Surface morphology characterization of laser-induced titanium implants: lesson to enhance osseointegration process

Javad Tavakoli¹ · Mohammad E. Khosroshahi^{2,3}

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Abstract

The surface properties of implant are responsible to provide mechanical stability by creating an intimate bond between the bone and implant; hence, play a major role on osseointegration process. The current study was aimed to measure surface characteristics of titanium modified by a pulsed Nd:YAG laser. The results of this study revealed an optimum density of laser energy (140 Jcm^{-2}), at which improvement of osteointegration process was seen. Significant differences were found between arithmetical mean height (R_a), root mean square deviation (R_q) and texture orientation, all were lower for 140 Jcm^{-2} samples compared to untreated one. Also it was identified that the surface segments were more uniformly distributed with a more Gaussian distribution for treated samples at 140 Jcm^{-2} . The distribution of texture orientation at high laser density (250 and 300 Jcm^{-2}) were approximately similar to untreated sample. The skewness index that indicates how peaks and valleys are distributed throughout the surface showed a positive value for laser treated samples, compared to untreated one. The surface characterization revealed that Kurtosis index, which tells us how high or flat the surface profile is, for treated sample at 140 Jcm^{-2} was marginally close to 3 indicating flat peaks and valleys in the surface profile.

Keywords Osseointegration · Surface characteristic · Surface roughness · Laser surface treatment · Titanium alloy

1 Introduction

An eventual bone formation at implant-bone interface involves a complex sequence of biological routes, a range of protein adsorption to biological recognition of the surface [1]. From biological point of view, mesenchymal stem cells (MSCs) and osteoblasts are responsible to provide mechanical stability by creating an intimate bond between the bone and implant [2]. After implantation, adsorption of water and lipid molecules facilitates blood protein's interaction with implant surface, some of which, i.e.

vitronectin and fibronectine, are involved in cell attachment process [3]. Subsequent to the blood platelets stimulation, formation of fibrin network on the implant surface facilitates cell migration and creates a biological coat over the surface [4]. The stronger the biological coating, the more robust bone formation is expected. Utilizing the enzymatic activity, MSCs travel through dense fibrin network to reach the surface and differentiate into osteoblast and fibroblast cells to start bone formation [5, 6]. Once differentiation process starts, two different fronts of contact (surface on the implant) and distance (surface of surrounding bone) osteogenesis can be distinguished at implantation site [7]. A rich in non-collagenous protein mixture is created by cells between the contact and distance fronts to facilitate osteoblast recruitment and maturation [8]. Bone remodelling process, at which osteoclast cells resorb the fresh formed bone to resolve micro-cracks and prime the surface of new bone formation, is the final stage of osseointegration process [9].

The impact of implant surface properties on successful bone formation is significantly high [10–13]. While osseointegration is in progress, the adhesive property of the

✉ Mohammad E. Khosroshahi
khosrom@mie.utoronto.ca

¹ Biomechanics and Implants Research Group, The Medical Device Research Institute, College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia

² Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON M5S 3G8, Canada

³ MIS-Electronics, Nanobiophotonics and Biomedical Research Lab, Richmond Hill, ON L4B 1B4, Canada

fibrin network including its coverage area and strength of attachment, bias of MSCs differentiation toward bone tissue and promotion of contact osteogenesis depends on implant surface properties. Upon selecting non-appropriate surface properties, formation of a fibrous tissue layer between implant and bone negatively affect bone formation results in eventual loosening of implant [14]. Local hardening and improvement of wear and corrosion resistance, composition, hydrophilicity and surface roughness are known factors to affect osseointegration process [15–19]. Among them, surface roughness seems to play an important role, while its optimization enhances hydrophilicity of the implant surface [20, 21]. Improvement of hydrophilicity followed by achieving smoother surface increases the accumulation of water molecules that results in higher blood protein's interaction with implant surface [22, 23]. It was explained that increasing surface roughness supports greater amount of fibrin network expansion on the surface or enhances its strength of attachment, both promoting a better cell migration towards implant [24, 25]. Therefore the implant surface characteristics at micro and sub-micro scale has to be well-thought-out to confirm long term osseointegration [26, 27]. Several surface modification techniques as well as their combination to alter micro-roughness, including acid etching, heat treatment, sand blasting, anodic oxidation were well discussed [28–32]. In spite of the reports that have revealed the techniques enhancing the process of osseointegration [33–35], when they compared to the relatively smooth surface, two important challenges need to be addressed. First, most of the mentioned techniques were shown to affect the chemistry of the implant surface, which may lead to undesired reactions, *in vivo*. The fact that among metallic implants, only titanium forms a protective thin layer of oxide that inhibits future corrosion addresses the limitation of the methods. Indeed, the application of moderately invasive methods to provide micro-roughness is limited. The second, comparing rough to smooth surfaces—within and/or between the methods—by themselves are not sufficient to establish an optimized surface roughness for bone formation. To the best of our knowledge, surface characterization of implant surface has been focused on measuring roughness and reporting arithmetical mean of height “Ra” or root mean square of deviation “Rq”. It seems that improving bone formation process based on surface topography requires further attention to develop a better modified techniques and improved analysis protocols for surface roughness optimization. Therefore, in this study we aimed to measure different surface characteristics of modified titanium surface by pulsed Nd:YAG laser to gain a better understanding about optimization of implants' surface parameters with respect to osseointegration process. Comparing the outcome of this study with previously

reported results, showing cell-surface response *in vivo*, provides new insights into the relation of surface morphology and cell response.

2 Materials and methods

2.1 Sample preparation

Rectangular-shaped Ti6Al4V (Ti64) samples (20 (L) × 10 (W) × 2 (t) mm) were purchased from Germany (Friadent, Mannheim-Germany-GmbH). The samples were divided into groups of untreated (7 samples), laser treated (14 samples). Prior to treatment, all samples were cleaned with 97% ethanol and subsequently been washed twice by distilled water in an ultrasonic bath (Mattachanna, Barcelona-Spain). A stereo-microscope with magnification of 20× was used to ensure that no particles were left on the sample surface.

2.2 Experimental setup

Titanium surface treatment was carried out with the same set up as described previously (20). A Nd:YAG laser with 1.06 μm wavelength, 200 μs pulse duration and pulse energy of 50 J was used. The output beam was suitably imaged on to the target surface in a 500 μm spot diameter where it scanned the surface at a constant velocity using a motorized XYZ translator. All the experiments were carried out in air at pulse repetition frequency of 1 Hz.

2.3 Scanning electron microscopy

All scanning electron microscopy (SEM) images were acquired from the exposed surface of each sample. For SEM imaging, the voltage was set at 5 kV and the distance from the sample to the beam source was kept constant for all performed imaging.

2.4 Surface roughness measurement

The surface micro roughness measurements were carried out using a non-contact laser profilometer (NCLP) (Messtechnik, Germany) equipped with a micro focus sensor based on an auto focusing system. Ra is the arithmetical mean of the absolute values of the profile deviations from the mean line. Five two-dimensional NCLP profiles were obtained for each surface over a distance of 3.094 mm with a lateral resolution of 1 μm using a Gaussian filter and an attenuation factor of 60% at a cut-off wavelength of 0.59 mm. The roughness parameters including arithmetic mean deviation (R_a), root mean square deviation (R_q), Skewness (R_{sk}) and kurtosis (R_{ku}) of

amplitude density function were calculated with the NCLP software similar to that described by Wieland et al. [36] and samples' surface profiles were compared.

2.5 Surface characterization

The SEM images that were prepared under similar magnifications were used for other surface characterization including measurement of overall surface orientation and distribution (histogram) of orientation as well as preparing interactive 3D surface plots and morphological segmentation, using ImageJ software.

The OrientationJ plugin was used to measure the orientation and coherency of surface structure in the input (8-bit) images, with Cubic Spline Gradient with Gaussian window = 1 selected as the structural tensor for fitting the data. The results of orientation distribution were presented as orientation (°) versus distribution of orientation (Count). The MorphoLibJ plugin was used for presenting sample morphology segmentation. The 8-bit border images were used as input and watershed segmentation with tolerance of 10 and connectivity of calculated dams equal to 8 were prepared. Both watershed lines and overlaid dams images were shown without any post-processing. Using watershed images the shape factor and elongation of the surface segments were measured and compared for all samples. For 3D reconstruction of surface, 3D plugin (interactive 3D surface plot) was used. Inverted grayscale images were modified by selecting grid size of 1024, smoothing and lighting of 0.0 and perspective equal to 1.0 and sample 3D surfaces were compared.

2.6 Statistical analysis

Independent samples t-tests were conducted (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.), having test variables of arithmetical mean height (Ra), root mean of deviation (Rq) and orientation within the untreated and laser treated samples using an alpha of 0.05.

3 Results

SEM images of Ti64 surface at different laser fluences are shown in Fig. 1. Different surface topographies were identified at various laser fluences. A range of planar platform (for untreated sample; Fig. 1a), dendritic patterns (Fig. 1b), relatively smooth surface (Fig. 1c) and disorganized surfaces suffering cellular cracks originated from plasma-induced degradation or thermo-mechanical shocks (Fig. 1d, e) were seen as laser fluence was increased from 0 to 300 Jcm⁻². The changes observed in surface

morphology seem to be consistent with variation of etch depth with laser fluence (Fig. 1f). The morphological changes occurred at about 70 Jcm⁻² where clearly some dendrite features are seen. However, by increasing the fluence some thermally-induced effects such as shock due to thermal gradient producing cracks and plasma causing some degree of melting at surrounding regions became more evident.

Ti64 surface plots of major axis of the laser imprints obtained at different fluences are presented in Fig. 2.

Different surface profile as consistently observed in SEM images indicated the changes in surface morphology after laser surface treatment. The surface of untreated sample was identified as the roughest surface among all samples. Its mean (95% CI) maximum profile height was 20.83(1.98) µm, with maximum valley and peak depth of -11(2.24) and 9.83(1.28) µm, respectively. The lowest values for maximum valley depth and maximum peak height were seen in samples that were irradiated at 250 Jcm⁻². The mean (95% CI) values for maximum profile heights were measured as 13.7(0.99), 13.6 (1.23), 11.3(0.71) and 7.7(0.32) µm at 70, 300, 140 and 250 Jcm⁻² laser fluences, respectively. The summary of the surface roughness characterization of Ti64 samples at different laser fluences are presented in Table 1. The surface characterization revealed that only for untreated and laser treated at 300 Jcm⁻² samples, the skewness were negative, while the kurtosis for all samples were less than 3. However, in the case of 140 Jcm⁻² the Rku is marginally close to 3 (Rku = 3 for Gaussian distribution).

The change in two important surface characteristic parameters i.e., the arithmetical mean height (Ra) and root mean square deviation (Rq), versus different laser fluences is illustrated in Fig. 3. As shown, both Ra and Rq values were lower than other samples with values of 1.95 (0.082) and 2.44 (0.095), respectively. The untreated samples and those treated at 300 Jcm⁻², were identified with highest Ra and Rq.

The surface texture of different Ti64 samples were characterized by measuring their orientation relative to the x-axis (along with the major axis of the laser imprints) as shown in Fig. 4. According to the orientation analysis, two different organized angle of orientations (approximately measured as ± 90° and 0°) were detected in untreated samples as well as those were treated at 250 and 300 Jcm⁻², with non-Gaussian distribution. Interestingly, the orientation of surface texture was seen to be altered by treatment of samples with laser at 70 and 140 Jcm⁻², where a more-Gaussian distribution of texture was identified. The overall effect of laser treatment on Ra and Rq was significant ($p \leq 0.02$), with post hoc comparisons revealing that the 140 Jcm⁻² surface characteristic parameters (Ra and Rq) were significantly lower than untreated

Fig. 1 Scanning electron microscopic images of Ti64 surface at different laser fluences (**a–e**). Variation of etch depth/pulse with laser fluences (**f**)

($p = 0.012$) and laser treated samples at 300 Jcm^{-2} ($p = 0.038$).

Table 2 shows the results corresponding to characterization of orientation for all five samples. The surface textures were found to be orientated at an angle of approximately 2.8° (0.31°) in laser treated samples at 140 Jcm^{-2} . For the lower and higher values of laser fluences than 140 Jcm^{-2} , the overall orientation of surface texture was increased, dramatically. The overall effect of laser treatment was significant ($p \leq 0.01$), with post hoc comparisons revealing that the 140 Jcm^{-2} orientation was significantly lower compared to the untreated and samples that were treated by laser at 70 , 250 and 300 Jcm^{-2} ($p < 0.03$).

Both watershed lines and overlaid dams images (Fig. 5), prepared from the segmentation analysis, support the findings of orientation of surface texture. The identified segments were different between samples; however, their

shape seemed to be more regular for 140 Jcm^{-2} sample. Smaller segments were found in both untreated and 70 Jcm^{-2} samples, while in 250 Jcm^{-2} sample they were more aligned along the crack axis. Deformation of surface segments after thermal ablation and crack formation (Fig. 5g) as well as the overall reduction of segments' size between dendritic patterns after partial surface remelting (Fig. 5c) was a frequent occurrence that were observed in all samples.

Reconstructed 3D surfaces for untreated and laser treated samples are shown in Fig. 6. Apparent differences in surface morphology was seen among all the samples, while laser treatment at 140 Jcm^{-2} led to more smooth surface relative to other sample (Fig. 6c). The untreated sample's surface was rough with relatively high profile height (Fig. 6a). Propagation of dendritic texture in radial direction (Fig. 6b, denoted by arrow) and thermal ablation of surface resulted in randomized deep valleys (Fig. 6d,

Fig. 2 Ti64 surface profiles obtained at different laser fluences **a** 0 Jcm^{-2} , **b** 70 Jcm^{-2} , **c** 140 Jcm^{-2} , **d** 250 Jcm^{-2} and **e** 300 Jcm^{-2}

Fig. 3 Effect of laser fluence on arithmetical mean height (Ra) and root mean square deviation (Rq) of Ti64 samples. The trends of Ra and Rq (best fit curves) indicating lower depth and reflecting smoother surface are shown by dashed and solid lines, respectively

denoted by *) was identified in 70 and 300 Jcm^{-2} samples, respectively.

4 Discussions

Understanding the impact of morphology of the implant on micromechanical properties and implant osseointegration is an important step towards developing more-accurate multi-scale computational models for implant function, which can lead to improved strategies for tissue engineering and repair. Based on our previous studies significant improvements in micromechanical and biomedical properties of Ti64 were reported, however, appropriate surface morphology characterization was not reported [20, 22]. Therefore the aim of this study was to characterize the morphology of Ti64 surface in order to achieve more information on surface texture alteration, which may affect osseointegration process after laser treatment. We found significant differences between arithmetical mean height (Ra), root mean square deviation (Rq) and texture orientation, all were lower for 140 Jcm^{-2} samples compared to untreated samples (Fig. 3 and Table 2). An apparent shift

Table 1 Mean (95% CI) of surface characteristic parameters obtained from surface plot analysis

Laser fluence (Jcm^{-2})	Max valley depth (Rv)	Max peak height (Rp)	Max profile height (Rt)	Skewness (Rsk)	Kurtosis (Rku)
0	− 11 (2.42)	9.83 (1.28)	20.83 (1.98)	− 0.125	2.248
70	− 5.5 (0.91)	8.2 (1.12)	13.7 (0.99)	1.006	2.412
140	− 5 (0.43)	6.3 (0.81)	11.3 (0.71)	1.062	2.893
250	− 2.9 (0.21)	4.8 (0.35)	7.7 (0.32)	1.201	1.706
300	− 7 (1.12)	6.6 (0.93)	13.6 (1.23)	− 0.647	1.950

Fig. 4 The orientation of surface texture **a** before laser treatment and **b–e** after treatment at 70, 140, 250 and 300 Jcm⁻², respectively

of surface morphology to more Gaussian distribution, which was consistent with created segments after surface remelting and 3D reconstructed surface, revealed a significant relation between surface morphology and laser treatment.

From micro-mechanical point of view, the previous finding of enhanced properties [16, 20, 22] after laser treatment at 140 Jcm⁻² compared to untreated, as well as 70 and 250 Jcm⁻² samples, were consistent with new finding of significant difference between samples' surface

Table 2 The summary of Mean (95% CI) overall surface texture orientation across all untreated and laser treated samples at different laser fluences

Laser fluence (Jcm ⁻²)	Overall orientation (°)
0	74.8 (8.1)
70	35.8 (4.2)
140	2.8 (0.31)
250	56.5 (13.4)
300	69.2 (17.8)

morphologies. The higher surface hardness (850, 377 HVN for 140 Jcm⁻² and untreated samples, respectively), enhanced corrosion resistance, lower contact angle (35° for 140 Jcm⁻² and 70° for untreated sample) and higher surface tension (60 mNm⁻¹) for 140 Jcm⁻² compared to untreated surface (39 mNm⁻¹) have been reported before.

Decrease in maximum height profile, maximum valley depth and maximum height peak after surface laser treatment at 140 Jcm⁻² (Table 2), identified a more relatively smooth surface, where the inclusions were disappeared and the scratches due to machining and polishing were sealed. The lower value of measured Ra for 140 Jcm⁻² samples indicated a decrease in surface roughness; however, it was impossible to differentiate between profile peaks and valleys by using this parameter, alone. Therefore Rq, as a more sensitive parameter that is able to distinguish between surfaces with different profiles and distribution of surface texture, was used. In general Rq has a slightly (10–20%) higher value than Ra, as seen in current study, however, it was identified to be lower for 140 Jcm⁻² sample compared to other samples. The lower Rq was consistent with the finding of relevant alteration for distribution of texture orientation in 140 Jcm⁻² sample (Fig. 2c). This indicated that surface segments (Fig. 5e, f) are more uniformly distributed with a more Gaussian distribution. The segments may reflect the reorganization of grain boundaries and more probably, attributed to grain refinement associated with laser melting and rapid solidification. The plasma-induced damage and crack formation, which became dominant throughout the surface at high laser fluence, were seen to affect the characterization of surface parameters including roughness, texture orientation and segmentation. More interestingly, distribution of texture orientation at high laser density (250 and 300 Jcm⁻²) were approximately similar to untreated sample (Fig. 4a, d, e).

From osseointegration point of view, the observation of change in density of cell network from mono-layer to multi-layer for 140 Jcm⁻² samples [20, 22] were consistent with the optimum height profile obtained in the sample (Figs. 2, 3). Also the finding of relatively low orientation of

Fig. 5 Watershed lines and overlaid dams images for **a**, **b** untreated and **c–h** laser treated samples. Laser fluence was set at **c, d** 70, **e, f** 140 and **g, h** 250 Jcm⁻², respectively

surface segments after laser treatment at 140 Jcm⁻² (Table 2) supports the previous finding revealed that there was no specific directional spread of attached cells. The enhanced bone healing, based on histology assessment performed at distance osteogenesis, may attributed to the contact angle decrease, is consistent with the current study results, which shows improved surface characteristics for carefully selected laser density in treated samples.

It was proved that the samples irradiated by laser beam can cause oxygen diffusion through the molten materials and thus to oxidize the titanium [37]. Also the variation of surface oxidation layer thickness depends on steam sterilization process and the time of exposure to air [38]. As the oxygen content of surface increases, the measured contact angle decreases. This is explained by the fact that surfaces with higher concentration of oxygen atoms and more incorporation of oxygen-base polar functionalities of surface exhibit higher wettability. Therefore, change in

surface oxide layer seems to be relevant to the result of the current study identifying different surface characteristics among samples.

Our study showed that surface texture can affect the osseointegration process, the more optimized surface roughness, the more effective will be the cell-surface interaction. In the current research, a surface roughness with maximum height profile of 10 µm (approximately) was achieved, which is expected to enhance both the micromechanical and biomedical properties. Another important finding of our analysis is that Rsk showed a positive value for laser treated samples, compared to untreated one. Rsk as a measure of asymmetry of surface height relative to the mean line, shows how peaks and valleys are distributed throughout the surface (i.e. for Gaussian distribution Rsk = 0). It seems that surface with negative Rsk are rough, while positive Rsk indicated more porous surface. As a matter of fact surfaces with the same

Fig. 6 3D reconstructed surface profiles of Ti64 sample at different fluence of laser **a** 0 Jcm^{-2} , **b** 70 Jcm^{-2} , **c** 140 Jcm^{-2} and **d** 300 Jcm^{-2} . The surface texture of samples at lower laser fluence are oriented radially (denoted by arrows). Increasing the laser fluence to 300 Jcm^{-2} resulted in thermal ablation inducing deep valleys (denoted by *) that randomly distributed on the surface of sample. The scale bar is the same for all images (a–d)

Ra and Rq can have different Rsk, which may affect osseointegration process. Our surface characterization revealed that Rku, which tells us how high or flat the surface profile is, for treated sample at 140 Jcm^{-2} was marginally close to 3. Rku less than 3 indicates flat peaks and valleys, while its greater values than 3 shows sharp peaks and valleys in a surface profile.

5 Conclusion

The present analysis demonstrates that the samples treated at 140 Jcm^{-2} provided an optimized surface characteristics, which have a direct influence on the osseointegration process. The significant effect of laser surface treatment on optimization of surface parameter, i.e., Ra, Rq and Rku makes it a prominent method to enhance implant-cell interaction.

Compliance with ethical standards

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

Ethical approval It is declared that no human or animal has been used at any stage during this experiment.

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