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Physicochemical Changes of Contaminated Titanium Discs Treated With Erbium-Doped Yttrium Aluminum Garnet (Er:YAG) Laser Irradiation or Air-Flow Abrasion: An In Vitro Study

Reza Amid[1], Mahdi Kadkhodazadeh[1], Seyed Massoud Mojahedi[2], Maedeh Gilvari Sarshari[3], Zeinab Zamani[3]

[1]Department of Periodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran
[2]Department of Laser, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Research Institute of Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Correspondence to**
Zeinab Zamani
Dentist, Post graduate student, Research Institute of Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Tel: +989127305245,
Email: zeinab_zamani@yahoo.com

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Abstract

Introduction: Peri-implantitis is a common complication of dental implant treatment. A cause-and-effect relationship has been previously documented between microbial plaque and peri-implantitis and implant failure. A difference has been reported in the disinfection efficacy of erbium laser irradiation and air-flow abrasion for contaminated titanium surfaces. Also, the surface changes caused by lasers and air-flow abrasion have not been well studied. Thus, the purpose of this study was to compare the surface changes of contaminated titanium discs following decontamination by erbium-doped yttrium aluminum garnet (Er:YAG) laser irradiation and air-flow abrasion.

Methods: Twenty-eight intact, sandblasted, and acid-etched (SLA) titanium discs were used. Twenty-four titanium discs were contaminated with *Escherichia coli*. Then, they were decontaminated by using Er:YAG laser irradiation and air-flow abrasion. Four discs remained intact. The mean and standard deviation of the contact angle and the weight percentage of aluminum, titanium, oxygen, carbon, phosphorus, and calcium were measured. Qualitative changes in surface topography of titanium discs were assessed by scanning electron microscopy (SEM).

Results: The mean weight percentage of carbon in the air-flow abrasion group (4.98%) experienced a significant reduction compared with the contaminated (positive control) group ($P=0.035$). The contact angles were 46.54° and 38.67° in the laser and air-flow abrasion groups respectively, which were significantly lower than the value in the positive control group (75.15°) ($P \leq 0.001$). SEM micrographs showed no significant change in the surface area in either technique.

Conclusion: Air-flow abrasion was more successful in improving the surface characteristics of titanium discs with no alteration in surface topography or elements, compared with Er:YAG laser irradiation. Further studies regarding the safety of the Er:YAG laser for the decontamination of titanium surfaces are recommended.

Keywords: Peri-Implantitis; Lasers; Decontamination; Wettability; SEM; Energy Dispersive X-ray Spectroscopy.

Introduction

Peri-implantitis is a common complication of implant treatment, which is characterized by an inflammatory process that affects the soft and hard tissues surrounding the osseointegrated dental implants. Peri-implantitis is caused by the activity of pathogenic bacteria.[1,2] Spirochetes and Gram-negative anaerobes are the most common bacteria causing peri-implantitis.[3,4] Therefore, surface debridement is the first step in the treatment of peri-implantitis,[5] similar to periodontitis.[6] The decontamination of dental implants is a difficult procedure due to the structure of the implant surface.[5,7] The conventional methods commonly used for implant surface decontamination include the use of plastic curettes, ultrasonic scalers, and air-flow abrasion.[8] However, the conventional methods cannot efficiently remove the bacteria from the implant surface due to limited access to the microscopic rough porosities on the implant surface.[5,9] According to some studies, air-flow abrasion can effectively remove the microbial plaque

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from the implant surface. In addition, it has been reported that using sodium bicarbonate or glycine powder is more effective, and it does not cause major damage to the sandblasted and acid-etched titanium implant surfaces.[10] Nevertheless, the use of glycine powder with a smaller particle size is preferred to sodium bicarbonate due to lower abrasiveness.[11,12] Another disadvantage of air-flow abrasion is its limitation for use in narrow vertical defects.[13]

The Erbium-doped yttrium aluminum garnet (Er:YAG) laser has also been studied for the treatment of peri-implantitis.[6] The Er:YAG laser exerts a high antimicrobial effect on rough implant surfaces without causing significant morphological alternations, and it does not cause a temperature rise over 47°C.[6,14]

The implant surface characteristics, such as roughness, composition, wettability, and titanium surface morphology can greatly affect cellular behavior. Data concerning the effect of the Er:YAG laser on the biological properties of titanium surfaces are conflicting.[5] Also, no standard recommendation exists regarding the type of laser, setting, or irradiation protocol for peri-implantitis treatment.[15]

According to the literature, no gold standard exists for the treatment of peri-implantitis, and the currently practiced modalities cannot ideally eliminate the inflamed tissue, debride the bone defects, or decontaminate the implant surfaces.[16] Also, limited, yet controversial information, is available regarding the surface changes caused by Er:YAG laser irradiation or air-flow abrasion. Therefore, the purpose of this study was to assess the physicochemical changes of contaminated sandblasted titanium discs treated with Er:YAG laser irradiation or air-flow abrasion.

Materials and Methods

Titanium Discs

Twenty-eight intact, sandblasted, and acid-etched (SLA) titanium discs (Servo-Dental, Germany), measuring 8 mm in diameter and 2 mm in thickness, were used in this experimental study. At each step of the experiment, UV-exposed containers were used to store the samples.

Contaminating the Discs

Escherichia coli (ATCC 25922) was cultured in 10 mL of Todd Hewitt broth to prepare a bacterial suspension with 0.5 McFarland standard concentration with an optical density of 625 nm containing 1.5×10^8 colony forming units (CFUs)/mL.

Twenty-four titanium discs were inoculated with *E. coli* suspension under aerobic conditions at 37°C for 24 hours. After completion of the incubation period, all 24 discs and 4 intact discs were randomly divided into two control (i.e. positive control and negative control) groups and two experimental groups (i.e. laser treatment group and air-flow abrasion group).

Laser Treatment

The Er:YAG laser (DEKA, Italy) with a 2940 nm wavelength and an MZ6 tip was used in this study with 150 mJ/pulse (1.5 W) energy density and 10 Hz frequency. Ten intact discs were randomly chosen for laser treatment. The discs were fixed by forceps, and the laser was irradiated with a sweeping motion from the top to the bottom and from the right to the left side for 30 seconds at 21°C. The laser beam was pointed to the disc surface using a non-contact tip from a 10 mm distance. The water flow rate was 50%. After laser treatment, the samples were transferred to a suspension of hydrogen peroxide and silver salt (Nanosile water, Kimia Faam Pharmaceutical Co., Iran) for 90 seconds. Then, they were placed in a container, which had already been exposed to UV radiation for 20 minutes. All procedures were performed by an expert clinician.

Air-Flow Abrasion

Ten intact discs were treated with an air-polishing device (Perio-Mate, NSK, Japan) and Perio-glycine powder (Perio-Mate, NSK, Japan). The nozzle tip was maintained at a distance of 2-5 mm from the fixed disc surface, and the disc surface was treated with sweeping motions from the top to the bottom and from the right to the left side for 30 seconds at 21°C. Afterwards, they were immersed in a suspension of hydrogen peroxide and silver salt (Nanosile water, Kimia Faam Pharmaceutical Co., Iran) for 90 seconds. Finally, they were placed in a container, which had already been exposed to UV radiation for 20 minutes. All procedures were performed by an expert clinician.

Surface Wettability Measurement

The wettability of the experimental disc surfaces was determined according to their contact angle. The contact angle of the disc surfaces was measured using CA-ES20 (Fars EOR Technologies CO, Iran). The contact angle was measured three times for each sample. Distilled water (1 g/cm^2) was added on the disc surface at 21°C using a needle with 0.793 mm diameter, and it was photographed immediately.

Energy Dispersive X-ray Spectroscopy

Elemental analysis of the treated surfaces was carried out using an energy dispersive X-ray micro-analyzer (Roentec Detector, Roentec©, Berlin, Germany). The phosphorus, carbon, oxygen, aluminum, calcium, and titanium contents of the surfaces were quantified in the experimental groups as such.

Scanning Electron Microscopy

The surface alterations of each experimental disc were evaluated using scanning electron microscopy (SEM). The discs were gold coated and observed under a scanning electron microscope (TeScan-Mira III, Czech) at ×1000, ×5000, ×25000, and ×50000 magnifications). Random SEM micrographs were also obtained to evaluate the morphological and topographic changes of SLA surfaces such as glossiness, melting, ripple patterns, cracking, and slip line formation.[17]

Statistical Analysis

The sample size was determined according to the studies by Ayobian-Markazi et al[5] and Giannini et al[18] (Figure 1). The mean and standard deviation of weight percentages of aluminum, titanium, oxygen, carbon, phosphorus, and calcium in the experimental groups were analyzed using the Kruskal-Wallis test. The Dunn-Q Bonferroni test was employed to compare the study groups. Moreover, the mean contact angle was analyzed using the one-way analysis of variance (ANOVA, Welch) robust equivalent test, and the Games-Howell post hoc test was used to compare the experimental groups. All statistical analyses were carried out using SPSS version 24.0.

Results

Surface Wettability Measurement

As shown in Table 1, the mean contact angle in the negative control group was larger than that in the experimental groups and the positive control group. Moreover, the results of the Games-Howell test (Table 2) indicated no significant difference in the contact angle between the air-flow abrasion and erbium laser groups ($P = 0.176$).

Table 1. Comparison of Contact Angle (in Degrees) Between the Four Groups of Air-Flow Abrasion, Er:YAG Laser, Negative Control, and Positive Control

Group	Contact Angle		
	Mean	Minimum	Maximum
Air-flow abrasion	38.67 ± 14.26	15.78	67.412
Er:YAG laser	46.54 ± 12.98	18.28	72.31
Negative control	83.52 ± 0.85	82.57	84.21
Positive control	75.15 ± 22.2	34.9	114.4
P value	0.0001	—	—

Table 2. Pairwise Comparisons of the Contact Angle (in Degrees) Between the Four Groups of Air-Flow Abrasion, Er:YAG Laser, Negative Control, and Positive Control

	(I) Group	(J) Group	*P* Value
Games-Howell	Air-Flow	Erbium	0.176
		Negative control	0.000
		Positive control	0.000
	Erbium	Air-flow	0.176
		Negative control	0.000
		Positive control	0.000
	Negative control	Air-flow	0.000
		Erbium	0.000
		Positive control	0.248
	Positive control	Air-flow	0.000
		Erbium	0.000
		Negative control	0.248

Results of Energy Dispersive X-ray Spectroscopy

The results of the non-parametric Kruskal-Wallis test demonstrated a significant difference between the weight percentages of the main elements of disc surfaces. The results of the Dunn-Q Bonferroni test revealed a significant difference in the percentage of phosphorus between the air-flow abrasion and Er:YAG laser groups ($P = 0.014$) and also between the air-flow abrasion and negative control groups ($P = 0.004$). Moreover, significant differences were noted in carbon and titanium percentages between the air-flow abrasion and positive control groups ($P = 0.035$ for carbon and $P = 0.048$ for titanium) and also between the negative and positive control groups ($P = 0.016$ for carbon and $P = 0.029$ for titanium).

The comparison between the Er:YAG and positive control groups demonstrated a significant difference in calcium content ($P = 0.005$). Although the carbon percentage in the air-flow treated group was slightly

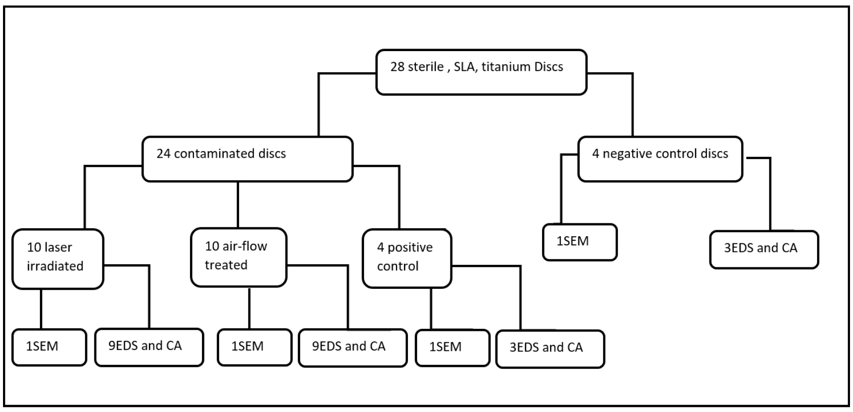

Figure 1. The Number of Samples Used at Each Stage of the Study.

lower than that in the laser group, this difference was not statistically significant. The mean weight percentage of oxygen was minimum in the negative control group and maximum in the positive control group. A comparison of laser and air-flow abrasion groups revealed that the mean percentage of oxygen was lower in the air-flow abrasion group by approximately 5.16 units. However, this difference was not significant ($P = 0.291$) (Table 3, Figure 2).

Scanning Electron Microscopy

At ×1000 to × 25000 magnifications, the highest level of similarity was observed between the surface of negative control discs and air-flow treated discs (Figure 3). No significant difference was found between the laser-irradiated and air-flow treated disc surfaces.

Discussion

The chemical composition and the titanium implant surface charge may vary depending on implant's mass composition and type of surface treatment. Chemical composition and electric charge of the surface are two important factors determining protein absorption and cell adhesion to surfaces. The hydrophilicity of the surface is determined by the chemical composition of titanium implant surfaces. The surfaces that are highly hydrophilic are apparently more favorable than hydrophobic surfaces and better interact with cells, biological fluids, and tissues.[19] Surface wettability, which largely depends on surface free energy, has a fundamental role in host tissue cell healing and regeneration. It also enables the implant surface interactions with the biological environment.[20] Furthermore, it enhances cellular adhesion, protein adsorption, and spreading.[21] Topographic parameters such as micro-texture and roughness and surface chemistry also affect wettability.[20] Borgs et al indicated a reduction in roughness in surface contact angles < 90° and an increase in roughness when the contact angle was greater than 90°.[22] In the present study, the mean contact angle in both laser-treated and air-flow abrasion groups was significantly smaller than that in the negative control group. This result indicates the success of both treatments in increasing the titanium surface wettability. The difference in the mean contact angle was not significant between the laser and air-flow abrasion groups. However, the mean contact angle was smaller in the air-flow abrasion group ($P = 0.176$). Strever et al aimed to develop a laboratory model of single-species biofilm on implant surfaces and find ideal Er,Cr:YSGG laser power settings to remove the biofilm without damaging the surface.

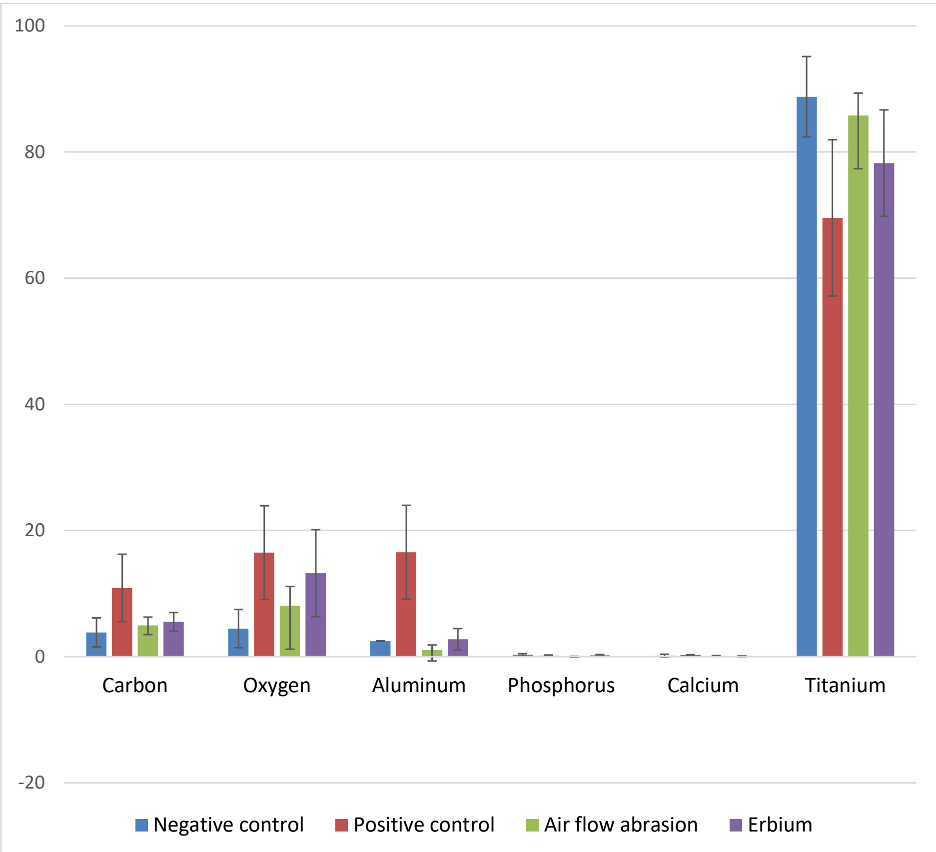

Figure 2. Comparison of the Weight Percentage of the Main Elements of the Disc Surfaces Between the Four Groups of Air-Flow Abrasion, Er:YAG Laser, Negative Control Group, and Positive Control Group.

Table 3. Comparison of the Weight Percentage of the Main Elements of the Disc Surfaces Between the Four Groups of Air-Flow Abrasion, Er:YAG Laser, Negative Control, and Positive Control

Group	Element						
	Carbon	Oxygen	Aluminum	Phosphorus	Calcium	Titanium	Total
Negative control	3.85 ± 2.29	4.45 ± 3.03	2.46 ± 0.03	0.33 ± 0.15	0.15 ± 0.24	88.76 ± 6.38	112.2 ± 1.7
Positive control	10.9 ± 5.34	16.56 ± 7.43	2.57 ± 1.11	0.17 ± 0.09	0.24 ± 0.08	69.55 ± 12.4	111 ± 1.1
Air flow abrasion	4.98 ± 1.29	8.08 ± 3.05	1.02 ± 0.83	0.02 ± 0.04	0.1 ± 0.07	85.79 ± 3.55	107.94 ± 2.35
Erbium	5.52 ± 1.48	13.24 ± 6.9	2.75 ± 1.71	0.22 ± 0.13	0.05 ± 0.06	78.24 ± 8.44	109.7 ± 2.52
P value	0.015	0.014	0.02	0.002	0.007	0.006	0.038

A single-species biofilm containing *Porphyromonas gingivalis* was formed on the surface of titanium discs. The Er,Cr:YSGG laser was applied with 0 W, 0.5 W, 1.0 W, and 1.5 W power with a radial firing tip. No permanent or repairable change in the contact angle of water was noted as a result of laser treatment on any surface.[23]

In the present study, significant differences were noted in weight percentages of all elements between the groups; thus, the null hypothesis was rejected. the mean weight percentage of calcium in the laser-treated group was significantly lower than that in the positive control group ($P = 0.005$). Also, calcium had the highest percentage (0.24% ± 0.08%) in the positive control group compared with other groups, which may indicate the presence of

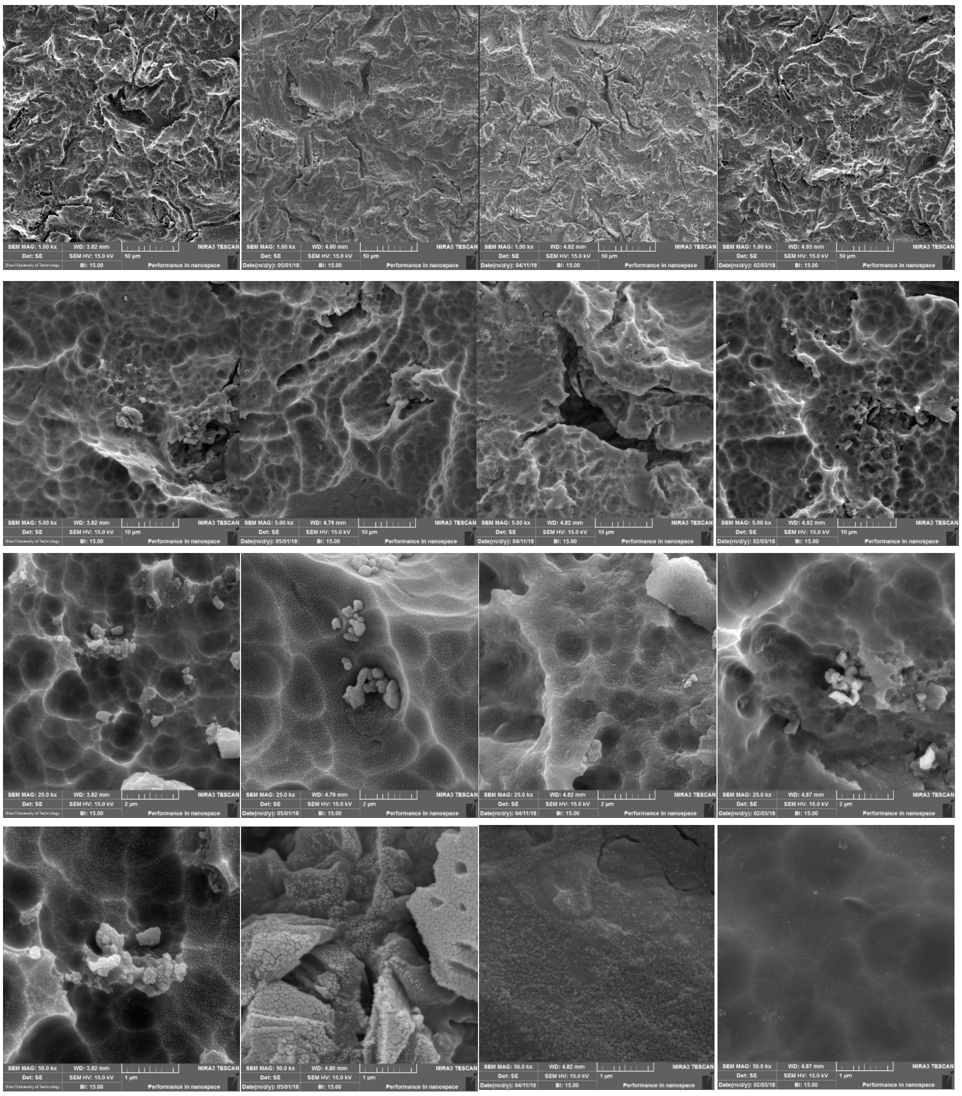

Figure 3. SEM View for different titanium disc surface are shown in rows from left to right including: Negative control, Positive control, Air flow abrasion, Erbium and different magnification levels are shown in columns from top to bottom including:×1000,×5000,×25000,×50000.

organic contaminants on the positive control discs and the success of the Er:YAG laser was in the decontamination of the discs. The amount of calcium was lower in laser-irradiated discs in comparison with air-flow treated discs, but not significantly.

The mean weight percentage of carbon in the positive control group was the highest, probably due to its high organic content. However, the percentage of carbon was the lowest in the negative control group. The difference in the mean weight percentage of carbon was significant between the two groups ($P = 0.016$). Moreover, the significant reduction in the mean weight percentage of carbon in air-flow treated and laser-treated groups suggests a probable reduction in surface contamination with hydrocarbons, although the percentage of carbon in the air-flow abrasion group was slightly, but not significantly, lower than that in the Er:YAG group. This result indicates the absence of carbonation and harmful side effects of the Er:YAG laser at a 2940 nm wavelength and 150 mJ/pulse energy to create a carbon layer on the surface of SLA discs.

Taniguchi et al showed that the use of laser beam with water spray decreased the carbon and oxygen contents, which indicates that the Er:YAG laser can successfully remove hydrocarbons (which prevent osseointegration). However, without the use of water spray, laser radiation can increase the oxygen content. This finding indicates the oxidation of the implant surface due to a temperature rise during calculus removal.[13] An increase in the oxygen content of the Er:YAG laser group, compared with the negative control group in our study, is probably due to the interference of the laser with water, although this increase was not statistically significant.

A comparison of the mean weight percentage of titanium between the positive control group and the air-flow abrasion group revealed a statistically significant difference ($P = 0.048$). The mean weight percentage of titanium was lower by about 16.24 units in the positive control group, compared with the air-flow abrasion group. Although the content of oxygen and titanium in the air-flow abrasion group was most similar to the negative control group, further studies are needed to study the changes in the thickness of the TiO_2 layer.

Similar to a review study by Tastepe et al, SEM micrographs of surfaces treated by air-flow abrasion in the present study were similar to those of the negative control surfaces.[24] Stübinger et al used an Er:YAG laser with 10 Hz frequency and 100, 300, 500, and 1000 mJ energy densities. The SLA surfaces did not demonstrate any change following laser irradiation with energy densities below 300 mJ. The disc surfaces did not change prior to Er:YAG laser irradiation by 500 mJ energy and 10 Hz frequency. However, following laser irradiation with 500 mJ energy and 10 Hz frequency, the cracks started to form at ×1600 magnification.[25] In a study by Kim et al, Osseotite implants were exposed to 1, 1.5 and 2 minutes of Er:YAG laser irradiation with 100, 140, and 180 mJ/pulse energy density and 10 Hz frequency. No considerable change was observed after 2 minutes of laser irradiation with 100 mJ energy and 10 Hz frequency. Moreover, no melting or cracking occurred in any of the samples in laser energies lower than 180 mJ.[26]

In a study by Taniguchi et al, five commercial implants (Osseotite®, Tioblast®, TiUnite®, RBM®, SLA®) were subjected to an Er:YAG laser. The laser was irradiated on each micro-structured surface in near-contact mode from 1 mm by a contact tip. At each radiation spot, the Er:YAG laser was irradiated for 5 s with 30, 40, and 50 mJ/pulse energy and 30 Hz frequency under water spray and 50 mJ/pulse energy without water spray. SEM indicated that 40 mJ laser irradiation under water coolant caused partial melting of the micro-structural edges of SLA® and RBM® surfaces. With 50 mJ irradiation with water spray, all surfaces melted except for Osseotite®. However, 50 mJ radiation without water spray created severe morphological changes on all surfaces. The TiUnite® implant degraded in the use of all levels of laser energy, even with water spray.[13] Their study emphasized the important role of implant type or titanium disc in surface changes caused by laser irradiation.

Although *Actinobacillus actinomycetemcomitans* is a common microorganism associated with peri-implantitis, it requires a microaerophilic atmosphere.[18] Thus, in the present study, *E. coli* as a Gram-negative aerobe was used, which can be considered as a limitation of this study. In addition, the internal pigments of black-pigmented species absorb red light and subsequently improve the laser efficacy.[17]

Since it is not possible to stabilize the distance of the laser beam from the implant surface in clinical conditions, we used an experienced clinician in this study to approximate this distance. Also, in most clinical protocols, the use of chemical methods such as rinsing with chlorhexidine or hydrogen peroxide is common as an adjunct; thus, hydrogen peroxide rinsing was performed equally for all discs in this study for better simulation of intraoral conditions.

According to Schwarz et al., the irradiation of the Er:YAG laser (100 mJ/pulse, 10 Hz, 60 seconds) was more efficacious than plastic curettes for microbial biofilm removal from the SLA surfaces and did not cause surface changes.[27] Shin et al showed surface changes following the use of all different setting times with 180 mJ/pulse laser irradiation.[28] In the present study, we used the Er:YAG laser (DEKA, Italy) with a 2940 nm wavelength and an MZ6 tip with 150 mJ/pulse energy (1.5 W) and 10 Hz frequency for 30 seconds.

Conclusion

According to the results, the mean weight percentage of surface elements following air-flow abrasion was more similar to that in the negative control discs. Moreover, surface contamination with carbon was low in the air-flow abrasion group, while the oxygen content was greater in the Er:YAG laser group. However, these differences were not statistically significant. The contact angle similarly and significantly decreased in both surface decontamination methods. Furthermore, according to SEM micrographs, the laser-irradiated and air-flow treated surfaces had no significant difference.

Ethical Considerations

This study has been approved by research institute of dental sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Conflict of Interests

The authors declare that they have no conflict of interest.

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