ORIGINAL PAPER

CHARACTERIZATION OF HA-AL FILMS OBTAINED BY MAPLE DEPOSITION ON TITANIUM SUBSTRATES

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Abstract. *In attempt to avoid prosthetic problems and possible side effects that may occur in long-term bisphosphonate treatment, a solution commonly studied in recent years is local bisphosphonate release. Thus, a higher dose can be administered in the region of interest with positive effects on reduction bone loss in the periprosthetic area, but especially on osteointegration reduction time and acceleration of prosthesis component secondary fixation. On the other hand, stable fixation is also achieved in the case of osteoporotic bone. In the present paper, we have proposed to obtain implant type structures by deposition of alendronate-hydroxyapatite compositions (HA-AL) on titanium metal substrates. The deposition of the composite directly on the metallic component was accomplished by pulsed laser evaporation technique (MAPLE).*

Keywords: *MAPLE, alendronate-hydroxyapatite, AFM*

1. INTRODUCTION

Hydroxyapatite (HA) was originally used for dental implants, subsequently, its use in various forms (powder, block) has increased. This was used in correcting bone defects as an alternative to conventional bone grafts. It has thus been found that healing time was shorter than when nano-hydroxyapatite was not used. HA is also used for medical implants [1].

Among the calcium phosphate-based bioceramics, crystalline hydroxyapatite was found to be the most stable phase in contact with body fluids, amorphous HA and tricalcium phosphate being less stable and faster resorbed by the body [2]. Due to innate biocompatibility, this material does not produce an immune response after introduction into the body. Another important property of HA is bioactivity. After implantation, it produces chemical species that support the adhesion of the implant to surrounding tissue by forming a functional connective structure [3]. Considering that the most commonly used drugs in the

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treatment of bone resorption-related diseases are in the bisphosphonate category, exist a huge interest to include these drugs on the surface of orthopaedics metallic prosthetic components [4].

Natural hydroxyapatite has a hexagonal structure with a $Ca_{10}(PO_4)_6(OH)_2$ formula unit. The hydroxyl ions (HO^-) can be replaced with F^-, Cl^- ions in the fibrous collagen matrix [5]. The local delivery of bisphosphonates implants was tested in various preclinical studies on laboratory animal models (rat, rabbit, dog) and the results were encouraging regarding the viability of the implant [6, 7]. The high affinity of bisphosphonates for calcium ion and the advantages of using hydroxyapatite (HA) as a coating for prosthetic implants led to the search for new solutions to include drugs on the prosthetic surface and the need for synthesis of hydroxyapatite-bisphosphonate composite, which could be deposited on the metallic implant [8].

Thus, in this study, we proposed to obtain implant-type structures by deposition of alendronate-hydroxyapatite (HA-AL) compositions on metallic titanium substrates. The deposition of the composite directly on the metallic component was accomplished by Matrix Assisted Pulsed Laser Evaporation (MAPLE) technique.

2. MATERIALS AND METHODS

2.1. MATERIALS

All chemicals were analytical reagent grade: $Ca(NO_3)_2 \cdot 4H_2O$ (Sigma-Aldrich, ≥99%); $(NH_4)_2HPO_4$ (Sigma-Aldrich, ≥99%); NH_3 (Sigma- Aldrich, 30 - 33%); alendronate sodium salt (Axxora, ≥ 97%); HPLC Water (LiChrosolv® Merck); hydroxyapatite powder (Sigma-Aldrich).

2.2. METHODS

In this research hydroxyapatite–alendronate composites were synthesized from calcium nitrate, diammonium hydrogen phosphate and alendronate by chemical precipitation method, according to equation:

$$10\ Ca(NO_3)_2 + 6\ (NH_4)_2HPO_4 + 8\ NH_4OH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 20\ NH_4NO_3 + 6\ H_2O$$

Initially were prepared the two solutions of 1.08M calcium nitrate tetrahydrate (12.75 g $Ca(NO_3)_2 \cdot 4H_2O$ in 50 mL solution) and 0.65 M diammonium hydrogen phosphate (4.292 g $(NH_4)_2HPO_4$ in 50 mL solution). The pH of this solution was adjusted to 10 with NH_4OH.

The calcium nitrate solution was initially heated to 90 °C, and then the diammonium hydrogen phosphate was added under continuous stirring (600 rpm). The addition rate of the second reagent was 0.1 mL/min, added with a peristaltic pump. The compounds containing alendronate were obtained by dripping the alendronate solution, 20 mM aqueous alendronate solutions (0.249 g in 50 mL H_2O), to the reaction environment immediately after the ammonium hydrogen phosphate was added. The composite product was maintained in the reaction environment for 5 hours at a constant temperature under constant stirring.

Afterwards, it was centrifuged at 10000 rpm for 10 minutes (Eppendorf 5804 centrifuge) and washed repeatedly with distilled water until no traces of ammonia remained. The powder was heated overnight at 37^{o}C and then triturated.

Table 1. Experimental parameters for the HA-AL 20 mM synthesis and the quantity of alendronate contained in the HA-AL composite.

No	**1**	2	3	4	5	6	7	8	9	10	11
Temperature [°C]	**90**	30	90	30	90	90	30	90	60	60	60
Flow [mL/min]	**0.1**	0.1	0.1	5	5	0.1	5	5	2.5	2.5	2.5
Speed [rpm]	**600**	200	200	200	200	1000	1000	1000	900	900	900
pH	**10**	10	10	10	10	10	10	10	10	10	10
Maturation (Days)	**0**	0	3	3	0	0	0	3	1.5	1.5	1.5
AL (mg) in 5 mg HA-AL (HPLC)	**0.0151**	0.0051	0.0060	0.0055	0.0017	0.0120	0.0011	0.0009	0.0027	0.0030	0.0025
Conc. AL [%]	**0.302**	0.102	0.120	0.110	0.034	0.240	0.022	0.018	0.054	0.060	0.050
Incorporation efficiency [%]	**9.258**	0.985	4.023	3.385	0.946	7.626	0.726	0.639	1.613	1.896	1.841
Size [nm]	**397**	590	368	259	732	356	2200	300	454	754	822

In order to optimize the synthesis process of the alendronate hydroxyapatite compositions, several parameters were varied: temperature (between 30 and 90 Celsius degrees), reactant addition rate (0.1-5 mL / min), and maturation of the samples (0-3 days), according to the data presented in Table 1 [9, 10]. A 20 mM alendronate solution was also processed to achieve the highest inclusion efficiency of this bisphosphonate in hydroxyapatite. The alendronate concentration (m/m) (%) and incorporation efficiency (%) were determined by the HPLC method [11, 12].

In order to immobilize/deposit the material on the metal surface of the collector, Ti discs with a diameter of 12 mm and a thickness of 5 mm polished and subjected beforehand to a chemical treatment to increase the active surface were used [13]. After preparing the suspension of material in water (0.22 g in 10 mL H_2O**),** the sample was homogenized and frozen at 77 K in liquid nitrogen. After this process was performed, the target was immobilized in the reaction chamber and rotated during the experiment in order to avoid overheating due to laser irradiation. During the irradiation, the target was continuously cooled with liquid nitrogen.Experimental conditions for the deposit of the 20 mM HA-Al composite (obtained from synthesis with the highest incorporation of alendronate synthesis - synthesis 1) are shown in Table 2.

Table 2. Experimental conditions for the deposition of HA-AL compounds.

Sample	**Substrate**	**Energy [mJ] 5Hz**	**Spot area [mm^2]**	**Pressure [mbar]**	**D_{t-5} [cm]**	**Nr. pulses**
HA-AL (synthesis 1) 0.22 g in 10 mL H_2O	Ti (7x) + 1xSi	180	12	10^{-1}	4.5	20.000

From the point of view of the HA-Al composite deposition on the titanium metal support, the material to be deposited is initially solubilized and the solution thus obtained is subjected to a freezing process and subsequently is irradiated.

By controlling the number and intensity of UV laser beam pulses (KrF *, λ = 248 nm, τFWHM $\geq$ 25 ns) passed through the magnesium fluoride lens, f = 30 cm, in the stainless steel

vacuum reaction chamber, was able to monitor the quantity of evaporated material deposited on the titanium metal support.

Radiation was focused on as many points as possible on the metal surface in order to achieve an even more uniform deposit. Morphological investigations were performed using atomic force microscope (Park Systems model XE7).

3. RESULTS AND DISCUSSION

Matrix-Assisted Pulsed Laser Evaporation (MAPLE) is a relatively new technique but has begun to be extensively used to the detriment of classical deposit methods because it provides a qualitative and quantitative transfer of various types of organic or inorganic materials on various substrates types. The formed films obtained are thin, stable and meet the requirements for further use in the medical field of bioimplants (Fig. 1).

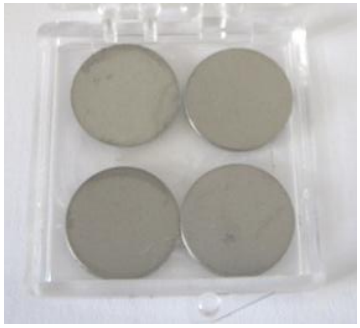


Figure 1. Titanium metal discs on which a 20 mM HA-AL microfilm was deposited through MAPLE technique.

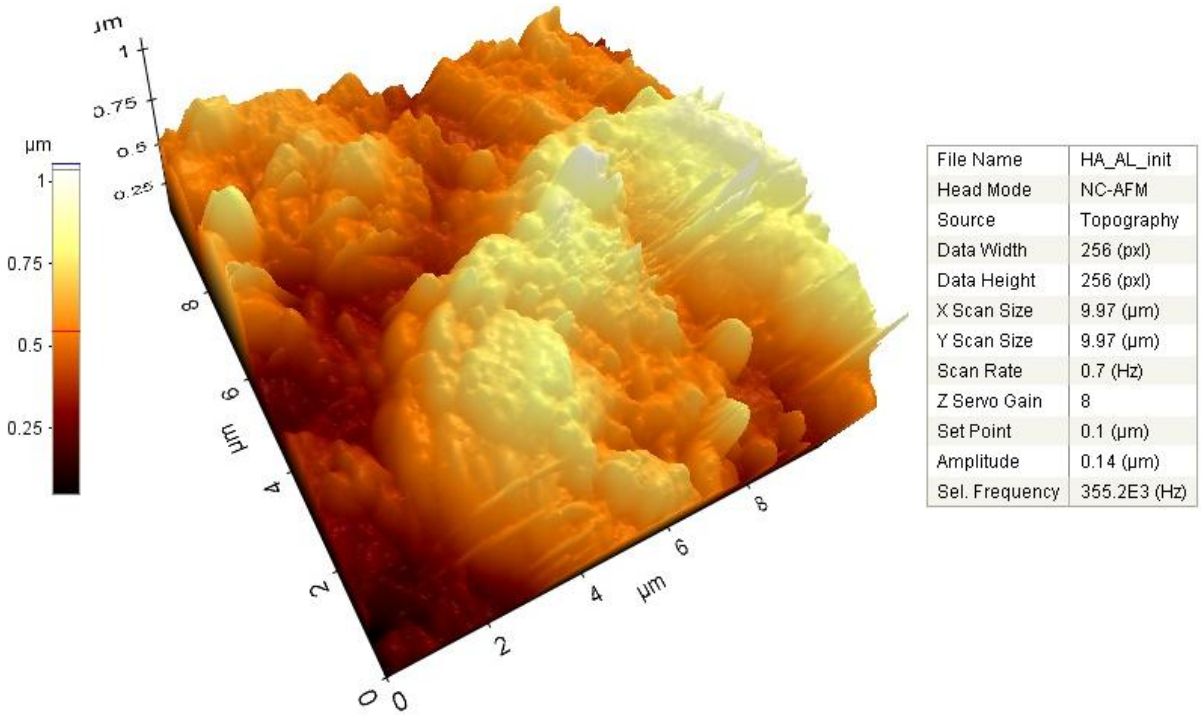
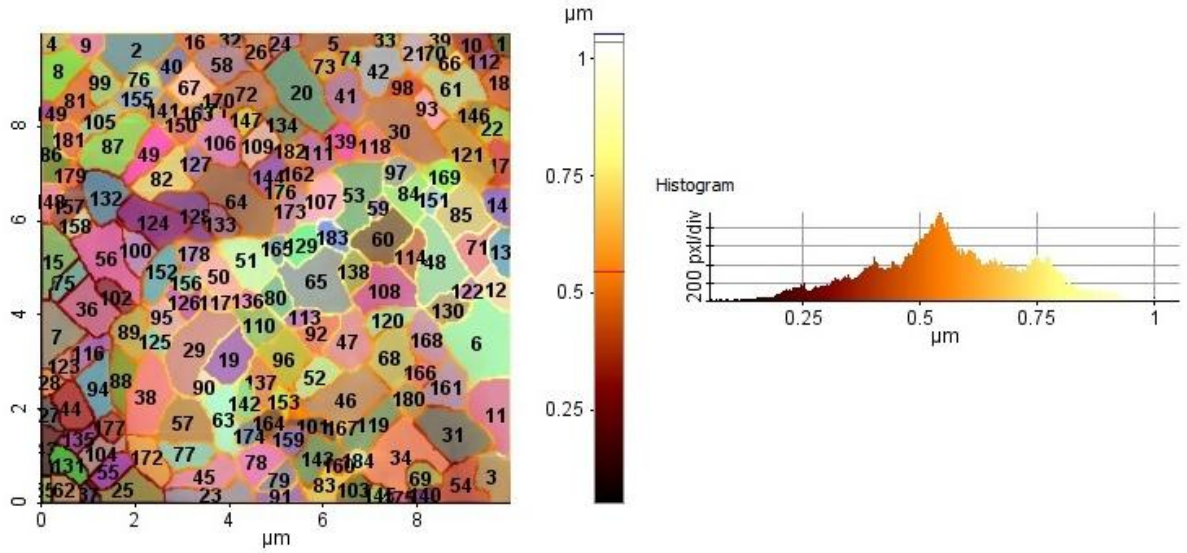


Figure 2. 20 mM HA-AL (synthesis 1) structure deposited on Ti by MAPLE (3D image).



Grain	Area(µm²)	Vol(µm³)	Length(µm)	Peri(µm)	Angle L(deg)	Angle R(deg)
Mean	4.997E-1	2.794E-1	1.057	3.247	0.000	0.000
Std.	3.552E-1	2.288E-1	0.413	1.312	0.000	0.000
1	1.426E-1	4.699E-2	0.608	1.645	0.000	0.000
2	9.845E-1	5.866E-1	1.742	4.983	0.000	0.000
3	6.462E-1	3.44E-1	1.232	3.731	0.000	0.000
4	1.79E-1	9.695E-2	0.639	1.870	0.000	0.000
5	5.112E-1	2.682E-1	1.566	4.313	0.000	0.000
6	1.817E0	1.368E0	2.055	6.632	0.000	0.000
7	7.964E-1	2.897E-1	1.482	4.529	0.000	0.000
8	7.236E-1	4.026E-1	1.297	3.851	0.000	0.000
9	2.806E-1	1.909E-1	0.752	2.278	0.000	0.000
10	2.928E-1	1.3E-1	0.862	2.654	0.000	0.000
11	1.022E0	7.528E-1	1.751	4.740	0.000	0.000
12	4.02E-1	3.59E-1	1.049	3.085	0.000	0.000
13	3.701E-1	3.123E-1	1.153	2.897	0.000	0.000
14	3.671E-1	2.347E-1	0.962	2.722	0.000	0.000
15	7.084E-1	1.848E-1	1.570	4.322	0.000	0.000
16	2.169E-1	1.153E-1	0.822	2.401	0.000	0.000
17	3.444E-1	1.845E-1	1.049	3.309	0.000	0.000
18	4.308E-1	1.924E-1	1.108	3.323	0.000	0.000
19	8.131E-1	6.398E-1	1.318	4.172	0.000	0.000
20	1.511E0	6.285E-1	1.938	5.975	0.000	0.000

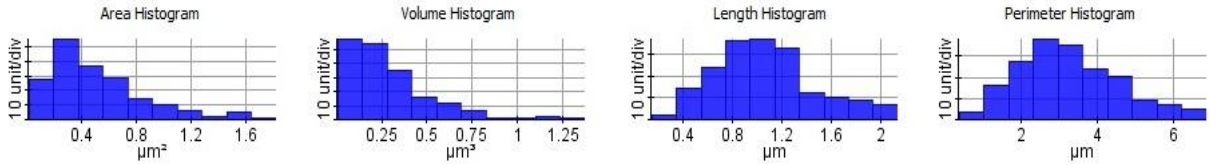


Figure 3. Volume / area distribution of 20 mM HA-AL (synthesis 1) composite deposited on Ti by MAPLE.

The incident laser beam initiates two photothermic processes in the array: evaporating the frozen composite and releasing the material into the room. The solvent molecules

evaporate and are discharged through the pumping system. The material molecules are loaded with sufficient kinetic energy from the collision with the evaporated solvent molecules to be transferred from the gaseous phase to the substrate. Water evaporation takes place during transfer and continues on the metal substrate, which explains the appearance of pores highlighted by the AFM images. Thin films of immobilized material on the metal surface have a high adhesion to the titanium substrate, noting the absence of delaminations or any other morphological defects (Fig. 2). Microparticles of active substance (alendronate) are incorporated into the crystalline hydroxyapatite network, the AFM not showing large structural differences between different titanium plates area where HA-AL composite has been immobilized. The films have a porous structure with pores of medium size of 1-4 μm. The specific morphology of these thin films could be characteristic of the deposit technique. Unlike PLD, MAPLE uses a cryogenic target (a mixture of diluted material to be deposited). The roughness parameters evaluated by AFM analysis are quite similar for different coating areas (Fig. 3).

4. CONCLUSIONS

In the present study, was performed the deposit of hydroxyapatite – alendronate composite synthesized on the surface of the titanium implant by the MAPLE technique. The method of synthesis of HA-Al composites by wet precipitation has a number of advantages that have been taken into account at the time of the choice: simplicity in performance, relatively low costs, slightly water-soluble reagents ($Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$), the pH of which can be readily adjusted to maintain the basicity of the reaction medium.

The application of the material was carried out by MAPLE, and the subsequent characterization of the deposited material was performed by AFM. Thin films of immobilized material on the metallic surface of titanium have high adhesion, noting the absence of delaminations or any other morphological defects. Microparticles of the active substance (alendronate) are incorporated into the crystalline hydroxyapatite network.

The methods used for the synthesis of HA-AL composite and the parameters used for titanium deposit can be considered as viable solutions for the inclusion of bisphosphonate on the surface of metallic prosthetic components used in orthopaedics.

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