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Orientation and confinement of cells on chemically patterned polystyrene surfaces

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Abstract

UV/ozone oxidation was combined with a photomasking technique to produce adjacent regions of different chemistry on polystyrene (PS) surfaces. The surface chemistry and topography were studied using AFM, XPS and contact angle measurements. The physicochemical patterns were visualised by the condensation of water vapour upon the surfaces and by the differential attachment of Chinese hamster ovarian (CHO) cells. The orientation of CHO cells on 55 and 125 μm wide oxidised PS strips were measured and found to be highly dependent on the width of the oxidised feature. CHO cells in relatively close proximity to a linear polar/non-polar border showed significant axial alignment along the border. CHO cells can also be confined to specific regions of the polymer surface. Cells attached to larger areas (75 μm × 75 μm) were found to have a smaller average cell size than cells attached to the smaller (56 μm × 56 μm) areas.

Keywords: UV/ozone; Cell culture; XPS; Water contact angle; Spatial control of cells

1. Introduction

Surfaces are now being designed at the molecular level to not only facilitate the attachment of different cell types but also spatially direct their growth [1]. Numerous bio-molecular electronic devices have been developed that have a number of practical applications [2]. For example, photolithographic techniques have been used to confine neurons to highly defined patterns and enhance their responsiveness in culture and demonstrate their potential applicability to biosensor and drug screening development [3]. UV/ozone modification is widely used to improve cellular attachment to polymer surfaces [4–10] and in particular PS, which does not support cell attachment unless pre-coated with adhesive serum proteins [8]. This modification technique combined with a photomask has only recently been used as a means of introducing chemical heterogeneity to a surface [11–13].

There are many possible applications of devices employing this technology in the study of cell behaviour, organisation and cellular interactions, as well as in the construction of novel biomedical devices. Such applications include medical implants for wound [14,15] or fracture healing [16,17], drug delivery systems [18,19], dental implants, artificial prosthesis [20–22], biosensors [23,24] and biochips [25,26].

Surfaces with micro- and nano-structured features can control biological activity (i.e. adhesion, proliferation and differentiation) if those surface features are of a similar size to the biological moieties they interact with [27–31]. Modification techniques such as micro-contact printing that produce micro- and nanometre sized domains on the surface of materials have been thoroughly investigated by several research groups [27–31]. Since cells and other biological systems react to chemical and topographical stimuli through specific receptors, micro- and nano-structured materials can be used for studies of cell physiology and function [32–34]. These include the influence of surface morphology and chemical composition on the adhesion and growth of specific cells and the ability to spatially control the growth of cells, which may ultimately permit the regeneration of biological tissue [35]. The presence of micro- or nano-structures on a surface allows the control and manipulation of two important external signals: the cell–substrate (adsorbed protein layer) [35] and cell–cell interactions [36].

A number of studies have reported that contact guidance can promote the alignment of cells on substrate grooves and

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ridges [32–34,37]. Methods that influence cellular orientation or morphology may be used to enhance the secretion of extracellular matrix and overcome the inhibitory nature of the scarring response at a site of injury and provide a pathway for regenerating neurones and other applications [37–39].

In this study, we utilise a UV/ozone treatment to modify polystyrene (PS) surfaces to produce chemically patterned surfaces that allow us to study the behaviour of cells when cultured on surfaces with heterogeneous surface properties. UV/ozone is an attractive alternative to other methods of surface modification as it does not require vacuum apparatus, sophisticated gas or liquid handling and has been shown to have controllability similar to oxygen plasma treatments [4,7–9] and is suitable for point-of-use by clinicians.

2. Experimental

2.1. UV/ozone treatment

Untreated polystyrene dishes (Nunc, Denmark) were used as-received in this study. The polystyrene dishes were treated in a commercial UV/ozone unit, described in more detail elsewhere [40]. The UV/ozone treatment unit consists of a low-pressure mercury vapour lamp that emits strongly at 184.9 and 253.7 nm wavelengths. These wavelengths are known to generate reactive oxygen species such as ozone and to photosensitise polymer surfaces. Before use the lamp was preheated for approximately 1 h and all surfaces were treated at 3 cm distance. Before cell culture, it was necessary to remove any low molecular weight photolysis products present on the PS surface [41]. This was done by washing with ~2 ml of doubly distilled water with gentle agitation on a rocking platform for 60 min. The dishes were dried for 60 min under ambient laboratory conditions before use.

2.2. Analysis of surfaces

Contact angles were measured using 20 μl drops of doubly distilled water placed on UV/ozone treated polystyrene dishes. No measurements were taken from unwashed surfaces due to dissolution of low molecular weight oxidised material influencing the measured wettability [41–43]. All contact angle measurements were made on initial contact between water and surface to avoid absorption and consequent swelling of the oxidised polystyrene. Surface energies were calculated from contact angles obtained from water and di-iodomethane using the Owens and Wendt equation [44]. Owens and Wendt proposed the division of the total surface energy of a liquid or a solid into two components: a dispersive force component and a polar component. By assuming that the polar interactions between the liquid and solid could be approximated by a geometric mean expression they obtained the following relationship:

$$\sigma_{sl} = \sigma_s + \sigma_l - 2(\sigma_s^d \sigma_{lv}^d)^{1/2} - 2(\sigma_s^p \sigma_{lv}^p)^{1/2} \quad (1)$$

where σ_l is the liquid surface tension and σ_s is the solid surface tension, or free energy. The 'd' and 'p' in the superscript refer to the respective dispersive and polar components. The 's' and '1' in the subscript denote the solid and liquid phases, respectively. Combining Eq. (1) with the Young–Dupré equation leads to:

$$\sigma_{lv}(1 + \cos\theta) = 2(\sigma_s^d \sigma_{lv}^d)^{1/2} + 2(\sigma_s^p \sigma_{lv}^p)^{1/2} \quad (2)$$

Thus, from measurements of the contact angles of two different liquids it is possible to determine the polar and dispersive components of the surface free energy. An estimate of the surface free energy can be obtained from the sum of these two components. All contact angles and surfaces energies were obtained using a First Ten Ångstrom contact angle analyser running FTA32_Video software Version 2.0, Build No. 160.

The surface chemistry of the UV/ozone treated polystyrene dishes were studied before and after washing by monochromated X-ray photoelectron spectroscopy. The experimental parameters used in this study are described in detail elsewhere [12]. Spectra were obtained with a constant pass energy of 20 eV. Charge neutralisation of the insulting polymer surfaces was achieved using a low energy electron flood gun. The instrument was operated in so-called hybrid mode, which uses a combination of electrostatic and magnetic lenses. The elemental compositions were calculated from peak areas obtained from the narrow spectra after subtraction of a linear background. The XPS quantification results presented in this paper are mean values of at least three repeat measurements and are given with the standard deviation of those measurements allowing accurate intercomparison of sample surfaces. Peak positions were corrected to the hydrocarbon signal at 285.0 eV. The carbon 1 s peak envelope was deconvolved into five Gaussian components: hydrocarbon (C–C, C–H) at 285.0 eV, alcohol/ether (C–O) at a shift of +1.5 eV, carbonyl (C=O) at a shift of +3.0 eV, carboxylic acid or ester (COOH/R) at a shift of +4.5 eV and π–π^* shake-up at a shift of ~7 eV [45,46].

FTIR spectroscopy was used to study the effect of UV/ozone treatment on free-standing polystyrene films. The films were produced by spin coating a clean silicon wafer with a solution of polystyrene in chloroform. After the spin-coated films dried they were detached from the wafer by soaking in water and affixed to a supporting frame and dried in ambient conditions for approximately 1 h prior to FTIR analysis. Transmission FTIR measurements were performed on a Perkin-Elmer Spectrum GX FTIR system with a resolution of 8 cm^{-1}, averaging over 25 scans. All FTIR measurements on oxidised surfaces were performed within 1 h of UV/ozone treatment.

Topographical changes resulting from the UV/ozone treatment were studied using a Digital Instruments (DI) Nanoscope IIIa SPM under ambient conditions. The surfaces were imaged in contact mode using silicon nitride tips. RMS roughness (R_q) for each sample was from at least three sample areas of 20 μm × 20 μm areas, the mean roughness RMS and SD reported.

2.3. Production of chemical micro-patterns

The patterning technique used to chemically micro-pattern the PS surface has been described previously [12]. Briefly, the surfaces were chemically patterned by placing a copper