



Stimulation of calcium phosphate crystal formation by implant surfaces with electret properties

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Oxide coatings with electret properties are investigated. The possibility of stimulation of the formation of calcium phosphate sediments near the electroactive surface is discussed. The exposure of implants with such coatings to solutions imitating blood plasma showed their high efficiency of biointegration due to activation of an exchange processes in living tissues by a negative superficial charge. The revealed effect amplifies with the growth of the thickness of the anodic oxide film.

Keywords: anodic oxide, coating, electret, electrostimulation, implant, osseointegration, titanium

1. INTRODUCTION

Of great interest for the production of medical implants is the use of those metals that form a protective oxide film, decreasing the chemical activity of the material [1–5]. For the oxide film to possess protective properties, it has to meet the following requirements: continuity—pore-free; good adhesion to the metal substrate; a coefficient of thermal expansion close to that of the metal; chemical inertness; high hardness and wear resistance [6–8]. The oxides of metals such as Ti, Zr, Nb and Ta fulfil most of these requirements [1, 9, 10].

It is possible to increase the protective properties of oxide films by anodic oxidation of the metal substrate that forces growth of a thick oxide using a high external electric field in a gas or liquid environment [9, 11, 12]. Such anodic oxides have a disordered structure and can maintain significant deformation (up to 50%) without disintegrating, and strongly adhere to the metal substrate [13]. Most of the anodic oxides are chemically inert and do not interact with osteal tissue [14].

Anodic oxide films of Ti, Zr, Nb and Ta with amorphous structures can pass into an electret state and, after switching off the external electric field, maintain polarization [12]. This polarization may be associated with both the ordering of dipolar oxide molecules and the redistribution of space charge in deep traps of the dielectric oxide film. The existence of an electret charge on a medical implant surface can activate processes of adjustment and stabilization after installation into bone tissue. It is defined by activation of the formation of calcium phosphate masses, including new hydroxyapatite crystals, in the zone adjacent to the implant [15]. Such electrostimulation tends to diminish any postoperative

wounding in the bone tissue and facilitates adaptation of the organism to the implant.

However, there are open questions connected with the influences of the sign of the surface charge of the oxide, the oxide thickness in the electret state, and metabolic processes near the implant surface.

The purpose of this work was to investigate the formation of crystalline calcium phosphate sediments and their nature near the oxide coating of an implant in a solution simulating the composition of blood plasma (inorganic components).

2. EXPERIMENTAL

Titanium plates imitating implants with different thickness of surface oxide were used. Oxide films were grown by anodic oxidation of titanium in a 0.01% aqueous solution of phosphoric acid [8, 9]. The resulting set of samples had oxide thicknesses varying from 50 to 300 nm.

To carry out model tests in a simulated biological environment, the metal plates imitating implants were placed into a solution whose composition of inorganic components was close to that of blood plasma. This composition involves the creation of calcium phosphate sediments and subsequently the formation of hydroxyapatite crystals. Introducing the implants into the solution induces sludge formation; its quantity and distribution over the surface of the plates were estimated. It was presumed that if the implant after anode processing will activate the formation of calcium phosphate on its surface, the stimulation of similar processes upon introduction of an implant to real bone tissue will lead to faster restoration of tissue at the implant site and, therefore, to faster healing of the wound.

The test solution was prepared to be equivalent to blood plasma at $T = 36.5$ °C and $\text{pH} = 7.4$. The

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concentrations of dissolved ions were: Na^+ , 142.0; K^+ , 5.0; Mg^{2+} , 1.5; Ca^{2+} , 2.5; Cl^- , 147.8; HCO_3^- , 4.2; HPO_4^{2-} , 1.0; and SO_4^{2-} , 0.5 mmol. The solution was prepared by dissolving the following reagents: NaCl , NaHCO_3 , KCl , $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 and Na_2SO_4 in distilled water [16]. To adjust the pH, $(\text{CH}_2\text{OH})_3\text{CNH}_2$ or 1 M hydrochloric acid solution was added.

Infrared (IR) spectra of the sediments in the solution were taken using a Perkin–Elmer Spectrum One Fourier transform spectrometer. Processing and analysis of spectra were carried out by means of software built-in to the instrument.

The X-ray diffraction phase and structural investigations were carried out using a DRON-2.0 diffractometer according to the Bragg–Brentano θ – 2θ scheme. To eliminate K_β –reflexions a manganese filter was used. The point-wise measurements were fulfilled with step 0.1° and 10 s exposure at each point in the angular range $2\theta = 15$ – 120° .

3. RESULTS AND DISCUSSION

The oxides of some metals (Ti, Nb, Ta, etc.) applied to the production of implants have electret properties and are able to accumulate and hold electric charge in their volume for a long time. This can be extremely useful as it allows the natural distribution of electric potentials on bone tissue surfaces, which can be disturbed by the introduction of an implant [15], to be restored. The distribution of natural potentials is defined by the piezoelectric properties of bone tissue. Restoration of the normal surface electric potential in the field of an implant allows activation of relaxation processes of the damaged bone tissue by transport in this zone of calcium phosphate components, with the subsequent formation of hydroxyapatite crystals and, therefore, stimulation of a more complete integration of the implant into living tissue than otherwise.

Determination of the sign of the surface charge that promotes the accumulation of calcium phosphate from solution onto an electrode, modelling blood plasma, was the initial task. For this purpose, two stainless steel electrodes were placed in the electrolytic cell filled with the prepared solution, and a voltage $U = 1$ V from an external source was applied. After one week of incubation at room temperature the solution was removed and the electrodes were dried. The end result of this experiment is shown in Fig. 1.

A white coating was formed on the negative electrode but not on the positive electrode, on which only traces of electrochemical corrosion of the metal were visible.

IR-spectra were taken and compared with the corresponding spectrum of native bone tissue (Fig. 2).

On both spectra there are bands at 3568.03 cm^{-1} , 3434.64 cm^{-1} , 3409.37 cm^{-1} and 3244.06 cm^{-1} corresponding



Figure 1. Photograph of the electrolytic cell at the end of the calcium phosphate sedimentation test (in colour online).

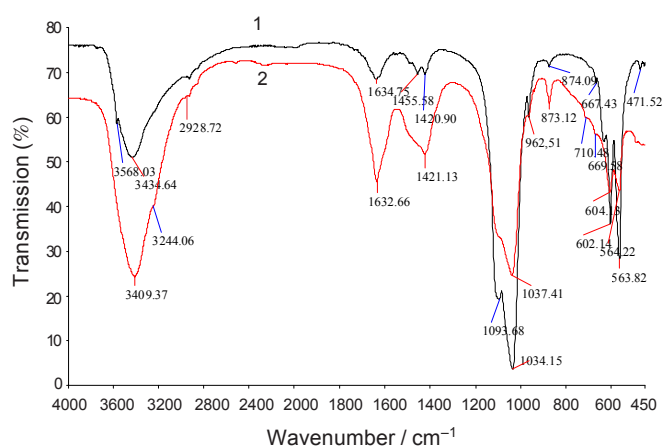


Figure 2. IR spectra of (1) native bone tissue and (2) calcium phosphate sediment from the solution imitating blood plasma.

to stretching vibrations of hydroxyl groups OH^- ; a band at 2928.72 cm^{-1} corresponding to CH_2^- groups; bands at 1634.75 cm^{-1} and 1632.66 cm^{-1} corresponding to the deformation vibrations of H_2O ; bands at 1455.58 cm^{-1} , 1421.13 cm^{-1} and 873.12 cm^{-1} corresponding to carbonate groups CO_3^{2-} ; and bands at 1093.68 cm^{-1} , 1037.41 cm^{-1} , 1034.15 cm^{-1} , 669.58 cm^{-1} , 602.14 cm^{-1} and 563.82 cm^{-1} corresponding to phosphate groups PO_4^{3-} . The main IR absorption bands of bone tissue coincide with those of calcium phosphate sediments on the sample surface. This indicates that the coating composition formed from the prepared solution is similar to the composition of native bone tissue, of which the main part is hydroxyapatite. Nevertheless, the intensity of the CO_3^{2-} bands is more in the case of calcium phosphate sediments. At the same time the signal from PO_4^{3-} is a little understated; also a higher background in the range 950 – 670 cm^{-1} is observed. These indicate a violation of the composition stoichiometry of hydroxyapatite crystals in the sediment and the presence of other calcium phosphate compounds—impurities—in comparison with the composition of native bone.

The X-ray diffraction pattern of the crystal sediments formed on the metal implant surfaces is presented in Fig. 3. Essentially all the lines on the X-ray diffractogram correspond to the hydroxyapatite lattice. Nevertheless, ascribing more weight to the indistinct bands of the IR spectrum corresponding to the calcium phosphate sediments and the intensive lines of carbonate radicals in comparison with the spectrum of native bone tissue, the apparent abundance of single-phase crystals is inconclusive. It is more probable that the sediments include the dominant hydroxyapatite phase and other calcium phosphate compounds in a highly dispersed or amorphous state.

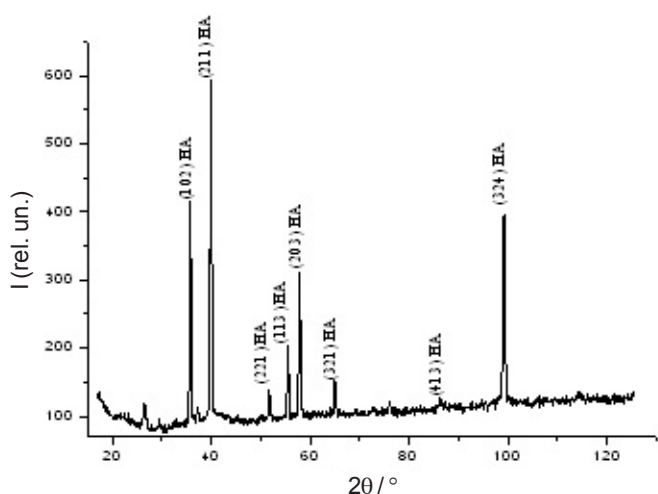


Figure 3. X-ray diffraction pattern of the crystal sediments formed on the metal implant surfaces.

Surface areas of sediments were measured using the optical microscope equipped with a special measuring grid in an eyepiece for surface area assessment. Images of samples with different oxide thicknesses after exposure in the simulation solution are shown in Fig. 4; the ratio of calcium phosphate sediment area S_s to implant plate area S_o , depending on the oxide thickness, is presented in Fig. 5. From the figure it is seen that as the oxide thickness grows, the amount of calcium phosphate sediment increases. Such results can be explained as follows. The oxide phase, having a disordered structure, is capable to trap electrons and, thus, accumulate negative charge in the bulk. This charge, in turn, influences the solution and promotes movement of calcium and phosphorus ions to the implant surface; on this base calcium phosphate is formed and, subsequently, the hydroxyapatite crystals. As the oxide thickness grows, the enlarged total volume of negative charge increases the rate of formation and the amount of sediment on the implant surface.

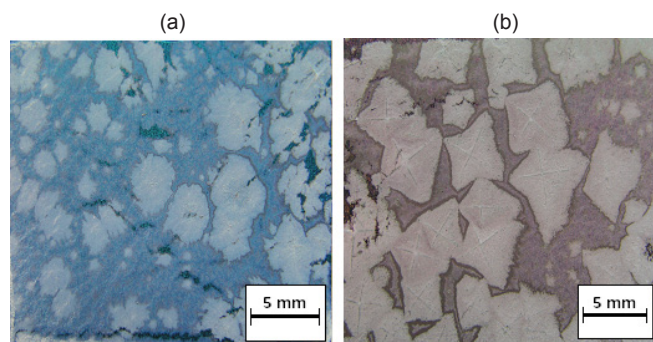


Figure 4. Micrographs of samples with thicknesses (a) 190 nm and (b) 290 nm after exposure to the solution imitating blood plasma.

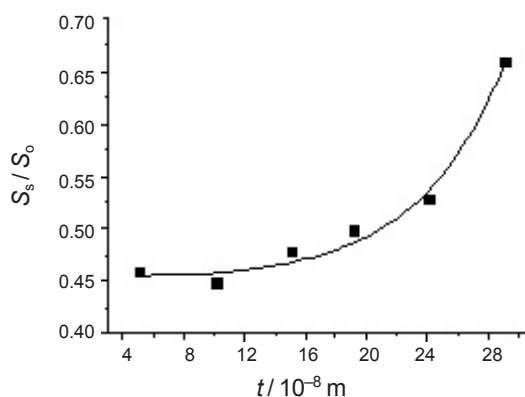


Figure 5. Dependence of the ratio of calcium phosphate sediment area S_s to implant plate area S_o on oxide thickness on titanium.

4. CONCLUSIONS

In order to achieve the formation of a protective coating on an implant surface it is necessary to select materials not only from the point of view of their chemical and electrochemical inertness; an important criterion is also the presence of active properties of such materials; for example, the ability to form and maintain in their volume negative electrical charge. Such charge activates the environment surrounding the implant and accelerates the formation of hydroxyapatite crystals, which are the basis for creating new bone tissue. This should diminish the time to restore damaged tissue and optimize the integration of the implant.

REFERENCES

- Schider, S. & Bildstein, H. Tantalum and niobium as potential prosthetic materials. In: *Advances in Biomaterials* (eds G.D. Winter, D.F. Gibbons and H. Plenk, Jr.), pp. 13–20. New York: Wiley (1980).
- Liu, X., Huang, A., Ding, C. & Chua, P.K. Bioactivity and cytocompatibility of zirconia (ZrO_2) films fabricated by cathodic arc deposition. *Biomaterials* **27** (2006) 3904–3911.
- Olmedo, D., Guglielmotti, M.B. & Cabrini, R.L. An experimental study of the dissemination of titanium and

- zirconium in the body. *J. Mater. Sci.: Mater. Med.* **13** (2002) 793–796.
4. Ungersboeck, A., Geret, V., Pohler, O. et al. Tissue reaction to bone plates made of pure titanium: a prospective, quantitative clinical study. *J. Mater. Sci.* **6** (1995) 223–229.
 5. Larsson, Cc., Thomsen, P., Lausmaa, J., Ridahl, M., Kasemo, B. & Ericson L.E. Bone response to surface modified titanium implants: studies on electropolished implants with different oxide thicknesses and morphology. *Biomaterials* **15** (1994) 106–274.
 6. Singh, A.V., Patil, R., Thombre, D.K. & Gade, W.N. Micro-nanopatterning as tool to study the role of physicochemical properties on cell-surface interactions. *J. Biomed. Mater. Res.* **101** (2013) 3019–3032.
 7. Lowe, T.C. & Reiss, R.A. Understanding the biological responses of nanostructured metals and surfaces. *IOP Conf. Series.* **63** (2014) 012172–5.
 8. Dub, S.N. & Starikov, V.V. Elasticity module and hardness of niobium and tantalum anode oxide films. *Functional Mater.* **14** (2007) 347–350.
 9. Starikov, V.V., Starikova, S.L., Mamalis, A.G., Lavrynenko, S.N. & Ramsden, J.J. The application of niobium and tantalum oxides for implant surface passivation. *J. Biol. Phys. Chem.* **7** (2007) 141–145.
 10. Galante, J.O., Lemons, J., Spector, M., Wilson, P.D. & Wright, T.M. The biological effect of implant materials. *J. Orthopaedic Res.* (1991) 760–775.
 11. Pauporte, T., Finne, J., Kahn-Harari, A. & Lincot, D. Growth by plasma electrolysis of zirconium oxide films in the micrometer range. *Surf. Coatings Technol.* **199** (2005) 213–219.
 12. Montero, I., Albella, J.M., Ortega, C. & Siejka, J. Space charge and electret behaviour in anodic Ta₂O₅ films. *Thin Solid Films* **167** (1988) 95–100.
 13. Maissel, L.I. & Glang, R. *Handbook of Thin Film Technology*. New York: McGraw Hill (1970).
 14. Kutsevlyak, V.I., Starikov, V.V., Starikova, S.L., Mamalis, A.G., Lavrynenko, S.N. & Ramsden, J.J. Influence of implant surface modification on integration with bone tissue. *J. Biol. Phys. Chem.* **8** (2008) 147–150.
 15. Tkatchenko, S.S. & Rutsikij, V.V. *Electrostimulation of Osteoreparation*. Leningrad: Meditsina (1989) (in Russian).
 16. Miyazaki, T., Kim, H.-M., Kokubo, T., Miyaji, F., Kato, H. & Nakamura, T. Effect of thermal treatment on apatite-forming ability of NaOH-treated tantalum metal. *J. Mater. Sci.: Mater. Med.* **12** (2001) 683–687.