Research Highlights

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Controlled movement of nanocolumns

Everyone knows the following phenomenon that we experience sometimes, particularly in the cold season: If the skin is exposed to cold air, the hairs will stand on end, causing goose bumps. The movement of the hairs is caused by the contraction of a tiny bundle of muscle fibres in the skin. It is an example of responsive behaviour, which is intrinsic to natural systems. For engineers it is a challenge to mimic such systems, and to create artificial materials that change appearance and properties in response to external stimuli. These advanced materials could be useful for a variety of applications, such as actuators, microfluidics, controlled surface wettability and controlled surface pattern, tuneable photonic structures, artificial muscles, and release systems. Joanna Aizenberg and co-workers have developed a dynamic actuation system that enables reversible actuation of rigid nanocolumns on a surface—like tiny hairs on a skin—by combining hard and soft elements.[1] They have fabricated high-aspect-ratio silicon structures (they named these structures "AIRS") with feature sizes between 100 to 300 nm and aspect ratios reaching 100 using the Bosch process. The nanocolums are embedded in a hydrogel to form a hydrogel–AIRS assembly ("HAIRS"), in which the nanocolumns have a defined pattern (Fig. 1). The hydrogel provides the responsive behaviour, *i.e.* it changes the volume depending on the humidity level. The silicon nanocolumns move upon contraction and/or swelling of the hydrogel. Two hybrid architectures are developed using either free-standing or attached nanocolumns. Free-standing nanocolumns are perpendicular to the surface in a swollen hydrogel. Upon drying, they move to a tilted state with a tilt angle of 60 to 75° (Fig. 1D). In the second design with nanocolumns attached to the surface, drying of the hydrogel results in bending of the nanocolumns (Fig. 1E). Moreover, the formation of reversibly actuated micropatterns with complex geometries is demonstrated. By introducing topographic patterns on the surface the tilt direction of the nanocolumns could be controlled. A highly uniform tilt direction is achieved by using a confining surface that is patterned with lines. Two more complicated patterns, a honeycomb-like pattern and a microtrap formed by several nanocolumns, are also presented. The time needed for the movement of the nanocolumns is evaluated. While switching to the vertical orientation due to swelling of the hydrogel is extremely fast ($\sim$60 ms), the reverse transformation to the tilted state caused by drying takes $\sim$4 s. The actuation system is very robust, no deterioration of the microchip is observed after several months. Furthermore, the mechanics of the actuation process is assessed in the study.

How to create a wrinkled surface

John W. Hutchinson and co-workers present a technique to form ordered wrinkles on polymer surfaces.[2] The patterns are formed by exposing a flat polydimethylsiloxane (PDMS) sheet to a focused ion beam (FIB) of Ga^+ ions. FIB irradiation induces the formation of a thin stiff skin that tends to expand in the direction perpendicular to the direction of the FIB irradiation. The resulting mismatch strain between the stiff skin and the PDMS substrate gives rise to skin buckling. By controlling the relative motion of ion beam and PDMS, a stiff skin exhibiting simple one-dimensional wrinkles as well as complex hierarchical nested wrinkles could be created. Wrinkles with wavelengths and

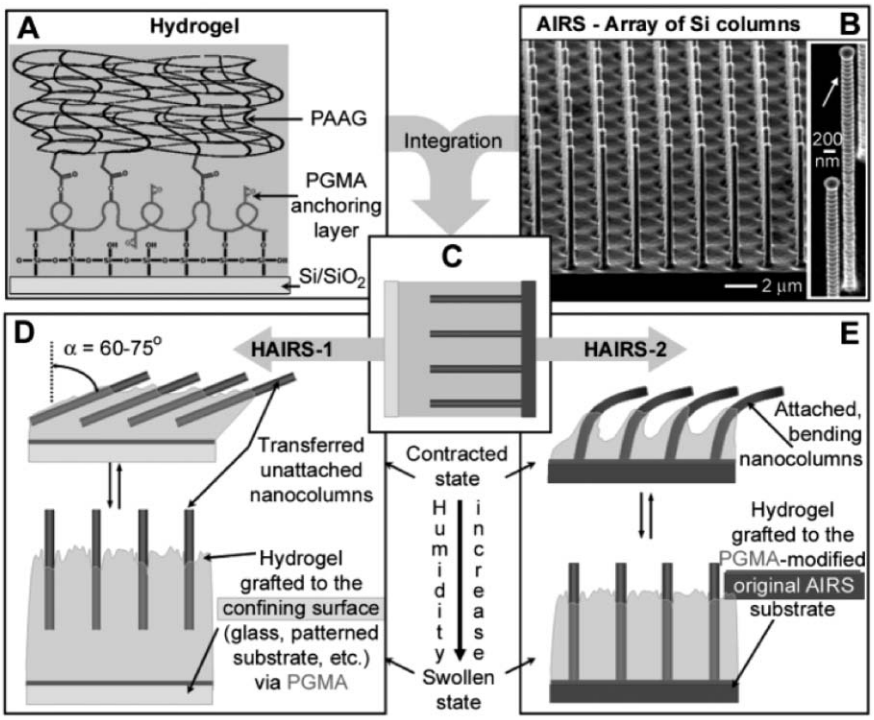

Fig. 1 Reversible switching of hydrogel-actuated nanocolumns: The actuation system is formed by a combination of rigid features (silicon columns) and a soft material (a hydrogel) that can be actuated by external stimuli. (A) The structure and composition of the hydrogel that is responsive to the humidity level is schematically shown. An array of silicon nanocolumns (B) is embedded in the hydrogel (C). Two hybrid architectures are realised: Nanocolumns that are free-standing in the gel (D) or attached to the substrate (E). Upon a change of the humidity level, the hydrogel swells or contracts, and hence, the nanocolumns are put in motion. (From Sidorenko *et al.*[1] Reprinted with permission from AAAS.)

amplitudes in the micrometre and sub-micrometre dimension can be achieved by varying the ion beam fluence. The authors investigated the topology of the exposed PDMS surface for various experimental parameters by scanning electron microscopy (SEM), and quantified the morphology of the wrinkle patterns by atomic force microscopy (AFM). They found that the surface morphology of the wrinkle patterns induced by FIB depends mainly on the exposed ion fluence. A weak fluence on the order of 1×10^3 ions per cm^2 results in straight, one-dimensional wrinkles with an average wavelength of ∼460 nm. Larger ion fluence induces the formation of complex hierarchical patterns, with primary wrinkles having a wavelength of ∼460 nm nested in larger secondary wrinkles with an average wavelength of ∼2 μm. The stiff skin that is formed on the polymer surface upon exposure to FIB has an altered chemical composition. Examination using Auger electron spectroscopy (AES) reveals that the stiff skin resembles amorphous silica. Such wrinkled surfaces with defined topology could be useful for the formation of micro-sized biological sensors, optical components and fluidic components.

Counting proteins in a single cell

During the last years, microchips have been shown to be a convenient tool for the handling and manipulation of single cells with the potential to give new insights in genomic, proteomic and metabolomic processes of a cell. In former studies, heterogeneities of cellular processes, particularly gene expression, with respect to time and cell population have been evaluated (see also *Research Highlights* from Issue 5 2006 and Issue 1 2007). A critical aspect for single-cell analysis is still the development of single-cell assays in combination with suitable detection techniques, since many target molecules are present in a cell only in low concentrations.

Richard N. Zare and co-workers developed a complex microfluidic chip in which cells can be captured and lysed. In a recent work, they have advanced the microchip, and succeeded in labelling, separating, and quantifying multiple low-copy-number proteins of single cells using single-molecule fluorescence spectroscopy.[3] The chip is made of PDMS and has three sections for cell manipulation, electrophoretic separation and counting of single fluorescent molecules (Fig. 2). Cells are captured in a reaction chamber between two valves, and a lysing and labelling buffer is injected. The labelling buffer contains fluorescently tagged antibodies that specifically bind to the target protein. The excess labelling reagent is then separated by electrophoresis. At the end of the separation channel, fluorescent molecules are detected as they pass a rectangular, curtain-shaped detection region that is generated across the channel by focussing a laser beam using a cylindrical lens. The number of fluorescence bursts above a preset threshold corresponds to the number of fluorescent molecules.

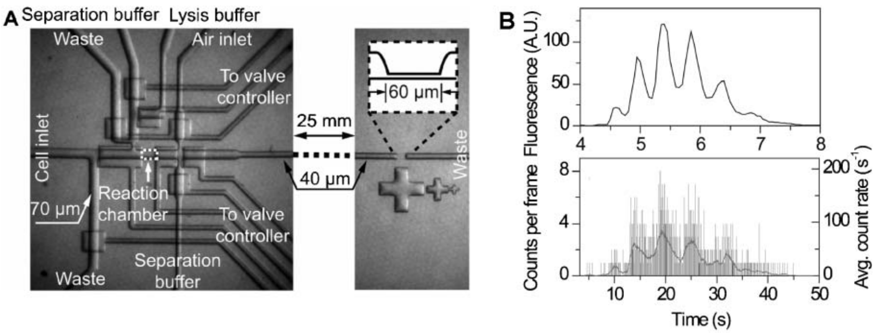

Fig. 2 (A) Layout of the device designed for cell analysis. The section for single-molecule counting is shown in the right part of the image. (B) Capillary electrophoresis (CE) and single-molecule counting in a microfluidic channel. In this example, Alexa Fluor 647 labeled streptavidin (A647-SA, concentration of 100 nM) is separated into multiple peaks using CE and detected with laser induced fluorescence (top). The different peaks correspond to different numbers of Alexa Fluor labels attached to the streptavidin. The CE separation is of 100 nM concentration of A647-SA. The line corresponds to the average molecule count rate in one-second time bins. ((A) From Huang *et al.*[1] Reprinted with permission from AAAS. (B) Courtesy of R. N. Zare, Stanford University, USA).

Proteins of two cell types are investigated. First, the number of a human transmembrane protein (β_2AR) expressed in an insect cell line (SF9) is determined. A short peptide sequence is genetically added to the transmembrane protein, so that it could bind to a fluorescently tagged monoclonal antibody. In the microfluidic chip, the number of proteins per cell is counted by single fluorescent molecule spectroscopy. The average number is in agreement with ensemble measurements, but the single-cell measurements reveal a high variance of the protein number from ∼2000 to ∼60 000. In a second application, the phycobiliprotein content in unicellular cyanobacteria (*Synechococcus* sp. PCC7942) is analysed. These cells comprise mainly two specific phycobiliprotein complexes (phycocyanin and allophycocyanin) to collect the light energy and transfer it to the photosynthetic reaction centres. The authors found significant differences in the levels of the two phycobiliproteins when the cells are grown under nitrogen-depleted conditions.

Bacteria support mixing

The movement of the living cells can enhance mixing in a microchannel. Min Jun Kim and Kenneth S. Breuer report on a microfluidic device which uses bacterial cells *E. coli* to improve spreading of high-molecular-weight tracer molecules.[4] Furthermore, the chemotactic behaviour of the cells, *i.e.* the movement of the cells in response to chemical stimuli, is exploited to adjust the strength and direction of the mixing enhancement.

The authors have monitored the concentration profile of the fluorescent tracer molecule TMR–Dextran (MW 2 000 000) that is fed into the middle arm of a three-junction microchannel at several positions along the microchannel (Fig. 3). The presence of bacterial cells in the same stream accelerates mixing of the TMR–Dextran molecules. The concentration profile of TMR–Dextran spreads faster, *i.e.* the effective diffusion coefficient is increased. If a chemoeffector is

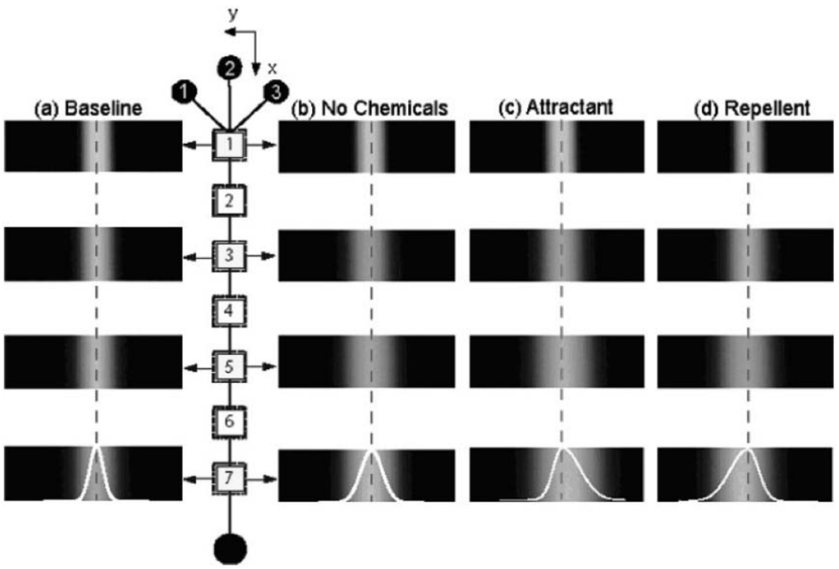

Fig. 3 Controlled mixing in a microchannel using bacterial cells. The fluorescence images illustrate the diffusion profiles of the tracer TMR–Dextran in a three-arm microfluidic device for varying conditions: (a) without and (b) with bacterial cells in the centre stream, (c) with bacterial cells in the centre stream and a chemoattractant in the right stream, and (d) with bacterial cells in the centre stream and a repellent in the right stream. (Reprinted from Kim and Breuer.[4] Copyright 2007 American Chemical Society.)

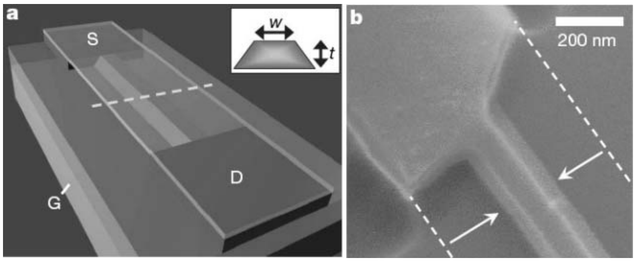

Fig. 4 Fabrication of nanowire sensors with CMOS field effect transistor compatible technology. (a) Scheme and (b) SEM image of the device after anisotropic wet etch. The silicon-on-insulator active channel is undercut etched. S: Source, D: Drain, G: Underlying backgate, w: width and t: thickness of the channel. (Reprinted with permission from Macmillan Publishers Ltd: *Nature*, Stern *et al.*,[5] copyright 2007.)

supplied in one side-arm of the microdevice, the cells modulate their motility in response to the chemical signals in their environment and preferentially swim to one side or the other of the microchannel. Thus, the enhanced mixing due to bacteria becomes asymmetric. The authors demonstrate both, the addition of a chemoattractant (aspartic acid) or a chemorepellant (nickel sulfate) in one of the control streams, which results in increasing or suppression of the effective diffusion of TMR–Dextran towards the respective side of the channel (Fig. 3(c) and (d)). The study is a simple demonstration how to utilise living organisms to power and control the streams in microsized devices.

Sensors for label-free immunodetection

In the last few years, several approaches for using semiconducting nanowires as sensors for highly sensitive, selective, and label-free detection of target molecules have been demonstrated. However, the fabrication processes of such devices are suboptimal leading often to a disappointing sensor performance and hindered their extensive use so far. In a recent publication, researchers from Yale University reported a novel fabrication procedure using complementary metal oxide semiconductor (CMOS) field effect transistor compatible technology to fabricate nanowire-type devices (Fig. 4).[5] Ultra-thin silicon-on-insulator wafers are utilised which require only two-dimensional active layer definition to achieve nanometre dimensions. The developed fabrication process involves an anisotropic wet etch to generate non-degraded devices that are narrower than their lithographic pattern definition. A variety of nanowire geometries can be fabricated, *e.g.* a six-point, Hall bar device. The quality of the devices is verified by electrical characterisation. A comparison of unfunctionalised nanowire sensors made by reactive-ion etching and by the novel wet etching procedure illustrates the superior performance of the wet etched device. Three bio-analytical applications show the efficacy of the nanowire sensor (i) for real-time live cellular response, (ii) for the detection of unlabelled macromolecules by functionalising the nanowire with a receptor for the target molecules, and (iii) for immunoassays capable of detecting antibodies of below 100 fM concentration. The strength of the developed sensors lies particularly in seamless integration with CMOS technology without the need of hybrid fabrication schemes, and hence, could facilitate widespread diagnostic applications.

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