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Effects of Er: YAG, 980 nm and 810 nm Diode Lasers Irradiation on Biocompatibility of SLA Titanium Disks Using SaOs2 Cells Morphology

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Objectives The use of lasers for the treatment of periimplant hard and soft tissues is now refmore than ever before. Achieving bone integrity. The aim of this study is to evaluate the effects of Er:YAG, 980 nm and 810 nm diode lasers irradiation on biocompatibility of SLA titanium disks by SaOs2 cells morphology.

Methods In this in-vitro study sixty sterile titanium disks with SLA surface were divided into four equal subgroups. One subgroup was used as a control group and the remaining three groups were irradiated with Er:YAG laser 980nm and 810nm Doide lasers, separately. After laser irradiation, all discs were autoclaved at 121° C and placed in 24 appropriate plates. The SaOs2 cells were, then, added to the plates at a density of 2×104. The cells were incubated in DMEM, CO2, and penicillin-streptomycin medium at 37 ° C for 3 days. Then, the samples were extracted from the culture medium for scanning electron microscopy. Finally, the photograph was taken by SEM at magnifications of 750, 1000, 3000, and 5000. The analyses were performed through Kruskal-Wallis and Mann-Whitney tests.

Results All three groups, irradiated by laser, and the control group have shown spreading cells with plentiful phylopodia, which means the morphology of a mature bone cell. The numbers were 20.7% (Er:YAG), 52.7% (980nm Diode), 48.8% (810nm Diode) and 38.7% (Control) groups, respectively, which were not statistically significant.

Conclusion Er:YAG, 980nm and 810nm diode laser irradiations with the parameters mentioned in this study do not have any negative effects on osteoblast cells attachment and their maturity on titanium implants.

Keywords Dental Implant, Osseointegration, Lasers, Laser Therapy

Introduction

Dental implants, like natural teeth, get infectious disease of soft and hard tissues around them[1]. One of the problems that occur following treatments around the implants, regardless of therapeutic techniques, is the change in surface properties and the loss of biocompatibility of the implants. These changes lead to a close and spherical shape presence but without attachment and stretching of bone cells in the vicinity of the implant. Therefore, along with the removal of the microbial plaque from the implant surface in the therapeutic procedures of infections around the implant, what ensures long-term success of the therapies is the lack of formation of adverse changes on the surface of the implant. In recent years, the positive role of laser has been reported for cleaning and decontamination of implant surfaces[2-4].Various laser systems such as Co2, Er-YAG and diode lasers are used for this purpose[5-7]. These lasers were chosen because of their wavelengths, which exhibit almost no absorption of laser irradiation in titanium and thus prevent excessive energy transformation in the form of heat development[8]. Therefore, if used correctly, these lasers are able to decontaminate the implant surface without changing its properties[9, 10]. Schwarz et al. and Yamamoto et al. have indicated that Er:YAG laser has been used as the most recommended method for cleaning of implant surfaces and induction of bone regeneration[11, 12]. Schwarz et al showed that Er:YAG laser irradiation with 100MJ energy and a frequency of 10Hz is safe and improves the tissue compatibility of titanium surfaces and the response of osteoblasts[13, 14]. Ayubian markazi et al. in an in-vitro study concluded that Er:YAG laser had no negative effect on biocompatibility of the surface of titanium implant. They recommended Er:YAG laser as a safe tool for cleaning of the implant surface[15]. In addition, Kotsakis et al. in a systematic review with meta-analysis, reported that correct use of diode laser is able to remove contamination from titanium implant surfaces without creating any significant changes[16]. Other investigations have shown that diode laser can be useful as an effective treatment in non-surgical pocket therapy of peri-implant diseases[17, 18]. Cell behaviors such as adhesion, morphology, and functional changes of proliferation and differentiation are strongly influenced by biocompatibility or the surface properties of the implant[19, 20].

The aim of this study was to investigate the irradiation of 810nm diode, 980nm diode and Er:YAG lasers on biological biocompatibility of titanium discs with SLA surface by investigating the morphology of human osteoblast-like cells (Saos-2).

Materials and Methods

A total of 60 titanium disks with SLA surface (Snucone Co.

LTD, Daegu, Korea) with a dimension of 1.5*5.3 mm were considered for this in-vitro study. At first, all disks were sterilized in an autoclave at 121 °C. Then, the disks were divided into 4 groups of 15, including three experimental groups and one control group. The experimental groups were exposed to three laser types of Er:YAG, 810 nm and 980 nm diode.

In this study, an Er:YAG laser device (Fotona, Fidelis plus, Ljubljana, Slovenia) with a wavelength of 2940 nm and a cylindrical fiber with a diameter of 940 micrometer was used. The energy was100 mJ/pulse and The frequency was 10 Hz.. Cooling flow was used at a rate of 5 ml/min. The parameters used for diode lasers (Fox, A.R.C. Laser GmbH, Germany) were 1 watt continuous wave (CW) and a 300µ fiber tip for 30 sec. First, the irradiation with the above parameters was applied to each disc after performing irradiation for all 15 disks of each group, the laser was re-irradiated on the disks for 30 seconds (total time of 60 sec per disk). In each irradiation, the fiber tip was placed at a distance of 0.5-1mm from the disk with sweeping motion from top to bottom and left to right (checkered) to cover the entire disk.After laser irradiations and before cell culture.

All disks in each of the four groups were autoclaved at a temperature of 121 °C so that then, each group was cultured separately for human osteoblast-like cells. Saos-2 human osteoblast-like cells (Sarcoma Osteogenic). These cells with NCBI CODE of C453 as a flask of cells were prepared from Pasteur Institute (Tehran, Iran), and then, incubated at 37 ° C in a humid atmosphere (95% air and 5% carbon dioxide). The culture medium contained Dulbecco's medium (DMEM) * 100 V/ml, 100 Mg/ml streptomycin-penicillin, and FBS 10% (Fetal Bovine Serum). The culture medium was changed 3 times a week. Titanium disks were placed in 24-well plates after being sterilized in autoclave at 121 °C and were incubated in the culture medium for 4 hours. The morphology of Saos-2 human osteoblast-like cells was examined by an electron microscope. The photography was done by electron microscope at magnifications of 750, 1000, 3000, and 5000[21].

In order to describe the data, data analysis was conducted by SPSS software ver.22 (IBM, Armon, NY, USA) Kruskal–Wallis and Mann–Whitney tests were adopted to compare the data .The p-value<0.05 was considered significant.

Results

At first, the average number of cells, counted on the total disks of each group, was statistically evaluated for the evaluation of cell density. For this purpose, kruskal-Wallis test was performed on the data, which showed no significant difference in cell density between the four groups (p-value=0.566).

After this, counted cells were statistically evaluated separately according to their morphology. Kruskal-Wallis test showed no significant difference between the four groups in broad and extended morphology (p-value=0.190), but the difference in round morphology was significant (p-value<0.001).

Mann-Whitney test was performed separately in the groups for further evaluations. The results of the analyses are presented in table 1 and table 2.

Table 1-Mean,Standard deviation and Median measurements separately by the groups and morphologies

Report					
		Round	**Spindle**	**Spread**	**Total**
Er	Mean	6.9333	10.4667	4.5333	21.9333
	Std. Deviation	7.08587	5.80476	2.87518	7.99524
	Sum	104.00	157.00	68.00	329.00
	Median	5.0000	11.0000	4.0000	21.0000
980	Mean	6.8000	5.4000	10.2000	22.4000
	Std. Deviation	6.80546	3.85079	10.60458	12.66491
	Sum	102.00	81.00	153.00	336.00
	Median	5.0000	5.0000	8.0000	20.0000
810	Mean	6.0667	9.6667	13.8667	29.6000
	Std. Deviation	8.20685	7.51823	14.59876	17.86777
	Sum	91.00	145.00	208.00	444.00
	Median	3.0000	8.0000	7.0000	21.0000
Control	Mean	.3333	14.0000	5.4667	19.8000
	Std. Deviation	.48795	5.55492	4.03320	5.58314
	Sum	5.00	210.00	82.00	297.00
	Median	.0000	14.0000	5.0000	20.0000
Total	Mean	5.0333	9.8833	8.5167	23.4333
	Std. Deviation	6.82211	6.46265	9.87291	12.25764
	Sum	302.00	593.00	511.00	1406.00
	Median	3.0000	8.0000	5.5000	20.0000

Table 2- P-value in the Mann-Whitney test separately by the groups

Two group compared	Er-980	Er-810	Er- Control	980-810	980- Control	810- Control
P-value	0.786	0.438	<0.001	0.232	<0.001	<0.001

The results of this study showed that the use of Er:YAG laser with wavelength of 2.94µm and 100mJ energy and a frequency of 10 Hz, 980nm and 810nm diode lasers each one with 1 Watt power and continuous wave (CW) creates no negative effects on the surface of the implant, and maturity of osteoblast-like cells on this surface is not damaged after application of these lasers.(Figures 1-4)

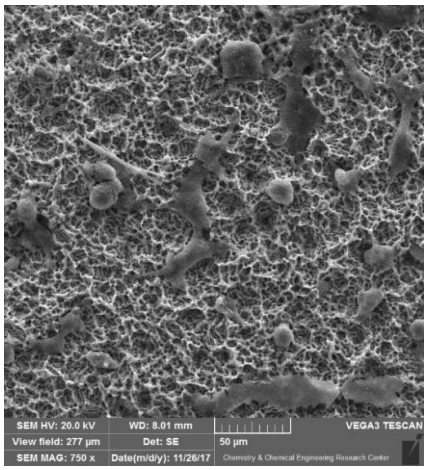

Figure 1- The image of the electron microscope associated with the Er:YAG laser group with a 750 magnification

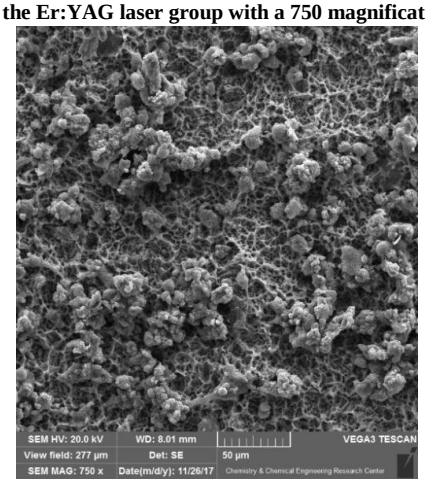

Figure 2- The image of the electron microscope associated with the 980nm diode laser with a 750 magnification

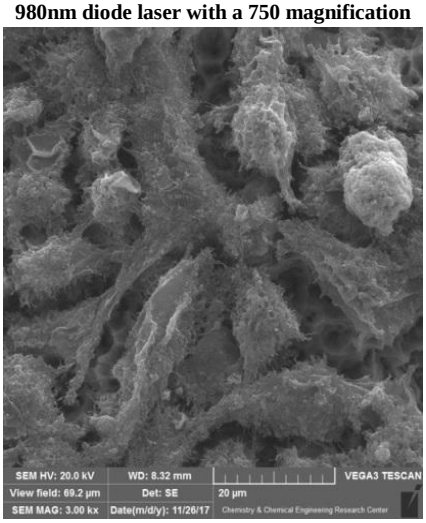

Figure 3- The image of the electron microscope associated with the 810nm diode laser group with a 3000 magnification

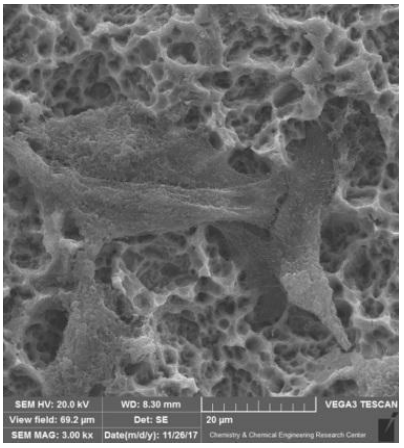

Figure 4- The image of the electron microscope associated with the control group with a 3000 magnification

Discussion

SEM evaluations showed that mean total cells, counted in all samples of each group (cell density) was not significantly different between the four groups (p-value= 0.566).

Subsequently, the analyses were carried out separately for each of the round, spindle and spread morphologies between the four groups, and showed that the differences in the extended and broad morphologies were not significant between the four groups (p-value=0.190). With regard to the fact that the formation of cells with broad and extended morphologies indicates the maturity of these cells and adhesion on the surface of the implant, the results showed that the effect of laser intervention with the parameters mentioned in this study on the surface of titanium implants is insignificant until these changes did not lead to negative effects on the maturity and adhesion of osteoblast-like cells on titanium surfaces, and perhaps, re-osseointegration after peri-mucositis and peri-implantitis treatments following the use of these lasers on implant surfaces is not affected by negative effect of the laser-induced surface changes.

Ayubian Markazi, et al. (2015) examined the effect of Er:YAG laser irradiation on biocompatibility of titanium disks and morphology of Saos-2 human osteoblast-like cells.In that study they concluded that Er:YAG laser had no negative effect on biocompatibility of the surface of titanium implant[15].

In a study by Romanos, et al. the adhesion of osteoblast cells on the surface of titanium disks after irradiation of Er:YAG and carbon dioxide lasers on titanium disks with machined surface, coated with hydroxyapatite, sandblasted and titanium plasma-spray was examined. Their findings showed that osteoblasts could grow on all of these surfaces, and filopodia and cells with extended and broad morphologies that exhibit cell maturity were observed on surfaces of titanium disk, exposed to laser irradiation[22].

The results of the present study were consistent with the results of the above studies.

In the present study, additional analyses, separated by morphology of cells, showed that there was a significant difference in the presence of round cells in the samples under laser irradiation with the control group (p-value<0.001). Meanwhile, there was no difference in this regard between the three groups under laser irradiation.

One of the problems that occur following the treatment of inflammation of hard and soft tissues around the implant (regardless of therapeutic technique) is the change in surface properties and the loss of biocompatibility of the implant surface[23]. These changes lead to the presence of spherical bone cells, but without attachment and elongations in the vicinity of the implant[24].

In the present study, although the irradiation of Er:YAG, 810nm and 980nm diode lasers in with the parameters used showed no difference in adhesion of broad cells to surfaces of titanium disks indicating the biocompatibility of titanium disks surfaces, the presence of round cells adjacent to these surfaces may in part indicate a difference in the structure of the titanium surfaces under laser irradiation, compared to the control group.

According to the research, conducted in recent years in this field, studies at the level of electron microscopy have shown that irradiation of Er:YAG and diode lasers with specified parameters used in this study does not change the surface roughness of titanium implants[14, 25]. However, studies, conducted by X-ray photoelectron spectroscopy (XPS), have shown that the use of different methods of cleaning the surface of the implant can change the chemical and atomic structure of the titanium surfaces[26]. Therefore, it is possible that the irradiation of the lasers, used in this study, in spite of the lack of a change in surface morphology under SEM, has altered the chemical elements to a degree that could affect the maturation of osteoblast-like cells and the occurrence of more round cells on the surfaces under the intervention.

One of the probable responses of the surfaces of titanium implants after laser irradiation is the thermal changes resulting from this irradiation, reported in recent years. If the irradiation occurs with the specified parameters, the resulting thermal changes do not lead to a change in the morphology and properties of implant surface but the damage to surrounding bone cells[27, 28].

So XPS-based studies is suggested to get some definite results.

Conclusion

According to the results of the present study, Er:YAG,980 nm and 810nm diode lasers irradiation with the parameters mentioned in this study, have no negative effects on osteoblast cells attachment and their maturity on SLA titanium implants.

Conflict of Interests

None Declared ■

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