

HYDROXYAPATITE PULSED LASER DEPOSITED THIN FILMS BEHAVIOUR WHEN SUBMITTED TO BIOLOGICAL SIMULATED TESTS

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(Received September 6, 2005)

Abstract. Structures of hydroxyapatite (HA) as thin films, with and without buffer layers, were deposited on Commercially Pure Titanium Grade 4 (c.p. Ti gr4) substrates by Pulsed Laser Deposition. They were afterwards heat treated in water vapours. In this study, processes taking place at the hydroxyapatite/Hank's Solution interface are investigated. The experiment consists of immersing the films in Hank's Solution for 21 days, at a constant temperature of 37°C. The composition and the morphology of the films are investigated before and after the experiment. The deposited layer was neither dissolved nor destroyed due to hydroxyapatite's good chemical stability and adherence to substrate. Moreover, at the hydroxyapatite/Hank's Solution interface the formation of a uniform Ca₂P₂O₇ thin layer is detected, which could lead to a better bio-integrability. Also, single micro-crystals of NaCl are observed on the surface of the samples after the experiment, demonstrating the good surface conditions for cell-adhesion. No Ca₂P₂O₇ is synthesized in the case of the reference sample (Ti without deposition), while the NaCl (Halite) crystals were very small. Our findings confirm the better biological simulated behaviour of hydroxyapatite films, demonstrating very convincing premises for bio-integration and bio-activity.

Key words: hydroxyapatite, laser deposition, interface processes.

INTRODUCTION

The use of different techniques to coat metallic implants with calcium phosphate-based materials is aimed to promote the combination of the strength properties of the metal with bioactive character of the ceramic, leading to better bonding with the newly remodelled bone [1]. Calcium phosphate (CaP, particularly HA) coatings are generally accepted to be biocompatible because their compositions are similar to the mineral content of natural bone. Different research groups [2, 3]

have shown that CaP coatings can promote early bone apposition and fixation of the implants by encouraging chemical bonding between new bone and the surface of these materials. Implants with HA coatings have been shown to have much better bone apposition compared with uncoated implants in the first several months after implantation [4]. The coating acts as active interface for bony tissue proliferation and provides compensation for lack of direct bony contact when the surgical fit is poor.

Titanium and its alloys are often used as metallic biomaterials because of their high corrosion resistance and excellent mechanical properties. However, titanium is sometimes detected in tissues around implants. Released titanium ions may combine with molecules such proteins and induce biological hindrances (*e.g.* toxicity and allergy) [5].

Some papers in literature sustain that introducing a buffer layer between the titanium substrate and hydroxyapatite film may result in a diminution of metallic ions released in the surrounding tissues [6]. Also, the buffer layers serve as a barrier during the deposition process, avoiding the hydroxyapatite film contamination with titanium atoms [7]. They also increase the adhesion tensions by improving the HA film adherence [8].

The deposition of hydroxyapatite thin films can be performed by different methods. The Pulsed Laser Deposition (PLD) is gathering interest due to its versatility and controllability, as opposed to the more conventional plasma spray and other techniques. The advantage of PLD technique is the aptitude to synthesize and deposit uniform, pure, crystalline and stoichiometric hydroxyapatite films [9, 10]. Up to now, UV excimer lasers generating at 193 nm, 248 nm and 308 nm have been used, allowing obtaining hydroxyapatite coatings with different structural and compositional properties.

In vitro studies of excimer laser deposited coatings have been documented, focusing on the compositional and structural changes [11, 12]. The present study is concerned with the use of a dissolution test to characterize the changes in the deposited hydroxyapatite coatings.

As known, Hank's Solution has almost the same ion concentrations as those of the human blood plasma and can well reproduce the *in vivo* surface changes. *In vitro* tests of plasma-spray HA-coated titanium have found new bone-like apatite layer formation on the surface of HA coating [13, 14].

EXPERIMENTAL

A hydroxyapatite target was ablated in an oxygen controlled atmosphere chamber by means of a KrF* excimer laser beam of 248 nm wavelength, generating pulses of $\tau_{\text{FWHM}} \approx 10$ ns duration. The laser source was operated at a

frequency repetition rate of 2 Hz. The spot area was between 4.5–5 mm², and the incident laser fluence on the target was 2.6 J/cm². In order to avoid target piercing during multipulse ablation, a rotation movement was applied. The coatings were deposited on 15 mm diameter disks made of high purity c.p. Ti (gr4), heated at 400°C. All the substrates were submitted to mechanical polishing. After polishing the substrates were cleaned in alcohol in an ultrasonic bath for 15 minutes. Prior to every film deposition, the chamber was evacuated down to a residual pressure of 10⁻⁴ Pa. Then a dynamical flux of 10 Pa O₂ was introduced and carefully monitored during deposition. For obtaining one film, we applied 15000 subsequent laser pulses. After deposition, the films were treated in water vapour enriched atmosphere at 400°C for 6 hours, in order to improve their crystalline state. This is explained by the crystal-level reorganization while hydroxyl groups are assimilated by the as-deposited hydroxyapatite structure. It is to mention that during the deposition process, OH groups are partially lost as a result of high temperatures involved in the ablation process. Different samples have been prepared by the insertion of a buffer layer. In this paper we shall refer to interlayers of TiN, deposited by PLD before the application of HA coatings. The thickness of TiN layer is estimated to ~ 200 nm, while the thickness of HA layers was estimated to be in the range 1–1.5 μm.

The samples used for experiments are summarized in Table 1.

Coated samples were immersed into separate vials containing 5 ml of Hank's solution, as shown in Fig. 1. A reference unbarred Ti substrate was directly submitted to immersion tests. The different ionic concentrations of Hank's solution are collected in Table 2.

The samples were kept at 37°C ± 1.5°C in a thermostatic oven for 21 days. To insure the homogeneity of the solution, the vials were stirred every 12 hours.

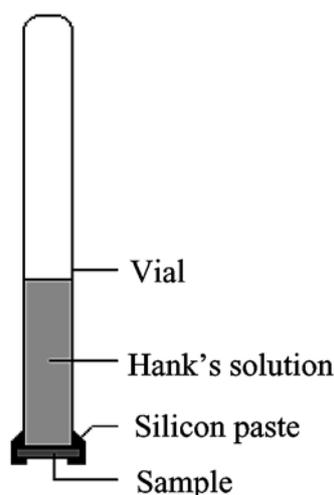


Fig. 1 – Schematic drawing of the experimental device.

Table 1

Codification of samples used in our experiment

Sample	Structure
Sample 1	CpTi (gr4)
Sample 2	HA / Ti
Sample 3	HA / TiN / Ti

Table 2

Ionic concentrations for Hank's solution and blood plasma

Ions	Hank's solution [ppm]	Blood plasma [ppm]
Na ⁺	142,0	142,2
K ⁺	5,0	5,0
Mg ²⁺	1,5	1,5
Ca ²⁺	2,5	2,5
Cl ⁻	147,8	103,0
HCO ₃ ³⁻	4,2	27,0
HPO ₄ ²⁻	1,0	1,0
SO ₄ ²⁻	0,5	0,5

The crystalline status of the deposited and treated thin films had been investigated by grazing incidence X-Ray diffractometry (GIXRD), using a Siemens Kristalloflex diffractometer using a Co K α (0.179 nm wavelength) radiation tube at 40 kV voltage and 30 mA current intensity. Morphological modifications were revealed by means of reflected light microscopy and scanning electron microscopy (SEM), using a Philips XL30 environmental scanning electron microscope. Due to the insulating behaviour of HA, the micrographs were acquired by exploiting the signal of the secondary electron beam. Compositional changes were determined by energy dispersive X-Ray spectrometry (EDS).

3. RESULTS AND DISCUSSION

From X-ray diffractograms shown in Fig. 2 it can be seen that the initial coatings are composed of partially crystalline HA. During and as an effect of immersion in solution, HA peaks ratio keeps relatively constant, but we noticed a slight decrease of their intensity. An important observation is the advent of Ca₂P₂O₇ peaks. It proves the synthesis of a new calcium phosphate crystalline layer at the solution – film interface. Another evidence is the formation of NaCl (Halite), as a result of salt precipitation phenomena.

As visible in the SEM micrographs (Fig. 3), the salt precipitated uniformly all over the film surface. We accordingly admit a uniform crystals growth. The presence

of Na, K and Cl elements was confirmed by EDS (Fig. 4), performed on the entire analyzed area. By dedicated EDS measurements we investigated the Ca/P ratio before and after immersion. We noticed that the effect of immersion of contact surface to Hank's solution is a decrease of Ca/P ratio, from 1.69 (very close to the stoichiometric value of 1.67) to 1.52 (Table 3). This loss of Ca is in our opinion in good accordance with the formation of $\text{Ca}_2\text{P}_2\text{O}_7$ film. We note that this Ca phosphate is characterised by a Ca/P ratio equal to 1 – *i.e.* more Ca atoms are consumed for the synthesis than P.

Going now in more details, we mention that on sample 1, only small NaCl crystals were observed, while no presence of Ca or P has been detected.

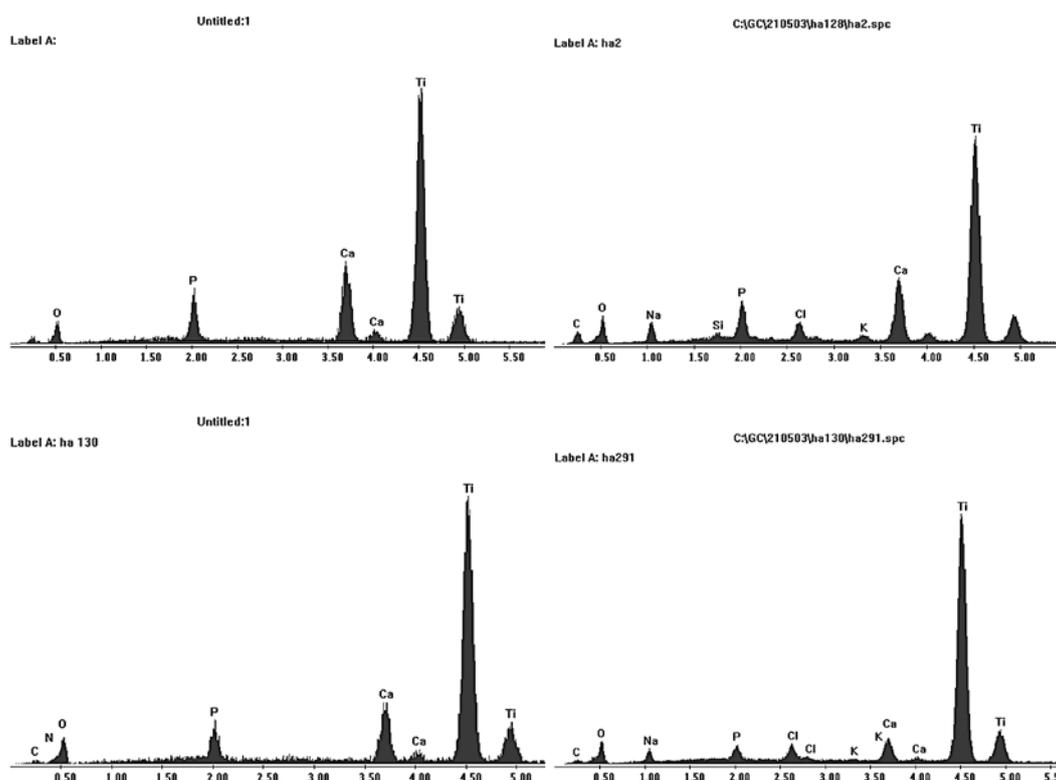


Fig. 4 – EDS analysis for Samples 2 and 3, before (left) and after (right) immersion.

Table 3

Ca/P ratio for Samples 2 and 3

	Sample2	Sample 3
Before	1.75	1.69
After	1.60	1.52

To observe in more details the $\text{Ca}_2\text{P}_2\text{O}_7$ layer we performed reflected light microscopy studies. For differentiation of the newly grown $\text{Ca}_2\text{P}_2\text{O}_7$ film, a sharp pin was used to slightly scratch the samples surface, avoiding any perturbation of the HA film. The differences between various samples are visible in Fig. 5.

By optical measurements, we did not detect any differences between samples coated with HA thin films. The behaviour was similar and crystals dimensions were comparable. Conversely, the reference sample is practically unmodified as compared with its initial state, while small salt crystals prove a rather weak adhesion to substrate.

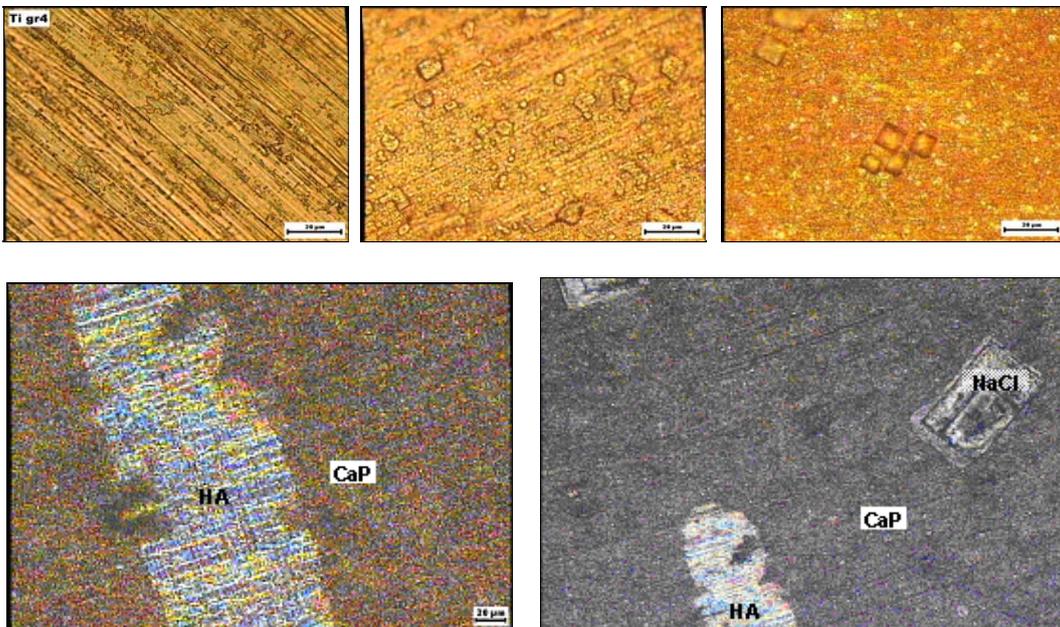


Fig. 5 – Optical microscopy images for Samples 1–3. The enlarged pictures show the difference between the initial HA film (lightened) and the formed CaP film (brownish).

B 4. CONCLUSIONS

We performed comprehensive studies of Ti substrates covered by PLD with HA films and buffers. One important observation is that immersion caused neither films destruction nor dissolution, but only surface modification by formation of a $\text{Ca}_2\text{P}_2\text{O}_7$ layer and large spread of NaCl (Halite) crystals. In particular, $\text{Ca}_2\text{P}_2\text{O}_7$ is synthesised by reactions between ions originating from both solution and HA film. $\text{Ca}_2\text{P}_2\text{O}_7$ crystalline film and NaCl crystals are absent on surface of reference sample (without HA deposition). This points to the essential role in the synthesis of

the two compounds of the HA layer which intervenes superficially in the respective reactions, but also prevents the flux of Ti atoms from substrate to solution. No large differences were however detected between samples covered with different layers (HA with or without TiN buffer layer). We further proceed to new experiments with prolongement of the immersion time from 21 days on. As an important application, we note the superficial growth of NaCl (Halite) crystals could prove very important for osteointegration of prosthesis in the living body. The new growing material on surface is expected to play an important role in the faster integration in simulated biological systems.

Acknowledgements. Romanian authors are grateful to Prof. Alain Cornet from INSA – Strasbourg and to Prof. Sorin Ciuca from the “Politehnica” University, Bucharest for their generous help in the preparation and achievement of the results reported here.

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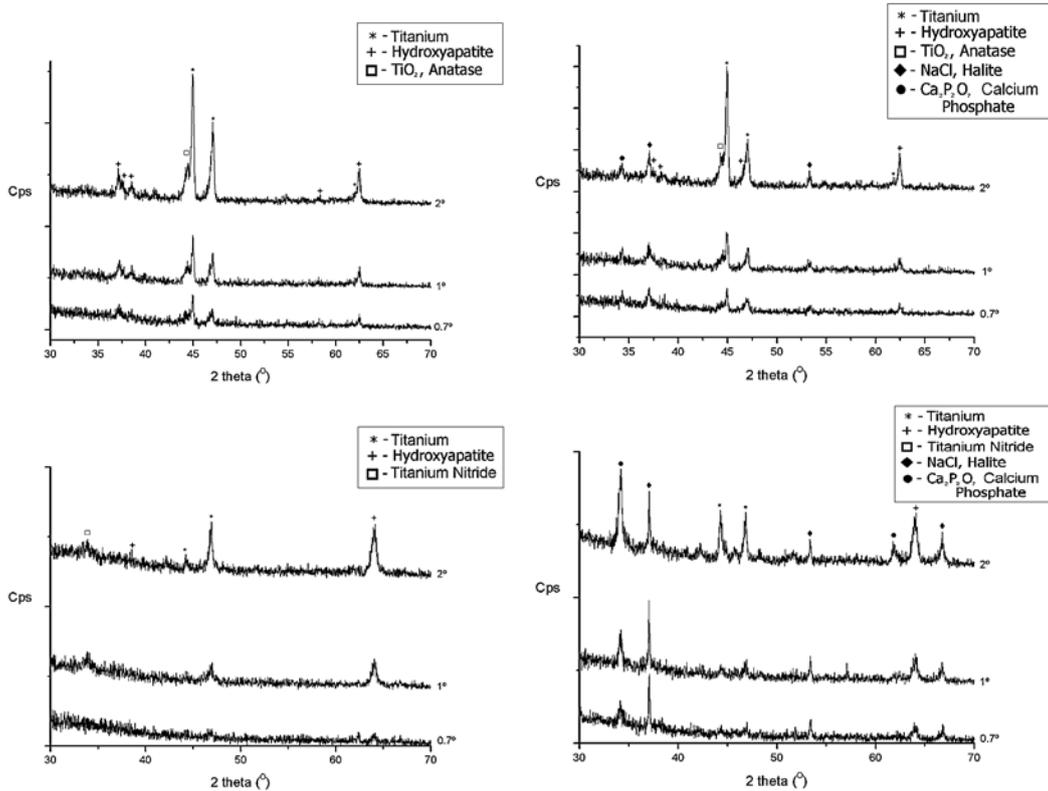


Fig. 2 – Diffractograms for Sample 2 and Sample 3, before (left) and after immersion (right).

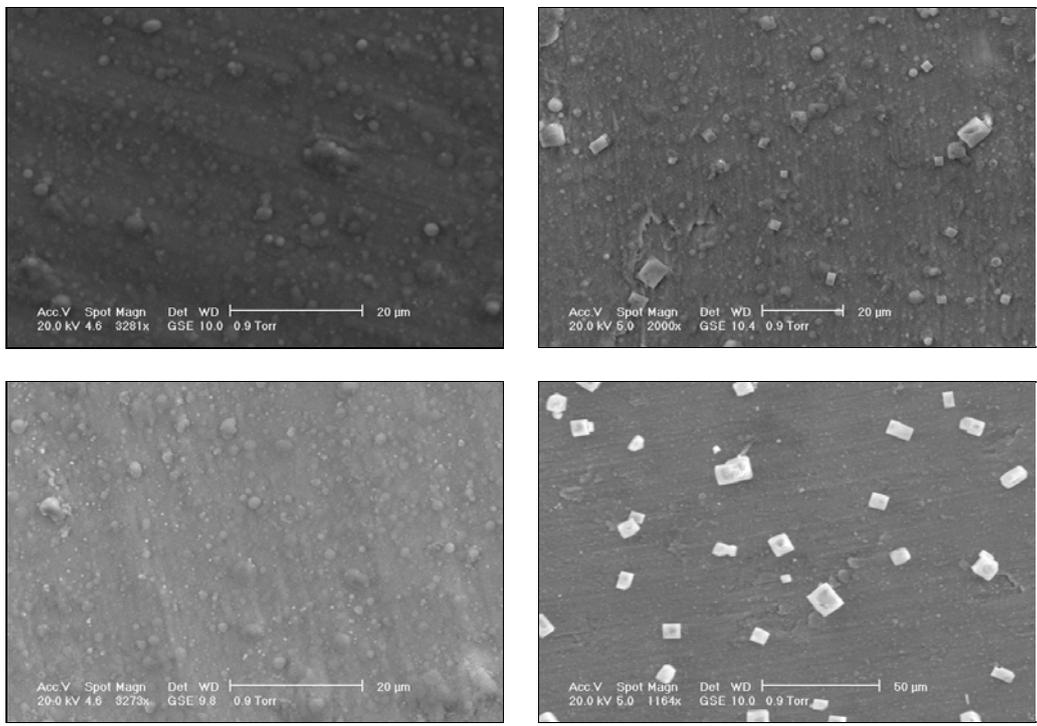


Fig. 3 – SEM images for Samples 2 and 3, before (left) and after (right) experiments.