Original Article

The Effect of Carbon Dioxide (CO_2) Laser on Sandblasting with Large Grit and Acid Etching (SLA) Surface

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Abstract:

Introduction: The purpose of this study was to investigate the effect of 6W power Carbon Dioxide Laser (CO_2) on the biologic compatibility of the Sandblasting with large grit and acid etching (SLA) titanium discs through studying of the Sarcoma Osteogenic (SaOS-2) human osteoblast-like cells viability.

Methods: Sterilized titanium discs were used together with SaOS-2 human osteoblast-like cells. 6 sterilized SLA titanium discs of the experimental group were exposed to irradiation by CO_2 laser with a power of 6W and 10.600nm wavelength, at fixed frequency of 80Hz during 45 seconds in both pulse and non-contact settings. SaOS-2 human osteoblast-like cells were incubated under 37° C in humid atmosphere (95% weather, 5% CO_2) for 72 hours. MTT test was performed to measure the ratio level of cellular proliferation.

Results: The results indicated that at 570nm wavelength, the 6W CO_2 laser power have not affected the cellular viability.

Conclusion: CO_2 laser in 6w power has had no effect on the biologic compatibility of the SLA titanium surface

Keyword: CO_2 laser; titanium; SLA

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Introduction

Dental implants enjoy high success in the partial or complete toothlessness[1]. However, within a five-year period of time, up to 14.4% of the dental implants have had signs of inflammation of the surrounding tissues together with bone loss[2]. A multicenter study including 151 patients and 588 implants indicated that within the 2nd and 3rd years after implants embedding, some 2% of the implants have failed, which occurred mainly in patients who suffered from high levels of aggregation of microbial plaque[3]. Infection of the tissues around the implants which is accompanied with progressive bone loss during treatment is known as Peri-implantitis[4].

For the time being, there is not enough evidence available to support any certain treatment strategy for Peri-implantitis[5,6]. In all the treatment methods, the primary purpose is to remove the microorganisms from the surface of the implants and its disinfection, so that the bone may be in contact with the surface of the

implants and reosseointegration made possible[5]. One of the problems which occur following cleaning the implants surface and Peri-implantitis is the change of the surface characteristics and loss of biological compatibility of titanium surface[7,6]. Such change, results in the close and global shapes presence of the osteoblast cells without any links and pulling of the cells besides titanium[8].

A variety of treatments for disinfection of the implants surface have been proposed. Metal curette and ultrasonic tools cause major changes on the implants surface, without being able to completely remove the microorganisms[9,10]. Although plastic curette generates the least destructive effects on the surface of the titanium, it may not remove the contamination of the implants surface[10-12]. Air polisher has successfully been applied to remove the contamination of the implants; however, the risk of emphysema as well as change in the implants HA coverage area limits its application[12,13]. Applying various antimicrobial agents is effective in the early stages of the disease, while using subgingival irrigation with local disinfector and local antibiotic therapy have limited applications and their long term results are unknown[14-16]. Various studies have shown the efficiency of laser irradiation in reducing the pathogen bacteria from the implant surface[17,18]. Various studies have shown that the Carbon Dioxide Laser (CO_2) application enjoys proper capability in removal of the bacteria from the surface of the implants without changing the superficial characteristics of the titanium and its biologic compatibility[19,20]. However, although CO_2 laser may be considered as a useful laser in this field, it may also be destructive as well, if not being used correctly and with suitable parameters. CO_2 laser physical characteristics may generate a variety of reactions on the surface of titanium as well as neighboring tissues[21]. Temperature changes during CO_2 laser irradiation indicate an increase in the temperature up to 50°C simultaneously with the increased CO_2 laser power and irradiation time. Therefore, it has been expressed that utilization of the CO_2 laser shall be under time limit, so that the implement and bone may be cooled down. Studies have shown that using CO_2 laser in the pulse mode produce less heat in comparison to the continuous mode on the implants[8]. On the other hand, other studies specify that continuous CO_2 laser has no undesirable effects on the chemical characteristics of the titanium surface[8,22,23]. Using the CO_2 laser shall be done at certain power, in a way that while having the capability to remove the microbial plaque and contamination from the implants surface, prevent any damaging to the implants surface and increasing its heat[22]. Considering the aforementioned findings, it is necessary that any kind of CO_2 laser undesirable effects on implants surface be studied prior to its application in the treatment of peri-implantitis. Using the CO_2 laser with low power and short irradiation time, though not harming the implants surface, may lack enough efficiency to remove the microbial plaque and disinfect the implants surface[23]. On the other hand, using high power laser may be useful in removal of plaque and disinfection of the implants surface, but may change the titanium structure and affect undesirably its biologic compatibility characteristics, which are part of the principal conditions for successful osseointegration[22]. Therefore, the purpose of this study was to compare the effect of various powers of CO_2 laser on the biologic compatibility of the Sandblasting with large grit and acid etching (SLA) surface titanium discs through studying the Sarcoma Osteogenic (SaOS-2) human osteoblast-like cells survival rate[21].

Methods

In this experimental study (performed at the department of biology of Kharazmi University), sterilized titanium discs were used together with SaOS-2 human osteoblast-like cells. The titanium discs are of SLA (Sandblasting with large grit and acid etching) type, which were used in making the dental implants. Some 14 titanium discs (2.5 × 10 mm) were used, while 6, 6 and 2 discs were respectively considered as group 1, group 2 and control group.

CO_2 Laser

6 sterilized SLA titanium discs of case group 1 were exposed to emission by CO_2 laser with a power of 6W, while the same was performed for case group 2 with a power of 8W and 10.600nm wavelength, fixed frequency of 80Hz for 45 seconds in non-contact manner. Irradiation was performed at a distance of 7mm from the top of the disc with 90 degrees emission angle, stain focal diameter of roughly 2mm and using sweeping movements. 2 sterilized SLA titanium discs of the control group were not exposed to laser irradiation. Following laser irradiation, all the discs were sterilized using autoclave and placed in 12-cell wells.

Cell culture

SaOS-2 (osteo-sarcoma cells) are of immortal cells provided by Pasteur Institute and were used to study the cellular survival rate on the titanium surface. The cells were incubated under 37°C in humid atmosphere (95% weather, 5% CO_2), while the culture medium included Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Germany) 100U/ml, streptomycin-penicillin 100 µg/ml, 10%FBS. The culture mediums were changed 3 times a week. These cells were removed from the culture medium after the cellular reproduction in the third passage from the cultured cells by taking benefit from Trypcin-EDTA sterilized solution and suspended inside the MCcoys 5A environment containing 10% FBS, 1% streptomycin, 1% penicillin (Gibco, Germany), and transmitted to the 12-wells plates by micropipette with a density of 2 × 104 cell/well containing titanium discs and the aforementioned culture medium was added for 0.5ml to each well containing titanium disc and cell. Wells were incubated for 72 hours under 37C in humid atmosphere (95% weather, 5% CO_2).

Determination of the cellular reproduction and survival rate

In order to do such measurement, the MTT (Method of Transcriptional and translational) test was used. MTT test is applied to measure the level of ratio of the cellular reproduction and performed based on the colorimetric method principled on the regeneration and breaking blue Formosan crystals, and enjoys a sensitivity of more than 98%. After completion of incubation, the MTT test was performed as following:

First of all the top culture medium was disposed and each of the wells containing the titanium discs and cells were washed completely using sterilized PBS, and so the cells were stripped. After that, 100 environment lamdas containing MTT (10 lamda 5mg MTT/1ml (PBS) + 90 lamda DMEM) were added to each well and placed into the incubator containing CO_2 for four hours under 37C. During incubation, MTT was regenerated by the Succinate dehydrogenase. Regeneration and breaking of such enzyme loop resulted in production of blue and non-soluble crystals of Formosan inside the cellular cytoplasm, which were quite obvious under microscope. After 4 hours, the liquid on each well was again removed and washed using sterilized PBS and then 100 lamda isopropanol acidic 0.4% together with HCl were added to each well. Incubation was performed for 10 minutes under room temperature. Alcohol caused slippage of the cellular cytoplasm and extraction of the insoluble Formosan from the same and solved the formed color which resulted in coloring of the environment. Eventually, the optical absorption of the final solution in 630nm as a reference wavelength and 570nm as the measurement wavelength were recorded to ensure the accuracy of the results in the Elisa Reader machine.

Statistical Plan

In order to finalize the study findings, the statistics which could be calculated for each of the quantitative variables applied in the study included: average and standard deviation; the ANOVA test was used as well.

Results

In this study, some 8 samples were studied (6 and 2 titanium discs were considered as experimental group and control group, respectively). The viability of the SaOS-2 cells in the studied groups have been given in table 1.

Considering the above table, having 570 nm wavelength, regarding group 1 (6W), the viability was 0.24±0.11, which was not statistically significant in comparison to the control group (0.33 ± 0) ($p < 0.7$). These results indicated that at 570nm wavelength, the 6W CO_2 laser power have not affected the cellular viability.

Discussion

Various laser systems have been used in the literature on various titanium surfaces. However, choosing the correct laser parameters for a certain level of implant to treat the infections around it is a difficult task. Therefore, the purpose of this study was to examine the effect of CO_2 laser irradiation in two 6 and 8W powers on the biologic compatibility of the SLA titanium surface, which is one of the most common titanium surfaces modifications in

Table 1. Cellular viability of the SaOS-2 cells as per the wavelengths and CO_2 laser power

Result of ANOVA test	Rate	Group	Wavelength
0	03±0.33	control	570
P<0.6	46	0.11±0.24	6wat	570
P<0.7	1.8	0.03±0.17	control	630
P<07	28.6	0.04±0.14	6wat	630

dental implant. The use of cell culture has made it possible to study the biologic compatibility of the surfaces. Various studies have shown the efficiency of the SaOS-2 cells culture to evaluate the effects of laser emission on the biologic compatibility of the titanium surface[8,11,17]. Although the use of mechanical methods for debridement of the implant surface such as plastic courts, rubber cap or chemicals are considered reliable and acceptable methods regarding protection and prevention of titanium from damage, but such methods lack efficiency in complete removal of bacteria and germs[8]. Another problem of these means is the uneasiness in accessing the implant surface, while the non-contact lasers, e.g. CO_2 laser, are recommended due to the easy application and high efficiency in removal of the microorganisms for disinfection of the implant surface. Meanwhile, in various studies, CO_2 laser efficiency in removal of the contamination as well as not changing and damaging the implant surface has been proved[19,20]. In vivo animal studies have shown good prospective results for reosseointegration after disinfection of the surface of the implant by CO_2 laser[24]. In this study, we used relatively high powers of 6 and 8W of CO_2 laser in pulse and non-contact mode on the SLA titanium surface, although implant surface changes were not studied by electronic microscope, even in case such changes happened, they had no negative effect on the SaOS-2 cells behavior and titanium biologic compatibility. However, in the in vivo conditions, the other important aspect of the CO_2 laser is the prevention of damage to the tissues around the implant and bone and eventually the increasing heath following laser irradiation, i.e. laser settings shall be in a certain level so that in addition to enjoying enough efficiency in removal of microbial plaque and disinfection of the titanium surface, not damaging the titanium superficial characteristics and its biologic compatibility, it does not increase the heat in the tissues around the implant more than the threshold. It has been expressed in various articles that CO_2 laser has no considerable absorption by the metal surfaces and is highly reflected, therefore following laser irradiation, the temperature in the implant and its surrounding tissues will not increase and has no negative effect on the reosseointegration[20]. In case of application of 6 and 8W powers, it is better to keep the surface moisturized, as they showed that the 8W power of CO_2 laser in pulse mode generates 3°C less heat on the moisturized implant surface, while such level is clinically acceptable (treating Peri-implentitis). Meanwhile, they emphasized that while applying CO_2 laser, the time limit during the irradiation shall be observed, so that the bone and implement may be cooled down. Romanos et al[8], following a revision of the articles related to the application of CO_2 laser in treatment of implant surfaces, recommend using 2-4W power of laser. In confirming such issue, Oyster 21) has reported that CO_2 laser in 2,4W powers generates partial heat increase under threshold level in continuous mode, while doing the same for 6W power in pulse mode (F-20 Hz)[21]. Kreisler et al[17] also showed that upon applying CO_2 laser, the time limit during irradiation shall be observed so that the bone and implant may be cooled down. They also expressed that CO_2 laser in 1-4W powers caused superficial changes in the form of making the SLA surface shiner, which resulted from superficial melting. Park et al[20] reported that CO_2 laser application in the pulse mode and non-contact form in 1,2W powers resulted in no change in the implant surface, notwithstanding the fact whether the surface is smooth or resorbable blast media (RBM). However, in 3.5, 5W powers, partial changes appeared in the form of tiny grooves and micromachine grooves[20]. It is probable that in the applied powers (6,8w), there is an increase in the melt and change of the titanium surface, while in that study the titanium superficial changes have been studied using electron microscope (SEM).

Romanos et al[8] expressed that CO2 laser emission in continuous mode up to maximum 7W results in no change in the sand blasted titanium surfaces of titanium plasma-sprayed (TPS), hydroxyl-apatite coated (HAC) and has no negative effect on the cells adhesiveness[8]. Some other scholars, e.g. Kato[19], recommend using CO_2 laser with 5W power to remove the microbial contamination without harming the implant surface and confirm the bactericidal effect of this laser[19]. Unlike the aforementioned scholars, Deppe et al[25] indicated that no changes were observed in the TPS implants and that the application of very low powered laser (2.5W) has enough efficiency to disinfect the surface of the implant[25]. Results of Romanos et al[8] study also approves CO_2 laser efficiency in 2W power to remove the microorganisms considerably from the titanium surface. The recent findings have shown that generation of morphologic changes on the titanium surface by laser result in an improvement in the titanium biologic compatibility in contrast with the surface changes through sandblasting. Cei et al[26] found that biologic compatibility and morphology of the SaOS-2 cells on

the surface of titanium engineered by laser emission improves in comparison to the SLA titanium surface. In approving this issue, it may be said that it seems that laser has caused certain changes in the surface of titanium resulting in improving in the biologic compatibility. We concluded that CO_2 laser in 6 and 8w powers for 30 seconds do not result in a change in the SLA titanium biologic compatibility[26]. Study was performed under in vitro mode, and therefore has had certain limitations. In vitro studies which may imitate the clinical state conditions is recommended, especially the other aspects of the CO_2 laser effects, e.g. heat changes, in various settings of CO_2 laser and superficial changes of titanium simultaneously. A correct protocol to apply the laser shall be generated for use in peri-implantitis, undesirable superficial changes of titanium as well as heat damages of the bone are prevented and the success chance in treating the infections of the tissues around the implement are increased.

Conclusion

CO_2 laser in 6w power has had no effect on the biologic compatibility of the SLA titanium surface.

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