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BIOCOMPATIBILITY OF HYDROXYL-APATITE THIN FILMS OBTAINED BY PULSED LASER DEPOSITION

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Abstract. Implant materials were made from metals such as titanium for a long period of time. Despite their good mechanical properties, metals and their alloys may be toxic for the human body. New materials have been developed for orthopedic implants and artificial teeth. Among these materials, Hap, which is known for its high biological activity and its unique property to integrate with bones, has attracted attention. Different techniques for HAp based biomaterials synthesis (mechanical methods, co-precipitation, biomimetic procedure, hydrothermal procedure) are described in literature. Hydrothermal synthesis offers many advantages over conventional and non-conventional ceramic synthetic methods. Hydrothermal synthesis is a technology for crystallizing materials (chemical compounds) directly from aqueous solution by adept control of thermodynamic variables (temperature, pressure, composition).
The objective of this paper is to demonstrate the enhanced biocompatibility of HAp thin films starting from hydrothermal synthesized powders.
Hydrothermal synthesized HAp was deposited on pure titanium substrates by PLD (pulsed laser deposition) at 400 °C in water vapor and in oxygen atmosphere, the pressure value was in the range from $3.5 \cdot 10^{-1}$ to 10^{-1} torr. Biocompatibility tests performed on coated titanium showed the ability of the HAp layer to promote cell growth on the surface.

I. INTRODUCTION

One of the most important objectives of the science is to develop new materials for bone substitution [1]. Hydroxyl-apatite, $Ca_{10}(PO_4)_6(OH)_2$ – HAp is very familiar in biomedical implant science and it is used as a hard tissue replacement material by virtue of its chemical and structural similarity to that of the mineral phase of bone and teeth. It is used in various physical forms such as granules, rods and coatings over metallic implants [2,3]. Its main applications include biocompatible phase/reinforcement in composites [4], coatings [5-7] and granular fill for direct incorporation into human tissues [8].

Although HAp is a promising implant material, its use under load bearing applications such as artificial joints have been restricted by the low toughness (0.8 – 1.2 $MPa \cdot m^{1/2}$) and low flexural strength (< 140 MPa) of the ceramic body [9-12]. Therefore, its medical applications are limited to small unloaded implants or low loaded porous implants. This status has been established for the past 15 years and there have been very little changes.

Multiple HAp-based composites (HAp/ceramic, HAp/metal and HAp/polymer) have been fabricated in order to make artificial hard tissue replacement implants, but only the HAp coated titanium alloys

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have found wide application at the beginning of bioceramics development. Among the others, the most promising seem to be HAp composites with biocompatible and biodegradable polymers (polylactide, polyglycolide, *etc.*), which are presently at an early stage of development [13].

From the point of view of mechanical properties and biocompatibility/bioactivity, microstructurally controlled HAp ceramics such as hydrothermal synthesized HAp, fibrous HAp-reinforced polymers, or biomimetically fabricated HAp/collagen composites seem to be the most suitable ceramic materials for the future hard tissue replacement implants [13].

Here, we report the fabrication of thin films based on hydrothermal synthesized HAp by PLD on different titanium substrates. The objective of this paper is to demonstrate the enhanced biocompatibility of HAp thin films starting from hydrothermal synthesized powders. The influence of PLD parameters on biocompatibility of HAP thin layers is also demonstrated.

It is obvious that biological and mechanical properties of the final product (implant, coating or granular fill incorporated into human tissues) depend on HAp powder as a precursor for thin film deposited on Ti substrate. Parameters such as particle size and shape, particle distribution and agglomeration vary substantially from one synthesis method to another [9]. For example, submicron HAp powders exhibits greater surface area. Nanometer sized HAp is also expected to have better bioactivity than coarser crystals [14,15]. These are just a few of many advantages that hydrothermal synthesis offers compared to mechanical methods or co-precipitation.

2. EXPERIMENTAL PROCEDURE

HAp powder was produced via hydrothermal method starting from Ca and P precursors. The experiment was carried out in an autoclave with a capacity of 1 L. Temperature and pressure were maintained constant being digitally controlled. The pH value was adjusted using 25% NH_4OH solution. The precipitate was thoroughly washed with distilled water, dried and then ground using mortar and pestle to obtain a white fine powder. The synthesis procedure is described in detail elsewhere [16].

White powder was sintered at 1100 °C for 2h. Opaque pellets of small size (d=10.2mm and h=1.3mm) represent the so-called target used in pulsed laser deposition procedure (PLD) for the preparation of HAp thin films onto Ti substrate. The powder and pellets thus obtained were characterized by chemical quantitative analysis (ICP – inductively coupled plasma, DCP – directly coupled plasma, AAS – atomic absorption spectroscopy), XRD analysis (modernized DRON 2 diffractometer connected to a computer for automatic data processing), SEM/EDAX to evidence the microstructure (LEO 1530 with Gemini column type), DTA–TG (MOM – Budapest Derivatograph, differential thermal analysis – thermogravimetry) and by specific surface area measurements (Gemini 2360 Analyzer). The thin layers obtained by PLD technique were characterized by transmission electron microscopy (TEM).

Sintered HAp was deposited by PLD on titanium substrates (grade 4) with different roughness of the surface. Substrate temperature was 400 °C. The distance between the target and the substrate was 4 cm. Part of the samples were thermally treated at 400 °C for 6 hours in water vapor atmosphere. The others were not. Gas pressure value of water vapor and oxygen atmosphere was in the range between 0.1 and 0.35 torr.

Biocompatibility tests were performed on coated titanium having ceaselessly to test the ability of HAp layer to promote or not HeLa cell proliferation on its surface.

3. RESULTS AND DISCUSSION

White fine powder as obtained in hydrothermal synthesis was pressed, sintered and examined both by chemical and physical analysis. Specific surface area of hydroxyl-apatite powder thus obtained was: S_{BET}=63.66 m^2/g compared to the measured specific area of standard hydroxyl apatite S_{BET}=33.00 m^2/g. This value leads us to the hypothesis that the chemical reactivity of the surface is enhanced and the substrate used for pulsed laser deposition could have good biocompatible properties.

Fig. 1 shows the XRD pattern of sintered HAp. It can be observed that the pattern matches with the standard HAp peaks. Specific surface area of hydroxyl-apatite powder also confirms its formation as the main constituent of the bulk. In accordance with X-ray results, chemical analysis shows the formation of pure HAp after hydrothermal treatment and sintering at 1100 °C. Values of Ca/P molar ratio before and after fabrication of thin films are presented in Table 1. It can be observed that Ca/P=1.65 is not influenced by the target sintering temperature and it is very close to theoretical value of this ratio given in literature for hydroxyl-apatite (1.667). EDX analysis performed on thin films showed a higher value for Ca/P molar ratio (1.82), due to the fact that this

Table 1. Ca/P ratio before and after sintering.

HAP pellet	Ca (g)	P (g)	Ca/P ratio	Method
Sintered HAP	40	18.75	1.65	Quantitative chemical analysis
Sintered HAP	38.75	16.42	1.82	EDX

Table 2. PLD deposition on Ti substrate.

Target sample name	Substrate	Gas pressure, torr	Substrate temp., °C	Pulse no. J/cm^2	Fluency,	Observations (treatments performed after PLD)
HAP1	Chemically etched Ti (grade 4)	0.35 torr, water vapor	400	15000	2	Thermal treatment, 400 °C/6h, water vapor atmosphere
HAP2	Mechanically polished Ti (grade 4)	0.1 torr, oxygen	400	15000	2	Thermal treatment, 400 °C/6h, water vapor atmosphere.
HAP3	Ti (grade 4)	0.1 torr, oxygen	400	15000	1.7	Without thermal treatment

technique is a semi-quantitative one. The differential thermogravimetric analysis for a standard hydroxyl-apatite and for a sintered hydroxyl-apatite hydrothermal synthesized is presented in Fig. 2.

For both samples it can be observed no phase transformation in the temperature range 25 – 1100 ° C. As a result, best HAp samples were selected with respect to their characteristics to serve as a target for pulsed laser deposition (PLD). PLD is a physical technique whose principle seems quite easy. In this method, one needs two main constituents: a target and a substrate. Light radiation emitted by a laser is pulsed onto the target surface so that small particles of the material which makes up target are directioned, transported and finally deposited onto the substrate surface.

We have chosen titanium as a substrate because surgical implants usually have a based stainless steel, Co-Cr alloy, Ti or Ti alloy part. The titanium substrate base is then coated with a ceramic layer

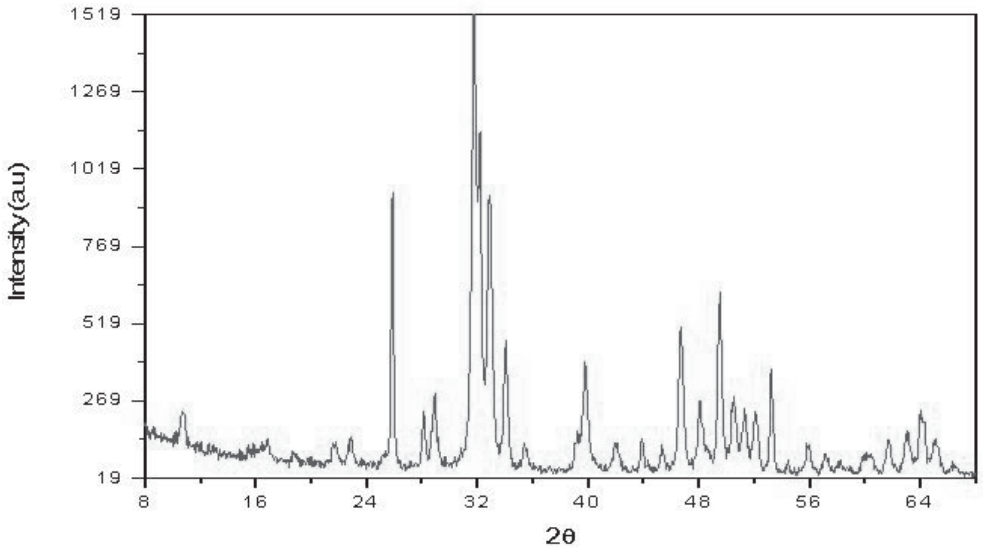


Fig. 1. X-Ray diffraction pattern of Hap.

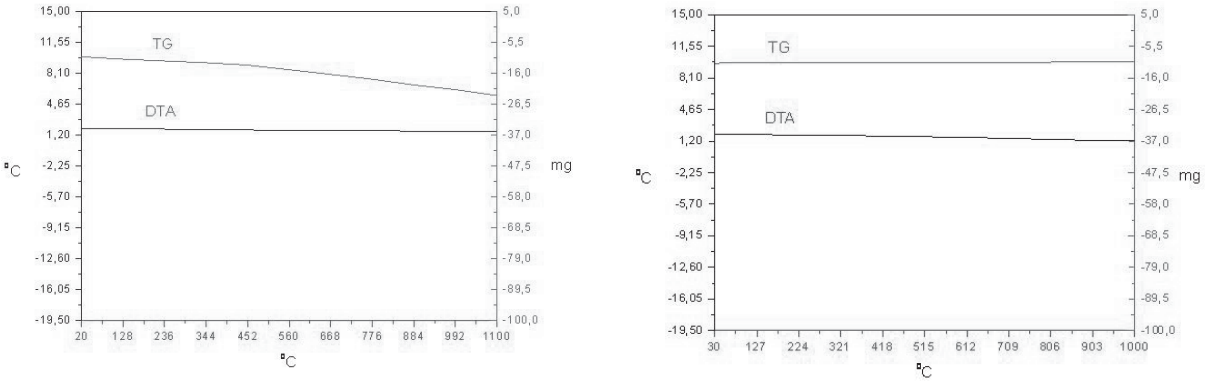


Fig. 2. DTA-TG diagram for (a) -hydrothermal synthesized Hap and (b) HAp standard.

consisting essentially of hydroxyl-apatite or calcium phosphate. Deposition conditions are given below in Table 2.

In order to study the influence of the deposition parameters on the biocompatibility of thin film of HAp, working parameters such as pressure, temperature, pulse, and the nature of vapor phase in work chamber were varied. Further determinations made on thin films produced by PLD technique showed that these parameters are as important as powder synthesis method and its conditions. As an example, Fig. 3 represents transmission electron micrographs of sample HAP3 (deposition in oxygen atmosphere, no thermal treatment after deposition). HAp layer is very thin but low adherent and non-uniform on Ti substrate. The thickness of the layer is not uniform on the substrate probably due to the O_2 atmosphere during deposition. Ti substrate surface was not processed before deposition and this can be an explanation for the low adherence of the HAp film.

It may be possible to correlate these results with SEM micrograph of the powder (Fig. 4). One can observe the crystallization of the ceramic phase; the surface presents some irregularities that could negatively influence the properties of HAp layer.

Biocompatibility tests consisted of ability of HAp layer to proliferate HeLa cells on its surface. The micrographs for the samples noted HAP1, HAP2, HAP3 visualised in fluorescence after they were marked with NBD C6 Ceramid are presented in Fig. 5. HAP1 and HAP2 samples recorded good results. If HAP2, which is the one deposited in oxygen atmosphere is partially covered by HeLa cells, HAP1-

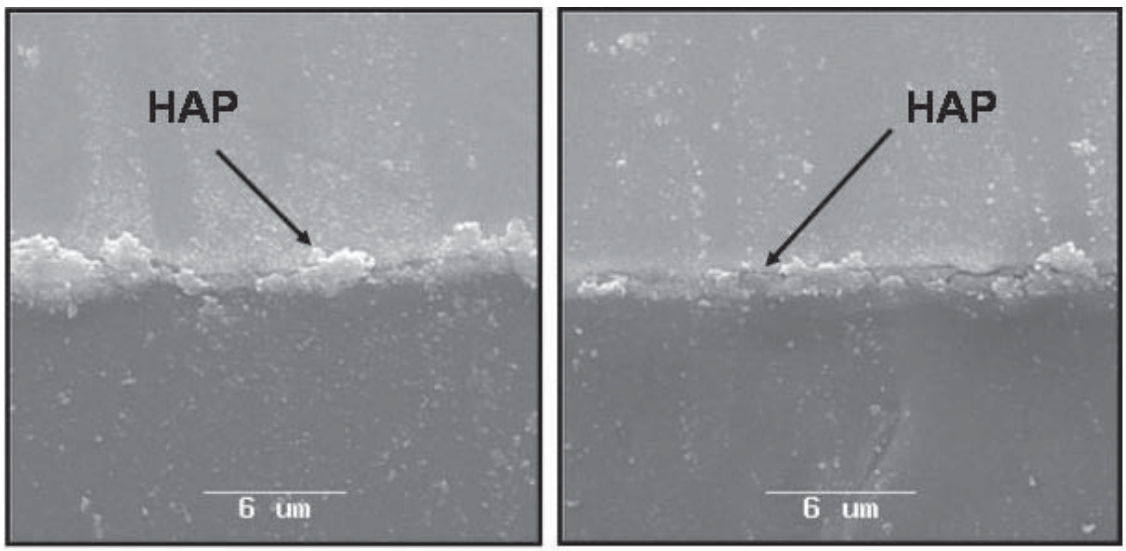


Fig. 3. TEM micrographs of HAP3 layer onto Ti substrate.

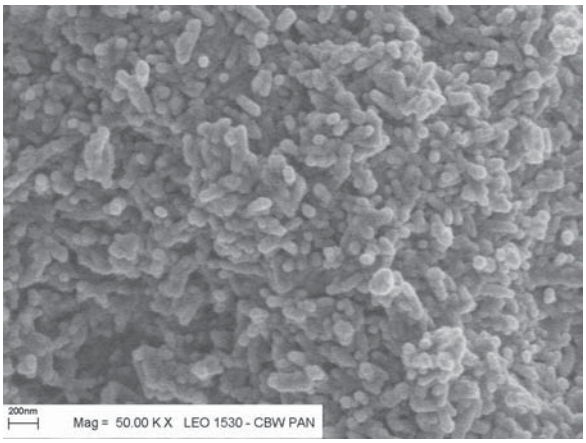


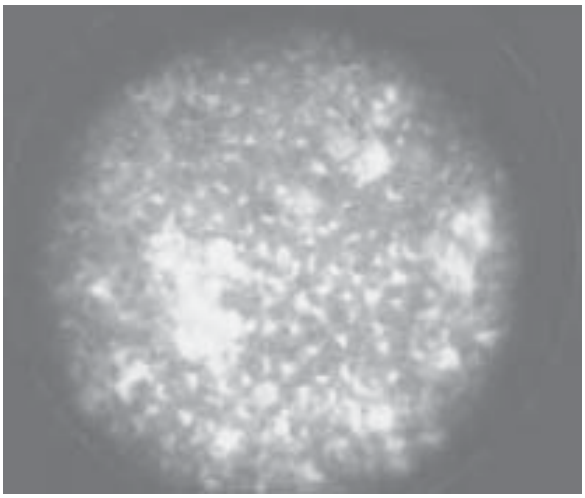
Fig. 4. SEM micrograph for HAP powder.

deposited in water vapour conditions represents a promising result for fabrication of implant materials being able to promote cell growth all over its surface, see Table 3. The difference between these two samples could be the quality of the substrate roughness. Chemically etched Ti corresponding to HAP1 sample posses a larger surface for HAp deposition than mechanically polished substrate used for HAP2 sample.

HAP3 sample was completely different from the first two samples. HeLa cells didn't proliferate on HAP3 surface acting as if only Ti substrate would be present. Both TEM characterization and biocompatibility test for HAP3 sample lead us to the hypothesis that atmosphere during deposition and Ti surface roughness are the main factors for obtaining a good biocompatible HAp thin layer. It is to be noticed that in the case of HAP layer titanium substrate was not processed by chemical or physical techniques while HAP1 and HAP2 were deposited on a pre-treated surface of titanium.

Table 3. Biocompatibility of HAp thin layer.

Sample	Layer Cell growth onto surface
HAP1	Totally covered by HeLa cells
HAP2	Partially covered by HeLa cells
HAP3	Not biocompatible because of the non-uniformity of HAP layer deposited on Ti surface; low adherence of HAP to Ti; metallic surface was unable to promote cell growth.



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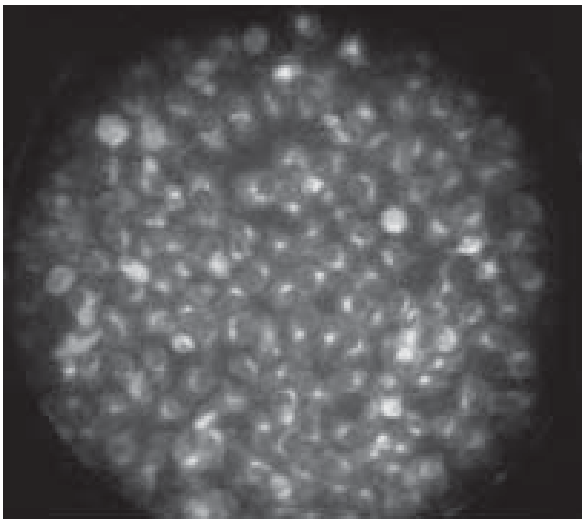

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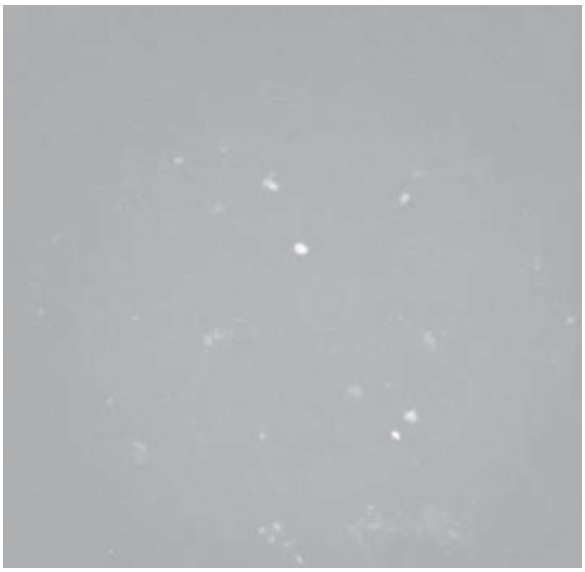

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Fig. 5. Biocompatibility tests: (a) HAP1; (b) HAP2; (c) HAP3.

4. CONCLUSIONS

Specific surface area of hydroxyl-apatite powder shows a good chemical reactivity of the substrate that was further used for physical deposition. The quality of the powder as a precursor for the preparation of biocompatible thin films is very important. Particle size, uniformity and homogeneity definitely influence cell growth on the surface of biocompatible material. That is why we believe hydrothermal synthesis is the best choice concerning ceramic powder preparation methods for further clinical tests (biocompatibility, bioactivity).

The experimental conditions during the hydroxyl-apatite deposition by PLD procedure are of great importance for the uniformity, the adherence and the thickness of films and substrate, so further improvements of this technique are required.

Future work will be performed to obtain a biocompatible material to be used in orthopaedics and dentistry by combining the best HAp powders as precursor for thin films with optimum parameters of PLD method.

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