Nanotopography for cell- and tissue engineering

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Deliberately micro- and nanostructured interfaces for biology and medicine have attracted much interest over the last 15 years. The manufacturing technologies developed for the fabrication of integrated circuits have readily been adopted for the creation of well defined topographically, chemically, or mechanically patterned interfaces[1]. These patterns are of interest for the tight control of cell/material interfaces in the areas of biosensors, implant, and cell biology and the methods applied have benefited from the drive in the electronics industry for ever-closer integration of features on integrated circuits. In the following I would like to concentrate on the fabrication of topographic patterns for various biomedical applications. We concentrate here on surface topographies as there have been a large number of papers suggesting that nano- and microstructured surfaces have useful effects on cells (i.e. to elongate, guide, control adhesion, and differentiate). Further points in favour of topography are that the underlying principle is based on surface physics/mechanics; topography has permanence, and is "easy" to fabricate. As biomedical applications are not uniform in their needs, the patterns required are not standardised . Therefore, the methods for the primary pattern definition at the submicron level vary considerably: phase separation of binary polymer mixes, or block-copolymers, which form tunable, but random patterns and colloidal lithography, holographic, and x-ray lithography as well as electron beam lithography have been applied. As the fabrication of primary patterns is usually time consuming and often expensive, a wide range of replication techniques were employed to create the number of topographic samples required for biological tests, see Figure 1 for further detail. We applied imprint technologies to replicate e-beam defined features in polymers[2] In earlier experiments we observed that a wide variety of cells responded to microtopography, and even more so to patterned adhesive chemistry[1].. We have developed techniques which allow the "fast" fabrication of e-beam defined regularly nanofeatures [2]. When the dimensions of pits or pillars on surfaces were reduced to submicrometric levels, various cell types (fibroblasts, epithelia, bone) responded by exhibiting a significantly reduced tendency to adhere and spread . These kind of features are known to influence the physicochemical surface properties of materials, and we investigated the changes in surface energy on nanopatterned hydrophilic, and hydrophobic model materials, and polymer imprints. The results showed that nanopatterning increases hydrophobocity, if the material had a water contact angle of around 70º, and that the same features made hydrophilic materials superhydrophilic[3]. This lead us to hypothesise, that the reduced adhesion, and proliferation of cells on these surfaces could be due to surface trapped air. As the most effective patterns had to be defined by e-beam, but cells live in a 3-dimensional environment we needed to develop methods to take the most effective nano-patterns and incorporate them into 3-D. The method developed is based on supported thin silicone membranes, which bend during embossing, to accommodate the viscous flow of the polymer, see Fig 2.

We have shown, that cells respond to topographic, chemical, and mechanical patterns of both micro- and nanometric size, with changes in cytoskeletal arrangement, behaviour, and gene expression. Although we are trying to establish the underlying physical principles of the cell surface interaction, there is so far not enough evidence to support a generalised theory which would allow the prediction of a cells reaction simply based on the surface design.

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Figures:

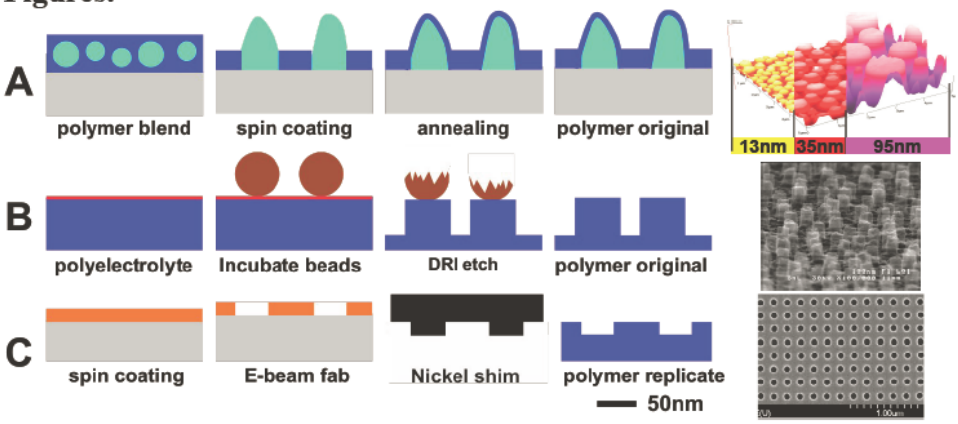

Figure 1: Various fabrication processes useful for the creation of bio-interfaces. A binary polymer blends. B Colloidal lithography. C electron beam lithography.

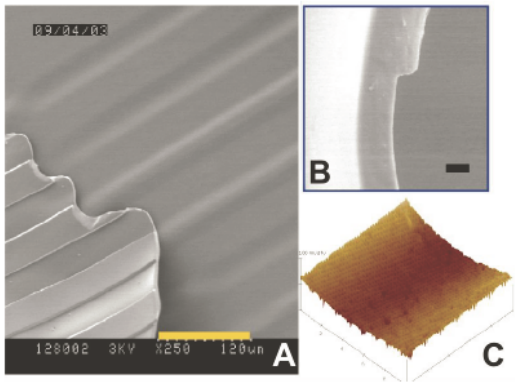

Figure 2: nano-µ embossing of nanopatterned, undulating patterns using supported nanopatterned PDMS membranes. A embossing membrane in situ. B crossection of embossing membrane (bar = 5µm). C AFM (contact) of nano-µ embossed polycarbonate x/y size 5x5µm, height, 300nm.