J Mater Sci: Mater Med (2011) 22:1671–1679
DOI 10.1007/s10856-011-4342-3

Laser surface modification of titanium substrate for pulsed laser deposition of highly adherent hydroxyapatite

P. Rajesh · C. V. Muraleedharan · Manoj Komath · Harikrishna Varma

Received: 14 February 2011 / Accepted: 9 May 2011 / Published online: 20 May 2011

Abstract Biomedical implant devices made out of titanium and its alloys are benefited by a modified surface or a bioactive coating to enhance bone bonding ability and to function effectively in vivo for the intended period of time. In this respect hydroxyapatite coating developed through pulsed laser deposition is a promising approach. Since the success of the bioactive ceramic coated implant depends mainly on the substrate-coating strength; an attempt has been made to produce micro patterned surface structure on titanium substrate for adherent hydroxyapatite coating. A pulsed Nd-YAG laser beam (355 nm) with 10 Hz repetition rate was used for surface treatment of titanium as well as hydroxyapatite deposition. The unfocussed laser beam was used to modify the substrate surface with 500–18,000 laser pulses while keeping the polished substrate in water. Hydroxyapatite deposition was done in a vacuum deposition chamber at 400°C with the focused laser beam under 1×10^{-3} mbar oxygen pressure. Deposits were analyzed to understand the physico-chemical, morphological and mechanical characteristics. The obtained substrate and coating surface morphology indicates that laser treatment method can provide controlled micro-topography. Scratch test analysis and microindentation hardness values of coating on laser treated substrate indicate higher mechanical adhesion with respect to coatings on untreated substrates.

P. Rajesh · M. Komath · H. Varma (✉)
Bioceramics Laboratory, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Trivandrum 695 012, Kerala, India
e-mail: varma@sctimst.ac.in; prhkvarma@gmail.com

C. V. Muraleedharan
Division of Artificial Internal Organs, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum 695012, Kerala, India

1 Introduction

Titanium and its alloys are widely used in biomedical applications and device components. These are commonly used in dental and orthopedic prostheses because of their desirable properties, such as relatively low modulus, good fatigue strength, formability, machinability, corrosion resistance, and biocompatibility [1–3]. However these implants cannot meet many of the clinical requirements such as direct bone bonding in lesser time periods and long term in vivo functionality. The inherently less bone bonding and mechanical integration with tissue can be improved by providing a bioactive surface of calcium phosphate ceramics such as hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. A coating of hydroxyapatite (HA) was proven to be efficient for better bone bonding and to provide long term in vivo functionality [4–9]. Plasma spraying technique is the commercially accepted technique for producing thick calcium phosphate coatings. However, this high temperature process mostly produces coatings with inhomogeneity in phase, micro cracks, etc. and is prone to failure under in vivo conditions [10–12]. A better alternate for plasma spraying is the pulsed laser deposition (PLD) where the stoichiometric transfer of sintered HA results to form a thin adherent bioactive coating onto titanium substrate [13]. The coating adhesion strength of HA to titanium depends on the surface chemistry as well as the microstructure of the substrate apart from the process parameters of PLD such as substrate temperature, laser power density, etc. [13–17]. Various surface modification techniques such as surface oxidation, nitridation, ion implantation, etc. have been used

to improve the metal-ceramic interface [18–22]. In recent years laser was shown to be a powerful tool for controlled micro-structuring and modifications of material surfaces. Lasers have also been used for modifying ceramic as well as metallic implant surfaces for improving bio-functionality. The use of a laser for texturing surfaces presents many advantages; for instance, it is rapid and extremely clean [23–27].

In this work an improvement of the coating adhesion strength was attempted by water assisted laser surface modification of titanium prior to PLD of HA. Micro patterning in water by short-pulsed laser provides clean grooves while normal processing in air results in grooves filled with molten material. The presence of water helps to minimize the surface heating effect thereby minimizes surface hardening effects, structural and mechanical deformations of the substrate surface. The surface etching rate can also be easily controlled as the bubbles formed in liquid carry the debris away effectively which enhances the etching rate in liquid while the bubbles scatter laser light, which lowers the etching rate in liquid [28–30]. HA was deposited over titanium substrate modified by irradiation of different number of laser pulses and were analyzed in comparison with HA coating on unmodified titanium substrate. A better control of the surface micro-structure of substrate along with mechanical adhesion of HA was possible by the laser modification of titanium substrate in an aqueous environment.

2 Materials and methods

2.1 Substrate, target, PLD system, laser surface treatment and HA coating

Commercially available Ti6Al4V (ASTM F1108, Manhar Metal Supply Corporation, Mumbai, India) was machined to discs of thickness 2 mm and diameter 9 mm. The titanium substrates were polished by tumbling method with different grades of SiC powders and alumina powders successively as grinding media to get an average roughness around 0.45 μm. These are cleaned ultrasonically with distilled water, rectified spirit, acetone, and then dried prior to laser treatment. HA powder was prepared in-house by a wet precipitation technique using $Ca(NO_3)_2{\cdot}4H_2O$ and $NH_4H_2PO_4$ (Rankem, India) at pH 11 and 80°C. HA target was prepared from calcined (300°C) and pulverized powder of particle size around 125 μm. Powder compaction was done at 200 MPa in a cold isostatic press (Cold Isostatic Press, EPSI, Belgium) and subsequent sintering at 1100°C for 2 h. The sintered target was mechanically polished by silicon carbide papers of grit size 240 and 600 (Buehler-Ecomet 3 Variable speed Grinder-Polisher, Buehler Ltd, USA). The target was then cleaned successively in distilled water, rectified spirit, and acetone followed by drying in hot air. A schematic of the laser treatment of titanium substrate in water and PLD system is shown in Fig. 1. For the surface treatment; substrate was positioned in the laser transparent container filled with deionized water. Substrate was inserted with a movable sample holder that can be suitably positioned or scanned to enable the whole surface to be treated by laser. A beam of 355 nm from a Q-switched Nd-YAG laser (Quanta systems, S.P.A, Italy) with a beam diameter of 10 mm was used to modify the surface. The laser delivered an output of 2 W at a repetition rate of 10 Hz. Six groups of titanium discs were treated with different number of laser pulses (500; 1,000; 3,000; 6,000; 12,000; and 18,000). PLD of HA was done in a custom designed deposition system (developed by Excel Instruments Mumbai, India). The 10 mm out put laser beam (355 nm) with 10 Hz repetition rate was focussed to the HA target at a spot size of 1 mm and an angle of 45°. The substrate was positioned face to face with the target at a distance of 35 mm. The chamber was evacuated to a base pressure in the range of 10^{-6} mbar by means of attached turbo molecular pump backed with a rotary pump and then filled with oxygen to a working gas pressure of 1×10^{-3} mbar for the deposition of HA. Deposition of HA was done for about 1 h at a substrate temperature of 400°C to get a thickness of around one micron assessed by a profilometer.

2.2 Structural and morphological characterization

The surface roughness of Ti6Al4V substrate and HA coating were measured by profilometry technique. The surface profile was obtained using Talysurf CLI 1000 (Taylor Hobson, UK) with the software Talymap Gold. The thickness of the coating was determined by step height measurement. The phase analysis of target and the deposited films were done by X-ray diffraction analysis (XRD). The samples were scanned between $2\theta = 20$ and 50° using Cu Kα1 radiation at a voltage of 40 kV and a current of 30 mA (Siemens D-5005, X-ray Diffractometer, Germany). Presence of functional groups –OH and $–PO_4$ was confirmed by Fourier transform infrared spectroscopy (FTIR) analysis performed on a Thermo Nicolet 5700 spectrometer (USA). The spectra were collected in the diffuse reflectance (DRIFT) mode. Samples were prepared by mixing the powder collected by scratching the surface of coated samples with optical grade KBr powder; while pure KBr was used as the background. The spectra were recorded at a resolution of 4 cm^{-1} and scanned between 400 and 4000 cm^{-1} with an average number of 64 scans. Surface of modified titanium substrate and the coatings were observed for microstructure morphology by a scanning electron microscope (ESEM-Quanta 200, Germany).

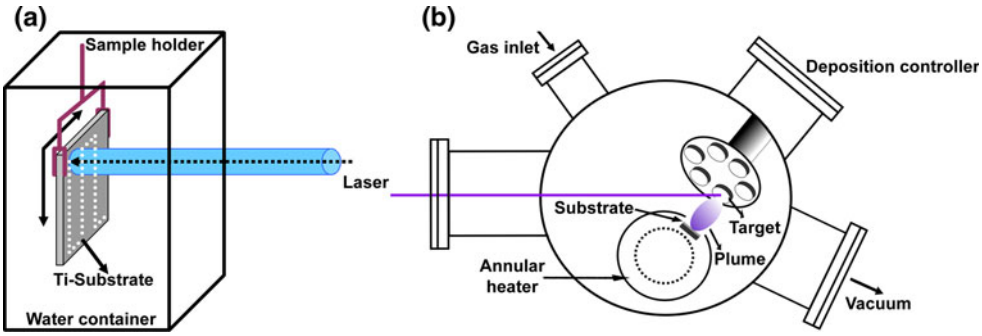

Fig. 1 **a** Schematic of laser surface modification of titanium substrate in water medium and **b** pulsed laser deposition system used for HA coating

2.3 Mechanical testing

The mechanical properties of the different coatings were compared by scratch test and indentation methods. The adhesion of HA to titanium substrate was examined with reference to ISO 20502:2005(E) [31] with a micro-scratch tester (Micro-combi tester; CSM Instruments, Switzerland) equipped with a diamond Rockwell tip of 100 μm radius, friction force measurement and an acoustic emission detector. The measurements were obtained at a progressive load of around 12 N/min for a scratch length of 5 mm and a speed of 6 mm/min. The scratch track was examined with an attached optical microscope to determine the critical failure points and further by SEM.

Microindentation hardness test was performed in accordance with ASTM E384-10e2 [32]. Four groups of samples i.e., polished titanium substrates, laser treated substrates, HA coated polished substrates and HA coated laser treated substrates were analyzed using a Vickers indenter (Shimadzu-HMV-WIN, Ver 1.03) under constant test load of 1.961 N with 14 S duration. The microindentation hardness (Vickers hardness value) was calculated as an average of six indentations per sample.

3 Results and discussions

3.1 XRD analysis

The XRD patterns of the titanium substrate and pulsed laser deposited HA film is shown in Fig. 2. The titanium substrate does not have any difference in preferential orientation or phase even after the surface treatment by laser in water within the detection level of XRD. The deposited HA film essentially had the XRD peaks indexed with the JCPDS No. 09-0432. The titanium substrates have the same pattern of JCPDS standard 44-1294. The preferred orientation [211] of the sintered HA target and deposited films are the same with decreased intensity compared to a sintered dense HA was observed. This indicates phase pure but less crystalline nature of deposited film as previously reported [20]. Due to the relatively small thickness of film,

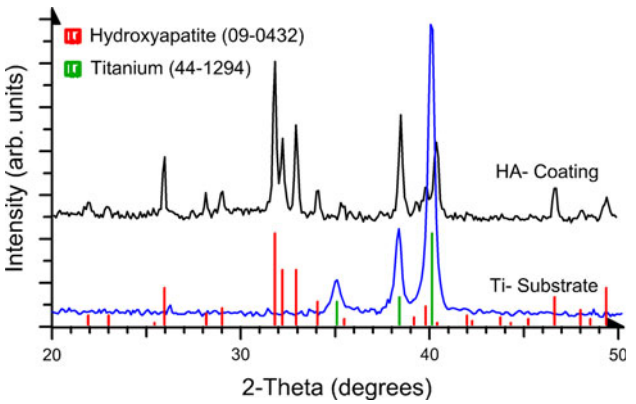

Fig. 2 XRD patterns of hydroxyapatite coating and titanium substrate indexed with corresponding JCPDS files

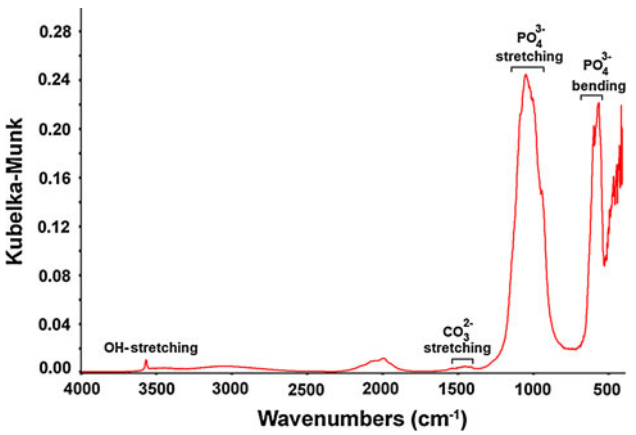

Fig. 3 FTIR spectrum of hydroxyapatite coating obtained over titanium substrate

the strong titanium substrate peaks acts to decrease the intensity of deposit in general and specifically the titanium peak at 2θ value of around 40.1° is found to mask the intensity of HA peak of [310] orientation around 39.8°. Similar trend was also observed with the most intense peaks of HA [211] and [300] leading to less resolved peak patterns. HA patterns obtained on modified and unmodified titanium are similar without any significant change in the

XRD spectra irrespective of the extent to which the modifications are done.

3.2 FTIR spectroscopy

FTIR Spectroscopy was used for the hydroxyl and phosphate functional group identification in HA target and coating. FTIR spectrum for the laser deposited HA coating onto titanium substrate is shown in Fig. 3. Spectra clearly illustrate vibrational modes associated with the –OH and $–PO_4$ groups. The coating appears to have bands for –OH stretching and vibrational modes at 3,570 and 630 cm^{-1}, respectively even after PLD of HA. PO_4 stretching vibrations are observed between 1200 and 900 cm^{-1} and those of O–P–O bending modes between 650 and 400 cm^{-1}. The spectra were similar in all cases of HA coating on laser modified surface. FTIR spectra thus showed that PLD does not impart any significant changes in hydroxyl and phosphate groups after ablation process except the slight decreased intense peaks in HA coating which was observed in similar cases [20, 33].

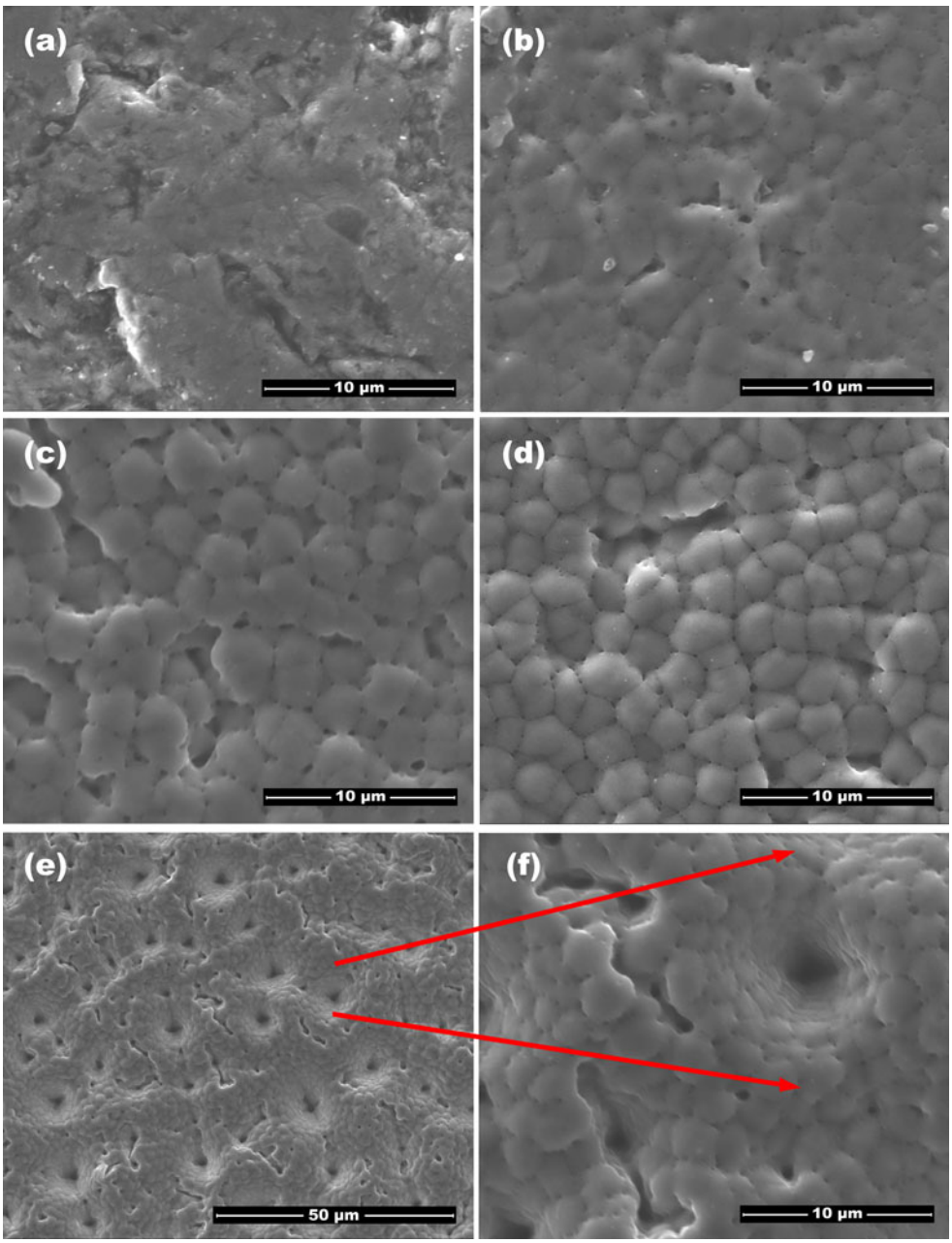

Fig. 4 Scanning electron micrographs of Titanium substrate (**a**) and laser irradiated surface with different laser pulses **b** 500; **c** 6,000; **d** 12,000; and **e**, **f** 18,000

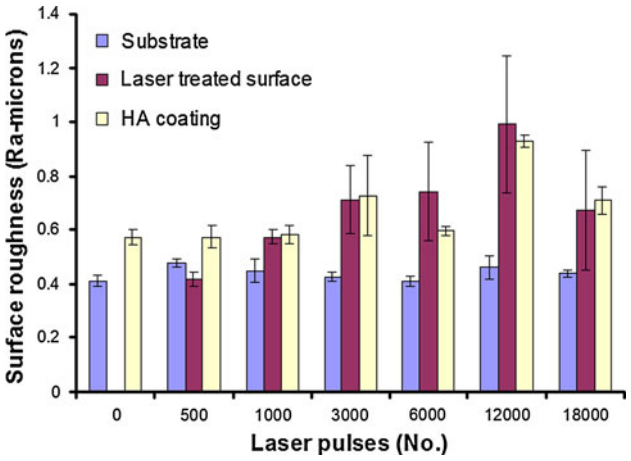

Fig. 5 Plot of average surface roughness of titanium substrates treated with different laser pulses and HA coating compared with control sample ($n = 4$)

3.3 SEM and surface analysis

The surface morphology of the substrates treated with different number of laser pulses and of polished titanium observed through SEM are shown in Fig. 4. All the samples were having an average surface roughness (Ra) of 0.45 μm measured by profilometer prior to laser treatment. The average surface roughness values measured after laser treatment and after HA coating along with their initial roughness are plotted in Fig. 5. The SEM micrographs clearly indicate the variation in surface microstructure with laser irradiation. The surface artefacts and non-uniformity occurred during the polishing stages happens to be removed at the initial stages of treatment with less number of laser pulses. The effect of laser to reveal the microstructure further continues with increased groves and depth along the boundaries of grain like structures similar as observed in many metallographic preparation by chemical etching. Finally a micro-patterned surface was achieved at about 18,000 laser pulses with periodic appearance of uniform groves having circular orientation and conical depth profile over the treated area. These kinds of microstructures are important in that the adhesion of ceramic coating to metallic substrate can be controlled by surface microstructure which provides mechanical interlocking. Once the substrate is treated by laser, the surface morphology changes and is less predominant with less number of laser pulses (500) giving random or less roughness due to surface is smoothening. As the number of pulses exceeds about 1,000; the roughness can be controlled by the number of laser pulses. Also the laser can produce micro-patterned surface at about 18,000 pulses with decreased Ra value which indicates the surface homogeneity at this micro level. The microstructure of HA coating on irradiated surface with 18,000 laser pulses is shown in Fig. 6. The substrate roughness effect also reflects in the coating surface morphology but to a lesser extend. The topography is well understood as the variation in film surface roughness with that of substrate. Thus the surface roughness which is an important criteria determining the properties such as cell growth, proliferation or tissue integration with the HA coated implants [34–36] can be tuned using laser treatment for better in vivo functionality.

3.4 Vickers hardness

Figure 7 shows the Vickers hardness values of laser treated samples and HA coatings onto the same substrates. The hardness values for the untreated titanium and the HA coating are also included in the figure. The plot indicates that there is no significant change in the mechanical behaviour of titanium samples after laser treatment in presence of water even though minimal local hardening on the surface layer due to laser action may happen. However,

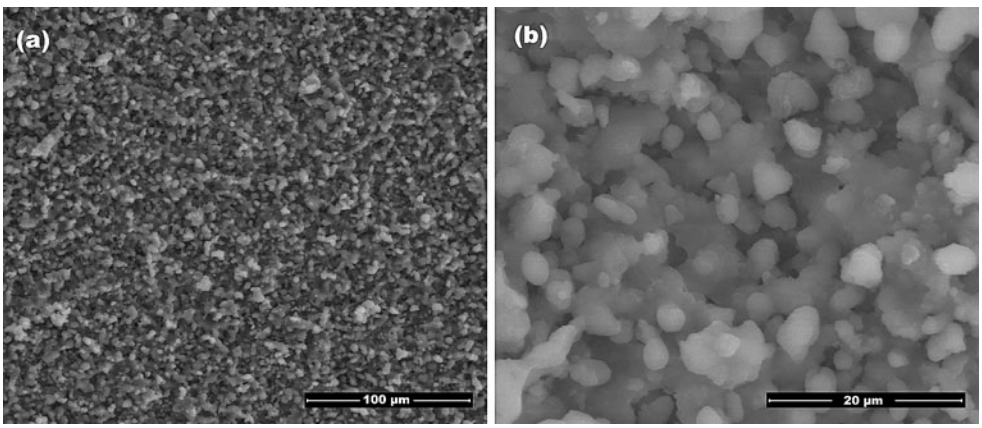

Fig. 6 Scanning electron micrographs of HA coating on laser irradiated titanium substrate with 18,000 laser pulses. **a** 1000× and **b** 6000× original magnification

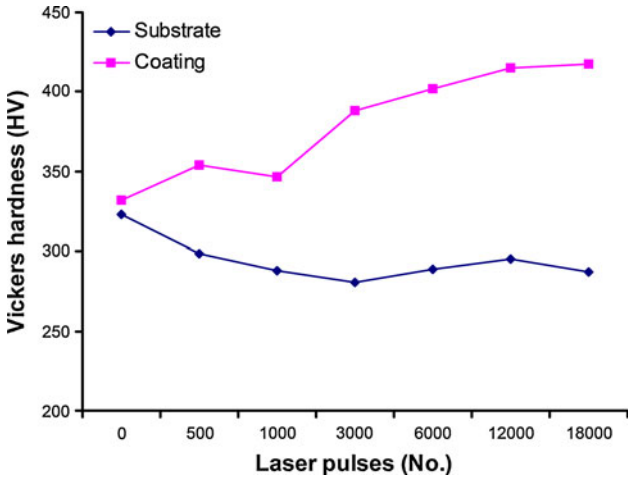

Fig. 7 Plot of Vickers hardness of control substrate, laser irradiated substrates and HA coated substrates

HA coating on samples differently treated with laser giving increased surface roughness was having improved hardness values. This increased value of HA coating towards the bulk hardness of sintered HA ceramics of about 450 N probably because of the mechanical integration of HA particles on the roughened and micro-structured surface due to larger metal-ceramic contact area.

3.5 Scratch test

The adherent nature of the HA coating on titanium substrates differently treated are obtained by scratch test. The driving force for the failure of the coating-substrate system in the scratch test are a combination of elastic–plastic indentation stresses, frictional stresses and the residual internal stress present in the coating. The failure of coating was assessed by the plot between applied load with the variation in acoustic signal and frictional force along with optical imaging of the scratch track of constant length of 5 mm. A number of consecutive coating-failure events are observed at increasing critical normal values which is the normal force at which failure occurs. The values of first crack which occurs as individual failure only at a point (denoted as Lc1), the second point from which a periodic failure points occurs through the track (Lc2) and the point of complete failure of the coating (Lc3) were located for each scratch run. Figure 8 clearly depicts various points of failure for the representative sample i.e., HA coating on untreated titanium substrate (Fig. 8a) and coating on substrate modified with 18,000 laser pulses (Fig. 8b). The SEM micrographs for the two types of coating are shown in Fig. 9; this clearly indicates the mode of failure at the metal-ceramic interface. HA coating seems to detach from

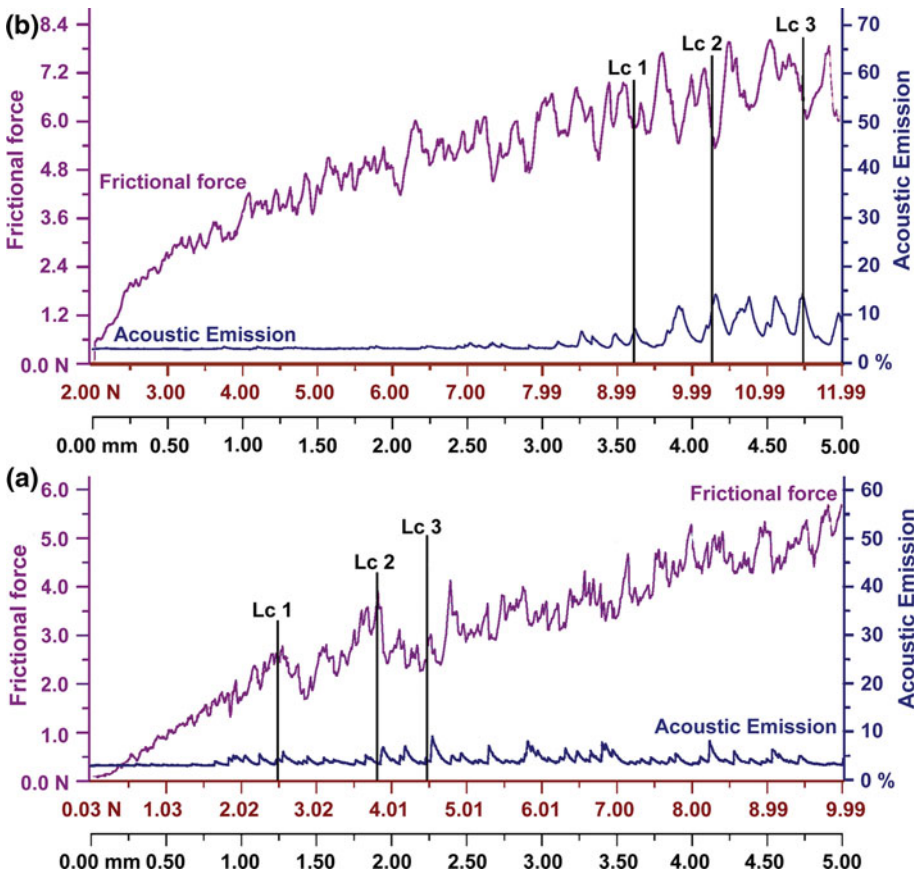

Fig. 8 **a** Scratch profile comparing acoustic and frictional force of HA coating on non-irradiated titanium substrate with **b** HA coating on laser irradiated (18,000 laser pulses) titanium substrate

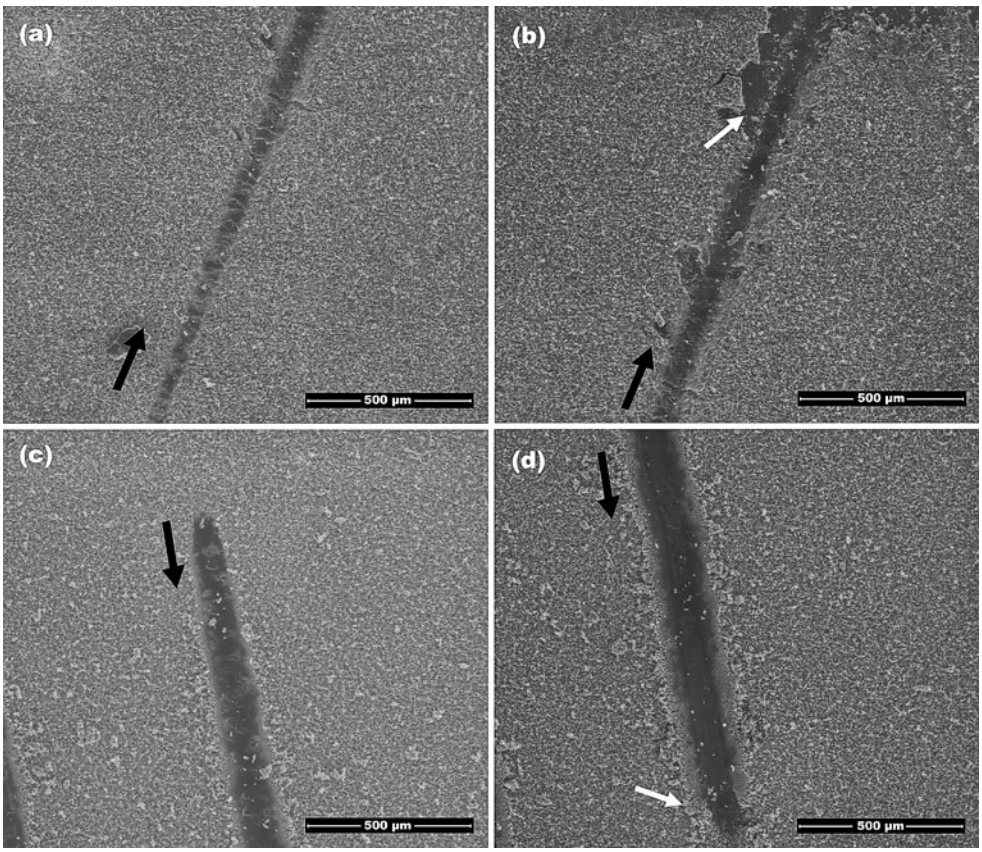

Fig. 9 Scanning electron micrographs of HA coating with direction of scratch indicated by *arrow*. **a** HA coating on non-irradiated titanium substrate at lower load, **b** region of complete delamination indicated by *arrow*, **c** HA coating on laser-irradiated titanium substrate (18,000 laser pulses) at low initial load and **d** towards complete delamination

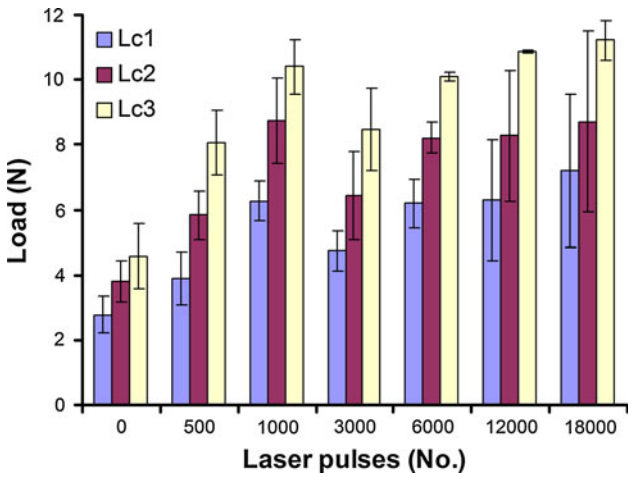

Fig. 10 Plot of failure values obtained by scratch test (Lc1, Lc2 and Lc3) for the HA coatings on differently irradiated and non-irradiated titanium substrate ($n = 4$)

the substrate as flakes at Lc3 in the case of untreated substrate, while the coating is adhered well to the laser treated substrate until delamination occurs (Lc3) through the scratch track. Figure 10 compares the adhesion strengths of HA coating on untreated polished titanium and those treated with 500–18,000 laser pulses. In all the cases HA coatings onto the laser treated substrate was found to have higher bonding strength. The value for the point of first failure (first crack, Lc1) increases as the number laser pulses increases as similar to the increase in roughness values. Similar is the case for consequent failure points which indicates that a maximum of adhesion can be achieved as indicated by the complete failure points (Lc3) at 10.87 and 11.21 N obtained, respectively of 12,000 and 18,000 laser pulses while that of untreated substrate falls to around 4.57 N only. Therefore PLD produces highly adherent HA coatings on titanium substrates subjected to controlled treatment with laser in a water environment.

4 Conclusions

Laser surface modification of titanium substrate in water can provide controlled substrate microstructure which in turn can improve the mechanical properties of HA deposited by PLD. The surface microstructure of the substrate and coating can be controlled by the number of laser pulses

without affecting the bulk mechanical property of titanium substrate. The results further confirm the deposition of crystalline phase pure HA with its characteristic chemical and compositional nature. The micro-scale pattern formed due to laser action is having significant role in increasing the metal-ceramic bond strength even though the detailed mechanism of the process is not well understood. Thus the laser surface modification for the deposition of HA by PLD can be most promising for implant fabrication compared to the unmodified titanium substrate. Laser induced surface topography changes can be further explored to improve the functionality HA coated implants.

Acknowledgments The authors gratefully acknowledge The Director and Head, Biomedical Technology Wing, SCTIMST for extending the research facilities to successfully carry out this work. Rajesh P acknowledges Kerala State Council for Science Technology and Environment (KSCSTE) for the research fellowship.

References

1. Schuler M, Trentin D, Textor M, Tosatti SGP. Biomedical interfaces: titanium surface technology for implants and cell carriers. Nanomedicine. 2006;1(4):449–63.
2. Niinomi M. Metallic biomaterials. J Artif Organs. 2008;11:105–10. doi:10.1007/s10047-008-0422-7.
3. Niinomi M. Recent research and development in titanium alloys for biomedical applications and healthcare goods. Sci Tech Adv Mater. 2003;4:445–54. doi:10.1016/j.stam.2003.09.002.
4. Lacefield WR. Material characteristics of uncoated/ceramic-coated implant materials. Adv Dent Res. 1999;13:21–6. doi:10.1177/08959374990130011001.
5. Wang G, Zreiqat H. Functional coatings or films for hard-tissue applications. Materials. 2010;3:3994–4050. doi:10.3390/ma3073994.
6. Borsari V, et al. Physical characterization of different-roughness titanium surfaces, with and without hydroxyapatite coating, and their effect on human osteoblast-like cells. Biomed Mater Res Part B: Appl Biomater. 2005;75B:359–68. doi:10.1002/jbm.b.30313.
7. Laursen MB, Nielsen PT, Soballe K. Bone remodelling around HA-coated acetabular cups: a DEXA study with a 3-year follow-up in a randomised trial. Int Orthop (SICOT). 2007;31:199–204. doi:10.1007/s00264-006-0148-1.
8. Ter Brugge PJ, Wolke JGC, Jansen JA. Effect of calcium phosphate coating crystallinity and implant surface roughness on differentiation of rat bone marrow cells. J Biomed Mater Res. 2002;60:70–8. doi:10.1002/jbm.10031.
9. Narayanan R, Seshadri SK, Kwon TY, Kim KH. Calcium phosphate-based coatings on titanium and its alloys. J Biomed Mater Res Part B: Appl Biomater. 2008;85B:279–99. doi:10.1002/jbm.b.30932.
10. Sun L, Berndt CC, Gross KA, Kucuk A. Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings: a review. J Biomed Mater Res (Appl Biomater). 2001;58:570–92. doi:10.1002/jbm.I056.
11. Yan L, Leng Y, Weng L-T. Characterization of chemical inhomogeneity in plasma-sprayed hydroxyapatite coatings. Biomaterials. 2003;24:2585–92. doi:10.1016/S0142-9612(03)00061-9.
12. Knowles JC, Gross K, Berndt CC, Bonfield W. Structural changes of thermally sprayed hydroxyapatite investigated by rietveld analysis. Biomaterials. 1996;17:639–45.
13. Lo WJ, et al. Physical, chemical and biological characterization of pulsed laser deposited and plasma sputtered hydroxyapatite thin films on titanium alloy. J Biomed Mater Res. 2000;50:536–45.
14. Zeng H, Lacefield WR. XPS, EDX and FTIR analysis of pulsed laser deposited calcium phosphate bioceramic coatings: the effects of various process parameters. Biomaterials. 2000;21:23–30.
15. Zeng H, Lacefield WR, Mirov S. Structural and morphological study of pulsed laser deposited calcium phosphate bioceramic coatings: Influence of deposition conditions, laser parameters, and target properties. Biomed Mater Res. 2000;50:248–58.
16. Ball MD, et al. Osteoblast growth on titanium foils coated with hydroxyapatite by pulsed laser ablation. Biomaterials. 2001;22:337–47.
17. Rau JV, et al. Physicochemical investigation of pulsed laser deposited carbonated hydroxyapatite films on titanium. ACS Appl Mater Interfaces. 2009;1(8):1813–20. doi:10.1021/am900356e.
18. Man HC, Chiu KY, Cheng FT, Wong KH. Adhesion study of pulsed laser deposited hydroxyapatite coating on laser surface nitrided titanium. Thin Solid Films. 2009;517:5496–501. doi:10.1016/j.tsf.2009.03.208.
19. Dinda GP, Shin J, Mazumder J. Pulsed laser deposition of hydroxyapatite thin films on Ti–6Al–4V: effect of heat treatment on structure and properties. Acta Biomater. 2009;5:1821–30. doi:10.1016/j.actbio.2009.01.027.
20. Rajesh P, Muraleedharan CV, Komath M, Varma H. Pulsed laser deposition of hydroxyapatite on titanium substrate with titania interlayer. J Mater Sci Mater Med. 2011. doi:10.1007/s10856-011-4230-x.
21. Kim H, Camata RP, Chowdhury S, Vohra YK. In vitro dissolution and mechanical behavior of c-axis preferentially oriented hydroxyapatite thin films fabricated by pulsed laser deposition. Acta Biomater. 2010;6:3234–41. doi:10.1016/j.actbio.2010.02.031.
22. Pelletier H, Nelea V, Mille P. Nano-scratch study of pulsed laser-deposited hydroxyapatite thin films implanted at high energy with N^+ and Ar^+ ions. J Mater Sci. 2004;39:4185–92.
23. Lahoza R, Espinos JP, de la Fuente GF, Gonzalez-Elipe AR. "In situ" XPS studies of laser induced surface cleaning and nitridation of Ti. Surf Coat Technol. 2008;202:1486–92. doi:10.1016/j.surfcoat.2007.06.061.
24. Delgado-Ruız RA, et al. Femtosecond laser microstructuring of zirconia dental implants. J Biomed Mater Res Part B: Appl Biomater. 2011;96B:91–100. doi:10.1002/jbm.b.31743.
25. Zavestovskaya IN. Laser nanostructuring of materials surfaces. Quantum Electron. 2010;40(11):942–54. doi:10.1070/QE2010v040n11ABEH014447.
26. Kurella A, Dahotre NB. Review paper: surface modification for bioimplants: the role of laser surface engineering. J Biomater Appl. 2005;20:5. doi:10.1177/0885328205052974.
27. Gaggl A, Schultes G, Muller WD, Karcher H. Scanning electron microscopical analysis of laser-treated titanium implant surfaces-a comparative study. Biomaterials. 2000;21:1067–73.
28. Tarasenko NV, Butsen AV, Nevar EA. Laser-induced modification of metal nanoparticles formed by laser ablation technique in liquids. Appl Sur Sci. 2005;247:418–22. doi:10.1016/j.apsusc.2005.01.093.
29. Reimers H, Gold J, Kasemo B, Chakarov D. Topographical and surface chemical characterization of nanosecond pulsed-laser micromachining of titanium at 532-nm wavelength. Appl Phys A. 2003;77:491–8. doi:10.1007/s00339-002-1477-6.
30. Kruusing A. Underwater and water-assisted laser processing: part 2-etching, cutting and rarely used methods. Opt Lasers Eng. 2004;41:329–52.
31. ISO 20502:2005(E). Fine ceramics (advanced ceramics, advanced technical ceramics)-determination of adhesion of

ceramic coatings by scratch testing. Geneva, Switzerland: International organization for standardization ISO; 2005
32. Standard test method for knoop and Vickers hardness of materials (Designation: E384-10e2). doi:10.1520/E0384-10E02.
33. Rehman I, Bonfield W. Characterization of hydroxyapatite and carbonated apatite by photo acoustic FTIR spectroscopy. J Mater Sci Mater Med. 1997;8:1–4.
34. Heinrich A, Dengler K, Koerner T, Haczek C, Deppe H, Stritzker B. Laser-modified titanium implants for improved cell adhesion. Lasers Med Sci. 2008;23:55–8. doi:10.1007/s10103-007-0460-z.
35. Rong M, Zhou L, Gou Z, Zhu A, Zhou D. The early osseointegration of the laser-treated and acid-etched dental implants surface: an experimental study in rabbits. J Mater Sci Mater Med. 2009. doi:10.1007/s10856-009-3730-4.
36. Hao L, Lawrence J, Phua YF, Chian KS, Lim GC, Zheng HY. Enhanced human osteoblast cell adhesion and proliferation on 316LS stainless steel by means of CO_2 laser surface treatment. J Biomed Mater Res Part B: Appl Biomater. 2005;73B:148–56. doi:10.1002/jbm.b.30194.