Matrix assisted pulsed laser evaporation of pullulan tailor-made biomaterial thin films for controlled drug delivery systems

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Abstract. We report the first successful deposition of cinnamate-pullulan polysaccharide thin films by Matrix Assisted Pulsed Laser Evaporation (MAPLE). Thin film depositions were performed in vacuum using a KrF* excimer laser source (λ = 248 nm, τ ≈ 20 ns) operated at a repetition rate of 10 Hz. The dependence on incident laser fluence of the induced surface morphology is studied. We demonstrated by Raman spectroscopy that our MAPLE-deposited cinnamate-pullulan thin films are composed of starting materials preserving their chemical structures, with no impurities.

1. Introduction
The delineation between biotechnology, pharmaceuticals, and medical devices has begun to decrease, and new techniques based on nanosciences and nanotechnology of processing knowledge-based multifunctional materials multilayers, and new production processes and device must be developed to accommodate the change through breakthroughs in new applicable knowledge and long-term research and technology development [1, 2]. A tremendous amount of work has been concentrated worldwide in the past two decades on the pharmaceutical and medical research and development of drugs with improved temporal and spatial site-specificity which can be embedded in medical devices like teeth fillings and drug eluting coronary stents, that is, targeted drug delivery systems [3-6]. Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the last one is released from the material in a predesigned manner.

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[7]. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external factors [8].

Recently a novel drug timed-release strategy based on a laser-assisted vacuum deposition technique, namely the matrix-assisted pulsed laser evaporation (MAPLE), has emerged [9]. Extension of MAPLE to proteins is interesting owing to its reliability and good control of deposition process parameters [10]. The MAPLE processing relies on achieving a modulatory release profile of drug particles by varying the thickness and composition of biodegradable polymer in a multilayer implementation. In the last few years there has been resurgence in interest in pullulan (a natural water-soluble polysaccharide), particularly for higher-value health and pharmaceutical applications. Various protocols have been adopted in order to perform the desired experiments, since pullulan, like most polysaccharides, has poor solubility in common organic solvents.

We report the successful deposition of cinnamate-pullulan derivatives by MAPLE. We demonstrate that we produced thin films whose composition as determined by Raman spectroscopy was similar to the starting materials suggesting that the functionality is preserved.

2. Experimental

2.1 Materials

We used in our experiments a new derivative of pullulan: cinnamate-pullulan synthesized by the Petru Poni Institute of Macromolecular Chemistry, Iasi, Romania, from pure pullulan made (P-20 type) using a patented original method by the National Institute for Chemical-Pharmaceutical R&D, Bucharest, Romania [11]. Cinnamate-pullulan was obtained by an interfacial esterification reaction between pure pullulan and cinnamyl chloride in a methyl-ethyl-cetone and toluene mixture medium (4:1 v/v). After preliminary tests this biopolymer is soluble in chloroform and methylene chloride, acetone, DMF and DMSO. In this study we used the chloroform (melting point of 209 K) as a solvent for the MAPLE experiments because of its good absorption at the 248 nm KrF* laser wavelength.

2.2 Method

The UV-MAPLE deposition setup has been described elsewhere [12]. Briefly, the set-up consists of an excimer laser source, directed into a vacuum chamber that encloses a substrate holder and a liquid nitrogen cooled target holder. After freezing, the target was rapidly mounted inside the deposition chamber and rotated at 0.25 Hz to avoid heating and possible drilling by the pulsed laser beam. Before deposition, the chamber was evacuated down to a residual pressure within the range of (5-9) 10⁻⁴ Pa. Films were grown by MAPLE arrangement at the IP ASCR, Prague using a pulsed KrF* excimer laser (248 nm, τ = 20 ns and operating at 10 Hz), and a rotated target holder cooled to LN temperature. The laser radiation was focused by a fused silica lens placed outside chamber. The laser beam incident angle was 45° with respect with target surface. The depositions have been done on fused silica for Raman spectroscopy. The target-substrate distance was 3 cm. The laser spot area was 20 mm². The number of subsequent pulses applied for a deposition of one film was (4,000–13,000). The laser fluence was set within the range of (75-500) mJ/cm². The overall MAPLE deposition conditions are collected in Table 1.

2.3 Films investigations

The morphological and structural characterization of all MAPLE films was carried out by Atomic Force Microscopy (AFM), Raman spectroscopy, and contact profilometry. AFM measurements were performed with a Quesant Atomic Force Microscope with a resolution of 500 cm⁻¹ at a 1 Hz scan frequency. A Renishaw in Via spectrometer (Renishaw, U.K.) was used to collect Raman spectra. Samples were excited with a HeNe laser (632.8 nm) focused on the sample with a 50x microscope objective. A 10 s integration time was used, and the signal was summed over 20 scans in the extended scan mode. After irradiation, the samples were visually inspected through the microscope, but no signs of laser damage to the sample were observed. Contact profilometry measurements were recorded with an Alphastep device.
Table 1. MAPLE deposition conditions for cinnamate-pullulan (N30-N34)

| Sample AFM symbol | Vacuum pressure [10^{-4} Pa] | Energy/pulse [mJ] | Fluence [mJ/cm²] | Number of pulses | Thickness [nm] | Deposition rate [Å/pulse] |
|-------------------|-------------------------------|-------------------|------------------|-----------------|---------------|--------------------------|
| N 30              | 8                             | 104               | 500              | 4,000           | 16,410        | 41                       |
| N 31              | 8                             | 64                | 300              | 6,000           | 7,793         | 13                       |
| N 32              | 9                             | 44                | 200              | 8,000           | 4,851         | 6                        |
| N 33              | 5                             | 23                | 100              | 8,000           | 648           | 0.8                      |
| N 34              | 9                             | 15                | 75               | 2,000 (@10 mJ) + 13,000 (@15 mJ) | 0 + 525 | 0.4 |

3 Results and discussion

As shown in Table 1, in the case of cinnamate-pullulan thin films MAPLE depositions, we found threshold fluence values of 75 mJ/cm² (N34). Decreasing fluence at slightly beneath this value no deposition was observed.

3.1 Atomic Force Microscopy

Figure 1 contains the AFM micrographs of cinnamate-pullulan thin films deposited by MAPLE. As in the case of triacetate-pullulan [13] the surface morphology strongly depends on the laser fluence and evolves from small globular structures to quite big clusters. As previously discussed at the fluences below 75 mJ/cm² no deposition was detected. At the fluence of 75 mJ/cm² we observe these small globular structures uniformly distributed having an average diameter of 300 nm (symbol N34) indicating an intact structure. At the fluence of 100 mJ/cm² (symbol N33) we observed conformational modifications and even an agglomeration tendency of globular structures visible at the lowest fluence (75 mJ/cm²). Further these agglomerated structures become larger taking a plate-like form (with an approximate dimension of about 1 μm) typical for a premelting/crystallization process at 200 mJ/cm² (symbol N32). This is indicative for a degradation trend. We also notice a formation of large clusters (having an average size of about 2 μm) typical for a melting/crystallization process at 300 mJ/cm² (symbol N31). This is typically illustrative for a degradation process. Further we observe quite big clusters typical for a global melting/crystallization process followed by solidification over large domains with an average dimension of about 6 μm (for 500 mJ/cm², symbol N30) indicating an advanced degradation process. AFM images show the possibility that cinnamate-pullulan has been modified by the impact of the cinnamate-pullulan clusters with the substrate at higher kinetic energies. Under these premises we decided to continue investigations on the thin films deposited at 75 mJ/cm².

3.2 Raman spectroscopy

Raman investigations are congruent with AFM images where the transformation from cinnamate-pullulan at a fluence of 75 mJ/cm² (N34) to residues at a fluence of 500 mJ/cm² (N30) change the morphology from globular material to large clusters. Raman investigations revealed that spectra of samples deposited at a fluence of 75 mJ/cm² were the closest to cinnamate-pullulan starting material spectra. The Raman spectra of the cinnamate-pullulan reference (starting material) and thin films deposited by MAPLE at 75 mJ/cm² are shown in Figure 2. The broad bands at about 810 and 605 cm⁻¹ in N34 correspond to the spectrum of the substrate. In our opinion, the Raman spectra for the samples N30-33 do not provide supplementary useful information as compared with the evidence already presented in Figure 2. We decided therefore to not introduce them in this figure in order to avoid any possible confusion.
Figure 1. AFM micrographs MAPLE-deposited thin films of cinnamate-pullulan at the fluence of 75 mJ/cm² (symbol N34), 100 mJ/cm² (symbol N33), 200 mJ/cm² (symbol N32), 300 mJ/cm² (symbol N31), and 500 mJ/cm² (symbol N30).
Figure 2. Raman spectra of cinnamate-pullulan starting material (symbol Ref) and thin film deposited by MAPLE at the fluence of 75 mJ/cm² (symbol N34).

4. Conclusions
We demonstrate in this work that MAPLE is suitable for producing cinnamate-pullulan thin films whose composition and likely functionality to be close to the starting structures. In case of cinnamate-pullulan thin films MAPLE depositions, we found the threshold fluence value of 75 mJ/cm². AFM investigations revealed in case of cinnamate-pullulan that the surface morphology strongly depends on the laser fluence and evolves from small globular structures uniformly distributed with an average diameter of 300 nm indicative for an intact structure (at 75 mJ/cm²) to quite big clusters typical for a global melting/crystallization process followed by solidification over large domains with an average dimension of about 6 μm (at 500 mJ/cm²). This suggests an advanced degradation process. Raman spectroscopy investigations revealed that spectra of cinnamate-pullulan samples deposited at 75 mJ/cm² were the closest to starting material spectra. We conclude that MAPLE can provide a better approach to growing high quality thin films of pullulan derivatives, allowing an accurate thickness control highly required in targeted drug delivery. The fluence plays an essential role in the optimization of the depositions of these polysaccharides. This new pullulan-tailor-made biomaterial with desirable functional groups can be used not only for innovative drug delivery systems but also as potential linings for artificial organs, as substrates for cell growth, and as agents in immunology testing.

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