

Surface Chemical Gradients to Optimise Substrata for Self-Renewal of ES Cells

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INTRODUCTION: Various reports detail how the culture conditions for mouse ES (mES) and human (hES) may be manipulated to maintain these cells in an undifferentiated state.^{1,2} Significant differences between mES and hES cells have been commented upon, as well as common mechanisms in maintaining self-renewal. It has been recently shown³ that the self-renewal of mES and hES cells can be promoted by restricting the degree to which these cells spread. This result implies that the self-renewal mES and hES cells can occur when their spreading is restricted by culture on weakly adhesive substrates. Herein, we show how using surface chemical gradients, of varying carboxylic acid density, an optimal chemistry is readily identified whereby cells can be maintained in compact small colonies, retaining cell-cell contact, without loss of the key ES cell markers alkaline phosphatase and Oct-4.

METHODS: Surface chemical gradients of carboxylic acid were fabricated by means of plasma deposition on 13 mm plastic coverslips.⁴ High functional group retention was achieved by the use of low plasma power. Exact control was maintained over the start and endpoints of gradients, and a batch of identical gradients was produced for this study. X-ray photoelectron spectroscopy (Kratos Ultra) with a monochromated x-ray beam was used to obtain line-scan spectra along the length of the gradient, from which the carboxylic acid gradient was reconstructed, and optimal acid density and spacing of acid groups calculated. mES and hES were seeded on chemical gradients and after 7 days, were examined by optical microscopy and stained for AP activity and Oct-4.

RESULTS: Fig. 1 shows hES on a surface chemical gradient of carboxylic acid. The cells were maintained in serum free media (Advanced media, Invitrogen). At the top end of the coverslip, where the surface is purely hydrocarbon, cells have failed to attach. Attachment occurs at the mid point, where the surface comprises a mixture of carboxylic acid and hydrocarbon. These cells have

formed compact colonies and retain tight cell-cell contact. At the bottom of the coverslip, cells are much more fully spread, and have reduced cell-cell contact. Cells at the mid-point have stained strongly for AP, whilst at the bottom the cells do not stain strongly. hES and mES cells have been cultured on homogeneous surfaces with the chemistries of the mid-point and various positions towards the bottom of the coverslip. Using stains AP and Oct-4, it is shown that on surfaces of increasing surface acid density, cells spread and there is loss of expression of AP and Oct-4.

Fig. 1: hES cells on a surface chemical gradient of varying acid density. At top, 0% carboxylic acids, at mid-point ca. 8%, towards the bottom ca. 12%. Cells stained for AP.



DISCUSSION & CONCLUSIONS: These preliminary results are strongly suggestive that the capacity of ES cells for self-renewal may be maintained by surface chemistry alone. If true, this has the important implication that geometric control (ie control over cells spreading) is an important factor in the maintenance of self-renewal. Surface chemical gradients are an ideal tool for rapid (high throughput) screening.

REFERENCES: ¹ A.G. Smith, et al, *Nature* (1988) **336**, 688-690 ² T Burdon et al. *Trends Cell Biol.*,(2002) **12**, 432-38 ³ Murray P et al, in preparation ⁴ J D Whittle et al. *Chem. Comm.*, (2003) **14**; 1766