



Lab-on-a-Chip and Microarrays: Discovery and Development

Cambridge Healthtech Institute's Fifth Annual Lab-on-a-Chip Conference.
February 13–14, 2003, Zurich, Switzerland

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Introduction

The theme of the Fifth Annual Lab-on-a-Chip Conference from Cambridge Healthtech Institute (CHI) was discovery and new developments in biochip technologies as well as their applications relevant to human health, such as molecular diagnostics. Biochip is used as a broad term for various technologies including lab-on-a-chip, DNA chip, protein chip, microarrays and nanoarrays. The attendees at this multidisciplinary meeting included biologists, engineers, bioinformaticians, database developers, as well as those involved in drug development and molecular diagnostics. This is a brief overview of selected presentations at this meeting. Biochip/microarray technologies are described in more detail elsewhere [1].

Advances in hybridization

Shear-driven flows have been used to generate a rapid lateral convective transport across the microarray surface to overcome diffusion limitation in the hybridization step of microarray experiments. Binita Dutta (Flanders Institute for Biotechnology, Leuven, Belgium) presented shear-driven microarray hybridization for the development of fast DNA and protein screening systems. This approach would yield reductions in the sample amount and the time of hybridization, enabling development of small arrays for rapid diagnosis of cancer, mutations, or other genetic diseases. Future work will be in the direction of switching to submicron channels and more complex probes.

Achim Wixforth (Advalytix AG, Brunthal, Germany) presented a novel microfluidic platform enabling the *in situ* monitoring of DNA, single nucleotide polymorphism (SNP), and proteomic hybridization events. The fluid in this case is actuated by surface acoustic waves, propagating on the planar surface of the computer-controlled microfluidic biochip. The chips are completely aimed towards the realization of programmable bio-processors for smallest liquid volumes. The commercially available Array-Booster™ for the hybridization of microarrays has a reduced timescale, enhanced signal intensity, and selectivity for hybridization assays compared to diffusion-limited microfluidics. This enables the direct observation of the dynamics of hybridization events and opens up applications in research as well as in diagnostics.

Vladimir Lazar described the development of a microarray facility at Institut Gustave Roussy (Villejuif, France) based on technologies that use cDNA as well as oligonucleotide microarrays to analyze the gene's steady-state level on the whole transcriptome level. Quality control of RNA specimens and of labeled targets is performed prior to any hybridization and enables correction, which improves the quality of experiments. The reproducible processes have a high potential in defining pertinent collection of genes related to pathways and treatment responses in cancer enabling the development of personalized medicine [2].

Improvements in array content and labeling

Analyses routinely performed in central laboratories are usually quantitative. The use of microarrays for diagnostic purposes would enable simultaneous testing of different markers, point-of-care diagnostics, and reduced time as well as reagent consumption with a reduction in overall cost. However, the quality of the spots in DNA and protein microarrays is strongly affected by the uncontrolled drying of the spotted solutions and the fluorescence readout is often equally subjected to fluctuations. Quantitative, sensitive, and reliable analyses require the formation of the array and the assay to be tightly controlled. David Juncker and colleagues (IBM Research Laboratory, Zurich, Switzerland) have developed microfluidic capillary systems and arraying strategies that provide the necessary control for each step from 'spotting' to the final assay readout [3]. These capillary systems can improve the quality and reproducibility of protein arrays and enable high-throughput screening as well as quantitative diagnostics. In an immunoassay, the capillary system was shown to reduce time by a factor of 10, reagent consumption by a factor of 10,000 and increased information per unit area by a factor of 100,000.

Søren M Echwald (Exiqon A/S, Vedbaek, Denmark) showed how the superior binding affinity and specificity of locked nucleic acid (LNA) has been exploited as it enhances specificity tenfold and sensitivity eightfold in expression arrays. LNA capture probes are applied in SNP arrays [4]. Since LNA is compatible with standard oligonucleotide synthesis and can be used in most standard molecular biology methods, LNA is expected to be widely used in enhancing existing technologies in the near future.

Signal amplification of protein arrays as a means of enhancing test sensitivity was discussed by Tito Bacarese-Hamilton (Imperial College of Science, Technology and Medicine, London, UK). A tyramide signal amplification (TSA) assay can detect < 1 fg of allergen-bound IgE in human serum. The clinical performance of the assay compares favorably with ELISA, enabling the use of microarray immunoassays for serodiagnosis of IgE in human serum.

Novel concepts in chip design

Several new concepts were presented for chip design. A novel chip platform for single- and multiple-cell analysis was presented by Jacques Jonsmann (Scandinavian Micro Biodevices A/S, Copenhagen, Denmark). This technology platform enables fast and more cost-efficient development of new chip systems. The chip contains both silicon and polymer microfluidics, with functions requiring true microfeatures placed in the silicon part and less critical structures located in the polymer shell. Microstructured coatings with feature sizes down to ~ 2 µm enable control of cell adhesion and repulsion (i.e., the controlled positioning of living cells).

With developments in glycobiology, there is an increasing awareness of the importance of glycans as biological information molecules. Nir Dotan (Glycominds Ltd, Lod, Israel) described his company's GlycoChip® technology for discovery, prioritization, and development of targets and drugs. This unique array enables unprecedented throughput for the analysis of glycan-protein (including antibodies, enzymes and lectins) interactions, opening the door for novel post-genomic approaches in biomarker identification, pharmacoglycomics and drug development. Serum anti-glycan Ig profiling has been used to develop 'pharmacoglycomics' personalized medicine tests for inflammatory diseases.

Molecular diagnostics

Molecular diagnostics is the most important clinical application of biochip technology. Bottlenecks for this

application were identified by the session chairperson Elaine Broomfield (Agilent Technologies, Bracknell, UK) as those related to sample-analyte preparation, population genetic studies, informatics and regulatory approval. According to the FDA, "Microarray targeted sequence analysis will not be adopted for screening blood samples until analysis is automated, cost effective and sensitive for viral gene expression." The presentations in this part of the session dealt with overcoming some of the difficulties with clinical applications of microarrays, as well as the use for personalized medicine.

NextGen Sciences (Huntington, UK) is developing a series of protein microarrays to investigate their use as a prognostic indicator in breast cancer studies. Linda Cammish of the company described solutions to a number of the major problems associated with use of protein microarrays including:

- attachment of functional protein to a biochip substrate
- achieving high levels of assay sensitivity
- developing a universal mechanism for attaching protein recognition molecules to a biochip substrate
- achieving reproducible assay results
- performing protein biochip processing in a high-throughput manner

A new microfluidics system is used to automate protein biochip processing. Protein biochips have been developed for breast cancer prognosis by protein expression analysis of complex breast cancer samples [5].

Andreas Schütz (Institute for Molecular Nano-Technology, Recklinghausen, Germany) described the application of the GeneStick® microarray platform for cancer diagnostics. The GeneStick comprises a stick-shaped biochip made of an optical-grade plastic material. Hybridization is performed in a closed tube with a heated standard laboratory shaker, ensuring temperature-controlled hybridization under constant mixing. It is optimal for enzymatic reactions on the chip, uses small sample volumes (50 µl–1 ml), and there is no evaporation. The readout

of the GeneStick is performed with a chemiluminescence detector. It has been used for cancer diagnosis: early detection of tumors, characterization of tumors, and selection of appropriate therapy and monitoring of the course of disease. For the detection of lung cancer, a microarray has been developed that allows the determination of the promoter methylation status of the five most frequently methylated promoters in lung tumors. Microarrays for detection of k-ras mutations from stool samples can be used for the early detection of cancer of the colon or the pancreas as well as for phenotyping of disseminated cancer cells by measuring the expression level of tumor-related genes.

Tissue microarrays for miniaturized high-throughput molecular pathology

Guido Sauter (University of Basel, Basel, Switzerland) described a tissue microarray to examine the clinical significance of a large number of genes that are suspected of playing a role in cancer biology. Using this technology, samples from up to 1000 different tumors can be arrayed in one recipient paraffin block, sections of which can be used for several *in situ* analyses [6]. A comprehensive evaluation of new potential biomarkers involves a two-step approach. First, genes of interest are analyzed on a normal-tissue microarray (TMA) and on a multitumor TMA containing > 3000 samples from 130 different tumor types. In a second step, organ-specific TMAs containing tissues with clinical end point information are used to test the clinical significance of the biomarker in these tumor entities where alterations were detected on the multitumor TMA. An advantage of the TMA method is the applicability of automated analysis for RNA *in situ* hybridization, immunohistochemistry, and fluorescent *in situ* hybridization (FISH). Tissue arrays can be used for the epidemiological evaluation of genes of interest, as well as for rapid evaluation of valuable targets. It is anticipated that 'tissue chips' will greatly accelerate the clinical application of the results of basic research.

DNA chips are valuable tools for the simultaneous detection of known SNPs in numerous genes. In the field of pharmacogenetics, the value of genotype-phenotype correlation in several drug-metabolizing enzymes is recognized, but parallel detection of SNPs has been laborious in the past. Daniela Massa (GeneScan Europe, Freiburg, Germany) presented Pharm-O-Kin Chip[®] that was designed as a rapid and reliable method of detecting SNPs in several genes of pharmacogenetic relevance. The current version of this chip can detect 39 polymorphisms from CYP2D6, CYP2C9, and CYP2C19, as well as NAT2 and MDR, which cover important enzymes from drug metabolism as well as a transporter protein known to play a role in the bioavailability of administered drugs. This enables a better clinical

trial design in terms of minimizing the risks for trial subjects, and also reduces costs of drug development.

Concluding remarks

This was an excellent conference and presented the state-of-the art as well as developing biochip technologies with potential applications in healthcare. Although not specifically identified in the themes of the conference, it was obvious that the miniaturization is moving to the nanoscale.

There were many examples of applications of microarrays in cancer diagnosis. Several of the new technologies will be useful for the development of personalized medicine. There were ample opportunities for interaction between the speakers and audience as well as between the academic researchers and the representatives of the industry.

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