P, Laganá DC, Campos TN (2008). Terapiafotodinâmica no tratamento da periimplantite.Implant News, ***5***(4):401-5

Marotti J, Tortamano-Neto P, Campos TT, Aranha ACC, Weingart D, Wolfart S, Haselhuhn K. (2011). Recent patents of lasers in implant dentistry. Recent Patents Biomed Eng ***4***(2):103–105.

Parker S. (2007). Surgical laser use in implantology and endodontics. Br Dent J,202(7):377-86.

RohitKarnik, SuchetanPradhan,(2012).Peri-implant Disease—A Clinical Overview (Part 1):Diagnosis, Etiopathology and Risk-Related Aspects. International Journal of Laser Dentistry, January-April ,2(1):18-25

Romanos GE, Everts H, Nentwig GH.(2000).Effects of diode and Nd:YAG laser irradiation on titanium discs: a scanning electron microscope examination. J Periodontol ,71(5):810-5.

Stubinger S, Henke J, Donath K, Deppe H. (2005).Bone regeneration after peri-implant care with the CO_2laser: a fluorescence microscopy study.Int J Oral Maxillofac Implants 20 (2):203–210.

Zitzmann NU, Berglundh T. (2008).Definition and prevalence of periimplant diseases. J ClinPeriodontol ,35(Suppl 8):286-91.

التأثير القاتل لليزر ثاني اوكسيد الكاربون على البكتريا المصاحبه مع أصابة زرعة السن: دراسه خارج الجسم

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الخلاصة : واحد من اكثر الاسباب شيوعا لأصابة الزرعة هو اللوح البكتيري.قد بينت دراسات سابقه التأثير القاتل للتشعيع بليزر ثاني اوكسيد الكاربون على البكتريا المصاحبة للانسجة الرخوة المحاطة للمواد الزرعة. لم نجد هنالك دراسات منشوره درست تأثير التشعيع بليزر ثاني اوكسيد الكاربون على البكتريا العنقودية الذهبية والمسبحية. هدف هذه الدراسة كان لتقيم التأثير القاتل لليزر ثاني اوكسيد الكاربون على البكتريا التى تسبب أصابة زرعة السن . نفذت هذه الدراسة على عزلتين من الانواع البكتيرية من اصل 25 عينة،عزلت من مرضى عندهم أصابات بالانسجة الرخوة حول زرعة السن.هذه العزلات النقية تتضمن البكتريا العنقودية الذهبية والمسبحية فحصت بواسطة الفحوصات المجهري،الصفات المزرعية،الاختبارات البايوكميائية ونظام الابي . معلق بكتيري 10^{-6} شعع بطول موجي 10600 نانومترلليزر ثاني اوكسيد الكاربون ،طور الانبعاث المستمر باستخدام كثافات طاقة مختلفة 500 الى 3000 واط على سنتيمتر مكعب مع ازمان تعرض مختلفة من 10 الى 60 ثانية لعزلة البكتريا المسبحية و 5 الى 30 ثانيه لعزلة البكتريا العنقودية الذهبية. بعد التشعيع 100 مايكرولتر من معلق البكتيري نشر على اطباق اكار وحضن عند 37 درجه مئوية بظروف هوائية ولا هوائية وفقا لطبيعة نمو البكتريا.عدد تكوين المستعمرة حسب وقارن مع مجموعه السيطرة ثم قيم التأثير القاتل لليزر ثاني اوكسيد الكاربون مع عدد تكوين المستعمرة للمجموعة السيطره. التأثير الاقصى القاتل في هذه الدراسه لليزر ثاني اوكسيد الكاربون على البكتريا المسبحية كان 100 % عند كثافه طاقية 2500 واط على سنتيمتر مربع مع زمن تعرض 50 و60 ثانيه، بينما أزال ليزر ثاني اوكسيد الكاربون 100 % من البكتريا العنقودية الذهبية عند3000 واط على سنتيمتر مربع عند زمن التعرض 25 و30 ثانية. تشير النتائج بأن التشعيع بالليزر ثاني اوكسيد الكاربون بطور انبعاث مستمر ربما يكون مفيد في تقليل اعداد تكوين المستعمره البكتيرية عند الكثافه الطاقية العالية والواطئة (مثلا كثافة طاقية 1000 واط على سنتيمتر مربع). وأيضا" نتائج هذه الدراسه تكشف عن تخفيض كاملة او شبه كاملة في اعداد البكتريا ويمكن ان يتحقق.