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INVESTIGATION OF BIO-FUNCTIONAL PROPERTIES OF TITANIUM SUBSTRATES AFTER HYBRID OXIDATION

In order to enhance bioactive properties of titanium 99.2 used in implantology and various biomedical applications, numerous methods to form tight oxide coatings are being investigated. Some of these interesting techniques for generating TiO_2 coatings include: electrochemical methods with anodizing, electric discharge treatment, plasma methods (PVD) and diffusive methods (i.e. oxidation in a fluidized bed). Each method aims to create a thin homogenous oxide coating characterized with thermal stability and repassivation ability in the presence of body fluid environment. However, new methods are still sought for increasing the biocompatibility of the substrate following a change in the intensity of depositing on the oxide coating compounds with high biocompatibility with body tissues, including hydroxyapatite, which constitutes the basis for subsequent osseointegration processes. The article presents investigation of HAp formation on titanium substrate surface after hybrid oxidation process. Hybrid surface treatments combine methods of fluidized bed atmospheric diffusive treatment FADT with the PVD surface treatment realized with different parameters (FADT – 640°C / 8h and PVD – magnetron sputtering with TiO_2 target). In order to investigate the effects of hybrid oxidation and the formation of HAp molecules, SEM-EDS, SEM-EBSD, STEM-EDS, RS, nanoindentation and Kokubo bioactivity tests (c-SBF2) were carried out. The hybrid method of titanium oxidation, proposed by the Author, presents a new outlook on the modification and development of the properties of oxide coatings in the area of biomedical applications. Combining the ways of Ti Grade 2 oxidation in the hybrid method highly improves the formation of hydroxyapatite compounds and shows the potential of applying such a technique in implantology, where the intensive growth of bone tissues is crucial.

Keywords: Titanium Grade 2, hybrid oxidation, bioactivity, hydroxyapatite

1. Introduction

Issues concerning the improvement in the bio- and chemical activity of metallic materials used in implantology and biomedicine, mainly for the regeneration and replacement of bone tissue are of great importance due to the elimination of toxic interactions between the bone tissue of living organisms and physiological fluids during operations of implants. Surfaces, characterized by the highly controlled and specific micro- and nanotopography, also possess a huge potential in the field of construction of biomaterials for the osseintegration processes. Increasing titanium materials' bioactivity has always constituted a challenge for biomedical and surface engineering. Titanium oxidation for biomedical applications is still a challenge for biomedical engineering in obtaining both good mechanical and physicochemical properties of thin oxide films as well as the required good adhesion to titanium substrates. In order to improve titanium properties for biomedical applications, numerous methods of forming oxide coatings are used, including, inter alia: electrochemical methods (anodizing), physical vapour deposition (PVD), laser methods (laser ablation), sol-gel, etc. Oxide coatings obtained with the use of the methods mentioned, however, have small thickness, and in case of coatings formed by the electrochemical method, they do not always have good adhesion, which depends on many factors, including the preparation of the titanium surface for the processes [1,2]. These methods, due to the conditions of physical and chemical interaction between the atmosphere and the surface, heat transfer and surface defect, have very limited influence on oxygen diffusion processes towards the surface layer. Thus, it is difficult to obtain the following correct arrangement: Ti_α substrate / $Ti_\alpha(O)$ solid solution / TiO_2 oxide layer. The analysis of titanium used in biomedicine shows that such favorable properties are typical for substrates obtained by diffusion methods including oxidizing in a fluidized bed (Fluid Atmospheric Diffusive Treatment FADT) [3]. The phenomenon of oxygen diffusion to the titanium substrate occurs by means of

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an interstitial mechanism with diffusion pathways resulting from the fluidized bed grain material continuously causing surface defects. Consequently, this enhances the change in the oxygen concentration profile, with the formation of the diffusion zone of oxygen solid solution in $Ti_{\alpha}(O)$ and a porous TiO_2 (rutile) oxide layer of a favorable substrate / layer stress state. This method of titanium oxidation is used as one of the ways of surface protection and improves mechanical properties of titanium. However, methods to improve the substrate's surface bioactivity are still being sought, in order to increase the activity of compounds having properties that enhance tissue regeneration, for example hydroxyapatite [4]. Therefore, the research carried out by the Author aims to develop a titanium substrate, whose surface zone is a solid solution of oxygen in $Ti_{\alpha}(O)$ saturated by means of diffusion, and the outer TiO_2 oxide coating is produced by surface processes i.e. PVD magnetron sputtering. The application of such hybrid methods makes it possible to use the advantages of continuous mechanical surface activation effect (as a result of the impact of an aeromechanical factor) in the fluidized bed (FADT), which occurs due to the recurrent defects of the substrate surface with simultaneous non-equilibrium oxidation process (PVD) mentioned [5]. There is expectation that such a combination of layers will ensure a synergistic increase in essential material functional properties, crucial from the point of view of the bioactivity of the implant – tissue system. The properties mentioned are mainly: the reduction in the stress gradient between the diffusion zone and the tight and homogeneous hybrid oxide layer, with a simultaneous morphology improvement and favorable phase composition of the surface [6,7]. Nevertheless, the mechanism of titanium oxidation differs from the oxidation of metals. This is due to the stability of the oxides: Ti_{α} – stable up to 882°C and Ti_{β} – stable over 882°C. Literature data shows that oxidation, its kinetics and mechanism depend mainly on the time and process type. A passive oxide nanolayer is formed at room temperature on the Ti surface 5-15 nm. However, the oxidation of titanium at an increased temperature (>400°C), leads to the formation of the crystalline oxide layer with zone structure which contains TiO /inter Ti_2O_3 layer / TiO_2 layer (rutile or anatase) directly interacting with the surrounding environments [8,9]. It was also found that the transport of oxygen in the layers depends highly on a defect level of the substrate structure. The density of defects and their type is connected with predominant inter-diffusion of oxygen in anionic vacancies. The predominant factor at 550°C during the oxidation process is the oxygen diffusion into the substrate, which increases its concentration at a greater distance from the surface, which in this case makes the oxygen concentration profiles smooth, homogeneous and stress stable. In turn, at high temperature, despite the fact that titanium oxidizes rapidly, thicker oxide layers are formed and it is worth mentioning that they are often poorly bonded (anchored) to the substrate, porous and thus they tend to delaminate and crack [10,11]. Such a substrate, with porous areas as mentioned before, also has limited applications in terms of improving the biocompatibility due to the insufficient adhesion of e.g. hydroxyapatite compounds. Therefore, the Author investigates the formation of the oxide coating to ensure its good adhesion with a simultaneous reduction of the stresses between the coating and the surface layer to be the fundamental substrate for subsequent hybrid processes. Another important role of the oxide layer on the surface of titanium in addition to the aforementioned properties is to improve osseointegration process rate [12,13]. For this reason, the Author will present an analysis that takes into account the phase and surface changes taking place in the FADT + PVD hybrid technology. The research includes the phase composition and concerns interactions between the components of the layer: rutile and anatase. The relationship between those oxides is highly important in the context of bioactive properties and their influence on the surface chemistry by lattice matching between rutile / anatase phase to apatite [14,15]. Research results show that the combination of oxide layers in the hybrid system allows for the synergetic functionalization of the surface, affecting the intensity of subsequent hydroxyapatite compounds' formation after 14 days of the Kokubo test, which is indicated by the Author as the main result of the investigation [16]. The adequate selection of hybrid oxidation methods parameters will make it possible to use the advantages of spontaneous mechanical surface activation in the fluidized bed FADT and rapid non-equilibrium PVD processes (hybrid process) to create smooth and bioactive surface which significantly affects the osseintegration.

2. Materials and methods

The specimens used to deposit the hybrid oxide layer were made of commercial pure titanium Grade 2 for biomedical applications (KOBE Steel Ltd – KS60, ASTM B265 Grade 2). Titanium had a α single-phase structure and was characterized by a banded structure after rolling. Substrates in the form of cylindrical samples of 5 mm-length, cut out from a 10 m diameter rod were mechanically activated (sandblasted) with a mixture of Al_2O_3+ZrO_2+Ti) [17]. Diffusive oxidation (FADT) process of the titanium substrate was performed in a fluidized bed reactor with Al_2O_3 as grain material at 640°C for 8hours. The air (flow 600 l/h) was the fluidizing factor in which oxygen was also a carrier of diffusion atoms. After the process, the samples were cooled down in the air. Subsequent PVD sputtering experiments were performed by reactive direct current (DC) magnetron sputtering with the use of a TiO_2 target at a pressure of 3×10^{-2} mbar, in an Argon (purity 99.995%) atmosphere. The deposition was carried out in constant power mode P = 350 W. Prior to the deposition, the target was sputter cleaned in an Argon plasma at a pressure of 0.5 Pa during 8 min. No heating was applied to the substrate holder. The target – substrate distance was 60 mm. The deposition time was 15-20 min. The effects of hybrid oxidation on titanium substrate microstructure were evaluated using SEM method (JEOL JSM-6700F). Oxygen distribution change was analyzed by STEM-EDS (FEI S/TEM TITAN 80-300) while structural studies and maps of oxygen concentration in Ti Grade 2 were realized with SEM-EBSD (HITACHI SU70) and TEM (FEI S/TEM TITAN 80-300) method. Analysis of TiO_2 thin layer was carried out by means of Raman spectroscopy (HORRIBA JOBIN YVON LABRAM HR MICRO-RAMAN SPECTROMETER). Excitation wavelength of 532 nm was used and the beam intensity was about 10mW at the surface of the sample. Acquisition time was set at 30 seconds. The test results obtained were used to identify the type of oxide coatings phase composition, thickness of the layers and the influence of crystallographic orientation on oxygen transport in the Ti substrate. The precise specification of the hardness of oxide layers, Young's modulus, as well as elastic and plastic energy was provided with the nanoindentation method (NANOTEST VENTAGE MICRO MATERIALS Ltd). After hybrid oxidation, the selected samples were tested for bioactivity response using a solution that simulates the environment of body fluids c-SBF2 – Kokubo test, under stringent conditions (I = 140 mM, $c_{0\ Ca^{2+}}$ = 2.5 mM, pH = 7.4, temperature = 37°C, CO_2 partial pressure = 0.05 atm) for 14 days.

3. Results and discussion

Titanium oxidation for biomedical applications is still a challenge for biomedical engineering in obtaining both good mechanical and physicochemical properties of substrates and thin oxide layers with good adhesion to titanium substrates. The surface treatment of titanium in fluidized bed oxidation FADT at 640 for 8 hours allowed to obtain oxide layers with a zone structure, comprising the top layer of TiO_2 (rutile) and internal fine grain diffusion layer of $Ti_\alpha/Ti_\alpha(O)$, whose microstructure and fracture are shown in Figure 1. According to the Author's previous results, the obtained diffusion layer was homogeneous with a thickness of ca. 10-12 μm. This occurs due to the fact that the treatment in a fluidized bed facilitates oxygen transport to the highly defected Ti substrate [18]. Obtaining such a fine diffusion layer reduces the difference in stresses produced through air cooling, caused by the difference in the coefficients of the thermal expansion of titanium and the top porous oxide layer formed. However, substrates obtained after FADT (fluidized bed) with highly porous areas have a limited use in terms of improving the biocompatibility due to the insufficient adhesion of hydroxyapatite compounds.

The statement above is well confirmed by the formation of dispersive particles of TiO_2 porous oxides and the oxygen distribution in the diffusion zone, observed with the STEM-EDS analysis (Fig. 2). The analysis of the obtained results indicates that the depth of oxygen layer is approximately 0.4 μm, with a significantly higher concentration of oxygen visible in the subsurface zone. Therefore, the mechanical activation zone of the fluidized bed corresponds to the distance in which oxygen concentration is higher than 35% at. of oxygen.

The effect of titanium strengthening caused by the saturation of octahedral voids with oxygen in FADT leads to an improvement in adhesive properties of the later deposited thin oxide

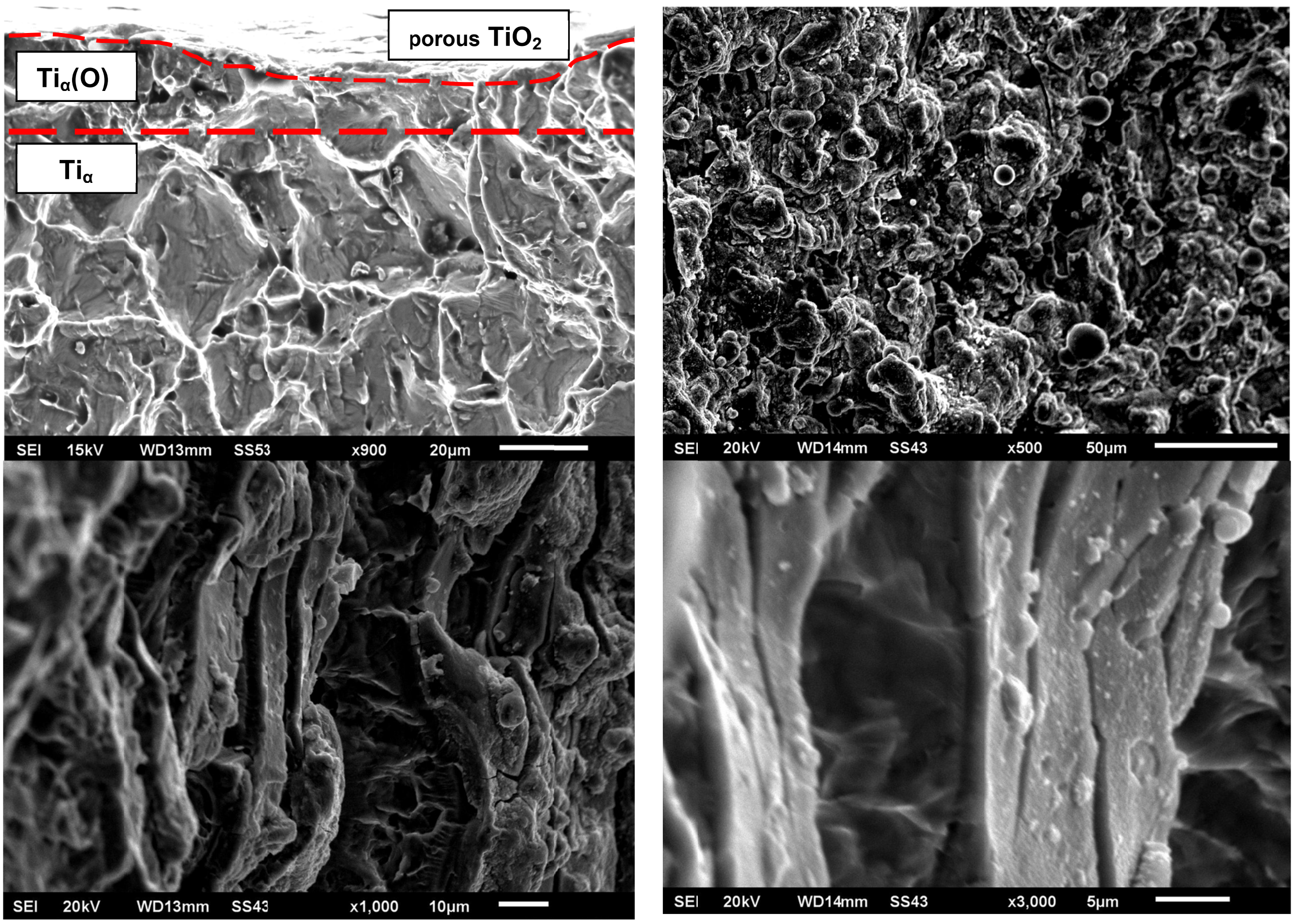


Fig. 1. SEM images of Ti Grade 2 substrate microstructure and fracture after fluidized bed diffusive treatment (FADT) at 640°C/8h

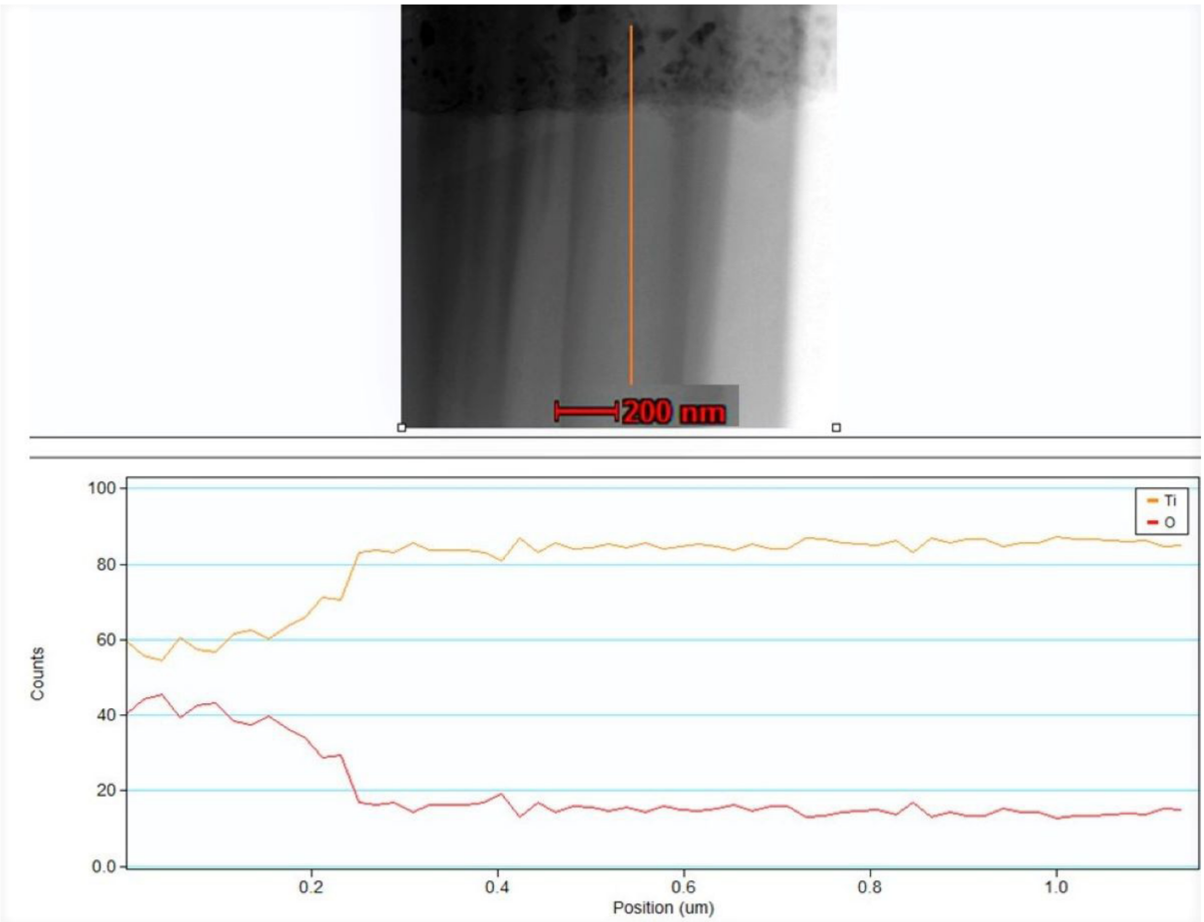


Fig. 2. STEM-EDS analysis of Ti Grade 2 substrate diffusion zone after fluidized bed oxidation (FADT) at 640°C/8h

layers for biomedical applications. Such a substrate will be the base material and substrate for hybrid processes of TiO_2 deposition realized with PVD – magnetron sputtering technique, which allows to obtain the oxide coating morphology with variable phase composition, favorable to the nucleation of biocompatible compounds (HAp). The hybrid FADT+PVD oxidation process successfully produced ultrafine highly homogenous and tight thin TiO_2 oxide layer. The FADT – PVD interface thickness was ca. 620 nm. In this zone there are visible areas of irregularly shaped nano-pores and varying in size from 20 to 60 nm. The pores distribution in the zone and their size are presented on the maps of oxygen concentration in Ti Grade 2, which also indicates that the areas with nano-pores are spots for easy anchoring of the TiO_2 particles deposited by the PVD method [19]. The STEM maps also showed the deposition of hybrid thin oxide layers resulting from the merger of TiO_2 rutile nanoparticles, which is shown in Figure 3.

However, the TEM results for titanium after hybrid treatment also revealed the presence of the TiO_2 mixed structure on the surface of the TiO_2 with anatase and rutile phase. During the PVD process, the plasma physio-chemical interaction with the substrate also involves a continuous bombardment (surface defect) and enhanced local heat transfer to control chemical reactions and sorption effects (chemi-, physisorption). Plasma composition with oxygen has a severe effect on local cleaning of the substrate surface (sputtering) and subsequent formation of stoichiometric TiO_2 thin layer (rutile) but also their mixture (rutile+anatase) [20]. In addition, the TiO_2 rutile and anatase phase mix was also indicated. Valuable results were also obtained by the studies of crystallographic planes orientation and their role in oxygen transport by means of electron backscatter diffraction (SEM-EBSD) presented in Figure 4.

The analysis of the results of EBSD maps leads to a conclusion that there is a differentiation of the crystallographic planes orientation of the oxygen phases at the surface and a correlation with the occurrence of (1010) oriented crystallographic planes and areas of (2110) crystallographic orientation in the diffusion zone. Thus, easier TiO_2 oxide phases nucleation in the diffusion zone occurs in the grains of (1010) orientation, which is caused by a change in the diffusion flux speed for this orientation, due to the incomplete filling of octahedral voids. Analysis of the TiO_2 duplex layers also revealed the texture in the (200) orientation which also suggests higher contribution of the TiO_2 rutile than anatase in the surface oxide layer. Rutile and anatase have tetragonal structures but rutile is a more stable phase at ambient pressure and temperature in macroscopic sizes while anatase is stable in nanoscopic size. The more compact structure of rutile (in comparison to anatase) causes important differences in physical properties. Rutile has a greater chemical stability than anatase. However, due to the metastable properties of anatase,

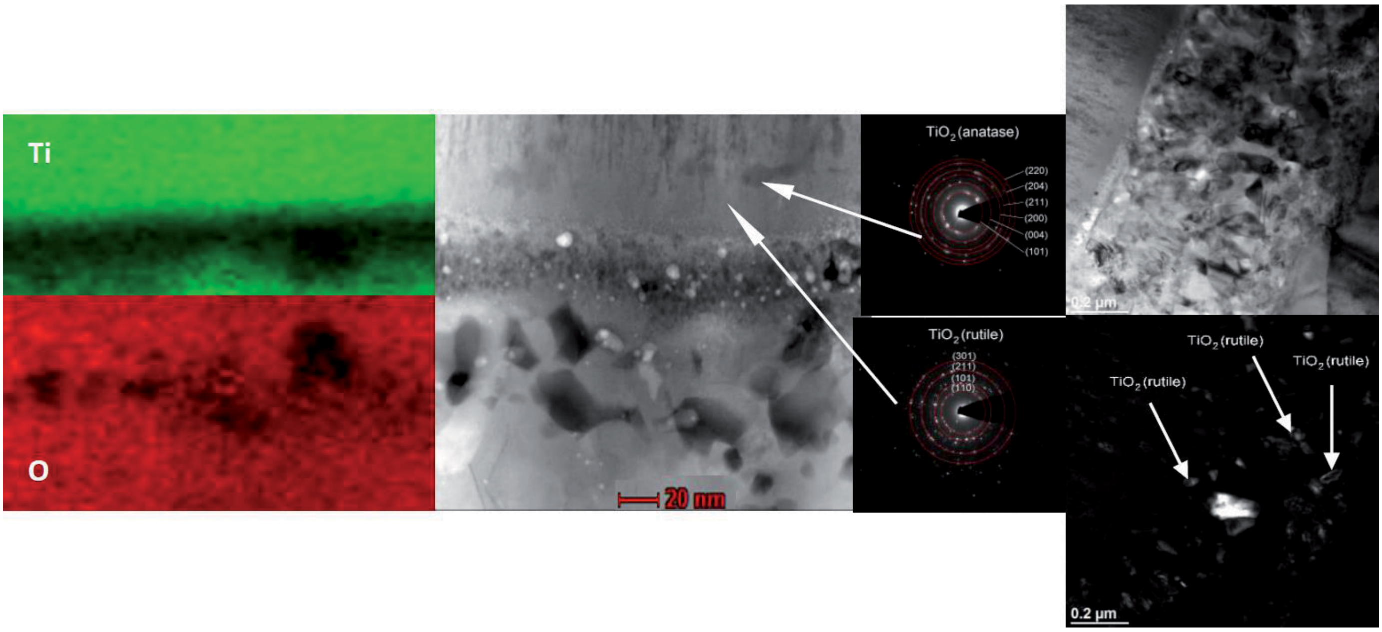


Fig. 3. TEM/STEM image and EDS maps of FADT – PVD TiO_2 interface, diffusion zone and TiO_2 rutile nanoparticles formed with hybrid oxidation

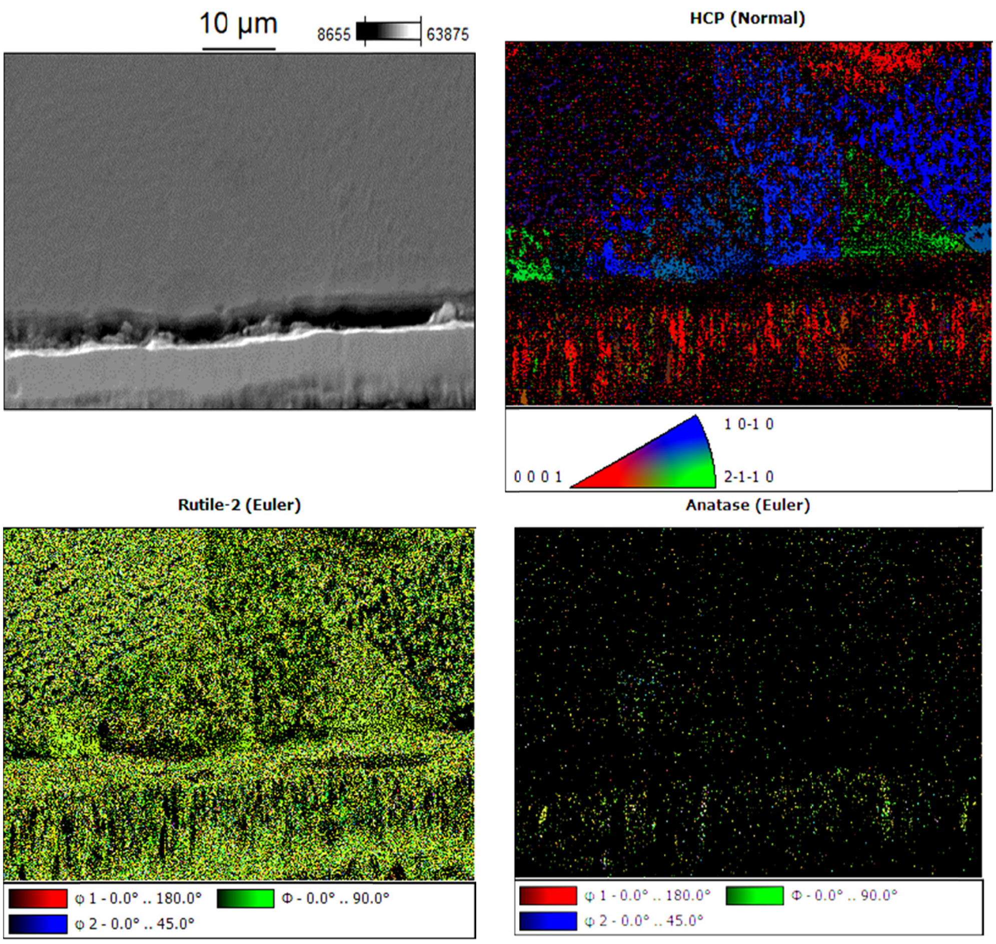


Fig. 4. SEM-EBSD maps of Ti Grade 2 crystallographic planes after hybrid oxidation

such phase mix layers perform an important role in inducing the apatite deposition because of the relationship of lattice matching between rutile/anatase to apatite. Literature data indicates that the adhesion of the titanium oxide layers to apatite is maximal when the magnitudes of the surface tension of both phase components are similar by minimizing interfacial stresses [21-23]. The phase gradient (rutile / anatase) in the biochemical affinity to osteogenesis and the mechanical properties in TiO_2 layer highly enhance the efficient bioactivity of titanium substrate affecting the first stage of vascularization of the tissues at the implant – substrate interface and significantly influence the rate of osseointegration. In fact, there are some literature data which show biomedical properties of anatase, also including the Author's previous works [24]. Furthermore, the Confocal Laser Scanning Microscopy CLSM and Raman spectroscopy results obtained for the substrate surface after hybrid oxidation allowed for a detailed analysis of 2D surface topography of the oxides that were created, which was also confirmed by TEM and SEM-EBSD results. Literature data indicate that for TiO_2 rutile, active vibrations are located at ca.: 143 cm^{-1} (B1g), 236 cm^{-1} (broad band), 447 cm^{-1} (Eg), 612 cm^{-1} (A1g), and 826 cm^{-1} (B2g) for rutile and six active bands for anatase: 144 cm^{-1} (Eg), 197 cm^{-1} (Eg), 399 cm^{-1} (B1g), 513 cm^{-1} (A1g), 519 cm^{-1} (B1g) and 639 cm^{-1} (Eg) [25]. Raman spectra obtained for titanium after hybrid treatment showed the presence of the strongest peaks coming both from the rutile and anatase phase. The visible peaks suggest the occurrence of a rutile and anatase mixed structure. Figure 5 shows a reference to Raman spectra for rutile and anatase phases and the spectrum after hybrid oxidation FADT + PVD. The visible band in the range of wavenumbers 143 cm^{-1} 395 and 515 cm^{-1} indicates the presence of a thin TiO_2 anatase layer. The other characteristic bands in the range of wavenumbers: 604 cm^{-1}, 438 cm^{-1} and 231 cm^{-1} come from the TiO_2 rutile phase which constitutes the dominant phase in the FADT+PVD oxidation. In addition, it was observed that the bands are shifted towards lower frequencies, suggesting the occurrence of compressive stresses in the TiO_2 layers after hybrid treament, confirming their phase differentiation.

The next step of the research was nanomechanical investigation of the PVD TiO_2 oxide layer. The results showed favorable strength properties of the PVD layers. A series of indentations (in nano- and micro- scale) was performed on pure titanium (raw substrates) and on the specimens after PVD hybrid treatment (Fig 6). This investigation allowed to find a correlation between mechanical parameters measured in nano and micro-scale for the TiO_2. Special attention was paid to mechanical properties of the interface of the FADT substrate / TiO_2 thin oxide layer whose zone plays a crucial role in the integrity of the whole hybrid system. The nanoindentation hardness (H), and the Young modulus (E) of the coatings were measured by a depth sensing technique using a Berkovich-type diamond indenter. Nanoindentation hardness and Young's modulus values measured for the hybrid TiO_2 were H= 15.21 GPa and E = 261 GPa, which are slightly higher values than the results of sputtered TiO_2 layers reported in the literature [26].

Considering the nanoindentation results, it might be stated that the hybrid treatment (FADT + PVD) favorably affects the

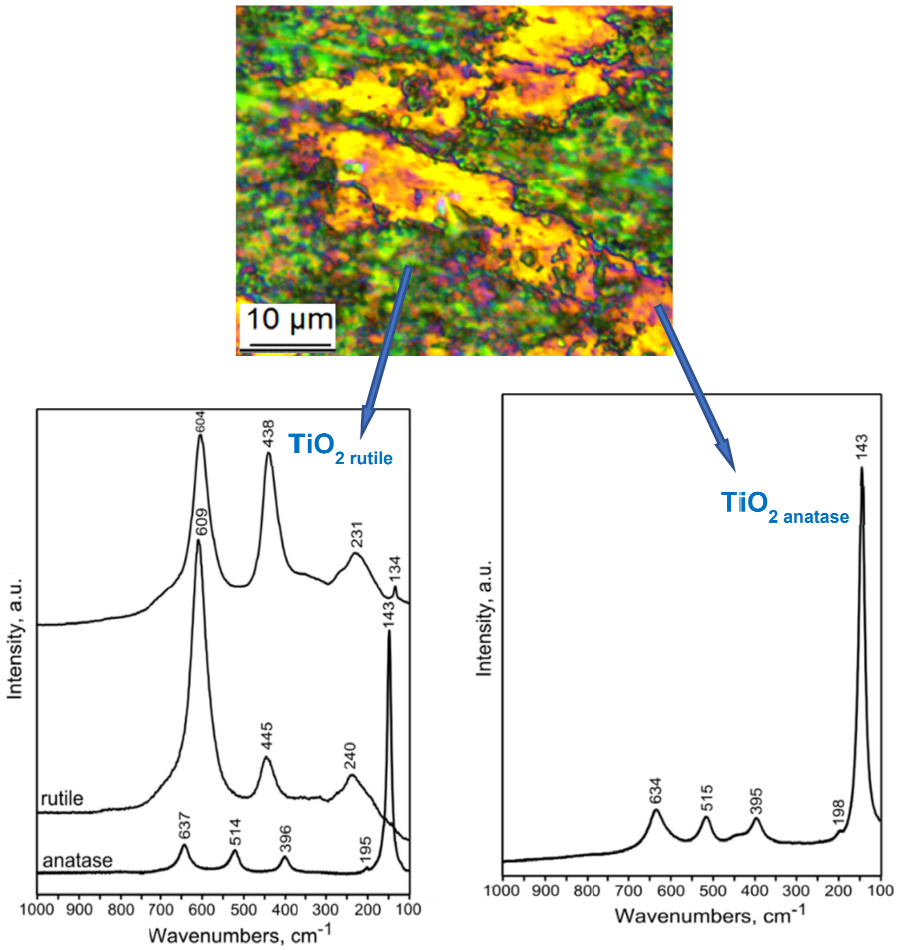
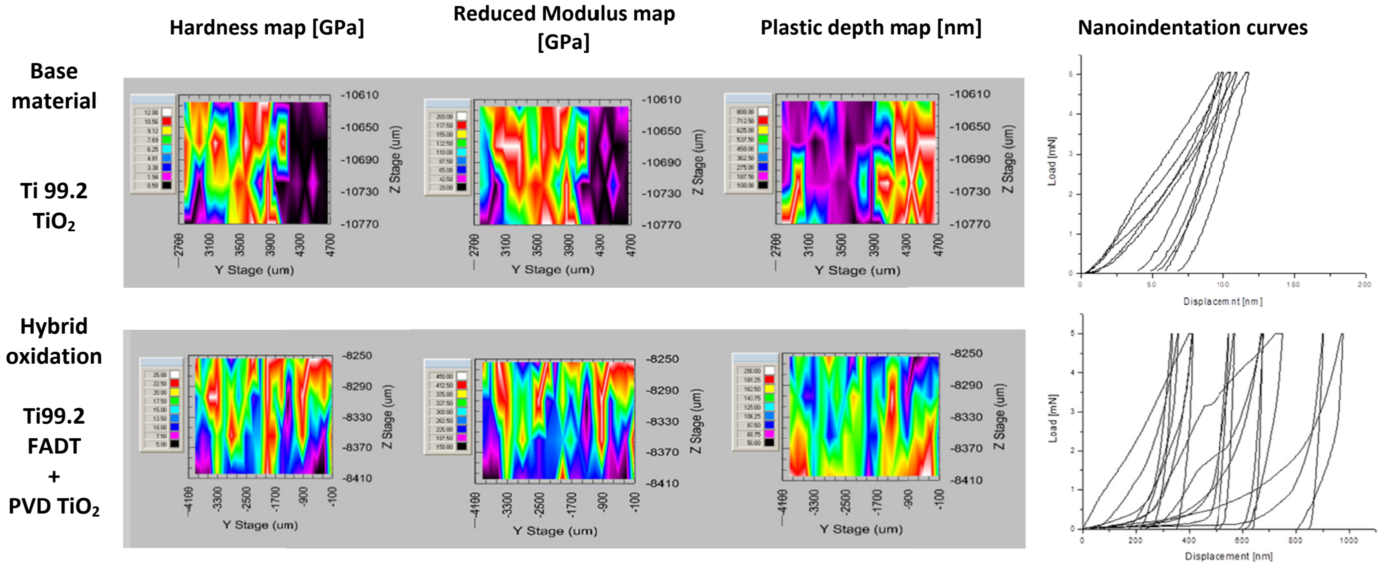


Fig. 5. Confocal Laser Scanning Microscopy (CLSM) 2D topography image and Raman spectra of Ti Grade 2 substrates surface after FADT+PVD hybrid oxidation



Sample name	H, Hardness [GPa]	St. Deviation	E_R, Reduced Young modulus [GPa]	St. Deviation	E, Calculated Young Modulus [GPa]	St. Deviation	Maximum depth [nm]	St. Deviation	Plastic depth [nm]	St. Deviation
Ti99.2 – TiO_2	9.33	4.14	160.00	60.30	148.34	55.91	204.08	57.41	167.17	54.36
Ti99.2 FADT+PVD TiO_2	15.21	6.04	281.83	87.79	261.28	81.39	144.90	28.87	119.20	27.37

Fig. 6. Nanoindentation results of Ti Grade 2 substrates after FADT+PVD hybrid oxidation

improvement of titanium surface hardness and strength as a result of the formation of a tight and homogeneous PVD TiO_2 layer. This is especially important in the subsequent intensified deposition of hydroxyapatite compounds, which was confirmed by the results of the Kokubo corrosion tests in the SBF environment for 14 days (Fig. 7).

The concept of bioactivity was firstly defined by Hench et al., who claimed that "A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material". The mechanism of tissue attachment of an implant is directly related to the tissue response at the implant

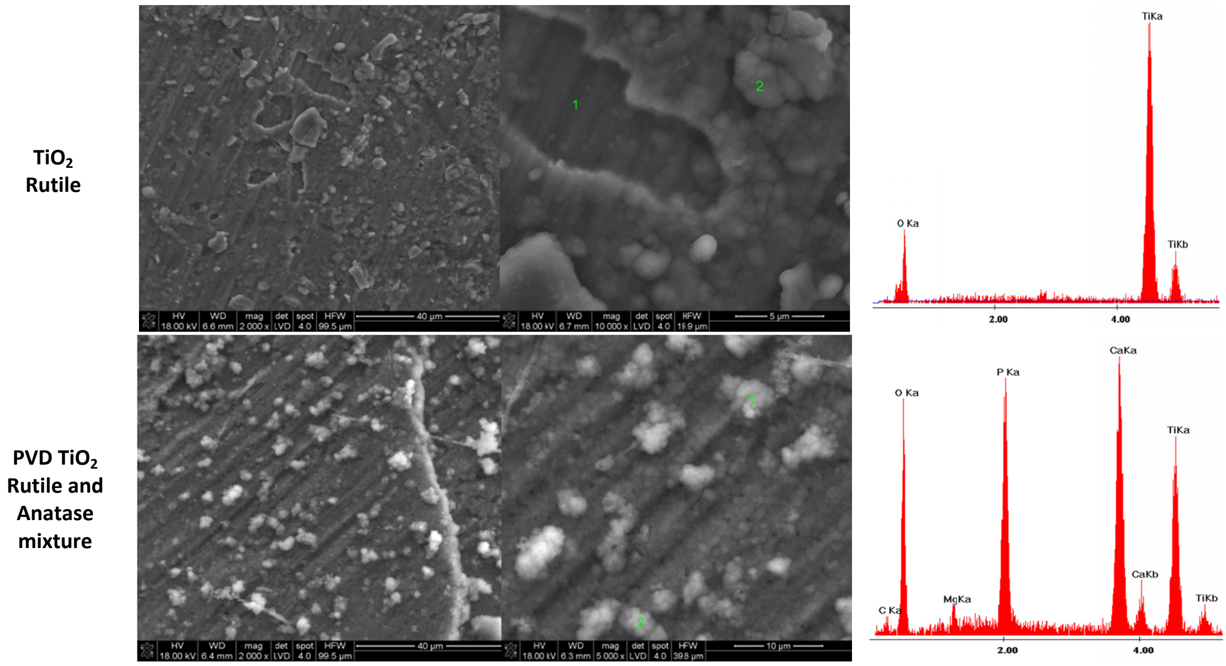


Fig. 7. SEM image and SEM-EDS analysis of hydroxyapatite (HAp) formed on the surface of Ti Grade 2 substrates after FADT+PVD hybrid oxidation after corrosion tests in SBF for 14 days

interface [27]. When a bioactive material operates in the body, a series of biophysical and biochemical reactions occur at the implant-tissue interface. The rate of development of the interfacial bond can be referred to as the level of bioactivity. Relative bioactivity and time dependence of the formation of interfacial bone bonding with bioceramics differs for a specific material and its level can be related to the time for more than 50% of the interface to be bonded [28]. The SEM-EDS study of hybrid oxidation (FADT+PVD) Ti Grade 2 substrates confirmed after 14 days in c-SBF2 solution the deposition of dispersive, globular hydroxyapatite (HAp) particles, formed at the surface of TiO_2 rutile / anatase interface and in the areas of surface defects after 14 days. HAp particles agglomerate and form clusters due to the stabilization and minimizing of surface tension and energy of the HAp particles, affecting the improvement in the biochemical activity of titanium substrates, which is beneficial for biomedical applications [29,30]. Intensifying the growth of hydroxyapatite particles and clusters shows the potential of applying the hybrid techniques presented herein for the growth of bone tissues, indicating the directions in forming biocompatible layers for medical applications.

4. Conclusions

The hybrid combination of oxidation methods (FADT+PVD) in the formation of bioactive substrates allows to obtain functional biomaterials for applications in bone implants by controlling the surface morphology parameters of the top surface oxide layers. The hybrid method of titanium oxidation presents a novel outlook on the modification and development of hybrid oxide coatings in the area of biomedical applications. The biological properties of thin oxide layers might be significantly improved by the controlling of their stress state and topography. Obtaining biofunctional surfaces will be ensured by the application of hybrid oxidation processes. Such a combined oxidation might significantly affect not only the character of the surface morphology, but also implement new properties of the layers in the field of mechanical parameters (interface anchoring), adhesion to the substrate and phase composition (the highly desired TiO_2 rutile+TiO_2 anatase mixture). It has also been found that the formation of oxide coatings with the use of the hybrid method (FADT+PVD), as opposed to conventional methods, leads to a change in oxygen concentration in the substrate as a result of its defects. In contrast, forming a tight homogeneous oxide coating on Ti surface improves the biocompatibility, which is particularly important in the context of biomedical applications. Such type of hybrid substrates might not only constitute an attractive solution for bone implants modification or the creation of modern biomedical materials, they might also constitute a candidate for numerous other applications requiring multifunctional materials. The study has also demonstrated that the tissue reaction changes in response to the gradient surface composition or the phase structure of materials. This implies the possibility to control the tissue response by a functionally graded structure of biomaterials. The conducted research has proved that by the implementation of hybrid oxidation processes, it is possible to obtain thin titanium oxide layers of differentiated physicochemical properties, ultimate state of stress and biofunctional parameters, including the ones which are significant in terms of medical applications. To sum up, the following conclusions from the conducted research should be indicated:

1. Hybrid titanium oxidation involves new ways to connect oxygen transport phenomena in oxide layers in terms of shaping favorable changes in the surface properties of titanium for biomedical applications. The intensified growth of bioceramics particles and clusters points to the applicability of the above mentioned techniques in the processes of bone tissue growth.
2. Combining the methods in the hybrid system suggested by the Author allows for a synergistic improvement in the surface effects, highly affecting the intensity of the subsequent formation of hydroxyapatite compounds after SBF Kokubo bioactivity tests.
3. In hybrid oxidation, PVD treatment after the FADT process enhances the formation of a homogeneous, tight, and continuous oxide coating with a mixture of TiO_2 rutile and anatase phases on the Ti Grade 2 substrate.
4. Ti Grade 2 hybrid treatment (FADT + PVD) favorably affects the formation of a tight and homogeneous TiO2 (PVD) coating. This is especially important in the subsequent intensified deposition of hydroxyapatite particles and clusters in globular form.
5. The Ti Grade 2 hybrid oxidation treatment presented herein is therefore a new solution to produce bio-compatible layers for later biomedical applications, for example in bone implants, to improve the processes of tissue osseointegration.

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