

## Micro-PIXE Analysis Of Bioconductive Hydroxyapatite Coatings On Titanium Alloy

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**Abstract.** Bioconductive materials and in particular implants using Ti alloy (Ti6Al4V) coated with hydroxyapatite (HAp) have proved to be a suitable surgical procedure. However, experience has shown that these implants not always have the required reliability to guarantee their expected life-span of approximate 15 years. In this research, experimental Ti alloy-implants coated with HAp and incubated in a simulated body fluid (r-SBF) under controlled physiological conditions were studied by nuclear microscopy. Selected HAp coatings, were analysed by nuclear microprobe (NMP) with protons of 1.5 MeV at the iThemba LABS facility. Major elements (Ti, Al, V, Ca and P) as well as trace elements (Si, K, Fe, Zn and Sr) were determined. The effect of longer incubation time was of particular interest. Results confirmed that secondary Ca-deficient defect hydroxyapatite precipitated from the simulated body solution onto the HAp coating surface after prolonged incubation. This newly formed layer is thought to be of vital importance for bonding of implants with living bone tissue.

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### INTRODUCTION

Hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] (HAp) coatings are widely applied to the stem of hip joint implants material in reconstructive orthopaedic or to dental root implants to enhance their biological fixation to the bone [1,2,3]. However, their utilization is severely limited due to poor intrinsic mechanical properties, which can lead to implants instability and substandard life span in the presence of body fluids [4,5]. Forming bio-functional composites between HAp and a metallic material is an effective way to overcome weak bonding strength between living tissue and implant [6]. Typically Ti6Al4V alloy having an alpha-beta microstructure is one of the most used metallic materials for the manufacturing of such composites by plasma spraying [1,7,8]. This process involves the introduction of hydroxyapatite powder to a plasma jet which melts the particles and propels them towards the Ti-alloy substrate. In this work, atmospheric plasma spraying (APS) [9] was applied to deposit HAp coatings onto Ti6Al4V substrate, followed by incubation in revised simulated body fluid (rSBF) [10]. Since there are still several unresolved issues about the quality and reliability of these coatings

[11,12,13], in particular the chemical and phase composition changes of the HAp coatings when brought into contact with living tissue, it is vital to further understand these changes to improve the reliability of HAp coatings for human implants application. Microanalysis of HAp coatings may help to quantify surface modifications of major components and/or trace metals in the coatings. Therefore, this investigation was focused on the micro-PIXE analysis and elemental mapping of HAp coatings, plasma sprayed onto Ti6Al4V after incubation in a revised simulated body fluid (rSBF) under controlled conditions.

### EXPERIMENTAL PROCEDURES

#### Materials

The materials used in this study were commercially available medical-grade hydroxyapatite powder as spraying powder and a mill-annealed Ti6Al4V alloy (6.12 at% Al, 4.20 at% V, 0.17 at% Fe, 0.01 at% C, 0.105 at% O, 0.005 at% N, 0.003 at% H, Ti bal.) as substrate material.

The machined samples (coupons of 20 x 20 x 2 mm) were prepared by pickling in acid to remove any surface oxide and degreasing by rinsing in acetone. In order to obtain a minimum roughness of 2  $\mu\text{m}$ , the samples were grit blasted with corundum. After blasting the samples were ultrasonically cleaned in a solution of 10% Tickopur<sup>TM</sup> in deionized water at 40 °C.

Medical-grade HAp powder was characterised as high purity HAp with a particle size range of 120 $\pm$ 20  $\mu\text{m}$ , a crystallinity of 85%, and HAp content of 97.6 $\pm$ 0.8 mass%. The remaining material consisted of 2.1 $\pm$ 0.8 mass% tetracalcium phosphate and 0.3 $\pm$ 0.5 mass% tricalcium phosphate as determined by Rietveld refinement.

### Coating Deposition

The HAp powder was deposited onto Ti6Al4V substrates preheated to 400 °C using an atmospheric plasma spray system (PT M-1000, Plasma-Technik Wohlen, Switzerland) equipped with an F4-MB plasmatron (Sulzer Metco (Deutschland) GmbH) [9,14]. The spraying pattern consisted of six horizontal passes of the plasmatron, each followed by an inter-cycle cooling period. To avoid significant heating during spraying, the samples were cooled with a stream of argon at the back. The Ti6Al4V substrates were coated with hydroxyapatite to a thickness of about 200  $\mu\text{m}$ .

### Incubation Process

To simulate the effect of a biological environment, the coated specimens were incubated for 28 and 56 days in a r-SBF solution under controlled physiological conditions (at 37 $\pm$ 0.5°C). The composition of the simulated body fluid was: Na<sup>+</sup>=142.0; Ca<sup>2+</sup>=2.5; K<sup>+</sup>=5.0; Cl<sup>-</sup>=103.0; Mg<sup>2+</sup>=1.5; HPO<sub>4</sub><sup>2-</sup>=1.0; SO<sub>4</sub><sup>2-</sup>=0.5; HCO<sub>3</sub><sup>-</sup>=27.0 mmol/g [10]. The samples were afterwards rinsed with deionised water, dried in air at 100 °C for 24 hours and then kept in a dry atmosphere until irradiation.

### Microprobe Analyses-PIXE Mapping

Carbon coated pieces of hydroxyapatite coating (top-side of coatings, ~1cm<sup>2</sup> in surface area) peeled off from the Ti6Al4V substrate were irradiated at the NMP facility at iThemba LABS [15,16]. Proton beams with energies of 1.5 MeV were selected for bombardment of particular micro-regions of the HAp coatings. Beam currents of 200-400 pA were used to ensure that no evaporation of constituents occurred due to a possible rise in temperature generated in the sample volume. Micro-regions were scanned with a proton-probe of 2-4  $\mu\text{m}$ . Areas of approximately 250 x 250  $\mu\text{m}^2$  were selected on the HAp coatings for analysis and scanning with a dwell time of 10ms, in a

map matrix of 128 x 128 pixels. True overlap-resolved elemental maps as well as total micro-PIXE data were obtained using the Dynamic Analysis (DA) method [17]. Off-line analyses of experimental data recorded as event-by-event computer files were obtained with the software package Geo-PIXE II [18]. In addition crystalline phase contents of the coatings were determined by x-ray diffraction (URD 6, Freiburger-Präzisionsmechanik). Measurements were performed using CoK $\alpha$  radiation generated by an x-ray tube operated at 40 kV and 35 mA with 2 $\theta$  angle ranging from 25° to 55° and at a scan step size of 0.03°. For quantitative data, Rietveld refinement was carried out using structural models of different calcium phosphate phases obtained from the ICSD (Inorganic Crystal Structure Database).

## RESULTS

### Average Elemental Concentration of HAp Coatings

PIXE spectra were obtained by irradiation with 1.5 MeV. Comparison of PIXE spectra of hydroxyapatite coatings incubated for different times (28 and 56 days) showed difference in intensity of P and Ca (main elements of HAp), the presence of minor elements such as Mg, K, and trace elements Fe, Co, Sr. Other elements such as Ru, Pd, Ag, and Cd were present in smaller amounts but were not quantified. Table 1 shows the concentration levels and uncertainty of the elements as calculated by the software package Geo-PIXE II.

**TABLE 1.** Concentration levels ( $\mu\text{g/g}$ ) of elements in incubated HAp coatings detected by PIXE.

Elements	Incubation time	
	28 days	56 days
Ca (%)	19.1 $\pm$ 0.2	29.5 $\pm$ 0.3
P (%)	6.6 $\pm$ 0.07	10.5 $\pm$ 0.1
Mg (%)	0.23 $\pm$ 0.04	0.48 $\pm$ 0.04
Ti (%)	n.d.	0.78 $\pm$ 0.013
S ( $\mu\text{g/g}$ )	184 $\pm$ 27	207 $\pm$ 27
K ( $\mu\text{g/g}$ )	n.d.	1,400 $\pm$ 127
Fe ( $\mu\text{g/g}$ )	89 $\pm$ 21	465 $\pm$ 31
Co ( $\mu\text{g/g}$ )	92 $\pm$ 19	113 $\pm$ 19
Sr ( $\mu\text{g/g}$ )	339 $\pm$ 92	346 $\pm$ 57

n.d.= not detected

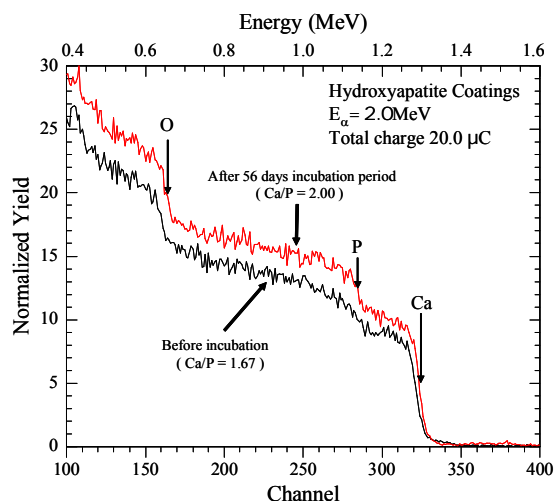
### Evolution of Ca/P ratio in HAp Coatings

The Ca/P ratio of HAp coatings was determined by Rutherford backscattering spectrometry with 2.0 MeV

alpha particles. These results showed that the stoichiometric Ca/P ratio of 1.67 at time zero (before incubation in rSBF) was depleted to a value of 1.25 after an incubation time of 7 days. Subsequent time of incubation at 28 and 56 days showed values for the Ca/P ratio of 1.68 and 1.93 respectively (see Figure 1).

### Elemental Mapping Analyses

Calcium elemental maps obtained from the coatings at 28 and 56 days showed a marked difference in the average Ca levels. The concentration of Ca was significantly lower in the coating incubated for 28 days (average concentration range was 5-20%) [19] than in the HAp coatings incubated for the longer incubation period of 56 days (average concentration range was 20-40%).



**FIGURE 1.** Comparison of RBS spectra for the hydroxyapatite coatings before and after 56 days.

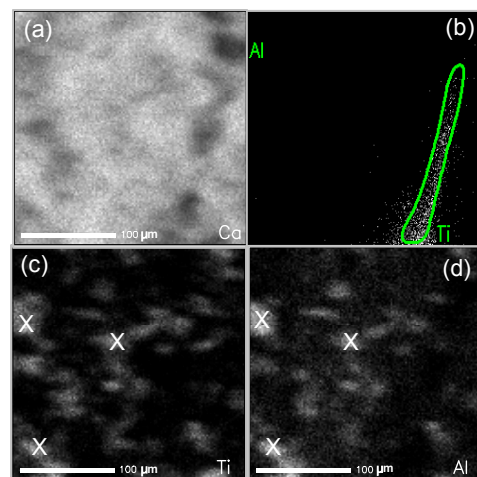
A similar behavior was observed for P the second major component of hydroxyapatite. The relative error of the analysis was of the order of 5%. This error includes experimental errors as well as uncertainties in the theoretical and experimental parameters used in the database of GeoPIXE II for the evaluation of the thick-target X-ray yields.

Considerable amounts of Ti were detected in the coatings particularly in the one incubated for 56 days. Figure 1b shows a correspondence between the Al and Ti maps obtained by GeoPIXE II (top right corner). A pronounced linear correlation (enveloped in the figure with a boundary line) between both Al and Ti is marked on each map (Al and Ti) with a cross (X) as shown in Figure 2 (c,d). Of relevance is the fact that Ca does not correlate with Al or Ti.

### DISCUSSION

Evolution as obtained by micro-PIXE analysis of the composition of HAp within the surface and near

surface of the coatings showed a marked increase of Ca with time of incubation. This was also confirmed by the calculated Ca/P ratio by alpha particle backscattering measurements, which showed a pronounced increase of the ratio to a value of ~1.92 way above the Ca/P stoichiometric value of 1.67. These findings correlate well with parallel investigations by XRD phase analysis on a similar set of HAp coatings [19] where results point to an excess of HAp precipitated onto the original stoichiometric matrix [19].



**FIGURE 2.** Elemental maps of HAp coating obtained by irradiation with 1.5 MeV protons. The coating was incubated in simulated body solution (rSBF) for 56 days. (a) Ca map; (b,c,d) Areas marked with X indicate enrichment of both elements in such locations.

In addition, considerable amounts of Ti were detected in the surface of coatings incubated for 56 days. A tentative explanation for this can be based on a possible change of coating structural morphology during prolonged incubation in rSBF [20], in particular the occurrence of cracks propagating along the surface of the coating [21] that would enable the Ti to diffuse to the hydroxyapatite coatings surface level. Several authors have measured the amounts of occasional Ti metal released into the surrounding bone tissue [22,23,24]. Secondly,  $Ti_nO_{2n-1}$  covering the surface of the Ti6 Al4V as a native non-stoichiometric oxide may dissolve in molten HAp and is thought to replace Ca ions to form compositions of the type  $Ca_{1-x}Ti_{x/2}(PO_4)_6(OH)_2$  [25,26]. On dissolution of the HAp coating these Ti ions will be mobilised and reprecipitated within the thin layer of secondary, *i.e.* bone-like HAp. However, the titanium alloy may also release ions to the solution, as pointed out by some researchers [27] who found that an *in vitro* metal release is induced by macrophages; however, no evidence of such diffusion *in vivo* has been found yet,

and most investigations, in which implant titanium was found, report only occasional local accumulation of the metal, without apparent adverse metabolic effects. The results of their study suggest that its presence in the implant tissue is inconsistent, and very likely due to localised micro-detachments, indicating a very low capability of titanium to diffuse at a distance [27].

## CONCLUSIONS

This research has demonstrated results which are relevant to improve the quality of bioconductive coatings for human joint implants. The most relevant findings of this study can be summarised as follows:

1) The stoichiometric value of the Ca/P ratio change with time of incubation. This point to the formation of secondary hydroxyapatite by biomimetic processes, *i.e.* a dissolution-reprecipitation mechanism thought to be governing the mechanisms of osseointegration of coated implants into living bone tissue.

2) Spatially resolved PIXE elemental maps further confirm that the levels of Ca and P in the precipitated HAp layer (56 days) increased with time of incubation.

3) Considering the titanium which has been found in the hydroxyapatite coating incubated for 56 days, it is reasonable to speculate about possible diffusion of Ti through the HAp coating. This can be explained by the areas marked X in Fig. 1 which represents enriched areas of Ti in the HA.

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