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Optimized allylamine deposition for improved pluripotential cell culture

E. Punzón-Quijorna [a,b], V. Sánchez-Vaquero [a,c], A. Muñoz Noval [a,c], D. Gallach Pérez [a], A. Climent Font [a,b], G. Ceccone [d], R. Gago [e], J.P. García Ruiz [a,c], M. Manso Silván [a,c,*]

[a] Departamento de Física Aplicada y Departamento de Biología Molecular, Universidad Autónoma de Madrid, Spain
[b] Centro de Microanálisis de Materiales CMAM, Universidad Autónoma de Madrid, Spain
[c] Centro de Investigaciones Biomédicas en Red, Bioingeniería, Biomateriales y Nanomedicina, (CIBER-BBN) Spain
[d] Institute for Health and Consumer Protection, European Commission, Ispra (Va), Italy
[e] Instituto de Ciencia de Materiales de Madrid, Consejo Superior de Investigaciones Científicas, Spain

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ABSTRACT

Deposition of allylamine (ALL) by plasma enhanced chemical vapor deposition has been optimized on silicon based models. Simultaneous energy recoil detection analysis and Rutherford backscattering spectra show that 100 W deposition is ideal in terms of polymerized film formation and H content while, lower or higher power induce reduced film retention or excessive cross linking, respectively. Surface composition of the ALL film was further probed by X-ray photoelectron spectroscopy revealing a monocomponent N 1s spectrum compatible with the presence of primary amines. Optimized ALL films were applied to polycaprolactone (PCL) surfaces after Ar plasma activation with implications in the chemistry and wettability of this biocompatible polymer. Human mesenchymal stem cells (hMSCs) were cultured on ALL coated PCL surface and controls. ALL functionalized PCL was found to be especially attractive for the formation of confluent monolayers of hMSCs after 72 h of culture.

1. Introduction

Surface modification has become a recurrent step in the processing of biomedical materials in order to adapt to the specific role that the surface will play. For biosensors a cascade may be necessary for immobilization of the analyte molecule (including proteins [1], polynucleotide [2] and aptamers [3] approaches). For tissue engineering, a cell adhesion oligopeptide (i.e. RGD sequence) [4] may be linked to improve cell colonization. The surface modification approach can rely in wet chemistry on in gas-solid transformations. The sol–gel route can be considered an intermediate process since initial precursors are in solution but condensation reactions leading to the activated surface take only place upon evaporation of the solvent [5].

Wet chemical routes imply a surface activation step performed normally in strong acid or basic conditions and rely generally on the adsorption capacities of thiols or silanes (depending on the metallic or semiconductor nature of the surface) to graft a saturating organic layer. Drawbacks from these procedures emerge from the fact that they generate chemical waste, they can be applied to a limited family of materials and the substrates have to be carefully prepared in order to control homogeneity (i.e. thiol adsorption dependence on exposed crystalline facets).

These drawbacks can be at least partially improved when gas processes are considered. A wide branch of materials can be the subject of plasma or ion beam treatments and, though these processes are energy consuming, they produce relatively low chemical waste. Concentrating on materials for cell culture, many different alternatives exist, which include simple plasma discharge activation [6], plasma grafting [7], plasma deposition [8–10] or transformations under ion beams in etching [10,11] or implantation regimes [12,13].

In the present work we concentrate on the modification of surfaces by plasma enhanced chemical vapor deposition (PE-CVD) of allylamine (ALL, $CH_2{=}CH{-}CH_2{-}NH_2$), which has already demonstrated to be a functional approach to modify homogeneous cell culture scaffolds [14] and cell guiding surfaces [10]. We apply the functionalization process to polycaprolactone (PCL, $O{-}(CH_2)_5{-}CO{-}$), a biocompatible and biodegradable polyurethane used in tissue engineering applications [15]. We focus on the search of adapted PCL surfaces for the culture of human mesenchymal stem cells, which are present in adult tissues and are involved in regeneration of bone, cartilage and tendon [16].

* Corresponding author. Departamento de Física Aplicada, Facultad de Ciencias M12-107, Universidad Autónoma de Madrid, 28049 Madrid, Spain.
E-mail address: miguel.manso@uam.es (M. Manso Silván).

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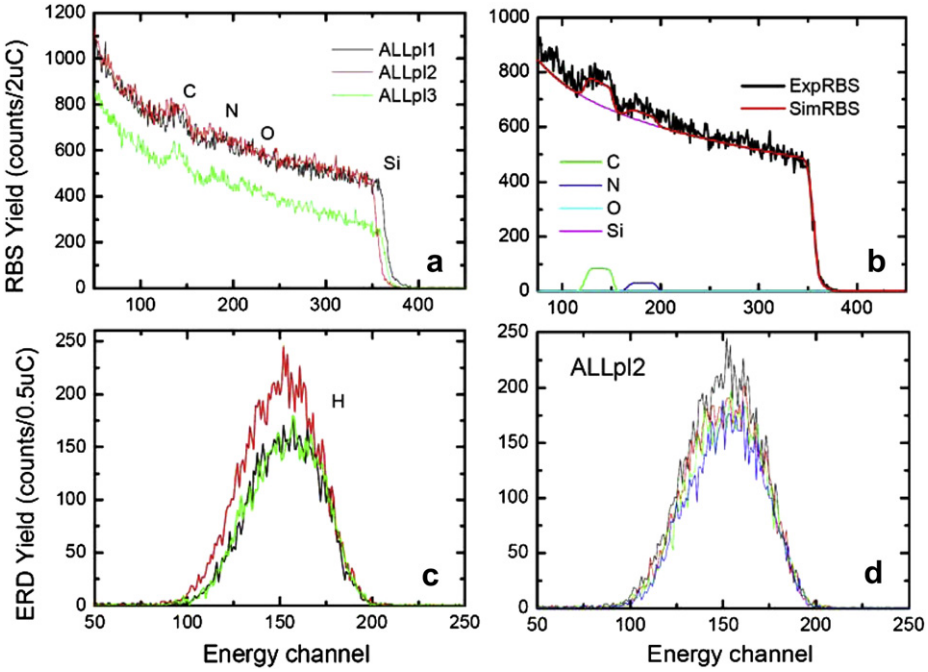

Fig. 1. a) RBS spectra of ALL films processed at increasing plasma power on Si substrates (pl1 = 50 W, pl2 = 100 W, pl3 = 200 W). b) RBS experimental and simulated spectra for the quantification of pl2 film. c) ERDA spectra of ALL films processed at increasing plasma. d) Consecutive ERDA spectra (0.5 μC each) for pl2 film.

2. Experimental

2.1. Substrate preparation and PE-CVD

ALL deposition optimization studies were performed in Si (100) substrates. The native oxide layer was eliminated by immersing substrates in 1:2 HF (49%):ethanol solutions at room temperature during 1 min, rinsing in ethanol and drying under N_2 flow. For studies performed onto PCL, films were grown on Si (100) and SiO_2 substrates by spin casting a 2% PCL (PCL 10000, Fluka) solution in chloroform.

Deposition of ALL (Sigma–Aldrich) was performed in a capacitively-coupled reactor equipped with a RF source (R.D. Mathis Co.) powered at 50, 100 or 200 W (samples labeled pl1, pl2, pl3 refer to increasing power, respectively). Initial chamber background pressure was 5×10^{-2} Pa and processing pressure was controlled by a chamber exit valve and set at 2 Pa. For the PE-CVD of ALL onto PCL, the samples were exposed to 2 min Ar etching and then exposed to supplementary 20 sccm of ALL (Fluka) (equivalent Ar flow) during 5 min.

2.2. Film characterization

Microanalytical techniques were used to obtain in-depth elemental information. A Cockcroft-Walton tandem accelerator located at Centro de Micro-Análisis de Materiales (CMAM, Universidad Autónoma de Madrid, Spain) was used for Rutherford backscattering spectrometry (RBS) and elastic recoil detection analysis (ERDA). RBS and ERDA measurements were performed simultaneously with a 2 MeV He^+ beam (incidence angle was 75° with respect to the surface normal). The RBS and ERDA spectra were acquired by using silicon surface barrier detectors placed at scattering angles of 170° and 30°, respectively. A 13 μm thick mylar foil was placed in front of the detector on the forward scattering angle to stop the He forward scattered particles and filter the H recoils. Four consecutive spectra for the same total ion dose were obtained for the ALL surfaces to evaluate the damage induced by successive exposure to the probing beam. All spectra were simulated using the SIMNRA code [17] to obtain the element in-depth composition.

The surface composition of ALL was studied by X-ray photoelectron spectroscopy (XPS). Spectra were acquired with an Ultra photoelectron spectrometer XPS, (KRATOS Analytical, UK) equipped with a 150 W monochromatic Al Kα source and obtaining the spectra at 90° take off angle and pass energy of 160 and 20 eV for the survey and core level scans, respectively. The chamber operating pressure was 2.66×10^{-7} Pa. An immersion magnetic lens system was used to compensate the surface charge. All peak energies refer to the binding energy (BE) of the hydrocarbon peak established at 285.0 eV. The spectra were peak fitted with a Gaussian/Lorentzian function after a linear background subtraction (Vision 2 software, Kratos, UK and CasaXPS, Surface Spectra, UK) and quantitative analyses were performed taking into account appropriate sensitivity factors (Kratos Analytical, UK).

Surface modification of PCL by ALL deposition was evaluated by optical and contact angle measurements. Transmittance (SiO_2 substrates) and reflectance (Si (100) substrates) UV–vis spectra were obtained using a Jasco V-560 double-beam spectrophotometer, equipped with an integrating sphere to avoid scattering losses. Water contact angle (wCA) measurements (milli-Q, 18 M Ω cm) were carried out in the dynamic mode in a KSV 101 goniometer. Data were obtained from mean values of advancing and receding contact angles at equivalent droplet volumes of 4 μl and 8 droplets for each surface.

2.3. hMSCs culture and staining

Bone marrow samples from healthy donors were provided by the Hospital Universitario La Princesa (Madrid, Spain) and collected as previously described for biomaterial adhesion studies [13]. The cells were isolated from bone marrow, collected by centrifugation on 70% Percoll gradient and seeded at 200 000 cm^{-2} in DMEM-LG