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The effect of biological environment on the surface of titanium and plasma-sprayed layer of hydroxylapatite

V. ĎALKA, J. IVAN, E. POŠTRKOVÁ, V. KOLENČIAK
Institute of Materials and Machine Mechanics, Slovak Academy of Sciences, Racianska 75, P.O. Box 95, 830 08 Bratislava 38, Slovakia

A. KRSEK
Department of Manufacturing Systems, Slovak Technical University, Namestie slobody 17, 812 31 Bratislava, Slovakia

I. INFNER
Military Hospital, Cesta mladeže 1, 833 31 Bratislava, Slovakia

H. K. KOERTEN
Laboratory for Electron Microscopy, Leiden University Rijnsburgerweg 10 (Building 54) 2333 AA Leiden, The Netherlands

The solubility of titanium samples with different surface coatings, i.e. hydroxylapatite (HA) powders, a two-layer coating of ZrO_5 + HA on a titanium substrate in solution and of tooth implants after long-term functioning in the human organism, was studied. A minimum difference in solubility of titanium samples with different surface finishes (polished or grit blasted) was established. For the HA powders and coatings, the lowest solubility was observed with a coarse-grained HA–B powder and a coating made of that powder. Clinical tests of tooth implants after long implantation times were performed. A titanium implant (implantation 12 y), a titanium implant with a two-layer coating of ZrO_5 + HA–A (implantation time 4 y) and a titanium implant with a two-layer coating of (Al_5O_3 + 3% TiO_2) + HA–A (implantation time 6 y) were studied. The results show that the titanium surface and HA–A layers were dissolved. Nevertheless, after 6 y implantation, total removal of HA–A coating from that part of implant set into the bone, was not observed.

1. Introduction

Hydroxylapatite, $Ca_{10}(PO_4)_6(OH)_2$, belongs to the group of surface-active bioceramics. The possibilities for its application in medicine are numerous. For example, as a filler material or as a coating on metallic substrates in stomatology and orthopaedics.

The main characteristics of apatite coatings are:

(a) they reduce the time necessary for bonding the implant to bond with bone;

(b) they enable good bonding of implant with the bone even with a less precise surgical technique;

(c) the coating is, after implantation, degraded within a certain time period.

A number of studies have been devoted to the solubility of HA and other phases in different environments [1–4]. It was demonstrated that HA powder, transported through the plasma beam, is subject to decomposition and new phases are formed [5]. The solubility of those phases is higher when compared to HA. For example, *in vitro* tests showed a decrease in the sequence tetracalciumphosphate–tricalciumphosphate–HA, while the effect of the medium is higher than the effect of pH [2]. By plasma spraying of HA, an amorphous phase is also formed and it exerts higher solubility than the crystalline HA [3]. The solubility of HA increases with a decrease in its crystallinity [6].

It must be taken into account that there is a considerable difference between the *in vivo* and *in vitro* conditions. The results of *in vitro* tests may correspond just a little or not at all to the *in vivo* conditions [2]. The coating integrity is also affected (as proved by a significant decrease in HA coating thickness) by the synergic effect of cyclic loading and an environment simulating the liquids in a human body [7].

The aim of present work was to study the effect of a biological environment on the dissolution of a titanium surface and HA layers produced by plasma

E-mail: postmast@umms.savba.sk

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spraying on a ZrO_2, (Al_2O_3 + 3% TiO_2) layer, which was deposited in advance by plasma spraying on a titanium substrate.

2. Materials and methods

For plasma spraying, the SNECMA equipment of PLASMA–TECHNIK with an F4-HB torch, was used. The grit-blasted sample surfaces of titanium (POLDI 45) were sprayed with HA from different producers:

(a) HA–A, mean grain size 3.8 μm (Merck, Germany);

(b) HA–B, mean grain size 88.2 μm (Amdry 6020);

(c) HA–C, mean grain size 11 μm (calcinated) and 6.9 μm (non-calcinated) (Fosfa, Břeclav, Czech Republic).

The distance of HA spraying was 65 mm. The plasma gases hydrogen (8.8 l min^{-1}), argon (43 l min^{-1}), carrying gas argon (3.8 l min^{-1}) were used. The spraying power was 24 kW. The titanium substrate was deposited with two layers, produced by plasma spraying of ZrO_2 (0.05 mm) and HA (0.05 mm) powders (designation: Ti + ZrO_2 + HA, while being aware that during plasma spraying of HA its decomposition takes place and thus in addition to the HA, other phases may also occur in the coating structure). The cylindrical samples were partially immersed in the infusion solution (Infundibile Natrii Chlorati isotonikum) which contained 154 mmol/l Na^+ and 154 mmol/l Cl^-. The presence of elements in the biological solution was determined using an atomic absorption spectroscope type Perkin–Elmer 2380. The surface of the implants was studied with a scanning electron microscope (Philips SEM525 M), equipped with a Voyager X-ray microanalysis system (Noran Instruments) and a JEM 100 C electron microscope with an additional scanning device ASID-4D and X-ray microanalysis system (Kevex) (IMMM-SAS). Some samples were sterilized in a hot-air sterilizer type HS-61 (Chirana) in the following cycle: heating to 160 °C for 30 min and holding at that temperature for 60 min; cooling down to during 20 °C in 6 h.

3. Results and evaluation

The effects of the biological environment upon the powder, substrate and coatings are summarized in Tables I and II. Table I represents the results after the following exposure times: 12 500 h, of which 2500 h were at 37 °C and 10 000 h at room temperature. The results presented in Table II correspond to the effect of the biological environment for 720 h at 37 °C and 4248 h at room temperature.

The results given in Tables I and II can be summarized as follows:

1. From the studied HA powders, the grained HA–B powder exerted the lowest solubility in a given medium. Table I also shows that calcined HA–C powder is significantly less soluble compared to non-calcined HA–C powder.
2. The lowest solubility of all the HA coatings was observed for the coating formed from HA–B powder (Tables I and II).
3. The solubility of HA coatings is higher than the solubility of HA powders used for their deposition (Table I).
4. There is just a slight difference in solubility between the titanium samples with polished and grit-blasted surfaces (Table II).
5. There was no significant difference in solubility between titanium samples and titanium samples coated with ZrO_2 + HA–A coating (Table II).
6. The surface roughness of the HA coating layer, as characterized by the R_a (μm) parameter after exposure to the biological medium, is lower in comparison to the surface roughness of an HA coating layer directly after plasma spraying (Table II).

TABLE I Solubility of HA powders and HA coating layers in a biological environment (μg ml^{-1})

Sample	Sample	Ca	P	Ti
1	Reference solution	0.55	< 0.1	< 0.1
2	HA–A powder	65.4	6.3	
3	HA–B powder	18	6.3	
4	HA–C powder non–calcinated	162.8	4.4	
5	HA–C powder calcinated	60.4	< 1	
6	Two-layer coating ZrO_2 + HA–A on Ti substrate	1367	< 0.1	< 0.1
7	Two-layer coating ZrO_2 + HA–B on Ti substrate	721.5	< 0.1	< 0.1

TABLE II Solubility of the substrate material (titanium) and of the coating layer (HA) in a biological environment

Sample		Solubility (μg ml^{-1})			R_a(μm)	
		Ca	P	Ti	After plasma spraying	After the test
1.	Ti-grit blasted surface			0.026	2.81 ± 0.32	1.94 ± 0.33
2.	Ti-polished surface			0.023	0.14 ± 0.01	0.15 ± 0.01
3.	Two-layer coating ZrO_2 + HA–A on Ti	88.11	3.73	0.026	4.24 ± 0.74	3.66 ± 0.1
4.	Two-layer coating ZrO_2 + HA–A on Ti + two sterlization cycles	50.84	2.4	0.024	4.23 ± 0.62	3.19 ± 0.29
5.	Two-layer coating ZrO_2 + HA–B on Ti	48.94	1.04	0.023	5.52 ± 0.28	3.16 ± 0.4
6.	Two-layer coating ZrO_2 + HA–C on Ti	147.1	4.66	0.024	5.44 ± 0.6	4.13 ± 0.4
7.	Reference solution	0.48	< 0.1	0.022		

The higher solubility of the fine-grained HA–A powder is mainly the result of the fact that HA–A powder degrades to the α-$Ca_3(PO_4)_2$, β-$Ca_3(PO_4)_2$, CaO and amorphous phase is also present due to the transfer through the plasma beam. It exerts lower crystallinity [5], and also the larger surface area of HA–A powder must be taken into account.

Due to incidental, irregular deposition of particles with variable measure of deformation on the coarse surface of the substrate, Fig. 1, the HA coating produced by plasma spraying is porous. In the case of an open porosity in the ZrO_2 and HA layers, the medium can even penetrate to the titanium substrate. This allows a medium/substrate reaction that can create the conditions which allow the coating to peel from the substrate. The fact that the titanium values (Table II) are approximately of the same level, indicates that the eventual dissolution of titanium substrate is low and that this does not exert any effect upon the results obtained under the given conditions.

It has been shown that different biological environments can exert different effects upon the solubility of an HA coating [2]. The results from this study suggest that the biological medium used does not have a significant effect as far as dissolution is concerned.

The solubility of non-calcinated HA–C powder is high compared to that of calcinated HA–C powder. This finding is in agreement with the results of other studies [6, 8] from which it can be concluded that the calcination of HA powder increases the crystallinity and thus reduces its solubility.

As already mentioned above, the results from *in vitro* studies on the effect of HA solubility may differ considerably from the results of *in vivo* experiments [2]. This depends most likely on the fact that the selected environment in the *in vitro* tests does not completely simulate the biological environment of the *in vivo* tests. The results of *in vivo* and *in vitro* studies on the solubility of HA coatings produced by plasma spraying have shown that dissolution and thus also reduction of HA coating thickness can occur [1–4, 7]. For example Dhert [4] reported that the HA coating on implants embedded in spongy and compact bone of the goat was almost totally vanished after 25 wk. Osborne [8] showed that HA degradation depends on cellular processes on the HA–bone interface, that are most active during the healing phase. After bone healing, the process of HA dissolution is slowed down or ceases completely [8, 9].

The results of a study on titanium (POLDI 45) tooth implants are given below. Implants with two-layer coatings (Al_2O_3 + 3% TiO_2) + HA–A or ZrO_2 + HA–A on a titanium substrate were tested in a clinical study, several years after implantation in the human jaw. The results of that study show that gaps remained visible after prolonged implantation time. The implants had to be extracted because of inflammation. Scanning electron micrographs of the surface of titanium implants prior to implantation and 12 y after implantation are shown in Fig. 2. The surface of an extracted blade-type implant (Fig. 2b) is coarse and articulated compared to unused implants (Fig. 2a),

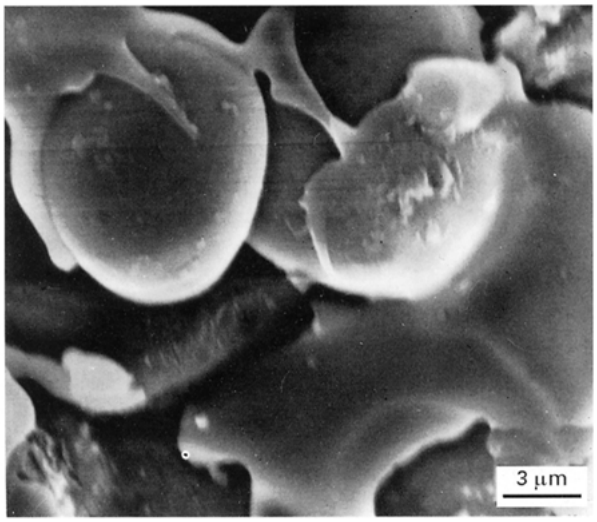
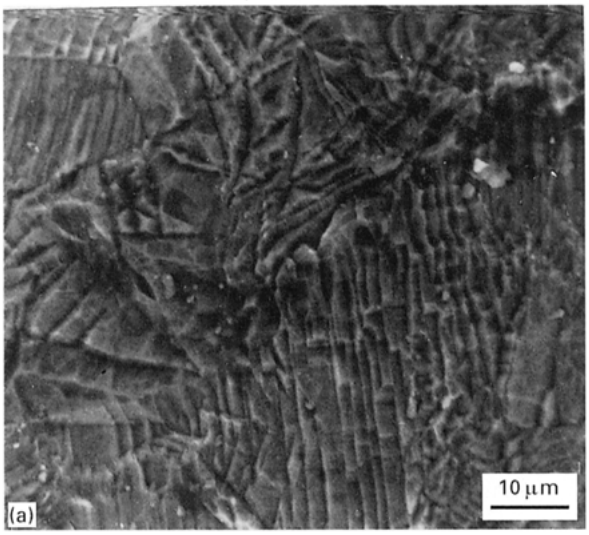


Figure 1 Scanning electron micrograph showing HA–A particles with different deformation on a titanium substrate.

and clear proof of dissolution of the titanium surface is visible.

Scanning electron micrographs showing the surface of a cylindrical implant with a two-layer ZrO_2 + HA–A coating after plasma spraying and an extracted implant, 4 y after implantation, are shown in Fig. 3. The original coarse surface of the HA–A layer (Fig. 3a) is dissolved and smoothed due to influences of the biological environment (Fig. 3b). X-ray microanalysis of the surface of the extracted implant demonstrated calcium and phosphorus, indicative of the presence of remnants of the HA–A coating (Fig. 4a). In other zones, the presence of zirconium was demonstrated by X-ray microanalysis. This zirconium is derived from the ZrO_2 layer (Fig. 4b). It is concluded that HA–A was removed from the sites where zirconium only was found. The seeming absence of an oxygen peak in the spectrum of Fig. 4a is explained by the proportionally low number of counts compared to the calcium and phosphorus signals.

The relatively distinct boundary between the HA zones and zirconium-rich areas suggests that removal of the HA layer was not due to the effect of the biological environment, but more likely due to destruction of the HA layer during the extraction of the implant. An X-ray microanalysis spectrum of an implant surface with a two-layer coating of (Al_2O_3 + 3% TiO_2) + HA–A on a titanium substrate, extracted 6 y after implantation, is shown in Fig. 5. The analysis of the implant surface was taken from morphologically different zones of the extracted implant. The spectra show, similar to the results on the ZrO_2 + HA–A coating, that the implant surface contains zones with HA–A rich layers, characterized by calcium and phosphorus peaks (Fig. 5a) and zones, where the HA–A layer was partially or totally removed (Fig. 5b). This means that the HA–A layer was not completely resorbed. Even after 4 or 6 y implantation in bone, including an inflammatory process and followed by extraction, identifiable remnants of part of the coating were still present. This is in agreement with the results of other authors [10], where, on extracted tooth



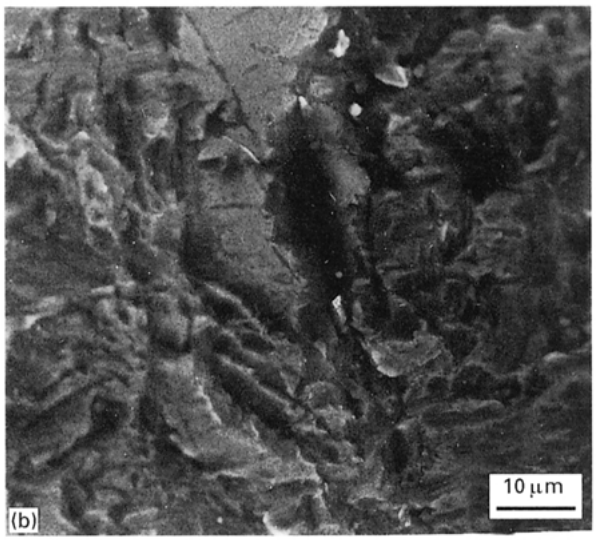


Figure 2 Scanning electron micrographs of the surface of a titanium implant (blade type) (a) prior to implantation, but after etching in 10% solution of hydrofluoric acid in distilled water, (b) 12 y implantation.

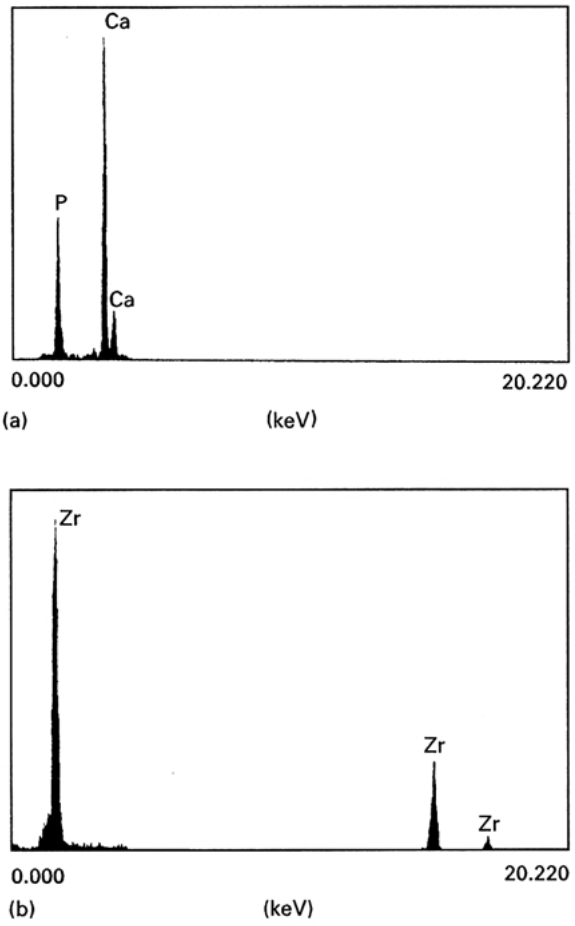
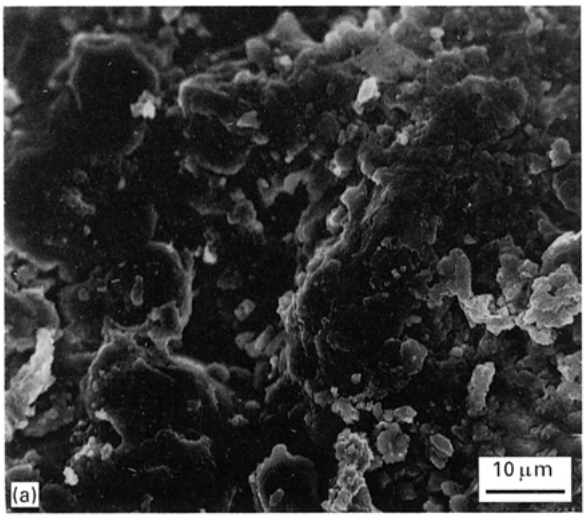


Figure 4 X-ray microanalysis spectra of the surface of extracted titanium implant, (a) from an implant with a ZrO_2 + HA–A coating demonstrating the presence of an HA–A layer, (b) spectrum of the surface of a ZrO_2 layer showing clearly the presence of zirconium.

implants thin, discontinuous zones of an HA layer were identified. The overall HA layer is discontinuous. However, the degree of discontinuity of an HA layer on the extracted implant cannot be translated to biological processes, because the implant coating will also be disrupted during the extraction of the implant. In



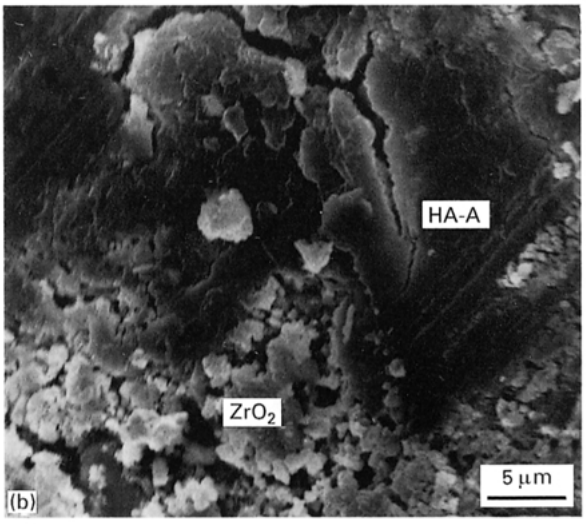


Figure 3 Scanning electron micrographs of the surface of cylindrical titanium implant with a two-layer ZrO_2 + HA–A coating, (a) directly after plasma spraying, (b) extracted 4 y after implantation.

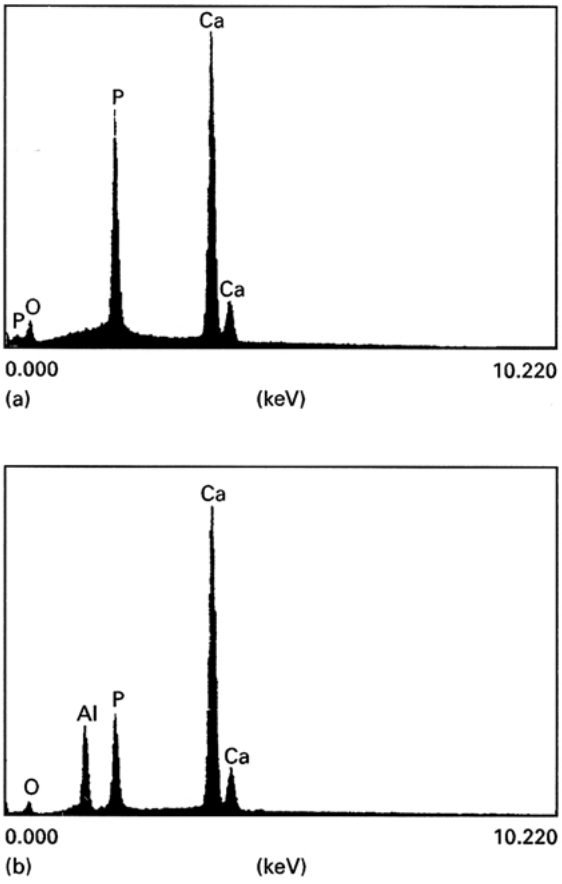


Figure 5 X-ray microanalytical surface analyses of an extracted titanium implant with (Al_2O_3 + 3% TiO_2) + HA–A coating; (a) spectrum from the surface of an HA–A layer, (b) spectrum from the surface composed of HA–A and Al_2O_3 + 3% TiO_2 layers.

the zone of implant contact with the soft tissue, the HA–A layer was fully resorbed. The presence of an HA layer on the implants extracted 4 and 6 y after implantation proves that the process of HA dissolution has markedly showed or possibly even ceased. This is in agreement with other studies [8, 9].

4. Conclusion

Experimental studies on the solubility of titanium samples with different surface finishes (polished, grit blasted) in the environment of an infusion solution have not proved any significant difference. Considering the powders and HA coating layers, the lowest solubility was observed with HA–B powder and coatings made of HA–B.

The clinical tests of tooth implants made of titanium (blade type), Ti + ZrO_2 + HA–A and Ti + (Al_2O_3 + 3% TiO_2) + HA–A (cylindrical type), have shown that the titanium surface and also HA–A layers on a titanium substrate with ZrO_2 and (Al_2O_3 + 3% TiO_2) coating layers were dissolved. However, even after 6 y implantation, total removal of a HA–A coating layer from the implant set into the bone was not achieved.

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