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Microscale valves drive progress in microfluidics

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A new microfluidics technology has been developed that uses novel miniaturized valves and pumps to overcome many of the drawbacks of current microfluidics devices. Microfluidics systems have been hailed as biology's equivalent of the silicon chip, the integrated circuit technology that revolutionized electronics. The goal is to develop an integrated 'lab-on-a-chip' that will perform complex assays.

Microfluidics is already in use in DNA assay and cell-sorting devices but progress has been held back by the limitations of the hardware available. Chips have been made from silicon or glass and materials moved around electrokinetically, by gas or water pressure, or by active peristaltic pumping. Although they are useful for specific tasks, the intrinsic properties of these systems limit the level of complexity that can be achieved. However, much work is going on to overcome these drawbacks and lab-on-a-chip devices based on these technologies are under development.

Elastomers replace silicon

Other groups working on microfluidics systems have abandoned silicon in favour of cheap elastomeric materials, such as polydimethylsiloxane. Patterns are incorporated by curing the elastomer on a micromachined mould, a technique known as soft lithography[1]. Features that can be made include channels, diffraction gratings and three-dimensional structures, such as coils and rings. These can be as small as 80 nm in diameter[2] but typical channel widths are 50–100 μm. Stephen Quake's group at the California Institute of Technology (Caltech; Pasadena, CA, USA) has extended this technique to produce multilayer structures[3]. Each layer is cast separately and bonded irreversibly to the next using the elastomer's chemical curing process, eradicating the problems of adhesion failure and thermal stress that occur when layering hard materials. This proprietary technology is now being commercialized by a new start-up company, Fluidigm Corporation (San Francisco, CA, USA).

In this technology, fluid movement is controlled mechanically using pumps and valves created from networks of channels (Fig. 1). Mechanical control eliminates several problems associated with electrokinetic methods, for example, the formation of electrolytic bubbles. Pneumatic pressure is applied to a control channel via a fluid-filled line, and the resulting deflection of the elastomer membrane closes the active channel below. At least 30 such valves can be packed into a square millimetre[3] and sequences of valves are used to create peristaltic pumps. 'Using elastomers has allowed us to produce the smallest valves with the lowest dead volume,' says Quake. 'These valves are of very high quality and can be actuated millions of times.' According to Quake, 'the technology enables us to produce devices with a degree of integration that is not currently possible with silicon-based techniques.' Fluidigm has produced chips with as many as 600 valves, which are controlled by only 12 lines.

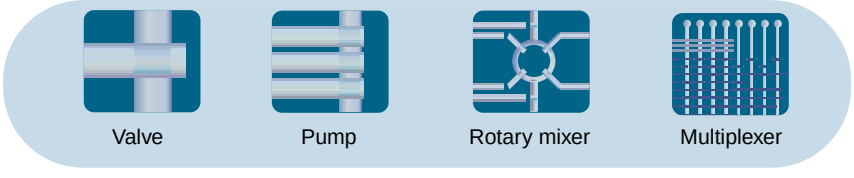

Figure 1. Fluidigm's on-chip devices can be used as tools to create basic or complex fluidic structures in an integrated system. Diagram supplied courtesy of Fluidigm Corporation, San Francisco, CA, USA.

Integrated bioassay chip

The Caltech group and Fluidigm are using multilayer soft lithography to produce an integrated bioassay chip that is capable of analyzing the contents of single cells. Actions are performed using a combination of valves, pumps, rotary mixers and multiplexers. The cell is first captured in a cage created by closing valves. It is then exposed to a drug candidate via a multiplexed input grid and, following incubation, travels to a fluorescence-based sorter[4] (Fig. 2). After sorting it travels to a second cage where it is washed with lysis buffer. The cell contents then pass through a narrowing channel where a reverse transcriptase-PCR (RT-PCR) cocktail is introduced; these reagents are subsequently mixed in a rotary mixer and RT-PCR is performed while the chip is put through a series of heating and cooling cycles. The resulting cDNA is detected using a laser,

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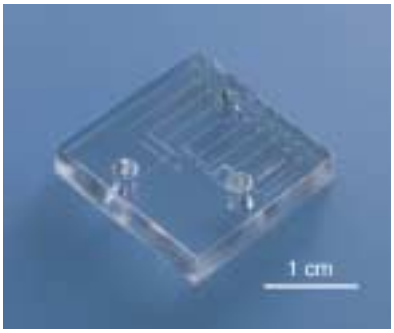

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Figure 2. A polydimethylsiloxane microfluidic chip designed for cell sorting. Diagram supplied courtesy of Fluidigm Corporation, San Francisco, CA, USA.

and then sized and sorted using a combination of pumps and valves before being pumped into a receptacle for further analysis.

The researchers have also carried out TaqMan™ assays using a similar device. Results were validated using conventional macroscopic analysis or the manufacturers' control protocols (Quake *et al.*, unpublished). 'The results have been very encouraging,' says Quake. 'We're quite optimistic that in the near future we will have a chip capable of performing single cell genetic and biochemical analyses.'

Users can design their own microfluidics systems using Fluidigm's computer-aided design (CAD) based software. Once a design is supplied, a mould can be printed and patterned within a day. Manufacturing is relatively easy because, unlike silicon, elastomers do not require clean room conditions for processing. Until now, innovation has been stifled by the length of time taken to produce new chip designs. 'A major benefit of this technology is that you can get very rapid turnaround times for prototypes,' Quake continues. 'You can find out quickly what works and what doesn't.' Standard polydimethylsiloxane adsorbs lipophilic compounds, but this problem can be overcome by either modifying the elastomer itself, or treating the device after manufacture to make it hydrophilic[5].

'This technology looks robust and easy to implement, even for non-experts,' says Sabeth Verpoorte of the University of Neuchatel's Institute of Microtechnology (Neuchatel, Switzerland). 'It will certainly open up routes to new microfluidic concepts based on pressure-driven flow. It will also be possible to expand the scope of applications to include many more types of solution matrices, since pressure-driven flow is not so sensitive to solution properties as electrokinetic flow.'

The next goal will be chips that are capable of more complex assays. Microfluidics is likely to find many more applications: these include genomic analyses; protein analysis, crystallization and purification; biochemical and electrophysiological assays; and gene expression and differential display analysis. It also has potential for use in drug delivery and diagnostics.

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Drug-eluting stents: flashy future or flash-in-the-pan?

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Recent data released from two clinical trials with different agents have highlighted the great potential for targeted drug delivery using drug-eluting stents in the reduction of clinical restenosis following angioplasty[1,2]. However, despite unprecedented results in some of these trials, some feel that the potential for these stents could be short-lived.

Restenosis

Percutaneous transluminal coronary angioplasty (PTCA) was first introduced in the late 1970s as an alternative to highly invasive coronary-artery bypass surgery to clear coronary vessels blocked by plaque. However, restenosis – the development of a new blockage from scar tissue – occurs in 30–50% of patients that are treated with balloon angioplasty[3].

Stents – cages of surgical-grade stainless steel resembling a scaffolding mesh – were then developed to eliminate elastic recoil and negative remodelling, and the widespread introduction of stents in the 1990s reduced the restenosis rate to 15–20%. However, restenosis continued

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