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Niobium based coatings for dental implants

G. Ramírez [a,b,*], S.E. Rodil [a], H. Arzate [c], S. Muhl [a], J.J. Olaya [d]

[a] Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de México, Circuito Exterior s/n, CU, México D.F. 04510, México
[b] Facultad de Química, Departamento de Ingeniería Química, Universidad Nacional Autónoma de México, México D.F. 04510, México
[c] Laboratorio de Biología Celular y Molecular, Facultad de Odontología, Universidad Nacional Autónoma de México, CU, México D.F. 04510, México
[d] Unidad de Materiales, Departamento de Ingeniería Mecánica y Mecatrónica, Universidad Nacional de Colombia, Cra. 30 45-03 Bogotá, Colombia

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ABSTRACT

Niobium based thin films were deposited on stainless steel (SS) substrates to evaluate them as possible biocompatible surfaces that might improve the biocompatibility and extend the life time of stainless steel dental implants. Niobium nitride and niobium oxide thin films were deposited by reactive unbalanced magnetron sputtering under standard deposition conditions without substrate bias or heating. The biocompatibility of the surfaces was evaluated by testing the cellular adhesion and viability/proliferation of human cementoblasts during different culture times, up to 7 days. The response of the films was compared to the bare substrate and pieces of Ti6Al4V; the most commonly used implant material for orthopedics and osteo-synthesis applications. The physicochemical properties of the films were evaluated by different means; X-ray diffraction, Rutherford backscattering spectroscopy and contact angle measurements. The results suggested that the niobium oxide films were amorphous and of stoichiometric Nb_2O_5 (*a*-Nb_2O_5), while the niobium nitride films were crystalline in the FCC phase (*c*-NbN) and were also stoichiometric with an Nb to N ratio of one. The biological evaluation showed that the biocompatibility of the SS could be improved by any of the two films, but neither was better than the Ti6Al4V alloy. On the other hand, comparing the two films, the *c*-NbN seemed to be a better surface than the oxide in terms of the adhesion and proliferation of human cemetoblasts.

1. Introduction

Dental implants require good mechanical properties because they are exposed to loads and fatigue cycles, which can only be achieved by the use of metallic materials. However, the biocompatibility of the metals is limited by their reactivity and degradation in the corrosive biological environment. Alloys such as CoCrMo and TiAlV are now commonly used as implant materials because they provide good mechanical properties and improved corrosion resistance; particularly Ti alloys have also some osteogenesis properties which make them very successful [1]. However, two key points are still needed to extend the lifetime of current implants: corrosion resistance and bone-bond ability (osseointegration). The corrosion resistance is of great importance, not only because it determines the device service life, but also because of the harmfulness of corrosion processes taking place in the living organism. The release of metal ions from some metal materials, e.g. aluminum (Al), nickel (Ni), iron (Fe), vanadium (V) and cobalt (Co), can generate adverse biological effects and affect cell metabolism [2]. On the other hand, the nature of the bone–implant interface is a result of the competition between bone regeneration and fibrous tissue formation [3]. For some bioactive materials, a thin bonelike apatite layer deposits on the implant surface after implantation into living tissues. Due to their chemical similarities, bone may not recognize the apatite layer as foreign, and therefore bonds directly with the implant, without the formation of the fibrous tissue [4]. Improvement of fixation between hard tissues and implants can be achieved by coating the metal surface with a bioactive and osteoconductive thin film to promote direct attachment to bone, such as the hydroxiapatite (HA) coatings [5]. However, HA coatings do not improve corrosion resistance [6].

Our proposal focuses on the surface modifications of stainless steel (SS) substrates by ceramic corrosion resistance coatings, which might also have bioactive properties [4]. The coatings were deposited on stainless steel because even though titanium alloys have good mechanical and biological properties, their extensive use in the third world countries is really limited due to the high cost of the implants. One possible solution for social care in third World countries might be the implementation of surface modifications to stainless steel or any other biocompatible metallic alloy of lower cost. The coatings were produced using magnetron sputtering; industrial process that has proven that well deposited films can extend the life time of industrial components, so it might be also

* Corresponding author at: Instituto de Investigaciones en Materiales, Universidad Nacional Autónoma de, México, Circuito Exterior s/n, CU, México D.F. 04510, Mexico. Tel.: +52 5556224734.
E-mail address: enggiova@hotmail.com (G. Ramírez).

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possible to apply this technology to medical implants. The objective is then to find a coating that performs similarly or better to the Ti alloys in terms of biocompatibility and osteogenesis properties, and that also improves the corrosion resistance of stainless steel. In this sense, we are studying different sputtered films based on refractory metals. Different studies have demonstrated the biocompatibility and corrosion resistance of the refractory metals, either as pure metals [7–9] or as stabilizing elements of the β-phase in titanium alloys [10–12]. Nevertheless, reports on the biomedical response of coatings based on refractory metals are more scarce and different response from Nb coatings have been reported [13]

In this paper, the response from niobium-based coatings in terms of corrosion resistance and biocompatibility are presented. The biocompatibility was tested in vitro using human bone-like cells and determining the adhesion, proliferation and viability of the cells in contact with the experimental surface. From our point of view, it is more relevant to evaluate firstly the biocompatibility by in vitro tests and secondly the bioactivity using Kokubo's based test. This test actually measures the capability of the surface to induce the growth of apatite layers when immersed in simulated body fluids (SBF), but not the cytotoxic effects. Although, it has been shown that it is a good indicator of the surface capability to induce rapid osseointegration [14].

There are some previous evidence that Niobium oxide films presented good biological compatibility as shown by Eisenbarth's group [15,16] and Ochsenbein et al. [17]. These groups produced Nb_2O_5 coatings using sol–gel techniques. Indeed, Miyazaki [4] also showed that sol–gel Nb_2O_5 coatings have good apatite-inducing ability by immersion in SBF. Niobium oxide films have been previously produced by magnetron sputtering showing the general trends between deposition conditions and physical–chemical properties, including the fact that as-grown films are amorphous, and that the composition depends mainly on the oxygen partial pressure [18] but no reports about the biocompatibility of these films were found. Similarly, no reports about the biocompatibility or bioactivity of NbN films were found in the available databases.

2. Materials and methods

2.1. Substrates preparation

Circular stainless steel substrates (AISI 316L) of $\phi = 14$ mm were cut from a 1 mm thick steel sheet. The pieces were grinded up to 600 grade SiC grit and then sandblasted using SiO_2 particles to produce a surface average roughness Ra of about 2 μm, measured using a profilometer (Ra measures roughness by comparing all the peaks and valleys to the mean line of a surface finish). This procedure has been tested before as a method to produce a uniform rough surface with improved osteoblasts attachment [19]. Then, the substrates were ultrasonically cleaned in acetone, isopropanol and de-ionized water for 30 min, respectively. Similarly, 14 mm samples of Ti6Al4V alloy were cut, sandblasted and cleaned to be used as a standard for the biological and corrosion resistance tests.

2.2. Film deposition

The Niobium oxide films were deposited using a commercially unbalanced magnetron (Teer Coatings Limited), from a Nb target (99.95% purity and 0.10 m diameter), in $Ar + O_2$ (both of ultra high purity) atmosphere. The base pressure in the chamber was less than 6×10^{-4} Pa and the substrate-target distance was 0.05 m. The argon–oxygen flow ratio, direct current (dc) power and the deposition pressure were previously optimized using the corrosion resistance of the coating as a parameter [20]. The conditions chosen for the biocompatibility studies reported in this work were 3 Pa, 10 sccm of Ar and 3 sccm of O_2 flow and 80 W. Under these conditions, the deposition time was adjusted to obtain 200 nm thick films. The maximum temperature reached by the substrate was about 80 °C.

The deposition conditions for the Niobium nitride films deposited using the same system and target were: substrate target-distance 0.05 m, 14 sccm of Ar, 2 sccm of N_2, working pressure 1 Pa, power 100W dc and film thickness 200 nm. These conditions were also previously optimized [21].

Some samples of both NbO and NbN were deposited on silicon substrates following their respective deposition conditions, in order to perform the physico-chemical characterization: hardness, composition and structure. While the samples deposited on SS were used for the biomedical testing, the corrosion resistance, water contact angle and surface morphology.

2.3. Film characterization

The structure of the films was analyzed by X-ray diffraction (XRD) using a Siemens D500 system in the Bragg–Brentano mode and CuKα radiation.

The corrosion resistance was evaluated by potentiodynamic anodic polarization performed using a PCI4/300 Gamry potentiostat. The test was performed on the bare and coated AISI 316 stainless steel substrates. The counter electrode was a platinum wire and the reference was a Saturated Calomel Electrode (SCE). The electrolyte was 8.9 g/l NaCl, pH 7.4. The sample was sealed to a wall of the electrochemical apparatus with a Viton O-ring leaving an area of 0.1 cm^2 exposed to the solution. The sample in contact with the electrolyte solution was kept at room temperature for 1 h without polarization to obtain the open circuit potential (OCP). Then, the voltage was scanned from −280 mV vs. OCP to +1280 mV vs. OCP at a scan rate of 20 mV/min. For the measurement of the average roughness R_a and film thickness, a profilometer (DEKTAK II) was used. Water contact angles were obtained using the sessile drop method using a Ramé-Hart Inc. system, model 100/07/00. Average values of the advancing angle were obtained after 10 measurements made at 37 °C and using 14 μl of water.

2.4. Biological tests

2.4.1. Cells

Cells derived from a human cementoblastoma were isolated by an explant technique, described previously [22]. The cells were subcultured in 75 cm^2 cell-culture flasks in "culture medium" composed of: DMEM, supplemented with 10% FBS and antibiotic solution. The cells were incubated in a 100% humidified environment at 37 °C in an atmosphere of 95% air and 5% CO_2. The cells used during this work were between the 2nd and 5th passage.

2.4.2. Cell culture

The biocompatibility of the Nb-based thin films was evaluated by quantifying the cellular attachment and the mitochondrial activity of the human cemetoblastoma derived-cells. Cementoblasts are derived from cells of the fibrous connective tissue that fills the space between the tooth and its bony socket and mediated tooth attachment to bone (periodontal ligament). Cementoblasts lay down cementum on the surface of root dentin, which is a heterogeneous mineralized tissue very much like bone that connects the periodontal ligament to the tooth root surface. The most important function of cementum is to anchor the principal periodontal ligament fibers, which span like a meshwork between the root and the alveolar bone, to the root. Other functions include participation in the maintenance of occlusal relationship, repair of root defects after re-sorption or fracture and protection of the pulp. From these functions, it is easy to understand the importance of

finding materials for odontological implants having good surface compatibility to cementoblasts and moreover that might induce cementogenesis. While there is a large body of information available on the regulation of bone formation in vitro, comparatively less information is available on the formation of cementum. In previous papers, Arzate et al. [23] has shown a procedure to obtain cementoblasts from a human cementoblastoma. These cementoblastoma derived-cells expressed several markers associated with mineral tissue formation and more important expressed cementun attachment protein, which is exclusive to cementum [23], demonstrating that the cementoblastoma derived-cells could serve as a suitable model to study the biological interaction of the cementum-phenotype cells with materials in vitro.

The samples were sterilized by autoclave prior to the biological tests. Niobium-based films (*a*-Nb_2O_5 and *c*-NbN), the bare stainless steel (SS) substrate and titanium-alloy samples were placed in 24-well culture plates and cells were plated at a density of 5×10^3/well and left to adhere for 3 h. After this time, 500 μl of culture medium were added.

All the following biomedical experiments were made in triplicate and the measurements were repeated three times. Statistical analysis was performed using Bonferroni's test and differences of significance $p < 0.001$, 0.01 and 0.05 are reported.

2.4.3. Cellular adhesion

For the attachment assay, the cells were incubated for 24 h in the culture medium at standard conditions. After this time, unattached cells were washed off three times with phosphate buffered saline (PBS) solution, and the remaining cells were fixed with 3.5% paraformaldehyde for 1 h and then stained using 0.1% toluidine blue for 3 h. The dye was extracted with 500 μl of 0.1% sodium dodecyl sulfate (SDS). The number of attached cells was quantified by measuring the optical density (or absorbance) at 595 nm, which correlates closely with cell number.

2.4.4. MTT test

The MTT test, also known as a cytotoxicity test, was used to assess cell viability/proliferation. The assay is based on the cleavage of the tetrazolium salt (3-[4,5-dimethylthiazolyl-2-y]-2,5-diphenyltetrazolium bromide) to formazan by cellular mitochondrial dehydrogenases. The formazan dye produced by viable cells was quantified by measuring the absorbance of the dye solution at 570 nm, which gives a reading directly proportional to the number of viable cells. For this, after the incubation period (24, 78 and 96 h) 50 μL of MTT were added to the medium and incubated for 3 h. Then, supernatant was removed and 500 μL of dimethyl sulfoxide (DMSO) were added to each well. After 60 min of slow shaking the absorbance (OD_{570}) of the dye solution was read.

3. Results

3.1. Film characterization

Fig. 1 shows the XRD spectra of both *a*-Nb_2O_5 and *c*-NbN films. The nitride film shows three main diffraction peaks related to the [1 1 1], [2 0 0] and [2 2 0] orientations of the FCC phase of δ-NbN (JCPDS no. 38-1155). The XRD signal from the *c*-NbN films confirms that the composition ratio Nb/N is close to 1, since no other phases are observed. On the other hand, Fig. 1 clearly shows that the niobium oxide films were amorphous, which is in good agreement with the results of other researchers that have shown that as-deposited niobium oxide films are amorphous. Physical–chemical characterization of the samples has been reported in previous papers. Briefly, the composition of the NbN was confirmed by X-ray photoelectron spectroscopy and the X-ray diffraction patterns [21,24], while the composition of the *a*-Nb_2O_5 was measured by RBS, giving a Nb/O

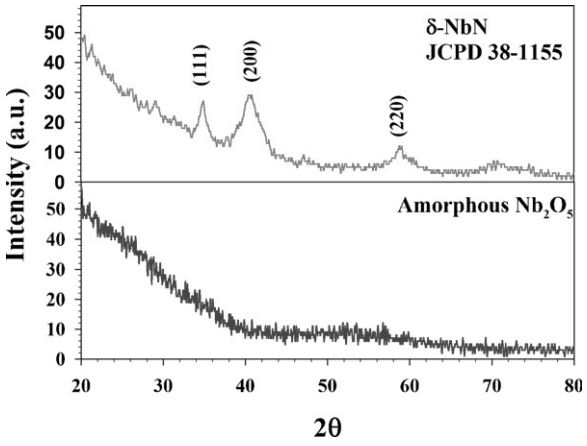

Fig. 1. X-ray diffraction patterns for *a*-Nb_2O_5 films and *c*-NbN films.

composition ratio of 0.418, which is within the ratio reported for stoichiometric Nb_2O_5 (0.39–0.43) [15,18]. The hardness values estimated from the nanoindentation analysis were 4.5 ± 0.78 GPa and 15 ± 0.8 GPa for *a*-Nb_2O_5 and *c*-NbN, respectively. These hardness values are higher than the hardness of stainless steel substrates, which was also measured in mirror-polished samples (~2 GPa), suggesting that there will be some improvement of the surface mechanical properties by the deposition of any of the films.

The water contact angles for the four surfaces are reported in Table 1. The water contact angle was slightly lower for the Ti-based alloy (~67°) in comparison to the other surfaces (~80°).

Fig. 2 shows the polarization curve for the four surfaces. It might be seen that both coatings behave similarly to the standard TiAlV sample, showing a more noble behavior than the SS substrate. The scans were made up to 1280 vs. OCP to observe the passivation region and failure of the surfaces. Fig. 3 shows that the minimum passivation current density was obtained for the *a*-Nb_2O_5 films, followed by TiAlV, *c*-NbN and SS316L. The results of the Tafel analysis made in the (−250 mV, 250 mV) interval around OCP and polarization resistance within the (−10, 10 mV) interval are shown in Fig. 3. It might be seen that the *a*-Nb_2O_5 coatings has the larger polarization resistance (R_p); while the corrosion current density

Table 1
Water contact angle measured at 37 °C for the four surfaces.

	SS 316L	Nb_2O_5	NbN	Ti6Al4V
Water contact angle (°)	78.5 ± 1.2	80.7 ± 2.1	78.7 ± 1.9	66.4 ± 1.1

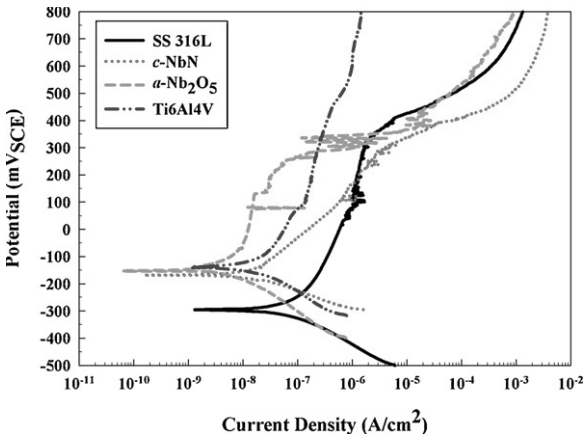

Fig. 2. Potenciodynamic polarization curves.

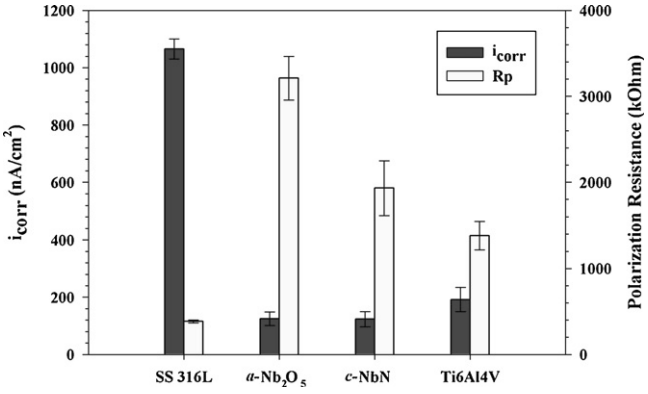

Fig. 3. Corrosion current density and polarization resistance of the surfaces.

(I_{corr}) was very similar for both coatings, but lower than for both SS and TiAlV. Moreover, using the following equation proposed by Yu et al. [25], $PI(\%) = 100 \times (1 - i_{corr}^{film}/i_{corr}^{substrate})$, one can estimate the protection efficiency of the coating, obtaining very similar values for both coatings (~88%).

3.2. Biocompatibility

3.2.1. Cellular adhesion

Fig. 4 shows the cellular adhesion represented by the absorbance measured at 595 nm for the four surfaces. The higher the absorbance, the larger the number of cells attached to the surface after 24 h. In this case, the Ti6Al4V surface shows a better response, followed by the *c*-NbN, stainless steel and the lowest value correspond to the *a*-Nb_2O_5 sample. Statistical analysis showed that the difference in cellular attachment among the four surfaces is significant, with a confidentiality of 95%.

3.2.2. MTT test

The cell viability was assessed by the MTT assay. The MTT is used as a marker of either cell enzymatic activity or cell growth [26]. Fig. 5 shows the absorbance at 570 nm, which is directly proportional to the metabolic activity of the cell and inversely proportional to the toxicity of the material. For all samples, there is a positive response attaining values above the medical grade stainless steel, indicating that none of the surfaces is toxic for human cementoblasts cells. MTT can also be used to determine the cell proliferation rate, since absorbance is directly proportional to cell number. From this data, we can see that after 96 h, the number of cells was five times the initial number in the Ti6Al4V surface, while

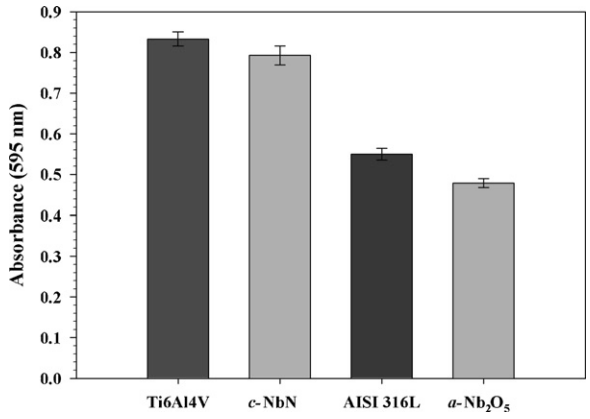

Fig. 4. Cellular adhesion test using spectrophotometric methods, where the absorbance is directly proportional to the number of attached cells on the surfaces.

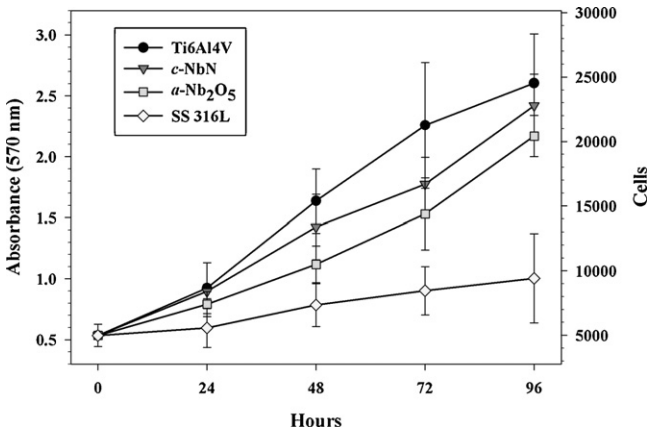

Fig. 5. Citotoxicity/proliferation test (MTT) where the absorbance (left axis) is proportional to the number of attached cells (right axis). The number of cells is obtained using a calibration curve previous to the experiment.

for the *a*-Nb_2O_5 the increment was only four-fold and the *c*-NbN was between these two values. On the other hand, the SS surface showed only a duplication of the number of cells after 96 h.

4. Discussion

The present study describes an in vitro model to study the response of cementoblastoma derived-cells to different Nb-based coating materials in comparison to stainless steel and Ti6Al4V alloy in order to evaluate the biocompatibility of the coatings and possible application for dental implants. The attachment of cementoblasts to *c*-NbN and *a*-Nb_2O_5 coatings and the metallic surfaces (Ti6Al4V and SS) was measured at 24 h after plating. Attachment of cells was larger in Ti6Al4V (probably related to the lower water contact angle) and *c*-NbN, but lower on both the steel and the *a*-Nb_2O_5. Nevertheless, the proliferation/viability test suggested that both coatings can improve the cellular growth on their surfaces in comparison to SS, since as observed in Fig. 5, the number of viable attached cells was superior on the *a*-Nb_2O_5 film than on the SS surface for the four periods, including the 24 h. So even that the attachment was lower on the *a*-Nb_2O_5 films in comparison to SS, the number of viable cells was higher on the oxide, meaning a better response. Moreover the proliferation rates suggested that both the Ti6Al4V and the Nb-based surfaces are more likely than SS to promote good cementoblasts differentiation and subsequent cementum formation [27].

The different biological response between the Nb-based coatings might be related to the structure and composition, since contact angle and roughness values were very similar, as shown in Table 1, i.e. the effect of surface topography is not the dominant one [28]. This might be an indication that the surface crystallinity, i.e. atomic ordering or the properties induced by the structure, play an important role concerning the cellular attachment or proliferation, which might be interesting to test in a future work. Actually, the niobium oxide films tested by Eisenbarth [15,16,29] were crystalline and they showed that the biological response of the oxide was similar or even better to the Cp–Ti, opposite to our results.

Concerning the corrosion resistance, the results suggested that both coatings provide the SS substrate with good protection efficiency, while the reactivity of the surface in the culture medium might be different explaining also the differences in the cellular–surface interaction. However, in order to be sure of the improvement in corrosion resistance, studies of the long-term stability of the coatings immersed in physiological fluids are in process.

5. Conclusions

In this work, crystalline *c*-NbN and amorphous Nb_2O_5 coatings were deposited on stainless steel substrates by magnetron sputtering. Evaluation of the cementoblasts attachment, viability and proliferation suggested that both coatings could improve the performance of stainless steel dental implants because the surface hardness, the corrosion resistance and the biological response were enhanced. Moreover, the biological evaluation showed that the biocompatibility of the SS could be improved by any of the two films. In terms of cell toxicity and proliferation, the response of the films was similar, although slightly lower, than the titanium alloy.

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References

[1] C.M. Cristache, M. Burlibasa, G. Tanase, M. Nitescu, R. Neamtu, A. Ciochinaru, Titanium as dental implant material, Metal. Int. 14 (2009) 14–16.
[2] A. Sarmiento-Gonzalez, J. Encinar, J.M. Marchante-Gayon, A. Sanz-Medel, Titanium levels in the organs and blood of rats with a titanium implant, in the absence of wear, as determined by double-focusing ICP–MS, Anal. Bioanal. Chem. 393 (2009) 335–343.
[3] B.D. Ratner, Foreword: the wound healing—biomaterials intersection, J. Biomater. Sci. Polym. Ed. 19 (2008) 953–954.
[4] T. Miyazaki, Development of bioactive materials based on bone-bonding mechanism on metal oxides, J. Ceram. Soc. Jpn. 116 (2008) 260–264.
[5] S.R. Paital, N.B. Dahotre, Calcium phosphate coatings for bio-implant applications: materials, performance factors, and methodologies, Mater. Sci. Eng. C 66 (2009) 1–70.
[6] J.H. Xie, B.L. Luan, Microstructural and electrochemical characterization of hydroxyapatite-coated Ti6Al4V alloy for medical implants, J. Mater. Res. 23 (2008) 768–779.
[7] H. Matsuno, A. Yokoyama, F. Watari, M. Uo, T. Kawasaki, Biocompatibility and osteogenesis of refractory metal implants, titanium, hafnium, niobium, tantalum and rhenium, Biomaterials 22 (2001) 1253–1262.
[8] E Eisenbarth, D. Velten, M. Muller, R. Thull, J. Breme, Biocompatibility of beta-stabilizing elements of titanium alloys, Biomaterials 25 (2004) 5705–5713.
[9] C.A. Scotchford, M. Ball, M. Winkelmann, J. Voros, C. Csucs, D.M. Brunette, G. Danuser, M. Textor, Chemically patterned, metal-oxide-based surfaces produced by photolithographic techniques for studying protein- and cell-interactions. II: protein adsorption and early cell interactions, Biomaterials 24 (2003) 1147–1158.
[10] R. Banerjee, S. Nag, J. Stechschulte, H.L. Fraser, Strengthening mechanisms in Ti–Nb–Zr–Ta and Ti–Mo–Zr–Fe orthopaedic alloys, Biomaterials 25 (2004) 3413–3419.
[11] A. Bigi, M. Fini, B. Bracci, E. Boanini, P. Torricelli, G. GiavareSi, N.N. Aldini, A. Facchini, F. Sbaiz, R. Giardino, The response of bone to nanocrystalline hydroxyapatite-coated Ti13Nb11Zr alloy in an animal model, Biomaterials 29 (2008) 1730–1736.
[12] X.J. Wang, Y.C. Li, J.Y. Xiong, P.D. Hodgson, C.E. Wen, Porous TiNbZr alloy scaffolds for biomedical applications, Acta Biomater. 5 (2009) 3616–3624.
[13] C.B. Johansson, H.A. Hansson, T. Albrektsson, Qualitative interfacial study between bone and tantalum, niobium or commercially pure titanium, Biomaterials 11 (1990) 277–280.
[14] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27 (2006) 2907–2915.
[15] E. Eisenbarth, D. Velten, M. Muller, R. Thull, J. Breme, Nanostructured niobium oxide coatings influence osteoblast adhesion, J. Biomed. Mater. Res.: Part A 79A (2006) 166–175.
[16] E. Eisenbarth, D. Velten, J. Breme, Biomimetic implant coatings, Biomol. Eng. 24 (2007) 27–32.
[17] A. Ochsenbein, F. Chai, S. Winter, M. Traisnel, J. Breme, H.F. Hildebrand, Osteoblast responses to different oxide coatings produced by the sol–gel process on titanium substrates, Acta Biomater. 4 (2008) 1506–1517.
[18] S. Venkataraj, R. Drese, O. Kappertz, R. Jayavel, M. Wuttig, Characterization of niobium oxide films prepared by reactive DC magnetron sputtering, Phys. Status Solidi A 188 (2001) 1047–1058.
[19] S.E. Rodil, C. Ramirez, R. Olivares, H. Arzate, J. Reyes-Gasga, C. Magana, Osteoblasts attachment on amorphous carbon films, Diamond Relat. Mater. 15 (2006) 1300–1309.
[20] G. Ramírez, S.E. Rodil, S. Muhl, D. Turcio-Ortega, J.J. Olaya, M. Rivera, E. Camps, L. Escobar-Alarcón3, Amorphous niobium oxide thin films, J. Non-Cryst. Solids (2010), doi:10.1016/j.jnoncrysol.2010.09.073.
[21] J.J. Olaya, S.E. Rodil, S. Muhl, Comparative study of niobium nitride coatings deposited by unbalanced and balanced magnetron sputtering, Thin Solid Films 516 (2008) 8319–8326.
[22] H. Arzate, M.A. Alvarez-Perez, M.E. Aguilar-Mendoza, O. Alvarez-Fregoso, Human cementum tumor cells have different features from human osteoblastic cells in vitro, J. Periodontal Res. 33 (1998) 249–258.
[23] H. Arzate, S.W. Olson, R.C. Page, A.S. Narayanan, Isolation of human tumor-cells that produce cementum proteins in culture, Bone Miner. 18 (1992) 15–30.
[24] S.E. Rodil, J.J. Olaya, S. Muhl, B. Bhushan, G. Wei, The influence of the magnetic field configuration on plasma parameters and microstructure of niobium nitride films, Surf. Coat. Technol. 201 (2007) 6117–6121.
[25] Y.J. Yu, J.G. Kim, S.H. Cho, J.H. Boo, Plasma-polymerized toluene films for corrosion inhibition in microelectronic devices, Surf. Coat. Technol. 162 (2003) 161–166.
[26] G. Ciapetti, D. Granchi, C.R. Arciola, E. Cenni, L. Savarino, S. Stea, L. Montanaro, A. Pizzoferrato, In vitro testing of cytotoxicity of materials, In: Biomaterial and Bioengineering Handbook, Marcel Dekker, New York, 2000.
[27] K. Anselme, Osteoblast adhesion on biomaterials, Biomaterials 21 (2000) 667–681.
[28] M. Bigerelle, K. Anselme, Statistical correlation between cell adhesion and proliferation on biocompatible metallic materials, J. Biomed. Mater. Res.: Part A 72A (2005) 36–46.
[29] D. Velten, E. Eisenbarth, N. Schanne, J. Breivie, Biocompatible Nb_2O_5 thin films prepared by means of the sol–gel process, J. Mater. Sci.: Mater. Med. 15 (2004) 457–461.