Chips to Hits: microarray and microfluidic technologies for high–throughput analysis and drug discovery

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Methods of performing experiments cheaper, faster, easier, as well as with a higher sensitivity and throughput, are desirable for many scientific endeavors and commercial applications. In particular, improved technologies are beneficial for the discovery of new drugs and the analysis of biologic systems, where many different molecules must be analyzed in a combinatorial and concentration-dependant manner. A major theme of the *2005 Chips to Hits* conference recently held in Boston, Massachusetts, USA revolved around the emergence of microarray and microfluidic technologies in addressing barriers to high-throughput experimentation.

DNA & protein microarrays

Microarrays can be used to perform high-throughput experiments by localizing tests to small regions of a 2D substrate. By restricting the location of individual experiments, microarrays can dramatically miniaturize assays. Microarray technologies have been widely used in academia and industry. It is estimated that these technologies currently have a market of approximately 2 billion dollars with more than 350 companies selling products. In addition, there are over 150 academic institutes and approximately 10,000 researchers conducting research in this field.

One of the most successful applications of microarrays is for RNA analysis. This technology, which was pioneered by Affimetrix, Inc., is now commonly used in many biologic and drug discovery studies to profile gene expression within cells. DNA microarrays can be used to conduct large-scale quantitative experiments that measure changes in gene expression for a particular tissue or cell type. The use of such technologies was a key aspect of the *Chips to Hits* conference, with many companies representing their latest technologic advancements and products. For example, Alan Sachs (Merck Research Laboratories) presented on the use of DNA microarray technology for enabling drug discovery by using animal models and tumor samples to identify drug targets.

Although gene arrays are good indicators of RNA expression within a cell, they are not necessarily representative of the level of protein expression, due to complicated dynamics of translational and post-translational modification processes. Therefore, complementing gene arrays with protein arrays is critical for producing a true picture of the state of the cell. It is envisioned that protein arrays can be potentially used to perform protein–protein, protein–DNA, protein–drug, or enzyme substrate screening assays in a sensitive, parallel and automated manner. As emerging tools for proteomics, protein arrays have a number of advantages over conventional methods of protein analysis using 2D gel electrophoresis and mass spectrometry, since they can be used to detect proteins with low concentrations in a high-throughput manner.

In general, there are two types of protein microarrays: antibody microarrays and functional protein microarrays. Antibody microarrays are miniaturized enzyme-linked immunosorbent assays that can be used to detect proteins with high sensitivity and selectivity. On the other hand, functional protein microarrays can be used to study protein interactions with other molecules. At *Chips to Hits*, Michael Snyder (Yale University, USA) presented on the use of yeast protein microarrays to identify novel partners within protein complexes, and to analyze the multifunctional nature of some proteins [1]. Using these assays, novel functions could be found for even well-studied proteins. Also, the use of protein arrays to understand phosphorylation mechanisms was demonstrated by studying the binding of yeast protein kinases to large protein microarrays. Using the data generated from protein–protein, protein–DNA binding and phosphorylation experiments, network maps could be generated that contain much of the interaction and regulatory pathways within a cell [2,3]. This ability to construct regulatory networks and to analyze biologic systems may have great implications in understanding the underlying biologic mechanisms and identifying candidate drugs.

Despite the remarkable potential of protein microarrays for understanding biologic systems, significant technological challenges remain. Current research thrusts in the area of microarrays involve increasing the sensitivity of the assays, minimizing false positives and increasing the throughput of the assays. For example, since protein–protein interactions are complicated by the existence of many

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post-translational modifications, such as phosphorylation and glycosylation, protein arrays must be fabricated that can sense such interactions by using post-translationally modified proteins or specific antibodies. Also, technological barriers exist for protein arrays that must be optimized to further facilitate their widespread use for biologic studies. Current research ranges from enhancing the functionality of proteins by using spacer molecules, to minimizing nonspecific protein adsorption through surface modification. For example, Jason Armstrong (Bio-Layer, Austria), presented the use of biomimetic surfaces to improve various assays in the range of 6- to 50-fold. These technologies may provide additional functionality and sensitivity to today's microarray technology.

Other polymeric microarrays

Microarrays fabricated from polymers or extracellular matrix (ECM) proteins can also be used to test extracellular conditions on cellular behavior or to test molecular libraries. Such libraries are useful for tissue engineering and have already yielded candidates that have been shown to induce osteogenesis [4] and cardiomyogenesis [5] from embryonic stem (ES) cells as well as the dedifferentiation of committed cells [6]. Recently, researchers in Robert Langer and Sangeeta Bhatia's groups (Massachusetts Institute of Technology, USA) have used robotic spotters capable of dispensing and immobilizing nanoliters of material to fabricate microarrays, where cell–matrix interactions can be optimized in a high-throughput manner. For example, synthetic biomaterial arrays have been fabricated to test the interaction of stem cells with various extracellular signals [7]. In this approach, thousands of polymeric materials were synthesized and their effect on differentiation of human ES cells [7] or mesenchymal stem cells [8] was evaluated. These interactions have led to unexpected and novel cell–material interactions. Although the molecular mechanisms associated with the biologic responses have yet to be clarified, such technology may be widely applicable in cell–microenvironment studies and in the identification of cues that induce desired cell responses. Also, the materials that yield desired responses could be used as templates for tissue engineering scaffolds. Such an approach is a radical change from traditional methods of developing new biomaterials, where polymers have been individually developed and tested for their effect on cells. In addition, the effect of natural ECM molecules on cell fate can be evaluated in a high-throughput manner. For example, combinatorial matrices of various natural ECM proteins were tested for their ability to maintain the function of differentiated hepatocytes and to induce hepatic differentiation from murine ES cells [9].

Other emerging microarray technologies include patterned polysaccharide and bead arrays [10]. Polysaccharide arrays can be used to study the interaction of chemical libraries or drugs with polysaccharides. Such interactions will be particularly important in finding new drugs that interact with the cell surface polysaccharide molecules, and are potentially important tools for understanding the interaction of sugars with other molecules in the emerging filed of glycomics.

Cell microarrays

Cellular micropatterning can be used to control cell shape and subsequent behavior (i.e., migration, proliferation, differentiation and apoptosis) [11,12]. Cell arrays are also a potentially powerful tool for performing high-throughput biologic studies. There have been a number of studies that have demonstrated the power of cell-based assays. For example, Sabatini and colleagues have demonstrated the parallel transfection of hundreds of genes in a microarray format [13]. In this technique, a printed array of full-length open reading frames of the genes in expression vectors, along with lipid transfection reagents, were used to transfect cells. The phenotypic effects of various genes can then be analyzed. One potential use of the cell microarrays may be to test the repression or silencing of genes in a sequence-specific manner using small, single-stranded antisense oligonucleotides, or small interfering RNA. Cell array systems can be utilized for identifying drug target interactions and for evaluating phenotypic changes resulting from the expression of specific proteins in the cells.

Other types of cell microarrays have also been developed in which cells are patterned on surfaces comprised of adhesive or nonadhesive regions. As adhesive cells are seeded on these substrates, they adhere to the patterned regions. Alternatively, microwells have been used, in which cells, including nonadhesive cells, are plated within the microwells and remain physically separated from each other. Cell arrays have been used to study cell behavior in a clonal manner [14,15] or to study the effects of drugs on cells in a high-throughput manner [16,17]. Current limitations with cell array technologies include lack of suitable technologies to assay for many different chemicals as well as maintaining proper cellular phenotype in culture. Methods of integrating cell arrays within microfluidic channels, generating patterned co-cultures and multi-phenotype cell arrays are emerging features which may solve these challenges. The cell array technology requires further development, but has the potential to lend itself to a broad range of functional ultrahigh-throughput cell-based assays.

Microfluidics

Microfluidics is a potentially powerful method of performing high-throughput experiments that encompass a series of techniques used for controlling the flow and reaction of minute amounts of liquids or gases. Microfluidic systems have a number of benefits, which include reduced waste, improved sensitivity/precision, reduced cost, reduced energy consumption, miniaturized experiments and an increase in the speed of the reactions by reducing diffusion times. With typical channel dimensions of 5–100 µm, devices comprised of complex networks of fluidic microchannels and interconnects can be generated around the size of postage stamps.

Many companies have been developing microfluidic technology for various high-throughput applications such as immunoassays, diagnostic devices, single molecule DNA and protein detection as well

as cell separation. Research is also being performed in many academic laboratories on novel applications of microfluidic technology. For example, researchers from the University of Chicago, USA, and other laboratories have demonstrated the use of two-phase droplet systems to generate droplets within microfluidic channels that could be used as microreactors [18,19]. These nanoliter plugs can be used for high-throughput screening of compounds and can be used to perform multiple chemical reactions. Microfluidic drops can also be used for studying crystal growth and nucleation, which could be used for many chemical screening or protein purification assays.

One of the limitations of microfluidic technologies has been the micro- to macroscale interface. In most applications, the number of inlets is limited by physical geometries of the tubing and syringe pumps. Research is currently underway in many aspects of interfacing microfluidic chips to their surroundings. A number of approaches have been developed in order to eliminate external pumps. In one scheme, microchannels sealed under vacuum have been formed in which the exposure of the liquid to the vacuum can be used to draw the fluids into the channels for subsequent analysis. Alternatively, hydrophilic surface modification of microchannels using plasma treatment, dextran or poly(ethelyne glycol) (PEG) have been presented as methods of spontaneously delivering fluids into the channels. Finally, methods of using gravitational forces have been used to drive the fluids within the microchannels. In one approach, forces generated by a rotating disk are used to drive the fluids through the microfluidic channels. The fluid is filled at the center of the disks and it is subsequently carried through the channels using microchannels.

To make microfluidic technologies cheaper and more mechanically robust, research is underway to improve microchannel fabrication. Traditionally, microchannels have been fabricated in silicon or glass using cumbersome lithographic methods. However, these techniques require extensive technologic expertise and access to clean rooms and microfabrication equipment. In order to alleviate these needs, soft lithographic approaches have been developed. In soft lithography, polymers are cured on a silicon master, which is microfabricated once and can be repeatedly used to generate molded replicas. Polydimethylsiloxane (PDMS), an elastomeric and transparent material, has been extensively used for this fabrication approach. However, there are potential disadvantages associated with PDMS, which include its hydrophobicity and inability to resist protein adhesion. In order to address these problems, PDMS surfaces have been coated with nonbiofouling molecules such as PEGs [20] and polysaccharides [21]. Alternatively, many microfluidic devices have used the physisorption of serum proteins, such as albumin, to minimize surface biofouling of functionalized microchips. Alternatives to PDMS include molding of polymethylmethacrylates-based [22] or fluoropolymer-based [23] materials. These alternative materials and other emerging techniques are advantageous with respect to their mechanical and protein biofouling properties.

Finally, the widespread use of microfluidic technologies and microarrays for high-throughput applications is limited by the detection methods. Research in detection methods has focused on increasing the sensitivity, portability, cost and size of these devices. Various modes of detection are currently in use. These range from fluorescence- and absorbance-based optical methods, to other approaches such as surface plasmon resonance (SPR) and diffractive optics technology. In many cases, fluorescence has been used as the desired source of detection due to its high sensitivity and accuracy. However, fluorescent detectors are not easily integrated within portable devices. SPR is an alternative method of detection that provides potential advantages. SPR works by measuring the binding of an analyte to a ligand or receptor on the surface. Since SPR measures the changes in the mass on the surface in a real-time and quantitative manner, it is not necessary to label the analyte. In diffractive optics technology, a flowing stream of sample is layered over the immobilized capture molecules, and binding events produce changes in the diffractive light signal. Using total internal reflectance diffraction, a pattern is generated in which the diffraction of light can be detected and quantified. Research in this technology is under way in academic and commercial sectors, and has significant potential to improve today's detection methods. Alternatively, microcantilevers can be used within microdevices for the detection of biologic molecules. Microcantilever biosensors are label-free, real-time and silicon-based tools in which the free end of the cantilever moves up or down upon a biorecognition event. Microcantilevers can be conjugated with proteins or DNA, and can be fabricated in a highly parallel and miniaturized fashion, making them useful for high-throughput analysis.

Commercialization

The commercialization of microarray and microfluidic technologies is an ongoing process as demonstrated by the emergence of many start-up companies. Throughout the conference, potential routes and challenges to commercialization were discussed. Richard Fisler (Beachhead Consulting) discussed the market for such technologies and noted that, as genomics technologies have shown, promising technologies often take longer to deliver market success than initially anticipated. Entrepreneurs need to realize that, even though it takes a relatively small amount of money to demonstrate the proof-of-concept for a product, it takes much more money and time to commercialize a successful product. Also, for a new technology to reach commercial success, it needs to be much more advantageous over existing technologies. One example is Affymetrix, who generated a new market based on their GeneChip® technology over a 12-year period. Despite the barriers to enter the market, the future looks promising. For example, despite the initial caution and hesitation in marketing protein microarray products or high-throughput microfluidic assays for drug discovery, certain applications

of these technologies have become tools that are routinely used in basic research and drug discovery.

Microarray and microfluidic technologies also face and pose unprecedented challenges to intellectual property law. As discussed by Kathleen Williams (Palmer & Dodge LLP), in the last decade, the number of patent applications in biochip or biotechnology lab-on-a-chip technology has increased from approximately 300 to 4000 per year. This increase has been difficult to handle for agencies such as the US Patent and Trademark Office, which has become a bottleneck for patenting. This increase in the number of patent applications is a further indication that the future brings many opportunities in a competitive market environment for emerging companies.

Conclusions

Microarray and microfluidic technologies have significant potential in increasing the throughput of existing assays for analyzing molecules (such as DNA, RNA, proteins and polysaccharides) as well as cells. These technologies could also be used for many diagnostic applications since they perform experiments with higher sensitivity while using less reagents. Current research revolves around increasing the sensitivity and the speed of these technologies, while minimizing their size and cost. The integration of microfluidic and microarray technologies may also lead to unmatched throughput for diagnostics and screening applications. With respect to commercialization, although commercially successful products have been generated, many opportunities remain that rely on improved technologies and niche markets.

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