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Protein-resistant polymer coatings obtained by matrix assisted pulsed laser evaporation

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ABSTRACT

Adsorption of proteins and polysaccharides is known to facilitate microbial attachment and subsequent formation of biofilm on surfaces that ultimately results in its biofouling. Therefore, protein repellent modified surfaces are necessary to block the irreversible attachment of microorganisms. Within this context, the feasibility of using the Poly(ethylene glycol)-block-poly(ε-caprolactone) methyl ether (PEG-block-PCL Me) copolymer as potential protein-resistant coating was explored in this work. The films were deposited using Matrix Assisted Pulsed Laser Evaporation (MAPLE), a technique that allows good control of composition, thickness and homogeneity. The chemical and morphological characteristics of the films were examined using Fourier Transform Infrared Spectroscopy (FTIR), contact angle measurements and Atomic Force Microscopy (AFM). The FTIR data demonstrates that the functional groups in the MAPLE-deposited films remain intact, especially for fluences below 0.5 J cm^{-2}. Optical Microscopy and AFM images show that the homogeneity and the roughness of the coatings are related to both laser parameters (fluence, number of pulses) and target composition. Protein adsorption tests were performed on the PEG-block-PCL Me copolymer coated glass and on bare glass surface as a control. The results show that the presence of copolymer as coating significantly reduces the adsorption of proteins.

1. Introduction

There is great interest in developing biomedical polymeric coatings that can resist protein adsorption for applications subjected to a humid environment, such as lab-on-chip systems or implant coatings. The main issues (i.e. inflammatory response or biofilm formation) for these types of applications are caused by the peculiar characteristics of the polymeric coatings surfaces onto which protein adsorption occurs.

The key parameters of an ideal adhesion-resistant polymer surface include: flexible, linear backbone for resisting undesirable interactions, a surface which is smooth at the molecular level to avoid infiltration of a biological adhesive leading to mechanical interlocking, high molecular mobility in the backbone and surface active side-chains, and a thickness which controls the fracture mechanics of the interface [1]. Within this context, two important issues must be envisaged: the characteristics of the materials and the method used to obtain the polymeric coatings.

Extensive studies have been performed for a large variety of materials with good resistance to protein adsorption, from coatings based on PEG polymer to dendritic or hyperbranched hydrophilic polymers, zwitterionic, self-assembled monolayers (SAMs) and polymer brushes [2–7].

The stability of the materials into aqueous media is related not only to its chemistry, but on the coating deposition technique as well. Various techniques used to obtain coatings have been reported, such as dip-coating, spray-coating, spin-coating, and solvent casting. However, these simple, fast and low cost techniques are characterized by a poor control of film homogeneity and thickness. More complex techniques, such as chemical grafting of molecules onto the biomaterial surface, SAMs surface-tethered polymers (polymer brushes), or multilayer coatings based on layer-by-layer assembly, have been reported in literature [8–10]. These techniques offer precise control of placement and orientation of chemical groups and biomolecules on the surface of the coating, but at the same time they exhibit difficulties in obtaining films with controllable thickness and good stability in harsh environment.

A technique which can provide control over coatings homogeneity and thickness, as well as over the chemical structure and physical stability in harsh environment of the polymeric coating, proves to be a laser based method (i.e. Matrix Assisted Pulsed Laser Evaporation – MAPLE). MAPLE is a laser evaporation technique for

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growing thin films of sensitive materials, such as proteins and polymers, and involves directing a pulsed laser beam onto a frozen target consisting of a compound dissolved at low concentration in a volatile solvent matrix. The matrix preferentially absorbs the incident laser radiation and the ejected molecules can be collected on a substrate to form a film, while the solvent molecules are pumped away [11–17].

Taking this knowledge into account, our approach was to combine the advantages given by the characteristics of a PEG based copolymer which contains functional methyl ether groups with the one of MAPLE process for obtaining stable and protein repellent coatings. The presence of methyl ether group is beneficial due to its preferential behavior of forming large numbers of hydrogen bonds with water molecules, so decreasing the ability of proteins to bind on the coating surface.

Herein the investigation of optimal MAPLE parameters for obtaining PEG-block-PCL Me copolymer thin films is reported. The correlation of coatings characteristic and protein-resistant properties of PEG-block-PCL Me copolymer coatings obtained by MAPLE was studied.

2. Materials and methods

2.1. Materials and target preparation

All the materials were obtained from Sigma-Aldrich, unless otherwise specified. Solutions consisting of PEG-block-PCL Me copolymer dissolved in chloroform (0.5–1.5 wt.%) were gently homogenized and rapidly frozen drop by drop in a liquid nitrogen cooled copper container which was then mounted on a cryogenic holder inside the deposition chamber. The target was rotated with a motion feed through driven by a motor to avoid local overheating and drilling following multiple pulses of laser irradiation.

2.2. MAPLE deposition system

A "Surelite II" pulsed Nd: YAG laser system (Continuum Company) working at a wavelength of 266 nm, having 6 ns pulse duration and 10 Hz repetition rate was used to irradiate the frozen targets. The laser spot size was measured to be 0.02 cm^2. The laser fluence was varied between 0.2 and 0.9 J cm^{-2}. In order to check the temperature, two thermocouples were placed at two different positions of the target holder. The background pressure ($2–3 \times 10^{-3}$ Pa) was adjusted by Pfeiffer-Balzers TPU 170 turbomolecular pump.

2.3. Substrates preparation

Two types of substrates were used: double polished Si (1 0 0) transparent in the IR (for FTIR post-characterization) and glass coverslips. The substrates were carefully cleaned in an ultrasonic bath in acetone, ethyl alcohol and deionized water and blow-dried with N_2 gas before use. All substrates were placed at a distance of 3.5 cm from the frozen target and kept at ambient temperature during the deposition. No post deposition annealing was carried out.

2.4. Structural and morphological characterization of the deposited copolymer thin films

Fourier Transform Infrared Spectroscopy was applied to study the characteristic vibrations of functional groups in the deposited thin films. The infrared spectrum of the native molecule was measured and compared with the thin film spectrum. The FTIR measurements were carried out with a Jasco FT/IR-6300 type A spectrometer in the 400–7800 cm^{-1} range, with a resolution of 4 cm^{-1} in absorption mode, by accumulation of 1024 scans, and using a Rosenfeld apodization function.

Morphology characterizations were performed by optical microscopy and Atomic Force Microscopy (AFM). The images were acquired with an Axiovert 200 Microscope coupled to a Carl Zeiss AxioCam MRm camera. AFM (XE 100 AFM setup from Park) measurements in non contact mode were performed to analyze the films surface roughness.

Contact Angle measurements were performed in static mode, by using a KSV CAM101 microscope provisioned with a video camera with FireWire interface allowing the acquisition of images having a resolution of 640×480 pixels. The sessile drop method was applied at constant room temperature (20 °C) using a syringe with double distilled water, which ensured droplets with a volume of 0.5–1 μl. The reported values for the contact angle were obtained upon averaging 5 measurements performed on different areas of the sample.

2.5. Protein adsorption studies

Protein adsorption studies were performed after immersing the coatings in 25 ml of phosphate buffer solution (PBS)/distilled water for 20 min. The coatings were subsequently incubated by placing 500 μl drops of 0.1 mg per ml fluorescein isothiocyanate (FITC) labeled BSA in PBS on top of the coatings. After 1 h of incubation, the coatings were rinsed with PBS and distilled water and imaged using a fluorescence microscope. Nitrocellulose and PEG surfaces were used as positive and negative controls.

The total fluorescence was determined using the built-in FITC filter of the microscope, with a constant exposure time, magnification, and image area for all the surfaces. Duplicate images of each coating before and after incubation with BSA were recorded using a CCD camera (ANDOR iXon DU897 E-CSO-UVB) and Olympus IX71 microscope.

To calculate the total fluorescence from BSA, the background fluorescence for each coating type was first subtracted using ImageJ software and the total fluorescence was calculated using the built-in plug-in.

3. Results and discussion

3.1. Copolymer structural characterization

FTIR analysis was applied to investigate the chemical structure of polymer films in order to identify the best experimental conditions for obtaining thin films without damage to the molecular structure of the polymers during their deposition by MAPLE.

The FTIR spectra of the films deposited at various laser fluences are presented in Fig. 1. A spectrum for the drop-casted PEG-block-PCL Me copolymer is included in each figure as a reference. One can notice the bond features corresponding to both PEG and PCL polymers as well as to the Me group. More specifically, the absorption band at 840 cm^{-1} is assigned to the PEG component in crystalline matrix, while the peak observed at 947 cm^{-1} is attributed to the symmetric stretching vibration of C—O—C bond. Three bands, positioned at 1060, 1113, and 1148 cm^{-1}, are characteristic of the skeletal vibrations of $—CH_2—O—CH_2—$ bonds of the PEG chains. On the other hand, the peaks at 1420 and 1725 cm^{-1} are attributed to $CH_2—C═O$ deformation vibration and, respectively, to the C═O stretching vibrations of the ester carbonyl group from PCL.

The CH_2 twisting and CH_2 wagging vibrations are present through the bands positioned at 1280 and 1361 cm^{-1}, respectively, the second one being superimposed to the O—H in plane deformation. The CH_2 symmetric and asymmetric stretching bonds centered at 2873 and 2942 cm^{-1}, respectively, correspond to the distinctive bands of the functional methyl ether.