

# The Particles Precipitation and Osseointegration of a TiO<sub>2</sub> Thin-Film Coating by Ion Beam Deposition - An *In vivo* Study

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## Introduction

Titanium-based materials are the most common materials implanted into the human body. Titanium and its alloys are widely applied as dental implants, bone fracture-fixation and artificial joints due to their good mechanical properties, favorable biocompatibility and excellent corrosion resistance [1,2]. The surface properties of Ti implants influence biological responses at the interface between bone tissue and implants and, consequently, their osseointegration [3,4]. Osseointegration is closely related to early interactions between the implant surface and its surrounding bone tissue. However, a surface modification of Ti is still required since spontaneously grown native TiO<sub>2</sub> has no strong bioactivity to osseointegrate with bone [5]. Histological evaluation has shown that significantly more bone formation is found on nanoparticle-coated implants compared to non-treated titanium implants [6]. However, long-term maintenance of osseointegration and stability of titanium-based implants is still a problem [7]. Since the efficacy of osseointegration is influenced by factors including the surface condition of titanium implant, varied surface modifications have been performed to improve the osseointegration, such as inclusion of natural organic compounds, chemical treatments and controlled formation of TiO<sub>2</sub> [8]. Beneficial modifications of titanium surfaces have been established, for example chemical etching of the surface in alkali solution or anodization at breakdown voltages (spark processing) [9-11]. For simple surface treatment of titanium, formation of TiO<sub>2</sub> nanostructure such as nanotube or with titanium oxide nanoparticle was studied [12]. TiO<sub>2</sub> coatings with various deposition techniques, including electrophoretic deposition, radiofrequency sputtering, and pulsed laser deposition are investigated and developed [13,14]. But precise control over thickness, chemical composition, and stoichiometry during the growth of biocompatible nano coverings remains an issue. Additionally, various complications involving TiO<sub>2</sub> coatings have been reported by dentists. Specifically, fragility of the thin-film and poor bonding strength between the film and substrate material are serious problems that can result in cracking of the coated film and the film-substrate interface during dental implant operation [15].

Ion beam deposition techniques are widely used in different thin-film applications for the deposition of oxides, include TiO<sub>2</sub> [16]. Since the mid-1970s, many surface modification techniques based on ion bombardment – such ion beam assisted deposition (IBAD), ion beam assisted deposition, and ion mixing – have been developed and are widely used to modify the surface of materials such as metals, polymers, ceramics, and biocompatible materials. Ion beam assisted deposition (IBAD) is a vacuum method that combines physical vapor deposition (PVD) with ion beam bombardment. The major feature of IBAD is bombardment with a certain energy ion beam during deposition of the coating. There are many parameters that affect the composition, structure, mechanical and chemical properties of the as-deposited coating in the IBAD process, among which ion bombardment is the key factor. The major processing parameters are coating materials, evaporation rate or sputtering rate, ion species,

ion energy, and ion beam current density. One of the most attractive characteristics of IBAD is that it is able to prepare biocompatible coatings with much higher adhesive strength to the substrate compared to a traditional coating method. This is assumed to be a consequence of interaction between the coating and substrate atoms that is aided by ion bombardment, which results in an atomic intermixed zone in the coating-substrate interface. It also possesses the advantage of low substrate temperature and high reliability and reproducibility, without adversely affecting the bulk attributes. Another attractive feature of the IBAD process is its superior control over coating microstructure and chemical composition [17-21].

In the present study, we investigated how nanostructured thin-film coatings of titanium dioxide affect the destruction of the implants and the integration potential of the TiO<sub>2</sub>-coated implant surface. TiO<sub>2</sub> thin-films prepared by IBAD on embedded implant samples were studied in the tissues of laboratory animals.

## Materials and Methods

### Sample preparation

Titanium dioxide films were deposited on a commercially available pure titanium substrate, a nylon substrate, a PTFE substrate, and a monocrystalline silicon substrate by IBAD under argon gas flow. The ion beam deposition system has been described previously [22]. It mainly consisted of one Kaufman ion source and one Hall ion source, one target holder, and one rotatable planet-type sample holder in the path of Ar ion source and deposited material. The deposition chamber pressure just before sputtering began was about 10<sup>-6</sup> Pa. The monocrystalline silicon substrate was used for RBS measurement. Ti samples were mechanically polished and ultrasonically cleaned with acetone and alcohol. Before deposition, all substrates were etched by 300 eV Ar ions in the deposition chamber to remove any contamination layers. Films were prepared on soft (nylon, PTFE) and solid (Ti, monocrystalline silicon) substrates by sputter-deposition of a commercially available (“Ligamet”, Russia) Ti target using an Ar ion beam of 1000 eV and 20 mA cm<sup>-2</sup> in a low pressure oxygen environment. Deposition time of TiO<sub>2</sub> was adjusted so that the thickness of deposited films was about 100 nm. The working pressure of the deposition chamber was 7 × 10<sup>-2</sup>

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Pa. All substrates for *in vivo* investigations were made in cylinders of diameter  $d = 1$  mm and 7 mm for Ti and  $d = 0.7$  mm and  $l = 7$  mm for PTFE and nylon.

For the AES in-depth analysis of TiO<sub>2</sub> on non-conductive samples, a silver (Ag) thin-film served as a conductive indicator layer and was synthesized by IBD using an Ar<sup>+</sup> beam of 1000 eV and 20 mA cm<sup>-2</sup> for 30 sec.

### Characterization of the TiO<sub>2</sub> films

Characterization of the thin-films was carried out by Rutherford backscattering spectrometry (RBS), scanning electron microscopy (SEM), and Auger electron spectroscopy (AES). The composition and thickness of prepared films were determined by RBS. The 1.5 MeV He<sup>+</sup> ions, accelerated by a linear Van de Graaff particle accelerator, were incident perpendicularly to the surface of the specimens and at the angle of  $\theta = 160^\circ$ . The topography of the titanium samples with prepared surfaces used in this study was characterized using a scanning electron microscope (JSPM, Japan). Images were collected using an acceleration potential of 30 kV. The in-depth profiles of the TiO<sub>2</sub> films were determined by AES (JSPM, Japan), with the acceleration voltage of AES fixed to 15 kV.

### Implants and treatments

For *in vitro* examination of TiO<sub>2</sub> integration effects, Wistar rats were used. The implantation was under general anesthesia by an injection of "Zoletil 100" (VetPharm) and a tropine with intramuscular administration. A skin cut and subcutaneous adipose tissue on the medial surface of the left shin 1.5 cm long was prepared. In the study of titanium particles precipitation processes, titanium samples with/without TiO<sub>2</sub> were implanted directly into the fascial sheath of shin muscles. In the study of osseointegration processes in PTFE/nylon implants with TiO<sub>2</sub> thin-film coating, the wound edges were fixed, the muscle layer and the periosteum were cut longitudinally, and the surface of the medial tibial diaphysis (part of the metaphysis) was dissected. The cancellous tissue bone groove was performed by drill with a spherical bur (7 mm long, 0.7 mm wide and 0.7 mm deep). The sterile samples were implanted in the resulting bone bed 6 mm long and 0.7 mm in diameter. The wound was sutured with noose stitches layer by layer, covering the implant completely. The skin was wound sutured with interrupted stitches catgut 4/0. Hemostasis was maintained during the operation. Throughout the experimental period the animals consume foodstuffs according to the norms of animal feeding.

**Ethics statement:** Surgery operation on rats was carried out in strict accordance with the directive of the Ministry of Health and Social Development of the Russian Federation #708n "On approval of the laboratory practices, "dated 23 August 2010. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Immanuel Kant Baltic Federal University (Order Number: 347-12).

### Particles precipitation analysis

The animals were withdrawn from the experience within 60 days. During autopsy, the tibiae were extracted from the area of implantation. After the experiments, a study of the non-specific toxicity of titanium implants was carried out that involved an integrated analysis of the tissues and organs of the operated animals. Atomic-adsorption spectroscopy analysis of the accumulation of metal in the liver, kidneys, spleen, skeletal muscles and small intestine (groups 1 and 2) was performed. The Ti samples with/without TiO<sub>2</sub> thin-film were used for atomic absorption spectral analysis.

### Histological analysis

Histological processing was carried out to evaluate the quality of osseointegration at the microscopic level. Generally accepted histological criteria were used in the morphological study of groups 3-4 (Table 1): quantitative and qualitative assessment of the characteristics of cellular elements and histological manifestation of the repair process of the implant (the reaction to the implant, the severity of the inflammatory response to the foreign tissue, the predominant type of tissue in the area of the implant). The bone segments with implants and the surrounding tissues were isolated using a circular saw. Bone samples prepared for histology were labeled with identification numbers referencing the surface area adjacent to the implant. Tissue samples were fixed in 10 % buffered formalin, and decalcification was performed in 10 % nitric acid. The degree of decalcification was evaluated by a wire dot test. Then standard histological techniques were applied to increase the concentration of alcohol and paraffin embedding. Serial sections 7 microns thick were prepared on a rotary microtome (Microm HM 325). The corresponding entries were then straightened on a slide and stained with hematoxylin and eosin. Identification was carried out in a light microscope with a 40-fold magnification. Histological study and microphotography were produced in the Axioplan 2 optical imaging system (Carl Zeiss, Germany) with a Canon 10C analog camera. The nylon and PTFE samples with TiO<sub>2</sub> thin-film coatings were used for histological analysis.

### Results and Discussion

#### Composition and thickness of the deposited TiO<sub>2</sub> thin-films

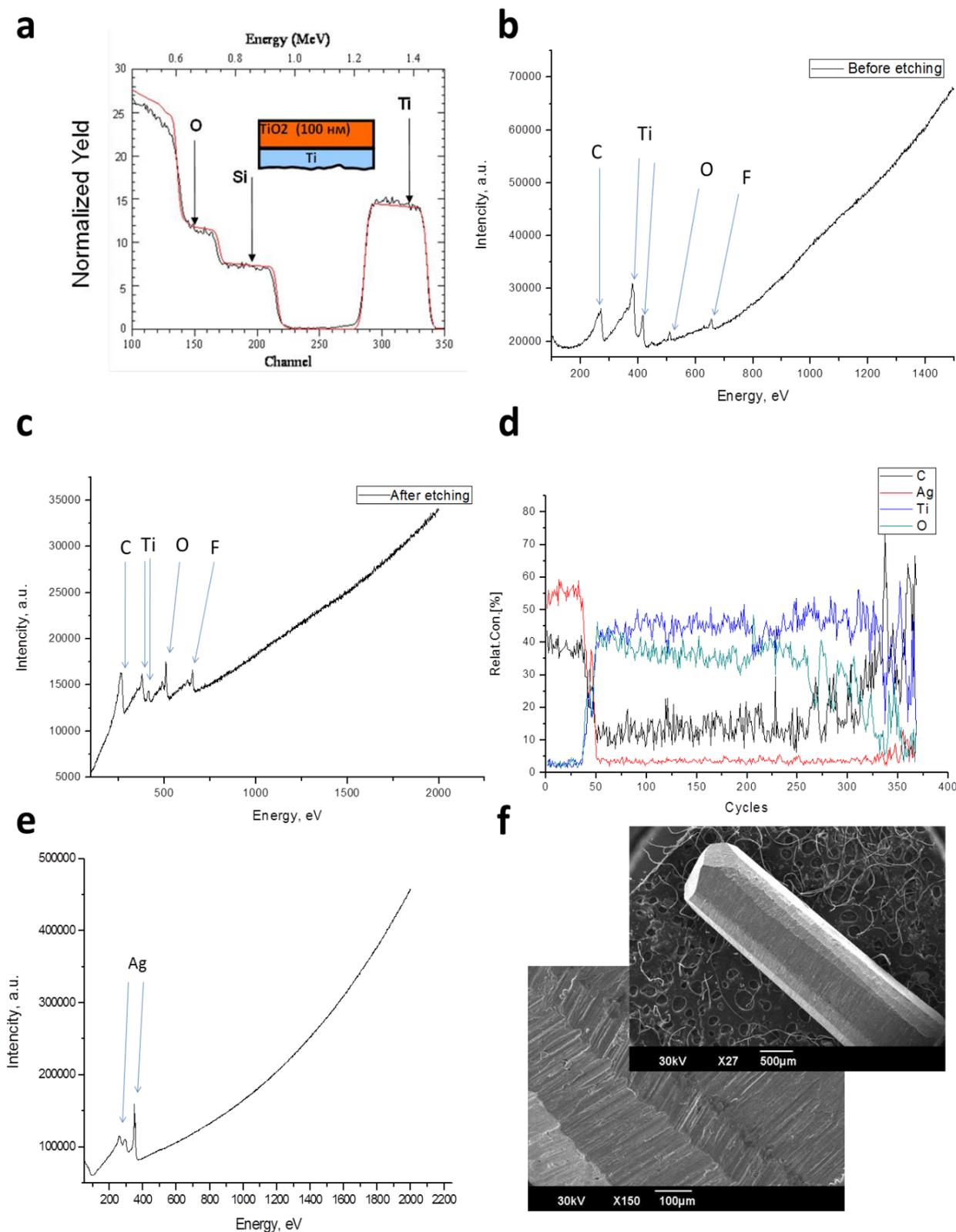
A typical RBS spectrum of a deposited film on monocrystalline silicon shown in Figure 1a. In order to separate Ti and O peaks and facilitate the analysis process, 1.5 MeV energy were applied. From the integrated intensities and widths of peaks, the quantity and thickness of the film were determined. A simulation of the RBS spectrum shown in the figure using a red line. The simulation fitting curve coincides with the experimental one at the layer thicknesses of 100 nm for TiO<sub>2</sub>, whereas stoichiometry rates are O – 60%, Ar – 8%, and Ti – 32%. A small argon impurity appeared in the film as a result of the Ar plasma used as anion beam.

#### Chemical properties of the deposited TiO<sub>2</sub> thin-films on PTFE and nylon implants

Figure 1b and 1c is showing the AES wide scan spectra of deposited TiO<sub>2</sub> on PTFE before ion etching and after ion etching, respectively. After ion etching at ~500 eV for 30 sec the intensities of Ti and O peaks are lower at the surface because of contamination by fluorine and carbon. This could be caused by a mixing layer formed between the film and substrate during the functional coating growth process. The AES in-depth profile of TiO<sub>2</sub> on nylon is presented in Figure 1d. After indicator layer (Ag) etches on TiO<sub>2</sub> the coating shows a decrease in Ag relative concentration after 30 cycles and an increase in TiO<sub>2</sub> relative concentration after 50 cycles. The contamination in TiO<sub>2</sub> thin-film is not observed, and the TiO<sub>2</sub>-nylon interface is smooth and sharp. We

Group 1 (n=20)	Titanium implant.
Group 2 (n=20)	Titanium implant with TiO <sub>2</sub> thin-film coating
Group 3 (n=5)	PTFE implant with TiO <sub>2</sub> thin-film coating
Group 4 (n=5)	Nylon implant with TiO <sub>2</sub> thin-film coating
Group 5 (n=5)	Control group without implants

Table 1: Groups and number of experimental animals.



**Figure 1:** a) The RBS spectrum of  $\text{TiO}_2$  deposited on monocrystalline silicon. b) AES wide scan spectra of  $\text{TiO}_2$  thin film surface on PTFE substrate. c) AES wide scan spectra of  $\text{TiO}_2$  thin film surface on PTFE substrate after ion etch. d) AES in-depth profiling of  $\text{TiO}_2$  thin film surface on nylon substrate. Observed thin film without any impurities e) AES wide scan spectra of Ag thin film indicator layer on nylon implant model coated by  $\text{TiO}_2$  thin film. f) SEM image of titanium implant for particles precipitation analysis.

suspect that contamination fluorine and carbon in TiO<sub>2</sub> thin-film on PTFE substrate are caused by weak molecular bond in PTFE. Nylon substrates are thus more suitable for the IBD thin-film synthesis method. However, a more detailed investigation is necessary to clarify the contamination source and use IBD method for creation thin-films on soft materials.

### Topography analysis

The topography of the random titanium sample for *in vivo* study compared in this work was investigated by scanning electron microscopy and is presented in Figure 1f. The titanium sample surface was treated with a bur using green electro corundum grit, with grain size of around 70 μm. After treatment some of Ti samples were coated with approximately 100 nm of TiO<sub>2</sub> thin-film via the IBAD method. The total Ti sample area for particles precipitation analysis was around ~25 mm<sup>2</sup>.

### Atomic absorption spectral analysis

Atomic absorption spectral analysis of tissue after total implementation and long-term (6-month) presence of the sample in the tissues is a highly informative method of evaluating of the results, and provides a means to determine the accumulation of trace elements in the tissues of the body, caused by electrolytic corrosion from wear and tear of the implant. The experiment compared the amount of atomic titanium in the tissues of laboratory animals, according to the type of implanted sample: uncoated titanium and titanium with TiO<sub>2</sub> prepared by IBD. The titanium content in five different tissues of rats was determined by atomic absorption spectroscopy. Organs of the reticuloendothelial system (liver, kidney, spleen), skeletal muscle, and small intestine (Table 2) were selected as the tissues for research. The first reaction that occurs between the implant and the surrounding tissues depends on the tissue fluid. It forms a layer of organic molecules and water, affecting the behavior of the cells as soon as they come to the surface. Then there is a series of interactions between the cells and the surface, which leads to the release of chemotactic factors and growth factors, and modulates the activity of the cells in the surrounding tissues. The use of atomic absorption spectroscopy allows us to predict the component resistance to wear. The spleen is the organ most capable to accumulate titanium [23]. According to the results it is clear that the content of titanium in spleen tissue is approximately 1.85 times greater for implanted samples without the TiO<sub>2</sub> coating than for those with it. However, it must be noted that the titanium content does not exceed the tissues' natural titanium content in all cases (0.02 mg per 1 kg of body weight).

### Histological study results

During the experiments on rats, we investigated the PTFE and nylon samples integration potential with TiO<sub>2</sub> thin-film prepared by IBD and implanted into the tibia. The main problem in the study of osseointegration is a complete impossibility to study microstructure of the interface between the implant and the bone. Therefore, the most up-to-date methods involve determination by histological techniques. However, histomorphological data of osseointegration from research conducted *in vivo* are not numerous. For example, studies by J. Lemons [23] were conducted on the material of dental implants, which were removed from the patients together with the adjacent bone tissue. However, the methodology applied in the study of histological processing (20-50 micron non-decalcified thin sections) prohibited study of the thin microscopic structure at the contact surfaces.

A second group of researchers conducted histological experiments *in vivo* using a hybrid implant model based on PTFE [24]. This new experimental model opened the possibility of depth study of the implant with the surrounding tissue structures.

According to the literature, osseointegration is recognized as the optimal form of implant integration into the bone – that is, the form of the implant contact with the bone lacking connective tissue [14]. On the 60<sup>th</sup> day of our study, we noted in group 3 the formation of direct intimate contact of the new bone tissue without an intermediate layer of connective tissue. Such contact is regarded as a morphological manifestation of Osseointegration (Figure 2a and 2b). In the field of implantation, intensification of the osteogenesis process was marked (Figure 2c). New formed trabecular bone structure surrounded the implant from all sides (Figure 2c). There was a rigid connection of the bone and the implant. This is also true for group 4, for which we observed the interface between bone and muscle (Figure 2e and 2f).

In addition to the models of hybrid implants based on PTFE, we took samples with the basis of nylon. This framework is easier to use due to the fact that, first, the TiO<sub>2</sub> thin-films lack additional impurities, and second, the material does not decay into the fibers when being histologically processed. A morphological manifestation of osseointegration in group 4 is presented on (Figure 2d).

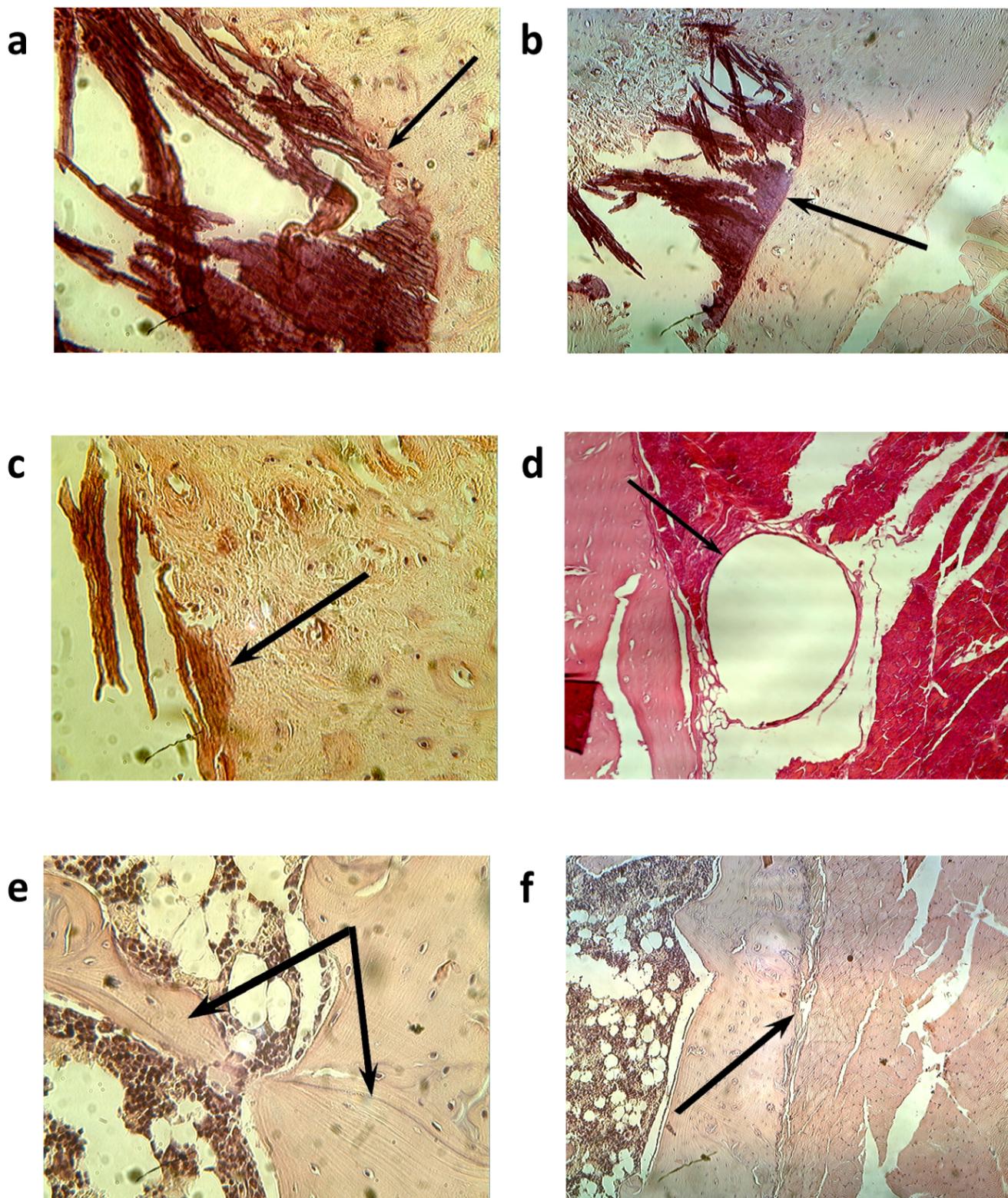
It should be noted that the pronounced pathological changes in the bone tissue contiguous to the implants, and – above all – its rarefaction in large areas, are not observed. The friable cell fibrous capsule with dense focal lymphoid macrophagal infiltration to the surface of implant is not adjacent, and multinucleated giant cells of foreign bodies are not detected (Figure 2c-2e). The area of defect is preserved to the structure typical of the organ. A neutrophil-exudative component was not found during the study period.

### Conclusions

The result of this study shows that the TiO<sub>2</sub> thin-film prepared by IBD can be used as implant biocompatible coatings. The high quality and functionality of TiO<sub>2</sub> thin-film prepared by IBD contribute much to its extensive use as a coating for different implants. The benefit of using implants with this coating is the presence of thin-film structure which prevents erosion of titanium particles by 1.85 times, and this reduces the negative impact of the foreign object implanted into the patient's body. Acceleration of osseointegration can be achieved by means of the snug fit of the coating to the implant surface and the absence of foci of destruction in the coating film; that is, acceleration of processes is due to the indissoluble contact between the tissue and the implant. This demonstrates a big promising application of IBD method

Group	Test sample №	Ti, mg/kg	
1. Implant without coating	1.1	The liver	0.39 ± 0.08
	1.2	The kidneys	0.35 ± 0.07
	1.3	The spleen	4.94 ± 0.99
	1.4	The small intestine	0.73 ± 0.15
	1.5	The skeletal muscle	0.19 ± 0.04
2. Implant with TiO <sub>2</sub> coating	2.1	The liver	0.25±0.05
	2.2	The kidneys	0.67 ± 0.13
	2.3	The spleen	2.67 ± 0.53
	2.4	The small intestine	0.56 ± 0.11
	2.5	The skeletal muscle	0.57 ± 0.11

Table 2: Ti concentration in the organs of experimental animals.



**Figure 2:** a,b) The new formed bone tissue without the elements of connective tissue and lymphohistiocytic infiltration adjoins the implant surface directly (arrow). c) Directly to the implant surface (arrow) adjoins the newly formed bone tissue without the elements of connective tissue and lymphohistiocytic infiltration. d) Histology of nylon sample e) Structure of intact bone. Snug fit of the muscle and bone (arrow). f) Structure of intact bone. Trabecular bone (arrow).

for TiO<sub>2</sub> thin-film formation as a barrier-inductive material used in combination with different types of implants.

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