Bull. Mater. Sci., Vol. 36, No. 5, October 2013, pp. 931–937. © Indian Academy of Sciences.

Effect of anodization on corrosion behaviour and biocompatibility of Cp-titanium in simulated body fluid

ARCHANA SINGH[1,*], B P SINGH[1], MOHAN R WANI[3], DINESH KUMAR[4], J K SINGH[5] and VAKIL SINGH[2]

[1]Department of Prosthodontics, Faculty of Dental Sciences, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India
[2]Center of Advanced Study, Department of Metallurgical Engineering, Institute of Technology, Banaras Hindu University, Varanasi 221 005, India
[3]National Centre for Cell Science, Pune University Campus, Pune 411 007, India
[4]Department of Zoology, Faculty of Science, Banaras Hindu University, Varanasi 221 005, India
[5]Corrosion and Surface Engineering Division, National Metallurgical Laboratory, Jamshedpur 831 007, India

MS received 18 December 2011; revised 2 March 2013

Abstract. **The objective of this investigation is to study the effectiveness of anodized surface of commercial purity titanium (Cp-Ti) on its corrosion behaviour in simulated body fluid (SBF) and proliferation of osteoblast cells on it, to assess its potentiality as a process of surface modification in enhancing corrosion resistance and osseointegration of dental implants. Highly ordered nano-porous oxide layer, with nano-sized pores, is developed on the surface of Cp-Ti through electrochemical anodization in the electrolyte of aqueous solution of 0·5% HF at 15 V for 30 min at 24 °C. The nano-porous feature of the anodized surface is characterized by field-emission scanning electron microscope (FESEM). Pores of some anodized samples are sealed by exposing the anodized surface in boiling water. Corrosion behaviour of the anodized specimen is studied in Ringer's solution at 30 ± 2 °C, using electrochemical impedance and cyclic polarization technique. Biocompatibility of the anodized surface is accessed using MG63 osteoblast cells. Both corrosion as well as pitting resistance of Cp-Ti in simulated body fluid are found to be highest in the anodized and sealed condition and followed in decreasing order by those of anodized and unanodized ones. Significantly higher MG63 osteoblast cell proliferations are found on the anodized surface than that on the unanodized one. Anodized Cp-Ti develops nano-size surface pores, like that of natural bone. It enhances corrosion and pitting resistance and also the process of osteoblast cell proliferation on Cp-Ti.**

Keywords. **Corrosion resistance; pitting resistance; anodization; biocompatibility; MG63 osteoblast cells.**

1. Introduction

As the health care is improving and longevity of people is increasing, demand of suitable materials for biomedical applications is increasing rapidly. Titanium is known to be a highly biocompatible material and is extensively used for several medical devices such as dental and orthopaedic implants, auricular episthesis and bone-anchored hearing aids. It has attractive bulk mechanical properties like low modulus of elasticity, high strength to weight ratio, excellent corrosion resistance, low rate of ion release combined with excellent biostability, and a high degree of biocompatibility (Keller *et al* 1994; Eliades 1997). Corrosion resistance of implants in body fluid is a critical factor as it can adversely affect biocompatibility and mechanical integrity of implants (Jacobs *et al* 1998). Corrosion and surface film dissolution are two important processes responsible for introducing additional ions in body from implants. Extensive release of metal ions from human body implant can result in adverse biological reactions and even lead to mechanical failure of the device (Brunette 2001). Anodic oxidation is a commonly used surface treatment especially for aluminium alloys for structured applications, to improve their corrosion resistance (White *et al* 1985). Shabalovskaya (2002) observed that corrosion resistance of Ni–Ti alloy is increased with formation of stable, adherent and protective titanium dioxide film. It was established by Huang *et al* (2009) that corrosion resistance of Ni–Ti alloy in Hank's based salt solution was significantly increased by anodizing in 0·075 M HF solution at 20 V for 1 h. Surface characteristics like wettability, surface topography and chemical composition play important role on biocompatibility of titanium. Surface smoothness at nano level has been shown to enhance cell adhesion and increase fibrous tissue encapsulation (Jiang *et al* 2006). A novel approach in design of the next generation implants has recently focused on development of nano topography on the implant surface (Campos *et al* 2007).

The process of anodization is an effective means of producing protective oxide layers on metals like aluminum

*Author for correspondence (archanasingh.bhu@gmail.com)

and titanium. Nowadays anodization has become a potential means of modifying the surface of titanium implants because of its effectiveness in changing the chemical composition as well as topography of the surface (Cai *et al* 2005; Chang *et al* 2005). Grimes *et al* (2001) observed well-aligned titanium oxide nanotube-like arrays on anodizing in 0·5% HF solution at 20 V for 20 min. The average tube diameter and tube length were 60 nm and 250 nm, respectively. It was also noticed that nanotube array was controlled by HF concentration and applied voltage. Relatively higher voltage was needed to achieve tube like structures in more dilute HF solutions. The final length of the tube was found to be independent of the duration of anodization.

Molecular and cellular interactions between bone and implant are governed, to a large extent, by surface properties of implant devices. A variety of surface properties including physicochemical as well as biomechanical are important. They are considered to be responsible for biological performance like protein absorption, cell attachment and subsequent osseointegration of titanium implants (Kieswetter *et al* 1996). In the present study, biocompatibility of Cp-Ti was assessed by using MG63 osteoblast cells. Osteoblasts are the main cells responsible for initial bone formation and remodelling, and help in osseointegration of bone to implant. Osteoblast cells arise from differentiation of osteogenic cells in the periosteum. They secrete proteins which form osteoid matrix, responsible for mineralization and thus regulate the growth of bone on the implant surface (Brunette and Chehroudi 1999). Consequently, alterations of titanium implant surface, to promote osseointegration and their biological responses have been of much interest in biomaterials, both from academic and industrial points of view. The present work was undertaken to study the effect of anodization of Cp-Ti on its corrosion behaviour in simulated body fluid and interaction with bone cell materials, using MG63 osteblast cells.

2. Materials and methods

2.1 *Electrochemical anodization of Cp-Ti*

Flat samples of Cp-Ti of 10 × 10 × 2 mm size were prepared. These samples were mechanically polished up to 4/0 grade of emery paper, cleaned in a mixture of HF and HNO_3 for 20 s, rinsed with ethanol and deionized water and finally dried in air. Samples were anodized in 0·5% aqueous solution of HF at 24 °C for 30 min at different anodizing voltages of 10, 15, 20 and 25. Anodization was carried out with two-electrode configuration, using Cp-Ti as working electrode (anode) and platinum sheet as counter electrode (cathode). The two electrodes were clamped in parallel by two copper clips fixed at a distance of 20 mm and immersed directly in the electrolyte. Temperature of the electrolyte was kept constant at 24 °C. Following anodization, the samples were rinsed with deionized water and dried at room temperature. Some anodized samples were sealed in hot water for 2 h.

2.2 *Corrosion testing*

Cp-Ti samples were cleaned ultrasonically in ethanol to remove oily/greasy material or dirt from the surface, if any, and were mounted in non-conducting resin. They were subjected to electrochemical impedance (EIS) and cyclic polarization in Ringer's solution (NaCl, 9 g/l; KCl, 0·42 g/l; $CaCl_2$, 0·48 g/l; $NaHCO_3$, 0·2 g/l). EIS studies were performed imposing 10 mV of sinusoidal voltage (with reference to open circuit potential) at working electrode and varying the frequency from 100 kHz to 0·01Hz. Cyclic polarization studies were performed in a flux cell exposing the working electrode area of 1 cm^2. Two graphite rods at two sides of the working electrode were fitted to act as auxiliary electrodes. The reference electrode was a saturated calomel electrode (SCE). A luggin capillary was used to provide electrolytic contact between the calomel electrode and electrochemical cell. All the tests were performed at 30 ± 2 °C. Electrochemical studies were conducted using GAMRY Potentiostat (M/S GAMRY instruments, USA).

2.3 *Invitro cell culture*

MG63 osteoblast cell line was procured from NCCS Pune, India and was kept in Dulbecco's modified Eagle's medium (DMEM GIBCO, Invitrogen Corp.). The medium contained high glucose with L-glutamine, pyridoxine HCl, sodium pyruvate, sodium bicarbonate and was supplemented with 10% fetal bovine serum (FBS, Biological Industries, Haemek, Israel), 100 IU/ml penicillin (Himedia), 100 μg/ml streptomycin (Himedia) and 20 μg/ml gentamycin (Nicholas). The cells were seeded into tissue culture flasks and were allowed to grow in a controlled humidified incubator with 5% CO_2 and 98% humidity at 37 °C. Before seeding of the cells, the samples of Cp-Ti in the unanodized and anodized conditions were sterilized by soaking in Extran MAO_3 phosphate free detergent solution (Merck Industries) and subsequently autoclaved at a pressure of 15 lb for 30 min.

2.4 *Cell morphology*

MG63 osteoblast cells were plated for cell-morphology study, at a density of 5 × 10^4 cells/cm^2, on each test sample, kept in a 12-well tissue culture plates. The growth of cells was examined at 24 and 48 h in a CO_2 incubator (Cytoperm Heraeus) at 37 °C in DMEM medium containing 10% FBS, 1% antibiotics. After 24 and 48 h cell incubation, cells were fixed in 4% v/v paraformaldehyde in PBS for 30 min and the samples were rinsed thoroughly with PBS thrice. Cells were dehydrated slowly in sequence with ethanol concentration starting from 50, 70, 95 and 100% v/v. Subsequently the samples were dried to a critical point, using acetone and hexamethyldisilazane. Samples were mounted on aluminium stubs, gold coated and examined under SEM (ZEISS-EVO).

2.5 *Cell viability*

The cell viability was examined after 24 and 48 h, following seeding of the cells, using a commercially available

MTT assay (Sigma). MTT was 4,5 dimethyl thiazol and 2,5-diphenyl tetrazolium bromide. 5 mg MTT was dissolved in 1 ml phosphate buffer solution (PBS, $Na_2HPO_4 \cdot 2H_2O$ – 1·149 g/l, NaH_2 $PO_4 \cdot 2H_2O$–0·29 g/l and NaCl–9 g/l in triple distilled water). 50 μl MTT solutions were added to 500 μl of the medium. Cells were incubated for 4 h at 37 °C in CO_2 incubator, in order to allow formation of MTT formazan. 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 sulphoxide, Sigma Aldrich Chem.). MTT solution of 100 μl was taken and duplicated in a 96 well plate. The optical density of each well was measured at 540 nm, using ELISA as reader.

2.6 *Statistical analysis*

Data were expressed as mean value (MV) and standard deviation (SD) of three independent experiments. One-way analysis of variance was used to determine significance among the mean levels.

3. Results and discussion

3.1 *Anodization and surface characterization*

SEM micrographs of Cp-Ti in unanodized and anodized condition are shown in figure 1. It may be seen that, as expected, there are no distinct features on the surface of unanodized specimen (figure 1(a)). On the other hand, there is porous type of structure, varying in size and distributions of pores, on the surface of anodized specimen. Porous feature is less distinct on the specimen, anodized at 10 V. Morphology of the pores is most distinct on the specimen, anodized at 15 V for 30 min. The pores may be seen to be quite uniform in size and shape throughout the anodized surface (figure 1(c)). Also the depth of these pores is considerably larger than those formed on other anodized surfaces. The average size of pores ranged from 50 to 110 nm, on the specimen anodized at 15 V for 30 min. The best anodized surface of the CP-Ti, in terms of uniformity in size, depth and morphology of porous structure, resulted from anodizing in aqueous solution of 0·5% HF at 15 V for 30 min of anodization.

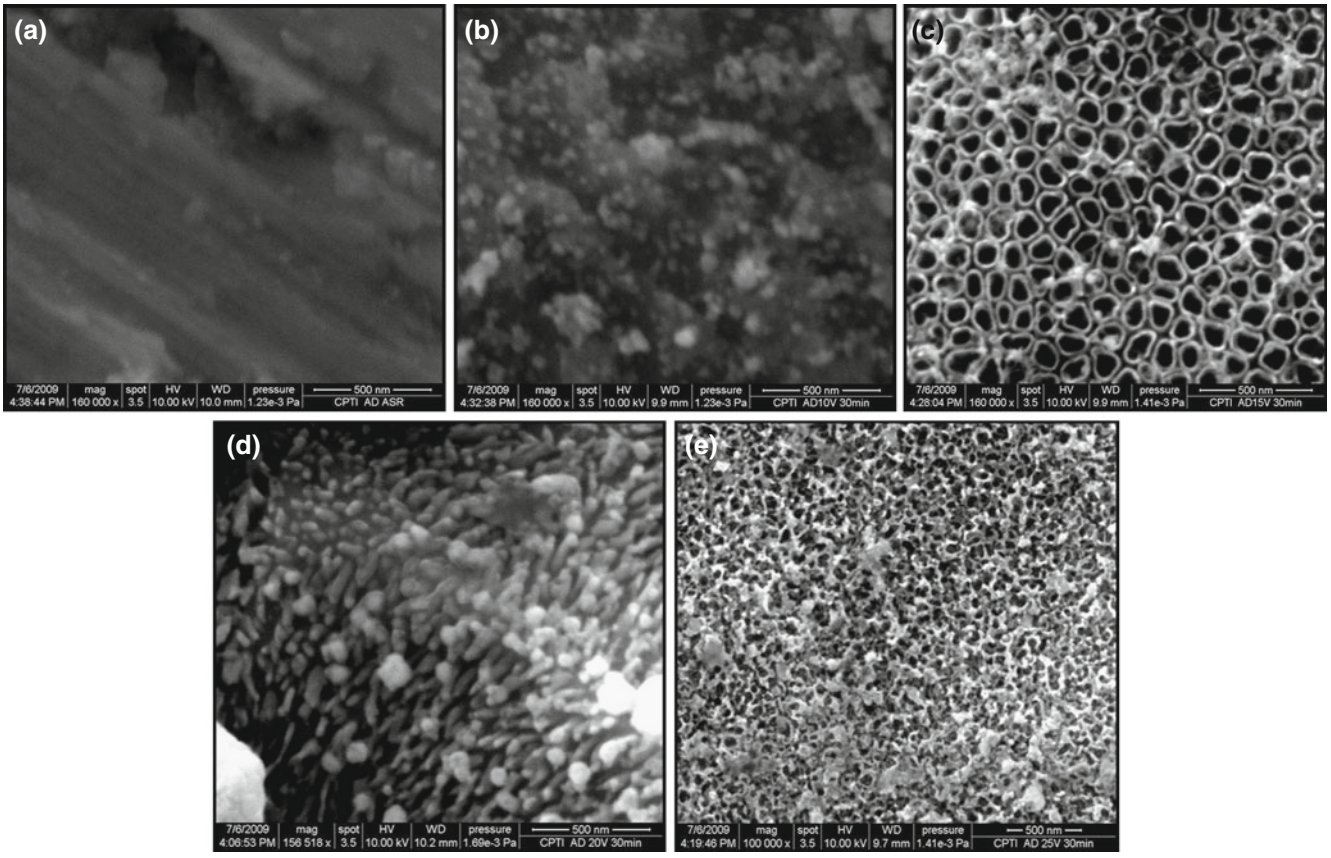

Figure 1. Scanning electron micrographs showing surface features of Cp-Ti in unanodized and differently anodized conditions: (**a**) unanodized, (**b**) anodized at 10 V for 30 min, (**c**) anodized at 15 V for 30 min, (**d**) anodized at 20 V for 30 min and (**e**) anodized at 25 V for 30 min.

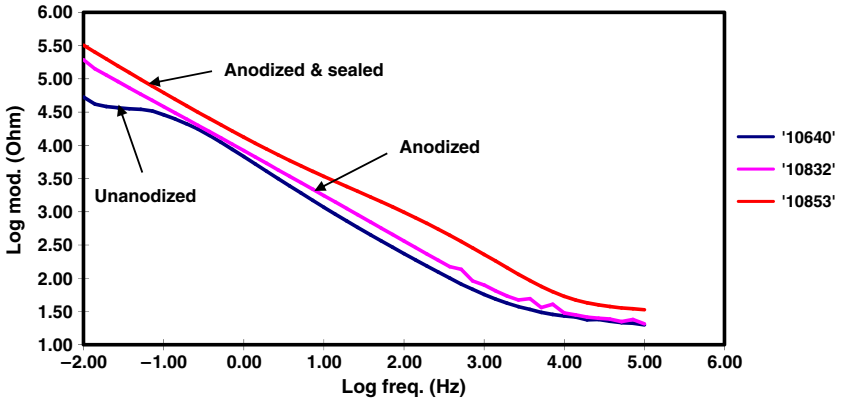

Figure 2. Electrochemical impedance of Cp-Ti in unanodized, anodized and anodized and sealed conditions after exposing for 1 h in Ringer's solution (simulated body fluid).

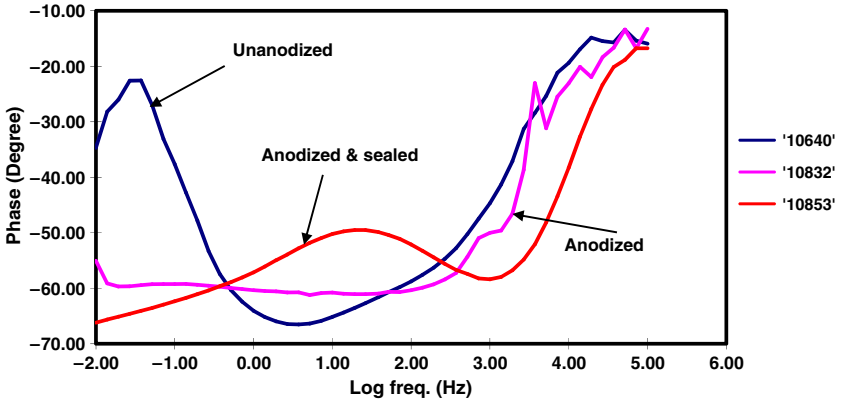

Figure 3. Log frequency–phase plots for Cp-Ti in unanodized, anodized and anodized and sealed conditions after exposing for 1 h in Ringer's solution.

3.2 *Corrosion behaviour*

Corrosion behaviour of the unanodized, anodized and anodized and sealed specimens is shown by electrochemical impedance (figures 2 and 3) and cyclic polarization (figure 4). Important electrical components and characterizing corrosion resistance, evaluated from impedance plots, are recorded in table 1. Impedance plots in figure 2 (log freq. vs log mod.), suggest that corrosion resistance in anodized and sealed condition is highest, followed in decreasing order by anodized and unanodized conditions. Cyclic polarization (figure 4) suggests that both anodized as well as anodized and sealed samples exhibit considerably lower passive current density, compared to the unanodized one.

3.3 *Cell attachment and morphology*

MG63 osteoblast cell attachment on unanodized and anodized surfaces was analysed using a scanning electron microscope. Figures 5 and 6 show MG63 osteoblast cell morphology, on unanodized and anodized surfaces, after 24 and 48 h of cell culture. Cells on the unanodized surface after 24 h, show flattened morphology with some filopodia (figure 5(a)). After 48 h, there is slight increase in cell growth. Cells are elongated, larger in dimension and more filopodia (figure 6(a)). In contrast, cells on the anodized titanium surface after 24 h show increase in attachment, cell spreading and proliferation (figure 5(b)). Cells are elongated with many filopodia extensions from the cell to the substrate. After 48 h, excessive amount of cell growth was noticed. Cells formed a three-dimensional network like structure and small calcified nodules were also found (figure 6(b)).

3.4 *Cell proliferation using MTT assay*

Cell growth behaviour of MG63 osteoblast cells on the unanodized and anodized samples, brought out by the plots of optical density (OD), as a function of duration of exposure is shown in figure 7. It may be seen that proliferation of osteoblast is much higher on the anodized surface than that on the unanodized one. Anodized surface showed significantly higher cell proliferation than the unanodized one ($p = 0{\cdot}009$).

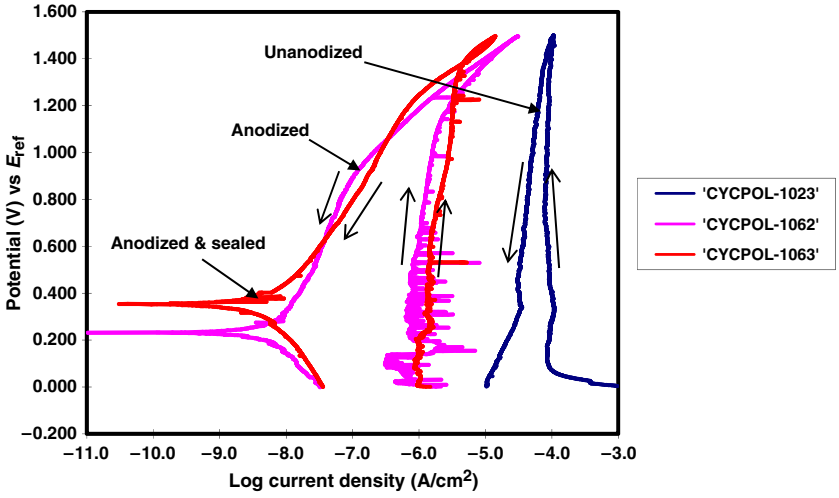

Figure 4. Cyclic polarization of Cp-Ti in unanodized, anodized and anodized and sealed conditions after exposing for 1 h in Ringer's solution.

Table 1. Various electrical components evaluated from impedance plots.

Sample ID	R_p (Ohm · cm^2)	R_c (Ohm · cm^2)	αEOC (V)	C_f (F · cm^{-2})
Unanodized	$5{\cdot}418 \times 10^4$	$2{\cdot}598 \times 10^4$	0·73–0·453	$1{\cdot}793 \times 10^{-5}$
Anodized	$1{\cdot}592 \times 10^6$	$2{\cdot}872 \times 10^4$	0·68–0·222	$1{\cdot}528 \times 10^{-5}$
Anodized and sealed	$1{\cdot}779 \times 10^6$	$5{\cdot}605 \times 10^4$	0·70–0·276	$1{\cdot}237 \times 10^{-5}$

R_p = Polarization resistance, R_c = charge transfer resistance; EOC = open circuit potential and C_f = capacitance.

Properties of the anodized oxide film formed on titanium surface depend on the processing parameters like electrolyte concentration, applied voltage, current density, pH, temperature and duration of anodizing. As mentioned in the previous section, the best anodized surface of Cp-Ti, in terms of uniformity in size, depth and morphology of porous structure resulted in the sample, anodized in aqueous solution of 0·5% HF at 15 V for 30 min. This observation is similar to that of Grimes *et al* (2001) in which small pores were observed in the specimen anodized at 15 V for 20 min. However, the pores were seen over approximately 50% of the anodized surface. Fully developed pores resulted from anodizing the sample at 20 V for 20 min. The size of the pores on the sample anodized at 20 V (Grimes *et al* 2001), was relatively smaller (25–65 nm) than that observed in the present investigation (50–110 nm) from anodizing at 15 V for 30 min.

It is obvious from the impedance plots (figure 2, log freq. vs log mod) and the value of impedance at the lowest frequency, that corrosion resistance is highest in the anodized and sealed condition, and is followed in decreasing order by those of anodized and unanodized ones. The impedance of the anodized and sealed specimen is highest at higher frequencies (10^2–10^5 Hz), reflecting its highest resistance, indicating development of thicker and nonporous passive film on it. Log frequency vs phase plot (figure 3) indicates that Cp-Ti in unanodized and anodized conditions exhibits only one maxima (in freq. range of 1–10 Hz), whereas in the anodized and sealed conditions it exhibits two maxima, one in freq. range of 0·1–1 Hz and the other at around 1000 Hz. These facts suggest that the anodized Cp-Ti after sealing has two log constants and corrodes under the influence of double layer.

Cyclic polarization shows that the Cp–Ti in anodized as well as in anodized and sealed condition exhibits considerably lower passive current density compared to the unanodized condition. The anodized specimen during anodic scanning of potential shows fluctuation in current density which is absent in unanodized and anodized and sealed conditions. These facts suggest that Cp-Ti simply in the anodized condition formed a passive film; however, it remained in unstable condition in contact with body fluid. Further, the transition potential from anodic to cathodic current is considerably higher for the anodized and sealed condition in comparison to that of simply anodized condition. These facts suggest that Cp-Ti in anodized and sealed condition develops superior corrosion as well as pitting resistance in comparison to anodized and unanodized condition. It may be noted that the loops formed during back scanning are in negative direction (towards lower current density region) in all the three

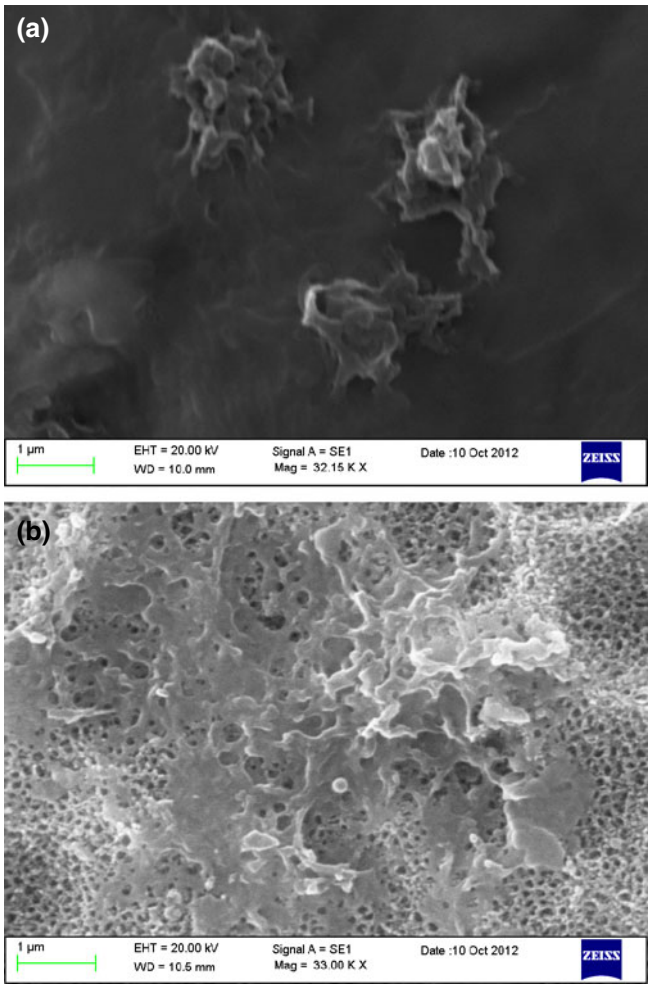

Figure 5. Scanning electron micrographs showing profiles of MG63 osteoblast cells on Cp-Ti after 24 h of culture: (**a**) unanodized and (**b**) anodized.

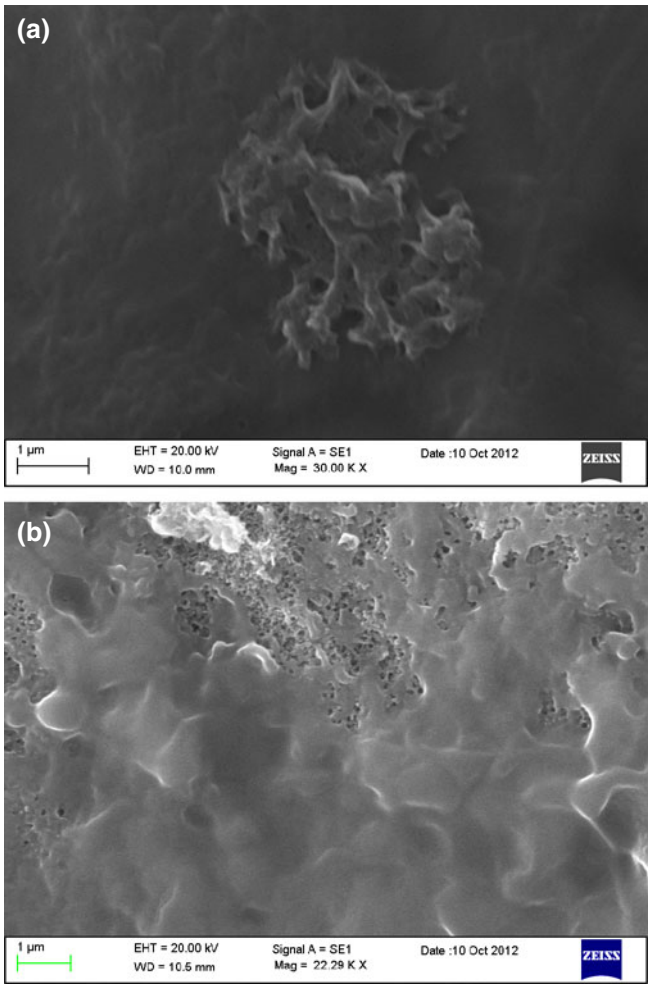

Figure 6. Scanning electron micrographs showing profiles of MG63 osteoblast cells on Cp-Ti after 48 h of culture: (**a**) unanodized and (**b**) anodized.

cases, however, in anodized and anodized and sealed condition, the loops are bigger as compared to that in the unanodized condition and indicate strengthening of passive film during back scanning. It is further proof that anodized and anodized and sealed Cp-Ti acquired higher resistance against corrosion and pitting. Shukla *et al* (2005) reported that based on their electrochemical impedance spectroscopy study, that corrosion of Cp-Ti in SBF was essentially due to dissolution of weak passive oxide layer by Cl^- ions that resulted in localized corrosion. The breakdown of these passive layer zones in localized regions enhanced the tendency for pitting corrosion.

Surface topography of implant plays a critical role in cell–material interaction. The surface which absorbs more proteins offers more focal areas for osteoblast attachment, and leads to faster bone in-growth and implant stabilization. It has been reported that TiO_2 nano-pores absorb more proteins and thus accelerate the phenomena of cell–material interactions and enhance cell adhesion and proliferation. According to Arnold *et al* (2004), separation of 54–73 nm, between

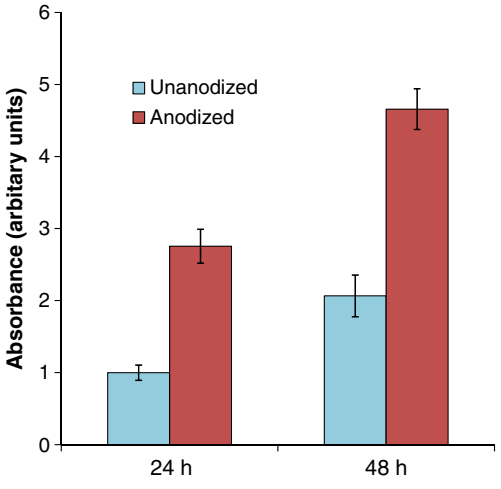

Figure 7. MTT absorbance value for MG63 osteoblast cells on unanodized and anodized surface. Cells are more viable on anodized surface compared to that on unanodized surface after 48 h of culture ($p = 0{\cdot}009$).

the nano-pores, is a gold standard length scale for clustering of integrin and activation thus enhancing cell attachment and spreading. Park *et al* (2007) report that nano-pores with dimension of 25–80 nm, enhance cell adhesion and proliferation. In the present study, pore size of nano tubes on the anodized surface was ∼50–110 nm and it showed a multilayered network like cell growth, which is in agreement with the above finding.

MTT assay involves conversion of tetrazolium salt in MTT agent into a soluble formazam product when incubated with viable cells. Thus, the absorbance of formazan reflected the level of cell metabolism. The growth behaviour of MG63 osteoblast cell on unanodized and anodized specimen was examined after 24 and 48 h. The anodized surface showed significantly more osteoblast cell proliferation than the unanodized surface ($p = 0{\cdot}009$). Thus, these results suggest that cells are more healthy and viable on anodized surface as compared to those on unanodized one. Thus, higher cell proliferation in the anodized condition, in the present investigation, is in agreement with the findings of earlier investigations (Baxter *et al* 2002; Das *et al* 2007; Li *et al* 2010). Baxter *et al* (2002) reported that both quantitative as well as qualitative assessment of cell morphology revealed that osteoblasts and fibroblasts reacted in similar manner to different surfaces tested. The greatest amount of spreading, in both types of cells, was observed on the APC–CaP and anodized titanium. Li *et al* (2010) showed that anodic oxidation of titanium surface by CH_3COOH electrolyte promoted osteoblast adhesion. Das *et al* (2007) demonstrated that cell proliferation, using MTT assay, showed that the best adherence and proliferation of human osteoblast were in the HF and H_3PO_4 anodized oxide surfaces compared with the H_2SO_4, a Ti-control surface. It was observed that higher surface roughness, low contact angle, improved wettability and high surface energy led to better cell attachment and proliferation on the above two anodized surfaces.

4. Conclusions

The present investigation is concerned with study of the effect of anodization on corrosion resistance in simulated body fluid and osseointegration of MG63 osteoblast cells on Cp-Ti. It is observed that the best anodized surface of Cp-Ti, in terms of uniformity in size, depth and morphology of porous structure, is achieved from 0·5% aqueous solution of HF at 15 V for 30 min. Under these conditions, the mean diameter of pores varied from 50 to 110 nm. Sealing the pores of anodized surface in hot water further improved corrosion and pitting resistance of Cp-Ti in simulated body fluid. Thus, both corrosion as well as pitting resistance of Cp-Ti in SBF are found to be highest in the anodized and sealed condition and followed by those in anodized and unanodized conditions in decreasing order. The osteoblast cell proliferation is found to be much higher on the anodized surface as compared to that on the unanodized surface.

Acknowledgements

The authors would like to thank Dr Ajit Sodhi, Department of Biotechnology, Faculty of Science, BHU, and Mr Rajeev Pandey, for their eventful cooperation and help in cell growth study.

References

Arnold M, Cavalcanti-Adam E A, Glass R, Blummel J, Eck W, Kantlehnar M, Kessler H and Spatz J P 2004 *Chem. Phys. Chem*. **5** 383

Baxter I C, Frauchiger V, Textor M, Gwynn I and Richards R G 2002 *Eur. Cell Mater*. **4** 1

Brunette D M and Chehroudi B 1999 *J. Biomech. Eng*. **121** 49

Brunette D M (ed.) 2001 *Titanium in medicine: Material science, surface science, engineering biological responses and medical application* (Germany: Springer)

Cai Q Y, Varghese O K, Paulose M, Mukherjee N and Grimes C A 2005 *J. Mater. Res*. **20** 230

Campos D M, Santos J R, Kuromoto N K and Soares G A 2007 *Rev. Mater*. **12** 150

Chang Y, Elliott B, Slamovich and Thomas J W 2005 *Mater. Res. Soc. Symp. Proc*. **845** 215

Das K, Bose S and Bandyopadhyay A 2007 *Acta Biomater*. **3** 573

Eliades T 1997 *Int. J. Oral Max. Impl*. **12** 621

Grimes C A, Gong D J, Varghese O K, Thi W, Singh R S, Dickey E C and Hen Z 2001 *J. Mater. Res*. **16** 3331

Huang C, Xie Y, Zhou L and Huang H 2009 *Smart Mater. Struct*. **18** 024003

Jacobs J J, Gilbert J L and Urban R M 1998 *J. Bone Joint Surg. Am*. **80** 268

Jiang X P, Wang X Y, Li J X, Li DY, Man C S, Shepard M J and Zhai T 2006 *Mater. Sci. Eng*. **429** 30

Keller J C, Stanford C M, Wightam J P, Draughn R A and Zaharias R 1994 *J. Biomed. Mater. Res.* **28** 939

Kieswetter K, Schwartz Z, Dean D O and Boyan B D 1996 *Crit. Rev. Oral Biol. Med*. **7** 329

Li X, Guangfu Y, Danhong Y, Xiaoming L and Zhongbing H 2010 *J. Mater. Sci. Mater. Med*. **21** 259

Park J, Bauer S, Vonder Mark K and Schmuki P 2007 *Nano Lett*. **7** 1686

Shabalovskaya S A 2002 *Biomed. Mater. Eng*. **12** 69

Shukla A K, Balasubramaniam R and Bhargava S 2005 *Intermetallics* **13** 631

White K C, Svare C W and Taylor T D 1985 *J. Prosthet. Dent*. **53** 815