Effect of Ultraviolet Irradiation on the Osseointegration of a Titanium Alloy with Bone

Abstract

Introduction: Attempt has been made to analyze the potential of titanium (Ti) alloy for osteointegration by the effect of surface photo functionalization in different aspects as follows: in Ringer's solution, in vitro cell growth, and in vivo study on rabbit. The present study was aimed to investigate the influence of ultraviolet (UV) light on surface topography, corrosion behavior, and bioactivity of indigenously manufactured samples of Ti alloy mini-implant. Materials and Methods: The study includes surface modification of Ti samples by UV treatment, corrosion testing of the specimens using Potentiostat (GAMRY System), qualitative examination of modified surface topography using scanning electron microscope, and cellular viability test on Ti alloy surface (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide ASSAY). To find the effect of UV light on implant bone integration, biochemical test was performed on the femur of rabbits. Results and Discussion: Corrosion resistance of untreated Ti alloy in Ringer's solution was found to be less, whereas corrosion rate was more. Corrosion resistance of UV-treated samples was found to increase significantly, thereby lowering the corrosion rate. Cell growth in UV-treated specimen was observed to be higher than that in untreated samples. It is important to mention that cell growth was significantly enhanced on samples which were UV treated for longer duration of time. **Conclusions:** There was a marked improvement in cell growth on UV-treated Ti alloy samples. Hence, it is expected that it would enhance the process of osseointegration of Ti with bone. Another important finding obtained was that the removal torque values of UV-treated implants were higher than that of untreated implants. The overall result reveals that UV treatment of implants does help us in speeding up the osseointegration process.

Keywords: Bone, irradiation, titanium alloy, ultraviolet

Introduction

With an increase in the number of aged population, in the near future, more people can be expected to suffer from the loss of teeth. The degenerative oral function due to teeth loss can be restored with the help of placement of dental implants.^[1] Various dental implants of different designs have been successfully placed in different locations in the mouth using a variety of surgical protocols. Various studies have demonstrated that for maxilla the long-term success rate was found to be 92% and for mandible it was 94%, after 5 years of implant placement. While for 15 years of follow-up, success rate was 78% in maxilla and 86% in mandible.^[2]

Titanium (Ti) and its alloys are generally regarded to have good biocompatibility. They are relatively inert, and with the presence of thin surface oxide of TiO_2 ,

For reprints contact: reprints@medknow.com

it has good corrosion resistance.^[3] They typically do not suffer from significant corrosion in any of the biological environment. Ti readily adsorbs proteins from biological fluids. For instance, some specific proteins including albumin, laminin V, glycosaminoglycans, collagenase, fibronectin, complement proteins, and fibrinogen have been found to be adsorb onto the surface of Ti.^[4,5]

Ti surfaces can also support cell growth and differentiation. Much work have been devoted to the investigation of different cell interactions with Ti surfaces.^[6] After the materials are implanted into a human body, neutrophils and macrophages are first noted onto the implants, followed by the formation of foreign body giant cells from activated macrophages. It is generally accepted that osteoprogenitor cells migrate to the implant site and differentiate into osteoblasts. The first stage in the reaction after the materials which has been implanted into

How to cite this article: Yadav A, Yadav R, Gupta A, Baranwal A, Bhatnagar A, Singh V. Effect of ultraviolet irradiation on the osseointegration of a titanium alloy with bone. Contemp Clin Dent 2017;8:571-8.

Ashish Yadav, Ranjana Yadav¹, Aratee Gupta², Akash Baranwal³, Atul Bhatnagar², Vakil Singh⁴

Department of Dental Surgery, Government Medical College, Azamgarh, ¹Department of Public Health Dentistry, Babu Banarasi Das College of Dental Sciences, Lucknow, ²Department of Prosthodontics, Crown and Bridge, Implantology, Faculty of Dental Sciences, Banaras Hindu University, ³Department of Conservative & Endodontics, Faculty of Dental Sciences, Banaras *Hindu University*, ⁴*Department* of Metallurgical Engineering IIT, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Address for correspondence: Dr. Ashish Yadav, Government Medical College, Azamgarh, Uttar Pradesh, India. E-mail: researchguide86@ gmail.com



This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

the body is nonspecific protein adsorption.^[7] The next step is neutrophil and macrophage integration with the implant. The macrophage interaction and cytokines released by the macrophages are believed to attract fibroblasts and drive the foreign body encapsulation process. In bones, Ti heals in close apposition to the mineralized tissues under proper conditions. However, Ti and bones are generally separated by a thin nonmineral layer, and true adhesion of Ti to bones has not been observed. Instead, the bond associated with osseointegration is attributed to mechanical interlocking of the Ti surface asperities and pores in the bones. To make Ti biologically bond to bones, surface modification methods have been proposed to improve the bioactivity of Ti.^[8-10]

Surface modifications

To obtain specific surface properties on Ti medical devices, we require conducting surface modification. For example, to accomplish biological integration, it is necessary to have good bone formability. The proper surface modification techniques not only retain the excellent bulk attributes of Ti and its alloys, such as relatively low modulus, good fatigue strength, formability, and machinability, but also improve specific surface properties required for different clinical applications.^[11,12]

According to the different clinical needs, various surface modification schemes have been proposed, as described below:

Mechanical treatment

Machining, milling, and threading is not really a surface treatment method but can be used to produce specific surface topographies. Machined implant surface is generally characterized by grooves and valleys, more or less oriented along the machining direction.^[13]

Grinding and mechanical polishing are identical methods in which they remove some of the surface materials with the help of hard abrasives. Grinding involves use of coarse particles as abrasive medium to remove the surface at a faster rate.

Polishing of the implant surface involves use of a fine abrasive material that is applied to a flexible wheel or a belt, after that the implant is brought into direct contact with the abrasive surface. Polishing is always carried out in the presence of lubricant.

Grit blasting, also known as abrasive blasting, is another technique which is used to create surface topographies on the implant surfaces. In grit blasting, surface of the implant is bombarded with hard and dry particle or particles suspended in a liquid at high velocity.

Chemical treatment

Solvent cleaning is mainly aimed at cleaning the surface of the implant from oils, greases, and fatty surface contaminants remaining after manufacturing process with the help of organic solvents (aliphatic hydrocarbons, alcohols, ketones, or chlorinated hydrocarbons), surface active detergents, and alkaline-cleaning solutions. This process does not have any effect on the surface of the implant.^[14]

Wet chemical etching dissolves the native surface layer of the implant material including the oxide layer and parts of the underlying metal. Chemical etching is also used to improve surface roughening as well as for producing an esthetically favorable surface finish.

Acid etching or pickling is used for removing oxide layer to obtain clean and uniform surface finish. The most commonly used etching solvents are an aqueous mixture of 10–30 volume% of nitric acid (69 mass unit) and 1–3 volume% of hydrofluoric acid (60 mass unit).^[15]

Alkaline etching is a simple technique used to modify the Ti surfaces. Treatment of Ti in 4–5 M sodium hydroxide at 600°C for 24 h has been shown to produce sodium titanate gel of 1 mm thick and irregular topography with a high degree of open porosity. When the alkali treatment is preceded by etching in hydrochloric acid/sulfuric acid, it increases the porosity of the final surface.^[16]

Passivation treatments are used for obtaining a uniformly oxidized surface to improve corrosion resistance. It is often the last step in the surface preparation of the implants. The most commonly method employed is immersion of the Ti for a minimum of 30 min in 20–40 volume% solution of nitric acid at room temperature. After the passivation, surface of the implant should be neutralized, by thorough rinsing and drying.

Electrochemical treatment

Electro polishing and anodic oxidation, also known as anodizing, are the most commonly used methods for Ti surface modification. They are based on different chemical reactions occurring at an electrically energized surface (electrode) placed in an electrolyte. The specimen to be treated is made by anode and by controlling the variables such as choice of electrolyte and other processing parameters such as electrode potential, temperature, and current; with these different effects on the sample (anode) surface are obtained.^[17]

Vacuum treatment

Glow-discharge treatment, also known as cold plasma treatment, is based on the action of a low-pressure electrical discharge on the surface of the implant. Two different types of plasma treatments are available such as plasma deposition method and plasma surface modification. In plasma deposition, by reactions in the gas phase, glow discharge is used to deposit the coating material from a separate solid target (sputter deposition). Plasma surface modification, on the other hand, is based on the exposure of sample surface to a glow discharge to obtain a specific modification of surface properties.^[12]

Plasma treatment increases the surface energy of the implant and thereby improves the wetting characteristics as compared to conventional implant surfaces that are cleaned using solvents or autoclaving.^[18,19]

In ion implantation method, surface of the implant is bombarded with high-energy ions (approximately 100 KeV to 1 MeV range). Ion implantation is controlled by varying the concentration of ions and their energy. Ion implantation is most commonly used on those surfaces of implants which are subjected to high wear conditions such as orthopedic devices to increase surface hardness and reduce the generation of wear debris. This process is also used on some of the dental implants to increase the corrosion resistance by forming Ti-N surface.^[20]

Thermal treatments

Commercially pure Ti was thermally annealed up to 1000°C to form oxide layer composed of anatase and rutile structures of TiO_2 . Thermal treatment at 600°C and 650°C for 48 h is considered appropriate for implanted materials.^[21]

Laser treatments

Laser is an emerging field for use as a micromachining tool to produce a three-dimensional structure at micro- and nano-meter levels. It is a method of choice for complex surface geometries. The technique generates short pulses of light of single wavelength that provides energy focused on one spot. It is rapid, extremely clean, and suitable for the selective modification of surfaces and allows the generation of complex microstructures/features with a high resolution. These advantages make the technique interesting for geometrically complex biomedical implants.^[22]

The Brånemark BioHelix Implant has surface modified with laser micromachining process to create micro- and nano-structured surface roughness in only the inner part of the thread. The inner part of the thread is believed to be more suitable for bone formation than the outer part.^[23] The laser technique has several advantages as it does not add any chemicals and can be used in routine manufacturing. Only the valley and parts of the flank of the implant threads were laser treated while the remaining part was left as machined. The idea behind this design is that the flack portion of the implant thread, which might have the higher risk to expose to the microorganism and plaque, is characterized by relatively smooth surface to minimize the incidence of peri-implantitis, whereas the valley part of the implant threads has the rougher surface.

The work done in this research work is on the effect of the process of ultraviolet (UV) irradiation of Ti alloy on its osseointegration, through a newer technique of UV photo functionalization. Attempt has been made to bring out the effect of surface photo functionalization of Ti alloy on different aspects of corrosive behavior in Ringer's solution, *in vitro* cell growth, and in particular *in vivo* study on rabbits to analyze its potential to osseointegration. The present study was aimed at investigating the influence of the UV light on surface topography, corrosive behavior, and bioactivity of indigenously manufactured Ti alloy mini-implant samples.

Materials and Methods

The study includes surface modification of Ti samples by UV treatment, corrosion testing of the specimens by Potentiostat (GAMRY System), qualitative examination of modified surface topography with the help of scanning electron microscope (SEM), and cellular viability test on Ti alloy surface (3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide [MTT] ASSAY). An implant biomechanical test was performed on femur of rabbits to find the effect of UV light on implant bone osseointegration.

Subjects for animal study

Nine adult white New Zealand rabbits were selected for this study.

Sample selection

A total of 48 indigenously manufactured Ti alloy specimens of identical dimensions (10 mm \times 10 mm) with thickness of 3 mm were used in the study.

Mechanical polishing of samples

Square specimens were mechanically polished with different grit sandpapers followed by bazaar cloth, mounted on a rotating wheel.

Ultrasonic cleaning of samples

All the square samples were cleaned in acetone for 3 min followed by ultrasonic cleaning in distilled water for another 3 min.

Surface treatments

All the samples were divided into groups of six samples each and each group was subjected to different surface treatment as described below:

- Group 1: As received untreated material 24 samples
- Group 2: Samples UV treated for a period of 5 h 6 samples
- Group 3: Samples UV treated for a period of 12 h 6 samples
- Group 4: Samples UV treated for a period of 24 h 6 samples
- Group 5: Samples UV treated for a period of 48 h 6 samples.

Sample preparation for Groups 2, 3, 4, and 5: After mechanical polishing and cleaning, all the Ti alloy samples were subjected to UV treatment. Samples were kept in Petri dish one at a time and UV exposure was done in a specialized UV chamber continuously for different periods.

Microstructural examination

Scanning electron microscopy

One sample from each untreated and treated group was randomly selected for the evaluation of its surface morphology. The selected samples were examined under SEM and surface scanning was done using SEM (Quanta 200 FEG) and the samples were photographed at different magnifications.

Corrosion test

Preparation of Ringer's solution

The electrolyte used in this study was Ringer's solution because it is known to simulate the human body fluid. It was prepared using laboratory grade chemicals and double-distilled water. The composition of Ringer's solution is given below:

- NaCl 9 g/l
- CaCl₂ 0.48 g/l
- KCl 0.42 g/l
- NaHCO₃ 0.2 g/l.

Procedure

The different chemical constituents were weighed using Sartorius balance and were mixed in Milli-Q grade water. Mixing was done with a SPINOT magnetic stirrer until the solution was clear. The pH of this solution was kept at 7.2 using the required amount of Tris-hydroxy methyl amino methane and 2M HCl. The final volume was adjusted to 1 l so that the ionic composition of the Ringer's solution becomes similar with that of the human body plasma.

Corrosion testing

The specimens for corrosion behavior were studied using Potentiostat (GAMRY SYSTEM). Six samples were received and treated for 5 h. Samples were cleaned ultrasonically in ethanol for 5 min to remove oily, greasy material, or dirt from the surface.

Electrochemical potentiodynamic polarization studies were carried out in Ringer's solution at 7.2 pH using a Potentiostat (GAMRY SYSTEM). The salt concentration in the Ringer's solution should correspond to the body fluid. A conventional three electrode system with saturated calomel electrode as reference electrode, high-density graphite as counter electrode, and the test specimens as working electrode was used. The GAMRY system was used to record anodic polarization curves at a scanning rate of 1 mv/s. The polarization scan was done from -500 to +1000 mv. Tafel extrapolation was used to determine the corrosion parameters, using a software-based approximation.

Cell study

In vitro cell culture

MG 63 (human osteoblast cell line) was obtained from NCCS Pune, India, and was kept in Dulbecco's Modified

Eagle's medium (DMEM, GIBCO, Invitrogen Corp). The medium contained high glucose with pyridoxine HCl, sodium pyruvate, L-glutamine sodium bicarbonate, and was supplemented with 100% fetal bovine serum (FBS, Biological Industries, Haemek, Israel), 100 IU/ml penicillin (Himedia), and gentamycin 20 μ g/ml (Nicholas). The cells were seeded into tissue culture flasks and were allowed to grow in a controlled humidified incubator with 5% CO₂ and 98% humidity at 37°C. All the samples of Ti alloy were sterilized by soaking in Extran MAO₃ phosphate-free detergent solution (Merck Industries). Subsequently, they were autoclaved at a pressure of 15 lbs for 30 min. Then apart from Group 1, samples of Groups 3, 4, and 5 were irradiated to UV treatment for 12 h, 24 h, and 48 h, respectively.

 0.5×10^6 osteoblast cells were seeded on each test sample kept in a 12-well plate. Each experiment was performed four times in triplicate, and standard deviation and variance were calculated. The growth of cells was examined at different time intervals of 12, 24, and 48 h in a CO₂ incubator (Cytoperm[®] Heraeus[®]) at 37°C in DMEM medium containing 10% FBS and 1% antibiotics.

Cell viability

Commercially available MTT assay (Sigma) was used for the investigation of the cell viability after 12, 24, and 48 h following seeding of the cells. The MTT was 4,5 dimethylthiazol 2,5 diphenyltetrazolium bromide, 5 mg. The MTT was dissolved in 1 ml of phosphate-buffered solution (Na₂HPO₄.2H₂O - 1.149 g/l, NaCl - 9 g/l in triple-distilled water). 50 µl of MTT solutions was added to 500 µl of the medium. To allow for MTT formazan formation, the cells were incubated for 4 h at 37°C in CO₂ incubator. In this process, MTT is reduced by the mitochondrial dehydrogenases of viable cells and the tetrazolium ring is cleaved and yields purple formazan crystals. After removing the medium from the well, the formazan crystals were dissolved in 500 µl of DMSO/ well (Dimethyl sulfoxide, Sigma Aldrich Chem, USA). A volume of 100 µl of MTT solution was taken in duplicate in a 96-well plate. The optical density of each well was measured at 540 mm using ELISA reader.

Ultraviolet treatment of the samples

All the experiments were performed in a clean room under controlled conditions of 20°C and 46% humidity. Ti alloy samples were treated by UV radiation in UV chamber for various periods of time up to 48 h under ambient condition compared with untreated control ones for surface properties and biological potential. UV light treatment was performed using a 15W bactericidal lamp; intensity: $\lambda = 360 \pm 20$ [Table 1].

In vivo study

The experimental work was done in the Experimental Surgical Research Laboratory, Institute of Medical

Sciences, Banaras Hindu University. Irrespective of their sex, matured, healthy New Zealand white rabbits, bred at the institutes' animal house, were chosen as the candidates for this experimental work. The weight of the rabbits for this work was between 1.5 and 2 kg. Animals had free access to water and food.

Rabbits were selected as the suitable candidates for this study because of their easy availability, large size, easy handling due to their docile nature, and a suitable anatomy for the present study.

A total of 18 miniscrew-shaped implants were used in nine rabbits. The implants were indigenously made of 9 mm of the total length. Spiral threads were of 6 mm length, the remaining 3 mm was modified according to the bit size of the torque-measuring gauge which would remain above the bone and facilitate for removal torque analysis.

Nine implants were exposed with UV radiation and nine implants were untreated. The femur of rabbits was selected for implant site. Two mini-implants were placed in femur of each rabbit: one UV treated and one untreated at a distance of around 1 cm apart.

Before surgical placement, the implants were sterilized in standard clinical autoclave at 121°C for 15 min under 15 lbs pressure.

Postoperatively, the rabbits were kept in different cages and fed on readymade animal feeds and vegetables.

Operative technique

The rabbits were anesthetized using 2 mg/kg of midazolam (Sedos, Claris Life Sciences Ltd) and 2 mg/kg of ketamine hydrochloride (Aneket, Neon Labs) supplemented with local 2% xylocaine with adrenaline (1:100,000) at operative site. Xylocaine (AstraZenca Pharma India Ltd.) acted as supplemental local anesthetic and addition of adrenaline acted to control hemorrhage at the site to be operated. The rabbits were operated in lateral position. The surgical area was painted and draped with standard aseptic precautions. Legs were shaved with commercially available hair-removing agents. Area was cleaned with the mixture of betadine and ethyl alcohol (70%).

Table 1: Site and type of implants used in the study				
Number of rabbits	Site - lateral aspect of the right femur			
	UV treated	Untreated		
Rabbit 1	1	1		
Rabbit 2	1	1		
Rabbit 3	1	1		
Rabbit 4	1	1		
Rabbit 5	1	1		
Rabbit 6	1	1		
Rabbit 7	1	1		
Rabbit 8	1	1		
Rabbit 9	1	1		

UV: Ultraviolet

A longitudinal incision was given at the lateral aspect of femur and skin fascia was exposed. The soft tissue was retracted and femur was exposed. The site of implant placement was marked, and another mark was placed 10 mm apart for the control. The cortex was penetrated under low speed and profuse saline irrigation with the pilot drill. The site was rechecked. Then again, the pilot drill was penetrated to the required length. Next, a 2-mm drill was used for the surgical osteotomy to the required length. Both the cortices were penetrated. The surgical site was irrigated with saline to remove clots and bone chips or bone dust if any. The implant selected for the study was then placed in the osteotomy site and the position and alignment were ascertained. The surgical site was closed in layers with the absorbable sutures for fascia and with nonabsorbable for skin. Sterile dressing was applied over the wound. Postoperatively, the rabbits were kept in separated cages and fed on standard diet. Postoperative antibiotic was administered. Dressing was removed after 7 days and was left open thereafter. The operative site was observed for any signs of infection or rejection.

Removal torque analysis

The removal torque value (RTV) in Newton centimeter (Ncm) reflects the interfacial shear strength between bone tissue and the implant.

The rabbits were anesthetized under aseptic condition. The implant site was exposed, according to the following scheme: three rabbits after 4 weeks of implantation, three after 8 weeks, and three after 12 weeks.

Femora site containing the implant was exposed. Removal torque test was performed using universal combo torque wrench with a measuring range of 10–50 Ncm, measuring accuracy of 1%. A single and experienced person performed the reverse torque.

Results

Corrosion study result

Ti alloy in as received and UV-treated condition in Ringer's solution shows polarization curves in different parameters [Figure 1]. The different parameters are summarized in Table 2.

The results indicate that there is highest resistance to corrosion in Ringer's solution in the UV-treated sample for 5 h. This implies that the UV-treated sample has low passivation current and low corrosion current density (55 nA). This happens in a certain range of potential where Ti surface forms oxide layer. The movement of metal ions is hampered by thin oxide film formed on the metal surface which reduces corrosion.

After recovery, treatment grain boundaries of high density are seen on the surface of UV-treated Ti alloy. This hampers the movement of ions from the surface of metal, thereby improving resistance to corrosion.

Cell viability

Plots of optical density reveal the osteoblastic cell growth behavior on UV-treated and UV-untreated Ti alloy samples. Standard deviation of each group against 12, 24, and 48 h was taken out after calculating the mean from optical density. Using parametric two-way ANOVA (Bonferroni test), the multiplication of adherent cells was distinguished among different groups.

Groups 4 and 5 displayed increase in cell proliferation than Group 1 that did not receive any surface treatment after 12 and 24 h of time interval. When statistically analyzed, difference between Group 1 and Group 4 was found to be significant. Results were found to be significant between Group 5 and Group 1 when group comparison was made using Bonferroni test after 48 h.

When each group was evaluated separately using "paired *t*-test" at different time intervals, i.e. 12, 24, and 48 h [Table 3 and Figure 2], results were found to be significant, i.e. cells were growing in number as a function of time.

It was found that Group 3, Group 4, and Group 5 specimens showed better proliferation than Group 1 specimens.

All implants resisted the reverse torque firmly and then loosened suddenly. The RTV decreased steeply. After reaching the peak torque, the maximum value was marked which implies the fusion of implant with the bone tissue, that is, osseointegration [Table 4].

The mean RTVs of untreated implants were not significantly higher than that of the UV-treated implants after 4 weeks of healing period (P < 0.178). But, after 8–12 weeks of implant in bone, it was observed that the



Figure 1: Polarization curves for as received and ultraviolet-treated Ti alloy in Ringer's solution

RTV was noticeably higher in UV-treated implants than in untreated ones (P < 0.05).

Discussion

In this study, it was observed that bone–implant integration is better in UV-treated implant after 8 and 12 weeks whereas after 4 weeks no significant results were seen.

In a study by Suzuki *et al.*,^[24] they observed that healing period of aged Ti implant increased, thereby reducing osseointegration rate. They also found that UV treatment

Table 2: Corrosion behavior of Ti alloy in different parameters						
Material	Ba (V/ decade) × e-3	βcussion (V/decade) × e-3	I _{corr} (nA)	E _{corr} (mV)	Corrosion rate	
Ti alloy	299.5	158.8	61.6	-416.0	21.11	
Ti alloy UV	538.2	131.0	55.0	-403.0	18.84	
Ti alloy UV	538.2	131.0	55.0	-403.0	1	

J	V:	Ultraviolet	

Table 3:	Effect	of u	ultravi	olet	treatment	duration	on	cell
proliferation								

1		
Period of UV treatment (h)	Group I sample	Group II sample
12	15.33	17.33
24	17.66	22.00
48	17.66	25.33
TV. Illiansi allat		

UV: Ultraviolet

Table 4: Effect of ultraviolet treatment on osseointegration				
Period after implant placement (week)	Group I (Ncm) (untreated)	Group II (Ncm) (UV treated)		
4	15.33	17.33		
8	17.66	22.00		
12	17.66	25.33		

UV: Ultraviolet



Figure 2: Effect of the duration of ultraviolet treatment on cell proliferation

of aged Ti implants increased in osseointegration property which was found to be same as the freshly prepared implants. The results showed that osseointegration property of Ti degrades with time, but UV treatment of aged Ti implants improves its osseointegration property same as the freshly prepared implant surface.

A study in the rat model by Aita^[25] showed that UV treatment of implant causes new bone formation without any soft-tissue intervening between bone and implant and this implant-to-bone contact maximizes by 100% after 4 weeks. Therefore, UV treatment hastens the process of osseointegration by four times. UV treatment causes catalytic removal of hydrocarbons from TiO_2 layer formed on the surface of Ti and therefore enhances its osteoconductive property. Overall, UV treatment hastens the treatment process by speeding up osseointegration.

Time-dependent biological degradation of Ti and chromium-cobalt alloy was started by Att.^[26] They treated Ti and cobalt chromium by UVC rays which removed hydrocarbons from the surface of Ti and cobalt chromium alloy, thereby increasing superhydrophilicity. Cell growth was found to increase in treated samples.

In another study by Miyauchi,^[27] it was found that UV treatment improved the adhesive property of osteoblast cells. This study also states that nano-thin TiO_2 is coated over non-Ti metal and is UV treated; it will enhance its bioactivity, forming a new development of functional biomaterial.

Ueno et al.[28] took as received Ti rods and UV-treated Ti rods and placed both of them in an animal with or without contact with cortical bone. After 2 weeks of healing period, they performed push-in test, took computed tomography scan of bone, and analyzed the surface elements. It was found that in gap healing model bone-to-implant contact was one-third of the contact healing model. In gap healing model, when UV-treated Ti rods were placed, osseointegration was almost equivalent to that which was found in contact healing model with untreated Ti rod. This treatment was found to increase bone formation over UV-treated Ti rod in gap healing model by 2-3 times. This phenomenon is attributed to osteogenic cells derived from periosteum and bone marrow locally and whose function increases due to UV treatment.

Microstructural examination

To observe the effect of UV treatment on the surface of Ti alloy, a sample of 10 mm \times 10 mm and 3 mm thickness was taken. It was then divided into as received and UV-treated groups. The surface structure and morphology were then observed under SEM. It was found that there was no significant and noticeable change between both the samples and the results were almost same as the samples were mechanically polished.

Conclusions

Microstructure of the untreated and treated Ti alloy did not show much difference in scanning electron microscopy as the samples were mechanically polished. Corrosion resistance of untreated Ti alloy in Ringer's solution was less and corrosion rate was more. However, corrosion resistance of UV-treated sample was found to increase significantly, thereby lowering corrosion rate.

Cell growth of UV-treated specimen was observed to be higher than that of untreated samples. It is important to mention that cell growth was significantly enhanced on samples which were UV treated for longer duration of time.

The removal torque test of UV-treated and UV-untreated implants was measured after 4, 8, and 12 weeks of implant placement. Test was performed in rabbit femur at the second surgery. It was found that there was comparative increase in removal torque of UV-treated implants than in UV-untreated ones.

There was a marked improvement in cell growth on UV-treated Ti alloy samples. Hence, it is expected that it would enhance the process of osseointegration of Ti with bone. Another important finding was that RTVs of UV-treated implants were higher than that of untreated implants. The overall result hence reveals that UV treatment of implants helps in speeding up the osseointegration process.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

- 1. Yang Y, Oh N, Liu Y, Chen W. Enhancing osseointegration using surface-modified titanium implants. JOM 2006;58:71.
- 2. Gokcen-Rohlig B, Yaltirik M, Ozer S, Tuncer ED, Evlioglu G. Survival and success of ITI implants and prostheses: Retrospective study of cases with 5-year follow-up. Eur J Dent 2009;3:42-9.
- 3. Van Noort R. Titanium: The implant material of today. J Mater Sci 1987;22:3801-11.
- McArthur MA, Byrne TM, Sanderson RJ, Rockwell GP, Lohstreter LB, Bai Z, *et al.* An *in situ* study of protein adsorption on combinatorial cu-al films using spectroscopic ellipsometry. Colloids Surf B Biointerfaces 2010;81:58-66.
- Tamura RN, Oda D, Quaranta V, Plopper G, Lambert R, Glaser S, *et al.* Coating of titanium alloy with soluble laminin-5 promotes cell attachment and hemidesmosome assembly in gingival epithelial cells: Potential application to dental implants. J Periodontal Res 1997;32:287-94.
- Zhu X, Chen J, Scheideler L, Reichl R, Geis-Gerstorfer J. Effects of topography and composition of titanium surface oxides on osteoblast responses. Biomaterials 2004;25:4087-103.
- 7. Chevallier N, Anagnostou F, Zilber S, Bodivit G, Maurin S,

Barrault A, *et al.* Osteoblastic differentiation of human mesenchymal stem cells with platelet lysate. Biomaterials 2010;31:270-8.

- 8. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE. Biomaterials Science: An Introduction to Materials in Medicine. United states: Academic Press; 2004.
- Henry PJ. Comparative surface analysis of two osseointegrated implant systems. Int J Oral Maxillofac Implants 1987;2:23-7.
- Textor M, Sittig C, Frauchiger V, Tosatti S, Brunette DM. Properties and biological significance of natural oxide films on titanium and its alloys. Titanium in Medicine. Berlin, Heidelberg: Springer; 2001. p. 171-230.
- 11. Desmet T, Morent R, De Geyter N, Leys C, Schacht E, Dubruel P, *et al.* Nonthermal plasma technology as a versatile strategy for polymeric biomaterials surface modification: A review. Biomacromolecules 2009;10:2351-78.
- Chu PK, Chen J, Wang L, Huang N. Plasma-surface modification of biomaterials. Mater Sci Eng R Rep 2002;36:143-206.
- Ellingsen JE, Thomsen P, Lyngstadaas SP. Advances in dental implant materials and tissue regeneration. Periodontol 2000 2006;41:136-56.
- 14. Schwartz M. Encyclopedia and Handbook of Materials, Parts and Finishes. United States: CRC Press; 2016.
- Kohles SS, Clark MB, Brown CA, Kenealy JN. Direct assessment of profilometric roughness variability from typical implant surface types. Int J Oral Maxillofac Implants 2004;19:510-6.
- 16. Wen HB, Liu Q, De Wijn JR, De Groot K, Cui FZ. Preparation of bioactive microporous titanium surface by a new two-step chemical treatment. J Mater Sci Mater Med 1998;9:121-8.
- Kim KH, Ramaswamy N. Electrochemical surface modification of titanium in dentistry. Dent Mater J 2009;28:20-36.
- Aronsson BO, Lausmaa J, Kasemo B. Glow discharge plasma treatment for surface cleaning and modification of metallic biomaterials. J Biomed Mater Res A 1997;35:49-73.

- Kilpadi DV, Lemons JE. Surface energy characterization of unalloyed titanium implants. J Biomed Mater Res 1994;28:1419-25.
- Shinonaga Y, Arita K. Surface modification of stainless steel by plasma-based fluorine and silver dual ion implantation and deposition. Dent Mater J 2009;28:735-42.
- Gemelli E, Scariot A, Camargo NH. Thermal characterization of commercially pure titanium for dental applications. Mater Res 2007;10:241-6.
- 22. Brånemark R, Emanuelsson L, Palmquist A, Thomsen P. Bone response to laser-induced micro- and nano-size titanium surface features. Nanomedicine 2011;7:220-7.
- Ballo AM, Omar O, Xia W, Palmquist A. Dental Implant Surfaces–Physicochemical Properties, Biological Performance, and Trends. Implant Dentistry – A Rapidly Evolving Practice. London: InTech; 2011.
- Suzuki T, Hori N, Att W, Kubo K, Iwasa F, Ueno T, *et al.* Ultraviolet treatment overcomes time-related degrading bioactivity of titanium. Tissue Eng Part A 2009;15:3679-88.
- Aita H, Hori N, Takeuchi M, Suzuki T, Yamada M, Anpo M, et al. The effect of ultraviolet functionalization of titanium on integration with bone. Biomaterials 2009;30:1015-25.
- Att W, Hori N, Fuminori, Yamada, Ueno T, Takahiro Ogawa. The effect of UV-photofunctionalization on the time-related bioactivity of titanium and chromium–cobalt alloys Biomaterials. 2009;30:4268-76.
- Miyauchi T, Yamada M, Yamamoto A, Iwasa F, Suzawa T, Kamijo R, *et al.* The enhanced characteristics of osteoblast adhesion to photofunctionalized nanoscale tiO2 layers on biomaterials surfaces. Biomaterials 2010;31:3827-39.
- Ueno T, Yamada M, Suzuki T, Minamikawa H, Sato N, Hori N, et al. Enhancement of bone-titanium integration profile with UV-photofunctionalized titanium in a gap healing model. Biomaterials 2010;31:1546-57.