Effect of fluorides on bioactivity of titania nanotubes in SBF solution – by EIS studies

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ABSTRACT

Purpose: The influence of fluorides concentration in anodizing electrolyte on deposition of calcium phosphates Ca-O-P on titania nanotubes immersed in simulated body fluid (SBF) has been examined.

Design/methodology/approach: The electrochemical impedance spectroscopy (EIS) was used to monitor the process of deposition of calcium phosphates on titanium foils covered with titania nanotubes formed by anodizing in 1 M H₃PO₄ with various amounts of fluorides ranging from 0.2 wt% to 0.4 wt%. The changes in impedance characteristics combined with results of SEM and EDS analyses were used to evaluate the bioactivity of nanotubes in SBF and find out the relation between the morphology of deposits and the concentration of fluorides in the anodizing electrolyte.

Findings: The obtained results confirmed that titania nanotubes strongly favour the deposition of calcium phosphates (HAp) during the first 24 hours. However, the behavior of titania nanotubes formed in the electrolytes of various fluoride content differ afterwards when immersed longer in SBF solution. Particularly, contrary to other samples, the amount of deposits on nanotubes formed in 1 M H₃PO₄+0.30% wt. HF decreases significantly about 72 hours after immersing and these observations are recorded by both the SEM/EDS examination and XPS results. The corresponding changes in impedance parameters are noticed.

Practical implications: Development of the method to cover titanium implant materials with nanoporous anodic layer, enriched in phosphates and fluorides- both ions highly supporting bioactivity, enables new applications in implantology and biosensing.

Originality/value: Bioactivity is highly desirable property of implant materials. The phenomena observed during immersion in SBF solution by the Electrochemical Impedance Spectroscopy are related to the amount of fluorine in titania nanotubes. The explanation of this behavior and its consequence to bioactivity is proposed.

Keywords: Titania nanotubes; Electrochemical impedance spectroscopy; Hydroxyapatite; Dynamics of deposition; Simulated body fluid

Reference to this paper should be given in the following way:
1. Introduction

For more than 20 years already, titanium and its alloys have been used as implant materials [1-3] due to their good biocompatibility and mechanical properties [4-6]. It was assumed that the above mentioned properties can be enhanced due to the developed surface, which is characteristic for nanotubular structures and the affinity of surface layers on titanium oxide to composition of physiology fluid. In future applications these enhanced properties could be used for example to develop biosensor matrix for bone ingrowth or deliver drugs.

Nanostructures can be formed by several ways, e.g. using sol-gel method [7] or anodizing [8-13]. At proper conditions of anodizing, which include time, potential scan rate [14], electrolyte composition [15] one can receive nanotubes of the specified diameters (60-120 nm) and length (e.g. ~500 nm), which depends on the time of anodisation. Apart from the conditions of nanotubes formation also surface finishing parameters effect the resultant morphology of nanotubes [15].

To assess in vitro the bioactivity of implant materials, the deposition of the HAp particles on the material surface after immersion in SBF solution is used first [16]. One of the most suitable methods to monitor the deposition process and evaluate changes of surface morphology is the electrochemical impedance spectroscopy (EIS) [17]. The obtained impedance spectra are used as models for surface layers observed on the investigated nanomaterials [18]. These models can be used to describe the structure of porous layers and to interpret the phenomena, which occur on a surface of samples together with results of SEM and EDS observations.

The aim of the work was to monitor the changes of impedance characteristics of titania nanotubes formed on titanium in electrolytes of different fluoride concentrations in order to evaluate the influence of these ions on titania nanotubes bioactivity in vitro. The combined results of EIS analysis with SEM and EDS observations used for this purpose allowed to reveal that fluorine present in titania nanotubes influences the process of Ca-O-P deposition and as a result may be also responsible for the formation of HAp layer on surgical implants. Such an intermediate layer between implant and bone is able to stimulate bone in-growth after implantation [1, 4].

Particularly, the amount of deposits on nanotubes formed in 1 M \( \text{H}_3\text{PO}_4 \) solution with addition of different amounts of fluoride ions (sample 1 – 0.2 wt% HF, sample 2 - 0.3 wt% HF, sample 3 – 0.4 wt% HF) at potentials ranging from the OCP (the open circuit potential) to 20V, followed by further 2h at 20V. The scan rate of 500 mV/s was applied in the potential-dynamic stage of the process. Anodizing was performed with potentiostat/galvanostat Autolab 302 N, equipped with potential amplifier.

Anodizing was carried out in 1 M \( \text{H}_3\text{PO}_4 \) solution with addition of different amounts of fluoride ions (sample 1 – 0.2 wt% HF, sample 2 - 0.3 wt% HF, sample 3 – 0.4 wt% HF) at potentials ranging from the OCP (the open circuit potential) to 20V, followed by further 2h at 20V. The scan rate of 500 mV/s was applied in the potential-dynamic stage of the process. Anodizing was performed with potentiostat/galvanostat Autolab 302 N, equipped with potential amplifier.

The evaluation of bioactivity of titanium samples covered with titania nanotube layer was carried out in vitro by immersing in the SBF solution and monitoring changes in impedance characteristics after 1, 24, 72 and 120 hours, followed by the microscopic observation of deposits on the surface of samples removed from the solution [19]. The impedance spectra were recorded at the open circuit potential (OCP) with the use of ATLAS 9823 FR Analyser with frequency ranging from 105 to 0.18 Hz, AC amplitude 10 mV at the temperature 37°C. The results of investigations presented with Nyquist and two Bode diagrams (for impedance and phase angle) were fitted to the electric equivalent circuits. After completing the EIS tests, the samples were removed from the solution, rinsed in DI water and dried with N2 stream and morphologically examined. Separate samples were also taken out of the solution after 1, 24, 72 and 120 hours since the immersion for the analysis of the morphology and the chemical composition of deposits on the surface of titania nanotubes performed with JSM-7600F, JEOL Inc.

3. Results and discussion

Fig. 1 shows SEM pictures of the obtained nanotube structure for the sample 2. Nanotubes formed by anodizing process were characterized by dispersion of diameters from 60 to 120 nm and thickness of the nanotube layer equal to about 500 nm.

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Fig. 1. SEM pictures of nanotubes for sample 2 obtained in 1 M \( \text{H}_3\text{PO}_4 \) with 0.3 wt% HF: a) top view, b) cross-section

The samples of titanium foil with vertically aligned titania nanotubes formed by anodizing in 1 M \( \text{H}_3\text{PO}_4 \) with 0.2-0.4 wt% HF after 1, 24, 72 and 120 hours in SBF solution were examined by SEM and EDS analyses.

In Fig. 2, as an example, the morphology of deposits observed on the titania nanotube surface of sample 2 after different periods of immersion in SBF solution was presented. For samples 1 and 3 the morphologies were similar.

It can be clearly seen that already after the first hour since immersion the nanotubes were coated with deposits (Fig. 2a) and the surface was completely covered after 24 h (Fig. 2b). However,
next samples taken out after 72h showed repeatedly significant
decrease of deposits on the surface (Fig. 2c). Signs of re-coating
were seen on the surface during the prolonged exposition to SBF
solution up to 120 hours, when the increase of deposits was
observed again.

Table 1 contains the results of EDS microanalysis for sample
2 showing the change of chemical elements concentration in
eposits on the surface of nanotubes (Area 1) and in the layer of
nanotubes without deposits (Area 2) for different periods of
immersion in SBF solution.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>P (wt%)</th>
<th>Ti (wt%)</th>
<th>Ca (wt%)</th>
<th>F (wt%)</th>
<th>O (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanotube layer with deposits (Area 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.501</td>
<td>59.592</td>
<td>0.549</td>
<td>1.717</td>
<td>38.188</td>
</tr>
<tr>
<td>24</td>
<td>0.445</td>
<td>56.634</td>
<td>0.336</td>
<td>2.038</td>
<td>40.547</td>
</tr>
<tr>
<td>72</td>
<td>0.742</td>
<td>50.010</td>
<td>0.565</td>
<td>3.037</td>
<td>45.646</td>
</tr>
<tr>
<td>120</td>
<td>1.623</td>
<td>45.791</td>
<td>1.173</td>
<td>1.511</td>
<td>48.422</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanotube layer without deposits (Area 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.147</td>
<td>61.208</td>
<td>0.120</td>
<td>2.283</td>
<td>36.236</td>
</tr>
<tr>
<td>24</td>
<td>0.150</td>
<td>78.996</td>
<td>0.100</td>
<td>0.365</td>
<td>20.389</td>
</tr>
<tr>
<td>72</td>
<td>0.222</td>
<td>68.187</td>
<td>0.137</td>
<td>1.755</td>
<td>29.699</td>
</tr>
<tr>
<td>120</td>
<td>0.416</td>
<td>68.437</td>
<td>0.211</td>
<td>1.703</td>
<td>28.839</td>
</tr>
</tbody>
</table>

In deposits (Area 1) on the surface titania nanotubes, titanium,
oxigen, fluorine, calcium and phosphorus have been found. It can
be seen from Table 1 that in deposits (Area 1) the amount of
titanium decreases, while the amount of calcium, phosphorus,
oxigen and fluorine increases with time of immersion in SBF
solution. It is related to the thickening of deposit layer on the
titania nanotube layer. The presence of titanium (Ti) originates
probably from the substrate layer under the nanotubes.

For the surface nanotubes without deposits (Area 2) mainly
titanium, oxygen and fluorine have been found. After the first
hour of immersion, there is about 0.1 wt% of calcium and
phosphorus in the surface layer of nanotubes, and then the amount
of elements Ca, O, P and F increases with time of immersion in
SBF (Table 1). There is more calcium, phosphorus, oxygen and
fluorine in deposits (Area 1) than in nanotubes (Area 2). The
amount of fluorine after 24 h in SBF is up to five times higher in
deposits than in nanotubes. However, after 72 hours it drops (is
only twofold), which confirms that fluorine present in deposits
was adsorbed from the nanotube layer [20].
Fig. 3 shows the results of EDS microanalysis of deposits (Area 1) present on the surface of sample 2 after 72 hours of immersion in SBF (Fig. 3). Energy spectrum for chemical elements shows that content of calcium (Ca), phosphorus (P), oxygen (O) and fluorine (F) in deposits on the surface nanotubes. According to observed level of fluorine and phosphorus in the nanotube layer it seems that both elements stimulate the deposit formation of calcium ions on the surface of nanotubes, leading to the formation of deposits of calcium phosphate, non-stoichiometric hydroxypatite and fluorapatite. Literature data [21-22] confirm that the similarity of ionic radii of F⁻ and OH⁻ (0.132 nm and 0.168 nm, respectively) allows easy substitution of fluorine by OH⁻ groups in hydroxypatite structure. Fig. 4 shows the changes of surface concentration of analyzed chemical elements during immersion of sample 2 in SBF solution.

In Fig. 4 gradual decrease of titania concentration and simultaneously increase of concentration of oxygen, phosphorus and calcium can be seen. After about 24 hours since the moment of immersion in SBF solution, a characteristic decrease of concentration of phosphorus and calcium and then slow increase of their values during further immersion the samples in the solution can be observed. Such changes of concentration of analyzed elements may indicate that at the beginning of immersion the deposition of calcium and phosphorus ions on the titania nanotube surface was an intensive process, but then the deposited phosphates started to dissolve slowly.

At first the precipitated calcium phosphates generally have amorphous or slightly crystalline character [21]. But during the maturing process the deposits are transformed into hydroxypatite which is generally non-stoichiometric. The moment of the transformation depends mainly on temperature. For most deposits, about 24 hours in room temperature is necessary, but in higher temperatures the process is much faster. The calcium phosphates can be put into order of decreasing solubility and diminishing speed of dissolution: amorphous calcium phosphates ACP > Tetra CP > tricalcium phosphate αTCP > βTCP > hydroxyapatite with deficiency of calcium Ap > HA [21-22].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>Ca</th>
<th>P</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 h</td>
<td>1.55</td>
<td>4.54</td>
<td>2.96</td>
</tr>
<tr>
<td>1</td>
<td>72 h</td>
<td>1.65</td>
<td>5.24</td>
<td>5.21</td>
</tr>
<tr>
<td>1</td>
<td>120 h</td>
<td>1.84</td>
<td>4.91</td>
<td>9.11</td>
</tr>
<tr>
<td>2</td>
<td>24 h</td>
<td>1.41</td>
<td>4.76</td>
<td>3.77</td>
</tr>
<tr>
<td>2</td>
<td>72 h</td>
<td>1.57</td>
<td>4.43</td>
<td>3.80</td>
</tr>
<tr>
<td>2</td>
<td>120 h</td>
<td>1.81</td>
<td>5.25</td>
<td>5.17</td>
</tr>
</tbody>
</table>

Fig. 6. Bode diagrams (impedance module) for (a) sample 1 and (b) sample 2.

Thus, amorphous, slightly crystalline phosphates of higher solubility than the solubility of HA precipitate from SBF solution at the beginning. After 1 hour of immersion of the sample in SBF solution, large amount of phosphates precipitates, but then they dissolve (kind of biodegradation and bioreosorption in a body), and simultaneously the residual precipitate transforms into HA. The process of transformation into HA is faster than the dissolution of phosphates. The electrochemical impedance spectroscopy observations were carried out during immersion. The example impedance curves for samples 1 and 2 are shown in Figs. 5-7.

Nyquist diagram shown in Fig. 5 illustrates the change of impedance of surface layer for samples 1 and 2 versus time of immersion in SBF solution. The chart gives the evidence of the deposits shown in SEM pictures (Figs. 1-2) and confirms that after about 72 hours the value of impedance of surface layer decreased. Thus, also the electric capacitance as well as the
amount of deposited HA decreased, and then started increasing again. The same tendencies of impedance changes are shown in Bode charts (Figs. 6 and 7) presenting the values of impedance and phase angle versus frequency. Two time constants in impedance characteristics of surface layer seen in Bode chart in Fig. 7 indicate that the surface layer is built of two sub-layers.

One is the layer of nanotubes formed in the process of anodizing, and the other may be the layer of deposits. The results of impedance investigations were fitted to the equivalent circuit (Fig. 8), which represents two-layer model - titania nanotubes and deposits. The circuit is composed of resistances R and capacitances C. The Rs element represents the electrolyte resistance. The serial connection of Ra and Cpa elements represents the resistance of electrolyte and the double layer of the inner precipitates inside the nanotubes [23], respectively. Table 2 shows the values of these elements for conducted measurements.

For samples with nanotubes formed in 1 M H₃PO₄ + 0.3 wt% HF, the intensive deposition of SBF solution components on negatively polarized nanotube surface can be observed. However, after about 72 hours the deposition process stops and HA starts ‘to dissolve’, which can also be observed in the impedance diagrams (Figs. 5, 6 and 7).

In case of other HF concentrations (0.2 and 0.4 wt% HF) – the value of impedance is the highest after 72 hours (Fig. 9). However, during next hours HA starts ‘to rebuild’ in both cases, which is probably caused by the effect of blocking being the result of salts deposing inside nanotubes.

The observed changes in elements concentration and results of impedance tests can be explained as follows. The increase of fluorine concentration in Ca-O-P deposits (Tab. 1, Area 1) can be observed up to 72 hours. The increase of fluorine concentration in deposits is probably caused by the growth of thickness of Ca-O-P precipitates with time of immersion in SBF solution (the more deposits the more fluorine in the deposited layer). The source of fluorine transferred into the deposits is the substrate titania nanotube layer (titanium oxide containing fluorine ions - Area 2, Fig. 3). Then, the rebuilding of calcium phosphates takes place and the fluoroapatite is formed. EDS analyses of surface layers show that after further 120 hours the amount of fluorine decreases. It can be supposed that the speed of diffusion and speed of in-building fluorine into the calcium phosphate lattice is lower than the speed of growing up of the deposits layer.

Table 2. Values of parameters of equivalent circuit for investigated samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time (h)</th>
<th>Rs (Ω·cm²)</th>
<th>Ra (Ω·cm²)</th>
<th>Cpa·10⁻¹⁰ (F/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 wt% HF</td>
<td>1</td>
<td>1.66</td>
<td>4.54</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.55</td>
<td>4.23</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>1.65</td>
<td>5.24</td>
<td>5.21</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>1.64</td>
<td>5.06</td>
<td>8.76</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 wt% HF</td>
<td>1</td>
<td>1.50</td>
<td>4.54</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.41</td>
<td>5.10</td>
<td>3.77</td>
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<td>1.25</td>
<td>5.26</td>
<td>8.62</td>
</tr>
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<td></td>
<td>120</td>
<td>1.55</td>
<td>4.98</td>
<td>8.25</td>
</tr>
<tr>
<td>Sample 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 wt% HF</td>
<td>1</td>
<td>1.71</td>
<td>4.56</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>1.57</td>
<td>4.43</td>
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<tr>
<td></td>
<td>120</td>
<td>1.84</td>
<td>4.91</td>
<td>9.11</td>
</tr>
</tbody>
</table>
substrate titania nanotube layer (titanium oxide containing and 0.4 wt% HF

Fig. 10. Changes of resistances Rs (a) and Ra (b) and capacitance Cpa (c) of equivalent circuit during immersion in SBF solution for layers of nanotubes formed in 1 M H₃PO₄ with 0.2 wt%, 0.3 wt% and 0.4 wt% HF.

The source of fluorine transferred into the deposits is the substrate titania nanotube layer (titanium oxide containing fluorine ions - Area 2, Fig. 3). Then, the rebuilding of calcium phosphates takes place and the fluoroapatite is formed. EDS analyses of surface layers show that after further 120 hours the amount of fluorine decreases. It can be supposed that the speed of diffusion and speed of in-building fluorine into the calcium phosphate lattice is lower than the speed of growing up of the deposits layer.

It is known from our XPS measurements [24] of titania nanotubes formed in 1 M H₃PO₄ with different HF concentrations that sample 2 (0.3 wt% HF) contains more fluorine and less phosphorus in comparison with the other samples (0.2 and 0.4 wt% HF). Fluorine changing conductivity of solid oxides, influences also the conductivity of the investigated samples. The increase of fluorine during 72 hours (Tab. 1, Area 1, Fig. 4b) explains the changes of equivalent circuit parameters, especially Rs (the biggest value of F and the smallest value of Ra for 72 hours) and Cpa, which are observed in Fig. 10.

4. Conclusions

Titania nanotubes formed in phosphoric acid solution (1M H₃PO₄ + 0.2-0.4% HF) and immersed in SBF solution showed that the developed porous structure considerably stimulates the process of deposits precipitation as soon as the immersion begins. Some deposits similar to hydroxyapatite particles precipitated on the layer of nanotubes.

The SEM and EDS results revealed that fluorine present in the nanotubes layer and transferred into the deposits from nanotubes is responsible for the stimulation of the process of calcium deposition on the surface of nanotubes. The most deposits can be observed up to 24 hours, but for 0.3 wt% HF the amount of deposits decreases afterwards. The dynamics of deposition of HA on three different samples varies in such a way that, at first, the intensive deposition of calcium and phosphorus ions can be observed for all three ones, but then for the 0.3 wt% HF the amount of precipitates drops up to 72 h due to changes of fluorine concentrations, which has the influence on conductivity of the layer.

Acknowledgements

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References

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Titania nanotubes formed in phosphoric acid solution (1 M H₃PO₄ + 0.2-0.4% HF) and immersed in SBF solution showed the layer of nanotubes. The SEM and EDS results revealed that fluorine present in the deposits layer. Then, the rebuilding of calcium substrate titania nanotube layer (titanium oxide containing 0.4 wt% HF) was finished. Then, the rebuilding of calcium substrate titania nanotube layer (titanium oxide containing 0.4 wt% HF) was finished. Fluorine changing conductivity of solid oxides, phosphorus in comparison with the other samples (0.2 and 0.4 wt% HF). Fluorine changing conductivity of solid oxides, phosphorus in comparison with the other samples (0.2 and 0.4 wt% HF). Fluorine changing conductivity of solid oxides, phosphorus in comparison with the other samples (0.2 and 0.4 wt% HF).

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References