Applied Surface Science 281 (2013) 54–59

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Evaluation of the functionality of biodegradable polymeric platforms for drug delivery systems

M. Gioti*, V. Karagkiozaki, A. Basgiouraki, P.G. Karagiannidis, S. Logothetidis

Aristotle University of Thessaloniki, Physics Department, Lab for Thin Films – Nanosystems and Nanometrology (LTFN), GR-54124, Greece

ARTICLE INFO

Article history:
Available online 26 April 2013

Keywords:
Drug delivery
Spectroscopic ellipsometry
Biodegradable polymers
Protein adsorption
Atomic force microscopy

ABSTRACT

We present the development of a drug-loaded triple-layer platform consisting of thin film biodegradable polymers, in a properly designed form for the desired gradual degradation. Poly(DL-lactide-co-glycolide) (PLGA (65:35), PLGA (75:25)) and polycaprolactone (PCL) were grown by spin coating technique, to synthesize the platforms with the order PCL/PLGA (75:25)/PLGA (65:35) that determine their degradation rates. The outer PLGA (65:35) layer was loaded with dipyridamole, an antiplatelet drug. Spectroscopic ellipsometry (SE) in the Vis-far UV range was used to determine the nanostructure, as well as the content of the incorporated drug in the as-grown platforms. In situ and real-time SE measurements were carried out using a liquid cell for the dynamic evaluation of the fibrinogen and albumin protein adsorption processes. Atomic force microscopy studies justified the SE results concerning the nanopores formation in the polymeric platforms, and the dominant adsorption mechanisms of the proteins, which were defined by the drug incorporation in the platforms.

1. Introduction

Drug-delivery nanosystems have greatly promoted the implants technology in the aspects of controllable release of therapeutic agents at the site of implantation. A wide range of inorganic and organic materials (biocompatible metals and their alloys, polymers and block copolymers) have been used to manufacture drug eluting coatings for potential clinical applications [1–5]. Nanoporous coatings with pore sizes less than 100 nm are considered to exhibit superior drug eluting capacities as porosity of such low dimensions contributes to the material's high active surface and drug loading [6,7]. In particular, nanoporous materials with a variety of nanopore characteristics (depth, density, and diameter) can serve as drug reservoirs with multiplex loading capacities.

Drug eluting stents (DES) is a major breakthrough in the field of interventional cardiology because of their efficacy in reducing in-stent restenosis of coronary arteries using local drug delivery [8,9]. Several drug candidates, such as immune-suppressive agents, anti-inflammatory, and cellular proliferation inhibitors, have been employed for stent coating and evaluated in clinical trials. Sirolimus-eluting (Cypher®; Cordis, Miami Lakes, FL) and paclitaxel-eluting (Taxus®; Boston Scientific, Natick, MA) stents have been extensively used for coronary angioplasty [10,11]. The study of Mehilli et al. [12] shows promising results, both short- and mid-term, with biodegradable rapamycin-eluting stent coating. However, there is still a big concern about their long-term safety, particularly with the late or very late stent thrombosis, which is though to be related to the stent platform design, the toxicity of the active drug, and the durable polymer coatings [13,14]. DES can benefit immensely from the use of biocompatible and biodegradable polymer coatings of specific nanostructure and multilayer architecture. Multilayer coatings enable multidrug release, in different directions and at different rates. The coating structure (e.g. number of layers, deposition order, drug–polymer association, type of polymer) has to be optimized in order to obtain a sustained release of the active agents.

In this study, we designed and developed biodegradable polymeric matrices in a triple-layer configuration for stent-coating needs. Two classes of poly(DL-lactide-co-glycolide) (PLGA 65:35, PLGA 75:25) and polycaprolactone (PCL) with different degradation rates constitute the nanolayers of the platform, which were deposited by spin coating. The control of the nanoporosity of the engineered nanomaterials targeted to the successive encapsulation and the optimum distribution of dipyridamole (DPM), an antiplatelet drug known to inhibit clotting [15], into the external layer of the polymeric matrix. The in vitro haemocompatibility of the degradable, drug-loaded platform was evaluated by assessing the adsorption processes and profiles of human plasma fibrinogen (FIB) and human serum albumin (HSA) proteins, in order to verify that that the final structure could be physiologically tolerable and not cause an adverse response after its administration.

* Corresponding author. Tel.: +30 2310 998103; fax: +30 2310 998390.
E-mail address: mgiot@physics.auth.gr (M. Gioti).

0169-4332/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsusc.2013.04.082

Spectroscopic ellispometry (SE) technique in the visible-far ultraviolet (Vis-FUV) energy range was applied for the optical and structural characterization of the drug-loaded polymeric samples. Furthermore, SE was the main tool for the evaluation of FIB and HSA protein adsorption. Atomic force microscopy (AFM) studies provided additional data for the interpretation and the justification of the SE results.

2. Materials and methods

2.1. Materials

PLGA with different lactide:glycolide contents (65:35 with average molecular weight [Mw] = 40,000–75,000 and 75:25 with average Mw = 66,000–107,000) and PCL (with average Mw = 48,000–90,000) were purchased from Sigma–Aldrich. DPM was obtained from Sigma–Aldrich. NaCl, KCl, KH_2PO_4, and Na_2HPO_4 were obtained from Merck (Darmstadt, Germany).

2.2. Fabrication of polymeric films

The samples were fabricated by spin coating onto crystalline silicon (c-Si) substrates inside a nitrogen-filled glovebox. For the fabrication of the sequential polymeric layers, a solution of the corresponding polymer was prepared with a total concentration of 10 mg mL^{-1} in chloroform. The solution was spin coated using a rotation speed 3000 rpm and a spinning time of 30 s. The substrates were cleaned prior to spin coating with isopropanol and methanol and blow-dried using N_2 flow. Each layer was left overnight to slow dry any residual solvent left before the deposition of the next polymeric layer.

For the case of the drug-loaded outer layer a solution of drug:PLGA (65:35) 1:3 (w/w) was prepared with a total concentration of 13.3 mg mL^{-1} in chloroform. The composition of the produced drug-loaded polymeric layer with the spin coating method was determined by SE studies.

2.3. Protein solutions

Protein solutions of HSA (Sigma–Aldrich) and FIB (Sigma–Aldrich) were prepared in phosphate buffer saline (PBS), with a PH of 7.4, at concentrations of 10 mg mL^{-1} and 1 mg mL^{-1}, respectively. This protein concentration analogy was used in order to simulate the real conditions in human blood.

2.4. Spectroscopic ellipsometry studies

The characterization experimental technique that was applied in this work is ellipsometry; an optical technique that measures changes in the reflectance and phase difference between the parallel (R_p) and perpendicular (R_s) components of a polarized light beam upon reflection from a material surface. Using the following equation:

$$\tan(\Psi)e^{i\Delta} = \frac{R_p}{R_s} \tag{1}$$

The intensity ratio of R_p and R_s can be related to the amplitude ratio (Ψ) and the phase difference (Δ) between the two components of polarized light [16].

All measurements were performed using phase modulated spectroscopic ellipsometer (by Horiba/Jobin-Yvon), capable of measuring from the Near IR to Far UV (FUV) energy region (0.7–6.5 eV), at variable angles of incidence (55–80°).

In situ and real-time experiments of protein adsorption were carried out using a liquid flow-cell (by Nanofilm), in the energy region 1.5–4.2 eV, at 60° angle of incidence. Measurements were performed under static conditions, i.e. no solution flowing. For the real-time multi-wavelength ellipsometry (MWE) measurements the sampling time was 200 ms (the time step between two successive measurements), and the integration time was as low as 100 ms (the total time for which the experimental data are collected and one averaged spectrum is acquired).

All the experimental data were fitted to model-generated data using the Levenberg–Marquardt algorithm with the appropriate fitting parameters depending on the specific model applied for each case. Differences between the experimental and theoretical data were assessed by the mean square error (MSE). Depending on the complexity of the model and the number of the applied fitting parameters the values of MSE was the criterion for good fitting results.

The dielectric function $\varepsilon(\omega)$ of the polymeric films, the drug and the protein layers was described using the Tauc–Lorentz (TL) oscillator dispersion equation, where the imaginary part of the dielectric function is given by [17]:

$$\varepsilon_2(\omega) = \frac{A\omega_0\Gamma(\omega-\omega_g)^2}{\left(\omega^2-\omega_0^2\right)^2+\Gamma^2\omega^2}\times\frac{1}{\omega}, \quad \omega > \omega_g \tag{2}$$

$$\varepsilon_2(\omega) = 0, \quad \omega \le \omega_g \tag{3}$$

and the real part $\varepsilon_1(\omega)$ is obtained by the Kramer–Kronig integration [16,17]:

$$\varepsilon_1(\omega) = \varepsilon_\infty + \frac{2}{\pi}P\int_{\omega_{g1}}^{\infty}\frac{\xi\varepsilon_2(\xi)}{\xi^2-\omega^2}d\xi \tag{4}$$

where ε_∞ is the pure real part of the dielectric constant, and accounts the contribution of electronic transitions occurred at higher energies, which are not taken into account in the $\varepsilon_2(\omega)$.

The fitting parameters in this model are the fundamental band gap energy ω_g, the amplitude A of the oscillator, the Lorentz resonant energy ω_0, and its broadening term C.

The real part of the refractive index (n) and the extinction coefficient (k) of the complex refractive index are related to the dielectric function by the following equations [16]:

$$\varepsilon_1 = n^2 - k^2 \tag{5}$$

and

$$\varepsilon_2 = 2nk \tag{6}$$

The Bruggeman effective medium theory (BEMT) was implemented for the characterization of the composite layers, i.e. the outer drug-loaded PLGA layer. Based on this theory the effective dielectric function of the medium ε consisting of i components, is given by the following formula [18]:

$$\sum_i f_i\frac{\varepsilon_i-\varepsilon}{\varepsilon_i+2\varepsilon} = 0, \tag{7}$$

and

$$\sum_i f_i = 1 \tag{8}$$

where ε_i is the dielectric function of the ith component with a respective volume fraction f_i.

2.5. Atomic force microscopy (AFM) experiments

The polymeric layers were imaged by AFM SOLVER P47H scanning probe microscope (NT-MDT) in ambient environment. AFM measurements were performed in tapping mode, using rectangular antimony doped silicon cantilevers with 6–10 nm nominal tip curvature (NSG01, NT-MDT). The cantilevers had a typical force