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Electrophoretic synthesis and characterization of bioactive HAp/TiO_2 thin films coated on stainless steel

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The aim of this study was to synthesize titanium dioxide (TiO_2) thin films coated on 316L stainless steel (316L SS) and to characterize hydroxyapatite (HAp) growing onto TiO_2 layer coated on the metallic substrate. These films were obtained by the electrophoretic synthesis (EPS) method using an acidic electrolyte containing H_2SO_4 and TiO_2 nanoparticles. The growth of HAp on TiO_2 thin films was achieved by immersion in simulated body fluid (SBF) during different periods of time in order to evaluate the bioactivity of structure *in vitro* study. The obtained coatings were characterized by XRD, SEM, AFM and EDX measurements. The XRD results reveal a crystalline phase (anatase) of TiO_2 nanoparticles. The presence of TiO_2 nanoparticles in the bath led to the preferential orientation of TiO_2 crystallites after [101] crystallographic direction. During the coating EPS process, the presence of TiO_2 provides more nucleation sites which are favourable to the adherence of nanoparticles on the metallic substrate. The surface morphology observed by SEM analysis showed that TiO_2 nanocrystals were attached on metallic surface. The appearance of the surface indicates porous structure and island-like cracked morphology with polygonal shapes. The roughness of the coating is in good agreement with standards for biomaterials which is proved by AFM measurements. The EDX spectra also confirm the presence of the Ca^{2+} and PO_4^{3-} ions in the structure obtained by EPS method.

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1. Introduction

Biomaterials have received much interest and intensive research during the last few decades due to their obvious use as replacement materials of various body parts or even organs. Metals are used in the human body mainly for orthopaedic purposes and their degradation by wear and corrosion must be negligible so that they can be used for various practical applications. 316L Stainless Steel (316L SS) is frequently employed to prepare the material for implants because of its low cost and acceptable biocompatibility [1].

In order to make the composite coatings still more corrosion resistance and to increase their tribological properties, nowadays there is growth interest in the co-deposition of the nanoparticles because of their increasing availability. Among the nanomaterials, titanium dioxide (TiO_2) is in great demand for the generation of composite coating on steel with other metals or alloys [2,3]. The TiO_2 nanoparticles are co-deposited successfully with Ni, Cu, Ag and Zn metals [4-6]. In recent years, TiO_2 thin films on metallic implants have received a great attention in the field of orthopaedics because of their excellent mechanical, osteoconductive and corrosion resistant properties. TiO_2 nanoparticles can also be functionalized with various chemical groups to increase their affinity towards target compounds. Recently, among nanomaterials a growth interest has been devoted to TiO_2 which are
low cost chemically stable and non-toxic compounds and possess high photocatalytic activity.

Recently, electrophoretic synthesis (EPS) has been proposed as an alternative route for the production of nanocrystalline TiO_2 thin films since it offers a number of advantages, namely simplicity of equipment, accurate control of layer thickness and cost-effectively applicability of complex shapes [7]. However, the amount and distribution of TiO_2 nanoparticles incorporated during the EPS process depend on a variety of working parameters (stages of coating, particle size and particle concentrations).

One of the most effective methods to improve the corrosion resistance and the biocompatibility of the metals is to deposit a protective bioactive ceramic coating layer on the metal surface. Another class of materials used as hard tissue (bone) implants consists of calcium phosphate materials, in particular hydroxyapatite (HAp). HAp, having the composition $Ca_{10}(PO_4)_6(OH)_2$, is widely used as a bioactive ceramic since it forms a chemical bonding to the bone [8-10]. The motivation for the selection of HAp is based on its suitability for biomedical applications. HAp is a bioactive ceramic with a chemical similarity to natural bone [11].

Various methods have been developed to deposit HAp on metallic surfaces such as plasma

spraying, sol-gel deposition, chemical precipitation, electrochemical deposition, etc. [12-16]. These methods develop products with varying levels of purity, size, crystallinity and yield.

The biomimetic methods, applied to produce calcium phosphate ($Ca_3(PO_4)_2$) coatings, have attracted considerable research attention in last years [17-21]. This method develops $Ca_3(PO_4)_2$ coatings by immersing metal implants in an aqueous solution containing calcium (Ca^{+2}) and phosphate (PO_4^{-3}) ions at physiological pH and temperatures. The solution usually used for this type of deposition is the simulated body fluid (SBF) which mimics the composition of blood plasma with inorganic ions. Kokubo's recipe and Hank's balanced salt solution are two commonly used SBF's and differ only in their concentrations of Ca^{+2} and PO_4^{-3} and their need for extraneous pH buffers [17-19].

In the present investigation, an attempt has been made to deposit porous TiO_2 thin films on 316L SS with two main objectives:

- to establish an efficient coating method at room temperature, the EPS process, to obtain thin layers of TiO_2 from aqueous solutions on 316L SS;
- to prove the bioactivity of coating depositions in terms of HAp forming capability after immersion in SBF solution.

The aim of this work-paper is to initiate a systematic study of preparation method to obtain *HAp/TiO_2 thin films coated 316L SS with compatible structure and composition for biomedical applications.* Following, the effect of certain process variables on the incorporation of titania nanoparticles, the surface morphology and structure of the deposited films have been examined. An alternative coating method based on biomimetic technique is designed to form a crystalline HAp layer in a similar way to the process of natural bone formation. The HAp formation on the surface of TiO_2 coated 316L SS pre-treated with NaOH solution is investigated.

2. Experimental

2.1 Selection and pre-treatment method of substrate

Commercially available 316L SS specimens having the composition Fe+Cr: 18.00; Ni: 12.00; Mo: 2.50; Mn: 1.70; P: 0.04; C: 0.02; S: 0.01; Si: 0.15 (wt. %) were used as the substrate in the present study [22]. Prior to making electrical contacts, the plates were mechanically polished using 600 and 1200 grit emery paper, organically degreased with acetone, etched in a 1:1 HCl:H_2O solution for 60 s, chemically degreased with ethylic alcohol for a few seconds and rinsed with distilled water. Then, the samples were activated cathodically at -1.1 V vs. SCE in a 0.1 M NaOH solution for 2 min and finally rinsed with doubly-distilled water.

2.2 Electrophoretic coating process

The EPS process of TiO_2 was performed in a small three-electrode cell (500 mL cell volume) on plate specimens (with an active area of 1 cm^2), using Pt wire as the counter electrode and Ag/AgCl electrode as a reference electrode. The process was carried out potentiostatically with a potentiostat/galvanostat model 273A (EG&G Princeton Applied Research, USA), connected to a computer.

All chemicals were purchased from commercial sources and have the highest purity available. They were used without further purification. The composition of the deposition bath was that of an aqueous solution containing 4:1 H_2SO_4:TiO_2 (Degussa P-25 TiO_2 nanoparticles), 0.03 M H_2O_2, 0.05 M HNO_3 and 0.05 M KNO_3, having a pH of 1.4. The deposition was performed at room temperature (23-25 ºC) at -1.43 V potential vs. Ag/AgCl electrode. There were three different stages of coating 30, 60 and 90 min and a deposition cycle of 3×30 min. The plating solution was mechanically stirred (300 rpm) using a magnetic stirrer. Before starting the coating process, every time the electrolyte solution was sonicated for 20 min to electrostatic charging TiO_2 nanoparticles from the solution. The deposited layer was then heated in air at 400 ºC for 1 h in order to obtain crystalline TiO_2 film [3,4]. The heat-treatment was recorded by means of a temperature controller type 201 Carbolite Tersid (Milan, Italy). The substrates were weighed prior to the coating and after annealing in order to determine the amount of deposited TiO_2 by means of an analytical balance with an accuracy of 0.0001 g. Nearly 15% mass reduction was observed after heat-treatment at 400 ºC for 1 h in air, due to water evaporation from the film. To prepare a multiple TiO_2 layers the EPS was repeated through three consecutive depositions followed by the heating processes at 400 ºC for 1 h in air per each cycle.

2.3 Evaluation of bioactivity of coating

A simple SBF medium with high Ca^{+2} and PO_4^{-3} concentrations was used for biomimetic coating study of HAp, with known composition from speciality literature and presented in Table 1 [17-19]. The SBF was prepared by dissolving all reagent grades (Table 1) in distilled water. The final solution was buffered at a pH of 7.5 with tris(hydroxyl)aminomethane [$(CH_2OH)_3CNH_2$] and HCl at 37±0.5 °C [23]. In order to simulate *in vivo* process, and thus evaluate the bioactivity, the coated specimens were soaked directly into SBF solution, taken out of the solution after 7 and 21 days of immersion, rinsed with deionised water and dried at 60 °C for 1h.

Table 1. The chemical composition of SBF solution.

Solutions	SBF (g/L)
NaCl	7.996g
$NaHCO_3$	0.350 g
KCl	0.224 g
$K_2HPO_4x3H_2O$	0.228 g
$MgCl_2x6H_2O$	0.305 g
HCl	40 cm^3
$CaCl_2$	0.278 g
Na_2SO_4	0.071 g
$(CH_2OH)_3CNH_2$	6.057 g

2.4 Microscopic, EDX and structural characterization of coating

The surface morphology of thin layers was examined by means of a scanning electron microscopy (SEM) and carried out using Cambridge Instruments Stereoscan 360 (Princeton Gamma-Tech, New Jersey, USA) and Zeiss EVO 50 (Zeiss, Germany) for high resolution. The chemical composition of coated samples was determined by means of energy dispersive X-ray spectroscopy (EDX). The crystallographic feature of the electro-synthesized TiO_2 and HAp/TiO_2 thin films was analyzed by X-ray diffraction (XRD) method by means of a Röntgendiffractometer Philips X'pert PW 3710 with Cu K_α radiation working at 40 kV and 30 mA. The diffractograms were obtained for 2θ ranged between 20 and 100°, at a scan rate of 0.02° min^{-1} and acquisition time was 0.5 s/step. The topography and the roughness of the surface were studied using atomic force microscopy (AFM) technique by means of Navitar Nova (ROCHESTER, New York, USA) instrument. The scans were made on 20 μm × 20 μm areas of the films in contact mode.

3. Results and discussion

3.1 Structural and microscopic characterisation of TiO_2 coatings

XRD technique has been used to identify the structure of thin films onto 316L SS substrates at different stages of deposition (Fig. 1). The annealing of the obtained layer at 400 °C for 1 h in air leads to the formation of anatase phase of TiO_2. The TiO_2 thin films are crystalline. Two types of crystallographic planes corresponding to the SS substrate and the anatase form of TiO_2. The anatase is formed at 2θ = 25.2°, 27.3º, 37.8º, 48.0° which are attributed to (101), (110), (004), (200) and (211) crystallographic planes (JCPDS 71-1169) [28]. For SS (111) and (200) are the specific planes. Compared with the database from the Reference Pattern the free energy of the surface-support-solution system allows the preferential growth of TiO_2 crystallites after the [101] crystallographic direction was observed.

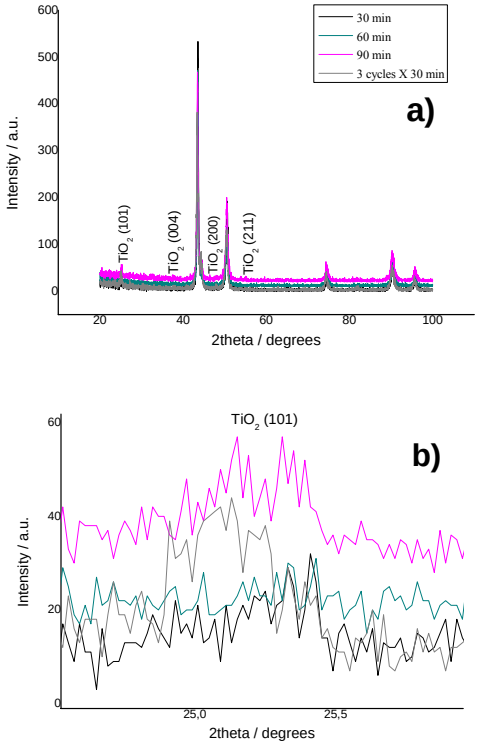

Fig. 1. X-ray diffraction profiles of a) deposited and annealed at 400 ℃ TiO_2 films coated 316L SS and b) a zoomed TiO_2 peak for (101) crystallographic plane.

Fig. 2 shows SEM micrographs of deposited and heat-treated TiO_2 coated on 316L SS at three stages of deposition. The observed morphologies vary from fine-grains to polygonal shape islands (Fig. 2a, b). Fig. 2a has a very porous structure compared with Fig. 2b which is more densely occupied by TiO_2 islands. A cracked morphology can be observed due to the shrinkage occurred after the drying process. The increase of time deposition leads to the formation of this structure which looks like islands with polygonal shapes (Fig. 2c). Careful observation from SEM pictures of the annealed samples reveals that each grain is made up with an aggregate of very small crystallites (Fig. 2d) [25]. This structure can occur due to the increased resistivity of multiple TiO_2 layer which leads to preferential NO_3^- reduction and consequent OH^- production. EDX analysis reveals that TiO_2 can be detected in the cracks which can prove the uniformity of the under layer of TiO_2 coated stainless steel (SS) substrate.

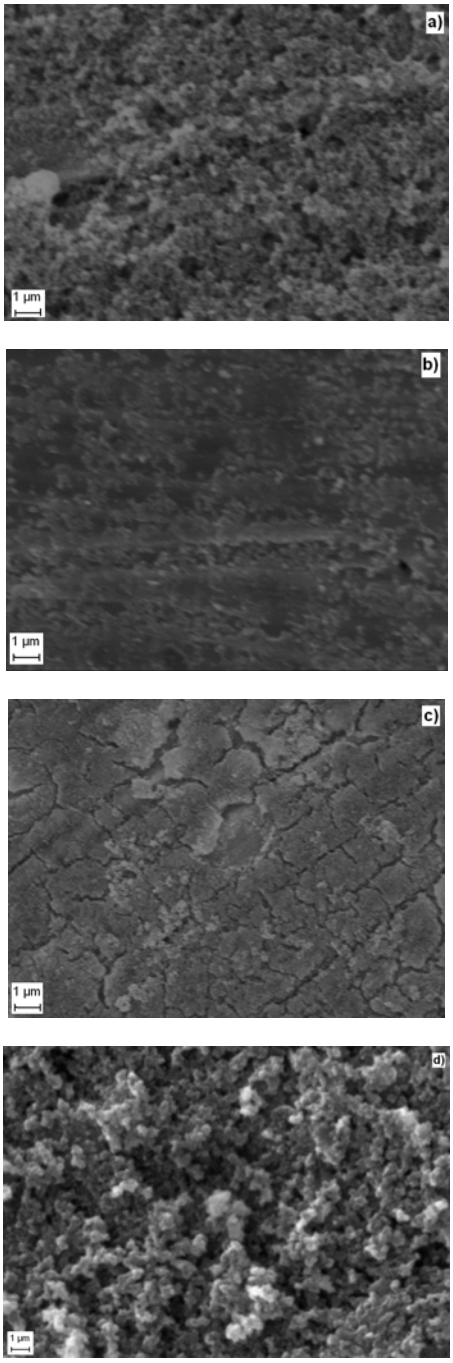

Fig. 2. SEM micrographs of electrophoretic µm synthesized TiO_2 films at different stages: (a) 30 min; (b) 60 min; (c) 90 min and (d) 3 consecutive cycles of 30 min each.

In order to further investigate the surface morphology in details, AFM observations for the electrosynthesized and heat-treated samples were carried out. Fig. 3 shows the 3D AFM images of deposited and then, heat-treated TiO_2 films. AFM images of the TiO_2 coated 316L SS revealed the changes in surface topography of thin films due to the heat treatment at 400 °C. Fig. 3c indicates a very rough surface due to the increase of time deposition (the values are presented in Table 2). Although the appearance of the AFM image of heat-treated film at different times (Fig. 3) is similar to the last one, the root mean square roughness value is increased due to the formation of aggregates of the nanoparticles by heat-treatment (Table 2).

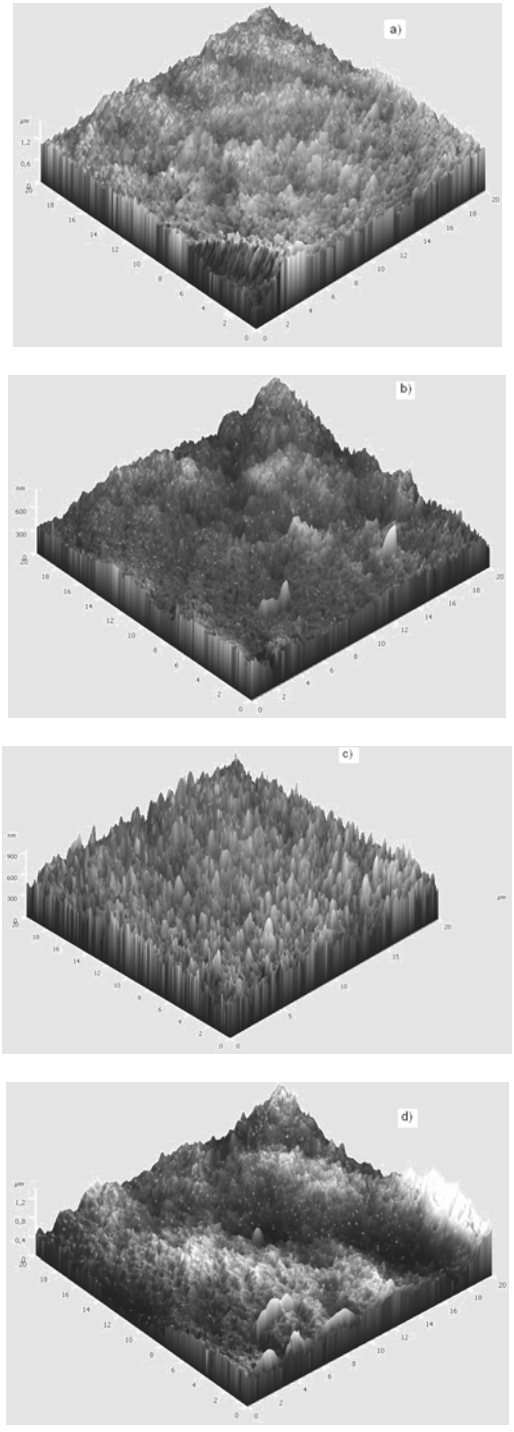

Fig. 3. AFM images of TiO_2 films at different stages of deposition: (a) 30 min; (b) 60 min; (c) 90 min and (d) 3 consecutive cycles of 30 min each

The weight of the coatings was measured before and after the heat treatment. The results reveal that the difference between the two values is caused by the evaporation of the solvent and organic components. Therefore, the surface roughness increases proportionally with time deposition. Also, this fact can be due to the agglomerations of nanoparticles revealed by annealing. Wennerberg et al. [26] suggested that the roughness can be described as smooth for abutments, minimally rough for roughness from 0.5 to 1 μm, intermediately rough from 1 to 2 μm and rough from 2 to 3 μm. Roughness has been shown to be a key feature for the quality of cell growth. From *in vitro* studies, the osteoblasts develop better on rough surface was found [27,28]. In contrast, epithelial cells and fibroblasts were better attached to the smoother surfaces than rougher ones. They better proliferate on smooth surfaces [29]. The surface roughness values for the samples are presented in Table 2. These results are in accordance with the values found for commercially available implant components.

Table 2. Characterization of surface roughness of TiO_2 coatings onto 316L SS.

TiO_2/316L SS samples Stage of deposition	R_a (μm)	R_q (μm)
30 min	0.043	0.051
60 min	0.384	0.392
90 min	0.479	0.489
3 consecutive cycles of 30 min each	0.566	0.587

R_a is the average surface roughness of all points from a plane fit to the rest part of the surface;
R_q is the root mean square of the average of the measured height deviation

3.2 Bio-growth and bioactivity

The biocompatibility of TiO_2 coated 316L SS was evaluated by soaking the substrate in SBF as mentioned in Section 2.3. The surface morphology of the biomimetically developed HAp coating (Fig. 4) was the clear evidence for the biocompatibility of the TiO_2 layer. The biomimetic deposition facilitated the natural growth of HAp from SBF solution. The TiO_2 thin layer coated acts as nucleation site for further HAp growth. Therefore, the adhesion of phosphate and Ca^{2+} components is obviously due to the acting as a negatively charged layer. This Ca^{2+} adsorption from SBF enhanced further apatite growth by adsorbing the corresponding phosphate moiety. The chemical composition of HAp coated TiO_2/316L SS is presented in Table 3. Also, the presence of HAp on surface is confirmed by SEM analysis (Fig. 4).

Table 3. EDX analysis of HAp/TiO_2 coated 316L SS.

Elements	TiO_2 on 316L SS (wt. %)
O	59.13
P	1.36
Ca	2.37
Ti	37.14

SEM micrograph (Fig. 4) indicates a good behaviour of TiO_2/316L SS system by immersing the samples in SBF solution due to the incorporation of Ca^{2+} and PO_4^{3-} ions during 7 and 21 days. From the EDX analysis a stoichiometric Ca/P ratio of 1.74 which agrees well with theoretical value of 1.67 was determined.

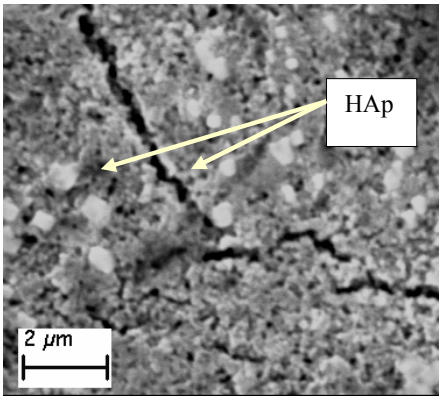

Fig. 4. The surface morphology of HAp growth after 21days of immersion in SBF

4. Conclusions

In the present work, TiO_2 thin films coated 316L SS have been successfully prepared by EPS method at different stages of deposition. 316L SS was modified through TiO_2 coating as a biocompatible substrate favourable for HAp growing. As stated above, the EPS process for synthesis the TiO_2 film is an innovative method. TiO_2 nanoparticles were uniformly dispersed in the solution due to the continuous stirring. Therefore, the nanoparticles are coated during the process using an aqueous solution. Surface characterisation studies reveal porous structure and cracked-mud structure made of islands in order to protect the SS substrates during EPS process. The TiO_2 coating onto 316L SS facilitated HAp growth from SBF solution for 7 and 21 days. The formation of HAp has been determined by SEM and EDX investigations. It is envisaged to extend the study of increasing the thickness of the film, the stability of the electrolyte and the biocompatibility properties. Also, the investigations will continue trying to show the effect of cell bioadhesion on the surface of coating depositions.

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