Cell Adhesion Guidance by Plasma Polymer Gradients

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Aim: Plasma polymerization has recently been shown to be a surface modification technique that is well suited for the control of cell adhesion in porous tissue engineering scaffolds.[1] Penetration of the plasma through a porous object is diffusion limited, resulting in a radial graduation in the polymer deposition rate. To facilitate more detailed study of the influence of such plasma polymer thickness gradients, planar samples were prepared and their surface properties related to cellular response.

Methods: Plasma polymer gradients were deposited on allylamine coated glass samples by diffusion from a hexane plasma under a fixed mask. The length of the gradient transition was controlled by the separation between the mask and the sample. The surface chemistry was studied by XPS and pico litre droplet water contact angle (WCA) and atomic force microscopy (AFM) was used to characterize the surface topography. Quartz crystal microbalance (QCM) experiments were carried out to model protein adsorption from serum using albumin and fibronectin. The cell response was tested with NIH 3T3 murine fibroblasts.

Results/Discussion: A steep and a shallow gradient of plasma polymerized hexane (ppHex) on plasma polymerized allylamine (ppAAm) were prepared with a wettability transition of 0.5 and 5 mm, respectively (Figure 1). The WCA stretches over a range of 30°, with the far ends of the transition corresponding to plain ppHex (85°) and ppAAm (50°). XPS confirms this is caused by the gradual increase in ppHex overlayer thickness on the more hydrophilic ppAAm surface.

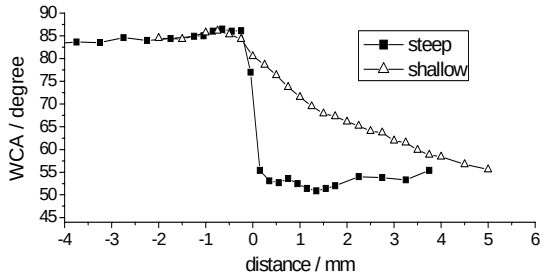

Figure 1 Pico-litre water contact angles on along the transition zone of the plasma polymer gradient.

The surface roughness of the samples was determined from AFM images and was found to be low and homogenous (rms < 0.8 nm) over the whole length of the gradients. Cell culture experiments on these samples showed that the cell density variation correlates with the wettability gradient, increasing from the ppHex to the ppAAm surface (Figure 2).

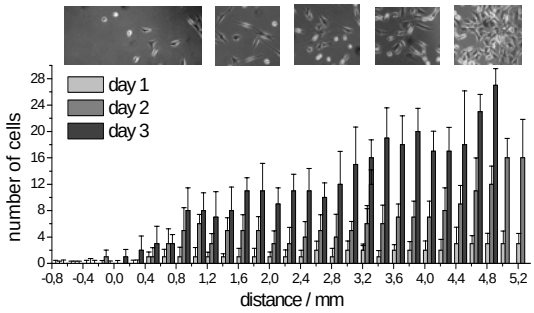

Figure 2 3T3 cell distribution along the shallow gradient. Initial adhesion occurred during day 1, after which the media was changed and proliferation was predominant.

Model protein adsorption experiments were carried out with cell-adhesive fibronectin and non-cell adhesive albumin on individual ppHex and ppAAm samples. When exposed to the surfaces separately, both proteins adsorbed in significant quantities (Table 1). In serum containing tissue culture media, however, the small and abundant albumin will adsorb first, possibly followed by a slower replacement of albumin by more massive proteins like fibronectin (Vroman effect). [2] Exposure of surfaces with adsorbed albumin to fibronectin showed that albumin strongly adheres to ppHex, so that only a small mass change could be observed (m_d). In contrast, a significant change in mass was observed on ppAAm, suggesting that albumin is more readily displaced by fibronectin on this surface. This offers a possible explanation for the different cell adhesion observed on ppHex and ppAAm.

Table 1 Adsorbed mass of albumin (m_A), fibronectin (m_F) and the measured mass change after displacement of albumin by fibronctin (m_d) on the ppHex and ppAAm.

	m_A / ng cm^{-2}	m_F / ng cm^{-2}	m_d / ng cm^{-2}
ppHex	230	80	5
ppAAm	275	105	45

Conclusions: The preparation of plasma polymer gradients by a diffusion controlled technique has been successfully employed to prepare wettability gradients of varying lengths and low surface roughness on flat glass substrates. 3T3 fibroblast cell response was shown to correlate to the gradient chemistry, with higher cell densities observed on ppAAm. Based on QCM data it is suggested that the cell response correlates to the nature of the proteins adsorbed on the surface which is mainly determined by the strength of albumin binding to the surface.

References:

[1] Barry JJ. Adv. Mater. 2006;18;1406-1410.
[2] L. Vroman, J. Colloid Interface Sci. 1986;111;391-402.