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Chemical Engineering at the Nanoscale

Last month, Nano.Cancer.Gov News provided an introduction to lab-on-a-chip technologies. This month, we delve into some of the technical challenges that researchers are addressing to build chip-based laboratories capable of working at the nanoscale.

In 1990, in a paper published in the first issue of a new journal on chemical sensors, Andreas Manz, Ph.D., who was then at Ciba-Geigy in Basel, Switzerland, proposed that it should be possible to construct a miniaturized "total chemical analysis system" that would integrate sample pretreatment, separation, and detection steps in a single device the size of then-current chemical sensors. Fifteen years later, through the collaborative efforts of chemists, engineers, physicists and biomedical researchers, such lab-on-a-chip systems are making a growing mark in biomedical research.

Already, relatively simple lab-on-a-chip devices are being used for some nucleic acid and protein analyses, but microfluidics technology may someday allow millions of automated biochemical experiments to be performed per day using miniscule quantities of reagents and biological samples such as blood or tissue. Eventually, individual analyses may be replaced by protocols in which tens to thousands of analytical measurements are made in parallel, either on the same or multiple samples. Such capabilities will be a boon to drug discovery and screening efforts, as well as for studies aimed at understanding the complexities of cancer. Such multiplexed chips may eventually help usher in an era of affordable personalized medicine.

Though that vision for the future of microfluidics is just that, a vision, it is a goal that helps motivate today's students and postdoctoral fellows. But so, too, do the successes now coming to the research market. For example, Agilent Technologies recently introduced a nano-scale LC chip system that integrates sample enrichment, separation and nanoelectrospray ionization onto a device smaller than a credit card. This integrated microfluidics device combined with high sensitivity tandem mass spectrometry will enable researchers to identify femtomolar levels of protein digests from complex cellular extracts (see Figure 1).

"The integration of trapping and separation channels together with an emitter for nanoelectrospray ionization on a compact microfluidic device greatly simplifies the inherent complexity and integrity of nanoscale chromatography for mass spectrometry analyses," explained Pierre Thibault, professor of chemistry and chair in proteomics at the University of Montreal, who collaborated with Agilent on the development of these devices. "The high sensitivity and reproducibility achievable with these microfluidic devices will undoubtedly play a significant role in mass spectrometry-based protein expression and identification platforms for biomarker and drug discovery programs." The new chip simply slides into a slot on an electrospray mass spectrometer, dramatically reducing the possibility of leaks and dead volumes, significantly simplifying workflow, and increasing sensitivity and reliability during analysis.

Courtesy: Agilent Technologies

Figure 1.
HPLC Chip

Shrinking what used to be bench-top sized equipment to the microscale and nanoscale is what the science of microfluidics is all about, and it is the challenge of making pumps, valves, sample preparation gear, detectors and other components small enough to move, separate, and analyze nanoliters volumes of sample-containing fluids. "How do you take a few milliliters of blood or urine, or a piece of tissue with some bacterial cells and get them into a device that can handle nanoliters or even picoliters of fluid?" asked Rashid Bashir, Ph.D., associate professor of electrical and computer engineering and biomedical engineering at Purdue University. "How do you move that fluid around? How do you mix it with various reagents? How do you detect the very few numbers of molecules in a sample that small?"

Those are just a few of the questions that researchers must answer when trying to create a usable lab-on-a-chip device, explains Rashid, with the laugh of some-

one who has experienced first-hand the difficulty of meeting those demands. "Then you have to integrate these components into a device that you can actually manufacture at a reasonable cost and that will perform as expected in the real world."

This last point is seconded by Richard Mathies, Ph.D., professor of chemistry at the University of California, Berkeley, who said, "A 90 percent success rate at making any of these components will be a failure if your intent is to make a device for commercial use. In my lab, it's not until a student can put 500 valves on a chip, for example, and not turn pasty and panicky that we start to think we have a useable component."

Meeting the challenges of chemical engineering at the nanoscale are hundreds of researchers in dozens of laboratories, both academic and commercial, around the world working in multidisciplinary teams. Here are some of the approaches that these investigators are taking to create the key components needed to build useful microfluidics devices. This summary is not meant to be an exhaustive review of the challenges that exist or of the many ways that researchers are solving this problem, but merely a cross-section of the innovative work occurring in this field.

Channels

The heart of any microfluidic device is the nanoscale channel etched or stamped into a substrate such as a silicon, glass or polymer wafer. Perhaps not surprisingly, investigators have developed many ways of making channels with well-defined characteristics, and the general sentiment is that channel construction is probably the area that the field is closest to mastering. Many of these techniques have been adapted from the photolithography methods used by the semiconductor industry. Basically, channels (and other features) are created in the substrate and then in a final step, a second piece of substrate is pressed onto the first to create a sandwich that seals the channels along their length.

Though simple in concept, explained Tony Owen, Ph.D., Agilent's marketing manager for liquid phase analysis platforms, "an important consideration with channels built to convey nanoliter and picoliter volumes is that the ratio of surface area to volume is much higher at the nanoscale than it is at the macroscale, so any surface defect effects are amplified many fold, and that can affect fluid flow dramatically." Currently, the causes of these surface defects - bumps, ripples and pits - are poorly understood and difficult to control. "This is not an impossible problem to solve," said Owen, "but we need to know more than we do now if we're going to increase the reliability and sensitivity of these devices." A better understanding of defects and their causes may also lead to an ability to make purposeful use of these surface features to more precisely control and shape fluid flow.

Valves

Another key component of any laboratory device is the valve, a simple device to stop, start or regulate the flow of liquid through a column. In the macro world, valves usually have moving parts that rotate or pivot - think of the handle on a water faucet - but fabricating such a structure in a microfluidic device is no simple matter. Not only are grossly moving parts difficult to construct on a chip, but the workings of the valve must themselves not trap any fluid within themselves. To create such "zero volume" valves, researchers have turned to constructs such as polymer membranes or flaps similar in design to the zero volume valves in the heart. Mathies' group, for example, uses a flexible, biocompatible polymer known as PDMS that is layered between channels etched in glass and a microscopic reservoir into which the membrane can stretch when it is opened in response to a finely controlled change in pressure applied into that chamber (see Figure 2).

Courtesy: Teris Liu and Richard Mathies, Ph.D., UC, Berkeley

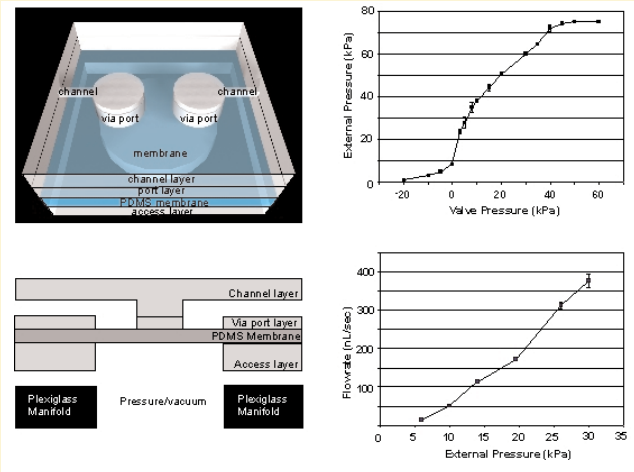

Figure 2.
Top left: 3-D schematic of a PDMS membrane valve
Bottom left: Cross-sectional schematic of a PDMS membrane valve
Top right: Breakdown pressure of the PDMS membrane valve
Bottom right: Flow characteristic of the PDMS membrane valve

Sample handling

Having a device with multiple, well-defined channels is one thing, but getting samples onto a microfluidics device in a reproducible manner presents its own challenges. "Reproducibly pipetting even a microliter of fluid to inject onto a chip is a difficult task," explained Owen. "It's an important bottleneck." Mathies agreed, saying, "if we can successfully automate sample preparation and put those functions on a chip, this field will really take off."

One straightforward approach, taken by microfluidics company Caliper Life Sciences, is to fabricate a tiny cannula, or thin flexible tube, that interfaces with the nanochannels on a chip and can sip small amounts of sample from the microtitre plates widely used in biomedical research laboratories. So far, the company has created a 12-channel sipper that can simultaneously load samples into a dozen microfluidics channels.

Another tack is to put whole cells into

some kind of chamber in a chip and then extract and purify biological samples on the chip itself. Investigators such as Mathies and Stephen Quake, Ph.D., professor of bioengineering at Stanford, and their collaborators are working on such approaches. Quake and his colleagues, for example, have created microfluidics chips that can isolate cells, lyse or break open the cells, and then purify and recover the DNA and mRNA in those cells. This device uses a series of valves that when lined up not only control when and what flows through a given chamber, but can operate as a peristaltic pump - opening a series of three valves in a row in sequence forces fluid through the connected channels. On-chip mixing chambers, reservoirs containing reagents, and pressure regulators complete the component list. Cells suspended in solution enter the chip and purified nucleic acids come out the other end, ready for analysis using other lab-on-a-chip systems for PCR or mRNA profiling, for example.

Courtesy: Mihri Ozkan, Ph.D., UC, Riverside

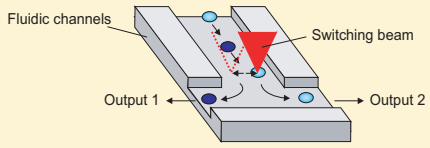

Figure 3.
Schematic of optical tweezers

Researchers are also developing methods for manipulating intact cells for analysis in microfluidic devices. For example, Mihri Ozkan, Ph.D., assistant professor of electrical engineering at the University of California, Riverside, and her collaborators have developed a technique that uses "optical tweezers" to apply pressure on cells in microfluidic channels (see Figure 3). An array of low-power lasers applies focused light beams onto the channels on a microfluidic device. The light generates a pressure on any dielectric or non-conducting object, acting as a handle that will trap such objects, including live cells. As the beam moves along the channel, it takes a trapped cell with it as if it were being manipulated with nanoscale tweezers. Using this setup, Ozkan has shown that cells can be directed to an optical trap where a particular property of the cell is measured. Based on the output of the measurement, the optical tweezers can direct the cell out of the trap to a specific output from a branched channel, drag the cell into a flowing fluid stream, and then release it to go on its way to another part of the microfluidic device.

Detection

The ultimate purpose of biomedical microfluidic devices is to analyze some biochemical reaction or detect the presence of important biomolecules, and accomplishing such analyses requires an ability to interrogate the contents of microfluidic channels. Today, most detection schemes rely on external recording and measurement devices, such as confocal microscopes capable of detecting fluorescent dyes, gold nanoparticles or quantum dots attached to the molecules or cells of interest.

But numerous groups are also working to add detection circuits to the microfluidics chip. "In general, this would allow you to detect specific molecules or cells without having to label them," said Bashir. His group at Purdue, for example, has used silicon nanoscale cantilevers created via photolithography to detect single virus particles flowing through microfluidic channels (see Figure 4). Making the cantilevers is straightforward, said Bashir. "The hard part is coating the cantilevers with the suitable reagents to capture whatever it is that you want to detect." One approach is to use a finely tuned inkjet printer to apply antibodies or other traditional capture reagents to the surfaces of the cantilevers.

Bashir and others, including Charles Lieber, Ph.D., professor of chemistry, engineering and applied sciences at Harvard University, are also working to incorporate nanowires into microfluidic channels. Lieber's group, for example, has used silicon nanowires to prepare individually crafted transistors capable of detecting the interaction of the anticancer drug Gleevec® with its protein target. As Bashir explained, nanowires can be more sensitive than cantilevers, particularly in fluids, but researchers must first develop a way to grow or construct nanowires in defined locations on the surface of a microfluidics device. "We and others are working on this problem, and I think it's now just a matter of time before such methods become available," Bashir said.

The likely result of these efforts to identify and solve such challenges is that researchers will continue to develop microfluidic devices capable of analyzing an ever-larger number of samples in an ever-shrinking amount of time. And as has been the case in the computer world, shrinking chip components and increasing processing speeds will decrease costs - in

Courtesy: Rashid Bashir, Ph.D., Purdue University

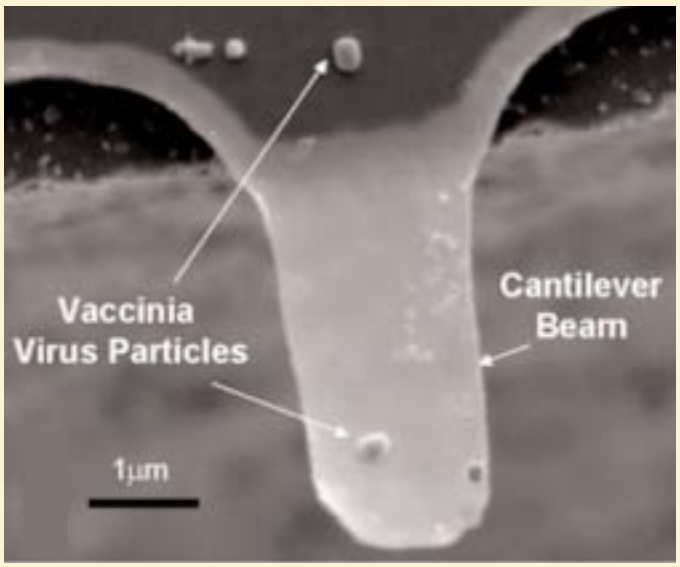

Figure 4.
Electron micrograph of individual virus particles on a cantilever

this case of processing samples and doing research - and open avenues to new applications for microfluidics in cancer research and clinical oncology.

In the near term, the research community should benefit from lab-on-a-chip devices that will automate routine molecular separations and analyses, and in the process, vastly reduce the amount of sample and expensive reagents needed for any given experiment and increase the number of experiments that a lab can perform at any one time. PCR, mRNA profiling, mutational analysis, and protein expression profiling are just a few of important laboratory tools that may soon be available as fully integrated lab-on-a-chip systems, which could enable cancer researchers to rapidly characterize tumor cells based on molecular characteristics.

Another likely benefit in the near-term could come from lab-on-a-chip systems designed to rapidly analyze how enzymes, receptor and even live cells respond to potential drug molecules. Such systems could speed drug development efforts, and could even find use in clinical trial applications as real-time monitors of therapeutic efficacy and potential side effects.

Researchers also envision a day in the not-too-distant future when a small sample of tumor, processed in a lab-on-a-chip, will not only reveal the genetic makeup of the cells in that tumor, but characterize the susceptibility of those cells to various therapies. Lab-on-a-chip systems are also expected to play a central role in driving down the cost and time for DNA sequencing to the point that sequencing an individual's genes may become the first step in both treating cancer and then preventing its recurrence.

Indeed, if the visionaries in the microfluidic and cancer research communities have their way, there could come a day when a yearly pin-prick of blood, parsed through dozens of microfluidic-based assays on a single chip, will spot cancer in its earliest manifestations, long before symptoms develop. Such a development would go a long way toward eliminating suffering and death from cancer. <

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