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STUDY ON BIOLOGICAL HYDROXYAPATITE DEPOSITION ON TITANIUM SUPPORT IN A BLOOD PLASMA-LIKE SOLUTION

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The overall objective of this study is to improve the quality of life by increasing the lifetime of implants and reducing the healing and rein
tegration period, generally following a maxillo-facial and reconstructive surgery. Thus the aim was to increase the lifetime of metallic implants by coating them with a micron layer of biological hydroxyapatite supposed to chemically react with the receiving bone bed as well as to determine the morphological characteristics, the physical and technological properties of the titanium implants, chemically coated with biological hydroxyapatite.

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Keywords: Blood plasma-like solution, Biological hydroxyapatite, Implant, Chemical bonds

1. Introduction

The biomaterials initially used to manufacture the implanting devices, on contact with the elements of the biological systems, were general-purpose materials, meeting to a more or less extent the specific requirements in the medical field, being known as first generation biomaterials. Such biomaterials combine the capacity of being tolerated by the body with some properties of the material, that are appropriate to the medical applications. In recent years, the second generation biomaterials have been developed which are synthesized considering the existence and control of the physical, chemical and biological processes at the implant/tissue interface, so as to stimulate the normal cellular processes (1, 2).

Metallic biomaterials are used in various applications, of which the most important are the orthopedic, dental and cardiovascular ones (3).

In dentistry, titanium and its alloys have emerged as an interesting alternative to the noble alloys, being present in the therapeutic arsenal since the 80s and initially used mainly in the fabrication of the implants and then even more frequently extended to dental prosthetics and orthodontics. The more and more use in dentistry of the titanium and its alloys is justified by some exceptional properties of these materials: high resistance to corrosion, excellent biocompatibility,

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low density, low thermal conductivity, low thermal expansion coefficient, X-ray translucence, odorless and tasteless property, low cost (4, 5).

While the technical fields use especially the mechanical and chemical properties of the material (high specific strength, low thermal conductivity, elasticity, special resistance to corrosion), the medical industry exploits mainly the biocompatibility of titanium, clinically tested for nearly half a century in various specialties like orthopedics, surgery and implantology. This propitious association of biocompatibility with certain advantageous mechanical properties of titanium justify its unprecedented spread in most medical specialties.

Titanium attracted the attention of the dental world through its particularly advantageous properties: biocompatibility, low thermal conductivity, low density, corrosion resistance, odorless and tasteless quality, the cost of the material being four times lower than that of gold. In order to extend the lifetime of titanium implants there were developed many methods for metal coating with bio-active layers intended to increase its resistance (6).

The objective of the study is the extension of metallic implants lifetime by coating them with a micron layer of biological hydroxyapatite able to chemically react with the receptor bone bed. At the interface is achieved a strong bond and is prevented the formation of a fibrous layer, being increased in this way the implant lifetime.

On worldwide scale, to fulfill this, researches are carried out by various methods. We chose a method of chemical deposition of hydroxyapatite from a blood plasma-like solution, on the surface of titanium or titanium alloys implants, previously activated by alkali treatment, followed by an appropriate heat treatment.

2. Materials and methods

We tried to develop a biomimetic method for producing a calcium phosphate layer at the junction of a metal support with the natural bone. This method consists in obtaining a biologically active layer of hydroxyapatite (a form of calcium phosphate existing in the bone structure) following the immersion of a metallic support, in this case, titanium or its alloys, in artificially prepared supersaturated solution of calcium and phosphorus, blood plasma-like (SBF), with a view to achieve a hydroxyapatite layer chemically bonded to the surface of the implant. When an implant is placed, osseointegration reactions usually occur faster and the bonds are much stronger and longlasting (7, 8).

The method allows the cold deposition of a layer of biological hydroxyapatite on titanium or titanium alloys support, by immersion in a liquid chemically similar to the mineral part of the blood plasma (SBF).

The biomimetic coating process is defined as a method thereby a layer of biologically active hydroxyapatite is formed after the immersion of a substrate in an artificially prepared supersaturated solution of calcium and phosphate ions, such as SBF. Considering that the most accessible method of obtaining titanium implants coated with hydroxyapatite is the biomimetic method of chemical deposition by keeping the metal substrate in a blood plasma-like solution (SBF), we present below the technological process:

For the experiments we used three titanium alloys of different purity: Ti_6Al_2NbTa, $Ti_{15}Mo_5Zr_3Al$ and Ti_6Al_4V. The first two samples were denoted by P1 and P2 and Ti_6Al_4V alloy by P3. P1 was cut as rings with a diameter of 5 mm and a thickness of 2 mm, P2 as a parallelepiped (6 x 6 x 2 mm) and P3 alloy as rings with a diameter of 10 mm and a thickness of 2 mm.

In order to change the morphology of titanium and titanium alloys surface we chose two methods: sandblasting and abrasion (9). After cutting, a part of the samples were sandblasted with SiO_2 grits to increase the reacting surface and to create some defects contributing to the osseointegration of the implant immediately after implantation, and the other part of the samples were abraded by polishing with diamond files, thus getting a surface with roughness of up to 0.5 mm.

The samples were cleaned with acetone, ethanol and distilled water and then their surface was attacked by alkali solution (10M NaOH) (9, 10, 11) at 60°C for 24 hours. On their surface a layer of sodium titanate was formed. Then the samples were washed with distilled water and dried

at 40°C for 24 hours. After that they were heated at 600°C in a programmed electric oven, as follows (Table 1):

Table 1. The heat treatment applied to samples.

Range	Speed
25 - 600°C	300°C/h
600°C level	1 hour
600°C - 30°C	100°C/h

As a result of the heat treatment, a density increase of the sodium titanate layer was obtained. After cooling, the samples were washed for 3 times with distilled water and then with 70% ethanol solution; then the surface was examined by electronic microscopy scanning (EMS).

For the deposition on the implants surface of a carbonated hydroxyapatite (HCA) layer, similar to the biological hydroxyapatite present in the bone structure, two solutions were prepared:

- a solution similar to the mineral part of blood plasma (SBF), as presented in Table 2 (12, 13, 14).
- a 1.5 SBF concentrated solution, as mentioned in the associated literature (12, 15).

Table 2. Chemical composition of the blood plasma and SBF

Crt. no.	**Ions**	**Human plasma**	**SBF**
1	Na^+	142,0	142,0
2	K^+	5,0	5,0
3	Mg^{2+}	1,5	1,5
4	Ca^{2+}	2,5	2,5
5	Cl^-	103,0	147,8
6	HPO_4^{2-}	1,0	4,0
7	HCO_3^-	13.5	4,2
8	SO_4^{2-}	0,5	0,5

For the preparation of the two solutions (SBF and 1.5 SBF), the following starting materials were used: $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$ and $CaCl_2$ dissolved in distilled water and stored in plastic containers. In our case, the pH value of the two solutions was 7.35 - 7.37, close to the human body value. In order to stop the bacterial growth, sodium azide was added in the solution (16). For buffering (keeping of the pH value within the desired limits), 50 mM of tris hydroxymethyl aminomethane $[(CH_2OH)_3CNH_2]$ and 45 mM of HCl were added in the solutions.

All the three samples obtained both by sandblasting and abrasion were immersed in SBF at a temperature of 37°C for one day. So, an ions exchange took place between the solution and the sodium titanate layer, leading to the formation of a titanium hydrogel able to induce the formation of hydroxyapatite crystal nuclei.

This is evidenced by the decrease of the concentrations of Na^+ ions and their replacement by hydronium ions, by ionic exchange with the surrounding fluid. The concentration of calcium and phosphate ions in the SBF decreases as a result of their deposition on the titanium gel, forming nucleation of hydroxyapatite. Once formed, they grow spontaneously by the consumption of calcium and phosphate ions in the surrounding fluid, giving rise to crystals of hydroxyapatite similar to those in the bone structure. The consumption of calcium and phosphate ions is also accompanied by a progressive decrease of the pH value of the surrounding fluid. To accelerate the hydroxyapatite formation process, the solution was replaced by another, with a higher concentration of the composing ions (1.5 SBF).

Two days later, the solution was changed by 1.5 SBF solution.

Further on, the metal implants were kept in a thermostatic chamber at 37°C ± 0.3°C for 21 days. Periodically, every 2-3 days, 1.5 SBF solution was completed to ensure losses by evaporation, but in particular to complete the loss of Ca^{2+} and PO_4^{3-} ions.

Chemical phenomena occuring at the surface of the titanium implant were followed by scanning electron microscopy measurements, pH measurements of SBF solution during deposition or gravimetric determinations of Ca and P concentrations.

Samples were collected at different times - 1, 3, 5, 10, 15 and 21 days and they were analyzed by electron microscopy and X-ray diffraction.

3. Results

The process of deposition of biological hydroxyapatitye by precipitation from a blood plasma-like solution is a mimetic process that is supposed to continue after implantation (17). The deposition of the hydroxyapatite layer on the surface was controlled by scanning electron microscopy and by X-ray diffraction.

The following of the process of obtaining and using of hydroxyapatite-coated titanium implants involves accurate physico-chemical and biological determinations. The implant is analyzed as concerns the physico-chemical properties of the surface before its implantation in living organisms. The chemical and mineralogical composition of the deposited layer is established.

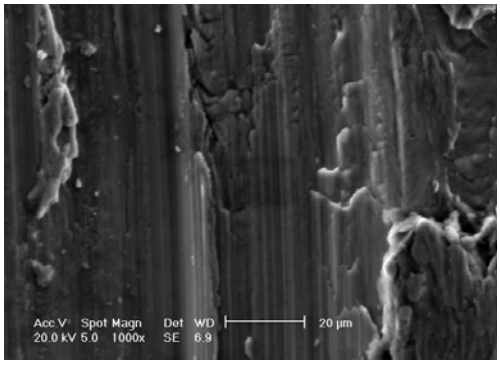

Fig. 1. SEM image of the surface of P1 titanium sample obtained by sandblasting

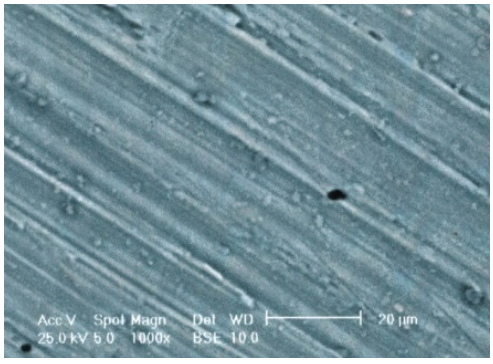

Fig. 2. SEM image of the surface of P2 titanium sample abraded by diamond files.

It was noticed that the samples obtained by sandblasting were impurified on their surface by a layer of SiO_2 which made them resistant to the action of NaOH, as can be seen in Fig. 3, 4 and 5 for all the three types of samples.

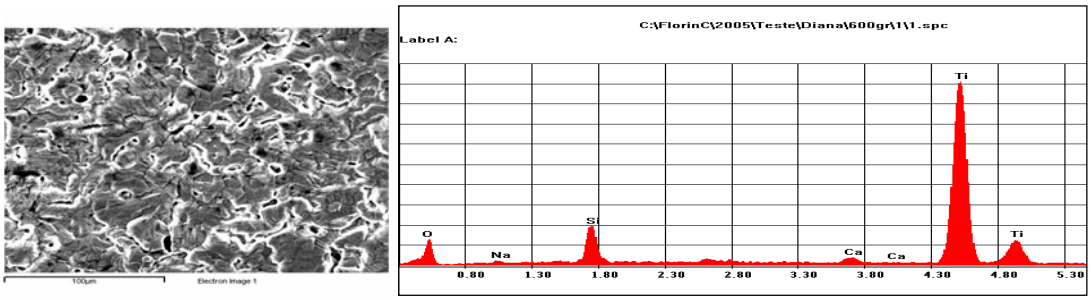

Fig. 3. SEM image and X-ray diffraction of the surface of P1 sample sandblasted with SiO_2, attacked by 10M NaOH solution for 24 h at 60°C and subjected to heat treatment for 1 hour at 600°C.

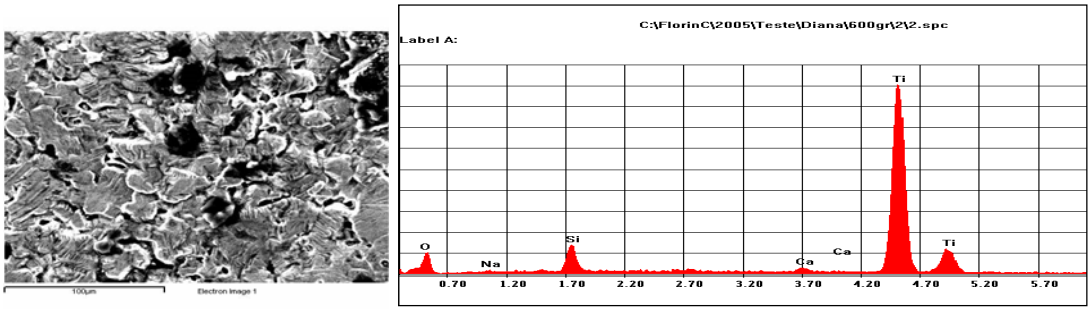

Fig. 4. SEM image and X-ray diffraction of the surface of P2 sample sandblasted with SiO_2, attacked by 10M NaOH solution for 24 h at 60°C and subjected to heat treatment for 1 hour at 600°C.

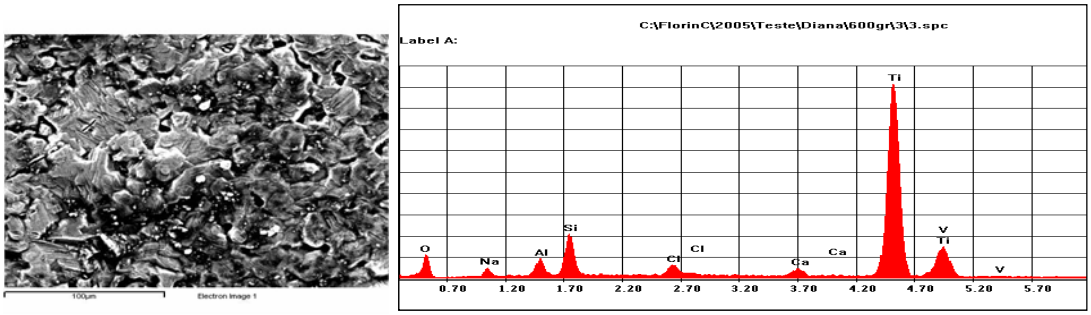

Fig. 5 SEM image and X-ray diffraction of the surface of P3 sample sandblasted with SiO_2, attacked by 10M NaOH solution for 24 h at 60°C and subjected to heat treatment for 1 hour at 600°C.

From the chemical analysis of the surface of the sandblasted samples, determined by EDX (Energy-dispersive X-ray spectroscopy), it is seen a large amount of silicon in the superficial layer (about 6%), regardless of the nature of the sample. On the right side it is showed the EDX analysis of the surface.

The cold deposition of the biological hydroxyapatite layer on the support is carried out by immersing the samples in the fluid that is chemically similar to the mineral part of the blood plasma (SBF).

The solution that is similar to blood plasma is prepared by the dissolution in water of the following reagents: NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$ and $CaCl_2$ in the required amounts (Table 2). The pH of the solution is adjusted to 7.3 with a buffer solution of tris-hydroxymethyl aminomethane and HCl at 37°C.

The scanning electron microscopy images shows the times of deposits formation: Fig. 6, Fig. 7 and Fig. 8 show SEM images of P1, P2 and P3 titanium samples prepared by abrasion after one day of immersion in SBF solution and 2 days of immersion in 1.5 SBF solution. On samples surface can be seen biological hydroxyapatite crystals deposited from the solution.

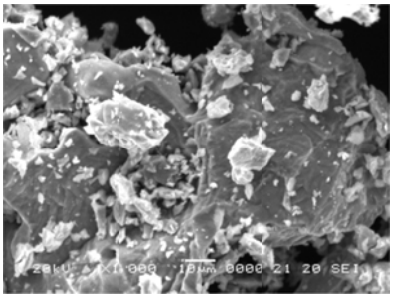

Fig. 6 SEM image of P1 titanium sample after 3 days of immersion in SBF (one day in SBF and two days in 1.5 SBF).

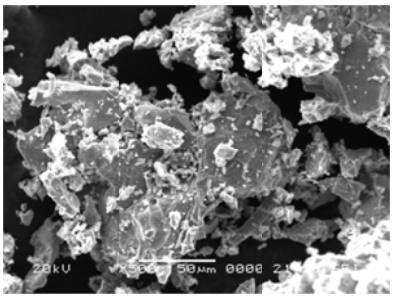

Fig. 7 SEM image of P2 titanium sample after 3 days of immersion in SBF (one day in SBF and two days in 1.5 SBF).

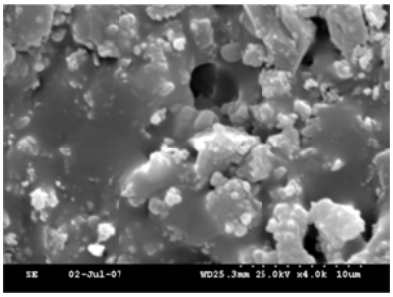

Fig. 8 SEM image of P1 titanium sample after 3 days of immersion in SBF (one day in SBF and two days in 1.5 SBF).

On the further immersion of the metallic implant in 1.5 SBF solution (Fig. 9, Fig. 10 and Fig. 11) it is found an increase of the hydroxyapatite layer formed by the ions supplied from the blood plasma-like solution (SBF).

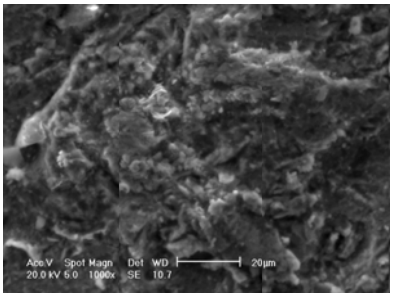

Fig. 9 Biological hydroxyapatite layer deposited after 15 days of immersion in 1.5 SBF on P1 sample surface (SEM image).

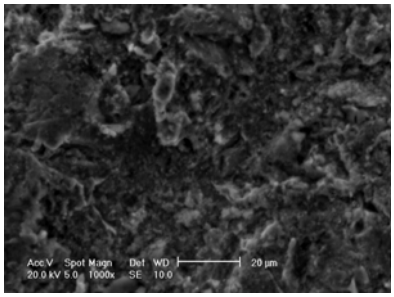

Fig. 10 Biological hydroxyapatite layer deposited after 15 days of immersion in 1.5 SBF on P2 sample surface (SEM image).

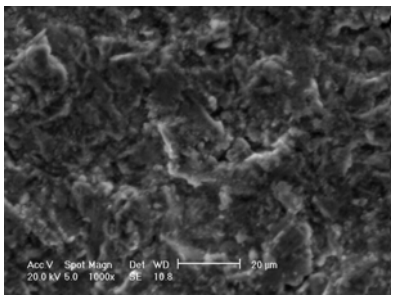

Fig. 11 Biological hydroxyapatite layer deposited after 15 days of immersion in 1.5 SBF on P3 sample surface (SEM image).

SEM images of the biological hydroxyapatite layer deposited after 21 days of immersion in SBF, on the surface of the three titanium samples: P1 (Fig. 12), P2 (Fig. 13) and P3 (Fig. 14).

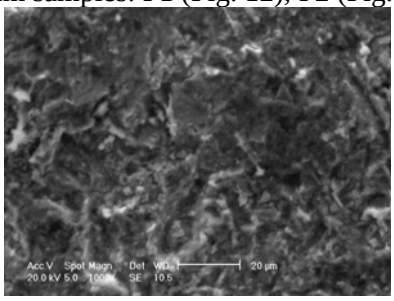

Fig. 12 Biological hydroxyapatite layer deposited after 21 days of immersion in 1.5 SBF on P1 sample surface (SEM image).

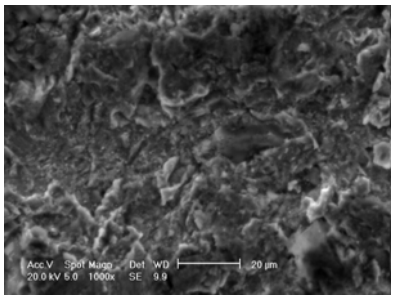

Fig. 13 Biological hydroxyapatite layer deposited after 21 days of immersion in 1.5 SBF on P2 sample surface (SEM image).

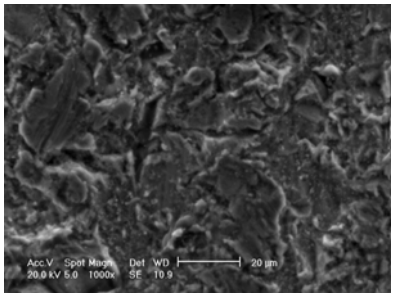

Fig. 14 Biological hydroxyapatite layer deposited after 21 days of immersion in 1.5 SBF on P3 sample surface (SEM image).

The nature of the crystals found in the layer on samples surface was established by X-ray diffraction and electron microscopy. Fig. 15 shows the X-ray diffractogram of a non-sandblasted P1 titanium sample and Fig. 16 shows the X-ray diffractogram of a non-sandblasted P2 titanium sample, after 15 days of immersion in 1.5 SBF. In both images is noticed the presence of some high-intensity black peaks, assigned to HA and of some red peaks, assigned to the support (Ti).

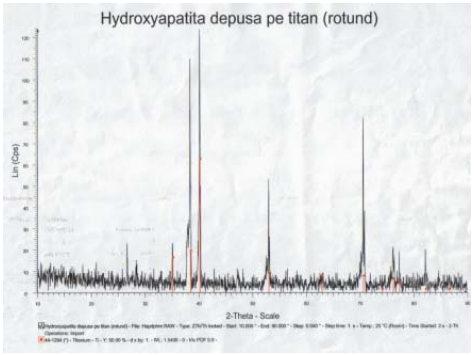

Fig. 15 Biological hydroxyapatite layer deposited after 15 days of immersion in 1.5 SBF on P1 sample surface (diffractogram).

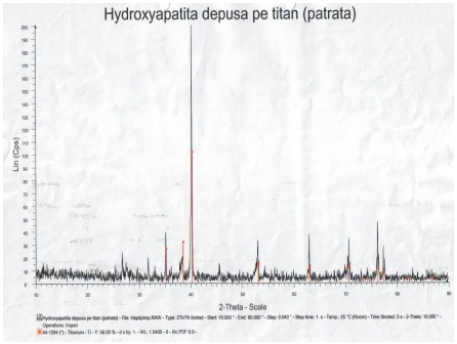

Fig. 16 Biological hydroxyapatite layer deposited after 15 days of immersion in 1.5 SBF on P2 sample surface (diffractogram).

The electron microscopy images in combination with X-ray diffraction allow a detailed analysis of the crystals on the surface of the titanium samples. On the material surface clearly appears the microcrystalline hydroxyapatite. It is characteristic the deposition area as layers of hydroxyapatite on titanium matrix of P1 sample obtained by abrasion, very well seen in SEM images x 500 (Fig. 17) and SEM x 4000 (Fig. 18). Quantitatively, EDAX diagrams (X-ray diffraction) reveal the peaks for the constitutive elements of the deposited layers (Fig. 19).

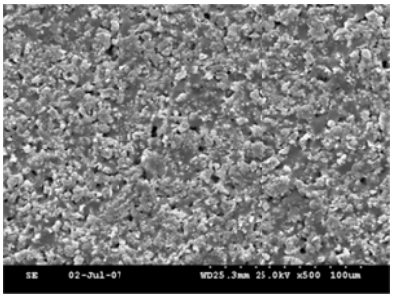

Fig. 17 Hydroxyapatite deposited on P1 sample (SEM image x 500).

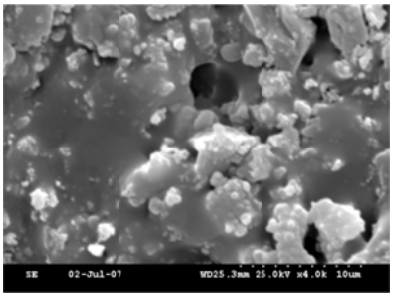

Fig. 18 Hydroxyapatite deposited on P1 sample (SEM image x 4000).

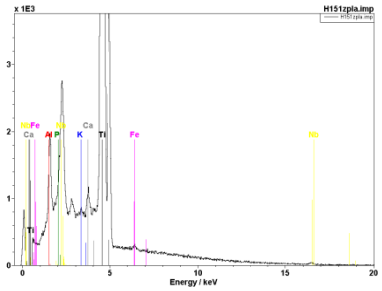

Fig. 19 Hydroxyapatite deposited on P1 sample (X-ray diffraction). The high-intensity black peaks are assigned to hydroxyapatite and the red ones are assigned to the titanium support.

4. Discussion

Hydroxyapatite is one of the most studied ceramics with medical implications in the field of reconstruction and regeneration of bony structures, for which researchers have shown a special interest. The variety of technological ways for its synthesis, such as processing of some hard tissues in the structure of mammals or corals and laboratory synthesis (chemical co-precipitation methods, solid state reaction synthesis, hydrothermal methods, sol-gel processes, microwave synthesis and so on), but also the multitude of forms in which this material can be used as compact or porous sintered powder for coating applications, as bioactive material of a bioinert support, as augmentation material in bone grafts or as hydroxyapatite-polyethylene composite material make from this bioceramics a material with a large applicability in the medical field (18).

According to some authors, biocompatibility and biofunctionality are two main characteristics of a biomaterial (19). The covering of these biomaterials with bioactive layers, respectively with thin layers of hydroxyapatite, provides the obtained biomaterial with both the required mechanical strength, by the presence of the titanium support and biological properties, respectively the ability to chemically react with living tissues, by the presence on the support surface of a bioactive layer of biological hydroxyapatite.

Currently, implants of titanium and its alloys gained ground, as they have a weight close to the bone and are more resistant to the biological fluids, having a surface coated with titanium

oxide by acid etch. In order to increase the contact surface, some implants are coated by plasma spraying with a rough titanium oxide layer (20).

The results of this study highlight the bioactivity of the material obtained by abrasion (grinding with a diamond instruments) and the remarkable opportunity to interact with living tissues.

As shown in Fig. 3, 4 and 5, from the chemical analysis of samples surface that were sandblasted with silicon oxide, determined by EDX, is noticed a large amount of silicon in the superficial layer (about 6%), regardless of the nature of the sample.

Fig. 20, 21 and 22 present SEM images of P3 samples, sandblasted, after one, five and ten days of immersion in SBF and 1.5 SBF.

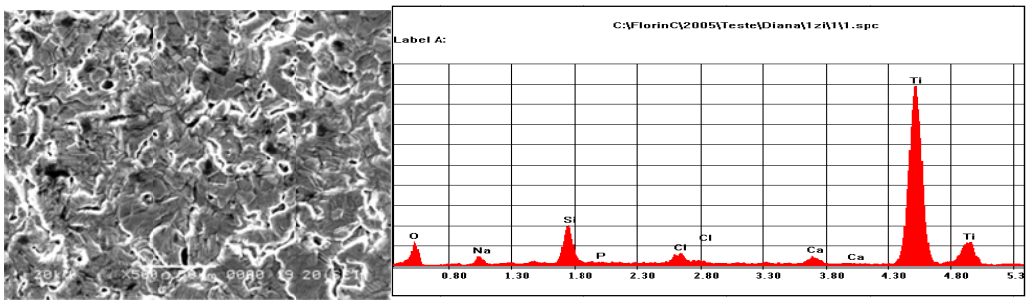

Fig. 20 SEM image of P3 sample surface, sandblasted, after one day of immersion in SBF and the composition of the superficial layer by EDX image

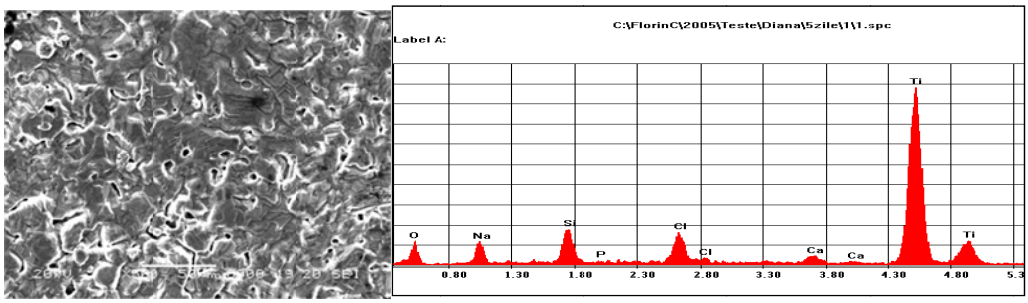

Fig. 21 SEM image of P3 sample surface, sandblasted, after five days of immersion in SBFand 1.5 SBF and the composition of the superficial layer by EDX image

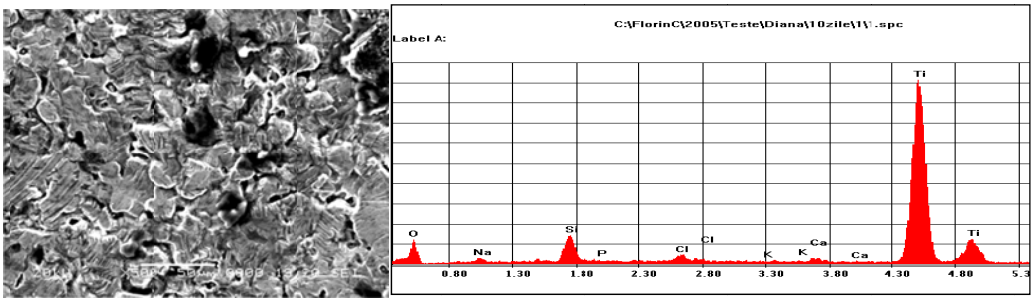

Fig. 22 SEM image of P3 sample surface, sandblasted, after ten days of immersion in SBF and 1.5 SBF and the composition of the superficial layer by EDX image

Changes in the chemical composition of the superficial layer of titanium samples that were sandblasted and subsequently placed in a solution similar to blood plasma for deposition of biological hydroxyapatite are shown in Table 3.

Table 3. Chemical composition of sandblasted titanium immersed in SBF

Chemical element	**Heat treated at 600°C**	**Immersed in SBF**				
		One day	**5 days**	**10 days**	**15 days**	**21 days**
O	31.19	29.71	26.74	30.28	28.97	29.39
Na	0.98	2.83	6.86	1.72	1.88	1.21
Si	6.43	6.34	5.76	4.7	5.62	4.05
P	0	0.41	0.25	0.19	0.4	0.43
Ca	1.01	1.46	1.55	0.81	1.22	0.40
Ti	60.39	57.45	54.02	61.03	60.43	63.58
Cl	0	1.80	4.81	1.06	1.27	0.57
K	0	0	0	0.21	0.21	0.36
Total	100.00	100.00	100.00	100.00	100.00	100.00

When analyzing the changes in the chemical composition of the superficial layer of a sandblasted titanium sample, on which were deposited large amounts of silicon oxide (Table 3), we notice the following:

1. An increase of the content of sodium in the first 5 days to the detriment of the titanium concentration, which involves the formation of sodium titanate.
2. A relatively constant concentration of calcium and phosphorus, which indicates the lack of deposition of crystallized biological hydroxyapatite.
3. A constant and relatively high concentration of silicon and implicitly of silicon oxide, as a result of the initial sandblasting.

Based on these results, it can be considered that sandblasting or alumina blasting of titanium and titanium alloys implants with a view to deposition of crystallized biological hydroxyapatite from SBF is not recommended.

The fixation of silica oxide to the implant surface leads to its passivation and therefore to an increased resistance to the attack by alkali solution for surface activation and finally to the impossibility of deposition of crystallized biological hydroxyapatite (12, 14, 21). Our research is consistent with data from the literature and highlights the importance of the nucleation process of crystallized biological hydroxyapatite on implant surface (12, 15, 16).

When it is made a choice for the coating process theyshould be considered the following final requirements: the coating material must not be altered irreversibly either chemically or structurally during the deposition process; the mechanical properties of the support should not be negatively affected by the coating process; the adhesion between the substrate and the material must be large enough to ensure the integrity of the surface during the lifetime of the implant (22).

The explantion of the mechanism of biological hydroxyapatite formation was established as follows: the titanium implant surface is coated with a titanium oxide layer and when the sample is placed in sodium hydroxide solution, at the metal surface are generated points of sodium titanate (Na_2TiO_3) and silica gel. In SBF solution, Na^+ ions migrate into the surrounding area; on the original titanium surface TiH_2 together with amorphous titanium oxide are formed. In this layer penetrate the Ca^{2+} and PO_4^{3-} ions and then the CO_3^{2-} ions, which form hydroxyapatite and cristallized biological hydroxyapatite. When the metallic implant is further on introduced in 1.5 SBF solution, it is seen an increase of the hydroxyapatite layer formed from the ions brought by the blood plasma-like solution (SBF).

After the formation of hydroxyapatite nuclei, the crystal growth and the formation of nuclei start (12, 14). The growth of the layer and of the hydroxyapatite crystals and cristallized biological hydroxyapatite lasts about 21 days and then the depositions are insignificant.

This mechanism of biological hydroxyapatite formation does not appear in the same measure for titanium samples processed by sandblasting, as if the sample is sandblasted, reactions

occur between the titanium oxide and the silica oxide granules which attach on implant surface leading to the passivation of this surface.

The achievement of the bond between titanium and hydroxyapatite following the chemical treatment of titanium surface with alkali, at high temperature, is explained as follows: titanium and its alloys can form a bond with the bone by means of the deposited hydroxyapatite layer, after immersion in solution similar to blood plasma, the metallic substrate being previously treated with alkali (NaOH) at high temperature. After these treatments, on the titanium implants surface apatite from blood plasma-like solution is formed. The formation of apatite layer on implants surface is considered a prerequisite to achieve the bond with the surrounding bone tissue (17, 23, 24). The amorphous sodium titanate generated by heat and alkali treatments results in the change of Na^+ ions with H_3O^-ions as well as in the formation of a hydrated titanium in SBF on implant surface. The Ti-OH groups of this hydrated titanium determine the occurrence of apatite nucleation process. The release of Na^+ ions into the solution also accelerates the nucleation process by decreasing the concentration of HO^- ions.

At high sintering temperature (600˚C), the implant surface becomes much more stable and the release of Na^+ ions from the substrate was much lower than that of the formed Ti-OH groups. At 25˚C, temperature at which there are formed a lot of Ti-OH groups on samples surface, it is easily formed apatite with a large grain size. Therefore, the titanate layer formed at this temperature was very slight bonded to the titanium substrate. To increase its adherence, following sintering it was treated with alkali (9, 10, 11).

From the experimental results it appears that at a temperature of 500°- 600˚C, the apatite layer is formed only 3 days after the soaking of the sample in SBF (Fig. 6 and Fig. 7) and the bond between sodium titanate and substrate is stable.

It is also noted the formation of small apatite crystals in the samples heat-treated at temperatures ranging between 500° and 600˚C, this being due to the reduction in the number of Ti-OH groups on the surface of the heat-treated samples. Thus, the titanium with a low content of Na^+ may be more appropriate for implantation in the human body, because the amount of ions released into the surrounding tissue is small and the speed of apatite formation is the same as in the case of titanium with a high content of Na^+.

5. Conclusions

The deposition of biological hydroxyapatite from a blood plasma-like solution on titanium or its alloys is possible if the surface of the biomaterial is attacked by alkali solution, so that on its surface appears a layer of sodium titanate, with formation of a titanium hydrogel able to induce the appearance of some nuclei of hydroxyapatite crystallization. This process of hydroxyapatite deposition is proportional to the duration of maintenance in the blood plasma-like solution.

So, we have demonstrated that titanium and its alloys can form a bond with the bone by means of a micron layer of deposited biological hydroxyapatite, able to chemically react with the receptor bed. The objective of this study was also achieved: at bone-implant interface is achieved a strong covalent bond that avoids the formation of a fibrous layer, thereby increasing the lifetime of the implant.

Disclosure

All authors made equal contributions to the study and the publication.

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