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Stable plasma-deposited acrylic acid surfaces for cell culture applications

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Abstract

Continuous and modulated glow discharges were used to deposit thin films from acrylic acid vapors. Different deposition regimes were investigated, and their effect on chemical composition, morphology and homogeneity of the coatings, as well as on their stability in water and resistance to sterilization. Stable films were utilized in cell adhesion experiments with human fibroblasts.

Keywords: Acrylic acid; Plasma deposition; Sterilization; Cell adhesion; Fibroblasts

1. Introduction

Plasma processes allow to tune surface properties of solids with negligible effect on their bulk [1–4]. The need of improving cell/surface interaction has decisively introduced plasma techniques in the field of biomaterials; with this aim, plasma-deposited fluoropolymers [5], polyethylene oxide (PEO)-like coatings [6,41] and nitrogen-containing plasma-grafted groups [7], among other surfaces, are extensively investigated.

Native and plasma-modified polymers with oxygen-containing surface groups generally support protein and cell adhesion; an exception to this statement are PEO polymers. Efforts were run to individuate the effect of each individual group (hydroxyl, carbonyl, carboxyl, etc.) on cell adhesion/growth, but the investigation is not over yet. Such groups favor the formation, through ion–ion, ion–dipole and similar interactions, of a protein layer on surfaces exposed to biological media, which drives the behavior of cells that, soon after, come in contact with. Negative charged groups, e.g. carboxylates, have been recognized as cell adhesion promoters [8–12]. It was shown that endothelium cells strongly prefer surfaces rich with carboxylic and/or hydroxyl groups [13]. MacNeil et al. [14] studied the attachment of keratinocytes to coatings deposited from acrylic acid (AA)/octadiene plasmas, containing carboxylic and hydroxyl groups, and found a strong preference of cells for the former groups. For the same coatings, Short et al. [15] reported an optimum concentration of surface carboxyl groups, above and below which the proliferation of osteoblast-like cells fails. Ertel et al. [16], instead, have shown that the growth of bovine aortic endothelial cells correlates with the density of carbonyl groups.

Limited information is available on the morphology of plasma deposited AA (pdAA) coatings [17]. This property becomes important when such functional layers are requested on geometrical/random nanopatterned substrates [18] to alter their surface chemistry and, at the same time, preserve their morphology. In these cases, extremely thin, conformal and homogeneous coatings are needed.

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Significant fragmentation of the feed generally occurs in glow discharges, as a result a wide range of functional groups appear in the coating [19,20]. The fragmentation can be minimized by carefully tuning parameters such as power input and duty cycle, to obtain a high degree of monomer structure retention in the film. In a previous work, we have shown how pdAA coatings with tunable carboxyl surface density, able to influence adhesion and spreading of keratinocytes within 24 h of culture [2], could be easily obtained in this way. Films with high carboxyl density, however, can result unstable in water due to swelling, leaching of unreacted fragments or/and de-lamination. Coatings that support cell growth for a certain time while they degrade may be attractive as biodegradable materials; more often, though, stable functional surfaces are requested, e.g. for long-term cell adhesion or for biomolecule immobilization. In any case, the stability of coatings intended for biomedical applications should be tested at least in water. Few papers deal with this topic for pdAA films [21–24].

Radiofrequency (RF, 13.56 MHz) glow discharges fed with AA vapors were used for this work, in different experimental conditions, to deposit pdAA coatings stable in water and to sterilization, are able to promote the adhesion of human fibroblasts [25].

2. Experimental

Polished silicon (Si), glass, polystyrene (PS) and polyethylenterephtalate (PET) substrates were cleaned in ethanol and dried at room temperature before being coated with pdAA.

A stainless-steel parallel plate plasma reactor was used. The upper electrode, shielded, was connected to a RF generator through a manual matching network; the lower electrode was grounded. Variable duty cycle, DC % = 100 $t_{on}/(t_{on}+t_{off})$, was used to modulate the discharge, with a period $(t_{on}+t_{off})$ of 100 ms; t_{on} and t_{off} are the pulse length and the off-time of the process. A rotary pump was used, equipped with a manual valve and a liquid nitrogen trap. The pressure was kept at 150 mTorr in all experiments. AA (99%, Sigma-Aldrich) was used after freeze-thaw degas cycles. An Ar/AA vapor blend (20/3 sccm; AA fed from a glass tank with a needle valve) was used to feed the discharges through an orifice in the RF electrode.

The substrates were always positioned at the ground electrode, and pdAA-coated in two very different fragmentation regimes, to deposit and compare coatings with a very different surface density of carboxylic groups. Low fragmentation conditions (20 W; 3% DC) led to coatings with a high carboxyl surface density (h-pdAA) [2]; a much lower density was obtained (l-pdAA) under more severe conditions (100 W, CW). Short deposition times were used (h-pdAA 1 min; l-pdAA 5 min), to investigate the early stage of growth of the coatings and obtain very thin layers. This approach allows to deposit conformal coatings of predefined thickness and composition on surfaces with nanometric morphological features [26,27].

Substrates were pre-treated in ammonia plasma to improve film/substrate interactions and obtaining homogeneous coatings. Such treatments, known for enhancing the adhesion of metals and polyelectrolytes on polymers [28–30], were performed in another plasma reactor (glass, tubular, internal parallel stainless steel electrodes; 13.56 MHz; CW 20 W; 10 sccm NH_3; 200 mTorr; 1 min) [31].

X-ray Photoelectron Spectroscopy (XPS) was performed with a PHI 5300 ESCA instrument with non-monochromatized AlKα radiation. Wide scan (0–1000 eV Binding Energy, BE) and high-resolution (C1s, O1s, N1s, Si2p) spectra were acquired at an electron take-off angle of 45° within 1 h after pdAA deposition. The hydrocarbon peak component in the C1s spectra was set at 285.0 eV to correct sample charging. C1s spectra were best fitted with four peaks corresponding to C-atoms with zero, one, two and three carbon–oxygen bonds: C0 (C–H, C–C; BE = 285.0 eV, reference), C1 (C–OH, C–O–C; BE = 286.6 ± 0.2 eV), C2 (O–C–O, C=O; BE = 288.2 ± 0.2 eV) and C3 (COOH, COOR; BE = 289.1 ± 0.2 eV). The best fitting was performed with a FWHM value of 2.00 eV for all components. Standard deviations were calculated on five replicates. Static water contact angle (WCA) measurements were carried out soon after each deposition, with a Ramé-Hart NRL goniometer (bidistilled water, 2 μl drops, room temperature). WCAs were measured on five different points of three replicates. The thickness of the coatings was measured with an Alpha-Step® 500 (KLA TENCOR) profiler. Their morphology was probed with an Autoprobe CP-Research scanning probe microscope, in contact mode atomic force microscopy (c-AFM; commercial Si_3N_4 tips).

To evaluate the effect of water (leach of soluble components, restructuring, de-lamination), duplicate pdAA samples were analyzed with XPS, WCA and AFM before and after soaking, from 50 h up to 3 months, in 7 ml of bidistilled water followed by overnight drying at room temperature. The effects of sterilization with absolute ethanol and autoclaving (120 °C, 20 min) were also evaluated.

The density of carboxyl groups per pdAA unit area was investigated with O-Toluidine Blue (Sigma-Aldrich), a basic dye [32–35]. The coatings were exposed to a water solution of the dye (5×10^{-4} M; pH 10; 30 °C; 5 h), then rinsed (NaOH 10^{-4} M) to remove unreacted molecules. Reacted Toluidine was then unbound from the pdAA carboxyl groups with a 50% v/v acetic acid solution, and dosed by colorimetry (absorbance at 633 nm). It was assumed that O-Toluidine Blue was