Research Highlights

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Engineering surface topologies

Soft organic surfaces with specific topologies are required for many different applications of microsystem technology, such as biochips (*e.g.* DNA arrays), biosensors, optics, and microfluidic systems. To control the surface topography, organic films are commonly adsorbed on a substrate, and structured using soft lithography or self-structuring processes. Julien Léopoldès and Pascal Damman from the Belgian University of Mons-Hainaut present a novel concept towards the three-dimensional structuring of a surface at the micrometre scale.[1] They describe the transfer of a two-dimensional pattern of a chemically structured surface with ''hydrophilic'' and ''hydrophobic'' domains to a three-dimensional topography, employing a self-driven process on the basis of selective inversion of a liquid–liquid bilayer through diffusion and nucleation/growth.

The substrate with the two-dimensional pattern is a gold-coated silica wafer, initially covered by a homogeneous monolayer of hexadecanethiol. Patterning of the surface is performed by a partial destruction of the hexadecanethiol monolayer with an ultraviolet lamp through a photomask. Chessboard or parallel line patterns with hydrophilic areas (Au surface) and hydrophobic areas (hexadecanethiol) are created. Afterwards, a hydrophobic layer (polydimethylsiloxane, PDMS) is spincoated on the pattern, and immersed in hydrophilic ethanol. Within just a few minutes, a morphological transformation can be observed on all Au patches simultaneously (Fig. 1). The trigger for destabilisation of the interface between PDMS and the Au domains is the diffusion of the ethanol through the PDMS layer. Ethanol molecules reach the substrate surface, and ethanol droplets nucleate, grow and coalesce on the Au domains, resulting in a confined replacement of PDMS (Fig. 2). After formation of the pattern, the PDMS films can be cured and lifted off the substrate. This process has no restriction regarding the shape of the Au/PDMS interface. Utilising an initial surface pattern with parallel lines of hydrophobic/hydrophilic domains, the droplets of ethanol appear first as short dashes that grow and coalesce. The final morphology is a set of hemispherical cylinders and lines of ethanol with a width that corresponds to the width of the Au domains. The authors investigated details of the process for different thicknesses of the initial PDMS layer. For thick PDMS films, ethanol droplets are formed in the PDMS layer as described above. Thin PDMS films, however, behave differently. The PDMS film self-destructs by a bursting mechanism which finally leads to a generation of PDMS droplets on each hydrophobic domain in an ethanol environment.

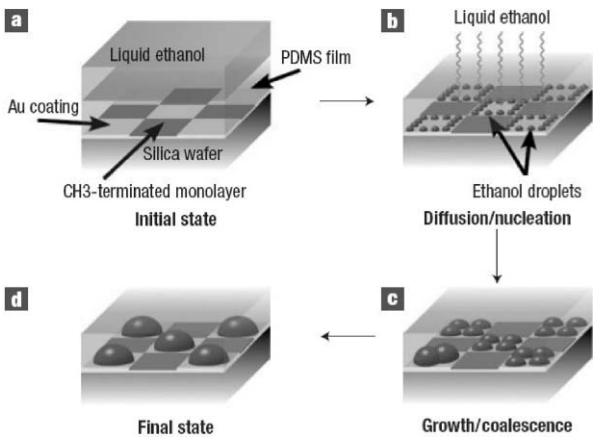

Fig. 1 Principle of the thin-film structuring. (a) Hydrophilic (Au) and hydrophobic domains (CH_3-terminated monolayer) are patterned on a silica wafer. Silicon elastomer (PDMS) is spincoated on the wafer, and immersed in ethanol. (b)–(d) After immersion, ethanol diffuses through the PDMS layer. Droplets of ethanol grow and coalesce until single ethanol droplets are located under the PDMS layer on each Au domain. (Reprinted with permission from Macmillan Publishers Ltd., *Nature*, Léopoldès *et al.*,[1] copyright 2006.).

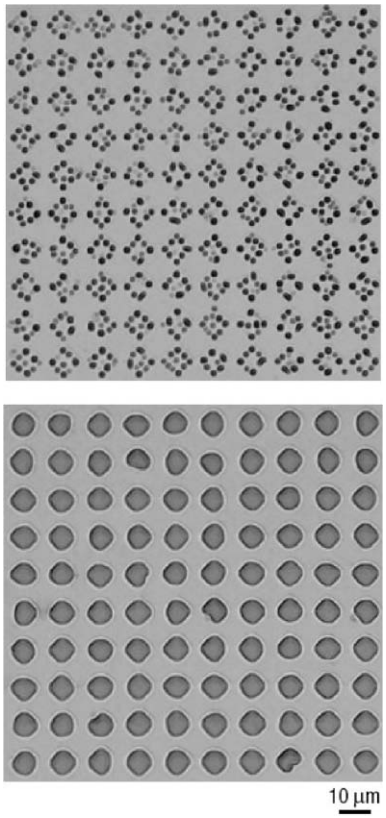

Fig. 2 Optical micrographs of the PDMS layer 1 min (upper image) and 20 min (lower image) after immersion in ethanol (compare to Fig. 1(b) and (d)). The dark regions correspond to the ethanol droplets. (Reprinted with permission from Macmillan Publishers Ltd., *Nature*, Léopoldès *et al.*,[1] copyright 2006.).

Additionally to the control of surface topology, the system is a promising approach for microfluidic applications to initiate mixing, reactions or filtration by controlling the diffusion coefficients, and the selective nucleation of droplets on various chemically structured surfaces.

Formation of lipid membranes on microchips

Cell membranes consist of lipid bilayers that function as a barrier to the external environment. Essential components of a cell membrane are membrane proteins, as they transfer chemical signals or transport ions and molecules selectively in and out of the cell. Since membrane proteins change their conformation and activity when isolated from cell membranes, they are more difficult to investigate than their water-soluble counterparts. To maintain their natural environment, they are often inserted in artificial, planar lipid bilayers, or spherical lipid vesicles.

In a recent work, Shoji Takeuchi and co-workers from the University of Tokyo describe a method to form a planar lipid bilayer in a microfluidic chip by contacting two monolayers that are assembled at the interface between water and organic solvent containing phospholipids.[2] Unlike most other approaches, the bilayer is formed in a vertical direction, allowing direct observation of membrane transport under the microscope. Two different microchip designs are tested. First, bilayer formation is performed in a double well chip that consists of two overlapping wells. The water/solvent/water interface is formed by injecting a water droplet into each well. Capacitance measurements revealed a bilayer thickness of 10 nm, indicating that a thin membrane was formed. The functionality of the bilayer membrane is proven by insertion of a reconstituted antibiotic peptide. The peptide ion channel used for this purpose (α-hemolysin from *Staphylococcus aureus*) is permeable for ions and small molecules which can be confirmed by recording the ion current across the membrane. In a second configuration, the lipid bilayer is formed in a cross-channel chip (Fig. 3 and 4), where the two interfaces controlled by syringe pumps are brought in contact at the crossing of two channels. Using this device, the membrane current is recorded when gramicidin A ion channels are incorporated into the bilayer. The bilayer formation and accompanying channel opening events of gramicidin A can be controlled in the microfluidic device simply by pushing and withdrawing the aqueous phase. Using multi-array lipid bilayer systems, the method will be a useful technology for high-throughput membrane protein analysis, *e.g.* for observation of membrane transport in physiological and pharmaceutical studies.

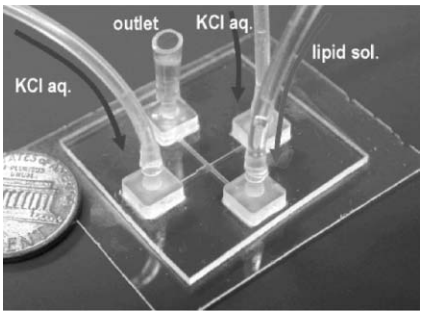

Fig. 3 Photograph of the "cross-channel-microchip" used for lipid bilayer formation. (Reprinted from ref. 2. Copyright 2006 American Chemical Society.)

A microfluidic maze for behavioural analysis

A maze is a common tool to investigate the behaviour and learning aptitude of vertebrate animals. In this issue of *Lab on a Chip*, Jinhua Qin and Aaron R. Wheeler introduce a miniaturized maze for the analysis of the behaviour of simple and tiny animals which could be useful for high throughput behavioural analysis and integration of neurochemical assays.[3] For the first time, the behavioural plasticity of an organism, here the worm *Caenorhabditis (C.) elegans*, is explored on a microfluidic platform. *C. elegans* is a well-studied model organism. The anatomy, development and genetics have been investigated extensively. It is one of the simplest organisms with a nervous system comprised of only 302 neurons. Nevertheless, it exhibits complex behavioural modalities, such as habituation, sensitisation and conditioning in response to stimulus. The size of this organism—about 1 mm long and 100 μm wide—matches perfectly with the dimensions of a microfluidic platform. The authors use simple microfluidic channels with one or more T-junctions, filled with air, to explore the behaviour of groups of worms and to investigate the capacity for associative learning of individual worms. The tendency of worms to explore novel

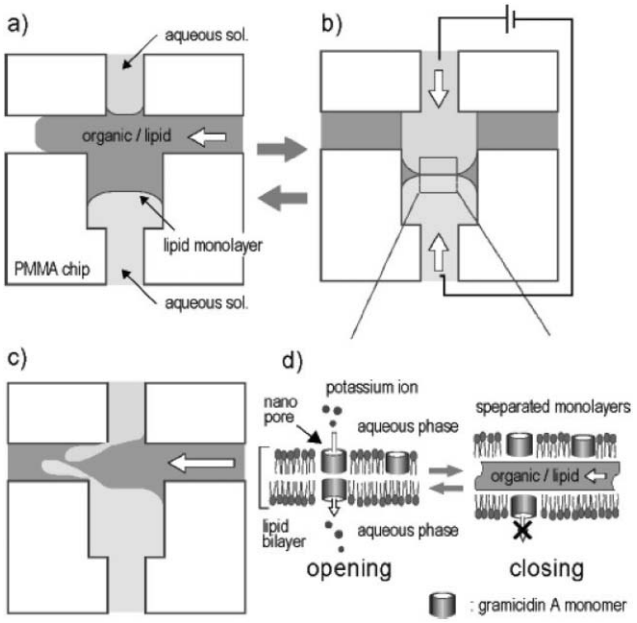

Fig. 4 Illustration of the bilayer formation in a microfluidic chip. (a) Two aqueous solutions are injected into opposite inlets of the channel system, and an organic solution containing the lipid fills is supplied by the crossing channel. (b) The aqueous solutions are brought into contact by fluidic control, and the lipid bilayer is formed at the interfaces. (c) The process can be performed repeatedly by flushing the channel with organic solution. (d) By insertion of proteins that form ion channels (*e.g.* gramicidin A), ions can pass through the membrane. The transport of ions can be interrupted by pushing or withdrawing the aqueous solution in the microfluidic channel, so that the organic/lipid phase disturbs the lipid bilayer. (Reprinted from ref. 2. Copyright 2006 American Chemical Society.)

environments is studied in the absence and presence of food (*i.e.* a bacterial suspension) in one of the outlet chambers. When no food is placed into the outlet chambers, the worms pass the microchannels, and enter the outlet chamber without any preference. However, when food is placed in one of the outlet chambers, the majority of animals are attracted to that outlet after one hour of observation time. In a second experiment, reward motivated learning of worms is demonstrated. Individual worms have to pass the microchannels in 10 successive trials, while the outlet chamber is filled (for the first five trials), or not filled with food. The time the worm spends inside the maze to choose its way toward the food-filled chamber decreases successively. Furthermore, the worm retains this choice in successive trials, when the outlet chamber is not filled with food any more. In all experiments, the influence of a neurotransmitter, dopamine, is studied additionally. Furthermore, the behaviour of wild-type worms and mutant worms is compared.

Cell-based protein detection

Protein microarray technology is an attractive approach towards high-throughput proteomic analysis. However, the availability of antibodies, which are most widely used to capture reagents, is limited. Furthermore, it is still a challenge to immobilise such capture ligands on a solid surface without reducing their activity. Sang-Hyun Oh *et al.* demonstrate a cell-based microfluidic protein sensor, wherein peptide displaying *E. coli* bacterial cells are utilised as capture reagents.[4] The *E. coli* cells are genetically engineered to express on their outer membrane surface

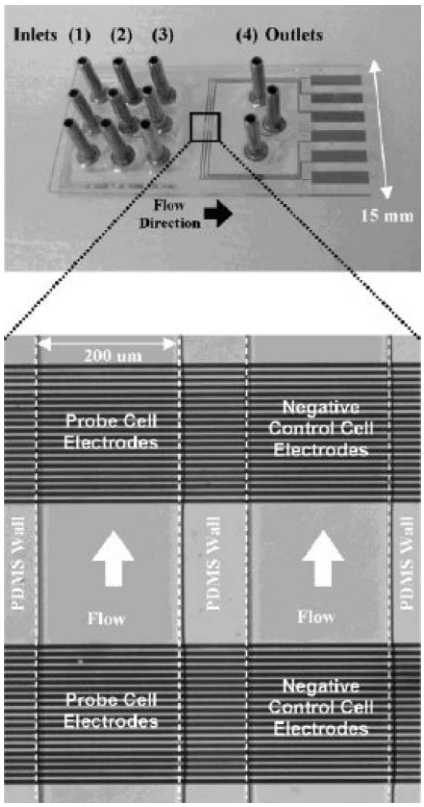

Fig. 5 Photograph of the microfluidic whole-cell protein sensor. Probe cells and negative control cells are supplied through inlet (1), and immobilised onto the microfabricated electrode array using positive dielectrophoresis (inset). The fluorescently-tagged target proteins and a wash buffer are introduced subsequently through inlets (2) and (3). The capture of target proteins is verified by fluorescence analysis. (Reprinted from ref. 4. Copyright 2006 American Chemical Society.)

thousands of copies of a unique peptide sequence, which functions as the capture ligand for the target molecules. The cells are electrokinetically immobilised on gold electrodes using positive dielectrophoresis (Fig. 5). Fluorescently labelled target molecules (here: streptavidin R-phycoerythrin) bind to the immobilised cells, and can be analysed by means of fluorescence spectroscopy within 10 minutes. In their study, the authors utilise *E. coli* cells that co-express the outer membrane protein, which binds to the red fluorescent target molecules, and the green fluorescent protein, which accumulates in the cytosol. By analysing the intensity of green and red fluorescence, both the number of immobilised cells on the electrodes and the affinity of target molecules to the capture ligands can be quantified.

This work is an important step towards whole-cell reagents as sensor elements, and is in particular useful for proteins that are difficult to purify and immobilise. The method may be applicable to other multiple affinity ligand display methods such as bacteriophage and yeast display technologies. The extension to larger, yet individually addressable dielectrophoresis trap arrays could enable a microsystem for versatile and programmable proteomic analysis.

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