Research Highlights

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On-chip Darwinian evolution

The principles of Darwinian evolution, developed by Charles Darwin more than 150 years ago, are fundamental for the understanding of biological organisation at the level of populations of organisms and for explaining the development of biological genomes and macromolecular function. Nowadays, it has also become a chemical tool for discovering and optimising functional macromolecules in a chemical laboratory. In contrast to natural evolution, man-made evolution in a test tube can be many orders of magnitude faster. However, the success of *in vitro* evolution relies on appropriate technologies that allow the precise control of reaction parameters and automation of the processes. Microchip technology greatly meets these requirements, which has been demonstrated in a recent work of Brian M. Paegel and Gerald F. Joyce from The Scripps Research Institute at La Jolla (CA, USA).[1] In this work, a microchip has been designed that provides the platform for directed evolution of RNA enzymes with RNA ligase activity (Fig. 1). In this assay, billions of RNA enzymes are challenged to catalyse the ligation of a promoter-containing oligonucleotide substrate to itself. In the case where an RNA molecule has acquired a promoter sequence as a consequence of RNA-catalyzed ligation, the RNA molecule is amplified by two polymerase enzymes. The selective pressure of the *in vitro* evolution is the efficiency of substrate utilisation. Under conditions of progressively lower substrate concentrations, only those RNA enzymes are amplified that react most efficiently with the substrate. This entire process of exponential growth phase and dilution is automated and relies on computer control. The readout parameter for RNA enzyme optimisation is fluorescence intensity. The reaction mixture contains a dye molecule that intercalates into nucleic acids and gives rise to a fluorescence signal upon laser excitation. When the fluorescence signal reaches a predetermined threshold, correlating with a 10-fold increase in the concentration of RNA, the reaction mixture is 10-fold diluted. Hence, evolution is observed in real time as the population adapt and achieve faster growth rates over time. After several hundreds of iterations, a set of 11 mutations of the RNA enzyme are evolved, with a 90-fold improvement in substrate utilisation.

In the automated chip-based system, evolution is achieved through a microfluidic algorithm. The continuous determination of fluorescence intensity provides a high-resolution record of an evolutionary trajectory, which can be obtained as a function of population size and heterogeneity, growth conditions and the availability of limiting resources.

Formation of giant unilamellar vesicles by microfluidic jetting

Vesicles formed by lipid membranes are widely used as chemical microreactors, drug delivery containers, and primitive cell models. Because a mixture of lipids and biomolecules will not spontaneously form into spherical vesicles, several appropriate methods have been developed to define the form and size of vesicles. However, it is still challenging to form giant vesicles with diameters above 10 μm that have a unilamellar membrane and enclose biomolecules with high efficiency and repeatability. Daniel A. Fletcher and

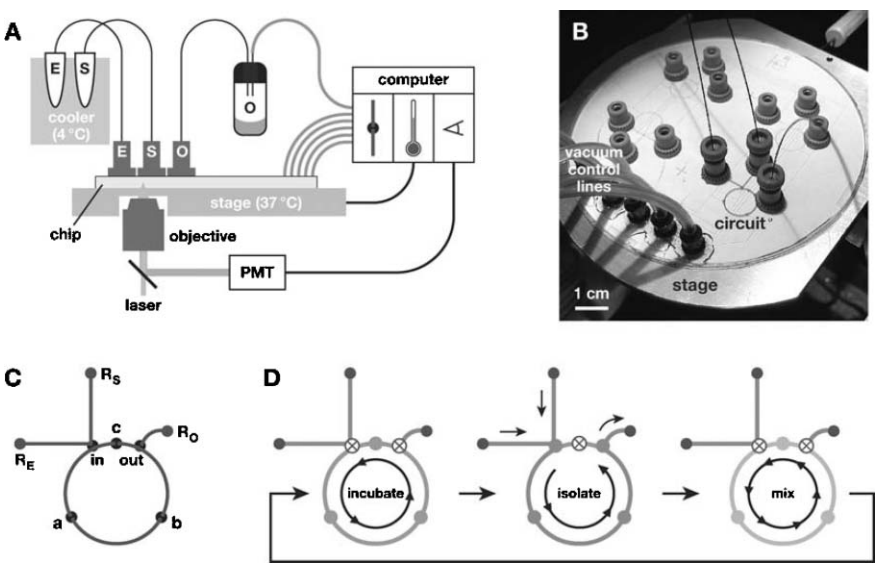

Fig. 1 Scheme and picture of the microfluidic evolution system. (A) The microchip is mounted on a temperature-controlled stage. Supply of reaction mixtures, temperature and data readout is computer-controlled. (B) Picture of the microfluidic device. An active circuit is shown in dark shading. (C) and (D) illustrate the dilution circuit and the actuation of the valves. (Reproduced from Paegel and Joyce[1]).

co-workers from University of California at Berkeley demonstrate in a recent article how these requirements are obtained.[2] The formation of giant unilamellar vesicles (GUVs) are achieved by microfluidic jetting induced deformation of a planar bilayer. A precisely controlled pulsatile liquid jet is directed into a unilamellar lipid bilayer that has been formed between two aqueous phases. For each pulse, a vesicle is formed that is filled with the solution from the jet. Using a nozzle with a diameter of 40 μm, GUVs with an average size of 208 μm are produced, varying by 2–3% for a given series of pulses. A high-speed camera is used to investigate the formation mechanism and the parameters determining the vesicle size. Furthermore, repeatable encapsulation of 500 nm particles is achieved into the GUVs, and the transport of molecules across the unilamellar membrane by addition of the protein pore α-hemolysin.

Nanoporous junctions for analyte preconcentration

A very simple method to fabricate nanoporous junctions in microfluidic channels with excellent repeatability has been presented by Sung Jae Kim and Jongyoon Han from MIT in a recent publication.[3] For this process, microchannels are imprinted in silicon elastomer (PDMS) (Fig. 2). By mechanical cutting across the channels, a small gap is created that is enlarged by bending the microchip, while a droplet of Nafion polymer is dropped into the gap. After curing at 95 °C, the Nafion junction is sealed tightly, without leakage, due to the elastic nature of PDMS. After removal of residual Nafion by taping, the microchip can be bonded to a glass plate after plasma treatment.

Nafion is a sulfonated tetrafluorethylene copolymer with pore sizes around 5 nm, widely used as a proton conductor for a proton exchange membrane. Due to anionic surface groups it is permeable to cations and polar compounds. The authors demonstrate the functionality of the nanoporous junction for ion and protein preconcentration. Charged dye molecules (BODIPY) and proteins (β-phycoerythrin) are preconcentrated with the aid of an electrical field, and preconcentration factors up to 10^4 are achieved within 15 and 22 min, respectively. The device can be operated in a faster mode by applying a pressure-driven flow. At a flow rate of 35 nL min^{-1} and an operation voltage of 120 V, the speed of preconcentration is doubled. The fabrication method is also suitable for large channels with dimensions of several hundreds of micrometres, in which high-throughput sample preparation can be achieved. The obtained plugs containing preconcentrated sample have volumes of nearly 10 nL, which makes the method suitable for connecting with commercial analytical systems such as mass spectrometry.

Biofuel production for molecular motors

In living cells, adenosine triphosphate (ATP) is a source of chemical energy that is consumed by biomolecular motors, which are omnipresent in cellular systems and serve various important functions, *e.g.* they are involved in active transport mechanisms inside the cell. On the other hand, molecular motors open fascinating opportunities for the construction of artificial nanoscaled systems. As in natural systems, these biodevices require the supply of chemical energy for continuous operation. In a current work, Jed Harrison and co-workers[4] demonstrate that the production of the "fuel" ATP can also be copied from cellular systems. They used mitochondria, *i.e.*, membrane-enclosed organelles that are sometimes also referred as the "cellular power plant" to generate ATP from high-density fuel sources such as succinate on a microdevice. The mitochondria are in suspension, or immobilised on microbeads, and are introduced into a microfluidic channel. In another section of the microchannel, separated by filter paper, the freshly produced ATP activates the kinesin motor protein, thereby moving microtubules that are attached to the kinesin motor protein. The authors anticipate that other high energy density fuel sources such as glucose can be used to produce many ATP directly on a microchip. Furthermore, the

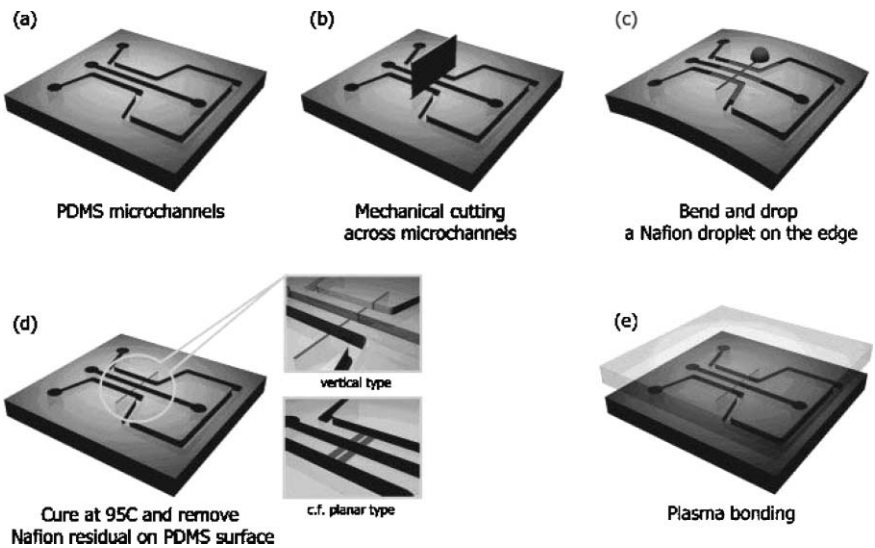

Fig. 2 A simple method to fabricate nanoporous junctions in microfluidic channels. Mechanical cutting of silicone elastomer microchannels and filling with a porous polymer solution (Nafion) provides leakage-free nanoporous junctions that can be used for efficient high-throughput preconcentration of analytes. (Reprinted from Kim and Han.[3] Copyright 2008 American Chemical Society.)

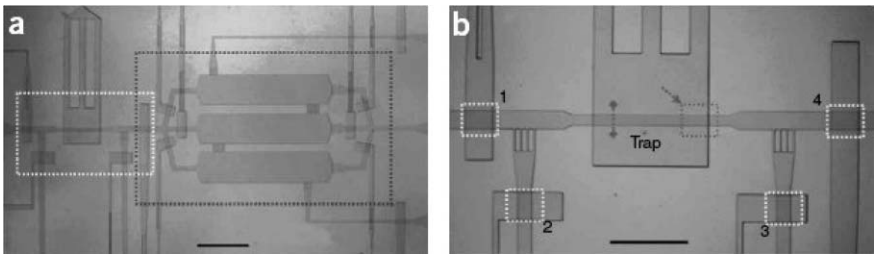

Fig. 3 Micrographs of the microdevice for nanoaxotomy of the worm *C. elegans.* (a) The bilayer microfluidic chip consists of a section to trap the worm (white rectangle) and a section with several feeding chambers (black rectangle). (b) Magnified view of the trapping chamber, in which the worm is immobilised, and the nerves are severed by several femtosecond laser pulses, and the recovery of the nerves is observed. (Reprinted with permission from Macmillan Publishers Ltd: Nature Methods, Guo *et al.*[5] copyright 2008.)

implementation of an enzymatic assay based on pyruvate kinase is shown, which is a promising approach in terms of recycling of ATP from ADP in the microdevice.

Nanosurgery and nerve regeneration studied on microchips

The fundamental understanding of nerve regeneration and degeneration is a key requirement to developing novel therapies for human neurodegenerative diseases. A well-suited model organism to study nerve regrowth after severing axons (so-called axotomy) is the nematode *C. elegans*, in which the injury of the nerves can be achieved by means of a well-focussed femtosecond laser. Of course, the worm has to be immobilized or sedated during the nanosurgery. In former studies, it has been shown that microfluidic approaches are ideal to study the behaviour of the small worm since trapping and manipulation can be achieved, while the worm is minimally affected, *i.e.*, without the need of chemicals for immobilization and without anaesthetics. Adela Ben-Yakar and co-workers have demonstrated now that microfluidic devices are also beneficial for femtosecond laser nanoaxotomy of the worm.[5] They designed a device in which the worm is trapped during nanosurgery (Fig. 3). The regrowth of nerves is observed by time-lapse imaging. The authors discovered a recovery time of nerves within 60–90 min, which is much faster compared to observations from studies where the worms are grown on agar pads and paralysed during axotomy; under these conditions, recovery times of several hours have been reported. Furthermore, the design of the microchip allows sorting of worms into different feeding chambers after nanosurgery and nerve regrowth imaging, where the worms can be observed for an extended period of time.

Petra S. Dittrich
ISAS, Dortmund, Germany
dittrich@isas.de

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