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Investigation on hydroxyapatite coatings formation on titanium surface

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Abstract. This study presents an alternative coating method based on biomimetic approach to grow the hydroxyapatite layers on the titanium surface. In order to increase the bioactivity of the titanium surface the anodization and alkali ÷ thermal oxidation pre-treatments have been applied. Titanium implants were then coated with a hydroxyapatite layer under biomimetic conditions by using a Simulated Body Fluid (SBF) solution. The apatite deposits were investigated by means of scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transformed infrared spectroscopy (FTIR). The obtained results confirmed that the hydroxyapatite coatings on the titanium surface were obtained.

1. Introduction

The metallic biomaterials such as titanium and its alloys play a significant role in the field of dental and orthopedic implants due to their properties. These materials have an excellent biocompatibility and greater mechanical behaviour compared to other metallic materials [1]. The biocompatibility of the titanium is ascribed to the titanium oxide film which form on the titanium surface [2]. This oxide film layer is very thin and easily to be destroyed. Thus, various attempts have been done to protect titanium oxide coating, such as: heating under atmospheric pressure or in vacuum, electrochemical oxidation, anodic oxidation, etc. [3-5]. The surface modification of the titanium implants in order to improve the tissue responses and osseointegration can be realised by different methods, such as: (i) coating with bioactive compounds that accelerate the bone formation and (ii) achieving rough surface of the metallic implants that allow a better anchorage of the implant [6]. Previous studies have recently proposed numerous surface treatment methods, including sandblasting, acid etching, electrochemical treatment, thermal spray coating, etc. [7-9].

The hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, a calcium phosphate ceramic, is widely used in biomedical application for load bearing implants and the dental industry due to excellent biocompatibility and bioactivity. The hydroxyapatite undoped or doped with foreign ions (Mg^{2+}, Zn^{2+}, Bi^{3+}, Ce^{3+} etc.) can be used as biomaterial pertinent to orthopedic and dental applications [10-13]. Unfortunately, the mechanical properties of the pure hydroxyapatite ceramic are poor. Investigations on various deposition techniques of hydroxyapatite coating on the titanium implants have been made over the years, in particular to improve its adhesion strength to the metal surface and its long-term reliability. A variety of methods have been developed for preparing the hydroxyapatite coatings on titanium implants, such as: vacuum deposition, plasma spraying, sol-gel and dip coating, electrolytic and biomimetic methods [14-18].

In this study, we obtained titanium oxide, sodium titanate and finally hydroxyapatite layers with different thicknesses and roughness on the titanium surface through a succession of treatments of the titanium implants, namely: anodization, alkali ÷ thermal oxidation and biomimetic treatments. The obtained results indicate that these treatments can induce the hydroxyapatite formation on the titanium surface.

2. Materials and methods

2.1. Materials

NaCl, $NaHCO_3$, KCl, $Na_2HPO_4{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$, Na_2SO_4, HF, HNO_3, NaOH, tris(hydroxymethyl)aminomethane $(HOCH_2)_3CNH_2$, methanol, ethanol and acetone were purchased from Sigma-Aldrich (Germany) and used without further purification. Experiments were performed with triply distilled water.

The Simulated Body Fluid (SBF) solution was prepared according the protocol described by Kokubo [19], by dissolving NaCl, $NaHCO_3$, KCl, $Na_2HPO_4{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, $CaCl_2{\cdot}2H_2O$ and Na_2SO_4 in 1000 mL deionized water, under vigorous stirring. The SBF solution was buffered at pH = 7.4 with tris(hydroxymethyl)aminomethane and 1 M HCl solution. The ion concentrations of the SBF solution were of 142 mmol/L Na^+, 5 mmol/L K^+, 2.5 mmol/L Ca^{2+}, 1.5 mmol/L Mg^{2+}, 1 mmol/L HPO_4^{2-}, 147.8 mmol/L Cl^-, 4.2 mmol/L HCO_3^- and 0.5 mmol/L SO_4^{2-}.

2.2. Pre-treatment of implants

In this study, the plate-shaped specimens fabricated from commercially pure Ti (c.p. Ti) were used. The titanium bar was cut as rectangular strips with typical dimensions of 10 mm x 10 mm x 3 mm.

The samples were mechanically gritted with silicon carbide paper and polished using 60- and 180-grit SiC paper followed by chemical etching in 1 % HF solution for 2 min. Afterwards, all the samples were cleaned with acetone (for 15 min), ethanol (for 10 min) and deionized water (for 5 min).

Then, in order to obtain a titanium oxide layer on the titanium surface, the samples were treated by an anodization process using an electrochemical cell. The surface area of the working electrode (Ti substrates) was 5 cm^2 and the counter electrode was made of two strips of pure Ti, each having a surface area of approximately 10 cm^2. The volume of the cell was about 2000 mL and the electrolyte for anodization was prepared by dissolving 24 mL of 50 % HNO_3 solution in 2400 mL methanol. Anodization of Ti sample was performed under constant cell voltage. The Ti substrates were introduced in the cell and the initial current density was 5 mA/cm^2. Afterwards, the samples were cleaned with deionised water.

The samples were then immersed into 0.5 M NaOH solution at 160 °C in a pressure chamber for 24 h with a heating rate of 5 °C/min. The samples were subsequently washed in deionized water for 5 min and finally heat-treated at 600 °C for 3 h in a furnace with a heating rate of 5 °C/min.

2.3. Coating solutions

In order to simulate the *in vivo* process, the pre-treated titanium samples were directly immersed into 200 mL SBF solution contained in a glass beaker kept at 37 °C in a shaking water bath. The SBF solution was refreshed every 6 h in order to keep the ion concentration stable. The titanium samples were taken out of the solutions after certain periods of immersion, rinsed with deionized water, followed by drying in an oven for 1 h at 37 °C.

2.4. Samples characterization

The morphology of the coatings was studied by scanning electron microscopy (SEM) with QUANTA 200 3D microscope (FEI, Netherlands). Silver sputtering was used to make the coating surfaces conductive for the SEM investigations. The coatings were characterized by X-ray diffraction (XRD) with X'PERT PRO MRD diffractometer (PANalytical, Netherlands) using monochromatic CuKα radiation (λ = 0.15418 nm), operating at 40 kV and 50 mA over a 2θ range from 2° to 70°. The FTIR

spectra of the coatings were recorded on a DIGILAB SCIMITAR-SERIES spectrophotometer with an attenuated total reflectance (ATR) accessory. Measurements were carried out in the attenuated total reflectance mode using a ZnSe prism, between 400 and 4000 cm^{-1}, with resolution setting 4 cm^{-1}.

3. Results and Discussion

Much research has been dedicated to the coating of the orthopedic and dental implants with porous ceramics, such as hydroxyapatite, to increase *in vivo* the hard tissue integration [14]. Several methods have been developed to obtain films of hydroxyapatite on metallic substrate, like the biomimetic method [19-21]. The biomimetic process is a physicochemical method in which a substrate is soaked in a solution that simulates the physiologic conditions, for a period of time enough to form a desirable layer of hydroxyapatite on the substrate. Chemical immersion in SBF solutions have been shown to obtain apatite coatings on the order of 10 - 100 μm of thickness, which are very homogenous and would be more favourable to the biological interaction of the titanium implants [19].

The coating method used in this study consisted in following major steps: mechanical, anodization and alkali ÷ thermal oxidation pre-treatments of the samples, and finally biomimetic treatment by samples incubation in the Simulated Body Fluid (SBF) solution.

Figure 1a shows the surface morphology of a titanium substrate after mechanical grinding with silicon carbide paper and chemical etching in 1 % HF solution for 2 min.

The surface morphology of the titanium oxide coating obtained by anodization method on the titanium substrate pre-treated in this way is shown in figure 1b. The surface appearance of the oxide coating was similar to that of the mechanical treated substrates, but was more round and smooth. This is attributed to the dissolution of titanium during the anodization process. The formation of the titanium oxide coating on the titanium substrate occurs by dissolution - precipitation mechanism. SEM micrographs indicate that anodization pre-treatment affects the surface roughness and surface area of the oxide coatings because the morphology of the oxide coatings is dependent on the morphology of the substrate. This is significant because the surface morphology is very important for the performance of the implants.

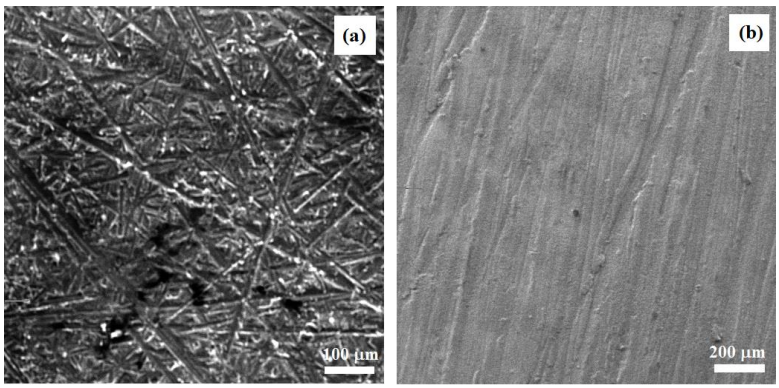

Figure 1. SEM micrographs of the titanium surface after mechanical grinding (a) and after anodization pre-treatment (b).

After alkaline pre-treatment, a sodium titanate ($Na_2Ti_5O_{11}$) hydrogel layer is formed on the surfaces of the titanium samples. The gel was found to be densified after heat treatment and the crystalline sodium titanate was formed on the titanium surface (figure 2a).

The structural characteristics of the coatings studied by XRD (figure 2b) indicate the sodium titanate presence on the titanium surface.

The FTIR spectrum of the sample after alkali ÷ thermal oxidation pre-treatment shows the existence of the sodium titanate on the titanium surface. Two strong bands at around 604 cm^{-1} and 1198 cm^{-1} can be seen in figure 2c which corresponds to the titanate groups. Also, two strong bands at around 855 cm^{-1} and 1468 cm^{-1} are due to Na–O bonds. Molecular water band appears at 1695 cm^{-1}.

The Ti–OH groups and the hydrogen bonds are characterized by a broad band in the range of 2700 – 3700 cm^{-1}.

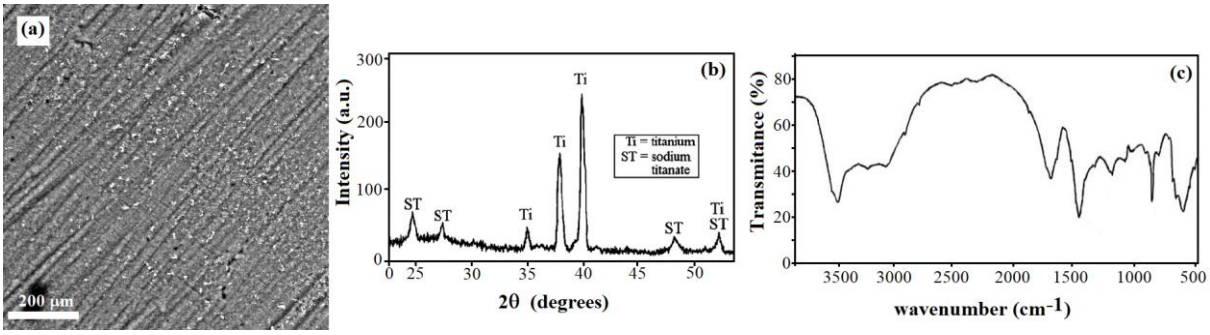

Figure 2. SEM micrograph (a), XRD pattern (b) and FTIR spectrum (c) of the titanium surface after alkali ÷ thermal oxidation pre-treatment.

The deposition of the hydroxyapatite layer on the titanium surface was realised by biomimetic treatment using a SBF solution. A series of micrographs illustrating the surface appearance after different periods of immersion in the SBF solution shows the different steps of the nucleation and growth of the hydroxyapatite layer on the titanium surface (figure 3). Firstly, a few hydroxyapatite nuclei appeared after soaking in SBF for 24 h (figure 3a). Once the first nuclei have appeared, it is found that the growth of the hydroxyapatite layer is fast enough. After soaking in the SBF for 168 h, a hydroxyapatite layer covering completely the titanium surface is observed (figure 3b). This layer consists of crystals of 1 – 1.5 µm in size showing petal rose-like morphology (figure 3c).

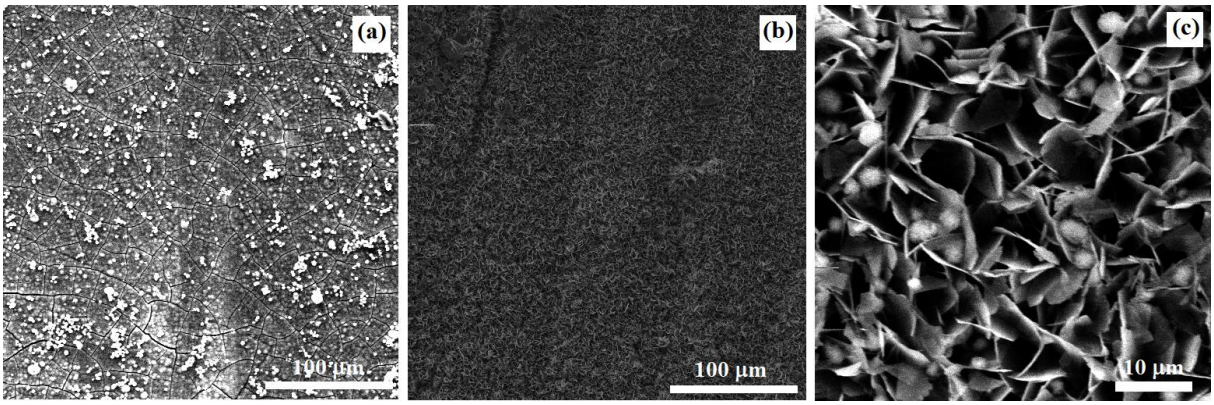

Figure 3. SEM photographs of the titanium surface after soaking in SBF solution for 24 h (a) and for 168 h (b and c) (low magnification and high magnification)

In the XRD pattern of the titanium surface after soaking in SBF solution for 168 h (figure 4a), the hydroxyapatite peaks can be observed at different degrees. The XRD patterns in figure 4a indicate that the sample has the characteristic peaks in the 2θ regions of 21–29º, 32–34º, 39–41º, 46–54º, in good agreement with the hexagonal (space group $P6_3/m$) hydroxyapatite phase (JCPDS Data Card09-0432). All peaks are very sharp, indicating that hydroxyapatite is well crystallized. The presence of the Ti peaks indicates that the deposited layer is thin, allowing penetration of X-ray beam up to the substrate surface.

The FTIR spectrum of the sample after biomimetic treatment in SBF solution shows the existence of the hydroxyapatite crystals on the titanium surface (figure 4b). The bands at 603 cm^{-1} and 569 cm^{-1} were assigned to the O–P–O bending mode. The bands in the 1000 – 1200 cm^{-1} region, due to PO_4^{-3}

groups, are characteristic to the hydroxyapatite, while the band at 1600 cm^{-1} is due to the absorbed water.

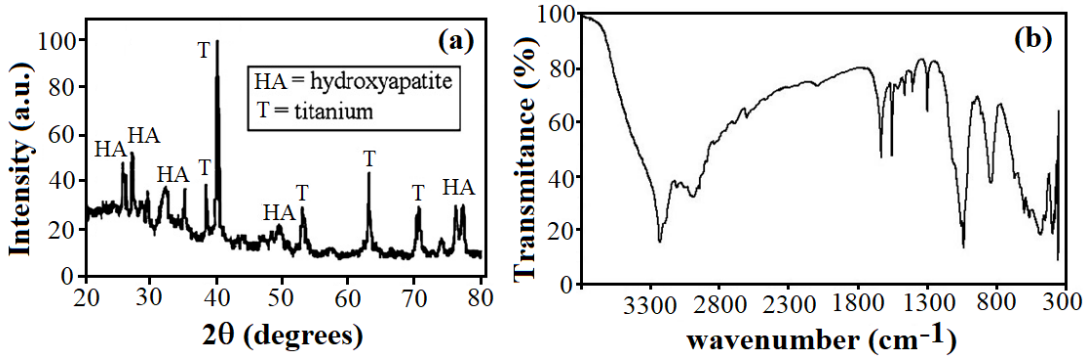

Figure 4. XRD pattern (a) and FTIR spectrum (b) of the hydroxyapatite crystals formed on the titanium surface after incubation in SBF solution for 168 h.

The hydroxyapatite formation on the titanium surface is an indicator of its bioactivity. The obtained results suggest that the method utilized in this work can be successfully applied to obtain hydroxyapatite coatings on the titanium surface.

4. Conclusions

This study presents a combined method based on biomimetic approach to grow the hydroxyapatite layers on the titanium surface. Firstly, the titanium oxide coatings were fabricated on the titanium surface by anodization in a methanolic electrolyte. The titanium oxide coated titanium samples were treated then in a NaOH solution and sodium titanate formation has been confirmed. The titanium samples after these pre-treatments were incubated in a simulated body fluid (SBF) solution for different period to study the hydroxyapatite formation. All the alkali-treated titanium oxide coatings could induce hydroxyapatite formation on titanium surface after incubation in SBF solution. The obtained results indicate that these treatments can induce the hydroxyapatite formation on the titanium surface.

5. References

[1] Saini M, Singh Y, Arora P, Arora V and Jain K 2015 Implant biomaterials: a comprehensive review *World Journal of Clinical Cases* **3** pp 52-57
[2] Tschernitschek H, Borchers L and Geurtsen W 2005 Nonalloyed titanium as a bioinert metal - a review *Quintessence International* **36** pp 523-530
[3] Sul Y T, Johansson C B, Kang Y, Jeon D G and Albrektsson T 2002 Bone reactions to oxidized titanium implants with electrochemical anion sulphuric acid and phosphoric acid incorporation *Clinical Implant Dentistry and Related Research* **4** pp 78-87
[4] Hamouda I M, Enan E T, Al-Wakeel E E and Yousef M K 2012 Alkali and heat treatment of titanium implant material for bioactivity *International Journal of Oral and Maxillofacial Implants* **27** pp 776-784
[5] Wang X, Li Y, Lin J Hodgson P D and Wen C 2008 Effect of heat-treatment atmosphere on the bond strength of apatite layer on Ti substrate *Dental Materials* **24** pp 1549-1555
[6] Osman R B and Swain M V 2015 A critical review of dental implant materials with an emphasis on titanium versus zirconia *Materials* **8** 932-958
[7] Ferguson S J, Broggini N, Wieland M, de Wild M, Rupp F, Geis-Gerstorfer J, Cochran D L and Buser D 2006 Biomechanical evaluation of the interfacial strength of a chemically modified sandblasted and acid-etched titanium surface *Journal of Biomedical Materials Research Part A* **24** pp 291-297

[8] Grosgogeat B, Reclaru L, Lissac M and Dalard F 1991 Measurement and evaluation of galvanic corrosion between titanium/Ti6A14V implants and dental alloys by electrochemical techniques and auger spectrometry *Biomaterials* **20** pp 933-941
[9] Heimann R B 2006 Thermal spraying of biomaterials *Surface and Coatings Technology* **201 (5)** pp 2012-219
[10] Kuroda K and Okido M 2012 Hydroxyapatite coating of titanium implants using hydroprocessing and evaluation of their osteoconductivity *Bioinorganic Chemistry and Applications* **volume 2012**, article ID 730693, 7 pages
[11] Ciobanu G, Bargan A M and Luca C 2015 New bismuth-substituted hydroxyapatite nanoparticles for bone tissue engineering *JOM* **67 (11)** pp 2534-2542
[12] Ciobanu G, Bargan A M and Luca C 2015 New cerium(IV)-substituted hydroxyapatite nanoparticles: preparation and characterization *Ceramics International* **41 (9)** pp 12192-12201
[13] Dorozhkin S V 2010 Bioceramics of calcium orthophosphates *Biomaterials* **31** pp 1465-1485
[14] Hung K Y, Lo S C, Shih C S, Yang Y C, Feng H P and Lin Y C 2013 Titanium surface modified by hydroxyapatite coating for dental implants *Surface & Coatings Technology* **231** pp 337-345
[15] Ciobanu M G, Pop G and Ciobanu O 2007 Hydroxyapatite coatings on titanium implants *Revista de Chimie* **58 (12)** pp 1313-1315
[16] Ciobanu G, Carja G, Ciobanu O, Sandu I and Sandu A 2009 SEM and EDX studies of bioactive hydroxyapatite coatings on titanium implants *Micron* **40 (1)** pp 143-146
[17] Ciobanu G and Ciobanu O 2013 Investigation on the effect of collagen and vitamins on biomimetic hydroxyapatite coating formation on titanium surfaces *Materials Science & Engineering C-Materials for Biological Applications* **33 (3)** pp 1683-1688
[18] Nazir M, Ting O P, Yee T S, Pushparajan S, Swaminathan D and Kutty M G 2015 Biomimetic coating of modified titanium surfaces with hydroxyapatite using simulated body fluid *Advances in Materials Science and Engineering* **volume 2015**, Article ID 407379, 8 pages
[19] Kokubo T and Takadama H 2006 How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials* **27** pp 2907-2915
[20] Coelho MFC, Cronemberger MER, Pereira JN, Nakamatsu S, Maestrelli SC, da Rigo ECS and Mariano NA 2014 A comparative study of biomimetic coatings on titanium and stainless steel *Materials Science Forum* **802** pp 440-445
[21] Barrere F, Layrolle P, van Blitterswijk C and de Groot K 2001 Biomimetic coatings on titanium: a crystal growth study of octacalcium phosphate *Journal of Materials Science: Materials in Medicine* **12 (6)** pp 529-534