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Developments in microarray technologies

David N. Howbrook, Anne M. van der Valk, Meg C. O'Shaughnessy, Dipak K. Sarker, Simon C. Baker and Andrew W. Lloyd

The focus of high-throughput drug discovery has progressed through the genome and the transcriptome and is now moving towards more difficult problems in assessing the proteome, glycome and metabolome. Microarrays are currently the major tool in the assessment of gene expression via cDNA or RNA analysis; however, they are also used to screen libraries of proteins and small molecules. Microarrays have helped to extract more information from smaller sample volumes and enabled the incorporation of low-cost high-throughput assays in the drug discovery process. The technology continues to develop and is being rapidly transferred into more challenging areas, with the potential to further aid and enhance the drug discovery process through the development of, for example, proteomic, glycomic and tissue arrays.

David N. Howbrook
Anne M. van der Valk
Dipak K. Sarker
Andrew W. Lloyd
School of Pharmacy and Biomolecular Sciences
University of Brighton
Moulsecoomb
Brighton, UK BN2 4GJ
e-mail: D.K.Sarker@brighton.ac.uk
Simon C. Baker
School of Biological and Chemical Sciences
Birkbeck University of London
Malet Street
Bloomsbury
London, UK WC1E 7HX
David N. Howbrook
Anne M. van der Valk
Meg C. O'Shaughnessy
ABgene
ABgene House
Blenheim Road
Epsom
Surrey, UK KT19 9AP

The advent of miniaturized technology for the study of molecule–molecule interactions has changed the way that new challenges are approached in cell biology. Drug discovery has moved away from bioinformatics-directed gene and protein hunting towards screening technologies that are able to cope with large numbers of small quantities of targets. Microarrays have provided a means by which microspots of DNA, protein or small organic compounds can be probed with possible binding ligands. Together with advances in techniques – particularly fluorescence-based approaches – for detecting the incidence of interactions, this has enabled the simultaneous analysis of thousands of variables in a single experiment.

Gene expression analysis

The first use of microarrays was in immunological assays [1,2] but the advent of genome sequencing created a demand for simultaneous multi-gene analysis. The 'DNA chip' combined advances in high-throughput oligonucleotide synthesis with PCR amplification to generate arrays of probes that are capable of detecting changes in the transcriptome via hybridization. Advances in detection and computation, and an increasing recognition that the expression of a gene was as important as its presence or absence, led to the refinement of systems that are capable of measuring changes in the concentration of mRNA of tens of thousands of genes on a chip only a few centimetres across.

Array-based gene expression analysis (immobilized DNA probes hybridizing to RNA or cDNA targets) has become an important primary tool in many research projects. The abundance of a particular species of mRNA is indirectly proportional to the amount of protein in the cell. However, the differing half-lives, efficiency of translation and other post-transcriptional events affecting each mRNA species, mean that it is difficult to correlate the abundance of two different mRNA species to their relative protein concentrations in the cell [3]. Protein arrays are now being used to examine enzyme–substrate, DNA–protein and protein–protein interactions (reviewed in [4]) and to some extent address this limitation. However, an integrated approach to array technology is required to reflect the true complexity of the transcription, translation and post-translational machineries. Current applications of microarrays include genome-wide genotyping [5,6] and expression profiling [7,8]. However, for DNA and protein microarrays to be reliable tools, they must possess probe sequences that hybridize with high sensitivity and specificity: results must be reproducible and quality control systems must be established. Potential applications of microarrays in the biomedical field include the assessment of RNA and protein alterations as diagnostic

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markers in clinical samples, most notably within cancer, (see Box 1 and [9]).

DNA microarrays

Expression analysis for the quantitative gene expression of many genes can be performed using either one- or two-colour fluorescent schemes. One-colour analysis is primarily used for arrays prepared by photolithography. Affymetrix (http://www.affymetrix.com) patented this process under the trade name GeneChip™ [10]. In this method, expression profiles for each sample are generated on a different chip using a single fluorescent label, such as phycoerythin, and the different images are then compared. A two-colour analysis protocol has subsequently been developed, whereby two RNA samples are labelled separately with different fluorescent dyes, for example cyanine 3 and cyanine 5 [8]. These labelled probes hybridize to a printed array of cDNA; when the microarray is scanned, the fluorescent signals can be overlaid to visualize genes that have been activated or repressed.

DNA microarrays can be fabricated using short oligonucleotides (15–25 nucleotides), long oligonucleotides (50–120 nucleotides) or PCR-amplified cDNA (100–3 000 base-pairs). *In situ* oligonucleotide synthesis on a solid support involves the use of photolithography to build up each element of the array, nucleotide by nucleotide up to 20 bases [11]. Alternatively, longer nucleotides and cDNA can be spotted directly onto glass slides. Between 10,000 and 30,000 spots can be mechanically deposited onto a single glass slide by robotic instrumentation designed to print from metal pins or ink-jets.

In the drug discovery and therapeutics arena, microarrays have been used for expression analysis of cells or tissues in different disease states, single nucleotide polymorphism (SNP) analysis, pharmacogenomics and toxicogenomics. The information obtained from these studies can be used to design arrays that assist in the selection of custom and rational drug design.

Current uses of DNA microarrays

Although the DNA microarray is being supplemented by other technologies, it is currently used, and will continue to be developed, in many areas of drug discovery. In the study of cancer, the ultimate goal will be to link the data obtained from DNA microarrays to the proteomic and metabolomic findings because the function of the cell is affected more by the proteins and metabolites within it, rather than the mRNA levels *per se*. A complete characterization of some of the more complex cancers will result in new drug targets, improved diagnosis and more successful treatment.

Box 1. Useful web links for microarray technologies

General links

Genomics Proteomics
http://www.genomicsproteomics.com
Lab-on-a-chip
http://www.lab-on-a-chip.com
Bio-IT World
http://www.bio-itworld.com
Pharmocogenomics Online
http://www.pharmacogenomicsonline.com
BioArray Software Environment – *BASE*
http://base.thep.lu.se

DNA microarrays

The Brown Lab, Stanford University
http://cmgm.stanford.edu/pbrown
Affymetrix
http://www.affymetrix.com

Protein microarrays

ESF Programme on Integrated Approaches for Functional Genomics
http://www.functionalgenomics.org.uk/sections/resources/protein_arrays.htm

Glycomics

NIGMS Consortium for Functional Glycomics
http://web.mit.edu/glycomics/consortium/
Glycominds
http://www.glycominds.com
GlycoSuite DB
http://glycosuite.com
University of New Hampshire Centre for Structural Biology
http://www.glycomics.unh.edu
Oxford Glycobiology Institute list of publications
http://www.bioch.ox.ac.uk/glycob/publications.html

Tissue arrays and cell microarrays

NHGRI Tissue Microarray Project
http://www.nhgri.nih.gov/DIR/CGB/TMA
UCLA Tissue Array Core Facility
http://www.genetic.ucla.edu/tissuearray
Whitehead Institute
http://www.wi.mit.edu/nap/features/nap_feature_sabatiniappt.html

DNA microarrays have had a significant impact on our understanding of normal and abnormal cell biochemistry and, thus, on the choice of targets for drug design. However, their use has not been restricted to human cell biology and is also being developed in many other drug-related fields. Community profiling of bacteria [12,13] could lead to new avenues of research in preventative medicine. In this area, the DNA microarray is an ideal tool for the identification

of bacterial species in a mixed population: the DNA or cDNA from an entire bacterial population can be isolated and hybridized to an array of 16S ribosomal DNA fragments giving information on both the abundance and identity of the bacteria in a particular environment [12]. The standard application of such microarray technology to the medical microbiology lab would certainly aid epidemiology and diagnosis [14], if costs can be reduced significantly.

Protein and peptide microarrays

DNA arrays are limited to providing information on the identity or amount of RNA or DNA present in a sample, providing that suitable controls are available. Translational products of genes can not be analyzed on such arrays and, therefore, require the use of polypeptide-based arrays. Most drug targets are proteins, therefore, protein and peptide microarrays are set to have an important impact on drug discovery. To date, such microarrays have not been used to their full potential due to difficulties with the technology [15]. An important challenge when producing protein microarrays is maintaining functionality, such as post-translational modifications and phosphorylation. An important consideration with protein-array surface chemistry is that the chemistry must permit immobilized proteins to retain both secondary and tertiary structure and, thus, biological activity. One of the first technical challenges with protein microarrays is to fix a protein or protein ligand to arrays in a biologically active form. Most arrays are either glass or silicon slides treated with an aldehyde to immobilize the protein [16]. Other immobilizing coatings include aluminium or gold, and hydrophilic polymers. Alternatively, proteins can be imprinted on a porous polyacrylamide gel, similar to that used in electrophoresis, and immobilized using a coupling agent that forms a covalent bond with amine groups on the protein molecules [17]. The gel provides a biocompatible aqueous 3D environment, so immobilized proteins can undergo binding reactions in solution. Tethered proteins or peptides are free to assume their native conformations and retain their functionality.

Overcoming degradation

Another alternative being developed is photolithography [18]. Miniature wells are etched onto the surface of silicon chips where the proteins or antibodies are located in flow chambers on the chip so that they are always in aqueous solution, preventing the protein being denatured. Proteins are then detected by fluorescence labelling using a rapid-scanning reader. This technique can be used to produce high-density arrays that are capable of detecting up to 10,000 proteins in parallel and could be suitable for target discovery and validation.

A protein *in situ* array (PISA) method has been described [19] in which proteins are synthesized *in vitro* by cell-free transcription and translation from PCR DNA, and directly immobilized onto suitable surfaces in array format. This method overcomes the problems of insolubility or degradation that are associated with existing protein overexpression systems.

Alternative strategies

Alternative strategies for protein microarrays involve the use of capture agents. Slides are often imprinted with antibodies, which can be monoclonal, polyclonal, antibody fragments or synthetic polypeptide ligands. However, the problem with this method is that large numbers of different antibodies are needed.

Other options include aptamers – single-stranded oligonucleotides that bind specifically to protein [20]. Aptamers have an advantage over antibodies because they can be imprinted using the same technology as mRNA expression arrays.

Another important consideration in the development of protein array technology is the choice of detection method. Those methods currently employed include ELISA-based assays with enzymatic or fluorescent labels, or universal protein stains. There are, however, disadvantages with these methods: ELISA-based assays can yield non-specific protein-antibody interactions, labelling proteins fluorescently can reduce the quantitative accuracy of assays, and universal protein staining is not an option if the capture molecules are themselves proteins (e.g. antibodies). Other methods of detection that have been optimized for use with protein microarrays include MS analysis and surface plasmon resonance [21].

Houseman *et al.* [22] described a novel approach to study protein phosphorylation and its potential inhibition in a peptide microarray format by the quantitative analysis of protein kinase activity. Previously, protein kinase activity required the purification and characterization of samples of enzymes from cell lysates. By contrast, Houseman's peptide microarray requires only small quantities of protein and can be used to develop high-throughput assays to monitor the activity of multiple protein kinases. The protein kinase family is the largest dominant group of oncogenic proteins [23], implicated in many pathologies, and are important drug targets.

Applications

Applications of protein and peptide microarray in drug discovery include the identification of protein–protein,

enzyme–substrate and protein–small molecule interactions [16]. Zhu *et al.* [24] analyzed the substrate-specific activities of 119 yeast protein kinases using 17 different test substrates with protein microarray technology, and MacBeath and Schreiber have provided further demonstrations of the potential of functional protein microarrays in drug discovery [16]. The advantages of protein microarrays over conventional methods, such as ELISA and western blotting, are that they use less sample and are relatively quick to perform. Protein arrays are thought to be 10–100-times faster than conventional methods (such as two-hydrid systems) [25].

The future of protein microarrays could be 'custom' arrays designed to capture a few proteins of interest – unlike DNA microarrays , which capture thousands of genes. Limitations of array density have been addressed to some extent by Fodor *et al.* [26], who have shown that it is possible to produce a high-density peptide microarray using photolithography.

Glycomics

Although peptide arrays are delivering increasing amounts of information, current arrays do not address the problem of post-translation modification. Many proteins and biomolecules are modified by the covalent attachment of sugar residues, known as glycans. Many biological processes involve sugar–receptor binding and microarrays will become an important tool for the study of such interactions in this rapidly expanding field.

Neoglycolipids, spotted onto nitrocellulose and PVD, provide an example of a carbohydrate micorarray [27]. The neoglycolipids were probed with proteins of known carbohydrate binding specificity to confirm identification of predicted protein–oligosaccharide interactions. Binding of the carbohydrates to the membranes was verified by the use of fluorescently labelled neoglycolipids or primulin stain for non-fluorescently labelled neoglycolipids. The carbohydrate composition of the bound moieties was identified using *in situ* analysis by MS. This array technology is interesting because the oligosaccharides to link to the lipid can be derived from diverse sources; for example, glycoproteins, proteoglycans, glycolipids, whole cells, organs and synthetic oligosaccharides. By binding known oligosaccharide sequences to the membrane, particular lectins or protein motifs can be identified. Alternatively, by binding unknown oligosaccharide structures to the membranes, proteins of known sequence can be screened to investigate which oligosaccharides they bind to.

An alternative approach might enable the identification of pathogenic micro-organisms due to unique glycans expressed on their cell surface [28]. Glycans have been spotted to the surface of nitrocellulose membranes and probed using antibodies raised against the micro-organisms from which the glycans were derived. The results were encouraging; the antibodies detected their specific glycans, indicating that from a clinical blood sample, specific infectious agents could potentially be identified.

Tissue and cell microarrays

Analysis of gene or protein expression levels can only begin to provide us with relevant information about the biological function of the gene, its potential clinical impact or its suitability as a drug target. Functional genomics enables the validation of targets that have been identified by microarray screening.

Conventional histological analysis of tissue specimens is a slow and labour-intensive process: tissues are first preserved in formalin before being embedded in paraffin for sectioning, staining and microscopic analysis on individual glass slides. The number of possible investigations that can be performed is restricted (to a few hundred) by the size of histological sample available.

In 1998, Kononen and Kalloiniemi [29] developed tissue microarrays (TMAs) whereby an ordered array of tissue samples are placed on a single slide. Once constructed, the TMA can be probed with a molecular target (DNA, RNA or protein) for analysis by immunohistochemistry, fluorescence *in situ* hybridization (FISH) or other molecular detection methods [30], enabling high-throughput *in situ* analysis of specific molecular targets in hundreds or even thousands of tissue specimens. The most significant advantage of microarray analysis is that all tissue specimens are treated in an identical manner. Thus, reagent concentrations are identical for each case, as are incubation times, temperatures and wash conditions [31]. Only small amounts of reagent (microlitre volumes) are required to analyze an entire array, offering greater consistency at reduced cost.

TMA construction and application

The construction of a TMA involves the assembly of up to 1000 needle biopsies taken from paraffin-embedded tissue specimens. The biopsies are embedded in a recipient paraffin block at defined array co-ordinates, to produce a master block that can then be sectioned into 200–300 slices (Fig. 1). In this way, ~200,000–300,000 individual assays can be produced from a single block [32,33]. The procedure causes such minimal damage to the original tissue block that it could still be used for subsequent large-section analysis. Archival specimens up to 60 years old have been used in this way [34] but RNA and antigens do not survive the process. By using frozen tissues in place of formalin-fixed tissues, antigens and RNA can be preserved [35]. To a large extent, validation of TMA studies is dependent on the

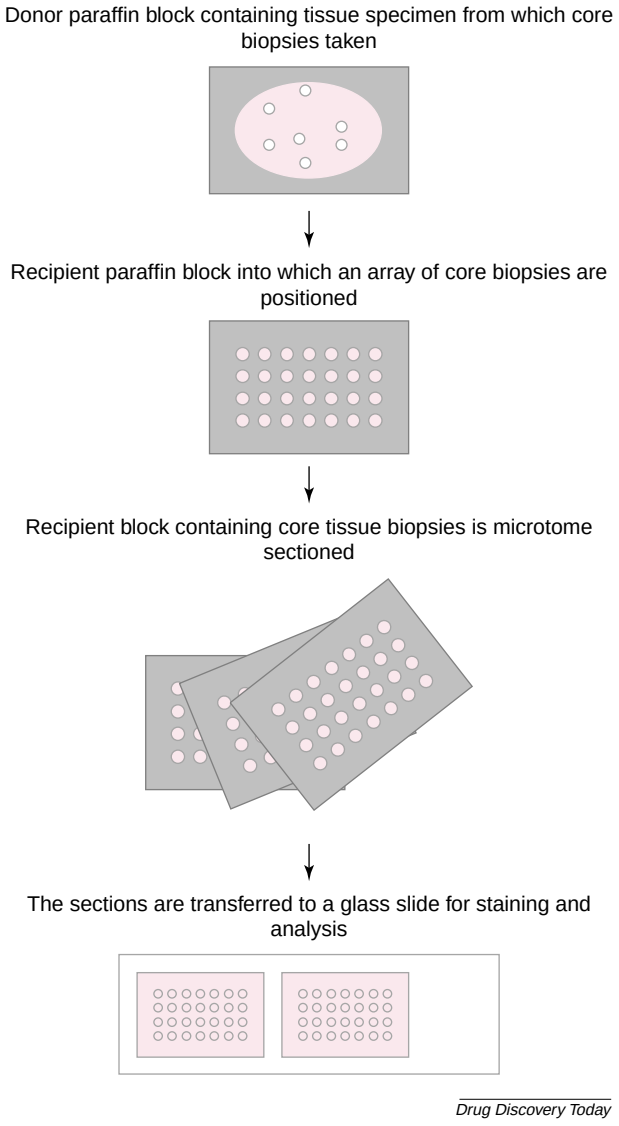

Figure 1. Schematic for the manufacture of tissue arrays

tissue type and marker under investigation. However, the greatest potential disadvantage of tissue microarray analysis remains the possibility of microdiversity of pathology within a tissue sample.

Potential applications of TMAs are, nevertheless, far reaching. In contrast to cDNA microarrays, in which up to 5000 genes from a single tissue can be analyzed simultaneously, in TMAs, a single probe can be used to analyze up to 1000 tissues. It is therefore possible to study gene targets that have been discovered by cDNA microarrays or other genomic studies. To date, almost all published TMA studies have been related to the analysis of tumours (for example, multi-tumour analysis, cancer progression analysis, or prognosis analysis in cancer studies). However, TMAs can also be applied directly to drug discovery for candidate gene distribution analysis and have also been used in the experimental analyses of cell lines [36].

An alternative to the use of whole tissue specimens is the use of live cell microarrays (Fig. 2). In this technique, first described by Ziauddin *et al.*[37], adherent mammalian cells are cultured on a glass slide that has been printed at defined locations with different cDNAs in an aqueous gelatin solution. Upon addition of a lipid transfection reagent, the cells take up the DNA and create spots of localized transfection within a lawn of non-transfected cells. These clusters of ~30–80 live cells actively express defined gene products that can be visualized using a variety of detection assays such as *in situ* hybridization, immunofluorescence or autoradiography.

Advantages

The advantage of this technique is, because the cell microarrays are printed with the same robotic microarrayer device as used to print conventional DNA microarrays, a similar magnitude of density can be achieved, (i.e. ~6000–10,000 spots per slide). And because such small quantities are used, potentially rare cell lines or biological samples can be used to assay many genes simultaneously. These transfected-cell microarrays can be used to identify potential drug targets by functionally characterizing large numbers of gene products in cell-based assays; to evaluate the specificity of candidate drugs; and to identify binding proteins for drugs of unknown mechanism-of-action or for candidates identified in phenotype-based assays [38]. These cell microarrays can also be used in loss-of-function analysis using plasmid-based small interfering RNAs (siRNAs). RNA interference is a useful genetic tool for the rapid and systematic silencing of genes of interest in mammalian cells (reviewed in [39]). By printing siRNA-plasmid constructs on slides in a manner analogous to cDNA constructs, it is possible to create cell microarrays in which each cluster of cells is deficient in a defined gene product. It is even possible to simultaneously create both cDNA and plasmid-based-siRNA microarrays on the same slide, or even within a transfected cluster, and thus genes of interest can be overexpressed and underexpressed in combination [40].

Antibody screening

A different approach for generating cell microarrays has been described by Schwenk *et al.* 2002 [41] and is used for the rapid screening of cell surface-specific antibodies to determine selectivity and cross-reactivity. High-density cell suspensions are directly printed onto glass slides using a robotic microarrayer device; glycerol is added to prevent complete dehydration of the printed cells, to conserve native protein structures and to ensure retention of the antigenicity

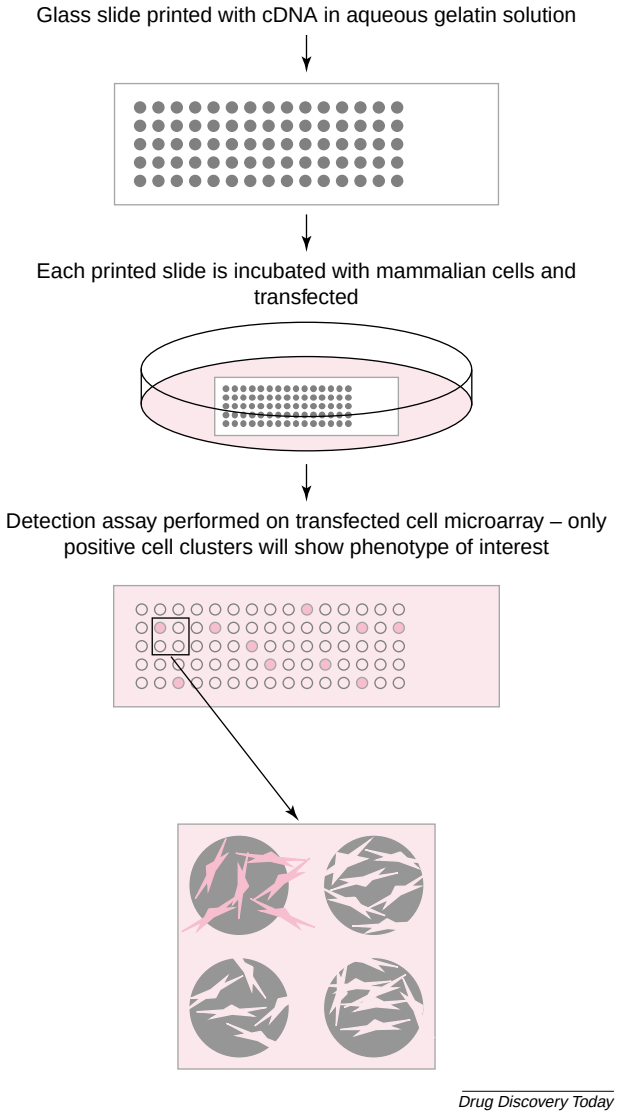

Figure 2. Schematic for the production of transfected cell microarrays

of the cell surface molecules; and the interaction of the antibodies subsequently bound to the cell microarrays are analyzed via immunofluorescence. The potential of such cell microarrays lies in the characterization of large numbers of antibodies, or other capture molecules, with respect to their binding abilities to conformationally well-defined preserved cell surface molecules [41].

The future of microarray technology

DNA-, protein-, glycomic- and cell-based microarrays continue to be developed to extract more data from smaller sample volumes. New technologies are now emerging to improve their overall design, to increase the speed of analysis and to reduce sample size even further. Box 2 indicates

Box 2. Novel platform microarray technologies

4-D Flow-Thru Chip™ for chemiluminescence detection of biomolecules
MetriGenix (http://www.metrigenix.com) have developed a patented, automated microfluidic biochip for the spotting and analysis of nucleic acids, RNA, DNA, proteins or cells in one-quarter of the time of conventional flatbed microarray systems.

Chips in Space for microgravity functional genomics
CombiMatrix (http://www.combimatrix.com) Corporation has developed a patented array processor system for the production of cost-effective custom oligonucleotide arrays using electrochemistry. The array is manufactured in a porous, 3D layer that sits on top of a semiconductor chip.

ProteinPrint™ polymer arrays and beads for sequence-specific protein capture
Aspira Biosystems (http://www.aspirabio.com) has developed a system based on base-pair hybridization in which a specific capture agent can be quickly and predictably generated against known and unknown proteins without purification of the target.

***In situ* synthesized peptide assays on microfluidic chips**
Xeotron (http://www.xeotron.com) Corporation has developed peptide/peptidomimetics arrays on a microfluidic platform (XeoChip™) using digital photolithography for the rapid screening of high affinity binding sequences.

Bio-CD® protein microarrays detected in a colorimertic assay
Advanced Array Technology (http://www.aat-array.com) has developed the Bio-CD®, a patented compact disc support for microarrays that provides a larger surface area than glass slides. Detection uses an adapted CD player that permits the simultaneous storage of data.

Resonance light scattering on protein microarrays
Genicon Sciences Corporation (http://www.genicon-sciences.com) has developed an application for resonance light scattering particles (RLS Particles™) in protein microarrays for high-sensitivity signal generation without enzymatic signal amplification to enable detection down to the femtomolar range.

Multiplexed biomolecular analysis using microcantilever arrays
The University of California, Berkeley (http://www.berkeley.edu) has developed a label-free technique based on a microcantilever that can be used to quantitatively detect DNA hybridization, protein–protein, protein–ligand and possibly DNA–protein interactions.

commercially available microarray technologies that use novel platform technologies.

In a drug development context, the analyst or laboratory requirement is to be able to undertake a fundamentally simple measurement, with the minimum number of interventions, in a technology that demonstrates extremely high substrate specificity, with the possibility of detection at low concentrations and with overall regulatory compliance. There is also a significant requirement for integration of a suitable chemometric data analysis and an information management system that can permit a measured data reduction, enabling the analyst to make the necessary key decisions [42,43]. Appropriate selection of a chemometric analysis pack is a pre-requisite in the future of HTS and the evaluation of new chemical entities. At present, this is an area where microarray technology is still in its relative infancy – the link between the micro- and macro-sensor devices must be strengthened, with better integration of chemistry and electronics [44]. In fact, future requirements will also involve a much closer marriage of data acquisition and data management and a greater all-encompassing linkage of individual components of any standard analysis. Fundamentally, for the pharmaceutical sciences, this needs to be in a form that can be validated clearly. Validation and global customer buy-in could be presently considered 'sticking' points in the wider application and acceptance of microtechnology.

In the future, a greater emphasis will be placed on point-of-care assessment and integrated analytical systems in such a way that a significant reassignment of the roles of healthcare professionals and scientists will emerge. It is conceivable that these systems will be used increasingly in biological and biomedical fields (such as regulating *in vivo* implants), for routine examinations and for health screening. Application will undoubtedly expand to include diagnostic testing and to encompass wider biotechnological applications (in environmental monitoring and identification of structural motifs relevant to the use and verification of genetically modified crops and foods [44]). The exact course of development is uncertain but what is a near certainty is that microarray technology will have a key role in the fabrication of novel devices and their adaptation for purpose, where demands push analysts for more information with increasingly more complex analyses.

Three inter-related techniques possess great analytical possibilities for the future and provide a route for 'total' lab-on-a-chip (TLOC) systems.

Microfluidics

Microfluidic device (MFD) technology – typically used to move small volumes of both liquids and gases – is a dynamic branch of analytical science with huge possibilities for bioanalysis [44]. Possibly the most assured application of microarray and MFD hybridization will be the creation of specific and adaptable 'micro total analytical systems' (μTAS) – MFDs will not simply be used as alternatives to microarrays but will be fused with them to produce fully integrated modular microfluidic microarrays [43,44]. The modular nature of the device means that individual portions could be linked and exchanged – the implications of which are huge. It would enable tailoring of a whole series of sub-tasks within an analysis and also coupling of this technology to microarray-based identification.

Structured MFDs are currently available that can involve micro-porous structure or fabricated channels on a scale of micrometers or sub-micrometers [45]. The formation of monolithic structures from resinous, polymeric or mineral matter, such as silicon and silicates or calixarenes [46] could be useful in ion-exchange, adsorption chromatography, micro-capillary electrophoresis [47] and flow cytometry. Other possibilities for these devices include uses in sample clean up, such as that undertaken in solid-phase extraction (SPE). In the future, several of these individual modules will be fused into a more sophisticated analytical device.

Chromatography [48], amperometric and ion-selective solid-state electrode sensors are potential applications for MFDs, where they exist as integrated complete (TLOC) systems that are equal in size, or smaller than whole cells and of notable interest to biochemists and molecular biologists. In itself, this has spin-off use in the production of artificial organs and micro-implants, and in molecular pharmacology. In this case, the use of nano-architectured devices or lithographic networks might provide a possibility for further miniaturization and provide a route for inclusion of multiple-module analytical devices and so-called nano-bots (nanometer-sized robots) in the body [42].

Other possibilities for future MFD technologies are metering devices for nano- or pico-litre dispensing systems [49], HTS and obvious augmentation in microarray micro-etching resolving power by selective surface engineering. MFDs using nanotechnology could also provide an alternative to cell and whole organism pharmaco-testing systems, although reliability and reproducibility would be obvious areas of concern. This would again be possible by mass manufacture of rigidly controlled etching and deposition of nano-features (chemical mosaics) and potential reagents, perhaps by using micro- or nano-particles or molecular imprintation onto what would essentially be a TLOC system. Box 3 indicates the inter-relationships of microfluidics and microarray technology.

Molecular imprintation

Molecular imprintation involves fabrication of a sensitized molecular layer. The technology has scope for use in many analytical systems but has received much attention for its use with piezo-electric technology, such as the quartz crystal resonance sensor (QCRS) or surface plasmon resonance (SPR) analysis. These techniques are considered useful in biofluid, environmental monitoring and quantitative gene and proteome profiling [50]. A possible use of molecular imprintation is in a polymerization process (MIP), where polymeric material is chemically sculpted into the shape of the target analyte molecule based on poly[meth(acrylate)] or poly(pyrrole) formation around molecular templates [51,52]. The ultimate application is the creation of synthetic biocatalysts for reaction engineering [53], affinity ligands, for use in flow-based systems, such as MFDs and biosensors. The potential selectivity of molecularly imprinted components means that they are often perceived as 'synthetic antibodies' that can be targeted directly at cell interiors and sub-cellular structures, for further use in techniques such as scintillation assay and magnetic resonance imaging (MRI) [54].

Nanotechnology

Nanotechnology is device chemical synthesis or structural engineering for end use on a nanometer scale. Applications closely mirror those of MFD technology and the ultimate applications of molecular imprintation. Nanotechnology might encompass atomic imprintation, and could be used in the formation of nanofluidic devices [49], optical and electronic sensors [50] and certainly in TLOC devices.

Other possibilities include the construction of appliances for molecular pharmacology and the hyphenation of systemic drug-delivery vehicles to TLOC analyses. Real promise lies in the scale of potential architectures and fabrication of 'never-seen' technology [55], incorporation of supra-molecular assemblies' [46] onto miniaturized complete sensors, with sufficient specificity to target individual molecule active centres and steric configurations. Examples in nucleotide research might include using ion-pair technology to form lipocomplexes (anionic DNA plus cationic lipids) or polyplexes, when using polymer ionophores. These have possible roles in terms of micro-coated wire electrodes for probing cell membranes and ion channels.

The technology might also be used for micro-scale solid-phase synthesis [56] as part of a TLOC system, or in the engineering and fabrication of nano-bots, nano-tubes, nano-circuits and several molecule-ion-selective electrodes [57]. In the future there could be a capability for nano-balances based on immobilized polymer molecules that specifically target complimentary substrates [57–60], microspheres [51], Langmuir-Blodgett films as membrane mimics and for use as self-assembled selective bilayers [61] and micro-reactors in the form of vesicle (artificial cells) [62] and micellar compartmentalized interiors [51]. Nanotechnology certainly has current and future applications in terms of surface patterning [59] and surface engineering [60] for roles in the manufacture of peptide-specific chips for proteome microarrays.

Now

Box 3. Inter-relationships of microfluidics and microarray technology

Microarray – gives selectivity and multiplicity of analyses

- Uses biorecognition surfaces
- Uses nano-engineered surface
- Uses surface with molecular imprintation of polymers
- Used for analyte targeting, identification and extraction
- Used as a a module, in stand-alone or in combination mode (a module being one unit operation or step)

When combined

Pro's

In situ, *in vivo* analysis system
Miniaturization of total analytical system
Portability, specificity
Labour-saving and cost effectiveness

Con's

Inhibitory development and unit cost?
Analytical validation concerns
Reliability under mass manufacture
Multiple use and recycling

Microfluidic system

- Exists as micro total analytical system (μTAS) with combined modules engineered to incorporate microarray mosaics with analyte guided over and dosed to the analyte specific surface
- Exist as a separate entity
- Used where little sample is available
- Combined with 'traditional' forms of analysis

Future – Multiple combined microfluidic systems?

Conclusions

Microarray technologies offer significant potential for advancements in both biochemical and pharmaceutical analysis. In principle, this is only bounded by the limits of

ethics or human imagination. The real challenge in the future comes in the form of application engineering, methodology validation and technology transfer from innovation generation to generation with the end-user in mind. This can only be achieved by rapid acceptance of new technology and by wider recognition of the benefits of miniaturization through nano-engineering, to enable the integration of microarray and TLOC technologies. The combination of these systems will not only impact the high-throughput drug-delivery market but will also provide major scope for the development of simple diagnostic systems for use in clinical practice. The future will yield opportunities to incorporate these technologies into 'smart' drug delivery systems that will enable therapeutic control in response to physiological changes in biochemical markers.

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