LASER BASED MAPLE DEPOSITION TECHNIQUE AND FILM GROWTH RATE; APPLICATION TO CRYOGOBULIN

M. Jelinek[1,2], R. Cristescu[1,3], T. Kocourek[1], J. Remsa[1], M. Vrbova[2], R. Frycek[2], L. Stamatin[4], D. Mihaiescu[5], C. Mirea[6], I. Stamatin[7], I.N. Mihailescu[3], D.B. Chrisey[8]

[1]Institute of Physics, AS-CR, Na Slovance 2, Prague 8, 182 21, Czech Republik.
[2]Faculty of Biomedical Engineering CTU, nam. Sitna 3105, Kladno 272 01, Czech Republik.
[3]National Institute for Laser, Plasma and Radiation Physics, MG-54, RO-77125, Bucharest, Romania
[4] Longhin Scarlat Dermato-Venerologic Hospital, Bucharest, Romania
[5]University of Agriculture Sciences and Veterinary Medicine, 59 Marasti, Bucharest, Romania
[6] National Institute of Forensic Expertise, Bd.Regina Elisabeta 53, Bucharest, Romania
[7]University of Bucharest, Faculty of Physics, P.O. Box MG-11, 3Nano-SAE, Bucharest-Magurele, Romania
[8]Naval Research Laboratory, Code 6851 Washington, DC 20375

jelinek@fzu.cz

Abstract: Increasingly stringent requirements on the thin film quality in terms of surface roughness and thickness are successfully met by a novel laser processing technique – Matrix Assisted Pulsed Laser Evaporation (MAPLE). We performed MAPLE deposition of thin films of some blood protein (cryoglobulin). using a KrF* excimer laser source (λ= 248 nm, τ = 20 ns) operated at a repetition rate of 10 Hz. We report herewith the influence of deposition parameters (laser fluence, number of pulses, target-substrate distance) on MAPLE films growth rate features. Potential biomedical applications of obtained structures is discussed.

Introduction

Functionalized materials include a large class of candidates that range from inorganics and simple polymers to enzymes, active proteins, antibodies, nucleic acids and living cells. Biomaterial transfer from target to substrate requires identically preserving molecular functions that is much stricter requirement than the retention of main peaks in case of passive materials. Because in case of functionalized materials the chemical bonds have assigned energies well below those corresponding to UV photon energies, some degree of photochemical decomposition is expected during the Pulsed Laser Deposition (PLD) processing [1]. New attractive solutions of this problem have emerged: UV Matrix Assisted Pulsed Laser Evaporation (UV-MAPLE) [2], Resonant Infrared-PLD (RIR-PLD) [3] or their combination RIR-MAPLE [4].

In PLD, the laser is used for ablation of bulk material, which is then collected on the substrate. RIR-PLD differs from PLD in using of an IR laser tuned to be resonant with a specific vibrational mode wavelength of the molecule. In this case the result is a non-harmful soft evaporation even for large molecules. The main drawback of this method is the demand of using an infrared laser, which must be tuned to a characteristic wavelength such as free electron laser (which is tunable from 2 to 10 μm [3-5]) or Er:YAG (2940 nm), which is efficient for alcohol as a matrix [6]. Also, basically MAPLE is very similar to PLD. It deviates from PLD by target preparation and deposition conditions, such as the laser fluence, which is often significantly lower. The frozen target (from 77 K to 240 K) consists of solute functionalized compound dissolved at low concentration (0.05 - 5 wt. %), in a volatile solvent (consequently the word „matrix“) that in the ideal case strongly absorbs at laser wavelength. Ideally, the laser energy is absorbed by the matrix and the solute is softly ejected from the target and carried towards the substrate while the solvent is pumped away and layers composed of large molecules are grown. Another reason why the solvent is not deposited is the “plume sharpening effect” [7]. This behavior known from matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) has been demonstrated in case of heavier molecules of functionalized materials. Then the angular distribution of the plume is narrower than that corresponding to the lighter solvent (matrix) [8].

Cryoglobulin is an interesting diagnostic blood protein that has the unusual property of precipitating from the blood serum when chilled to 0-4 C (hence the "cryo-") and redissolving when it is rewarmed to 37 C [9]. The physician must interpret the cryoglobulin result along with other test results and the patient’s clinical condition and medical history. The increased complexity to obtain a certain result requires supplementary clinical tests. In order to examine different amounts of cryoglobulin, these proteins must be transferred onto assays for further structural and morphological investigations as fast as possible. Meanwhile, it is essential that specimens submitted for evaluation be properly collected and processed. For

ISSN: 1727-1983

further medical investigations, maintaining cryogenic conditions against fragmentation, rearrangement, and degradation is mandatory. MAPLE processing offers an attractive alternative because of advantages, such as compatibility with noncontact masking techniques and minimum possible degradation.

We review relevant data about the growth dynamic of UV- and RIR-MAPLE deposited thin films. Also deposition details on cryoglobulin, including films characterization, are given.

MAPLE growth rate

The PLD growth rate (nm/pulse) is influenced by laser intensity incident on target, spot size, target-substrate distance, ambient atmosphere, optical and mechanical properties of target, substrate temperature and target-substrate-laser beam geometrical configuration. Typical PLD deposition rate is about 1 nm/pulse [1]. In MAPLE additional parameters such as properties of solvent, concentration of material/solvent in the target and target temperature are necessary to be taken into account.

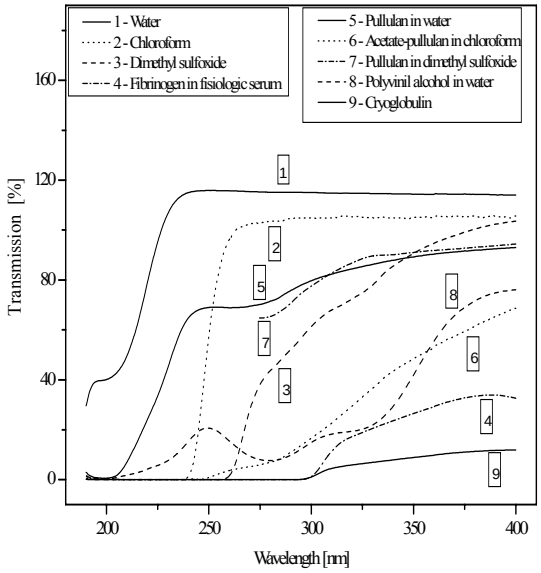

Figure 1: Transmission spectra of frequently used materials and solvents.

Examples of transmission spectra of some pure solvents and prepared solutions are presented in Figure 1. We see that there is a difference in absorption properties of solvents for usually used excimer lasers (i.e., ArF – 193 nm, KrF – 248 nm, XeCl – 308 nm).

A comparison between MAPLE and PLD deposited thin films performed under nearly identical experimental conditions shows that MAPLE is about 4-8 times slower than PLD [18]. It has been shown that in both cases (PLD, MAPLE), the growth rate increases roughly as $1/d_{ts}^2$ with decreasing target-to-substrate distance [26] As the laser fluence in MAPLE is very low, because the high fluence usually causes degradation of deposited material. On the other hand an important parameter is the ablation threshold that corresponds to a value after which the amount of ejected material from the target rapidly grows [17]. This has been observed as a minimal fluence, which is necessary for deposition [16]. The simplest way to create a usable target from a solution is to freeze it below the solvent melting point. The liquid nitrogen is frequently used as a medium, but when temperature going from 223 K to 113 K the growth rate is dropping 3−5 times [18].

Table 1: Summary of deposition conditions and film growth rate for various experiments using MAPLE and RIR-MAPLE technique * = mg/ml, LN – liquid nitrogen temperature, N/A – not available

MAPLE-deposited material	Solvent	Fluence [J/cm^2]	λ [nm]	Pressure [Pa]	Growth rate [Å/shot]	Ref
cryoglobulin	blood plasma	0.3	248	10^{-4}	0.15-1.21	This work
fibrinogen	physiologic serum	0.1-0.4	248	5-15, N_2	0.01-1.93	10
triacetate-pullulan	$CHCl_3$	0.1-0.5	248	10^{-4}	0.03-11.12	11
InAcAc	acetone	0.07-0.15	248	3, N_2	0.06-0.69	12
PhNi	DMSO	0.15-0.5	248	3, N_2	0.02-0.58	13
CuTTP	$CHCl_3$	0.1/0.2	248	3, N_2	0.11/2.50	13
CuTTP	$CHCl_3$	0.05-0.1	248	3, N_2	0.12/0.19	13
PhCo	DMSO	0.3/0.5	248	3, N_2	2.06/1.54	13
SXFA	$CHCl_3$	0.1-0.2	193	0.1-6.7, N_2	0.05	14
Mn_{12}-acetate	3-butanol	0.8-1.8	248	0.13-13	0.01-0.03	15
PEG	$CHCl_3$ / H_2O	0.22-0.23	193	10-3 - 10-4	0.1-0.21	16
glucose, sucrose, dextran	H_2O	0.05-0.25	193	6.65, Ar	0.05	17
SXFA	3-butanol	0.2-0.3	248	6.65, Ar	0.03	17
HRP	PBS	0.1-0.2	193	10^{-2} - 10^{-3}	0.2-0.6	18
PGA - PLA	ethyl acetate	0.165	193	10^{-4}	0.024	19
PEG	H_2O	2.5-10	355	10^{-4}	0.06-0.29	20,21,22
MAP analog	H_2O	0.41-0.62	193	10^{-4}	0.03-0.05	23,24
P_3HT	orthoxylene	0.2	266	10^{-4} - 10^{-2}	0.009-0.05	25
fluoropolyol	alcohol	3	2940	<0.66	10	6

Another important growth rate parameter is concentration of the material in solution. Recent studies [20,21] showed that in the range from 0.5 to 4 wt. % the yield is linear proportional to the material concentration. However it is necessary to take into consideration the very high fluence value used to compensate low absorption of the solvent at the laser wavelength. This reveals one of the main issues of MAPLE: finding the proper solvent suitable for material and laser. The material absorption at the incident laser wavelength could influence the growth rate in a critical way. MAPLE-depositions are performed for very low laser fluences, compared to PLD, where typically 2 Jcm^{-2} are applied (up to 33 Jcm^{-2} [27]).

ISSN: 1727-1983

We collected deposition data concerning MAPLE deposition, but most of experiments are focused on quality of deposited films than on film growth rate. The articles where the growth rate was directly reported or where we were able to calculate it are summarized in Table 1. We notice that the MAPLE growth rate ranges from 0.001 nm/pulse to 1.1 nm/pulse. The value of 1.1 nm/pulse is quite high for MAPLE, because usually the growth rate does not exceed 0.2 nm/pulse. The deposition rate for RIR-PLD and RIR-MAPLE is approximately the same (about 1.0 nm/shot) [3-26]. It is higher due to the better efficiency of energy transfer between laser pulse and material.

We can conclude therefore that MAPLE is less efficient and more expensive than conventional PLD for at least from the point of view of the growth rate of obtained structures. To compensate somehow we further discuss an example that in our opinion demonstrates which are the reasons to further apply and develop the MAPLE technique for deposition of an important class of functionalized materials. This is the unique capability of MAPLE to preserve the starting composition and structure of any material including biomaterials with delicate and/or large molecules. To this aim we first conducted experiments with cryoglobulin and we further give convincing results in support of these capability and performances.

Cryoglobulin deposition

An essential condition for proper evaluation of cryoglobulin level is the clean and accurate manipulation/transfer. This prerequisite is successfully met by laser processing.

The protein investigated in this work was the high quality type II cryoglobulin provided by the Longhin Scarlat Dermato-Venerologic Hospital, Bucharest, Romania. At room temperature, 0.15 mg/ml solutions were obtained by dissolving the cryoglobulin in blood plasma. The resulting solution was introduced in a stainless steel cup of 3.1 cm diameter and 3 mm height and frozen at the liquid nitrogen temperature (77 K). Films were grown by MAPLE arrangement at the IP ASCR, Prague (KrF excimer laser, rotated target holder -cooled to LN temperature) [28]. Deposition conditions are included in Table 1. Films were characterized by FTIR.

In Figure 2 we present typical transmission FTIR spectra recorded for the starting material as dropcast and for the MAPLE-deposited cryoglobulin thin films. We identified in our spectra several distinct bands. A broad absorption in the 3330 cm^{-1} region may be assigned to the NH stretching vibration of the secondary amide. It has an absorption band around 3075 cm^{-1} which is the overtone of the –NH absorption around 1550 cm^{-1}. Secondary amides show the carbonyl absorption around 1650 cm^{-1}. An absorption around 1550 cm^{-1} is indicative of the –C(O)NH- structure in aliphatic secondary amides. Absorptions around (1150-1000) cm^{-1} may be indicative of the –CN stretch in aliphatic ethers. For aliphatic hydrocarbons in the (2950-2750) cm^{-1} region are assignable to the –CH, $-CH_2$, and $–CH_3$ carbon/hydrogen stretch vibrations. Absorptions around 1460 cm^{-1} are indicative of CH_2 and $–CH_3$ groups while those at 1380 cm^{-1} are indicative of -–CH_3 groups. In general, we remark that in case of low fluences (Fig. 2a), the MAPLE-obtained cryoglobulin thin films preserve the typical IR signature/fingerprint of amides groups. Typically when all the important functional groups are maintained and there are no new degradation peaks, it can be assumed that the protein functionality is preserved in the film. On the other hand it can be noticed that the aforementioned amides groups degenerate at higher fluences forming aldehydes and carboxylic acids (Fig. 2b-c).

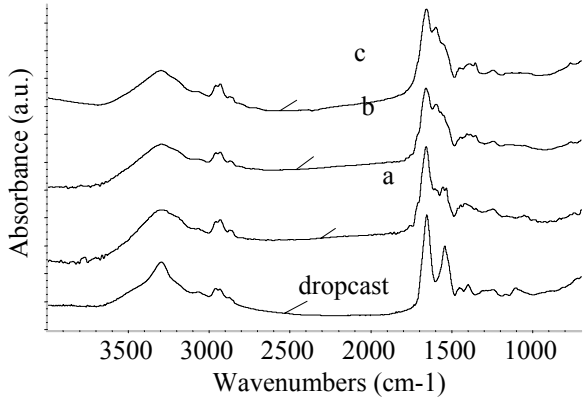

Figure 2: Typical transmission FTIR spectra of type II cryoglobulin for the starting material (dropcast), (a) for thin film obtained by MAPLE at a fluence of 100 $mJcm^{-2}$, (b) for thin film obtained by MAPLE at a fluence of 200 $mJcm^{-2}$, and (c) for thin film obtained by MAPLE at a fluence of 300 $mJcm^{-2}$.

Figure 3 contains the SEM micrographs of cryoglobulin thin films deposited by MAPLE technique. Fig. 3a. demonstrates that at lower fluencies (100 $mJcm^{-2}$) the surface exhibits a specific morphology where the aspect with a continuous and uniform cryoglobulin placement is clearly visible. It preserves the structural integrity on the blood plasma matrix. In Fig. 3b-c when the fluence is increased we can notice pores and large endonurial cryoglobulin units formed by denaturation and even agglutination/contraction tendency (200 $mJcm^{-2}$) and finally to disordered and decomposed structures made of fragments of cryoglobulin (300 $mJcm^{-2}$).

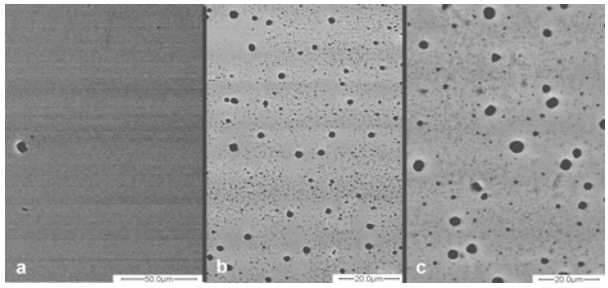

Figure 3: SEM patterns of cryoglobulin thin films obtained by MAPLE (a) at a fluence of 100 mJ/cm^2, (b)

ISSN: 1727-1983

at a fluence of 200 mJ/cm^2, and (c) at a fluence of 300 mJ/cm^2.

Conclusions

We have summarized a large amount of prior experimental data we could conclude that the growth rate in MAPLE is lower compared to PLD with MAPLE growth rates ranging from 0.001 nm/pulse to 1.1 nm/pulse. The material absorption at the incident laser wavelength could influence the growth rate in a critical way.

Further, we demonstrate in this work that MAPLE is suitable for producing cryoglobulin thin films with close resemblance to the starting cryoglobulin structure. FTIR revealed that spectra of samples deposited at fluence of 100 mJcm^{-2} were the closest to dropcast spectra. Our FTIR studies only of cryoglobulin amides groups evidenced a large degeneracy of the amide groups and their conversion to aldehydes and carboacids fragments when increasing fluence.SEM investigations have shown that for MAPLE-deposited thin films the morphology evolves from a continuous uniform aspect, preserving the structural integrity (100 mJcm^{-2}) to disordered and decomposed structures consisting of fragments of cryoglobulin (300 mJcm^{-2}) with increasing fluence. These results are an important step for fabricating novel test arrays potentially used for a faster, cleaner and more accurate diagnosis.

Acknowledgement

This project was supported by the GA ASCR, Project No. A1010110 and the Institutional Research Plan AV CR No. AVOZ 101000522. Our thanks should be also expressed to CTU Research Project No. 6640770030 and No. 88/1. One of us (DBC) gratefully acknowledges financial support from the Office of Naval Research.

References

[1] CHRISEY D. B., and HUBLER G K (Eds.) (1994): 'Pulsed Laser Deposition of Thin Films' (John Wiley & Sons, New York)

[2] MCGILL R. A., and CHRISEY D. B. (2000): 'Method Of Producing A Film Coating By Matrix Assisted Pulsed Laser Deposition', Patent No. 6,025,036

[3] BUBB D. M., HORWITZ J. S., CALLAHAN J. H., MCGILL R. A., HOUSER E. J., CHRISEY D. B., PAPANTONAKIS M. R., HAGLUND R. F., GALICIA M. C., and VERTES A (2001): 'Resonant Infrared Pulsed-Laser Deposition of Polymer Films Using a Free-Electron Laser', *J. Vac. Sci. Technol.* A, **19(**5), pp. 2698-2702

[4] TOFTMANN B., PAPANTONAKIS M. R., AUYEUNG R. C. Y., KIM W., O'MALLAEY S. M., BUBB D. M., HORWITZ J. S., SCHOU J., JOHANSEN P. M., and HAGLUND R. F. (2004): 'UV and RIR matrix assisted pulsed laser deposition of organic MEH-PPV films' *Thin Solid Films,* **453-454**, pp. 177-181

[5] CHRISEY D. B., PIQUE A., MCGILL R. A., HORWITZ J. S., RINGEISEN B. R., BUBB D. M., and WU P. K. (2003): 'Laser Deposition of Polymer and Biomaterial Films' *Chem. Rev.* **103** pp. 553-576

[6] BUBB D. M., O'MALLEY S. M., ANTONACCI C., SIMONSON D., and MCGILL R. A. (2004): 'Matrix-Assisted Laser Deposition of a Sorbent Oligomer using an Infrared Laser', *Journal of Applied Physics,* **95(**4), pp. 2175-2177

[7] CALLAHAN J. H., GALICIA M. C., and VERTES A. (2002): 'Atmospheric pressure matrix-assisted laser desorption ionization as a plume diagnostic tool in laser evaporation methods', *Applied Surface Science,* **197-198,** pp.130-137

[8] NARAYAN R., PATZ T. M., and CHRISEY D. B. (2005): 'Time Released Drug Coatings Using Excimer Laser Processing' *Lambda Physic Highlights,* Spring 2005, No. **65**

[9] FOERSTER J. (1993): ‚Cryoglobulins and Cryoglobulinemia', in G.R. Lee, T.C. Bithell, J. Foerster, J.W. Athens, and J.N. Lukens (Eds): ‚Wintrobe's Clinical Hematology', (Lea & Febiger., Philadelphia, London) p. 2284

[10] STAMATIN L.**,** CRISTESCU R., SOCOL G., MIHAIESCU D., STAMATIN I., MIHAILESCU I.N., and CHRISEY D.B. (2005): 'Laser Deposition of Fibrinogen Blood Proteins Thin Films by Matrix Assisted Pulsed Laser Evaporation', *Applied Surface Science* **248, pp.** 422–427

[11] CRISTESCU R., DORCIOMAN G., RISTOSCU C., AXENTE E., GRIGORESCU S., MOLDOVAN A., MIHAILESCU I. N., KOCOUREK T., JELINEK M., ALBULESCU M., BURUIANA T., MIHAIESCU D., STAMATIN I., and CHRISEY D. B. (2005): 'Matrix Assisted Pulsed Laser Evaporation Processing of Triacetate-Pullulan Polysaccharides Thin Films for Drug Delivery Systems', *Applied Surface Science* in print

[12] FRYCEK R., JELINEK M., KOCOUREK T., FITL P., VRNATA M., MYSLIK V., and VRBOVA M. (2005): 'Thin Organic Layers Prepared by MAPLE for Gas Sensor Application' *Thin Solid Films* in print

[13] FRYCEK R., JELINEK M., VRBOVA M., KOCOUREK T., FITL P., VRNATA M. and MYSLIK V. (2005): 'Matrix Assisted Pulse Laser Deposition of Large Molecules and Gas Sensors

ISSN: 1727-1983

Applications' Will be printed in 2005 EMBEC Conference Proc.

[14] PIQUÉ A., AUYEUNG R. C. Y., STEPNOWSKI J. L., WEIR D. W., ARNOLD C B, MCGILL R. A., and CHRISEY D. B. (2003): 'Laser Processing of Polymer Thin Films for Chemical Sensor Applications', *Surface and Coatings Technology,* **163-164,** pp. 293-299

[15] MEENAKASHI V., TEIZER W., NAUGLE N. G., ZHAO H, and DUNBAR K. R. (2004): 'Films of Mn_{12}-Acetate by Pulsed Laser Evaporation', *Solid State Communications,* **132(7)**, pp. 471-476

[16] PIQUÉ A., MCGILL R. A., CHRISEY D. B., LEONHARDT D., MLSNA T. E., SPARGO B. J., CALLAHAN J. H., VACHET R. W., CHUNG R., and BUCARO M. A. (1999): *'Growth of Organic Thin Films by the Matrix Assisted Pulsed Laser Evaporation (MAPLE) Technique', Thin Solid Films,* **355-356**, pp. 536-541

[17] YNGLING Y. G., ZHIGILEI L. V., GARRISON B. J., KOUBENAKIS A., LABRAKIS J., and GEORGIOU S. (2001): 'Laser Ablation of Bi-component Systems: A Probe of Molecular Ejection Mechanisms', *Applied Physics Letters* **78** pp. 1631-1633

[18] BUBB D. M., WU P. K., HORWITZ J. S., CALLAHAN J. H., GALICIA M., VERTES A., MCGILL R. A., HOUSER E. J., RINGEISEN B. R., and CHRISEY D. B. (2002): 'The Effect of the Matrix on Film Properties in Matrix-Assisted Pulsed Laser Evaporation', *Journal of Applied Physics,* **91,** pp. 2055-2058

[19] WU P. K., RINGEISEN B. R., KRIZMAN D. B., FRONDOZA C. G., BROOKS M., BUBB D. M., AUYEUNG R. C. Y., PIQUÉ A., SPARGO B., MCGILL R. A. and CHRISEY D. B. (2003): 'Laser Transfer of Biomaterials: Matrix-Assisted Pulsed Laser Evaporation (MAPLE) and MAPLE Direct Write', *Rev. Science Instr.,* **74 (4)**, pp. 2546-2557

[20] RODRIGO K., CZUBA P., TOFTMANN B., SCHOU J., and PEDRIS R. (2005): 'Water Ice as a Matrix for Film Production by Matrix-Assisted Pulsed Laser Evaporation (MAPLE), *Appl. Surf. Sci.* in print

[21] TOFTMANN B., RODRIGO K., SCHOU J., and PEDRIS R. (2005): 'High Laser-Fluence Deposition of Organic Materials in Water Ice Matrices by ''MAPLE''', *Applied Surface Science,* **247,** pp. 211-216

[22] RODRIGO K., TOFTMANN B., SCHOU J., and PEDRIS R. (2004): 'Laser-induced ion emission during polymer deposition from a flash-frozen water ice matrix', *Chemical Physics Letters,* **399**, pp. 368-372

[23] PATZ T., CRISTESCU R., NARAYAN R., MENEGAZZO N., MIZAIKOFF B., MESSERSMITH P. B., STAMATIN I., MIHAILESCU I. N., and CHRISEY D. B. (2005): 'Processing of Adhesive Polymer Thin Films by Matrix Assisted Pulsed Laser Evaporation', *Applied Surface Science,* **248**, pp. 416-421

[24] CRISTESCU R., PATZ T., NARAYAN R., MENEGAZZO N., MIZAIKOFF B., MIHAIESCU D. E., MESSERSMITH P. B., STAMATIN I., MIHAILESCU I. N., and CHRISEY D. B. (2005): 'Processing of Mussel Adhesive Protein Analog Thin Films by Matrix Assisted Pulsed Laser Evaporation', *Applied Surface Science,* **247**, pp. 217-224

[25] GUTIÉRREZ-LLORENTE A., HOROWITZ G., PÉREZ-CASERO R., PERRIÉRE J., FAVE J. L., YASSAR A., and SANT C. (2004): 'Growth of Polyalkylthiophene Films by Matrix Assisted Pulsed Laser Evaporation', *Organic Electronic,* **5**, pp. 29-34

[26] RINGEISEN B. R., CALLAHAN J., WU P. K., PIQUÉ A., SPARGO B., MCGILL R. A., BUCARO M., KIM H., BUBB D. M., and CHRISEY D. B. (2001): 'Novel Laser-Based Deposition of Active Protein Thin Films', *Langmuir* **17(11)**, pp. 3472-3479

[27] BONELLI M., MIOTELLO A., MOSANER P., CASIRAGHI C., and OSSI P. M. (2003): 'Pulsed Laser Deposition of Diamondlike Carbon Films on Polycarbonate', *J. Appl. Phys.*, **93(2),** pp. 859-865

[28] JELINEK M. (2005): 'Habilitation work at CTU FJFI', Prague

ISSN: 1727-1983