Drug Discovery – Advances in Microfluidics

a report by

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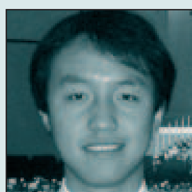
Targeting a unique molecule out of a large pool of similar chemicals with desirable efficacy is a tedious task in drug discovery, and is often time-consuming, labour-intensive and expensive. The idea of fast and reliable methods that can accelerate the drug discovery process while also reducing labour costs is highly appealing, and microfluidics seems to offer both of these aspects. Microfluidics has emerged as a state-of-the-art technique with the capability of manipulating small amount of fluids (10^{-9} to 10^{-18} litres).[1] A typical microfluidic chip consists of microchannels (1–100μm in size) that are connected with large pools of liquid supply, driven by syringe pumps and integrated with sensors and valves for dynamic control. It offers a variety of means for handling small volumes of liquids: filtering, synthesising and conducting highly sensitive analysis. Upon scaling down and optimisation, a conventional reaction system can be miniaturised onto a microscale chip, and many such microsystems can be performed in parallel; as a result, the processing rate is greatly increased. Microfluidics needs only a small aliquot of sample in operation, so it is a natural partner with chemistry and biology where a large quantity of sample is either too expensive or inaccessible. It is also possible to mimic the *in vivo* microenvironment of cells in microfluidic channels by patterning the cells and culture medium in 2D or 3D structures, which will improve the prediction of *in vivo* cell functions using the *in vitro* cell experiments.[2] In this article, we discuss several examples of advances in microfluidics in recent years – including active cell patterning, integrated biosensors and bioanalysers, chemical gradient generation and droplet manipulation – to demonstrate their potential applications in drug discovery.



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Cell Manipulation

One catalyst for the rise of microfluidics is the demand in molecular and cell biology for analytical methods with higher throughputs, higher sensitivity and higher resolution than conventional methods. A revolution in these areas could greatly benefit many fundamental techniques in drug discovery, such as high-throughput screening, bioanalysis, single-cell manipulations and toxic tests. Microfluidics, in particular poly-dimethyl-siloxane (PDMS)-based microfluidics, is a natural fit with these demands, and PDMS-based devices currently dominate the study of microfluidics. It is easy to design and fabricate PDMS devices, and the cost of materials and equipment is relatively low (e.g. a clean room is not necessary) compared with other microfabrication approaches; in addition, PDMS has an outstanding optical transparency, a low toxicity to biomolecules and a high permeability to oxygen and carbon dioxide, making it an ideal culture medium for cell growth, perturbation and observation. Therefore, PDMS-based microfluidic devices have gained a large degree of popularity, and many advances have been made towards their use in biological applications.

Microfluidic devices could be utilised to improve the conventional well-plate system to achieve high-throughput cell arrays.[3,4] A scalable experimental platform combining microfluidic addressability with quantitative fluorescent imaging has been developed to obtain realtime characterisation of gene expression in living cells. This approach could analyse hepatic inflammation by acquiring ~5,000 single-timepoint measurements in each automated experiment.[4] In addition to bulk experiments in cell behaviour studies, single-cell manipulations – e.g. trapping, releasing, separation and sorting – have been invented; single-cell arrays have also been demonstrated. A microfluidic bioreactor has been implemented to conduct long-term observation and regulate bacterial behaviour via the feedback from the bacterial population.[5] Combinations of optical detecting and optical trapping techniques with microfluidics also have their unique value. Optical tweezers could trap and move individual cells inside the microfluidic channels and enable the study of rapid cytological responses of living cells to environmental changes. Because the trapped cells are not in physical contact with the apparatus, this method provides a contaminant-free working condition due to reduced disturbance.[6,7]

Cell culture patterning is another cutting-edge topic in microfluidics. With cell culture medium patterned in 3D structures, it is possible to produce artificial extra-cell-matrices (ECMs) and mimic *in vivo* cell microenvironments. The latter will be crucial for improving the prediction of drug functions in the human body by *in vitro* experiments.[8] Scanning confocal or conventional fluorescence microscopy could be used to polymerise the photocurable materials delivered into microfluidic

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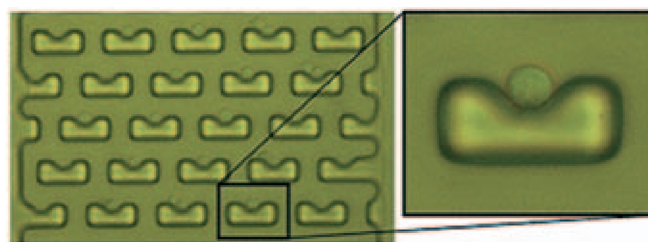
channels, and monolithic 3D structures could be fabricated and spatially aligned using dozens of different materials.[9] Another impressive experiment comes from the University of California, Berkeley, where ordered single-cell array (see *Figure 1*) is achieved without chemical treatment of surfaces.[10] The cells were loaded by flowing them into microfluidic channels; they were then trapped and protected from shearing forces by arrays of moulded PDMS wells. Cell–cell communications by contact or diffusion are controllable in this device, and single-cell study of metabolism and drug toxicity could be facilitated.

Materials other than PDMS have also been exploited for their potential significance in biology. One example is a re-circulation flow system that has been developed to accelerate DNA microarray hybridisation in a microfluidic device made of laminating Mylar and glass.[11] Microfluidic channels can be integrated with semiconductors to create a compact disposable optical-trapping microchip.[12] Electromagnetic fields are also powerful tools for manipulating living cells. For example, a prototype of single molecule sorting and steering has been realised using an electrical field on silica substrate.[13] Hybrids of microfluidic channels and complementary metal-oxide semiconductor chips bring the speed and programmability of integrated circuit (IC) chips to biology, with the aim of producing a universal cell manipulator.[14] One drawback of PDMS devices is their swelling in common chemical solvents, which hinders their applications in organic chemical synthesis. One counterpart is a solvent-resistant microfluidic DNA synthesiser based on per-fluoro-polyether (PFPE).[15] Although only a small batch of DNA was produced in the preliminary experiment, it is hoped that this technique can be combined with other biochemical amplification methods in order to realise whole-gene synthesis, sequencing and screening on a single chip (see *Figure 2*).

Chemical Gradient Generation

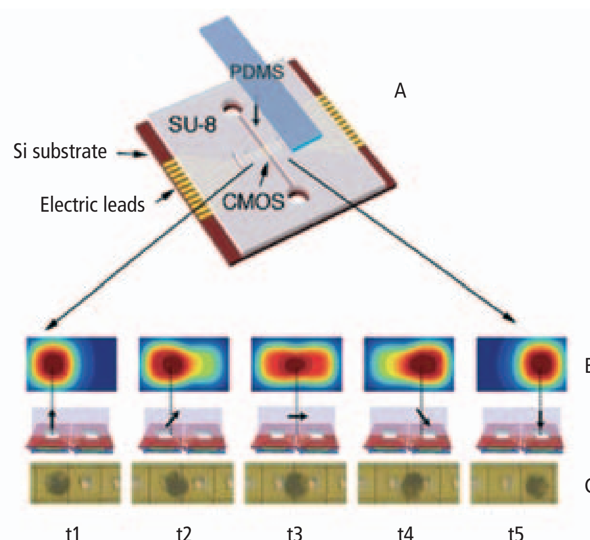
Generating a stable concentration gradient of desired chemicals is an important step for various applications in chemistry and biology, especially for the study of chemotaxis and toxic testing of cell responses in drug discovery. Due to the intrinsic laminar flow characteristics of microfluidic channels, convective transport of chemicals along the channel is much faster than diffusive transport across the channel width. Therefore, microfluidic channels are ideal tools for generating and maintaining stable concentration gradients. The simplest way to create a concentration gradient is to flow solutions of different concentrations simultaneously into the microchannels at the same speed. The side-by-side streams will establish a stable concentration gradient that will not be disturbed by diffusive flux over a relatively long distance. While some successful applications have been made in this fashion, the technique always produces sigmoidal concentration profiles and is sensitive to the flow speed and the position along the channels. The Whitesides group at Harvard has introduced a successful device that gets around these difficulties.[16] The key is using a gradient-generating network of microchannels that typically has a pyramidal shape. The network is fed with two source solutions that are split and delivered to the outlet in a number of streams, forming a concentration profile. This pyramidal network was later modified to deliver complicated shapes, such as exponential, linear or parabolic profiles.[17] In the modified design, the number of splitting and mixing stages increases logarithmically with the number of scales in the outlet gradient, compared with a linear increase of stages with the number of scales in the original 'Harvard' design. Thus, the modified design allows a much more compact array and provides a better gradient resolution. The chemical gradient can also be

Figure 1: Single-cell Array



Source: Di Carlo et al., 2006.[10]

Figure 2: Complementary Metal-oxide Semiconductor/ Microfluidic Hybrid Cell Manipulator



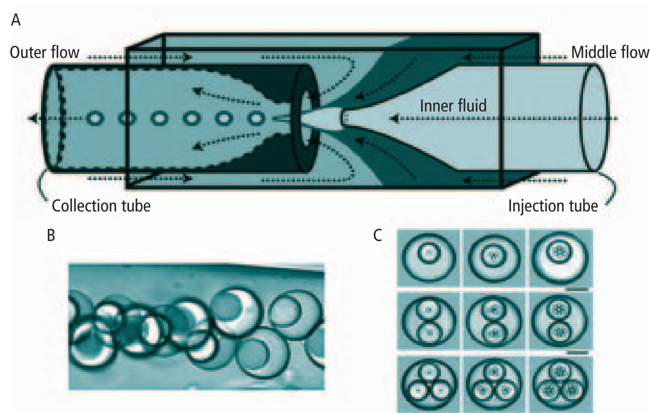
(A) Schematic. (B) Time-dependent magnetic field patterns; arrows show force direction. (C) A single magnetically tagged cell is trapped and moved using the magnetic field. Source: Lee et al., 2007.[14]

applied to pattern biomolecules – e.g. proteins, oligonucleotides and artificial polymers – on surfaces.[18] This method could be useful for immobilising biomolecules, studying the migration and polarity of cells and screening phenotypes.

Droplet Operation

The formation of droplets inside microchannels is a unique component of microfluidics, extending the continuous flow of microfluidics to a discrete state and permitting additional handling of sample liquids.[19] This could be an effective tool for improving current techniques in drug discovery, e.g. cell arrays, mass spectroscopy, protein crystallisation, micromixers and microreactors in organic synthesis. Since the upsurge of soft-lithography and flow-focusing techniques, these techniques have become useful tools for generating uniform nanolitre-size droplets at a frequency as high as 1kHz. These small droplets are perfect candidates for microreactors: their high surface-to-volume ratio allows very rapid thermal transfer, while their tiny size permits very fast mixing (usually in a zigzag channel or electrical field), promoting a homogeneous microenvironment for chemical reactions. Furthermore, in the steady laminar flow along the microchannels, the position of each droplet exactly corresponds to an individual timepoint, indicating the residence time since the mixing or reaction started, i.e. the time at which the droplet was formed. Therefore, the temporal variation of a reaction can be observed in space, and the kinetics of the whole reaction can be obtained at a single time. This kind of

Figure 3: Multiple-emulsion Capillary Device



(A) Geometrical set-up. (B) Double-emulsion droplets.[27] (C) Triple-emulsion droplets.[28]

experiment has been performed in a matter of a few milliseconds.[20] Another interesting example is the Phase Chip,[21] which includes many distinct components that can control the amount of water inside a droplet and thus adjust the solute concentration. Protein crystallisation rates are enhanced inside the manipulated droplet, as are the kinetics of nucleation and crystal growth. These novel techniques demonstrate the advantages of droplet-based microfluidics on chemical analysis and protein crystallisation, which could play an important role in drug discovery.

Early experiments in droplet-based microfluidics applied only simple regulations on the flows and droplets; as a result, the droplets moved passively in the flow field, where their motions were controlled by the channel network geometry for most of the time. In order to realise complex microfluidic platforms that will eventually lead to a total analysis system and microfactory on-chip, researchers are seeking versatile means of integrating compact and effective control modules into microfluidic systems, for example using electrical fields to manipulate or separate droplets.[19,22,23] Previously, novel designs and regulations in microfluidic platforms were mainly based on intuition and trial-and-error. As more and more components are integrated into the system, the complexity exponentially increases, and the mass production of microfluidic platforms necessitates the standardisation and modularisation of individual components. In recent years, the theoretical study and computational simulation of microfluidic channels has increased quickly,[24–26] paving the way for the rapid development and optimisation of microfluidic networks for industrial applications.

Besides the adjustment and external confinement of microfluidic channels, new research opportunities could also come from the droplets themselves. Compared with continuous flow, the high number of interfaces present in the emulsions is a particular characteristic of droplet-based microfluidics. By tailoring the surface property of the droplets, a large number of new features could be assigned to the multiphase emulsion systems. Uniform double- and triple-emulsion droplets have been generated using a clever design of microcapillary devices.[27] The core geometry of these multiple-emulsion droplets provides various useful applications, such as producing microcapsules to protect living cells or building microstructures to mimic *in vivo* environments (see *Figure 3*).

Summary

Microfluidics is a potent tool that could boost productivity in drug discovery. The development of microfluidics brings the hope of miniaturising the total synthesis, analysis and testing system onto a small chip. Parallel productions and nanoscale-volume manipulations are easily realised in microfluidics, which would promote the efficiency and cut the cost of drug discovery. With microfluidics, techniques for single-cell manipulation and dose testing have advanced rapidly. Significant efforts are still needed to transfer laboratory results to industrial and commercial products, such as developing standards for components, improving the biocompatibility and chemical resistance of materials and simplifying the operating process. Despite these challenges, the bright future of microfluidics in drug discovery cannot be underestimated. ■

Acknowledgement

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1. Whitesides GM, The origins and the future of microfluidics, *Nature*, 2006;442(7101):368–73.
2. Dittrich PS, Manz A, Lab-on-a-chip: microfluidics in drug discovery, *Nat Rev Drug Discov*, 2006;5(3):210–18.
3. Yu HM, Alexander CM, Beebe DJ, A plate reader-compatible microchannel array for cell biology assays, *Lab on a Chip*, 2007;7(3):388–91.
4. King KR, et al., A high-throughput microfluidic real-time gene expression living cell array, *Lab on a Chip*, 2007;7(1): 77–85.
5. Balagadde FK, et al., Long-term monitoring of bacteria undergoing programmed population control in a microchemostat, *Science*, 2005;309(5731):137–40.
6. Eriksson E, et al., A microfluidic system in combination with optical tweezers for analyzing rapid and reversible cytological alterations in single cells upon environmental changes, *Lab on a Chip*, 2007;7(1):71–6.
7. Eriksson E, et al., Optical manipulation and microfluidics for studies of single cell dynamics, *Journal of Optics A: Pure and Applied Optics*, 2007;(8):S113.
8. Toh YC, et al., A novel 3D mammalian cell perfusion-culture system in microfluidic channels, *Lab on a Chip*, 2007;7(3):302–9.
9. Cheung YK, et al., Direct patterning of composite biocompatible microstructures using microfluidics, *Lab on a Chip*, 2007;7(5): 574–9.
10. Di Carlo D, Wu LY, Lee LP, Dynamic single cell culture array, *Lab on a Chip*, 2006;6(11):1445–9.
11. Lee HH, et al., Recirculating flow accelerates DNA microarray hybridization in a microfluidic device, *Lab on a Chip*, 2006;6(9): 1163–70.
12. Cran-McGreehin S, Krauss TF, Dholakia K, Integrated monolithic optical manipulation, *Lab on a Chip*, 2006;6(9):1122–4.
13. van den Heuvel MGL, De Graaff MP, Dekker C, Molecular sorting by electrical steering of microtubules in kinesin-coated channels, *Science*, 2006;312(5775):910–14.
14. Lee H, et al., Integrated cell manipulation system – CMOS/microfluidic hybrid, *Lab on a Chip*, 2007;7(3):331–7.
15. Huang YY, et al., Solvent resistant microfluidic DNA synthesizer, *Lab on a Chip*, 2007;7(1):24–6.
16. Dertinger SKW, et al., Generation of gradients having complex shapes using microfluidic networks, *Anal Chem*, 2001;73(6): 1240–46.
17. Campbell K, Groisman A, Generation of complex concentration profiles in microchannels in a logarithmically small number of steps, *Lab on a Chip*, 2007;7(2):264–72.
18. Jiang XY, et al., A general method for patterning gradients of biomolecules on surfaces using microfluidic networks, *Anal Chem*, 2005;77(8):2338–47.
19. Joanicot M, Ajdari A, Applied physics – Droplet control for microfluidics, *Science*, 2005;309(5736):887–8.
20. Lucchetta EM, et al., Dynamics of Drosophila embryonic patterning network perturbed in space and time using microfluidics, *Nature*, 2005;434(7037):1134–8.
21. Shim JU, et al., Control and measurement of the phase behavior of aqueous solutions using microfluidics, *J Am Chem Soc*, 2007;129(28):8825–35.
22. Link DR, et al., Electric control of droplets in microfluidic devices, *Angewandte Chemie-International Edition*, 2006;45(16): 2556–60.
23. Cho SK, Zhao YJ, Kim CJ, Concentration and binary separation of micro particles for droplet-based digital microfluidics, *Lab on a Chip*, 2007;7(4):490–98.
24. Garstecki P, Stone HA, Whitesides GM, Mechanism for flow-rate controlled breakup in confined geometries: A route to monodisperse emulsions, *Phys Rev Lett*, 2005;94(16).
25. Muradoglu M, Stone HA, Mixing in a drop moving through a serpentine channel: A computational study, *Phys Fluids*, 2005;17(7).
26. Jensen MJ, Stone HA, Bruus H, A numerical study of two-phase Stokes flow in an axisymmetric flow-focusing device, *Phys Fluids*, 2006;18(7):10.
27. Utada AS, et al., Monodisperse double emulsions generated from a microcapillary device, *Science*, 2005;308(5721):537–41.
28. Utada AS, et al., Dripping, Jetting, Drops, and Wetting: The Magic of Microfluidics, *MRS Bulletin*, 2007;32:702–8.

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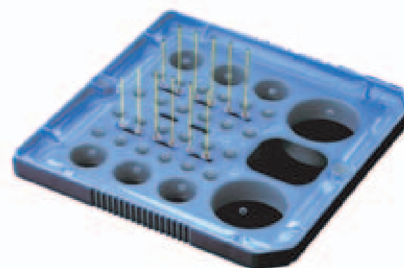

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