IN VITRO CHARACTERIZATION OF HYDROXYAPATITE LAYERS DEPOSITED BY APS AND HVOF THERMAL SPRAYING METHODS

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Titanium alloys are successfully used in medicine as implants due to their high mechanical properties and good biocompatibility. To improve implant osseointegration of titanium alloys, they are covered with hydroxyapatite because of its bioactive properties. Coating the implants with hydroxyapatite by thermal spraying, due to the temperatures developed during the deposition process, the structure can be degraded, leading to formation of secondary phases, such as TCP, TTCP, CaO. The paper presents the experimental results of hydroxyapatite layers deposition by two thermal spraying methods: Atmospheric Plasma Spraying (APS) and High Velocity Oxy-Fuel (HVOF). The microstructure of the deposited layers is characterized by X-ray diffraction analysis and electronic microscopy. The bioactivity of the hydroxyapatite layers was investigated in Simulated Body Fluid (SBF) by immersing the covered samples deposited by the two thermal spraying methods. In both cases the coatings did not present defects as cracks or microcracks. X-ray diffraction performed on hydroxyapatite deposited layers shows that the structure was strongly influenced by plasma jet temperature, the structure consisting mainly of TCP ($Ca_3PO_4)_2$. The samples deposited by HVOF after immersing in SBF lead to formation of biological hydroxyapatite, certifying the good bioactivity of the coatings.

INTRODUCTION

Titanium and its alloys are widely used in the manufacturing of dental and orthopedic implants due of their superior mechanical properties, low density, high corrosion resistance and excellent biocompatibility [1].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) is a bioactive ceramic material that has the chemical composition and structure similar to human bone, thereby facilitating integration of the implants and prostheses in bone tissue [2]. Because of its low mechanical properties, hydroxyapatite can not be used to bulk implants but it is used to cover them by various methods such as thermal spraying [3], laser [4], and sol-gel [5], electrochemical [6]. A common feature of ceramic materials with bioactive properties is the modification of their surface reactivity immediately after implantation; on the surface it forms a layer of carbonated hydroxyapatite (CHA), biologically active, forming a connection interface with the bone [7]. CHA phase, so formed, is chemically and structurally equivalent mineral phase with the bone that is responsible for formation of a stable bonding at implant-bone interface [8]. Clinical studies show that a bioactive hydroxyapatite layer will allow a bonding osteogeneses which is able to bear the complex forces that occur during implant using [9]. In this case, there are two processes of ossification, the first one manifested by development of bone to the implant and the second one from the implant to the bone tissue [10]. It was demonstrated that the existence of long-term stable bioactive hydroxyapatite coating of 150μm will elicit a specific biological response at the interface of the implant material by controlling its surface chemistry through adsorption of non-collageneous proteins such as osteocalcin, osteonectin, silylated glycoproteins and proteoglycanes [11]. This will create a strong osseoconductive bond between the implanted biomaterial and the natural tissue [12]. Another advantage of using bioactive coatings is that the material protects the body of metal ions releasing from the metallic implant. The release of metal ions can lead to effect of body defense by creating antibodies and forming a membrane around the implant (implant isolating). This membrane prevents the implant fixation in the body thus leading to implant failure [13]. On the apatite surface layer, bone-producing cells (osteoblasts) may proliferate rather than the fibrous tissue cells (fibroblast) as long as the structure and composition of the apatite layer is similar with the bone apatite [14]. Consequently, the surrounding bone can grow and can come in direct contact with the apatite layer, without the intervention of the fibrous tissue. When this occurs, it forms a chemical bond between the layers close to bone apatite, reducing the interfacial energy [15].

The performance of hydroxyapatite coatings depo-

sited by thermal spraying methods depends by the porosity, degree of particles deformation, the presence of cracks and microcracks, the residual stresses in coating-substrate interface, the biochemical resistance against aggression fluid in the body depending on the crystalline property of hydroxyapatite coating and the presence of amorphous calcium phosphate (ACP) [16]. In many cases, the failure of hydroxyapatite-coated implants is due to the amorphous calcium phosphate layer which is formed due to rapid cooling of molten or semi-molten particles droplets, a phenomenon that is mainly observed in the processes that develop high temperatures (plasma thermal spraying) [17]. This continuous layer of ACP has high solubility in biological environment, leading to a decrease in implant-tissue interface connection, leading to implant failure [18].

Past research has shown that plasma spraying normally leads to undesirable phase change to the hydroxyapatite. The coatings resulted by this method contain many bioinert or bioresorbable phases such as tetracalcium phosphate (TTCP), tricalciumphosphate (TCP), calcium oxide (CaO) and amorphouscalcium phosphate (ACP) [19]. These phases are rapidly soluble in human blood plasma and can cause implant instability after some time of implantation [2]. HVOF method is also used for realization HA coatings, the degree of the secondary bioresorbable phases is lower compared with plasma spraying [3].

Optimization HA coatings can be achieved by controlling the thermal spraying parameters. However, decomposition of hydroxyapatite during the thermal spraying process is inevitable because its melting temperature of 1570°C [22].

By using HVOF thermal spraying methods, together with thermal spraying gun specially designed to develop lower temperatures compared to other HVOF spraying guns but at supersonic speeds, it is estimated that the structure of hydroxyapatite coatings realized by this method will suffer smaller structure changes. The results will have an important influence to bioactivity evaluation of the coatings.

EXPERIMENTAL

Materials used

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) powders were used as deposition materials, with average particle size ranging between 5 to 15 μm provided from Sigma-Aldrich firm. As for the substrate titanium alloy (Ti6Al4V) discs Ø 30×10 mm provided from Bibus Steel Company were used.

Before spraying, the titanium samples were blasted with alumina with the average particle size of 1 mm at the pressure blast of 6 bars and distance of 50-60 mm. After blasting, the samples were cleaned with ethylic alcohol.

Thermal spraying equipments

Atmospheric Plasma Spraying (APS) from Sulzer Metco SUA and High Velocity Oxygen Fuel spraying HVOF from Thermico Germany GmbH equipments were used to perform HA coatings. The parameters used for depositions of HA layers by atmospheric plasma spraying are presented in Table 1. As plasmagen gas Ar + 6 % H_2 was used and as transport gas argon was used.

Table 1. Parameters used for depositions of HA coatings by APS.

Plasma current (A)	Plasma voltage (V)	Primary gas flow (l/min)	Carrier gas flow (l/min)	Powder feed rate (g/min)	Spray distance (mm)
400-500	60-75	40-60	10-15	10-15	90

For the deposition of HA layers by HVOF method was used ID Cool Flow spraying gun, which operates at lower power levels than other HVOF spraying guns, ensuring a lower temperature of the powders and the substrate but the supersonic speeds of the gas stream will assure dense coatings with good adhesion. Thus, it is estimated that by using this type of thermal spraying gun, the hydroxyapatite structure will suffer small degradations. The parameters used for deposition of the HA layers by HVOF method are showed in Table 2.

Table 2. HVOF spraying parameters of HA coatings.

Oxygen (l/min)	Hydrogen (l/min)	Kerosene (l/h)	Carrier gas N_2 (l/min)	Spray distance (mm)	Deposition rate (g/min)
300-320	90-95	2.8-3	15-20	70	15

The thickness of hydroxyapatite coatings deposited by APS and HVOF method has values of about 150 μm. The average surface roughness of the coatings has values of 5.23 μm for APS and 5.11μm for HVOF method.

Characterization of surface morphology

Scanning electron microscope (SEM) Inspect S was used to characterize the surfaces of the coatings. The phase composition of the deposited layers was investigated by X ray diffraction (XRD) using Dron 3 equipment. The working conditions were 40 kV and 30 mA, using copper radiation with the wavelength λ = 1.541Å. The microlayers thickness was determined using Easy Check F-N device and the surface roughness determination was made by Surftest 201 (SJ-201) device from Mitutoyo.

Soaking in the simulated body fluid (SBF)

The Kokubo simulated body fluid (SBF) [23] (pH = 7.40) was used for the in vitro incubation for 21 days at 37°C. The solution is composed of 142.0 mM Na^+, 5.0 mM K^+, 1.5 mM Mg^{2+}, 2.5 mM Ca^{2+}, 147.8 mM Cl^-, 4.2 mM HCO_3^-, 1.0 mM HPO_4^{2-}, and 0.5 mM SO_4^{2-}. The samples were immersed in polyethylene bottles and after immersion were cleaned with distilled water and investigated by SEM and XRD. Investigating the biological behaviour of biomaterials in this simulated body fluid is considered as the most efficient and economical way to predict their bioactivity in body environment [24].

RESULTS AND DISCUSSIONS

XRD characterization

Figure 1 shows the X ray pattern of hydroxyapatite powder used for the deposition by the two spraying methods.

The X ray pattern shows that hydroxyapatite powder does not contain amorphous phases like TCP, TTCP and CaO. Figure 2 presents the X ray pattern of hydroxyapatite layer deposited by APS before and after immersion in SBF.

It is noted that after the deposition of the HA layers by APS method, the structure has undergone significant changes, it was decomposed into amorphous phase leading to the formation of TCP ($Ca_3(PO_4)_2$). This is due to high temperatures during the thermal spraying process (≈ 15 000°C). Once the critical point is exceeded there is complete and irreversible dehydration of hydroxyapatite [25].

Hydroxyapatite has a high stability at pH values above 4.3, with bioinert an inhibitory effect on cell proliferation. Decomposition of hydroxyapatite leads to formation of amorphous calcium phosphate (ACP), tricalcium phosphate (TCP), tetracalcium phosphate (TTCP) and calcium oxide (CaO) and dehydroxylation produces oxyhydroxyapatite (OHA) and oxyapatite (OA) that are suitable solubility in the liquid that simulates human body [26].

Dissolution of unstable phases in the coatings is undesirable because it leads to reduced mechanical resistance of the coating process which can lead to implant failure. Analyzing the results of X-ray diffraction of the hydroxyapatite layer deposited by atmospheric plasma spraying and immersed in SBF for 21 days it can be observed that the structure presents no notable modification, due to the decomposition of hydroxyapatite in TCP amorphous phase. Figure 3 shows the X-ray diffraction analysis of hydroxyapatite coatings deposited by HVOF thermal spraying method, before and after immersing in SBF for 21 days.

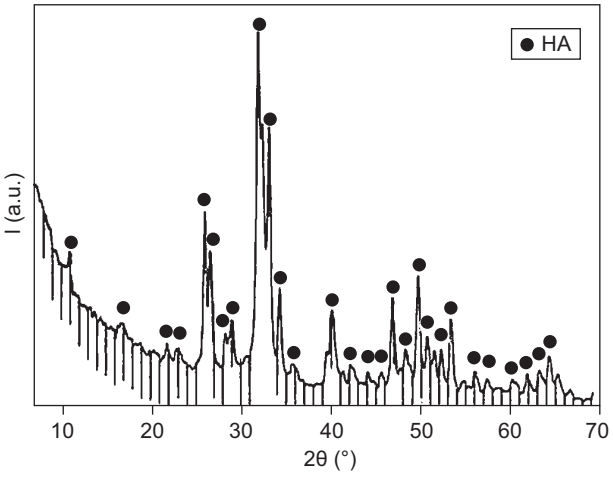
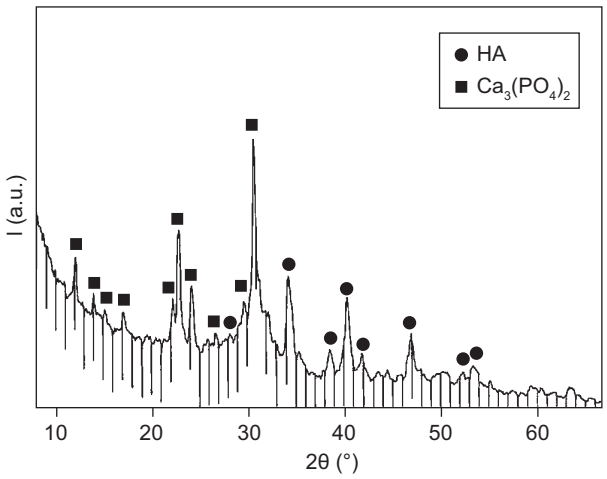
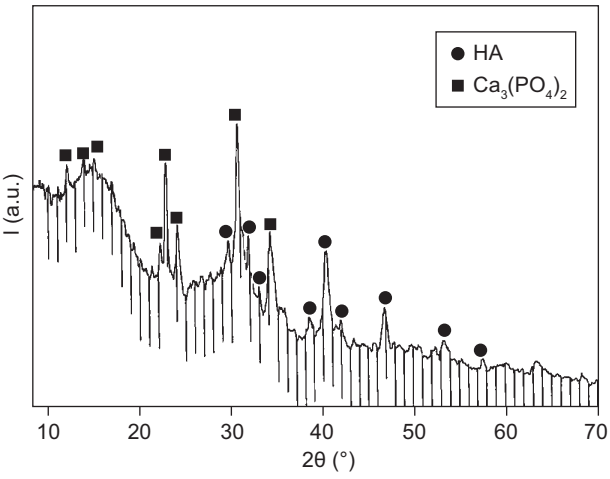


Figure 1. X-ray diffraction pattern of hydroxyapatite powder.

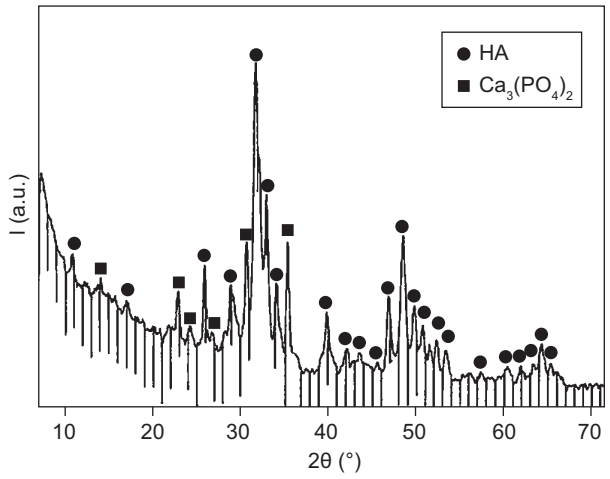


a)

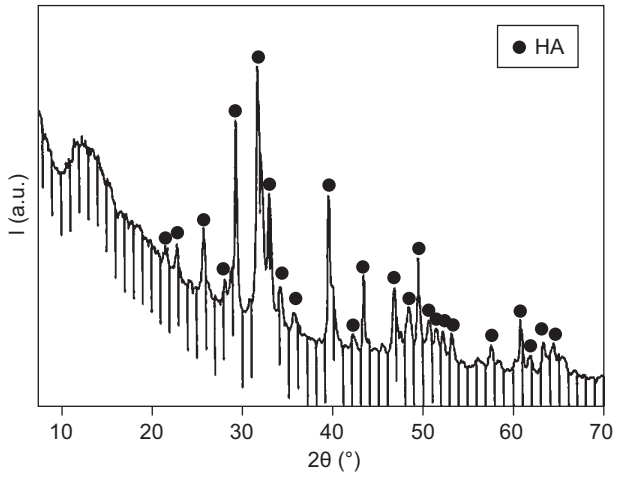


b)

Figure 2. X-ray diffraction pattern of the HA coating: a) deposited by APS, b) after 21 days of immersion in SBF.



a)



b)

Figure 3. X-ray diffraction pattern of the HA coating: a) deposited by HVOF, b) after 21 days of immersion in SBF.

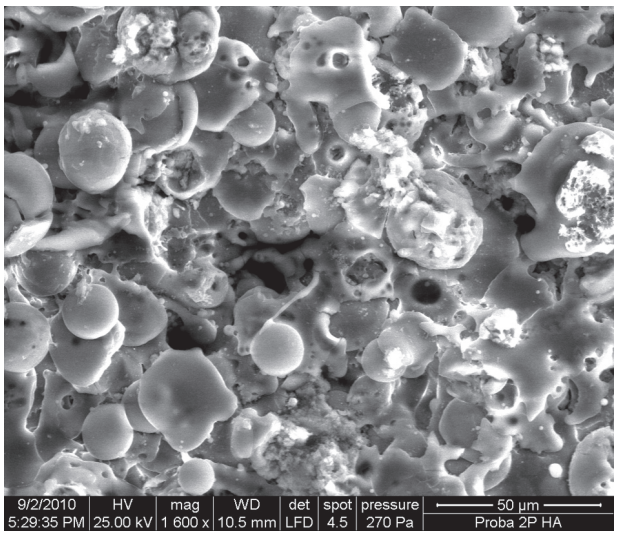


Figure 4. SEM image of as-sprayed HA coatings by APS.

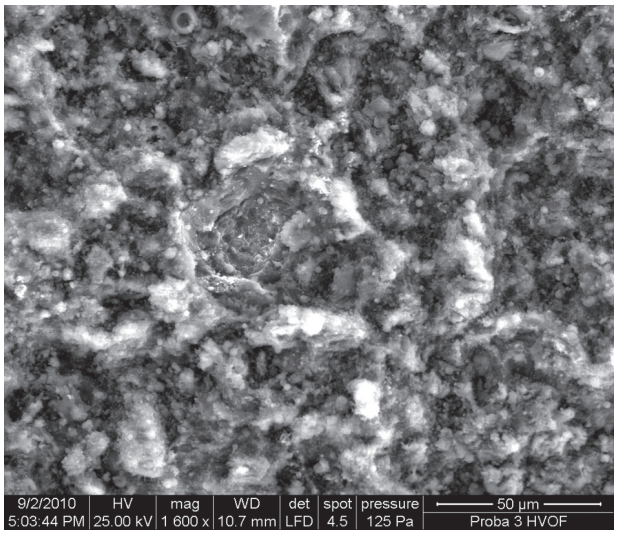


Figure 5. SEM image of as-sprayed HA coatings by HVOF.

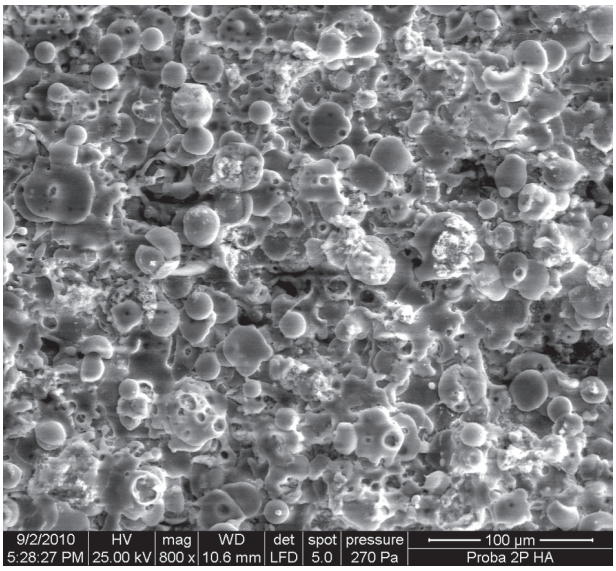
It is observed that after deposition of the HA layers by HVOF method the structure suffered minor modifications in comparison with the structure obtained by APS depositing, by forming small amounts of tricalcium phosphate (Figure 3a). After immersion of the HA coatings deposited by HVOF in SBF for 21 days the X ray analysis showed the presences of hydroxyapatite (Figure 3b). This is due to reactions of the deposited layer and the elements from the SBF reactions which lead to the formation of biological apatite, showing a good biocompatibility of the layers obtained in this way.

The microstructure of hydroxyapatite coatings

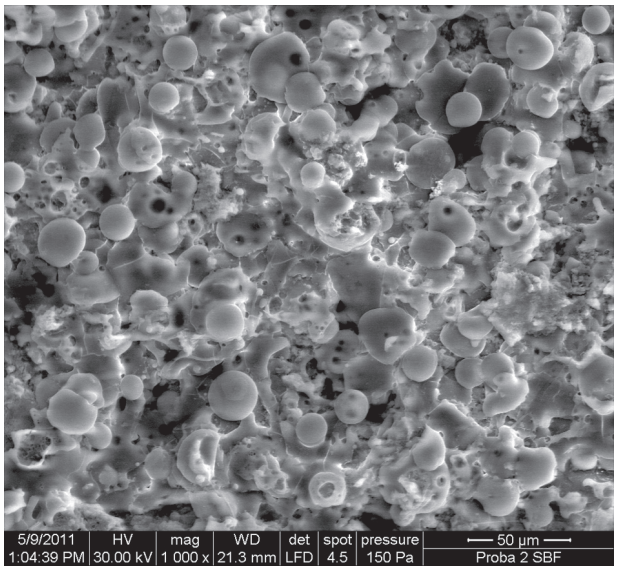
Figure 4 shows the SEM image of HA coatings deposited by APS and in Figure 5 is presented the SEM image of HA coatings deposited by HVOF.

Analyzing the surface morphology of HA coatings deposited by the two methods it can be seen that the layer deposited by APS consists of spherical particles and lamellar particles, while the layer deposited by HVOF method consists of flattened particles. This is due to the high speeds during the thermal spraying process, resulting a lamellar structure. Figure 6 shows the SEM images of HA coatings and cross-section deposited by APS after immersion in SBF for 21 days.

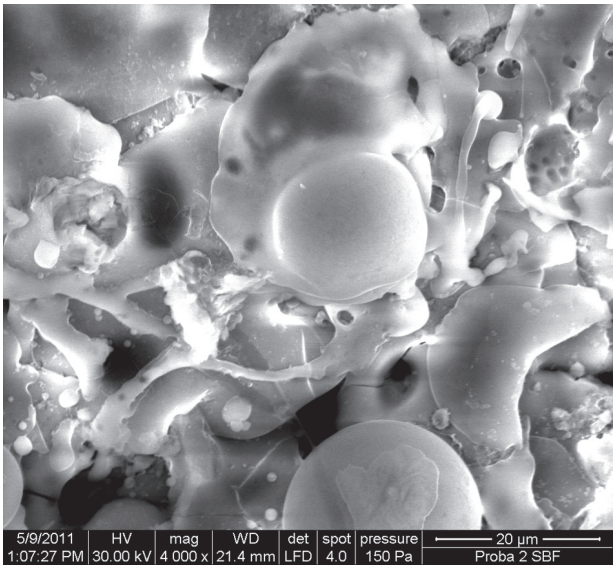
SEM images of hydroxyapatite coatings deposited by plasma spraying shows that the particle morphology remained unchanged compared with the structure before SBF test. This is due to the presence of TCP in the structure in a high percentage compared to that of hydroxyapatite. It was also observed a small germination of hydroxyapatite at higher magnification. Figure 7 shows the SEM images of hydroxyapatite coatings deposited by HVOF method after immersion in SBF for 21 days.



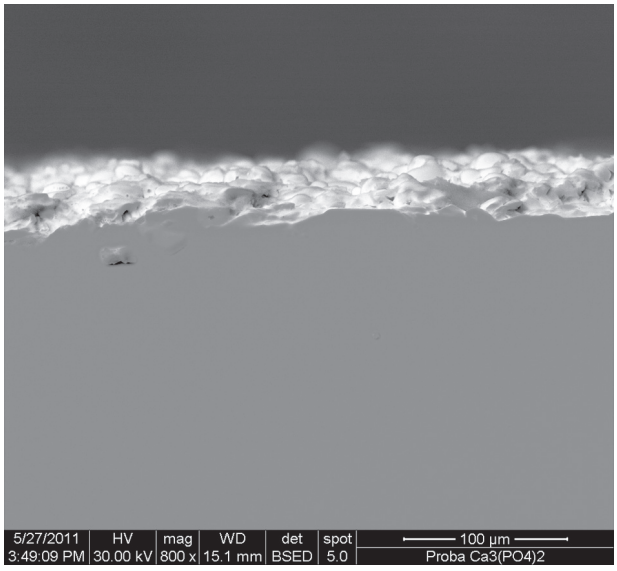
a) 800×



b) 1000×



c) 4000×



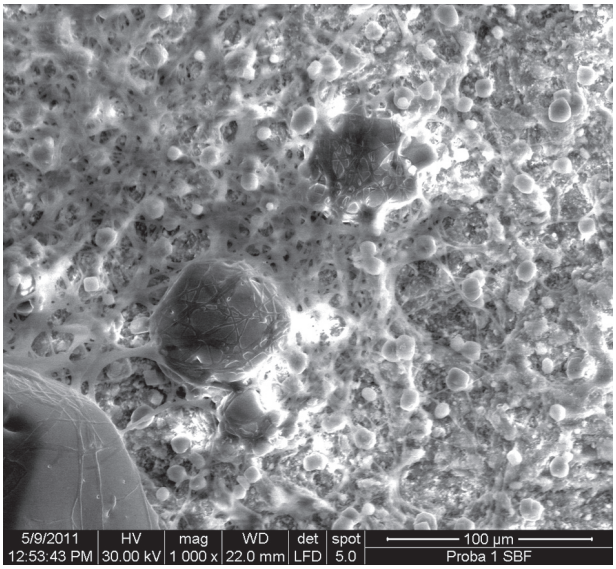
d) cross section, 800×

Figure 6. SEM images of HA coatings deposited by APS after SBF 21 day of immersion in SBF.

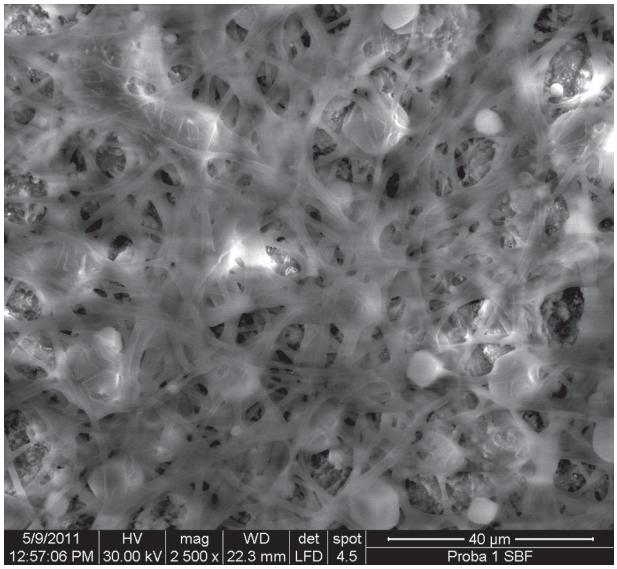
SEM images show the germination of the biological hydroxyapatite after the immersion in SBF. Tests have shown that the formation of the superficial layer of apatite starts immediately after immersing of the samples in SBF. After 21 days of SBF immersion, the microscope images show that on the surface of the coatings grew hydroxyapatite crystals of different dimensions. By developing of the biological hydroxyapatite it is shown that the layer deposited has a good bioactivity attesting that this method is suitable for deposition HA bioactive coatings. This highlights the fact that an essential condition required to coated implants with bioactive materials, to obtain their contact with the living tissue, is the formation on their surface of an apatite layer which is similar to bone, after the introduction in SBF. Once the apatite crystals are formed, they can grow spontaneously by consuming calcium and phosphate ions from the surrounding fluid. Also on the surface apatite layer formed are observed interconnected pores which will have a favourable effect of anchoring of the prosthesis to the bone, preventing implant separation.

CONCLUSIONS

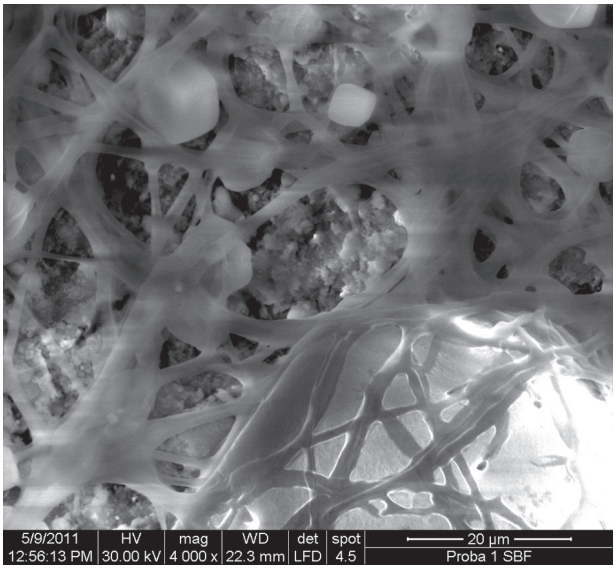
1. X-ray diffraction analysis of hydroxyapatite coatings deposited by APS thermal spraying method shows that due to the high temperatures during the spraying process resulted in a significant degradation of the hydroxyapatite structure, which decomposed into TCP. Using the HVOF method it is noted that



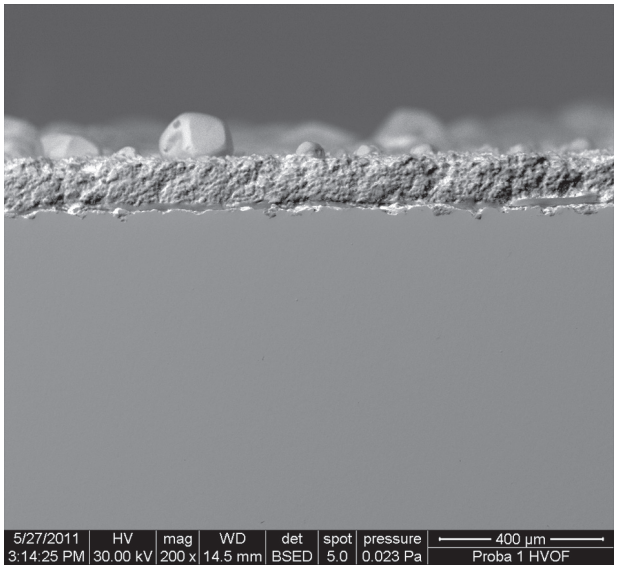
a) 1000×



b) 2500×



c) 4000×



d) cross section, 200×

Figure 7. SEM images of HA coatings deposited by HVOF after SBF 21 day of immersion in SBF.

HA structure presents small degradations during the thermal spraying process, leading to small quantities of TCP. This is due to lower temperatures during the HVOF thermal spraying process.

2. Hydroxyapatite coatings deposited by both methods (APS and HVOF) showed no thermal spraying defects such as cracks or exfoliations. The morphology of the coatings made by HVOF method consists of flattened particles, due to the high velocity of the gas stream.
3. Biocompatibility tests carried out by immersing in SBF of hydroxyapatite coatings showed that the coatings deposited by APS method, due to the decomposition of hydroxyapatite in amorphous phase (TCP) after immersion in SBF a reduced germination of hydroxyapatite was observed compared with hydroxyapatite coatings deposited using the HVOF method where after immersion in SBF was observed the formation of biological hydroxyapatite, which indicates a good biological activity of the layer.
4. In both spraying methods the roughness values of the surfaces is of about 5 µm, which will ensure a good osseointegration of the implants due to increased contact area between the bone tissue and implant.

Acknowledgement

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