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Combinatorial Matrix Assisted Pulsed Laser Evaporation of a biodegradable polymer and fibronectin for protein immobilization and controlled release

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ABSTRACT

Defined protein quantities were embedded *in situ* in a biodegradable polymer coating during simultaneous laser vaporization of two targets. Fibronectin (FN) and poly-DL-lactide (PDLLA) were transferred and immobilized concomitantly by Combinatorial Matrix Assisted Pulsed Laser Evaporation onto solid substrates. The film surface with gradient of composition was characterized by optical, scanning electron microscopy and profilometry. Micrometric FN packages were visualized in the polymeric matrix by confocal microscopy. The composition of FN was investigated by FTIR and μFTIR analyses in a polymeric matrix with different thickness.

1. Introduction

Matrix Assisted Pulsed Laser Evaporation (MAPLE) is a deposition technique of organic molecules on solid substrates with minimal chemical and thermal degradation [1]. It evolved and confirmed with the development of new pulsed laser sources. Thus, peak-to-peak energy stability of the laser pulses controls better the evaporation of biomolecules and fabrication of functionalized coatings. MAPLE was applied to obtain stable glassy polymers with kinetic stability at high temperatures [2], single-step double layer configuration [3], compositional gradient films [4] as well as biodegradable polymeric films [5,6] and proteins with biologically active properties for implant [7,8] or surface acoustic wave biosensor applications [9].

There is a general interest in developing biodegradable matrices with incorporated active substances. The time release could be controlled either by monitoring the amount of incorporated substance or thickness of the matrix. The release kinetics of delivery is preferable in a localized, spatiotemporal manner similar to the natural wound healing process [10]. The selection of the carrier should take into account a matrix-mimicking bio-environment. Poly-DL-lactide (PDLLA) is generally applied as thin film for drug delivery [11,12]. The chemical structure of PDLLA was found undamaged after laser evaporation with a near-infrared (1064 nm) irradiation [13]. It was expected that such fragile biomolecules are easily damaged by UV radiation. However, other studies demonstrated that using the appropriate laser fluence, PDLLA porous films with the same composition and structure as starting materials can be obtained by MAPLE using a KrF* excimer laser at 248 nm [14]. The same laser wavelength was used by irradiating cryogenic targets consisting of PDLLA and gentamicin mixtures dissolved in ethyl acetate [15]. On the other hand protein immobilization is still a challenging task [16]. Laser was used to transfer and deposit active fibronectin (FN) onto Si substrates [7]. Vitronectin deposited by MAPLE on a nanostructured layer of calcium phosphate was demonstrated to improve the overall structure biological properties [8].

Recently we have proposed and implemented Combinatorial MAPLE (C-MAPLE) in order to combine two polymers in a thin film configuration with variable composition [4]. The versatility of the method was demonstrated for the synchronized laser transfer in order to develop a thin composite coating uniform in thickness and with controllable biocompatible areas.

We propose herein C-MAPLE application for obtaining a composite coating composed of a biodegradable polymer, PDLLA, and a protein, FN, in a single step procedure. The two compounds have been chosen because the FN is recognized to mediate the

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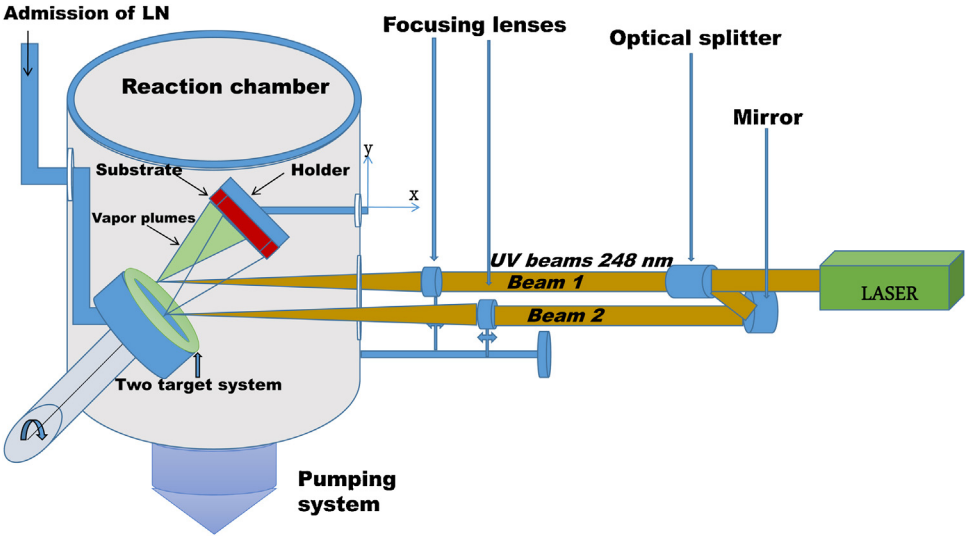

Fig. 1. Schematic of the C-MAPLE set-up: two solutions are frozen and form the targets (left, inside the reaction chamber) in a concentrically two target system cooled constantly by a liquid nitrogen flow. The laser beam is divided into two beams which simultaneously irradiate the frozen targets, vaporizing the materials and consequently depositing them on a facing substrate.

initial attachment of cells to surrounding tissues while PDLLA is the matrix capable to provide the degradable properties in biological environments. It is accordingly attained the immobilization of both compounds on solid substrates while the protein is embedded in the polymeric matrix for applications based on its controlled release. The challenge with this study was to correlate the two laser beams of different energies with the thermo-physical properties of the two organic compounds in order to obtain a composite active coating avoiding the liquid phase issues of mixtures such as specific solvent for particular active substance.

2. Experimental

2.1. Preparation of cryogenic targets

FN was purified from human cryoprecipitated plasma [17] while fluorescent FN was prepared as described elsewhere [18]. Solutions of FN dissolved in distilled water and saline buffer (50 mM Tris, pH 7.4, 150 mM NaCl) were homogenized following an established protocol [7]. The PDLLA powder was purchased from Sigma–Aldrich, Inc., St. Louis, MO, USA. We have dissolved 0.04 g PDLLA powder in 10 ml chloroform to obtain the second homogenous solution. The two solutions were poured in a two concentrically copper container and frozen in a liquid nitrogen (LN) Dewar to obtain two cryogenic targets. The container was placed in a reaction chamber to be used in the laser vaporization experiments.

2.2. Combinatorial MAPLE set-up

A detailed description of the C-MAPLE protocol in presented in Ref. [4]. A KrF* excimer laser beam ($\lambda = 248$ nm, $\tau = 25$ ns) was divided by an optical splitter into two beams. The beam splitter was placed in front of the incident laser beam at 45° so that one beam pass through (Beam 1) and the other is deviated at 90° (Beam 2) from its path. A mirror is placed very close to the splitter in order to reflect the Beam 2 toward the same direction and parallel with the Beam 1. Thus, the two beams are focalized by lenses such as the two laser spots are directed to the surfaces of the two concentrically cryogenic targets. The set-up is presented in Fig. 1.

The laser set-up allows for easily optimizing the laser fluence since two focusing lenses are used separately. This stands for an important advantage since the two different solvents are vaporized using the same laser beam. It was adjusted for each compound and fixed at 0.7 J/cm^2 for FN and 0.55 J/cm^2 for PDLLA respectively. Five thousand laser pulses were applied for the irradiation of the two targets to obtain one composite structure on either glass or silicon of 50 mm × 10 mm. In Fig. 2 we depicted schematically the structure which exhibits a gradient of composition obtained after C-MAPLE application. It was found that in the glass slice corners we have 100% FN and 100% PDLLA respectively. In-between mixtures of the two compounds are both interlinked and immobilized *in situ* during the process.

2.3. Physical–chemical characterization

The coating thickness and topography were monitored and estimated by profilometry using a stylus profiler XP2 from Ambios Technology; 0.01 mm/sec speed; 1 mm working distance and range 10 μm.

Surface morphology was studied with a Zeiss Optical Microscope (Zeiss Axio Imager Z1M with an Axio Cam MRc 5-HR).

Thin films morphology was also investigated using a Scanning Electron Microscope, FEI Co. (model Inspect S), 0–30 kV accelerating

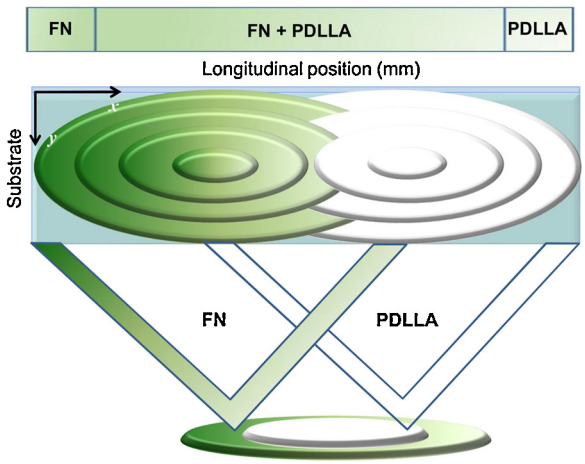

Fig. 2. Schematic representation of the thin film gradient structure growth and components intermixing developed by C-MAPLE.