THE MICROFLUIDIC MILIEU

Labs-on-chips may be the next step toward finding new drugs and drug targets

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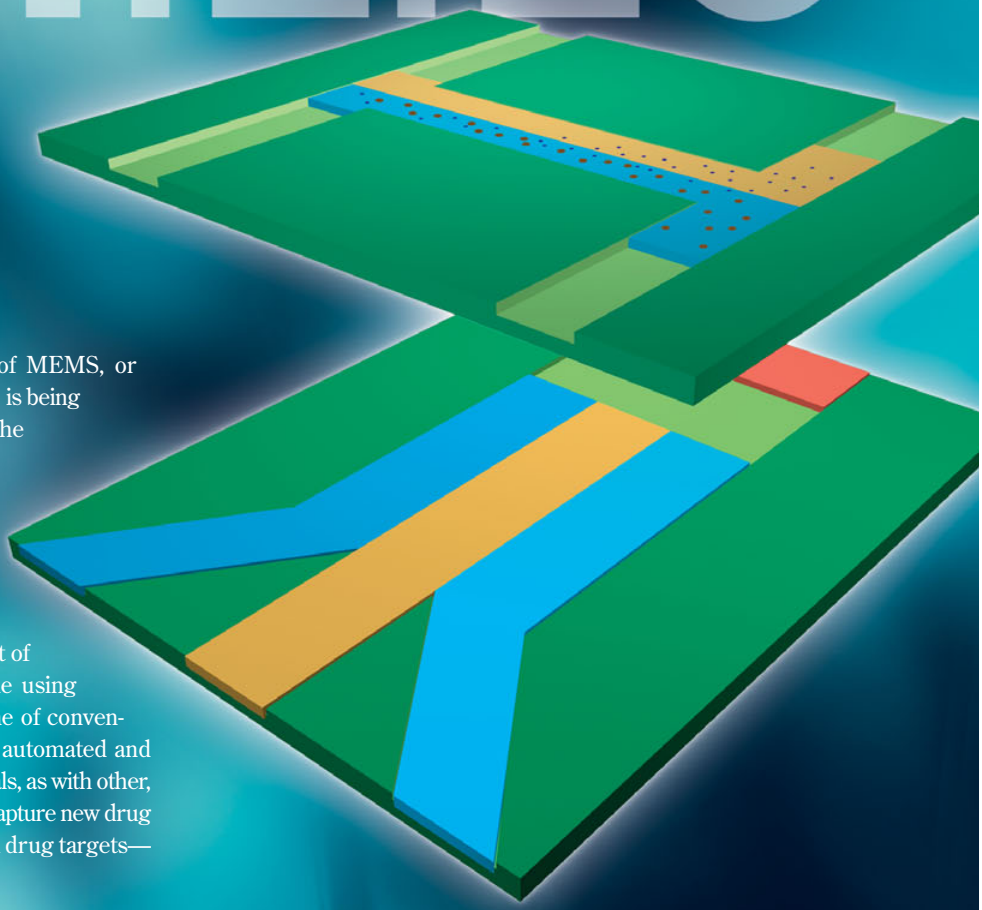

Microfluidics (the liquid subset of MEMS, or microelectromechanical systems) is being applied to a variety of steps on the drug discovery pathway. The coming microfluidic wave may be thought of not as a form of nanotechnology, but rather as the first wave of microlaboratories, sometimes referred to as μTAS (micro total analysis systems) (*1, 2*). Miniaturized instruments on microchips can perform the gamut of standard analytical and synthetic processes while using minuscule volumes that take a fraction of the time of conventional instrumentation and in a way that is easily automated and that can be made massively parallel. The ultimate goals, as with other, more standard pharmaceutical technologies, are to capture new drug candidates for clinical trial and lock down potential drug targets—a large task for these smallest of players.

Benchtops in a bottle

Almost every laboratory research or processing instrument is becoming a candidate for "chipdom". From genomics (DNA analysis, sequencing, PCR amplification) to proteomics (protein isolation and characterization, immuno- and enzyme assays) to cell isolation and clinical diagnostics, lab-on-a-chip technology looks to change how discovery is done. It is the ultimate step in the move toward smaller-footprint devices. The benefits include greater speed, lower cost, greater control, and modularity. Small sample size is particularly important in the area of high-throughput screening (HTS) of combinatorial libraries.

Typical enzyme-inhibition and ligand–receptor assays often require expensive reagents, and the drug target receptors can be difficult to obtain, regardless of price, especially when screening unknowns. In addition, the smaller the volume of material needed to test a combinatorial library, the more efficient the library production and assay become.

The structure of these microfluidic chips varies from traditional silicon and glass to a variety of polymers (including poly(dimethylsiloxane), or PDMS) chosen for their binding and interactive characteristics with the individual molecules or chemistries being

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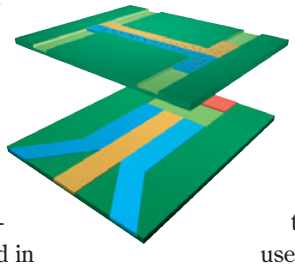
operated. These labs-on-a-chip can vary greatly in size, depending on the type of instruments or reactions incorporated—some look like a standard microchip, others like fabricated plastic sheets the size of a playing card.

Of course, fabricating these microlabs is an art form in itself, relying primarily on the same etching and molding technologies used for producing computer microchips (*1*). A wide variety of microvalves, mixing chambers, input junctions, pumping mechanisms, and detection systems are also becoming available for incorporation into these devices, as researchers attempt to mimic the capabilities of full-size laboratory instruments. Commercial devices have only recently become available, with the first microfluidics instrument, the Agilent 2100 Bioanalyzer, based on technology developed by Caliper Technology Corp., being introduced in 1999. Table 1 lists corporate websites where a wide variety of architectures can be seen detailing some of the ways that typical analytical chemistry reactions have been engineered to microscale.



Researchers in both industry and academia have designed chips to perform almost any imaginable operation, including affinity chromatography, capillary electrochromatography and electrophoresis, HPLC, and isoelectric focusing (*2*). Systems have even been designed to act as an electrospray sample injection unit for feeding nanoliter samples into a mass spectrometer for characterization (*3*).

Going with the flow

One of the key requirements of any piece of chemical instrumentation is the need to move materials through the system. Samples, reagents, and carriers must be able to enter, pass through, and leave efficiently, allowing sufficient time and appropriate conditions for reactions and analysis. This is comparatively easy to do in conventional instruments working at sizeable fractions of milliliter-to-liter volumes. But when the entire system is operating at the micro- and nanoliter scale, the degree of control needed to move samples and solvents is extreme—the entire system may be operating below the volumetric error rates of standard fluid-handling methods. The complexity of dealing with this problem at miniature scales is the very reason that microfluidics became such a dynamic field of study.

Simple pumps and gravity do not function as efficiently in the microfluidic realm. Instead, new means of moving solutions are required. Some of the most common methods rely on electrokinetics, more controlled versions of pressure pumps, and more recently, centrifugation. Electrokinetic methods of controlling liquid flow are becoming extremely sophisticated. These include electroosmosis and electrophoresis.

Electroosmosis refers to the motion of an ionic solution induced by an electrical field. By differentially changing the current at various end points in a multichannel junction, fluids can be directed wholly or partly into any of the channels desired, as experimental conditions require. This can be sufficient to create highly reproducible nanoliter or picoliter injections or mixing and can act to distribute materials to a variety of analytical chambers on the same chip in different proportions. One particular advantage of electroosmotic pumps is that they have no mechanical moving parts and have no specific location in the circuitry, thus providing an even flow through the entire length of the capillary channels involved.

Electrophoresis refers to the motion of particles through a solution induced by an electrical field. When used as a pumping mechanism, electrophoresis can provide similar distribution capabilities to those seen with electroosmosis.

Using physical pressure is still a valid option at the microchip level, if it is properly done. Microsolenoids, for example, can be used to trigger miniature syringe pumps to dispense uniform, small volumes. A variety of piezoelectric pumps have been demonstrated as useful adjuncts to microfluidic systems.

An innovative approach that works particularly well in microfluidic systems is a method of pumping through the production of a localized, controllable gas bubble or bubbles. Materials can be pushed through the system by increasing the size of the bubble. Localized thermal control to increase or decrease the size of a bubble can be used for very sophisticated forward-and-back pumping control.

Centrifugal methods have also been developed for moving liquids through microfluidic systems. These use hydrophobic regions as valves, which allow aqueous samples to pass only when rotational pressure exceeds capillary pressure (*1*).

Genomics

As might be expected in the era of genomics, manipulating nucleic acids has become one of the most intensely studied areas in microfluidic technology. Of course, standard DNA microarrays can be considered simple versions of lab-on-a-chip technology—and in this sense, their utility is unquestionable. But the newer methods of dealing with nucleic acids are far more sophisticated—so much so that a wide variety of standard laboratory bioanalytical and processing techniques for DNA and RNA have been miniaturized.

Microfabricated silicon PCR systems have been coupled to capillary electrophoresis microchips to create integrated DNA analysis systems. Researchers have developed such systems to contain mul-

Table 1

Some sources of microfluidic products for the drug discovery market

Aclara BioSciences	www.aclara.com
Advalytix AG	www.advalytix.com
Agilent Technologies	www.chem.agilent.com
Biosite Inc.	www.biosite.com
Caliper Technologies	www.calipertech.com
Cepheid Technology	www.cepheid.com/pages/fluidics.html
Fluidigm	www.fluidigm.com
Micronics	www.micronics.net
Motorola Life Sciences	www.motorola.com/lifesciences
Nanolytics	www.nanolytics.com
Nanostream	www.nanostream.com
Upchurch Scientific	www.upchurch.com

tiple PCR microchambers, integrated heaters and temperature sensors, gel electrophoresis channels with built-in electrodes, and on-chip photodetectors for fluorescence analysis of products. Similarly, monolithic chromatographic devices have been designed to perform automated restriction digests of DNA with separation and visualization of the fragments.

DNA probes can be immobilized in micofluidic channels via photopolymerization in a polyacrylamide matrix. Fluorescently tagged DNA fragments of interest can be electrophoresed through these hydrogel plugs. Hybridization can then be monitored using standard fluorescence detection. By creating sequential sets of different DNA plugs, multiple hybridization analysis of a DNA sample can be done in a single electrophoretic run (*4*).

These examples of just a few of the microfluidic systems used for genomic analysis demonstrate the profound versatility of the lab-on-a-chip approach above and beyond simple microarrays.

Proteomics

Lab-on-a-chip methods in proteomics can be divided roughly into those methods that seek to isolate and analyze proteins and those that use proteins as analytical devices and manipulative tools. As with DNA, protein microarrays are an initial form of this technology, but they are only the tip of the iceberg. Microfluidics can also be used to develop immobilized enzyme or receptor assay systems. For example, researchers at Texas A&M University have used biotinylated phospholipid bilayer-coated PDMS channels to immobilize streptavidin-conjugated alkaline phosphatase. Enzyme kinetic studies were performed using rapid and multiple substrate dilutions on the chip, allowing Lineweaver-Burke analysis to be performed in a single experiment with all the data collected simultaneously (*5*).

Similarly, researchers at Los Alamos National Laboratory and the University of New Mexico (*6*) have demonstrated the detection of soluble compounds of interest—in this case, small peptides—that bind to their specific receptor-bearing beads sequestered in microchannels. This was, simply, a standard form of affinity chromatography, although miniaturized. In another example of interest to the proteomics field, sample analysis of the protein melittin was demonstrated using nanoelectrospray mass spectrometry on tryptic digests performed directly on chips (*2*).

Life in the fast (and small) lane

Perhaps the smallest and currently most complex microfluidic device yet available is the living cell. Incorporating cells as vital (both figuratively and literally) components of laboratory microchips holds the most promise for MEMS in leading to the discovery and development of new pharmaceuticals. Because of size, complexity, and often the need to maintain functional physiologies during an experimental run, the processing of living cells in such systems is often a far greater challenge than using standard chemistries.

In an effort to develop cell-based microfluidic assay systems for potential use in high-throughput drug screening, researchers Won-Gun Koh and colleagues at The Pennsylvania State University and Texas A&M University developed a method of encapsulating viable, nonadhering mammalian cells in cylindrical microfluidic channels using a poly(ethylene glycol)-based hydrogel to create a 3-D matrix through which test samples of candidate drugs could be passed. By manipulating the size of the microchannels to only 50 µm, they could isolate 1–3 cells per channel and were able to monitor them using microscopy (*7*).

In another example, Eric Schilling and colleagues at the University of Washington, Seattle, developed a microfluidic system for lysing bacterial cells and extracting a large intracellular enzyme (β-galactosidase). Quantitative analysis was performed using a fluorogenic enzyme assay incorporated into the system (*8*).

Ultimately, the question might be asked: Will the development of microfluidics lead lab-on-a-chip technology to the forefront of lab analysis for most bioanalytical chemistry in drug discovery, or is it simply another of those boutique or niche phenomena that crop up every so often in science, promising the world but delivering far less? Only time will tell, but it is hard to dismiss the value of the potential savings in time, space, and sample and reagent materials that such developments bring. Furthermore, the potential levels of control, integration, and automation, as well as the "black-boxing" of the most sophisticated analytical techniques into systems that operators can handle competently with only minimal training, seem extremely difficult to ignore.

Dealing with diagnostics

Microfluidics is obviously not just for drug discovery. It is potentially applicable anywhere chemistry of any kind is important, from environmental analysis to industrial processing. And throughout the scientific health care industry as a whole, from diagnostics to therapeutic delivery, the technology is showing promise. In one example, researchers used polymer-coated capillary electrophoresis on a microchip to screen for breast cancer susceptibility genes using single-strand conformation polymorphism analysis. In another, microfluidic electrophoretic separation coupled to fluorescence was used on urine samples to detect high levels of amino acids associated with metabolic disease (*2*). The ability to automate and pyramid these types of microfluidic tests, their extreme accuracy and speed (separations and detection take place within seconds compared to hours), and their ease of standardization and use potentially make microchips the next greatest thing in clinical diagnostics. Furthermore, in the area of therapeutics, researchers are studying the use of microfluidic techniques using reservoir chips inserted in the human body for defined delivery of drug doses.

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