Biochips: from chipped gels to microfluidic CDs

The first NMHCC conference on *Molecular Arrays: Microfabrication for Biochips* convened at the Hyatt Sainte Claire in San José, CA, USA on the 13–14 July 1998. Explorations at the interface of biology and microelectronics have existed for some time. In the 1980s, Genex explored the possibility of using proteins to build microchips; however, in the 1990s, Affymetrix and Affymax have turned the tables by using semiconductor manufacturing technologies for the microfabrication of DNA and peptide chips [Pirrung, M.C. (1997) *Chem. Rev.* 97, 473–488]. The Chair, Marc Madou (Ohio State University, Columbus, OH, USA) began the meeting by asking if the two technologies, molecular arrays [Case-Green, S.C. *et al.* (1998) *Curr. Opin. Chem. Biol.* 2, 404–410] and microfluidics [Kopp M.U. de Mello, A.J. and Manz, A. (1998) *Science* 280, 1046–1048] – which have evolved independently, might be successfully linked in the future.

Technology

Molecular arrays

A tenuous connection between molecular arrays and microfluidics was provided by Kalim Mir (University of Oxford, UK) who described one of the earliest methods for synthesizing combinatorial molecular arrays [Southern, E.M. (1988) PCT/GB89/00460]; it involves a microfluidic method in which selective pumping of reagents through a series of channels (500 μm wide) creates a two-dimensional library of oligonucleotides on a polypropylene surface (~500 μm^2 feature size). Using the same combinatorial principle, William Chiang (Sarnoff, Princeton, NJ, USA) described a means for synthesizing a spatially addressable library of drug lead compounds.

A novel application for porous silicon (silicon wafers interfused with non-connecting pores), as a substrate for DNA array fabrication was described by Eric Eastman (GeneLogic, Gaithersburg, MD, USA). GeneLogic print their medium-density DNA arrays by using drop-on-demand robot technology. They find that porous silicon, with its large surface area, allows for increased probe attachment, and that hybridization analysis can be performed more effectively because of fluid flow through the perforations. Chris Boles (Mosaic Technologies, Boston, MA, USA) described the coupling of DNA-probe arrays with a more common flow-through method – electrophoretic separation in polyacrylamide gels. Probes are initially fixed in layers of gel by proprietary acrydite phosphoramidites, which copolymerize with acrylamide. Nucleic acid targets that are electrophoresed through the gel become retarded in gel layers containing complementary probes, but pass through layers without complements. Hybridization and washing is combined in a single process. It is a very effective means for discriminating mismatches.

Microfluidics – lab on a chip

Various types of laboratory manipulations and analyses would be more convenient and efficient if done in parallel or in a miniature format. Microfluidic devices are being developed for such applications using a toolbox that includes micromachining, photolithography and a variety of other technologies used for making MEMs (micro-electromechanical systems). Thomas Schulte (Micronics, Redmond, WA, USA) uses computer-aided design (CAD) to direct laser micromachining of a thin plastic film originally developed for audio-visual applications. When this film is sandwiched between two uncut sheets of plastic, channels are created.

Components such as pumps, valves, dispensors, reactors, mixers and separators can be emulated. Fluids are dispensed to different areas in the chip where specific processes are carried out. The movement of fluids can be visualized in real-time and this was used to demonstrate various means of controlling fluid movement. The liquid-handling approaches were described by Andrea Chow (Caliper Technologies, Palo Alto, CA, USA) and Pamela York (Orchid Biocomputers, Princeton, NJ, USA) included electro-osmotic, electrophoretic and electrohydrodynamic movement of fluids.

Madou presented the adaptation of a common technology. The conventional type of compact disc or CD found in most homes for musical playback was adopted as the platform for a microfluidic device based on centrifugation. A plastic microfluidic construct was attached to the surface of a CD. When the disc is spun, centrifugal force overcomes capillary forces and the fluid is propelled from the centre towards the edge of the disc. Control of fluid transfer from one compartment to another is achieved by manipulating the spin velocity of the disc. Each compartment will implement a particular processing step such as separation or mixing. An attractive feature of the approach is that the CD player's optics could be used to read informatics residing in the optical layer of the disc as well as to observe colour changes associated with reactions.

The interface of microfluidic devices with industrial/commercial applications, and indeed with molecular arrays, is an important area of development. Allen Northrup (Cepheid, Sunnyvale, CA, USA) stressed that to be compatible with

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many biological specimens, miniaturized devices needed microlitre and millilitre sample-handling provisions as well as sub-microlitre capabilities. For example, in expression analysis, nanolitre or picolitre sample-handling capabilities may not be able to represent the total diversity of mRNA species found in a particular anatomical tissue sample.

Re-usable versus disposable chips

The ability to re-use a chip is an important question that was addressed at the conference. Molecular arrays, which rely on molecular binding, often cannot be reliably re-used. Nevertheless, the research arrays described by Mir and Richard Murray (Eos Biotechnology, South San Francisco, CA, USA) may be used several times. Microfluidic devices by their nature are easier to re-use, and the research community in general demands re-usable devices to minimize costs. However, disposable diagnostic devices have gained acceptance in the general population (e.g. pregnancy testing kits) and are more commercially viable.

Applications

Gene expression chips

Gene expression analysis has become the first major area of application of DNA arrays. Moreover, the current demand from the bio-industry seems likely to increase given the scale of interest from manufacturers and the research community evident at the meeting. There are two approaches, the first of which is Affymetrix's genechip – this uses a set of 20 oligonucleotide probes to address each gene in a sample population. Jerry Olsen (Oncormed, Gaithersburg, MD, USA) presented reproducibility results for Affymetrix's 250 gene cancer chip. Several chips from the same batch (made in parallel using a single mask for photo-exposure) were hybridized to the same sample. A significant degree of variability was found between chips: 19 genes were reported to be differentially expressed (according to the manufacturer's suggested criteria) where none should have been.

Dari Shalon (Synteni, Fremont, CA, USA) described the second approach to gene expression analysis which involves hybridization to chips composed of long DNA probes (up to 1000 nucleotides) robotically spotted onto a glass substrate by capillary deposition. It was suggested that the Synteni approach offers several inherent advantages – certainly, one probe per gene and two-channel recording provides easier data interpretation – but there was no impartial evaluation of Synteni chips presented. Murray and Eastman also described arrays printed with long DNA fragments (>50 nucleotides).

Genotyping

Tim Woudenberg (PE Applied Biosystems, Foster City, CA, USA) described a 'credit card' format for an array of TaqMan™ PCR assays for SNP (single nucleotide polymorphisms) mapping – an application that would require many genomic sites to be assessed simultaneously [Livak, K.J., Marmaro, J.J. and Todd, J.A. (1995) *Nat. Genet.* 9, 341–342]. A 1024-well (100 nl volume per well) card was described. Reactions in each well could be multiplexed; three different TaqMan probes can currently be resolved. Maryanne O'Donnell (Sequenom, San Diego, CA, USA) described the marriage of DNA arrays with mass spectrometry [Little, D.P. *et al.* (1997) *Nat. Med.* 3, 1413–1416]. Samples spotted onto a surface undergo enzymatic extension reactions to determine the identity of SNPs as well as repeat numbers of STRs (short tandem repeats – another type of DNA marker).

The p53 gene, which carries regions with high densities of mutations, is currently the touchstone for evaluating mutation-detection technologies. Olsen presented an evaluation of Affymetrix's p53 genechip and although it was found to be narrowly beaten by ABI Sequencing for accuracy, it appeared to be better at revealing heterozygosity in the sample. Joerg Baier (Hyseq, Sunnyvale, CA, USA) and O'Donnell also presented methods for evaluating p53 mutations – these technologies were geared towards mutation analysis service providers.

Point-of-care devices

Hand-held biosensors for measuring glucose levels are commonly used by diabetics. One direction for biochip technology, is the development of devices that could be deployed in a doctors' office for a variety of gene-based analyses. Several presentations addressed this area. Schulte aims to do blood viral-load testing using a hand-held device, while Northrup described a PCR-in-a-briefcase for field analysis for military applications. The latter has evolved into a hand-held device in which sample preparation is performed 'in the field' and analysis is achieved by an accelerated PCR (35 cycles in 20 min).

Concluding remarks

Molecular arrays are highly informative means for analysing the presence, absence and identity of sequences within DNA or RNA. They are also useful for measuring the interactions of a library of ligands such as peptides, antisense oligonucleotides and small-molecule drugs with a target macromolecule. Various levels of sample preparation are required before analysis with molecular arrays can proceed so it would be convenient to integrate such preparations on a chip. This meeting offered a good opportunity for cross-pollination of ideas for people in the microfluidics and molecular array fields as well as being a good primer for those outside these fields. Many participants were very generous in sharing their experiences in developing and using biochips. It is likely that in the coming years, microfluidics and molecular array technologies will become integrated together into various types of miniaturized total analysis systems [μTAS; Kopp M.U. de Mello, A.J. and Manz, A. (1998) *Science* 280, 1046–1048].

Kalim U. Mir
Department of Biochemistry
University of Oxford
South Parks Road
Oxford, UK OX1 3QU
e-mail: kalim@bioch.ox.ac.uk