

## A NEW APPROACH TO MICRO AND NANO PATTERNING FOR CELL STUDIES AND PROTEOMICS APPLICATIONS

D.Falconnet, D.Pasqui, F.Assi, A.Koenig & M. Textor

Laboratory for Surface Science and Technology, Dept of Materials,  
ETH Zürich, Switzerland.

**INTRODUCTION:** Patterning of surfaces into bioadhesive and non-adhesive areas has attracted increasing interest over the past 10 years. The motivation for the production of such patterned surfaces ranges from basic investigations of cell adhesion, cell function and cell-cell interaction to cell based sensor development for drug testing. Designed scaffolds for tissue engineering also benefit from the development of these biologically designed surfaces. The common goal is to produce geometrically defined patterns that promote cell attachment in a background that is resistant to protein adsorption and cell interaction. A more recent topic of interest is the production of submicrometer patches containing proteins or bioactive ligands to study ligand-receptor activation and focal contact formation. Furthermore, in the area of microarray chips for sensing DNA/RNA (genomics) or proteins (proteomics), chemical patterns may be useful to better control spatial arrangement of recognition units and improve spot quality as well as signal to noise ratio.

**METHODS:** A simple photoresist lift-off process is exploited in conjunction with the spontaneous assembly of cationic poly(L-lysine)-grafted-poly(ethylene glycol) (PLL-g-PEG) onto negatively charged metal oxide surfaces. The process is termed molecular assembly patterning by lift-off (MAPL). A positive photoresist on a metal-oxide-coated substrate (e.g. niobium oxide) is developed resulting in a pattern of resist and bare metal oxide areas. Bio-functionalized (biotin or cell-adhesive peptide) PLL-g-PEG is adsorbed onto the pattern and immobilized at the bare metal oxide areas. The photoresist is lifted-off in an organic solvent without affecting the integrity of the adsorbed functionalized PLL-g-PEG monolayer. Subsequently the background is backfilled with protein- and cell-resistant PLL-g-PEG. Various functionalizations of the PEG chains are possible, in this study part of the PEG chains were modified either with biotin to specifically bind to streptavidin or with Arg-Gly-Asp for integrin mediated cell attachment. Pattern quality was investigated with AFM, XPS, ToF-SIMS and fluorescence microscopy.

Standard photolithography was used for structures  $\geq 1 \mu\text{m}$  and nano-hot embossing for producing sub-micron features.

**RESULTS:** Patterns of constant quality can be achieved over several centimeters with excellent contrast as shown in Fig. 1.

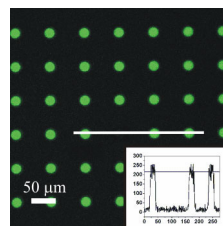


Fig. 1: PLL-g-PEG/PEG-biotin immobilized in the spots and PLL-g-PEG in the background. Fluorescent signal: alexa-488 labeled streptavidin. One spot has been bleached for comparison with the background fluorescence intensity.

The ligand surface density can be decreased by diluting with non-functionalized PLL-g-PEG. It was quantified through the measurement of the adsorbed polymer mass with the optical waveguide lightmode spectroscopy (OWLS) technique.

Exposing cells to patterns with different peptide densities is believed to be interesting for cell surface interactions studies. Pattern shape and area are also relevant parameters for a better understanding of cell mechanism.

MAPL was also successfully applied to produce nano-patterns of bioactive molecules (100 nm lines) in a non-fouling background.

**DISCUSSION & CONCLUSIONS:** MAPL is a simple technique for patterning interactive micro and nano structures in an inert background. When peptides are patterned cells attach to these ligands and not to the PLL-g-PEG background. A further potential application of MAPL are protein chips with high spot definition and homogeneity.

**ACKNOWLEDGEMENTS:** H. Schiff, S. Park, C. Padeste, M. Horisberger for providing the nano-embossed sample and metal oxide coatings. S. Pasche and F. Durmaz for the different PLL-g-PEG.