Going, going, gone: from microscale to nanoscale analysis

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The *EuroBiochips 2003 Conference* was held in London from 21–22 May 2003 at the Queen Elizabeth II Conference Centre. The event, organised by IBC Life Sciences, highlighted how emerging technologies, advancements within the field of protein arrays and the application of microarrays and microfluidics in medical and clinical diagnostics is impacting the pharmaceutical and biotech industries.

In the beginning

Roger Ekins (University College London Medical School; http://www.ucl.ac.uk) opened the conference by discussing the principles involved in miniaturised analysis. He stated that, contrary to apocrophyl dicats, the concepts and theories underlying microarray technology state that reducing analysis volume can increase sensitivity. In the mid 1980s, Ekins conceived and patented the Microspot®, an ultrasensitive, miniaturised assay for protein, DNA and RNA measurement [1]. This fundamental concept has permitted the development of systems for the massive parallel analysis of hundreds to thousands of samples and has resulted in the explosion of a technology that has the potential to become a multi-billion dollar industry.

The evolution of biology towards microelectronics

Since their commercialisation by Affymetrix and Agilent in the 1990s, *in situ* synthesis microarrays [2] and spotted microarrays [3] have become well established in DNA, RNA and protein analysis. Many speakers discussed the new platform technologies that are evolving as a result of the combination of microelectronics with biology. Physicists and engineers are now entering the field and are taking the development of microarray technologies in a new direction.

Of particular interest were those presentations outlining the application of miniaturised systems to the manipulation and analysis of whole, living cells. The first cell-based microarray was manufactured in a manner similar to spotted cDNA microarrays [4], but Nicolo Manaresi of Silicon Biosystems (http://www.siliconbiosystems.com) demonstrated how a microfluidic system, the DEPArray™, has been developed to enable the movement and separation of individual cells or functionalised micro-beads. Cells can be manipulated and moved about independently on a silicon chip by dielectrophoresis (DEP), where an electric field is generated across a silicon chip, causing neutral particles to experience a net force, directing them towards locations of increasing or decreasing field intensity.

Several presentations centred on the application of microfluidic devices to enable high-throughput cell patch-clamping. This is of special interest because ion channels are important in drug target development and reliable testing of the influence of active compounds is determined by performing patch-clamping methods on live cells. Until recently, it was not possible to do this in a high-throughput manner and it was consequently a time consuming process that could only be carried out by highly trained technicians. Jia Xu (Aviva Biosciences; http://www.avivabio.com) and Alfred Stett (Cytocentrics CCS GmbH; http://www.cytocentrics.com) described the development of SealChip™ and CytoPatch™, respectively, whereby individual cells from a flowing suspension are immobilised and positioned on planar openings by suction, thereby contacting with patch pipette contact openings.

SAW technology

Christoph Gauer (Advalytix AG; http://www.advalytix.de) gave a presentation that was particularly inspiring. Enhanced by the use of 'movies', he demonstrated the use of Surface Acoustic Wave (SAW) technology to precisely control chemical reactions on the surface of a biochip. One movie clip demonstrated that a reaction could be performed by manipulating different reagent droplets on a chip without the need for mechanical patterning. SAW technology was also shown to be applicable to sample agitation in either microtitre plates or on slides. In this way, incubation time, and therefore overall reaction time, can be reduced. It was also claimed that signal intensity and homogeneity could be increased in DNA microarray analysis, and the phenomenon of false positives reduced.

It's all about proteins

With the completion of the Human Genome Project, the scientific community has moved towards

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proteomics. Larry Gold (SomaLogic; http://www.somalogic.com) discussed the use of photoaptamers in proteomic studies. An aptamer is a single-stranded DNA or RNA molecule that can bind a target molecule with extraordinary affinity and specificity. When folded, an aptamer can take on a complex, sequence-dependent, three-dimensional shape that can bind a target protein in a tightly bound complex, analogous to an antibody–antigen interaction. By substituting thymidine with brominated deoxyuridine, aptamers are able to cross-link with specific sites on target proteins. These photoaptamers are thus capable of recognising the complex shape and charge distribution of target proteins, and the presence of specific amino acid residues at specific sites.

Proteins as biosensors were also discussed. Richard Palmer (Nanoscale Physics Research Laboratory, Birmingham University, http://www.nprl.bham.ac.uk) explained how organic molecules, such as light harvesting porphyrins, can be used to fabricate functionalised nanostructured systems that can be used to explore the self-assembly of molecules on deposited, size-selected clusters. In this way, 'designer binding sites' are formed that are suitable for single protein molecules and represent the ultimate limit in biosensing. This technique can be used to produce a detailed understanding of the interaction between an adsorbed molecule and a solid surface, as well as the assembly of a molecule into its functional structures.

Diagnostics

Crucial to the analysis of drug safety and efficacy, target identification, diagnostics and disease modelling, is the evaluation of the response of organisms to disease, drugs and the environment. Richard Wooster, Leader of the Cancer Genome Project (The Wellcome Trust Sanger Institute, http://www.sanger.ac.uk/CGP) outlined how the application of DNA microarrays is currently enabling the systematic mapping of human cancer genes.

Michael Spain (Rules-Based Medicine; http://www.rulesbasedmedicine.com) also discussed how species-specific multi-analyte profiles (MAPs) – microspheres impregnated with fluorescent dyes and coated with reagents that bind to target substances in the blood – are being used to characterise how biochemical pathways are altered in response to a stimulus. It was claimed that hundreds of biochemical markers can be analysed (in 10–20μL volumes) making it possible to accurately and precisely measure individual markers of cancer, infectious disease, autoimmunity and cardiovascular risk, as well as hormones, cytokines and/or chemokines, acute-phase reactants and other blood components.

Another interesting aspect was the application of carbohydrate microarrays in the recognition of pathogenic signatures. Denong Wang (Columbia University; http://genome4.cpmc.columbia.edu) described how this technology could be used in antibody fingerprinting to facilitate the recognition and identification of pathogen-specific antigenic polysaccharides. The advantage of this system over classical diagnostic tests, such as agglutination and immunoprecipitation, immunofluorescence, radioimmunoassay and enzyme-immunoassay, is that the detection of infectious disease is not dependent on the clinician selecting the correct diagnostic test. With its sensitivity, specificity and power of broad-range detection, Wang claimed that carbohydrate microarrays might enable the monitoring of unexpected microbial infections and microbial warfare agents as they emerge.

The future: Point-of-Care Testing?

Of course, it can only be speculated as to how microarray technologies will continue to impact on life sciences in the future [5]. With engineering solutions becoming the platform from which new technologies are being developed, the greatest potential for protein array technology, as suggested by Kevin Johnson (Cambridge Antibody Technology; http://www.cambridgeantibody.com), is within HealthCare – the ultimate market being routine health testing. Indeed, this was also the subject of the presentation by Larry Kricka (University of Pennsylvania, Medical Center; http://www.uphs.upenn.edu). Point-of-Care testing (POCT) devices are already widely available for use by the trained medic at the bedside and in the clinic, or by the untrained person in the home. Current examples include simple dipstick tests such as blood and urine glucose testing, pregnancy testing and drug abuse testing. More complex test devices, such as the i-STAT, Glucowatch, Metrika and Persona Personal Contraceptive System are also used. However, it was suggested that the future is in the development of hand-held, portable analysers for personal use. Although there could potentially be applications in genetic and pharmacogenomic testing, at present, companies are focusing their research towards pharmaceutical and research-based applications, with little effort being directed towards the development of clinical tests and POCT devices.

Concluding remarks

Microarray technology has rapidly evolved beyond the simple glass slide covered with DNA, proteins, carbohydrates or cells, and now provides technologies that can be used across a spectrum of fields that will undoubtedly expand over the coming decade. Those attending and presenting at the conference covered numerous areas of

scientific expertise – medics, physicists, engineers, chemists, biochemists and molecular biologists. It will be the multidisciplinary collaborations that arise as a consequence of such meetings that will provide the high-sensitivity microarray technologies of the future.

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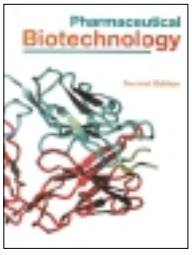

Pharmaceutical Biotechnology (2nd Edition)

Edited by Daan J.A. Crommelin and Robert D. Sindelar, Taylor and Francis, 2002, 425 pages in paperback, ISBN: 0-415-28501-1

In the past decade, pharmaceutical biotechnology has rapidly evolved at the interface of molecular biology, molecular genetics, bioengineering, (protein, sugar and nucleic acid) chemistry and pharmaceutical sciences to become a multidisciplinary field on its way to providing treatments for serious life-threatening diseases (e.g. cancer, viral infection, hereditary deficiencies). For such an ambitious goal, pharmaceutical biotechnology has to integrate solid pharmaceutical science and a strong industrial implementation, exemplified by an eightfold increase in worldwide total sales of the US pharmaceutical industry in the past twelve years. Conceiving an up-to-date pharmaceutical biotechnology textbook that trains next-generation pharmacy students, as well as updating pharmacists and pharmaceutical scientists on basic research and biopharmaceutical manufacturing topics is a formidable challenge, and one which the editors, together with their various academic and industrial experts, have accomplished to a high standard with the 2nd Edition of *Pharmaceutical Biotechnology*.

This textbook comprises 20 chapters, many of which contain industrial case-studies of blockbuster drugs, thus giving this textbook an industrial scope that is difficult to find elsewhere. All the chapters are easy to read and well illustrated to aid understanding of the interdisciplinary content. Key scientific information is referenced for the advanced reader. Each chapter is authored by experts in the field and contains a good introductory section and an in-depth discussion of each topic, as well as a well-designed Q&A section, which enables self-assessment or facilitates the integration of each chapter's essentials. Although the topics and scientific information flow well from chapter to chapter, each chapter is conceived in a stand-alone manner, allowing one to consult specific topics.

Chapter 1 contains a concise yet complete overview of molecular biotechnology essentials, including gene expression in pro- and eukaryotic cells, recombinant DNA technology and specific DNA techniques, as well as an introduction to the key systems that are used for biopharmaceutical manufacturing – microbial, animal and plant cell cultures.

Chapter 2 provides an insight into the biophysical, biochemical and structural analysis of recombinant proteins. The factors at work during protein folding are described, as are the standard technologies (immunoassays, electrophoresis, chromatography and mass spectrometry) that are essential for the quality control of protein pharmaceuticals.

The production and downstream processing of biotech products is covered in chapter 3. This chapter touches upon production issues, including expression and cultivation systems and medium components, together with the problems associated with contaminants. Because of the argument that prokaryotes are unable to provide the desired glycosylation of protein pharmaceuticals, the authors focus on eukaryotic production systems, thereby neglecting certain advantages that microbial configurations have shown in the production of non-glycosylated protein therapeutics. Downstream processing is well covered, taking advantage of the introduction to basic purification technologies included in chapter 2.

Chapters 4 and 5 provide a detailed insight into the biopharmaceutical considerations of protein pharmaceuticals, as well as their pharmacokinetics and pharmacodynamics. Controlled and site-specific delivery technologies are well described.

Chapter 6, entitled 'Genomic, proteomics and additional biotechnology-related techniques' provides an extensive coverage of diagnostics, therapeutic molecular interventions and drug discovery. General topics, including transgenic animals, tissue engineering and glycobiology are smoothly integrated

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