Marvellous microplates

The dynamic surge of interest in genomics and drug discovery has resulted in a cry for new technologies that will enable scientists to find the needles in the haystack. High-throughput screening (HTS), combinatorial chemistry (CC) and polymerase chain reaction (PCR) are the primary analytical and clinical tools. This is where the engineers meet the chemists and biologists to provide solutions. With economic and time constraints, it is becoming tougher for research to be carried out in petri dishes and test tubes. This is why the microplate is playing a pivotal role in these fields of research. This report represents the fruits of a recent visit to Whatman Polyfiltronics (Rockland, MA, USA), where I met Roy Manns (founder & Chief Technical Officer) who has pioneered many innovations in microplate design, and discussions with Dr Keld Sorensen, a microplate specialist at Pierce Chemicals (Rockford, IL, USA). Some major suppliers in the microplate technology field are listed in Box 1.

Anatomy of a microplate

The advent of the microplate has been a vital enabling technology for high-throughput laboratory applications, but what is it? The microplate is an injection-moulded rectangular plastic tray, the length and height of which are set at a standard of 5 by 3 3/8 inches (12.76 by 8.56 cm). The plate contains rows and columns of cells/wells (resembling a honeycomb). The volume of each cell can vary from 20 µl to 5 ml. The number of wells in microplates in general use can be as low as 6 or as high as 7,912. The most common format is 96 wells, but 384-well plates are now easily available and other sizes are in the pipeline. All of the formats are accommodated within the standard footprint. There are two basic types of microplate. The more common microplates are those used for holding liquids in each well. These 'collection' plates replace traditional test tubes. The other type of microplate is more complex, and is designed to filter contents through a medium within each well.

Possible applications for microplates seem never-ending. In how many ways can a standard test tube or 1.5 ml tube be used? Now imagine doing that in an 8 × 12 tube format! Among the projects that Manns has been involved with are the development of a 'see-thru' plate (ViewPlate™ 24–384-well blackwall with clear bottom), 96-well plates containing glass-fibre filters, a deep-well (800–2,000 µl) filter plate for DNA sequencing, a 48-rectangular-well plate with 1.5 ml wells for cell culture, a 384-conical-well PCR plate, and more recently a filterplate with 'Polykryptonite™' media for combinatorial chemistry. According to Manns, Polyfiltronics receive requests for many configurations; one strange request called for a plate that takes up the classic microplate footprint but contains one large well (75,000 µl), and NASA wanted a plate to perform microbiological studies in space that was the size of a 35 mm slide containing 48 wells with a filter.

History of filterplates

Historically, receptor binding assays involved the manipulation of radioactive samples, with the associated issues regarding safety, handling and time constraints. The amount of binding to the receptor was represented by how much radioactivity bound to protein that was trapped on circles of glass-fibre membranes. The membranes would be transferred to tubes and counted in a radiation detector. With the development of filterplates this is now performed in a 96-well format, where the glass-fibre membrane is encapsulated in the filterplate. This has greatly decreased handling requirements, and automation of the process has decreased the overall assay time. The lower reagent volume required in a 96-well vs test-tube format results in a more economic assay with less radioactive waste material generated. This transition from single tube to microplate arrays has mostly occurred at the analytical and research levels, and it will still take time for clinical laboratories to catch up.

Current technology

Because of the great interest in high-throughput methodology, much effort has been directed toward the analysis of a large numbers of small samples. Many standard colorimetric methods have been scaled down to the 96-well format. This is usually an easy task, provided that the chemicals involved do not damage the plate itself; some solvents can 'melt' plates. To circumvent this, Manns has developed 'stronger' collection and filter plates that can accommodate most solvents indefinitely. Some groups are developing glass microplates that are resistant to most solvents. Both types of plate will be an essential tool for combinatorial chemistry approaches, where many harsh solvents are used.

With the constant need to speed up and scale down, many microplates are now finding their way into diagnostic, assay and sequencing kits. Pre-aliquoting of

Box 1. Web sites of microplate suppliers and organizations referred to in the text

Advanced Genetics Technology Corp.	http://www.agtc.com
Amersham Life Sciences	http://www.amersham.com
Millipore Corp.	http://www.millipore.com
MipTec'97	http://www.miptec.com
Pierce Chemical Co.	http://www.piercenet.com
QIAGEN, Inc.	http://www.qiagen.com
Whatman	http://www.whatman.com
Whatman Polyfiltronics	http://www.polyfiltronics.com

reagents in the wells speeds up and simplifies the process for many molecular biology techniques, a common example being manual or fluorescence-based DNA sequencing.

Where are microplates heading?

Microplates will soon be regarded as a precise tool for research, as vital as the petri dish or test tube but in a high-density format and available with a range of surfaces. Manns has coined the term 'intelligent microplate' because the plates he is involved in designing will no longer be 'dumb' plastic receptacles, but will play an extended role through the use of activated surfaces and media. A recent example of intelligent plates would be the Cytostar-T™ plates from Amersham Life Sciences (Little Chalfont, UK), which have scintillant mixed into the moulded plate.

It will not be long before we see a new generation of plates. One example could be a liquid-format pap smear test, the liquid could be removed with a filter membrane and the testing components 'built into' the membrane. The membrane could then be transferred and used directly on a microscope and the cells analyzed. Others have already developed simple tests where activity of certain protein kinases can be visualized colorimetrically on the filter membrane. Before long we could see pregnancy and drug tests made compatible for microplate formats.

96-well microplates are the *de facto* standard, but the 384-well plates are replacing the 96 wells in areas such as DNA sequencing and high-throughput screening. The pragmatic limit for density of wells, in a 96-well footprint, could be 9,600 wells. The technology is certainly available in microplate design to achieve this, but practical issues associated with handling and analytical instruments are the limiting factors. Industrial R&D applications are driving the switch to the higher density formats, with the clinical field lagging far behind. Only about 10% of the clinical market are using 96-well systems, probably because the transition would require retooling/upgrading of old equipment, and also because the use of single tests allows for rapid turnaround of results. The clinical labs that have moved to a higher density screening format are usually centralized diagnostic facilities with higher volume throughput.

Plates in space

Polyfiltronics have a diverse client base, including NASA. Microplates with the footprint of a microscope slide and a density of up to 96 wells are being developed for use in various microbiological studies. This is a closed system that allows the user to centrally distribute fluids to each well. From previous experience, the company anticipates that approximately half of their work from the microplate collaboration with NASA will be applicable to the user on Mother Earth.

Record-keeping

The use of filterplates and media will play a key role in mediating record-keeping of test results from microplates. They provide a useful storage medium, enabling the operator to retain the membrane for archiving after drug testing, DNA testing or bacterial testing. According to Manns, this will become routine procedure for many laboratories.

What are the immediate advances for microplates?

According to Keld Sorensen, the widespread use of the 96-well format has opened up some new uses for gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE). The familiar round sample trays used in GC, HPLC and CE instruments are, in some cases, being replaced with sample handlers that use a 96-well format.

But what are the possibilities of performing chromatography in a 96-well format? Using their UniPlate™ 2000 system (2 ml well volume, 96-well blocks), Polyfiltronics has already made a number of plates containing Whatman's chromatography media. There are other examples in the molecular biology market, such as the 96-well DNA purification columns produced by Advanced Genetic Technologies Corp. (Gaithersburg, MD, USA) and QIAGEN, Inc. (Chatsworth, CA, USA). Advances in microplate sample preparation are in large part due to the companies producing separation media. Whatman, Schlecher & Schleuer, Millipore and 3M are some of these pioneers. Rapid advances in genome sciences, combinatorial chemistry, high-throughput screening and diagnostic markets mean that forthcoming developments will be very interesting.

Further information/MipTec'97

I thank Roy Manns of Whatman Polyfiltronics and Keld Sorensen of Pierce whose assistance has been invaluable in compiling this report. Manns and Sorenson will be co-chairing a session on microplate design and materials at MipTec'97, which will be held in Arlington (VA, USA) on 23–25 June. The meeting also includes the following sessions: applications in analysis, diagnostics and handling; applications in genome projects; robotics in laboratory automation; information management; biomolecular screening and library generation. For more information, visit the MipTec'97 Web site at www.miptec.com.

Martin Leach

In short...

Phytera, Inc. (Worcester, MA, USA) has acquired **Auda Pharmaceuticals**, the Danish combinatorial chemistry company based in Copenhagen. According to Dr Malcolm Morville, Phytera President and CEO, "Auda brings to Phytera excellent proprietary combinatorial chemistry technologies that fit extremely well with our existing lead identification approaches, as well as the outstanding scientific leadership of Dr John Nielsen, one of the founding scientists at the Technical University of Denmark in Copenhagen". Mr Neil Goldsmith, Auda MD, will continue to manage Auda and will also have a role in the management of Phytera's two other European subsidiaries. Phytera applies novel cell culture technologies and combinatorial chemistries toward the discovery and development of plant- and marine microorganism-based drugs.