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Microstructure and Biocompatibility of Titanium Oxides Produced on Nitrided Surface Layer Under Glow Discharge Conditions

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The disadvantages of titanium implants are their low wear resistance and the release of titanium elements into surrounding tissue. These can be eliminated by modifying the surface by surface engineering methods, among them nitriding under glow discharge conditions which allow to produce diffusive surface layers. Their combining with an oxide layer might be valuable for biological events occurring at the bone implant interface. The aim of this study was to enhance the titanium biomaterial performance via combining nitriding and oxidizing treatments in one process under glow discharge conditions. The oxynitrided surface layers were produced at 680 °C. The obtained layer was $TiO + TiN + Ti_2N + \alpha Ti(N)$ type and about 4-μm thick and was of diffusive character. This layer significantly increased wear resistance and slightly corrosion resistance compared to that of the reference titanium alloy. The produced titanium oxide was about 400-nm thick and built from fine crystallites. This oxide exhibits bioactivity in SBF (simulated body fluid). Osteoblasts of Saos-2 line incubated on this surface exhibited good adhesion and proliferation and ALP release comparable with cells cultured on the reference titanium alloy and $TiN + Ti_2N + \alpha Ti(N)$ surface layers. A quantitative analysis of blood platelets adhering to this layer revealed their highest amount in comparison to that on both the nitrided surface layer and titanium alloy. The presented study provided a simple and reproducible method of combining oxidizing and nitriding under glow discharge in one process. Experimental data *in vitro* suggests that titanium alloy oxynitriding under low temperatures at glow discharge conditions improves titanium alloy properties and biocompatibility and tissue healing. Therefore, the layer of $TiO + TiN + Ti_2N + \alpha Ti(N)$ type could be valuable for long-term bone implants.

Keywords: Titanium Oxides, Nitriding, Microstructure, Biocompatibility.

RESEARCH ARTICLE

1. INTRODUCTION

Titanium and its alloys are widely used for bone implants due to the spontaneous formation of oxides which play an important role in their biocompatibility and osteointegration.[1] However, this naturally passive coating of about 10 nm is relatively thin and its properties depend on its formation conditions. It also does not prevent metal ion release or protect from wear.[1] Therefore, titanium oxides of increased thickness and controlled nanostructures have been produced using different methods such as anodic oxidation, chemical vapor deposition, magnetron sputtering, sol–gel process.[2] In spite of these developments, an average of 25% of patients are still undergoing a revision surgery[3] and the functional lifetime of an endoprosthesis is limited to 10–25 years.[4] Recent observations suggest that time-dependent titanium degradation and corrosion can cause clinical complications,[5–7] since both processes directly affect implant bioactivity and fixation.[5,7] Further functionalization of the implant surface is expected by achieving an improvement in oxide properties, increase in wear resistance of titanium alloys and protection against the release of elements from the titanium alloy into surrounding tissue. One option that could eliminate the mentioned disadvantageous properties of titanium alloys would be producing oxide on sub-layer. The surface layer that fulfills these requirements could be a nitrided surface layer produced under glow discharge

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conditions $TiN + Ti_2N + \alpha TiN$ type which has diffusive character and exhibits high corrosion and wear resistance and prevents the release of titanium alloy elements.[8–10] Studies of endoprostheses with TiN layer, explanted post mortem, have shown their *in vivo* passivation[11] and incorporation of calcium.[12] Thus, the TiN sub-layer produced in our study are expected to improve bone implant durability while oxides could increase surface biocompatibility and bioactivity.

Therefore, to upgrade the performance of bone implants a glow discharge oxynitriding process realized at low temperatures has been elaborated. Our study examines the feasibility of such surface modification aimed to facilitate bone implant durability and bioactivity and biocompatibility.

2. MATERIAL AND METHODS

2.1. Material Processing

The samples of titanium alloy Ti_6Al_4V type, ground with a grade 600 abrasive paper were nitrided at 680 °C and than oxidized at the same temperature for 5 min under glow discharge conditions. The method of oxynitriding under glow discharge conditions has been described in details in a previous study.[13]

2.2. Material Properties

The surface layer microstructure was investigated using a TECNAI G^2 FEG Super TWIN (200 kV) transmission electron microscope equipped with a High Angle Annular Dark Field (HAADF) detector and integrated EDAX Energy Dispersive Spectroscopy (EDS) system. The thin foils of the surface layer cross-section were cut using Focused Ion Beam (FIB) Quanta 3D with Omniprobe pick-up attachment. Then, a ~ 50-nm-thick carbon coating was deposited to separate the surface layer from platinum used in this technique as a mask protecting the surface features and maintaining the image contrast.

Quantitative chemical composition was analyzed by WDS method in Cameca Semiprobe SU-30 X-ray micro-analyzer under energy of 10 keV and beam current of 0.3 μA for 180 s. Phase analysis was performed using a Brucker D-8 Advance X-ray diffractometer with CoKα lamp.

Oxide thickness was measured with scanning microscopy (SEM-Hitachi 3500N) on cross-sections of the samples under magnification ×6000.

Surface topography and morphology were characterized by a scanning profilometer of Talysurf Taylor Hobson Form and atomic force microscope (AFM) respectively.

The frictional wear resistance was determined by the "three rollers + taper" method under 400 MPa load according to PN-083/H-04302 Polish standard.

The corrosion resistance was examined in Hank solution by the potentiodynamic method using Atlas 0531 EUSIA set.

2.3. Bioactivity

The bioactivity of the surface layers was investigated by soaking them for 14 days in SBF at 37 °C and then analyzing by X-ray photoelectron spectroscopy (XPS), Esclab-210 apparatus of VG Scientific firm).

2.4. Biocompatibility

The biocompatibility was verified *in vitro* with osteoblast-like cells of Saos-2 line and human blood platelets. All specimens were plasma-sterilized prior to experiments.

Osteoblasts suspended at a density of $4.5 \times 10^4/50$ μL were placed on samples for 30 min and then incubated on samples in 500 μL McCoy medium (ATTC, USA) supplemented with 15% fetal calf serum and antibiotics (penicillin and streptomycin 1:100, Gibco BRL). Cells were incubated in a culture chamber at 37 °C for 48 h, 6 days, or 12 days.

Cell adhesion, spreading, proliferation, and metabolic activity were evaluated by analysis of cell morphology under scanning electron microscope (Hitachi S-3500N), performing MTT assay and measuring alkaline phosphatase activity (ALP test) respectively. The 3-[4,5-dimethylthiazol-2yl]2,5-diphenyltetrazolium bromide was added to the culture solution in MTT assay and the quantity of formazon produced due to activity of mitochondrial dehydrogenase was measured using a plate reader (ELISA) at $\lambda = 545$ nm. The ALP activity was measured by Dimension Ar at $\lambda = 405$ nm.

Platelet adhesion was investigated by incubating samples with 400 μL/sample of human platelet-rich plasma (PRP) for 2 hours at 37 °C under static conditions. The morphology, distribution, and the number of adhered platelets were assessed under a scanning electron microscope (Hitachi S-3500N) under magnification ×250 and ×1000.

Osteoblasts and platelets adhering to the sample surface were fixed for scanning electron microcopy examinations in 2.5% glutaraldehyde in PBS for 1 h and dehydrated in a graded series of ethanol and then sputter-coated with gold.

Samples of Ti_6Al_4V in both type experiments, i.e., with osteoblasts and PRP, were used as controls.

3. RESULTS

3.1. Material Characterization

Surface layers produced in a low-temperature glow discharge oxynitriding process were about 4 μm thick (Fig. 1) with high concentration of oxygen in external zone. Distribution of the elements Ti, O and N in this surface layer is shown in Figure 1. TEM examinations revealed zonal

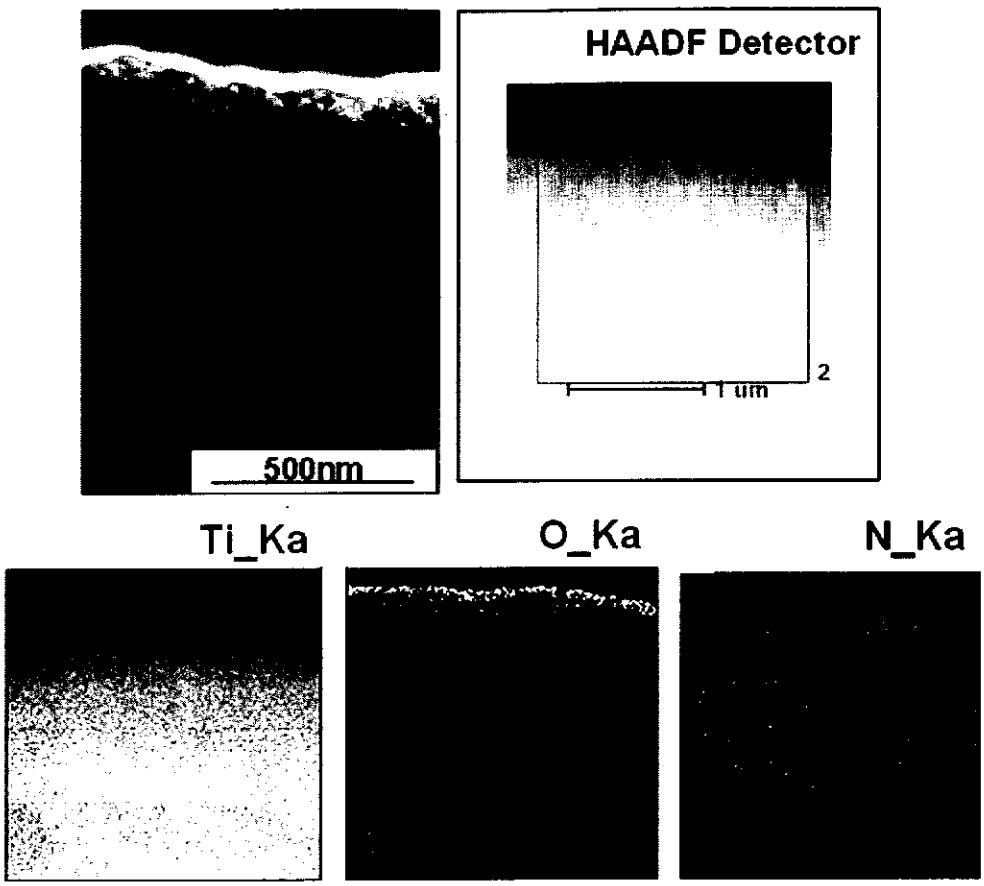

Fig. 1. Microstructure and distribution of the elements in an oxynitrided surface layer produced on titanium alloy Ti_6Al_4V type.

structure of the oxynitrided surface layer: (i) the upper zone was built of nanocrystallites; (ii) directly below there was a finecrystlline zone; (iii) the third zone consisted of columnar grains; (iv) the final zone was built-up of irregular cristallites (Fig. 2). The upper zone was about 400 nm thick and characterized by high density of the upper and porous structure of the bottom part. A diffraction analysis of this zone in the ring selected area showed that it is built-up of TiO (Fig. 2). The HRTEM imaging additionally showed that the zone consists of randomly oriented, relatively dense crystallites whose lattice spacing and angle measurements using Fast Fourie Transform (FFT) match those of TiO (Fig. 3).

Investigation of the oxynitrided surface showed its nanocrystalline structure, while measurements of topography revealed increased roughness profile parameters, e.g., R_a increased from 0.111 μm of the reference titanium alloy to 0.835 μm (Fig. 4 and Table I).

In comparison to titanium alloy Ti_6Al_4V examined in Hank's solution, an oxynitrided surface layer showed superior corrosion resistance (a corrosion potential increase from −141 mV of the titanium alloy to −82 mV after oxynitriding) and slightly increased current density (from 0.21 $\mu A/cm^2$ for the titanium alloy to 0.37 $\mu A/cm^2$ for the oxynitrided layer) (Fig. 5). The developed topography of the oxynitrided layer also has an influence on the value of current density. This surface layer is also characterized by a substantially increased frictional wear compared to that of titanium alloy which is only slightly weaker than a highly resistant nitrided layer (Fig. 6).

3.2. Bioactivity

During their 14-day exposure to SBF oxynitrided samples exhibited the presence of hydroxyapatite characterized by the P energy of 133.71 eV and the Ca energy of 347.8 eV (Fig. 7).

3.3. Osteoblast Adhesion and Activity

Osteoblasts of Saos-2 line incubated on oxynitrided samples were regularly spread. Cell populations consisted of intensively flattened and polymorphic osteoblasts with numerous pseudopodia (Fig. 8(A)). During the observation, cells intensively proliferated and released ALP. However no statistically significant differences among these parameters were found for populations incubated on an oxynitrided surface layer in reference to titanium alloy and nitrided bulk material (Figs. 8(C, D)).

3.4. Platelet Adhesion

Platelet adhesion to a surface with oxide in the external zone was higher in comparison to that to a titanium alloy Ti_6Al_4V. Platelets were regularly distributed on the surface and did not aggregate, however; their morphology was characterized by the formation of tiny pseudopodia (Fig. 8(B)). A quantitative analysis of blood platelets adhering to tested materials showed their highest amount on the oxynitrided surface layer when compared to the nitrided surface layer and the titanium alloy.

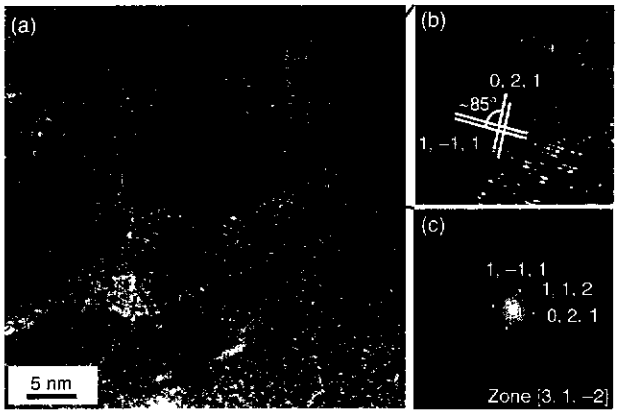

Fig. 3. A HRTEM image of the TiO zone of the oxynitrided surface layer (A) with a magnification of one of the crystallites (B) and corresponding FFT (C).

4. DISCUSSION

This study was motivated by further implant and endoprosthesis requirements for improving their integration with

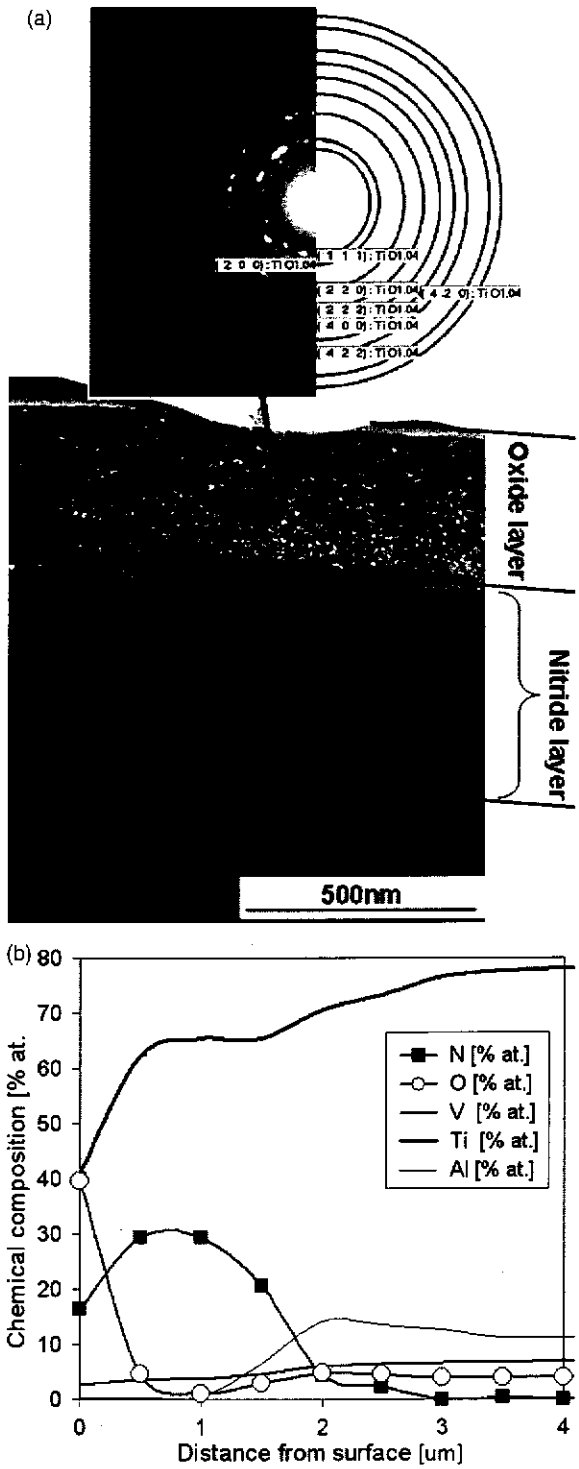

Fig. 2. TEM microstructure with diffraction analysis of a selected area of the top zone (a) and WDS line scan of a cross-section (b) of the oxynitrided surface layer.

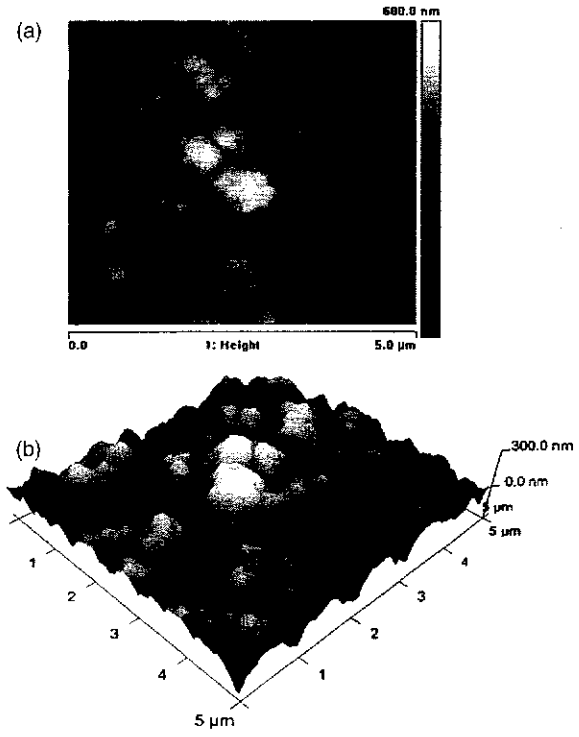

Fig. 4. An 2D (a) and 3D (b) AFM image of surface morphology of an oxynitrided surface layer produced under glow discharge conditions.

Table I. Stereometric parameters of the Ti_6Al_4V alloy before and after glow discharge oxynitriding.

Material	R_a[μm]	R_q[μm]	R_p[μm]	R_v[μm]	R_t[μm]
$TiO + TiN + Ti_2N + \alpha Ti(N)$	0,835	1,08	3,09	4,99	8,89
Ti_6Al_4V	0,111	0,15	0,941	1,49	2,43

R_a-arithmetic mean deviation of the roughness profile, R_q-square mean deviation of the roughness profile; R_p-maximum height of the roughness profile; R_v-maximum depth of the roughness profile; R_t-maximum distance between the lowest and the highest points of the roughness profile ($R_t = R_p + R_v$) (in according with ISO 4287/1/1984)

bone in long-term use. We employed a process of oxynitriding under low-temperature glow discharge conditions to produce a diffusive surface layer. The $TiN + Ti_2N + \alpha Ti(N)$ layer has been formed as a result of the diffusion processes activated by the cathode sputtering applied when the Ti_6Al_4V samples were heated to nitriding temperatures (680 °C). A thin TiO surface layer (of about 400 nm) was formed as a result of the oxidizing process of the nitrided layer under glow discharge conditions.

Produced at 680 °C, the nitrided surface layer exhibits advantages of the $TiN + Ti_2N + \alpha TiN$ layer produced at 850 °C, which has been presented and discussed by our group in the past.[8–10] The surface layer produced at 680 °C exhibits lower thickness but the same microstructure and similar properties as the one produced at 850 °C. Both surface layers are also characterized by a similar intensive osteoblast adhesion and proliferation and ALP release.[9] These findings are in agreement with the findings of bioactivity of nitrided surface layers *in vivo* presented in the literature for explanted post mortem endoprosthesis.[11–12, 14] Data revealed that bioactivity of the nitride surface layer are related to oxinitrides formed deep within the layer.[12]

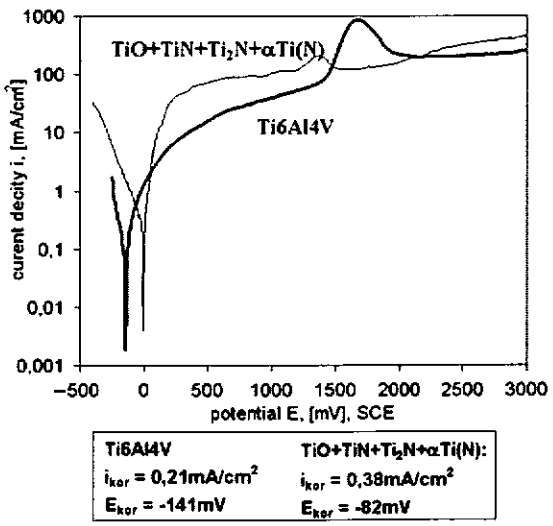

Fig. 5. Polarization curves (A) and values of the corrosion potentials and corrosion current densities (B) of an oxynitrided surface layer in comparison with those of reference titanium alloy Ti_6Al_4V

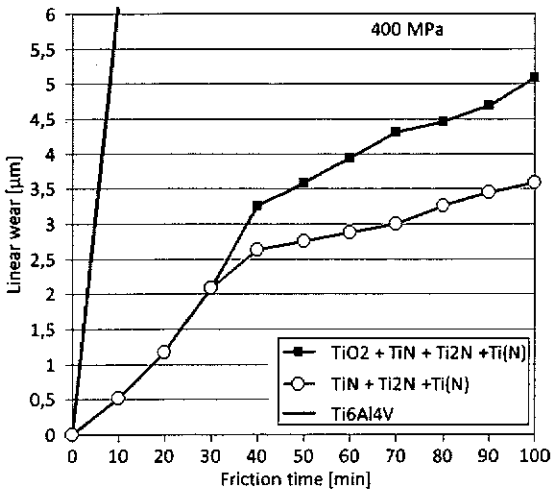

Fig. 6. Linear wear of an oxynitrided surface layer in comparison with reference titanium alloy Ti_6Al_4V and a nitrided surface layer produced under glow discharge conditions.

The nanocristalline structure of the TiO zone present as a part of the outer zone of the surface layer produced in the process elaborated by our group could be significant for both the surface bioactivity and cell behavior. Experiments

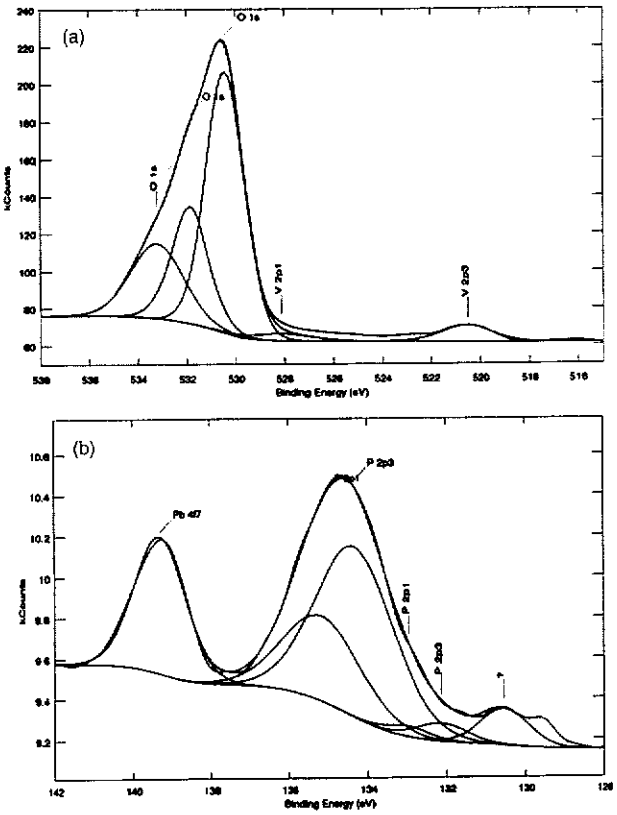

Fig. 7. The XPS analysis: the binding energy of phosphorous (P) (a) and calcium Ca(b) characteristic for an oxynitrided surface layer produced on titanium alloy under low-temperature glow discharge oxynitriding.

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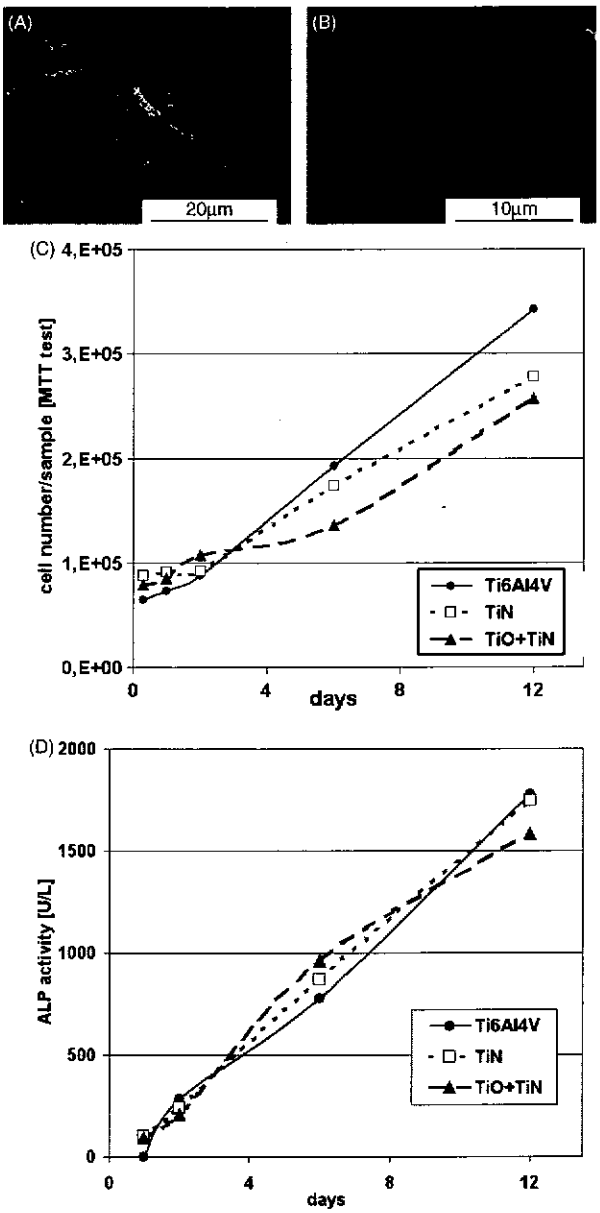

Fig. 8. Osteoblasts Saos-2 line (A) and human platelets (B) adhering to an oxynitrided surface layer, and osteoblast proliferation (C) and ALP concentration in the cell culture media (D) when cells were incubated on an oxynitrided surface layer produced on a titanium alloy under low-temperature glow discharge assisted conditions (TiO) and reference titanium alloy (Ti_6Al_4V) and nitrided bulk material (TiN) under glow discharge conditions

in SBF have shown that an oxide zone incorporates calcium and phosphorus and hydroxyapatite is formed. The fact of nucleation of calcium phosphates by oxides is in agreement with the data described in literature.[15–17] Thus the presence of calcium ions might further promote adsorption of calcium-binding proteins and cell differentiation and bone regeneration. In our studies, the TiO layer keeps osteoblasts proliferation and ALP activity comparable to these incubated on reference titanium alloy, but exhibits increased platelet adhesion. Platelets adhering to the oxide surface formed tiny pseudopodia indicative on their early activation stage. Activated platelets are known to release thrombin which directs the formation of fibrin further stabilizing adsorbed cells and they are considered to be a source of growth factors present in the α-granules which promote early bone healing.[18] Thus, increased platelets adhesion and activity in response to the oxynitrided surface layer $TiO + TiN + Ti_2N + \alpha Ti(N)$ type seem to be feature that potentially could enhance bone formation.

5. CONCLUSIONS

In conclusion, oxynitriding under glow-discharge conditions at low temperatures is inexpensive and simply method that can be use to enhance the surface wear and corrosion resistance. Additionally, produced an oxynitrided surface layer has a diffusion character therefore tightly bonds with the titanium alloy substrate. Both nanocristaline oxide zone and fine grained nitrided sub-layer are known to be naturally bioactive and as revealed recent studies also exhibit several biological phenomenon including good osteoblasts adhesion and proliferation and activity comparable to titanium alloy and increased platelets adhesion. All mentioned features are essential for implant or endoprosthesis successful clinical behavior. Thus produced the surface layer $TiO + TiN + Ti_2N + \alpha Ti(N)$ type of controllable microstructure seems to be promising to optimize the design of novel bone implants for long-term use.

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References and Notes

1. M. Geetha, A. K. Singh, R. Asokamani, and A. K. Gogia, *Progress Mat. Sci.* 54, 397 (**2009**).
2. L. Le Guehennec, A. Soueidan, P. Layrolle, and Y. Amourig, *Dental Mat.* 23, 844 (**2007**).
3. American Academy of Orthopedics Surgeons. Available from: http://www.aaos.org:2005
4. J. S. M. de Aragon and K. S. Keisu, Clin. *Orthop. Relat. Res.* 454, 133 (**2007**).
5. D. Zaffe, C. Bertoldi, and U. Consolo, *Biomaterials* 24, 1093 (**2003**).
6. D. G. Olmedo, D. R. L. Cabrini, and M. B. Guglielmotti, *Int. J. Oral Maxillofac. Surg.* 37, 1032 (**2008**).
7. W. Att, N. Hori, M. Takeuchi, J. Ouyang, Y. Yang, and M. Anpo, *Biomaterials* 20, 5352 (**2009**).
8. E. Czarnowska, T. Wierzchon, A. Maranda-Niedbala, and E. Karczmarewicz, *J. Mat. Sci: Mat. Med.* 11, 73 (**2000**).
9. E. Czarnowska, A. Sowinska, B. Cukrowska, J. R. Sobiecki, and T. Wierzchon, *Mater. Sci. Forum* 475–479, 2415 (**2005**).
10. A. Czyrska-Filemonowicz, P. A. Buffat, E. Czarnowska, and T. Wierzchon, *Mater. Sci. Forum* 513, 15 (**2006**)

11. K. Uetani, H. Kajiyama, A. Takagi, I. Tokomoto, Y. Koizumi, K. Nose, Y. Ihara, A. Kato, K.-ichi Onisawa, and T. Minemura, *Mater. Trans.* 42, 403 (**2001**).
12. S. Pisanec, L. C. Ciacchi , E. Vesselli, G. Comelli, O. Sbaizero, S. Meriani, and A. De Vita, *Acta Mater.* 52, 1237 (**2004**).
13. J. R. Sobiecki and T. Wierzchon, *Vacuum* 79, 203 (**2005**).
14. F. Esaka, K. Furuya, H. Shimada, M. Imamura, N. Matsubayashi, H. Sato, A. Nishijima, A. Kawana, H. Ichimura, and T. Kikuchi, *J. Vac. Sci. Technol. A* 15, 2521 (**1997**).
15. W. A. Badawy, A. M. Fathi, R. B. El-Sherief, and S. A. Fadl-Allah, *J. Alloys Compounds* 475, 911 (**2009**).
16. T. Y. Xiong, X. Y. Cui, and H. M. Kimtal, *Key Eng. Mater.* 254, 375 (**2004**).
17. J. Kunze, L. Müller, J. M. Macak, P. Greil, P. Schmuki, and F. A. Müller, *Electrochim. Acta* 53, 6995 (**2008**).
18. T.-M. You, B.-H. Choi, J. Li, J.-H. Jung, H.-J. Lee, S.-H. Lee, and S.-M. Jeong, *Oral Surg. Oral Med. Pathol. Oral Radiol. Endod.* 103, e8-e12 (**2007**).

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