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ORIGINAL ARTICLE

In vitro bioactivity of laser surface-treated Ti6Al4V alloy

Afife Binnaz Hazar Yoruç[1] · Ergün Keleşoğlu[1] · Harika Ekşioğlu Yıldız[1]

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Abstract
The effects of lasing parameters on the precipitation of hydroxyapatite (HA) on the commercial Ti6Al4V alloy in simulated body fluid (SBF) were investigated. Ti6Al4V plates were polished and ultrasonically cleaned in acetone and ethyl alcohol, respectively. The specimen surfaces were treated with Er:YAG laser using super short pulse (SSP, 50 μs) and very short pulse (VSP, 100 μs) modes. Surface roughness was measured before and after laser treatment. The specimens were immersed in simulated body fluid (SBF) for 1, 3, and 7 days and, then the amount of Ca and P precipitation on specimens was determined using SEM/EDS analysis. An average roughness varying between 0.19 and 0.81 μm in surface roughness was detected in all laser-treated specimens depending on the lasing parameters. The highest surface roughness and Ca precipitation were found in VSP group (20 Hz and 5 W). Laser treatment of specimen surfaces has dramatically increased the HA precipitation due to the increasing surface roughness. It is also concluded that the immersion time was effective on the HA precipitation as well.

Keywords Titanium · Er:YAG laser · Roughness · SBF · In vitro bioactivity

Introduction

Clinical demands for biomaterials are rapidly increasing with the increase in the elderly population. Biomaterials are artificial or biological samples which can be introduced into the body tissue as part of an implanted medical device (such as metallic implants, ceramic crowns) or used to replace an organ (such as heart valves, stents).

Grade 5 titanium (Ti6Al4V) is the most commonly used titanium alloys due to its biomedical applications. The main uses of pure Ti and Ti6Al4V based materials are widely used in medicine due to their stability, biocompatibility, mechanical performance, and long-term durability [1, 2]. In addition, it is highly inert and insoluble in the body fluids and forms a protective oxide layer on the surface [3, 4]. On the other hand, long-term in vivo applications can cause corrosion and resulted with the loss of strength. Moreover, leachable titanium could be debris in the host tissue and might be detrimental to human body [3, 5]. Previous studies have confirmed that biocompatibility and bioactivity of biomaterials are associated with surface topography [6–8]. It has been also proved that surface roughness and chemical composition of implant surface layers are significant for biological activities at the tissue-implant integration [3, 7, 9, 10]. Physical or chemical treatment on the implant surface can initiate bonding between bone and implant interface [3, 11]. Therefore, surface treatment of titanium alloys is very important to provide higher corrosion resistance, acceptable mechanical properties, and low toxicity [3, 5]. In addition, surface treatment provides osseointegration as well as increasing the contact area around the newly formed calcified bone tissue and corrosion resistance [12–14]. Many different surface modification techniques such as grit-blasting [1, 15–19], chemical etching [1, 18–20], electrochemical treatment [1, 21], plasma-spraying [1, 18, 19, 22], laser treatment [1, 22–24], and the combinations of the various methods [17, 19, 25, 26] are used to change the surface topography of titanium implants. Compared with the conventional methods, laser surface or pulsed-laser treatment has higher efficiency and excellent bonding strength between coating and substrate, as well as the ability to precisely control the thickness of coating (0.01–1 mm) [27, 28]. Moreover, laser-assisted techniques, which are among these techniques, have superior properties due to the fact that they do not cause contamination and the process variables can be easily controlled [8, 29]. In the past decade, several studies have been carried out to determine the best laser treatment techniques for the surface modification of

✉ Afife Binnaz Hazar Yoruç
afife.hazar@gmail.com

[1] Department of Metallurgical and Materials Engineering, Yildiz Technical University, Istanbul, Turkey

Table 1 Surface-treated sample codes, laser process parameters, and average roughness values before and after laser surface treatments

Sample code	Laser process parameters		Average surface roughness (Ra)	
	SSP (super short pulse) (50 μs)	VSP (very short pulse) (120 μs)	Before laser treatment	After laser treatment
1	10 Hz 2 W SSP		0.056	0.21
2	10 Hz 2 W SSP		0.077	0.19
3	10 Hz 2 W SSP		0.073	0.22
4	10 Hz 5 W SSP		0.066	0.51
5	10 Hz 5 W SSP		0.074	0.53
6	10 Hz 5 W SSP		0.097	0.52
7	20 Hz 2 W SSP		0.061	0.25
8	20 Hz 2 W SSP		0.086	0.24
9	20 Hz 2 W SSP		0.090	0.26
10	20 Hz 5 W SSP		0.077	0.43
11	20 Hz 5 W SSP		0.086	0.39
12	20 Hz 5 W SSP		0.154	0.42
13	20 Hz 8 W SSP		0.080	0.57
14	20 Hz 8 W SSP		0.072	0.55
15	20 Hz 8 W SSP		0.066	0.56
16		10 Hz 2 W VSP	0.071	0.51
17		10 Hz 2 W VSP	0.075	0.47
18		10 Hz 2 W VSP	0.084	0.49
19		10 Hz 5 W VSP	0.094	0.79
20		10 Hz 5 W VSP	0.083	0.77
21		10 Hz 5 W VSP	0.063	0.78
22		20 Hz 2 W VSP	0.079	0.23
23		20 Hz 2 W VSP	0.081	0.25
24		20 Hz 2 W VSP	0.085	0.24
25		20 Hz 5 W VSP	0.067	0.35
26		20 Hz 5 W VSP	0.065	0.38
27		20 Hz 8 W VSP	0.069	0.36
28		20 Hz 8 W VSP	0.077	0.79
29		20 Hz 8 W VSP	0.091	0.80
30		20 Hz 8 W VSP	0.076	0.81
31	Control group	C	0.090	
32	Control group	C	0.094	
33	Control group	C	0.089	

titanium alloys [27, 28, 30]. Long-pulse laser Nd:YAG [1, 8, 30], diode laser [8, 31], CO_2 [8, 32], and various excimer laser systems are the most common laser types for surface modification of the Ti-implants. Similarly, many studies have been carried out on the surface morphology as well as on the laser parameters, the environment, and the phases formed [14].

However, the effect of the laser techniques on the hydroxyapatite (HA) precipitation in simulated body fluids (SBF) on Ti6Al4V alloys has not been sufficiently described in the literature. SBF is a protein-free solution with an ion concentration nearly equal to that of human body blood plasma. SBF is known to be a metastable buffer solution [33, 34] and even a small undesired variance in both of the preparation steps and the storage temperatures may drastically affect the phase purity and high-temperature stability of the produced HA powders, as well as the kinetics of the precipitation processes [34].

Although there are many studies containing different surface techniques such as grit-blasting, chemical etching, electrochemical treatment, plasma-spraying, and the combinations of these methods to change the surface topography of Ti6Al4V based implants, the surface modification of Ti6Al4V alloys using laser treatment for the enhancement of bioactivity properties in SBF have not been yet sufficiently reported. Therefore, the present study is focused on the effects of laser surface treatment on in vitro biocompatibility of Ti6Al4V in SBF. In this study, Er:YAG laser was used to surface modification of the Ti6Al4V samples. Laser treatments were applied using two different pulse modes, three different lasing powers, and two different frequencies. The surface-treated Ti6Al4V specimens were studied following the Er:YAG laser surface treatment (λ = 2940 nm, max. power = 20 W, max. frequency = 50 Hz, pulse duration = 50–1000 μs). Average surface roughness of all samples before and after laser treatments was determined. After laser treatment, samples were immersed in simulated body fluid (SBF) for 1, 3, and 7 days and the amount of Ca and P deposition on the metal surfaces were determined by SEM and EDS analyses.

Experimental procedure

Commercial Ti6Al4V (Grade 5) plates (donated) were used in this study. The Ti6Al4V specimens are consist

Table 2 Preparation of simulated body fluid (SBF)

Compound	Specification	Amount	
		Weight (g)	Volume (cm^3)
NaCl	min. %99.5	7.996	
$NaHCO_3$	min. %99.5–100.3 (dry)	0.350	
KCl	min. %99.5	0.224	
$K_2HPO_4.3H_2O$	min. %99.0	0.228	
$MgCl_2.6H_2O$	min. %98.0	0.305	
1 kmol/m^3 HCl	HCl (%35.4)		40
$CaCl_2$	min. %95.0	0.278	
Na_2SO_4	min. %99.0	0.071	
$(CH_2OH)_3CNH_2$	min. %99.9	6.057	
1 kmol/m^3 HCl	HCl (%35.4) (pH adjustment)	–	–

of 6.20% Al, 4.1% V, 0.15% Fe, 0.07% C, 0.07% O, 0.008% N, and balance Ti by weight. Before the surface treatment Ti6Al4V plates were cut into thin sheets (10 mm × 10 mm × 7 mm), the surfaces of thin sheets were grounded with 400 grit SiC paper and polished with 1 μm alumina suspension and then washed with pure acetone and ethanol in an ultrasonic bath. In this study, Er:YAG laser was used to surface modification of the Ti-sheets. Laser treatments were applied using two different pulse modes (super short pulse: SSP and very short pulse: VSP), three different lasing powers (2 W, 5 W, and 8 W), and two different frequencies (2 and 10 Hz).

Ten different test groups ($n = 3$) were prepared as shown in Table 1. The surface-treated Ti6Al4V specimens were studied following the Er:YAG laser surface treatment (λ = 2940 nm, max. power = 20 W, max. frequency = 50 Hz, pulse duration = 50–1000 μs). Surface topography was examined by scanning electron microscopy (SEM-Hitachi, TM 1000 Model) operating at 15 kV and × 50, × 500, and × 1000 magnification. The sample codes, laser process parameters, and average surface roughness (Surftest 301, Mitutoyo) before and after laser treatments were given in Table 1.

After laser treatment, samples were immersed in simulated body fluid (SBF) for 1, 3, and 7 days and the amount of Ca and P deposition on the metal surfaces were determined by SEM and EDS analyses. SBF was prepared according to the Tas's method [34]. The chemical composition of synthetic body fluid (SBF) solutions was given in Table 2.

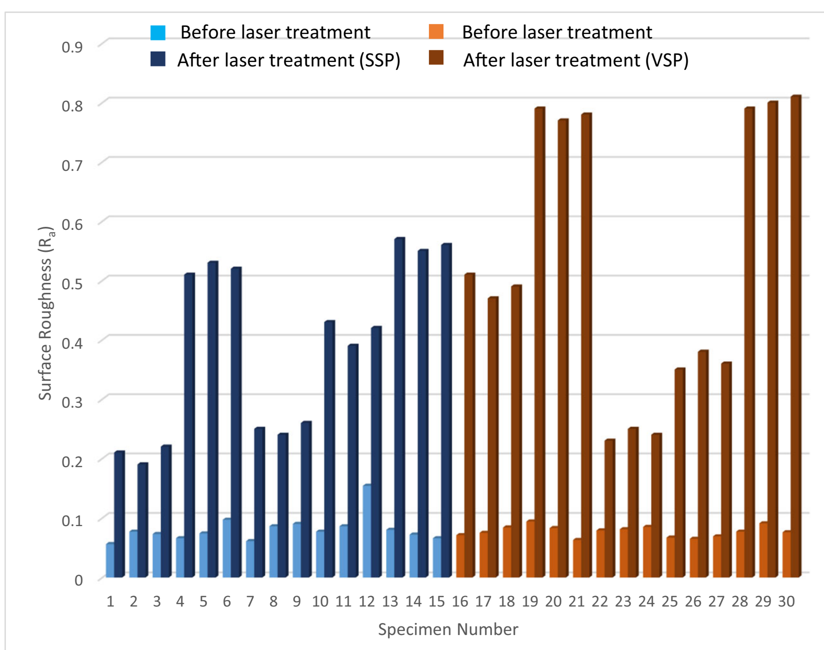

Fig. 1 The surface roughness (R_a) values in SSP and VSP modes before and after laser treatment

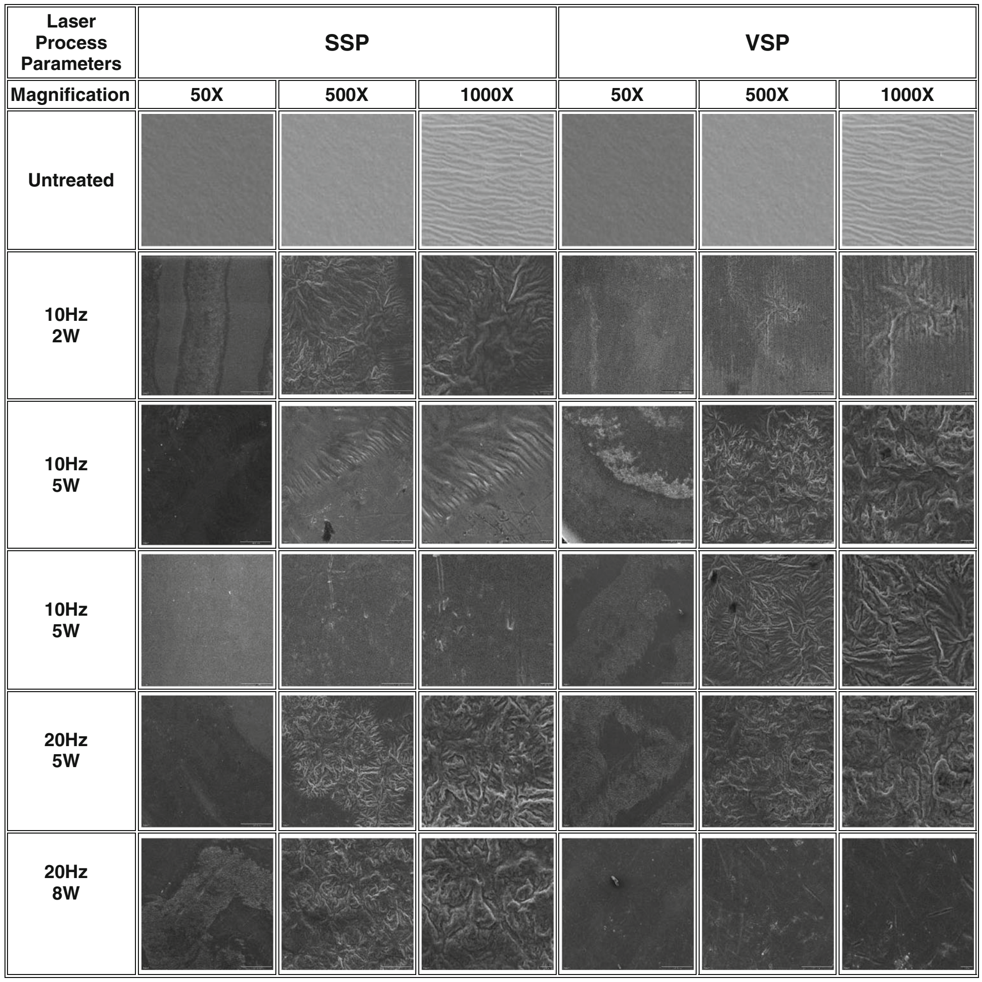

Fig. 2 SEM images of laser-treated surfaces with different laser frequency, power value, and pulse modes (×50, ×500, ×1000)

Results

Surface roughness and SEM examination

Ten different combinations of laser process parameters were used in experimental studies. The order of surface roughness values of ten different combinations is presented below (Table 1 and Fig. 1):

> Ra: (20 Hz 8 W VSP) > (10 Hz 5 W VSP) > (20 Hz 8 W SSP) > (10 Hz 5 W SSP) > (10 Hz 2 W VSP) > (20 Hz 5 W SSP) > (20 Hz 5 W VSP) > (20 Hz 2 W SSP) > (20 Hz 2 W VSP) > (10 Hz 2 W SSP)

Surface properties of the laser-treated samples were investigated using scanning electron microscopy (SEM) at ×50, ×500, and ×1000 magnifications. SEM images were presented in Fig. 2. The surface properties of the specimens treated in SSP and VSP modes were compared with the untreated sample surfaces. It was found that treated samples with the highest power value (20 Hz, 8 W, SSP) were more roughened and exhibited the highest surface roughness value for SSP mode (Fig. 2). The order of the surface roughness values of specimens treated in SSP mode is given below:

> Ra in SSP mode: (20 Hz 8 W) > (10 Hz 5 W) > (20 Hz 5 W) > (20 Hz 2 W) > (10 Hz 2 W)

The surface-treated specimens in the highest laser power (20 Hz, 8 W, VSP) were more roughened and the values of highest surface roughness were obtained for VSP mode (Fig. 2). The order of the surface roughness values of the surface-treated samples in VSP mode is given below:

> Ra in VSP mode: (20 Hz 8 W) > (10 Hz 5 W) > (10 Hz 2 W) > (20 Hz 5 W) > (20 Hz 2 W)

In vitro bioactivity in SBF and SEM/EDS examination

All surface-treated samples were immersed into SBF for 1, 3, and 7 days at 37 °C, washed with ultra-pure water, and then

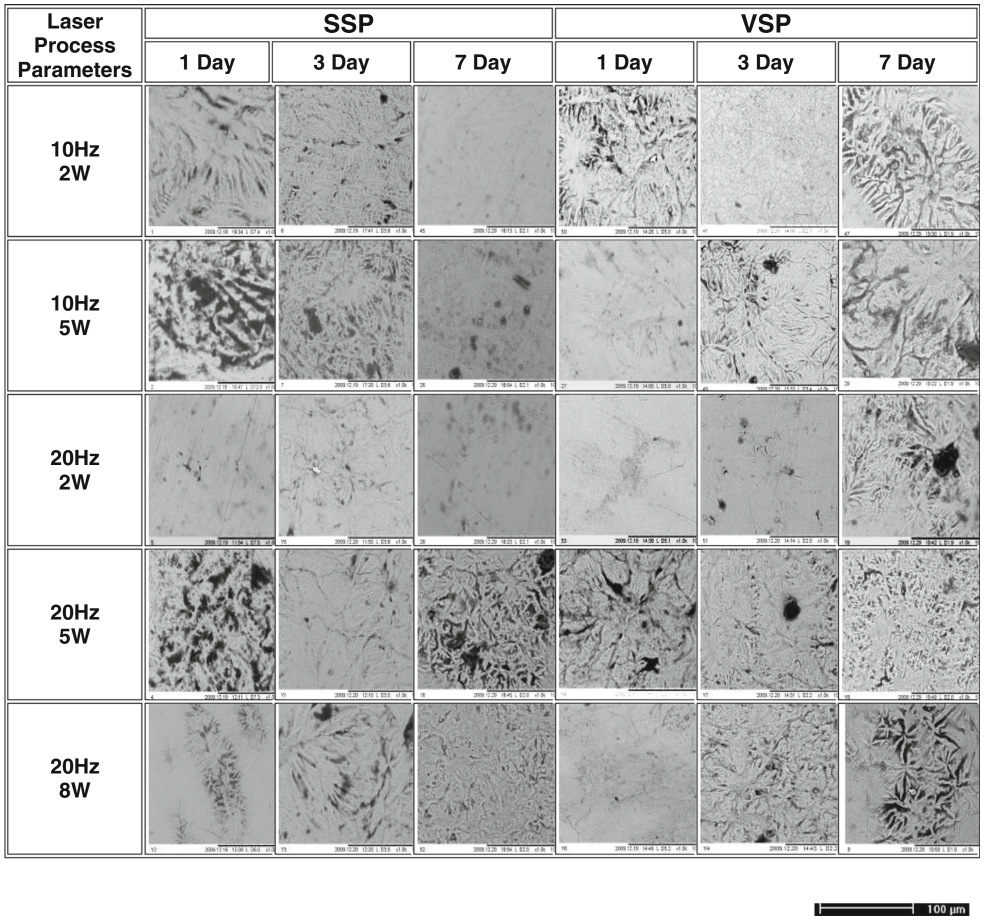

Fig. 3 SEM images of laser-treated surfaces after immersion in SBF (×1000)

dried in the oven at 37 °C. Surface-treated specimens were examined by SEM and EDS. SEM images were presented in Fig. 3.

Deeper hollows and higher amount of HA precipitation were detected on specimens 13 (20 Hz 8 W SSP), 18 (10 Hz 2 W VSP), 5 (10 Hz 5 W SSP), 12 (20 Hz 5 W SSP), 29 (20 Hz 8 W VSP), and 15 (20 Hz 8 W SSP).

In contrast, samples 1 (10 Hz 2 W SSP), 19 (10 Hz 5 W VSP), 23 (20 Hz 2 W VSP), and 2 (10 Hz 2 W SSP) had smaller grooves in pitting form and less HA precipitation on their surfaces according to the SEM images (Fig. 3).

EDS results of laser-treated surfaces were presented in Table 3. Ca/P ratios were then calculated according to the EDS results. Sample 25 that was immersed 7 days in SBF solution has shown the highest calcium precipitation (2.66%

Table 3 EDS analysis of laser-treated samples with two different pulse modes (SSP and VSP) and three different pulse durations (1 day, 3 days and 7 days)

Sample code	Laser process parameters	Ca %(wt) 1 day	P %(wt)	Ca %(wt) 3 days	P %(wt)	Ca %(wt) 7 days	P %(wt)
1–3	SSP 10 Hz 2 W	0	0.3	0.1	0.5	0.1	0.2
4–6	SSP 10 Hz 5 W	0.1	0.4	0.4	0.4	0	0.6
7–9	SSP 20 Hz W	0.2	0.2	0.1	0.2	0	2.1
10–12	SSP 20 Hz 5 W	0.1	0.4	0	0.3	0.2	0.5
13–15	SSP 20 Hz 8 W	0	0.7	0.2	0.4	0.4	0.1
16–18	VSP 10 Hz 2 W	0.2	0.8	0.1	0.2	1.4	2
19–21	VSP 10 Hz 5 W	0.2	0.2	0.1	0.3	0.8	1.5
22–24	VSP 20 Hz 2 W	0.2	0.8	0.1	0.2	0.4	0.9
25–27	VSP 20 Hz 5 W	0.1	0.4	0.2	0.2	2.1	1.7
28–30	VSP 20 Hz 8 W	0	0.7	0.3	0.5	0.2	0.3

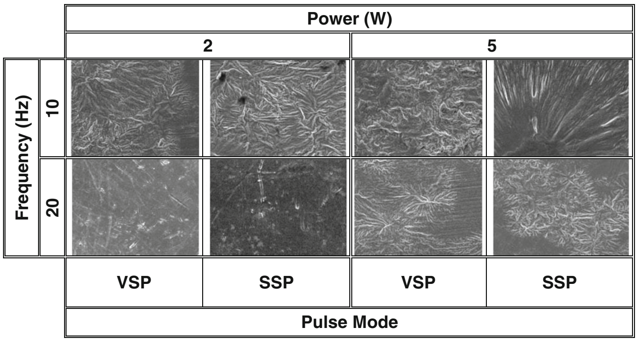

Fig. 4 Comparison of process parameters on laser-treated surfaces

Ca, Ca/P = 1.70). Ca/P molar ratio was changed between 1.55 and 1.70.

The results have shown that VSP and SSP pulse modes have exihibited different surface morphologies depending on the lasing power. Increase in lasing power has created more cavities and deeper hollows (Fig. 4). While the power of the laser operation was constant, increasing the frequency value did not increase the number of cavities, although they created deeper cavities.

Discussion

The results have clearly shown that the laser treatment on implant surface has changed the surface roughness (Ra) dramatically. Wavelength, frequency, and power settings have increased the surface roughness of all samples (Table 1 and Fig. 1).

The highest surface roughness (avg. 0.80 μm) was obtained on the samples, which the surfaces treated with 20 Hz, 8 W, and VSP pulse mode conditions (specimens 28–30 in Table 1). In contrast and as expected, the lowest surface roughness (avg. 0.20 μm) was detected on the specimen surfaces that were treated with 10 Hz, 2 W, and SSP pulse mode (specimens 1–3 in Table 1).

After the laser treatment process, SEM images have shown that the topographic structure of specimen surfaces was changed depending on the frequency and pulse duration of applied laser beam. Increasing the lasing power was more effective than increasing the frequency on the surface roughness which plays a determining role on the surface topography.

Increasing the deposition of calcium and phosphorous ions in SBF could effectively reduce the nucleation energy of hydroxyapatite that could lead to rapid precipitation of HA. Generally, it can be concluded that the Er:YAG laser wavelengths with pulse in the 120 μs at the maximum power = 5 W and maximum frequency = 20 Hz can effectively be applied for improving titanium alloy/implant roughness, thus improving its biointegration. Therefore, laser treatment has a great potential to future application on the surface treatment of Ti6Al4V alloys.

Rønold HJ and Ellingsen JE [35] suggested the relations between titanium surface topology and mechanical properties of titanium, also first bone tissue bonds in titanium implants are varying from 0.5 to 1.5 μm Ra surface roughness in the range of values. In the present study, similar results were achieved (Table 3: sample codes 1,4,7,10,13, 16, 19, 202, 25, 28).

The results show that rough surfaces were created on Ti6Al4V, and their Ca deposition capacity was also improved after surface laser treatment. In addition, it was found that the tendency to form Ca and P in the SBF of the laser-treated samples was much more effective than that of the original Ti6Al4V surface. A layer of bone-like apatite can be rapidly formed on the specimens when immersed in SBF solution for only 1 day, indicating its favorable biomimetic coating efficiency.

Conclusions

The experimental results were summarized below:

1. Laser treatment increased the surface roughness of the samples.
2. Different laser power increased Ra values of all samples at constant frequency (10 Hz and 20 Hz) and pulse modes (VSP and SSP).
3. The average roughness varies between 0.19 and 0.81 μm depending on increasing of laser power.
4. Highest Ra is obtained in VSP group (20 Hz and 5 W).
5. Maximum Ca deposition was obtained in the sample laser surface treated in VSP mode (20 Hz and 5 W) and then immersed in SBF for 7 days.
6. It was observed that a thick layer of HA was successfully coated on the surface of Ti6Al4V immersed in SBF for 1, 3, and 7 days after laser treatment.

The wavelength (λ, nm), pulse energy (W), frequency (Hz), and pulse duration (μs) are crucial parameters for laser treatment. It has been concluded that laser treatment might be used instead of mechanical surface treatments since it provides great control on both process parameters and surface properties.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval None.

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