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Assessing embryonic stem cell response to surface chemistry using plasma polymer gradients

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ABSTRACT

The control of cell–material interactions is the key to a broad range of biomedical interactions. Gradient surfaces have recently been established as tools allowing the high-throughput screening and optimization of these interactions. In this paper, we show that plasma polymer gradients can reveal the subtle influence of surface chemistry on embryonic stem cell behavior and probe the mechanisms by which this occurs. Lateral gradients of surface chemistry were generated by plasma polymerization of diethylene glycol dimethyl ether on top of a substrate coated with an acrylic acid plasma polymer using a tilted slide as a mask. Gradient surfaces were characterized by X-ray photoelectron spectroscopy, infrared microscopy mapping and profilometry. By changing the plasma polymerization time, the gradient profile could be easily manipulated. To demonstrate the utility of these surfaces for the screening of cell–material interactions, we studied the response of mouse embryonic stem (ES) cells to these gradients and compared the performance of different plasma polymerization times during gradient fabrication. We observed a strong correlation between surface chemistry and cell attachment, colony size and retention of stem cell markers. Cell adhesion and colony formation showed striking differences on gradients with different plasma polymer deposition times. Deposition time influenced the depth of the plasma film deposited and the relative position of surface functional group density on the substrate, but not the range of plasma-generated species.

1. Introduction

The cellular response to biomaterial surfaces, such as attachment and proliferation, is known to be mediated by the material's surface chemistry. Indeed, surface chemistry is also able to instruct cell function and direct cell differentiation [1–10]. However, it has proven difficult to predict cell fate outcomes based on the molecular structure of the polymer substrate [11,12]. The advent of high-throughput screening techniques including cell microarrays and surface-bound gradients has precipitated a paradigm shift in the field [13–17]. Surface chemistry gradients are a format that is particularly well suited to study subtleties in cell response to surface chemistry. The format permits one variable, such as functional group density, to be continuously changed with respect to the position on a test surface whilst other parameters are held constant [18–22]. These platforms also require significantly lower cell numbers, lower quantities of culture medium and sample materials than ad hoc testing of surface chemical properties with discrete samples.

Plasma polymerization lends itself to surface gradient formation, since it supports the introduction of a wide range of surface chemistries and forms well-adherent layers on a range of substrates [23–25]. The technique also allows surfaces to be modified independently of the underlying substrate, with little change to surface topography. Several methods using plasma polymerization to create gradients of surface functionality have been described, such as changing the input feed stock while a mask occluding part of the substrate is moved concurrently, or using a knife edge electrode, resulting in non-uniform plasma glow discharge [26–29]. Whilst these techniques are effective in depositing plasma polymer gradients, sophisticated and expensive reactors or permanent modifications are required. The diffusion of monomer underneath a solid mask can also be exploited to create plasma polymer gradients [30,31]. The distance of the mask from the substrate can control the depth of the plasma film and the slope of the gradient [31,32]. Furthermore, plasma polymerization time can be varied to influence the depth of the plasma film deposited. Such a diffusion-based gradient deposition process can be implemented in almost any reactor setup for negligible cost.

Recent work on carboxylic acid plasma polymer (octadiene-acrylic acid) gradient surfaces demonstrated a relationship between retention of "stemness" in mouse embryonic stem (ES) cells and

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