

# PULSED LASER DEPOSITION OF BIOLOGICAL AND BIOCOMPATIBLE THIN LAYERS

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**Abstract:** The pulsed laser deposition (PLD) technology was extensively studied as possible way for production of biological and biocompatible thin films for different purposes. Thin layers were deposited by irradiation with ArF excimer laser ( $\lambda=193$  nm, 30 ns pulse duration) of targets pressed from human tooth, pepsin, poly(tetrafluoroethylene) and poly(hydroxybutyrate) powder, respectively. The fluence was varied in order to obtain films with appropriate chemical and physical properties. The substrate materials were KBr, glass, gold, silver and titanium depending on the deposited material and the measurements to be performed. The chemical composition of the deposits was studied with Fourier-transformed IR spectroscopy, the morphology was investigated with optical- and electronmicroscope and atomic force microscope. The results showed that good quality films can be produced under appropriate deposition conditions from all the studied materials.

## Introduction

The pulsed laser deposition (PLD) technology was originally developed for preparation of inorganic thin films [1]. Combined with a stoichiometry transfer between target and substrate this allows depositing many kinds of different materials. The main advantages of PLD are that this method is free of solvent-solute and solute-substrate interactions, and accurate control of thickness is possible. In last few years, many investigations have been performed in order to produce organic and biomaterial thin films by this method. In case of complex molecules the possibility of irreversible decomposition of the functional groups caused by the laser irradiation is higher, however in some cases the deposition parameters can be optimised to obtain good quality thin films. Thus, PLD was found to be also suitable for deposition of several types of biological and biocompatible thin layers [2].

The main aim of our experiments was to widen the range of biological and biocompatible materials deposited by PLD.

## Materials and Methods

A focused beam of an ArF excimer laser ( $\lambda=193$  nm, 30 ns pulse length) was used to irradiate the target (pressed from powdery material) placed in a vacuum chamber (Figure 1). The ablated material was deposited onto substrates facing the target. The target-substrate distance was set to 3 cm, the pressure in the chamber was around  $5 \times 10^{-3}$  Pa. Thin films were prepared by ablating pellets pressed from human tooth, an enzyme (pepsin), a biocompatible polymer (polytetrafluoroethylene, PTFE, Teflon), and a biodegradable polymer (polyhydroxybutyrate, PHB) powder, respectively.

Different substrate materials were used: KBr for Fourier-transformed infrared spectroscopy (FTIR), glass for morphological and adhesion strength measurements. In the cases of PTFE and tooth material the substrates were heated during deposition, the layers were post-annealed in some cases.

The chemical composition of the thin films was studied by Biorad FTS-65 FTIR spectrometer in  $4000-400$   $\text{cm}^{-1}$ , the morphology was investigated with TopoMetrix 2000 atomic force microscope (AFM), optical microscope and Hitachi S2400 scanning electronmicroscope (SEM).

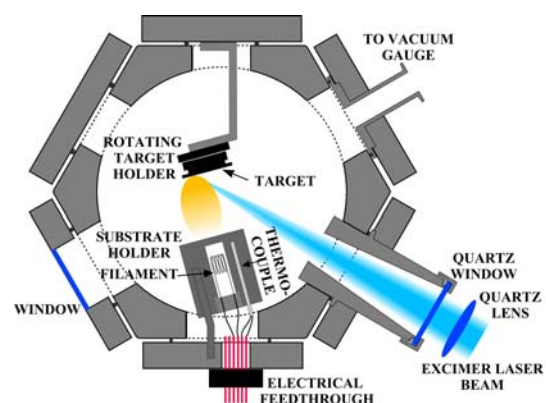


Figure 1: Experimental setup used for Pulsed Laser Deposition of thin films.

## Results

### Bioceramic thin films

Hydroxyapatite (HA),  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , with crystallographic and chemical properties similar to tooth and bone mineral is a promising coating material for increasing the biocompatibility of dental and orthopaedic implants [3]. Synthetic HA coatings have been successfully produced by a wide range of deposition techniques including pulsed laser deposition [4], plasma spraying [5], ion implantation [6], etc.

Biological apatites differ from synthetic stoichiometric HA due to the content of additional ions, especially carbonate ions, therefore these are referred to as carbonated hydroxyapatites (CHA). CHA films were produced with pulsed laser deposition from a sintered synthetic HA target in a water atmosphere [7].

The basic characteristic features of pulsed laser deposition of biological apatite thin films from pressed tooth powder was studied, as a possible alternative for replacing the synthetic material.

Targets were pressed at different pressing pressures between 150 and 450 MPa for 1 min from powder obtained by grinding roots of human tooth. The applied laser fluence was varied between 0.75 and 4.5 J/cm<sup>2</sup>, the ablated area was approximately 1.5 mm<sup>2</sup> and the number of pulses was in the 2000–20 000 range. Thin layers were deposited onto heated (250 °C) KBr, glass, and titanium substrates. Some of the deposited films were post-annealed at 550 °C for two hours.

The deposition of bioceramic films with appropriate stoichiometry has a crucial importance regarding its medical applicability, therefore the composition of the deposits were investigated as a function of laser fluence. Figure 2. shows the IR spectra of the target and thin films deposited at different fluences indicating the absorption bands corresponding to  $\text{PO}_4$  and  $\text{CO}_3$  groups and  $\text{H}_2\text{O}$ . The comparison of the spectra shows that the deposited layers are carbonate ion deficient and the water content is also reduced, the higher fluence resulted in higher deficiency. This can be interpreted as a result of the release of  $\text{H}_2\text{O}$  and  $\text{CO}_2$  from CHA material at elevated temperatures caused by laser irradiation. By post annealing for two hours at 550 °C in normal atmospheric conditions, the carbonate content of the layers could be increased, probably by interaction with  $\text{CO}_2$  molecules of the air. To maintain the right chemical composition, PLD in a water atmosphere can be favourable as in the case of synthetic CHA deposition [7].

For morphological investigations thin films were prepared using 1.5 J/cm<sup>2</sup> fluence. The layers had rough surface, the dimensions of the observed structures (particles) were below 3 μm, as shown in a typical AFM image (Figure 3), while the grain size of the tooth powder was up to 20 μm. This indicates that the plume contained large amounts of grains originating from the pellet and that these fractured during laser irradiation. The roughness of the surface was characterized by a  $R_a$

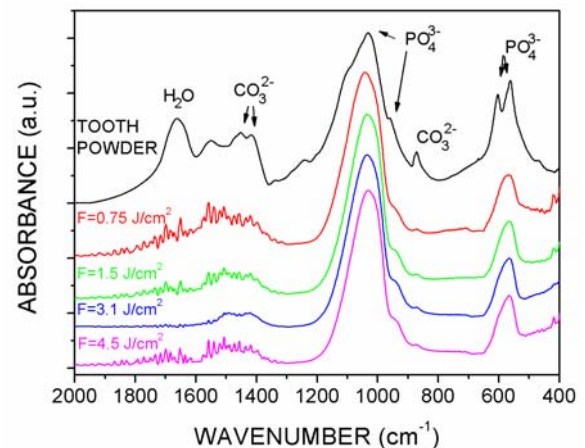


Figure 2: IR spectra of tooth powder and “tooth” thin films deposited at different fluences.

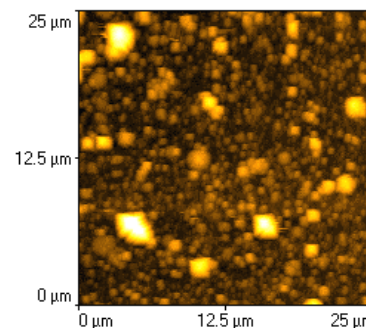


Figure 3: Atomic force microscopic image of a pulsed laser deposited “tooth” thin film. The number of laser pulses was 13000,  $F=1.5 \text{ J/cm}^2$ .

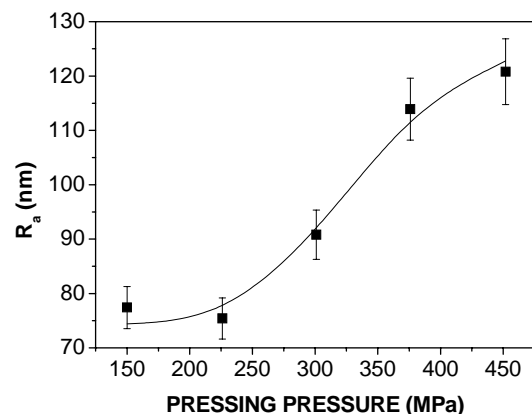


Figure 4:  $R_a$  roughness parameter of a 300 nm average thickness layer as the function of the pressing pressure of the targets.  $F=1.5 \text{ J/cm}^2$ .

mean roughness parameter, which was determined by the software of the AFM. Figure 4. illustrates that for layers having the same average thickness, the roughness of the surface increased with an increase in the pressing pressure of the used targets. This can be interpreted again as due to better cohering grains, i.e.

larger clusters of grains were removed during ablation. Therefore the surface roughness is determined by both the removed cluster size and the fracturing effect. HA films deposited by PLD from synthetic material [4] compared to these layers are relatively smooth, micrometric, spherical or horseshoe shaped droplets, embedded in the films. This difference is probably due to the different pellet preparation methods: in our case no post annealing or hot pressing was applied, therefore the grain structure in the pellets remained intact and led to rough deposits as discussed above. The layers deposited on glass and titanium substrates could be easily scratched with a steel needle, and when performing a Scotch-tape test, the upper, loosely bound grains of the layer were removed. When repeating the Scotch-tape test for the second time the amount of the removed material was negligible. This indicates that the film is denser and harder near to the substrate-film interface. The post-annealed layers become more resistant against mechanical actions, as the Scotch tape removed only negligible amount of material, and the films became scratch resistant.

#### *Pepsin thin films*

Thin films of a digestive enzyme, pepsin, were deposited with laser fluences between 0.25 and 5.1 J/cm<sup>2</sup>. The targets were pressed at 245 MPa pressure, the ablated area was 1.67 mm<sup>2</sup>.

Figure 5. shows the optical microscopic image of a film deposited on glass substrate at 1.22 J/cm<sup>2</sup> fluence. It can be seen that the layer was contiguous, and its surface appeared granular.

In order to check how the molecular structure is preserved FTIR spectra of films at 0.24, 0.5, 0.8, 1.3 and 5.1 J/cm<sup>2</sup> was compared to that of the original pepsin. The spectra of the films deposited at 0.5, 0.8 and 1.3 J/cm<sup>2</sup> fluences are nearly identical with the starting material over the entire studied spectral range, while those of the films deposited at 0.24 and above 1.3 J/cm<sup>2</sup> were different. This indicates that good conservation of the molecular structure can be obtained during the deposition process in the range of a 0.5 and 1.3 J/cm<sup>2</sup>.

The IR spectra proving the similarity of the functional groups are still not enough to establish the functionality of such a complex biomolecule. For this reason we had to study the biological activity of the thin films in the interest of demonstration that our deposited thin films behave as the pure pepsin. To prove this three samples deposited onto glass substrates at 0.38, 1.22 and 2.43 J/cm<sup>2</sup>, respectively, and a 10 mg crystalline pepsin pellet as a reference were placed into a chamber filled with vapour of 0.4% HCl solution and held at 37 °C temperature corresponding to the temperature of the human stomach. A 20 µm thick layer of boiled egg white was placed onto every single thin film and also onto the control pepsin pellet (Figure 6.). After 16 h layers of the boiled egg white disappeared from the pepsin pellet and the pepsin thin

films deposited at 0.38 and 1.22 J/cm<sup>2</sup> indicating digestion ability. We suppose that between 0.38 and 1.22 J/cm<sup>2</sup> the films would also show digestive ability. Our assumption is that the active pepsin of the layers is mostly contained in the grains of the films visible in optical microscopic images. At low fluences (approximately 0.25 J/cm<sup>2</sup> or less), the material is transferred in form of vapour leading to destruction of pepsin molecules. At higher fluences, grains having size of several micrometers are also removed from the target and transferred to the substrate. Above approximately 1.3 J/cm<sup>2</sup>, the grains are almost completely missing from the substrate probably due to the strong damage effect of the laser irradiation.

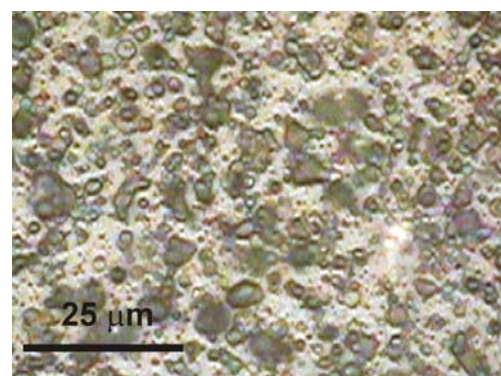






Figure 5: Optical microscopic image of a pepsin thin film.

-  laser deposited pepsin thin film
-  crystalline pepsin
-  20 µm thick boiled egg white
-  digested egg white

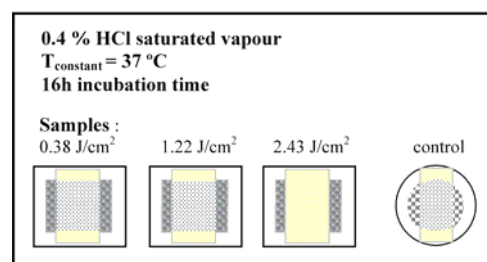


Figure 6: Test on digestion ability of the deposited pepsin layers.

#### *PTFE thin films*

Allergic symptoms induced by some metals, like nickel, mercury, chromium, lead, titanium, silver, gold, etc., are very frequent [8,9]. These can be due to metal objects that are in contact with the human skin, for example jewels, bijoux, backs of watches, frames, zippers, cutlery, etc., or metal implants and tooth fillings built into the human body. The simplest

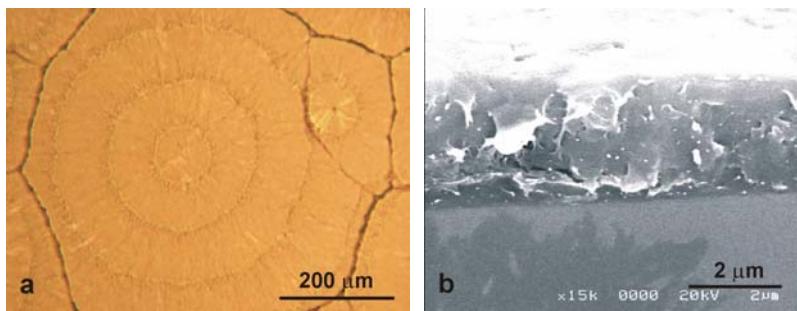


Figure 7: Optical microscopic image of a PTFE thin film post-annealed at 360 °C showing spherulitic structure (a) and scanning electron microscopic image of its crosssection (b).

solution to this problem is to avoid the contact with the objects containing allergenic metals. According to our assumption protective layers could prevent the contact between the allergenic materials and tissues. As a material of the protective coatings poly(tetrafluoroethylene) was chosen because it is a relatively strong material having a 7–28 MPa adhesion strength depending on the fabrication methods and it is hydrophobic and oleophobic as well. Due to its biological harmlessness, Teflon is already applied in several areas of the medical sciences. Implantation of PTFE prostheses into the human body is routine in surgery.

The chemical composition, morphology and crystallinity of pulsed laser deposited PTFE thin films were studied in several papers [10]. These deposition parameters were chosen on the basis of our earlier experiments in which PTFE thin layers were deposited onto the surface of glass substrates [11]. The targets were pressed PTFE powder pellets (Goodfellow, grain size 6–9 μm, pressing pressure 470 MPa, annealed at 250 °C for 10 h). The irradiated area was 0.87 mm<sup>2</sup>; the applied fluences were around 7.5–7.7 J/cm<sup>2</sup>. Teflon thin films were deposited on fourteen-carat gold (Au: 58.5%; Cu: 19.75%; Ni: 14.69%; Zn: 7.06%), silver (Ag: 92.5%; Cu: 7.5%) and titanium plates mounted on a heatable holder. The substrate temperature was 250 °C during the deposition. The post-annealing of the 10 μm thick films was carried out in atmospheric air at 320, 360 and 500 °C temperatures. The thickness of the as-deposited films was halved (~5 μm) by the post annealing due to structural transformations induced by heating.

The results were similar for both the gold and silver substrates. As the consequence of the heat treatment at 360 °C a nearly transparent coating was obtained, while above this temperature the films became visibly brownish. The microscopic investigations showed that post-annealing at 360 °C resulted in a semi-crystalline, spherulitic, contiguous, smooth PTFE thin layer. Furthermore, this film was very compact and pinhole-free (Figure 7.). In the view from above the crystal-like structure of the film can be seen (a) and the cross section shows morphology from lateral view (b). The as-deposited Teflon thin films, and those annealed at 320 °C, were granular, above 420 °C a melted and re-solidified morphology was observed.

From the point of view of the effective and reliable application of the protective Teflon layers, it is very important information to know how much these adhere to the different substrates, in the present case to the surfaces of the investigated gold and silver plates. Therefore a quantitative investigation was performed to determine the adhesion strength between the post-annealed Teflon layers and the metal substrates. In these experiments the samples (substrate and layer systems) were glued between two plexiglass cylinders by a two-component epoxy resin (Figure 8.a). Since PTFE is oleophobic and hydrophobic the sticking was improved by irradiation of PTFE films with a very low laser fluence (<1mJ/cm<sup>2</sup>) in the presence of a photoreagent (1,2-diaminoethane) vapour resulting in surface chemical modification of the layers. The cylinders were pulled by gradually increasing forces in opposite directions. The adhesion strengths were determined as the ratio of the force at which the coating separated from the surface and the area of the removed part (Figure 8.b). It was found that its value can exceed 1–4 MPa depending on the treatment temperature in the cases of both gold and silver samples. The lowest adhesion strengths (around 1 MPa) were reached for untreated PTFE layers, while the highest values (between 3.5 and 4 MPa) were obtained for post-annealed films at 360 °C. This can be due to the dependence of the film structure and the adhesion properties between the layer and the substrate on the treatment temperature.

The chemical isolation ability of the Teflon layers was also tested. For this purpose thin film was used to separate the two halves of a plastic container, filled with electrical conductive CuSO<sub>4</sub> solution and distilled water (low conductivity), respectively. The electrical conductivity of the distilled water was measured as the function of the time. The great difference between the

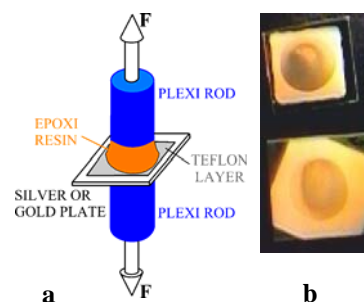


Figure 8: Experimental arrangement for adhesion strength measurements (a) and images of a gold and silver plate after removal of PTFE layer (b).

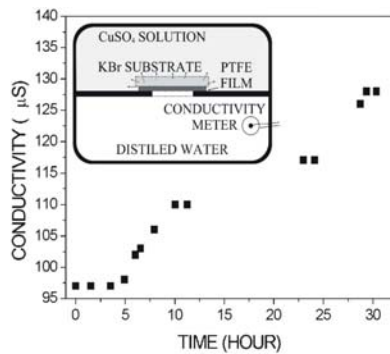


Figure 9: Conductivity changes of the probe liquid (distilled water) as the function of time. The measuring arrangement is shown in the inset.

conductivities of the copper sulphate solution (35 mS) and distilled water (97 μS) increased the sensitivity of the measurement. It was found that there was not a measurable change in the conductivity of the poor water for a 5-h time, afterwards a weak increase could be observed (Figure 9). However, the conductivity change of the probe liquid did not exceed 30% even after 30 h, which is less than 0.4% of the control CuSO<sub>4</sub> solution. This means that by using Teflon protective layers the allergenic objects can be chemically isolated from the human tissues with a relatively high efficiency.

#### *Poly(hydroxybutyrate) thin films*

PHB – a naturally form of polyesters – is an intracellular biomolecule synthesized by many bacteria as intracellular carbon and energy storage compound. Its monomer is a normal constituent of the human blood. PHB and PHB-based copolymers degrade comparatively slowly because of their hydrophobic nature and, therefore, are less popular compared with polyglycolic acid (PGA), polylactic acid (PLA), and polylactic-co-glycolic acid (PLGA) for tissue engineering applications [12]. Poly(hydroxybutyrate) was originally investigated as a compostable packaging material. The biocompatibility and resorbability have led to research on the use of PHB and its blends in medical science for example as temporary stents [13], bone plates, nails and screws, pericardial patches [14]. Thin films were deposited by irradiating pellets pressed at 440 MPa pressure with laser fluences between 0.05 and 1.5 Jcm<sup>-2</sup>.

FT-IR analysis of the starting material and the deposited films showed that the chemical composition of the material was preserved during deposition when using laser fluences up to 0.12 J/cm<sup>2</sup>, above that destruction of the molecular chain was observed. The analysis also indicated semi-crystalline structure of the starting material while the deposited films showed amorphous characteristics. Optical microscopic images showed that the films have rough surface micrometer sized grains were embedded into the contiguous layers

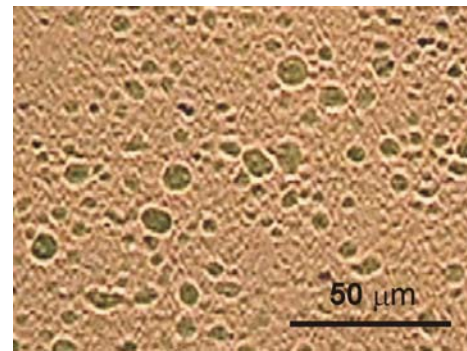


Figure 10: Optical microscopic image of a pulsed laser deposited PHB thin film.

(Figure 10.). The maximum lateral size of the grains was about 30 μm in the central part of the thin films while the size of the particulates was smaller than 3 μm on the periphery (about 1.5 cm far from the central part).

As an application possibility, preliminary experiments for use of PHB films as absorbing layer in Absorbing Film Assisted Laser Induced Forward Transfer procedure (AFA-LIFT). AFA-LIFT is a direct write method suitable for transfer of wide range of biomaterials (conidia, living cells). In this method biologic material is spread on a transparent plate covered with an absorbing layer. When the absorber is irradiated with laser light through the transparent plate, it transfers the photon energy into kinetic one and transfers the biomaterial to an acceptor plate facing it. (Figure 11.a) For AFA-LIFT, approximately 1 μm thick biopolymer thin films were deposited onto fused silica plates by PLD. The threshold fluence for complete removal of the films was found to be about 0.65 Jcm<sup>-2</sup> when using ArF laser. Single laser pulses were applied for the transfer of living *Astroglia* cells spread onto the deposited biopolymer thin film to the acceptor glass plate. The experiments were carried out in ambient air at room temperature. The distance between the donor and the acceptor surfaces was 600 μm. The applied laser fluence was 0.74 Jcm<sup>-2</sup>, slightly above the determined threshold value. The ejected material was deposited onto the glass acceptor

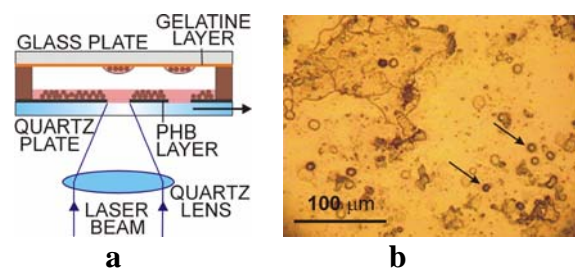


Figure 11: Experimental setup of AFA-LIFT procedure for transfer of living cells (a) and optical microscopic image of the transferred material (b).

substrate. Figure 11.b shows the optical microscopic image of the transferred material. It can be seen that the transferred material is composed of Astroglia cells (two of them signed with black arrows in the image) and debris of the PHB film. Other round-shaped particulates can be solidified PHB droplets transferred in molten phase and/or re-condensed polymer vapour. This means that the absorber layer was not completely evaporated during the AFA-LIFT process at this fluence therefore its several fragments were transferred along with the living cells. The results show that the deposited PHB thin films can be used as absorbent layers for cell printing by the AFA-LIFT procedure.

### 3. Conclusions

The results of our experiments proved that PLD was suitable for production of thin films from a wide range of targets (from bioceramic to more complex organic materials) preserving their chemical structure and even functionality.

### 4. Acknowledgements

The authors gratefully acknowledge the financial support of Hungarian scientific Research Foundation OTKA (TS 049872) and the Hungarian Ministry for Culture and Education (NKFP 3A/071/2004). B Hopp is indebted for his MTA Bolyai scholarship.

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