

Research Highlights

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Investigation of sickle cell disease

Patients with sickle cell disease, a genetic disease caused by a point mutation, have abnormal haemoglobin (HbS) that sticks to each other if partial oxygen pressure is low. This polymerization of HbS changes the stiffness and morphology of the red blood cells, *i.e.* the cells get a rigid surface and a sickle shape. As a consequence, sickling red blood cells slow down in narrow vessels, and eventually get stuck. This so-called vasoocclusion deprives the downstream tissue of oxygen and causes a vaso-occlusive crisis of the patient, resulting in ischemia and cell damage.

The processes involved in vasoocclusion occur on different time scales. Polymerization of HbS and change of cell morphology happen within milliseconds and seconds, respectively, whereas the macroscopic occlusion of vessels takes several minutes (Fig. 1a). Some of the important parameters that influence vasoocclusion are the pressure gradient in the vessels, the vessel diameter, the red cell concentration (hematocrit), the intracellular HbS concentration and the oxygen concentration. A research team from Harvard University, Harvard Medical School and MIT studied these geometrical, physical, chemical and biological determinants on vasoocclusion by using a microfluidic device that mimics a vascular network (Fig. 1b).¹ The researchers showed that the occlusion events could be evoked, controlled and even inhibited in the device. They first investigated the dependence of occlusion events on channel width, total hydrostatic pressure difference across the channel network, and the ambient oxygen concentration. In these experiments, blood samples of patients suffering from sickle cell disease are supplied to the microdevice, and the time of occlusion is recorded for varying parameters. The occlusion event occurs within minutes. The variability of the characteristic occlusion time is high though. This is attributed to the fact that vasoocclusion is a collective event, *i.e.* multiple cells are required to form a percolating network across the channel before there is a significant reduction

in flow velocity followed by a jamming event. On the other hand, the unjamming event, induced by oxygen supply, is a non-collective process, and is found to happen on a much shorter time scale (faster than a minute) with less variability.

Besides these more fundamental studies, the properties of the device for clinical application are demonstrated. The effect of therapeutic red blood cell exchange, in which blood from sickle cell patients is partly replaced by healthy red blood cells, is investigated. Moreover, the impact of small-molecule inhibitors of polymerization is studied, and it is found that

carbon monoxide, present in low concentration, can prevent an occlusion of vessels. These experiments demonstrate that this microfluidic approach to mimic vasoocclusion allows the measuring of the efficacy of treatments individualised for sickle cell patients, and may help to identify new therapies.

Electrochemical micropatterning of conductive polymers

Conductive polymer-based devices exhibit unique advantages with respect to tunable conductance, chemical specificity,

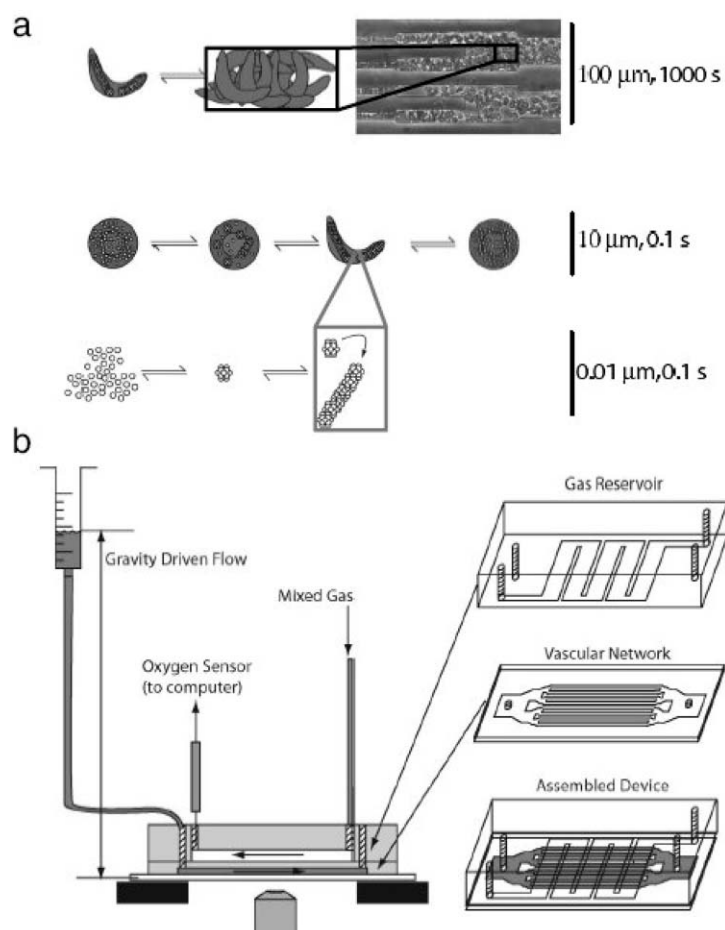


Fig. 1 Investigation of vasoocclusion in sickle cell disease by means of a microfluidic device. (a) Scheme of the collective processes of vasoocclusion. Polymerisation of abnormal haemoglobin and the following deformation of red blood cells occur on short time scales, while the jamming of vessels is a process of minutes. (b) Drawing of the microfluidic device used to mimic vasoocclusion in human vessels. (From ref. 1. Reprinted with permission. Copyright 2007 National Academy of Sciences, USA.)

flexible modification and low fabrication cost. In a recent work, Hou *et al.* introduce a method to form conductive polymer micropatterns in a microfluidic setting,² in which a hydrodynamically focused laminar stream of monomer precursor solution was generated and guided towards an array of individually addressable electrodes. The focused precursor stream (pyrrole solution) serves as a dynamic template for deposition of polypyrrole micropatterns across the microelectrode junction gaps, where a constant current (between 200 and 800 nA) is applied (Fig. 2). Micropatterns that are 1–5 μm wide and 0.1–1.2 μm thick are deposited across the 10 μm electrode junction gaps within 100–250 s.

The authors employed the dynamic, site-specific deposition method to fabricate a sensor array, in which two different types of conductive polymer micropatterns are created. Subsequently, several polypyrrole micropatterns as well as several carboxylic acid substituted polypyrrole micropatterns are deposited within the same device. Two micropatterns are chosen for sensing a collection of 12

saturated organic solvent vapours, and the resistive response of this binary sensor is recorded upon exposure of these vapours. The results indicate that the collective sensing responses are informative enough to identify a wide range of organic vapours with high sensitivity and a negligible memory effect. To expand the capability of the microelectronic device, the deposition method could be widely applied to micropatterning of other redox-active materials.

Bacteria to pump microflows

In microfluidic systems, typically external devices, *e.g.* syringe pumps, are used to pump the fluids through the channels. On the other hand, integration of micropumps, based on piezoelectric, pneumatic, electrokinetic, or electromagnetic effects, has been achieved. These approaches are convenient and work properly, but often bulky hardware is required, which negates the advantages of handheld microscale devices. An exciting alternative approach is the exploitation of biological systems as an energy source in an engineered mi-

crosystem. The principle concept has been demonstrated in the past for molecular motors such as the F_1 -ATPase rotary motor, the movement of microtubules along kinesin molecules, and for bacteria to rotate a micro-sized motor. Min Jun Kim and Kenneth S. Breuer have taken one step forward, and presented in a recent work the utilisation of bacterial carpets to generate useful work in a microfluidic system.³ The key finding of this study is the self-organisation of thousands of independent bacterial cells resulting in a collective motion of the flagella capable of pumping fluid through microchannels at speeds as high as 25 m s^{-1} . This is achieved by a repeated pumping and settling cycle of a bacteria suspension in a microchannel so that cells adhere to the channel walls (made by polydimethylsiloxane) and the glass cover. The cell tends to adhere with a clear orientation preference, corresponding to the flow direction, and additionally, most of the cells stuck to the surface by their bodies with several flagella free to rotate in the flow. After ~ 50 min, a high fill factor of 83% is measured, and the external flow is switched off, and the velocity inside the channel is determined by particle tracking velocimetry. It can now be observed that the bacterial carpet spontaneously self-organises and pumps fluid through the channel. The velocity increases rapidly for the first period of 20 min after carpet formation, reaches a maximum, and decays slowly afterwards. The performance of the pump is controlled by varying the concentration of glucose in the buffer. Although the rising time of pump velocity remains unchanged for all conditions, the maximum pumping velocity can be increased for high glucose concentration and the pump operation is maintained for a longer time. Additionally, the microchannel width (channels between 50 and 800 μm width are tested) influences the performance of the bacterial pump. While narrow microchannels support fast rising times and high maximum pumping velocities, there is barely any pumping activity in the widest channels at all. The authors explain the pumping ability of the bacterial carpet by interactions of many rotating flagella in a neighbourhood that leads to formation of flagella bundles and results in coordinated fluid motion that is most efficient in small channels. As a result, the work underlines the opportunity to use biological organisms

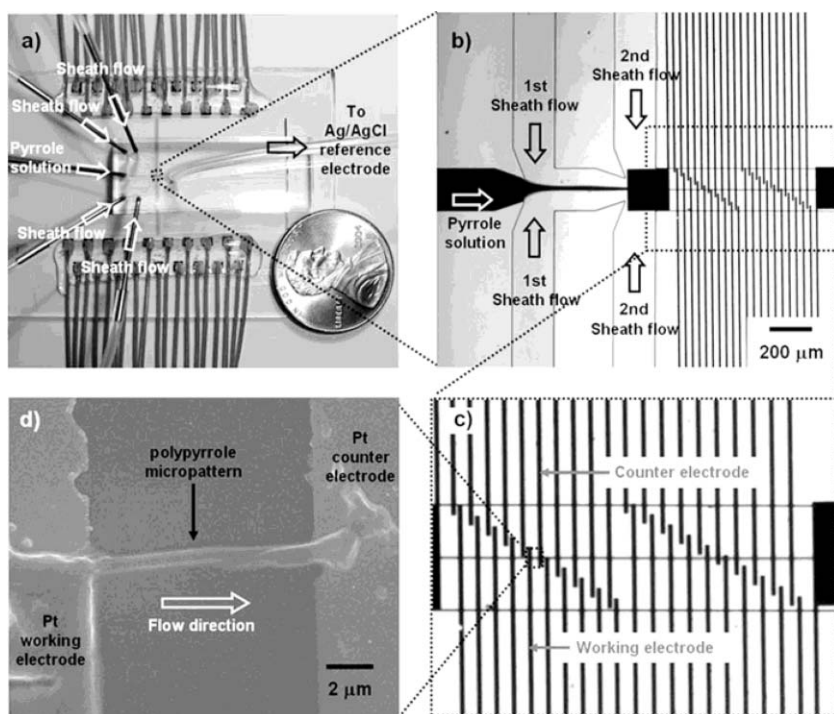


Fig. 2 Microfluidic-aided electrochemical micropatterning of conductive polymers. (a, b) The monomer precursor solution (pyrrole) is hydrodynamically focussed twice and guided towards an electrode array. (c) Design of the Pt electrodes. The gap between counter and working electrodes is 10 μm . (d) SEM image of the conductive polymer micropattern deposited between the electrodes. (From Hou *et al.*² Copyright Wiley-VCH Verlag GmbH & Co.KGAA. Reproduced with permission.)

as mechanical actuators in microscaled engineered systems, in which modulation and regulation can be achieved by external chemical stimuli or temperature that affect the motility of the bacterial cells.

Moulding of hydrogel particles in stretched cavities

The usage of shrinkable material to fabricate microscaled patterns in a fast and simple way without the need of photolithographic techniques has recently been presented.⁴ Flexible material that can be stretched and relaxed can also serve as a versatile mould to create particles. This approach to form hydrogel particles has been developed by researchers from Nanyang Technological University (Singapore).⁵ The top-down micromoulding method is designed to achieve formation of hydrogel particles with monodisperse micrometre and submicrometre size and controllable shape. Such particles are extremely promising drug delivery systems and facilitate high loading of water soluble protein drug, while the destruction of biomacromolecule drugs is minimal during the gentle process of particle formation. The novel method named SCAMP—stretched cavity-assisted moulding of particles—involves several process steps (Fig. 3). First, an elastic polydimethylsiloxane stamp with cavities is fabricated. In a second step, the stamp is stretched, and the cavities are filled with precursor solution. Afterwards, by exposure to ultraviolet light, the droplets in the cavities are hardened. The cured particles are then partly ejected out of the cavity by releasing the tension on the mould. The particles are now half inside the mould, and half outside. Finally, the detachment is achieved by encasing the particles in ice, while the elastic mould is peeled away. The

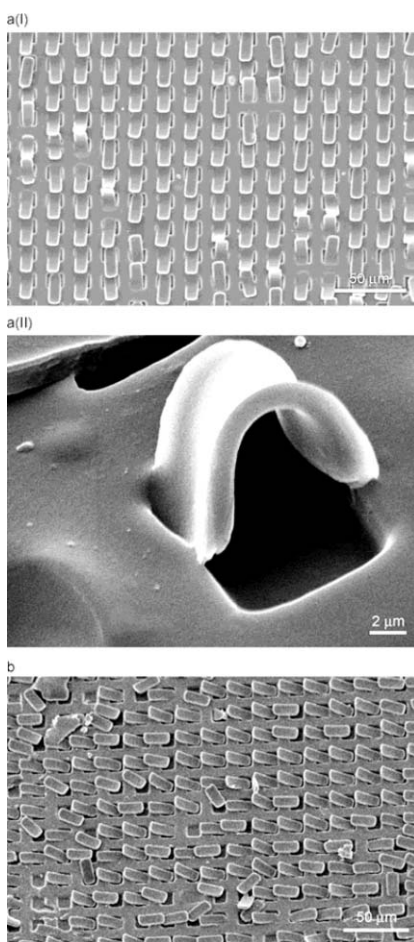


Fig. 3 Scanning electron microscope images of 10 µm sized particles made from polyethylene glycol diacrylate that are formed by stretched cavity-assisted moulding of particles (SCAMP). The top image shows how the particles are breaking away from the cavity walls, when the stress of the flexible mould is reduced and the cavities are relaxing. In the middle image, a unique buckled particle can be seen. Bottom image: In this experiment, the mould was stretched in two-axes, which enables complete detachment of the particles. (From Laulia *et al.*⁵ Copyright Wiley-VCH Verlag GmbH & Co.KGAA. Reproduced with permission.)

potential of the SCAMP method is characterised in terms of the incorporation of fluorescent proteins such as Green Fluorescent Protein (GFP) and fluorescein isothiocyanate-conjugated bovine serum albumin (FITC-BSA), and the distributions are determined by fluorescence microscopy. Furthermore, to demonstrate the preservation of biological activities of entrapped proteins after employing the SCAMP method, an avidin–biotin binding assay is performed. It could be confirmed that avidin entrapped in the particles is bound to biotin that is exposed afterwards. The authors suggest that the method could also enable subsequent coating steps to produce multilayered or targeted drug-delivery particles.

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