

NANOSTRUCTURE INFLUENCES CELL ALIGNMENT AND MORPHOLOGY

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INTRODUCTION: The interaction of cells with surfaces has been a subject of interest over a number of years. The effect of topography has been widely studied and in particular the alignment of cells to micro and nanoscale steps and grooves is well known. Although there are a number of proposed mechanisms for this alignment no clear picture remains, especially for alignment to nanometer step edges. In this work we address the importance of the sharp edge of the step in the alignment process. Nano and microfabrication have been used to define a range of model surfaces which have step edges which are continuous or discontinuous.

METHODS: A combination of photolithography and colloidal lithography have been used to define a range of model substrates. Silicon wafer chips were coated with thin films of oxidized titanium to ensure that the surfaces had homogeneous surface chemistry. Grooved samples were fabricated using photolithography. The exposed wafers were coated with titanium before lift-off processing, the groove/ridge width was 15 μ m, the groove depth was varied from 40 to 400nm. Nanostructured samples were fabricated by colloidal lithography [1], polystyrene particles were adsorbed to surfaces and subsequently coated in titanium films by electron beam induced evaporation. Some nanostructured samples were fabricated from flat substrates and some from grooved substrates. All samples were oxidized in a reactive ion etcher (O₂ 0.5Torr 200w 120s) and treated with UV/ozone for 20 minutes before cell culture. The dimensions of the grooves were determined with a profilometer and scanning electron microscope (SEM). SEM images of the nanostructured surfaces were analysed with scion image software.

Mouse mammary gland epithelial cells (HC11) were cultured on the substrates in RPMI 1640 medium containing 10% FCS and 1% PEST for 10h and 24h at 37°C. At 10h and 24h the cells were fixed and stained for nuclei and actin (cytoskeleton). Images were taken using a fluorescence microscope at 40X mag. Digital image analysis was used to classify cells as 'round', 'rectangular' or 'spool' shaped. The classification was based on training groups including the parameters elongation, dispersion and form C. The

alignment of the cells was also measured. Only single cells were analysed.

RESULTS: The morphology and alignment of cells was strongly affected by the surface topography presented to them. The effects were more visible after 10 hours of culture compared to 24 hours. The shape of the cells was systematically dependent on the size of nanoscale features (nanostructure size or groove depth). Alignment of cells correlated to shape and to nanometer surface structure. Alignment of cells to grooves with continuous edges was both higher and more persistent over time than for cells on grooves with discontinuous step edges.

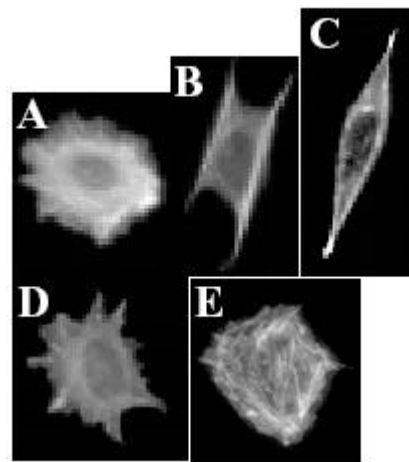


Fig. 1: The cells marked A,B,C are examples of cells classified as 'round', 'rectangular' and 'spool' respectively. The cells marked D and E are examples of cells which remain unclassified. Cell C is around 30 microns long

DISCUSSION & CONCLUSIONS: The alignment of cells to nanometer deep (40-400nm) grooves appears to depend on the presence of a 'continuous' step edge on the nanometer scale. The morphology of cells on 'discontinuous' topographically structured surfaces is different from cells on 'continuous' flat controls and is systematically effected by the scale of the topographic structure.

REFERENCES: ¹ P.Hanarp, D.Sutherland, J. Gold and B. Kasemo (1999) *Nanostr. Mat.* **1-4**:429-432.

ACKNOWLEDGEMENTS: This work has been funded by the SSF foundation in Sweden under the biocompatible materials programme.