Improved Osseointegration

Influence of Picosecond Laser Induced Topographical Modification on *in-vitro* Osseointegration of Ti6Al4V Bio-alloy

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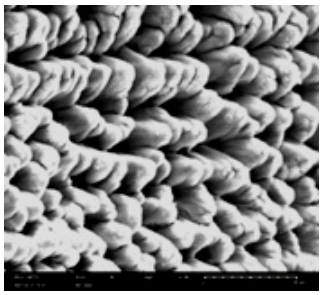

Picosecond laser induced cones on Ti6Al4V bio-alloy

ABSTRACT

With increase in the demand for artificial body implants, efforts expended towards overcoming implant failure have also increased. Laser assisted surface micro structuring of biomaterials is known to increase their biofunctionality. This work is aimed to qualify the best suitable microstructure among six unique patterns generated on Ti6Al4V bio-alloy by employing a 30ps pulsed laser. Time dependent in vitro osseointegration was evaluated from the rate and quality of hydroxyapatite (HA) growth on the samples. The SEM and EDS analysis confirmed good quality of HA and micro-Raman analysis revealed ~4.6 fold enhancement in the growth of HA on one of the laser treated sample in comparison to pristine sample.

KEYWORDS: Osseointegration, Picosecond laser surface modification, Micro structuring, Cell and protein adhesion, HAP growth

Introduction

Equipped with unique combination of high strength and low density, grade-5 titanium alloy (Ti6Al4V) has been considered a suitable biomaterial for dental and orthopedic implants applications [1]. In spite of excellent biocompatibility and corrosion resistance, this material exhibits fatigue failure due to poor osseointegration in the body [2]. The osseointegration is a complex process [3] that begins with the growth of hydroxyapatite (HA: $Ca_{10}(OH)_2(PO_4)_6$), a bone like material, on the surface of artificial implant that joins the implant to the body tissues. During interaction with blood plasma, dissociation of H_2O on the surface of implant leads to the formation of OH ions that subsequently stimulate absorption of Ca^{2+} ions and promote nucleation of HA. A mature HA layer is basically calcium orthophosphate salt with a Ca/P molar ratio about ~1.677 [4]. Growth of HA and further osseointegration is greatly influenced by the surface properties of the implant and hence suitable modification of the surface of an implant is a proven method to improve its biofunctionality.

Among various techniques such as texturing, coating, alloying, and oxidation, the laser surface texturing is attracting the attention of researchers for achieving the desired surface modification of biomaterials. This is because, laser assisted surface texturing is an easy and contamination free process involving minimum steps and can be achieved remotely [5]. The three factors that play a significant role here are the laser parameters, the substrate material properties and the experimental conditions. For a given material, change in laser power, wavelength, pulse duration and irradiation time can result in substantial topographical variation on the sample surface [6-7]. While absorption by the surface depends on its roughness and the wavelength of the incident radiation, the rise in temperature depends also on the laser pulse duration, repetition rate and fluence. Menciet et al. reported effect of laser wavelength and pulse duration on the surface structure of β Ti-alloy [6]. Morales et al. discussed effect of change in laser fluence and irradiation time on surface structure of Ti6Al4V sample [7].

In this work, a picosecond pulsed Nd-YAG laser operating on 532 nm at 10Hz repetition rate was employed to irradiate the surface of Ti alloy biomaterial. The laser power and sample scan speeds (along x and y directions) were varied. Surface melting coupled with varying extent of overlap of successive pulses resulted in the generation of several unique patterns on Ti6Al4V bio-alloy. Of the many patterns thus generated, six unique ones with distinctly different microstructures were chosen for *in vitro* testing of HA growth. Laser textured and pristine samples were immersed in simulated body fluid (SBF) for 24 hrs, and the laser treated sample with maximum number of nucleation sites of HA as revealed by scanning electron microscope (SEM) image was chosen for further studies. The change in wettability of the samples was studied by water contact angle (WCA) measurement. Energy dispersive X-ray spectroscopy (EDS) and micro-Raman analysis were used to confirm growth of HA on the samples. The results revealed a significant improvement of about 4.6 folds in the growth of HA on laser treated sample in comparison to pristine sample.

Material and Methods

Ti6Al4V bio-alloy sheets (~1mm in thickness) were procured commercially and cut into square pieces of ~10 mm x 10 mm and were mechanically polished using 180, 400 and 800 grit papers in that sequence and ultrasonicated in acetone, ethanol and water in that order for 10 min.

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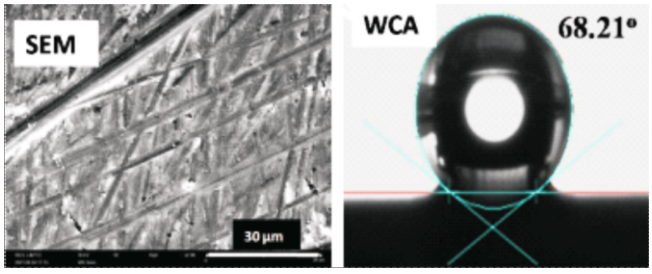
Table 1. Samples and experimental parameters

Sample	Laser power (mW)	Scan speed (µm/sec)	Overlapping (µm)
S1	2	2.5	100
S2	10	2.5	20
S3	10	2.5	80
S4	10	10	20
S5	20	5	50
S6	20	50	50

For laser surface modification experiments, the second harmonic emission from a picosecond Nd:YAG laser (Model # N311, Ekspla make) with 30 ps pulse duration and 10 Hz pulse repetition rate was focused on to the surface of the sample with a 10 cm focal length lens and the focal spot was ~30 µm. Different combinations of laser power, sample scan speed and lateral overlapping were tried to generate a variety of microstructures on Ti6Al4V sample surface. Experimental parameters of six samples that were chosen based on their significantly different surface patterns are listed in Table-1. Topographical modification of the samples was studied using SEM (M/s. SEC Co.). For wettability, about 1 µL water drop was put on the sample using a computer controlled micro-syringe and photo of the drop was recorded for WCA estimation using an inbuilt CCD camera.



(a) (b)

Fig.1: (a) SEM image and (b) WCA of pristine Ti6Al4V sample.

Table 2. Amount of reagents in the order they were used in the preparation of SBF

Order	Reagent	Amount in gm/L
1	NaCl	6.547
2	KCl	0.373
3	$NaHCO_3$	2.268
4	$Na_2HPO_4.2H_2O$	0.178
5	$MgCl_2.6H_2O$	0.305
6	$CaCl_2.2H_2O$	0.368
7	Na_2SO_4	0.071
8	$CNH_3(CH_2OH)_3$	6.057

To study in *vitro* osseointegration, pristine and laser treated samples were immersed in SBF which was prepared in-house by following a standard protocol [8]. To prepare 1 L of SBF, the reagents listed in Table-2 were dissolved in the mixture of de-ionized water (950 ml) and 1 M HCl (50 ml) on a magnetic stirrer. The pH of the fluid was maintained at 7.4 and each sample was immersed separately in 50 ml of SBF for 24 hrs and maintained at 37°C. The growth of HA on sample surface was analyzed using SEM, EDS and micro-Raman spectroscopy.

Results & Discussion

Fig. 1a and 1b are the SEM and WCA of pristine Ti6Al4V sample. Rough surface and hydrophilic nature with WCA of 68° were observed for this sample.

Laser surface treatment

Figs. 2a and 2b show SEM images of laser treated samples (S1 - S6) at two different scale bars of 30 µm and 5 µm. The laser treated area can be seen to contain micron size features covered with nanostructures, such hierarchy structures being very important for superior osseointegartion, cell adhesion and bacterial inhibitition [9-11]. The samples are significantly different from each other in sub-micron level and this is clearly visible in the magnified images. The WCA of laser treated samples increased in comparison to WCA of pristine

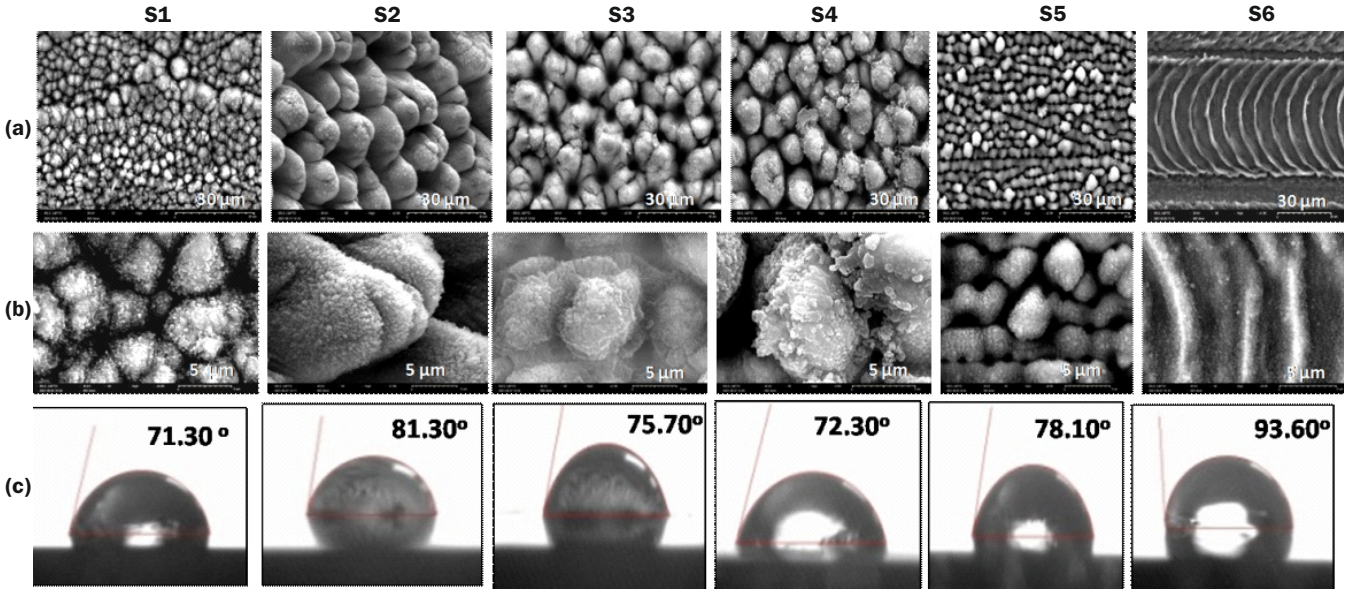


Fig.2: SEM images of laser treated samples at scale bar of (a) 30 µm and (b) 5 µm and (c) WCA of laser treated samples.

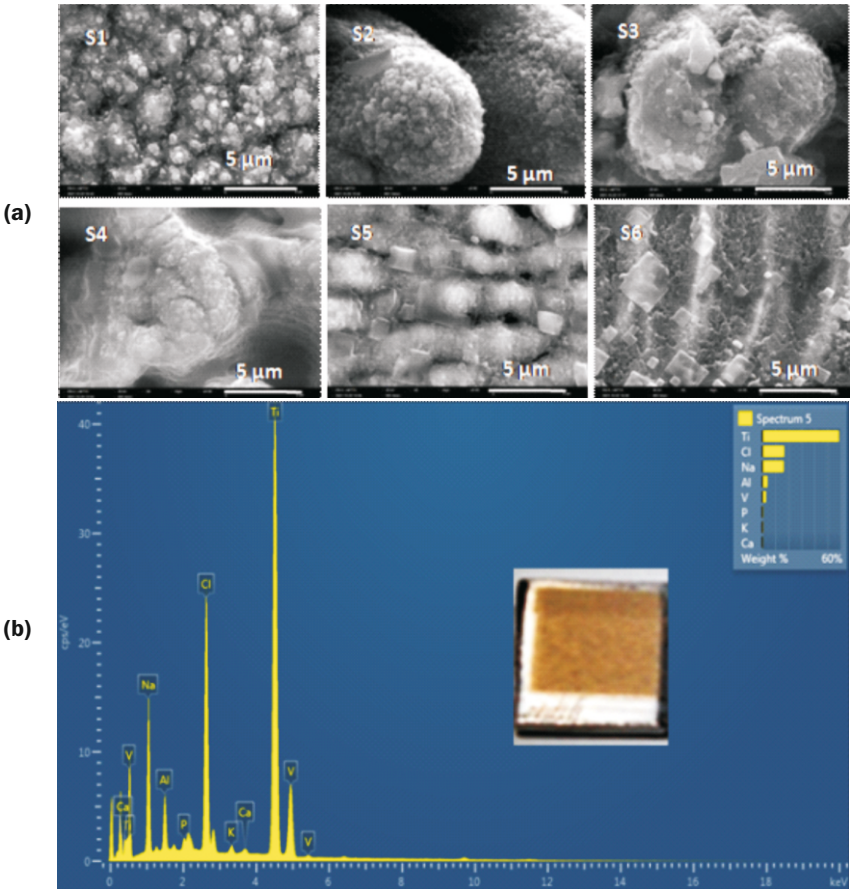


Fig.3: (a) SEM image of laser treated samples and (b) EDS of S6 sample after immersion in SBF for 24 hrs; inset is a photograph of S6 sample in which the golden part is laser treated region.

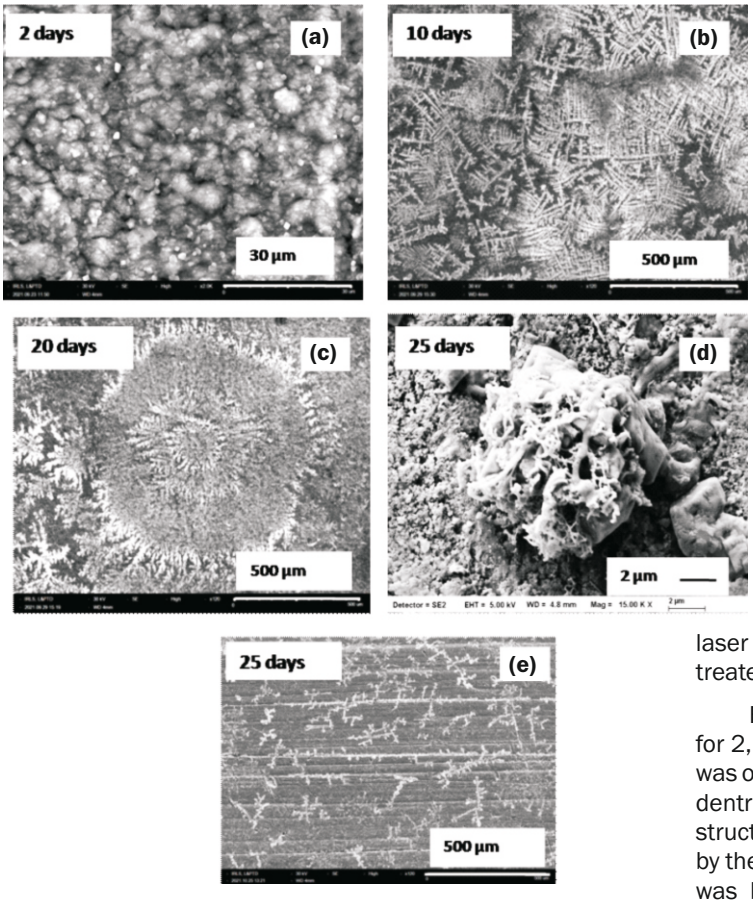


Fig.4: SEM image of S6 sample immersed in SBF for (a) 2 days, (b) 10 days, (c) 20 days, (d) 25 days and (e) Pristine sample immersed in SBF for 25 days.

Ti6Al4V and sample S6 showed hydrophobic nature. The decreased wettability is due to air that gets locked in the pockets of microstructures on laser treated samples that, in turn, restricts spreading of water on the surface of the sample.

Fig. 3(a) shows the SEM images of laser treated samples immersed in SBF for 24 hrs. Deposition of crystalline structures was observed on the surface of all samples. The EDS analysis of S6 sample shown in Fig. 3b revealed that the crystals were rich in sodium and chlorine; however calcium and phosphorus were also traced in small amount. Presence of Ca and P on the sample indicated initiation of osseointegration within 24 hrs. Density of the crystals and hence, the nucleation sites for growth of HA were highest on S6 sample as seen from SEM images and therefore, S6 was chosen for further study of day dependent growth of HA. For this, about four samples of S6 were made and immersed separately in SBF for pre-decided number of days viz., 2, 10, 20, and 25 days. Inset of Fig. 3b shows a photograph of S6 sample where golden region is the laser treated area. Growth of HA was compared on laser treated and untreated area for all cases.

Fig. 4a-4d shows the SEM images of S6 immersed in SBF for 2, 10, 20 and 25 days, respectively. Crystalline deposition was observed after 2 days and the morphology changed to long dentrite structures on the sample after 10 days. With time, the structure enlarged and took shape of concentric flowery circles by the end of 20 days (Fig. 4c). The Ca/P ratio of the structure was between 1.18 - 1.35 on this sample. After 25 days, spherical agglomerations were observed on surface of S6 sample (Fig. 4d) while HA growth in the form of linear structures was seen on the surface of pristine sample (Fig. 4e).

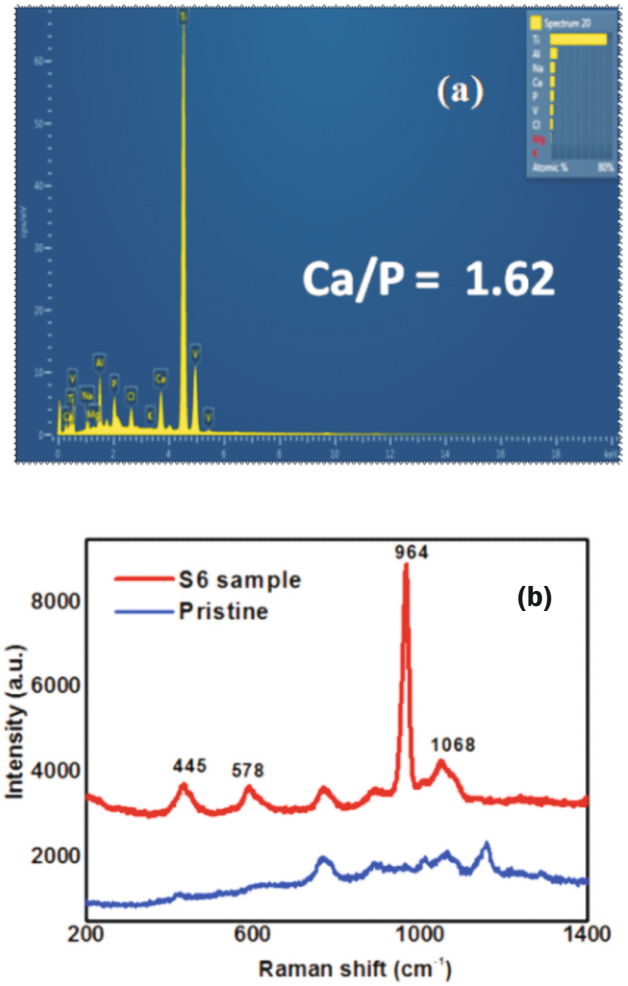


Fig.5: (a) EDS of HA grew on S6 sample and (b) Raman spectrum of HA grew on pristine and S6 sample after 25 days immersion in SBF.

Fig. 5a shows EDS analysis of HA grew on S6 sample after 25 days of immersion in SBF. The Ca/P ratio was found to be 1.62 which is close to the Ca/P ratio of matured HA [4]. For the same time interval the Ca/P on the pristine sample was about 1.22. This indicated the quality of HA grew on S6 sample was superior to HA on pristine sample. Fig 5b shows the Raman spectrum of HA on pristine and S6 samples after immersion in SBF for 25 days. The strongest peak at 964 cm^{-1} is attributed to the symmetric stretching mode of PO_4 tetrahedron and this is the characteristic peak of HA. The second peak at 1068 cm^{-1} can be assigned to the stretching vibration mode of CO_3^{2-} bond [12]. A significant improvement of ~ 4.6 fold in the intensity of characteristic peak of HA on S6 sample indicated faster and superior growth [12], that in turn, points to superior osseointegration. This can be attributed to the generated micro-nano structures on the surface of Ti6Al4V post laser treatment that increase the surface area, create nano voids, resulting in an increased interaction of the sample with body fluid.

Conclusions

A wide variety of micro-nano structures were generated on the surface of Ti6Al4V bio-alloy by controlling two experimental parameters viz., laser power and sample scan speed. The samples exhibited significant changes in the microstructure as revealed by SEM images and wettability as indicated by WCA measurements. More number of nucleation sites were observed on one of the laser treated sample (S6) within 24 hrs of immersion in SBF and ~4.6 fold improvement in the growth of HA was observed after 25 days as compared to the pristine sample. Thus the study indicates that laser surface treatment, for judiciously chosen laser parameters, is a viable method for improving the osseointegration of biomaterials in human body.

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References

[1] D. Li, J. Zou, R. Xie, Z. Wang and B. Tang. Surface texture-Based surface treatments on Ti6Al4V titanium alloys for tribological and biological applications: A mini review. Materials, 2018, 487, 11, 1-28. https://doi.org/10.3390/ma11040487.

[2] C. W. Lin, C. P. Ju, J. H. C. Lin. A comparison of the fatigue behavior of cast Ti-7.5Mo with c.p. titanium, Ti-6Al-4V and Ti-13Nb-13Zr alloys. Biomaterials, 2005, 26, 2899-2907. http://doi.org/10.1016/j.biomaterials.2004.09.007.

[3] P. Vaidya, S. Mahale, S. Kale and A. Patil. Osseointegration-A review. IOSR-JDMS, 2017, 16, 45-48. DOI: 10.9790/0853-1601014548.

[4] P. Prochor and Z. A. Mierzejewska, Bioactivity of PEEK GRF30 and Ti6Al4V SLM in simulated body fluid and Hank's balanced salt solution. Materials, 2021,14,2059–1-17. http://doi.org/10.3390/ma14082059.

[5] S. Shaikh, S. Kedia, D Singh, M. Subramanian and S. Sinha, Surface texturing of Ti6Al4V alloy using femtosecond laser for superior antibacterial performance. J. Laser. Appl., 2019, 31, 022011, 1-10. http://doi.org/10.2351/15081106.

[6] G. Menci, A. G. Demir, D. G. Waugh, J. Lawrence and B. Previtali, Laser surface texturing of β-Ti alloy for orthopedics: Effect of different wavelengths and pulse duration. Appl. Surf. Sci., 2019, 489, 175-186. http://doi.org/10.1016/j.apsusc.2019.05.111.

[7] A. I. A. Morales, S. Alamri, T. Kunze and A. F. Lasagni. Influence of processing parameters on surface texture homogeneity using direct laser interference patterning. Opt. Laser Tech., 2018, 107, 216-227 .

[8] T. Kokubo and H. Takadama, How useful is SBF in predicting in vivo bone bioactivity. Biomaterials, 2006, 27, 2907-2915. doi:10.1016/j.biomaterials.2006.01.017.

[9] N. Sirdeshmukh and G. Dongre. Laser micro & nano surface texturing for enhancing osseointegration and antimicrobial effect of biomaterials: A review. Materials Today: Proceeding, 2021, 44, 2348-2355. http://doi.org/10.1016/j.matpr. 2020.12.433.

[10] B. S. Moon, S. Kim, H. E. Kim and T. S. Jang. Hierarchical micro-nano structured Ti6Al4V surface topography via two-step etching process for enhanced hydrophilicity and osteoblastic responses. Mat. Sci. Eng. C, 2017, 73, 90-98. http://doi.org/10.1016/msec.2016.12.064.

[11] D. J. Cohen, A. Cheng, K. Sahingur, R. M. Clohessy, L. B. Hopkins, B. D. Boyan and Z. Schwartz. Performance of laser sintered Ti- 6Al-4V implants with bone-inspired porosity and micro/nanoscale surface roughness in the rabbit femur. Biomed. Mater., 2017, 12, 025021. https://doi.org/10.1088/1748-605X/aa6810.

[12] S. Shaikh, S. Kedia, A. K. Singh and S. Sinha, Surface treatment of 45S5 bioglass using femtosecond laser to achieve superior growth of hydroxyapatite. J. Laser Appl., 2017, 29, 022004-1-8. http://dx.doi.org/10.2351/14979304.