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ScienceDirect
Biomaterials 28 (2007) 2932–2945
Biomaterials
www.elsevier.com/locate/biomaterials

Behaviour of SH-SY5Y neuroblastoma cell line grown in different media and on different chemically modified substrates

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Received 30 November 2006; accepted 16 February 2007
Available online 27 March 2007

Abstract

Among the parameters that can be tested in experiments on neuronal cell culture the use of different culture media and substrates represents a powerful assay to influence cell adhesion and differentiation. In this work, plasma-enhanced-chemical vapour depositions (PE-CVD) from acrylic acid and allylamine vapours have been performed to deposit coatings bearing oxygen (O)- and nitrogen (N)-containing functional groups on polyethylenetherephtalate (PET) surface. Human neuroblastoma SH-SY5Y cells were grown on plasma modified substrates and in presence of media containing different amount of fetal calf serum (FCS) or in serum-free medium containing cAMP. Our results showed that N-containing substrates improved cell adhesion, while the neurites sprouting was influenced by cell culture media. Interestingly, the presence of carboxylic groups on the modified surface can influence the expression of a differentiation marker, neurofilament-200 (NF-H), in cells grown in serum-containing media.

Keywords: Neural cell; Surface modification; Plasma polymerisation; Cell morphology

1. Introduction

Neuronal differentiation is a fundamental event in the development of the nervous system as well as in the regeneration of damaged nervous tissue. Neurite initiation and guidance are accomplished by positive (permissive or attractive) and/or negative (inhibitory or repulsive) signals that may arise from the contact with extra cellular matrix (ECM) components or with the surface of other cells and by interactions with diffusible cell ligands [1–7]. When cells are cultivated in vitro, their adhesion, proliferation and differentiation depend on both chemical and topographical cues arising from the substrate and on cell culture media influence. The surface functionalities densities [8–9], their spatial distribution [10–11], as well as their conformation [12], charge [13] and presence of hydrophilic and hydrophobic domains [14] have shown to be important cues in affecting cell behaviour.

A number of different physical and chemical techniques have been developed to modify the surface of materials in order to influence cell–surface interactions in vitro [15]. Primary and neuronal cell line have been cultured on different synthetic polymers. Micropatterned polystyrene (PS) substrates, on which laminin has been covalently bound, showed a selective enhancement of neuronal differentiation and neurite alignment [16]. Random copolymers of oligoethyleneglycol methacrylate and methacrylic acid [poly(OEGMA-*co*-MA)], printed on standard tissue culture dishes, guided neurite extension of SH-SY5Y neuroblastoma cell line [17]. Micropatterns and gradients of poly(acrylic acid) associated to gradients of laminin enhanced the neurite outgrowth of PC12 cells, while the carboxyl groups affected negatively PC12 and C17.2 cell adhesion and neurite outgrowth [18,19]. Allylamine (AAm) pulsed-plasma-polymerised films on

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doi:10.1016/j.biomaterials.2007.02.022

hydrophobic polysiloxane substrate allowed the adhesion of neuronal cells with no need of polylysine adsorption [20].

It has also been extensively shown that the composition of cell culture medium, i.e. the presence of particular "active" molecules, is of outmost importance in neuronal differentiation in vitro. Low serum-containing media, retinoic acid or nerve growth factor treatments, respectively induce C-1300, P19, NT2 and PC12 neuronal cell line differentiation [21–26]. In serum-free medium and in the presence of various differentiation factors, SH-SY5Y human neuroblastoma cell line [27] acquire a neuronal phenotype and exhibit long neuritic processes. They respond to retinoic acid [28,29], brain-derived neurotrophic factor (BDNF) [29] and cAMP [30–32] by forming a complex network of neuritic prolongements and expressing neuronal markers, including low molecular weight isoform of the tau protein, neurofilaments, neuron specific enolase and growth-associated protein (GAP-43) [29–33].

Plasma-aided processes are often used to deposit functional coatings onto otherwise inert polymers like PS and PET [34–36]. By means of PECVD it is possible to obtain coatings that exhibit a tenacious adhesion to the substrate [34]. Hydrophilic plasma-deposited coatings with verifiable surface density of COOH/R groups [37–42] or NH_2 groups [42–44] have been investigated as cell adhesive layers. Efficient amino-functionalisation (i.e. a high density of amino-groups) is achieved when AAm is used as gas feed [43]. On the other hand, acrylic acid (AA) is generally used to obtain carboxylate surfaces due to the presence of a COOH group in its chemical structure [40]. Unsaturated "monomers", like AA and AAm, are preferred to saturated ones since the presence of a double bond (C = C) in the "monomer" has a positive effect also on the deposition rate of the coating which results in fast surface modification processes [37], as well as in a high retention of the chemical structure of the monomer.

This study was therefore carried out to investigate adhesion and differentiation levels of SH-SY5Y neuroblastoma cell line, treated with culture media containing 2% or 10% serum or cAMP (1 mM), and grown onto plasma-modified PET by mean of PE-CVD. For this purpose AAm and AA were used as plasma gas feed at a power value of 50 and 150 W. Four different coatings, characterized by nitrogen (N)- and oxygen (O)-containing groups, were obtained. The amount of elemental composition and of functional groups in the coatings were studied by X-ray Photoelectron Spectroscopy (XPS) analysis and differences in terms of hydrophilic nature was determined by means of Water Contact Angle (WCA) analyses. The area of substrate covered by adhering cells, the length of neurites and the expression of neurofilament-200 (NF-H) were evaluated in cells grown onto PET coated with chemically different plasma-deposited functional coatings and in different culture media.

2. Materials and methods

2.1. PET samples and cell culture dishes

Ten millimeters diameter PET (Goodfellow, UK) circular samples were sonicated for 30 min with ethanol before chemical deposition and when used as control substrates for cell culture experiments. Cell behaviour on PET and PET-modified substrates was always compared with that on the 24 well plates usually used for cell culture experiments (cell culture PS, CCPS) (IWAKY, UK).

2.2. PE-CVD of a coating from AA and AAm

Depositions were carried out in a stainless steel parallel plate vacuum chamber (Fig. 1). An inner parallel plate configuration was used: an upper electrode powered by a 13.56 MHz RF source through a matching network and a grounded lower electrode serving as sample holder. AA and AAm (Aldrich Chemical Co.) were the "monomers" used along with Argon (Ar) as carrier gas [43,44].

The vapours of the "monomers" (AA or AAm) were delivered to the reactor through calibrated needle valves and mixed with Ar. The gas feed flowed through 2 separated holes (1 mm in diameter) in the middle of the RF electrode. This configuration was used to avoid occurrence of any contaminations due to different monomers during plasma deposition when one of them was used. Pressure was maintained at 100 mTorr and power values of 50 and 150 W were delivered during discharges. The flow rate was fixed at 4 sccm (standard cubic centimeters per minute) for the monomers and at 5 sccm for Ar.

Four different coatings were obtained: pdAA50W, pdAA150W, pdAAm50W and pdAAm150W, where pd stands for plasma deposited. The thicknesses of the deposited films, measured by means of an ALPHA STEP500 KLA-TENCOR profilometer, were of 60 ± 2 nm for all the discharges considered.

2.3. X-ray photoelectron spectroscopy (XPS) measurement

XPS measurements were performed with a Theta Probe Thermo VG Scientific instrument (base pressure 1×10^{-10} mbar) equipped with a monochromatic AlKα radiation ($h\nu$: 1486.6 eV) operating at 300 W. The analyses were carried out at a 53° take-off angle, corresponding to ~10 nm of sampling depth, by means of a 200 μm wide X-ray spot. Samples were neutralised for the electrostatic charging by means of a flood gun (Mod. 822-06 FG) operating at 1 eV and 2×10^{-7} mbar to minimise the risk of differential or non-uniform charging. The high-resolution spectra were shifted to their correct position by taking C1s spectrum centred at 285.0 eV [45–47]. The hydrocarbon peak, C0, was fixed at 285.0 eV; the next peak, CN, with a contribution of amine C–N, at 285.9 eV. The peak C1 at 286.5 eV is due to C–O, from alcohol and ether, and nitrile groups; imine groups, C = N, may also contribute to this peak, although there are no reliable literature values for this functional group. Carbonyls and amides contribute to the peak C2 at 287.8 eV, the peak C3 at 289.2 eV is due to carboxylic and ester groups.

2.4. Water contact angle (WCA) measurement

Static WCA (2 μl drops of double-distilled water) measurements were performed with a Ramé-Hart A-100 goniometer to probe the hydrophobic character of the substrates used in cell culture experiments. The sessile drop was deposited onto the surface with a manual microsyringe. Each value is the mean ± SD of 5 measures on the same sample.

2.5. Cell culture

The SH-SY5Y human neuroblastoma cell lines (courtesy of G. Cibelli, University of Foggia, Italy) were routinely grown in Dulbecco's modified