In vitro Corrosion Study by Electrochemical and Surface Analysis Techniques of the Hydroxyapatite Sol-Gel Deposition on Commercially Pure Titanium

KAMEL EARAR1, CRISTIAN BUDACU2*, DANIEL MARECI1, LUCIA CARMEN TRINCA3*, JULIA CHISCOP1, CORINA CIUPILAN2, ALEXANDRU ANDREI ILIESCU6
1Dunarea de Jos University, Medicine & Pharmacy Faculty, Dentistry Dept., 47 Domneasca Str, Galati, 800008, Romania
2Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, Department of Anatomy, 16 Universitatii Str., 700115, Iasi, Romania
3Gheorghe Asachi Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, 73 D. Mangeron Blvd., 700050, Iasi, Romania
4Ion Ionescu de la Brad Agricultural Sciences & Veterinary Medicine University, Horticulture Faculty, Exact Sciences Dept. 3 Mihail Sadoveanu Alley, 700490, Iasi, Romania
5Craiova University of Medicine and Pharmacy, 2 Petru Rares Str., 200349, Craiova, Romania

Developments in the field of surgical implant materials are in progress which involves covering the metal implants with a layer, which may limit the leakage of metal ions and permitting a natural bonding to living tissues. The aim of this work is the production and characterization of sol–gel coatings for protection and bioactivation of metals used as standard surgical implant materials, such as commercially pure titanium (Cp-Ti). These films should both prevent degradation of the substrates by corrosion for inducing the formation of a hydroxyapatite (HA) rich layer onto the material surface. Formation of HA layers can be observed by soaking the material in phospho-calcium gel using scanning electron microscopy (SEM). Fourier transform infrared spectroscopy (FTIR) analysis was performed in order to emphasize the formation of the hydroxyapatite layer onto the Cp-Ti surface. The performance of the coated metal was evaluated in vitro by electrochemical impedance spectroscopy (EIS).

Key words: Cp-Ti, hydroxyapatite, FTIR, SEM, EIS

Alongside stainless steel and cobalt alloys, titanium and its alloys are increasingly used as metallic biomaterials in bone tissue engineering, for orthopedic prostheses, because of their low density, high mechanical strength, good corrosion resistance and biocompatibility [1-4]. However, titanium and titanium alloys, which are used for load bearing applications, are bioinert materials which do not bond to living bone tissue after implantation. Therefore, surface modification is required to improve the bioactivity of these materials [5-6]. In order to modify the surfaces of metallic biomaterials, a considerable interest has done to their coating with bioactive ceramics [7]. This concept combines the mechanical benefits of metal alloys with the biocompatibility of ceramic material, an attempt to accelerate bone formation on initial stages of osseointegration, thus improving implant fixation [8]. Among the ceramics, hydroxyapatite ((Ca₉(PO₄)₆(OH)₂), HA) and calcium deficient hydroxyapatite (Ca₉(HPO₄)₂(OH) - CDHA), are most widely used due to its osteoconductivity, bioactivity and chemical similarity to the inorganic component of human bone [9-11]. Among them, products containing calcium deficient hydroxyapatite are the closest to the structure of bone mineral.

Coating with calcium phosphate compounds have been done by a variety of techniques including plasma spray [12-14], laser ablation [15-16], electron beam evaporation [17-18], radio frequency sputtering [19], electrophoretic deposition [20] and sol-gel deposition [21-24]. Certain of these methods suffer from various drawbacks. In plasma spraying method, which has been commercial method for hydroxyapatite coatings, the particles are melted at a high temperature and deposited onto a roughened target to produce layers of solidified flattened droplets. The high temperature decomposes the sprayed material into tri- and tetra-calcium phosphate and hydroxyapatite, while the high cooling rate prevents crystallization, and an amorphous calcium phosphate matrix. Also, in radio frequency sputtering an amorphous and a phosphorous deficient material is obtained. These latter causes fast resorption of material in vivo. The ion beam sputtering is not able to coat odd shaped objects. The electrophoretic methods have problems with poor adhesion and formation of some other phases. The sol-gel method is a chemical procedure capable of producing calcium phosphate compounds from solutions. The advantages of the sol-gel technique include: an increased homogeneity due to mixing on the molecular level, reduced fritting rate due to the small particle size having high surface areas, ability to produce uniform fine grained structures and ease of application to complex shapes. Thus, the use of sol-gel processes for coating of titanium implants with hydroxyapatite became an important research objective. Sol-gel method has previously been used both for preparing hydroxyapatite [25-26] and for its submission on media [27]. In the present study, the Ca deficient hydroxyapatite (CDHA) was coated on the titanium surface by a sol-gel process. Also, corrosion behavior of uncoated and coated Cp-Ti samples was evaluated in Hank’s balanced salt solution (HBSS) by electrochemical impedance spectroscopy (EIS).
Experimental part
Sample preparation
Mixing precursors in the sol-gel technique takes place in a colloidal gel or a macromolecular network. Macromolecular gel or solution covers the substrate in thin film. Subsequently, the solvent is evaporated obtaining a solid film with fine porosity, which gives a very high specific surface, and thus a higher surface energy. After drying, the first layer may be deposited other layers to achieve a desired thickness of the final deposit.

In this study we used a gel-based calcium acetate / phosphoric acid where the molar ratio Ca : P was 1.69. Colloidal solution for coating was obtained by mixing 50 mL aqueous solution of Ca (CH3COO), in ethylene glycol 0.35 M with H3PO4 solution 1M. Concentrations of components in the mixture were: c (acetate) = 0.283 mol / l and c (H3PO4) = 0.167 mol / l. Before use, the mixture was ultrasonated for 30 minutes and let stand for 70 hours.

The samples as cylindrical electrodes were obtained from Cp-Ti ingots. For better adhesion between substrate and hydroxyapatite coating, the surface of Cp-Ti substrate was roughened by chemical etching in mixed acid (H2SO4 48% + HCl 18%) at 65 °C, for 60 min. Before treatments all samples were polished using abrasive SiC papers down up 2000 grit specification, degreased with acetone and washed in distilled water. After chemical treatment each sample was rinsed well with distilled water and dried in air at 140 °C.

In order to cover, the specimens were placed vertically in phosho-calcium gel and extracted very slowly, at a rate low enough to avoid accumulation of gel at the bottom. Speed of extraction in this case was 2.5 mm / min. After final drying, the samples were calcinated at 800 °C for 15 min. The next layer has been deposited in the extraction from gel, samples were dried in an oven at 130 °C for 15 min. The number of layers deposited: sample 1: 3 layers, the sample 2: 5 and sample layers 3: 10 layers.

Sample characterization
Sample surface characterization
The samples were characterized by FTIR and micro-FTIR spectroscopy, scanning electron microscopy (SEM) and EDX microanalysis, both after deposition and drying, and after calcination.

Micro-FTIR spectroscopy was operated on a TENSOR 27 spectrometer for mid-infrared range coupled with a HYPERION 1000 microscope from Bruker Optics-Germany. The FTIR spectra of the calcinated samples were registered with a Bomem MB-104 spectrophotometer (Quebec, Canada). The spectral resolution used was 4 cm⁻¹. Fifty scans were added per sample spectrum, and Fourier transformation of the spectra was done using the Win Bomem Easy 3.5 software package.

The microstructure and micro-chemical composition of the deposited material were examined using a Scanning Electron Microscope (SEM), VEGA-TESCAN type, equipped with a Microanalysis system. Calcium to phosphorus ratios were evaluated by Energy dispersive X-ray spectrometry (EDX) (QUANTAX Bruker AXS Microanalysis system, GmbH, Berlin, Germany).

Electrochemical tests
The corrosion medium was Hank’s balanced salt solution (HBSS) at 37 ± 1 °C. The chemical composition of HBSS is reported in previous work (Mareci et al., 2010c). The test specimens were placed in a glass corrosion cell, which was filled with freshly electrolyte. A saturated calomel electrode (SCE) was used as the reference electrode and a platinum coil as the counter electrode. All potentials referred to in this article are with respect to SCE.

The variations of the corrosion behavior of the samples (coated and uncoated) were studied using the electrochemical impedance spectroscopy (EIS) technique. Electrochemical impedance spectroscopy measurements were performed using a potentiostat manufactured by PAR (Model PARSTAT 4000, Princeton Applied Research, USA). The instrument was controlled by a personal computer and specific software (VersaStudio, PAR, USA).

EIS measurements were performed after the coated samples 3 were immersed in HBSS at 37 ± 1 °C, at open circuit potential, for different times (1-h, 1-day, 2-days and 7-days). The alternating current (AC) impedance spectra for samples were obtained with a scan frequency range of 10 kHz to 10 mHz with amplitude of 10 mV.

In order to supply quantitative support for discussions of these experimental EIS results, an appropriate model (ZsimpW in-PAR, USA) for equivalent circuit (EC) quantification has also been used. The usual guidelines for the selection of the best-fit EC were followed: a minimum number of circuit elements are employed and they χ² error was suitably low (χ² < 10⁻⁴), and the error associated with each element was up to 5%. Instead of pure capacitors, constant phase elements (CPE) were introduced in the fitting procedure to obtain good agreement between the simulated and experimental data [28-29].

| Fig. 1. Micro FT-IR spectra for sample coated with three layers (1), sample coated with five layers (2), and (3) sample coated with ten layers |
| Fig. 2. FT-IR spectra for calcinated samples: (1) three layers, (2) five layers, (3) ten layers |
Results and discussions

Figure 1 presents the micro-FTIR spectra for the three samples, comparative with the hydroxyapatite spectrum according to OPUS software library.

These spectra relieve basic bands characteristic for apatite compounds, reflecting the vibrations in the PO4 group of the apatite in the range 960...1100 cm⁻¹ and around 600 cm⁻¹. The bands group located in the domain 1426 - 1567 cm⁻¹ in the FTIR spectrum of the sample 3 is due to the acetate groups absorbed in the covering layer, which is or thick than in the case of the other two samples. After calcinations these acetate bands are almost completely attenuated and the apatite bans are clearly emphasized (fig. 2).

In figure 3 are shows the SEM micrographs and the 3D feature of the sample 2 surface. SEM analysis and of the coated surface point out that, despite to the simplicity of the coating method, the microstructure of the superficial coat is quite elaborate.

After drying and calcining, appears a porous structure on the surface of titanium. However, on this area can distinguish two distinct porous structures. The first type of structure is shown in images a, b and d. They were obtained by aggregation of microparticles of about 2 cm. This aggregation leads to the formation of tubular pores. The second type of porous structure, shown in images c, e and f, is achieved through agglomeration of microparticles in irregular blocks and random packing of their, creates grooving goals.

To identify the nature of microparticles which forming both types of structure, EDX analysis was performed at three points in this structure (1, 2 and 3 in figure 4, image d, points that are microparticles of the composition from the first type of structure). EDX spectra obtained are shown in figure 5. The same is shown and EDX spectrum for the area from image e, where compact blocks are prevalent. Quantification of these spectra led to following values of ratio Ca : P: 1.48; 1.49; 1.51 and 1.53. One can see that on the entire surface of the sample, the ratio Ca : P = 1.5. This means that the phospho-calcium compound, which covered the surface of the support, is deficient in calcium hydroxyapatite: Ca,(HPO4)(PO4)5OH. More, a quantity of 15...20 % titanium have been present, most probable as TiO2, explaining thus the structural feature of the deposed layer.

Entirely different appears the calcinated surface of the sample 3, covered with ten layers, as can be seen in figure 5.

The quantification of the EDX spectrum leads to a Ca : P ratio equal with 1.51 and a titanium percent of only 2.5%. Titanium is very well covered with a consistent layer of calcium deficient hydroxyapatite, with grooves and holes which penetrate up to titanium support.

EIS measurements offer useful information regarding the superficial layer of the coated sample. EIS measurements were performed at the open circuit potential in artificial saliva over 7 days immersion time. Figure 6 gives the Bode plot from the sample 3 covered with ten layers and calcinated at 800 °C, over different immersion times in the HBSS. Generally, three frequency regions referring to the high, medium and low frequency values are distinguished from impedance spectra. The high
Fig. 4. EDX spectra in 1…3 points and for „e“ area from figure 3

Fig. 5. SEM image, 3D feature and EDX spectrum for sample 3, covered with ten layers and calcinated at 800 °C

Fig. 6. Measured (discrete points) and fitted (solid lines) Bode impedance spectra of coated Cp-Ti samples exposed to HBSS for different immersion times
frequency plateau of the impedance values at frequency higher than $10^3$ Hz, yields the value of the solution resistance ($R_{sol}$). In the Bode plot, impedance at medium frequency represents the response of the coating, while at the low frequency limit, the information about process is related to the reaction on the substrate/electrolyte interface [31].

According to the impedance diagram, after 1-h immersion, the Bode-phase plots are in agreement with an EC with one time constant (fig. 7a). In fact, 1-h immersion period is too short to reveal any degradation of the substrate.

The impedance spectra were fitted using the ZSimpWin software and the resultant EIS parameters are given in table 1. The fitting quality of EIS data was estimated by the chi-square ($\chi^2$) test (between $10^4$ and $10^5$) values and the comparison between error distribution versus frequency values (+5% for the whole frequency range) corresponding to experimental and simulated data. The $R_{pore}$ and $Q_c$ (c means coating) parameters describe the processes occurring at electrolyte/coating layer. $R_{pore}$ is the charge transfer resistance associated with the penetration of the electrolyte trough the pores or pinholes existing in the coated layer and $Q_c$ corresponds to capacitance of the coating layer. $R_{sol}$ is the ohmic resistance of the electrolyte.

The high $n_c$ value of the immersion time of 1-h indicated a capacitive response between HBSS and Cp-Ti.

When the immersion period is greater than 1-day, the phase shift is different to that of 1-h. The low-frequency domain evidences the processes taking place at the substrate/electrolyte interface. Such behavior is typical for a metallic material covered with a porous film, which is exposed to an electrolytic environment.

In this case, the electrochemical interface can be subdivided into sub-interfaces: electrolyte/coated layer and electrolyte/substrate. This type of EIS spectra is best modeled by an equivalent circuit (EC) proposed by Baltat-Bazia et al., 1992 [32] for the two sub-electrochemical interfaces, as shown in figure 7b.

The parameter $R_p$, coupled with $Q_s$ (s means substrate) describes the processes at the substrate layer at the electrolyte/substrate layer interface. $R_p$ is the polarization resistance at the electrolyte/substrate interface in the pores or pinholes and $Q_s$ to the capacitance at the electrolyte/substrate layer interface which seems to be associated to the double layer formation. The values of the electrochemical parameters from equivalent circuits that fit best the experimental data are presented in table 1. The $R_{sol}$ of the test electrolyte, equals $60 \pm 5$ W, was observed for the specimens, indifferent of the immersion time, and was not inserted in table 1.

The decrease of $R_{sol}$ as immersion time increases imply that increased number of opening pinholes and pores the coating layer became more conductive. And moreover the improvement of conductive property for hydroxyapatite coating layer caused the decrease of $R_{sol}$. Corrosion may occur at pores as the result of the metal being directly exposed to the aggressive attack of the electrolyte. Pores in the coating may act as paths for the electrolyte attack to the metal beneath. The value of polarization resistance indicates that the sample maintained 7-days in HBSS is still highly resistant to corrosion [33].

For the comparison, the EIS spectrum of the uncoated Cp-Ti sample recorded after 7-days in the HBSS was also recorded and is given in figure 8.

In this case of uncoated Cp-Ti samples the Bode-phase plots are in agreement with an EC with one time constant. The EC (fig. 7c) is characterized by one parallel combination terms ($R_{Q_s}$) in series with the resistance of the solution ($R_{sol}$). Again, the values of the parameters ($R_p$, $Q_s$) obtained with the fitting procedure are reported in table 1. Polarization resistances ($R_p$) are higher for coated Cp-Ti material than for uncoated Cp-Ti sample, revealing more resistant protective films.

Table 1

<table>
<thead>
<tr>
<th>Cp-Ti samples</th>
<th>Immersion time</th>
<th>$10^3 Q_s$ (S cm$^{-2}$ s$^{-1}$)</th>
<th>$n_c$</th>
<th>$10^3 R_{pore}$ (k$\Omega$ cm$^2$)</th>
<th>$10^3 Q_p$ (S cm$^{-2}$ s$^{-1}$)</th>
<th>$n_x$</th>
<th>$10^3 R_{sol}$ (k$\Omega$ cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated</td>
<td>1 hour</td>
<td>1.7</td>
<td>0.81</td>
<td>33.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>8.7</td>
<td>0.80</td>
<td>2.3</td>
<td>2.3</td>
<td>0.09</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>8.9</td>
<td>0.79</td>
<td>1.9</td>
<td>2.7</td>
<td>0.68</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>9.1</td>
<td>0.78</td>
<td>1.4</td>
<td>2.4</td>
<td>0.65</td>
<td>3.2</td>
</tr>
<tr>
<td>Uncoated</td>
<td>7 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
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Table 1: Electrochemical parameters obtained from EIS spectra of uncoated Cp-Ti sample exposed to HBSS for 7 days.
Conclusions
The sol-gel method can create an effective hydroxyapatite layer on the surface of Cp-Ti. The FT-IR spectra of the calcinated samples show the typical apatite compounds bands. Two different physical models with related equivalent circuits are devised to simulate the EIS results for coated Cp-Ti. After 7 days exposure to HBSS, the solution penetrated into interface through the opening pinholes and pores and the coating layer became more conductive. However, after 7 days immersion time in HBSS, the coated Cp-Ti had higher polarization resistance than the uncoated suggesting that the coatings exhibited a protective character.

References