Chemical & Engineering News

Cover Story

September 10, 2007
Volume 85, Number 37
pp. 14-19

Mimicking Biological Systems

Control of the chemistry and physics of cell-populated microenvironments reveals new biology

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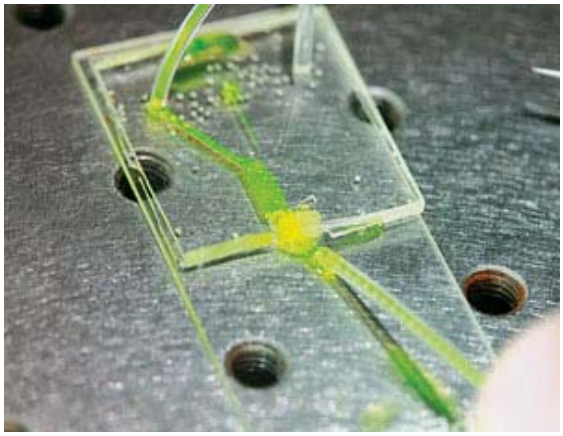
FOR THE PAST CENTURY,
the petri dish has been the best thing available to study cells and tissues outside the body. But cells in a petri dish inhabit an environment that looks nothing like their natural milieu. Engineers and physical scientists, in collaboration with biologists, are demonstrating that the channels and wells of microfluidic devices can mimic the chemistry and physics of biological systems in ways that reveal hitherto unknown biology.



Courtesy of Justin Williams
Brain Control This microfluidic device allows researchers to control the chemical environment of a slice of brain tissue.

Microfluidic devices are systems for manipulating fluids in micrometer-scale channels and wells. Small and ever more readily produced, they can be made of silicon, taking advantage of decades of fabrication technology from the semiconductor industry, or they can be made of polymers such as polydimethylsiloxane (PDMS), using the "soft lithography" technology pioneered by George Whitesides at Harvard University.

"A lot of our body is microfluidic, little tubes and ducts and blood vessels," says Shuichi Takayama, a biomedical engineer at the University of Michigan, Ann Arbor. "It makes intuitive sense that microfluidics are useful for mimicking different parts of the body."

Some parts of the body fit this description particularly well, such as the micrometer-scale capillaries of the circulatory system and the finer, similarly sized airways in lungs. For mimicking other cellular systems, the micrometer-length scale provides precise control of the environment surrounding cells.

Microfluidic systems provide a "bridge between the classic in vitro study and the classic in vivo study," says Dana Spence, an analytical chemist at Michigan State University. "Microfluidic systems are going to allow people to do studies that mimic the in vivo system in a controlled—emphasis on controlled—in vitro platform." For his part, Spence is using microfluidic devices to learn more about the roles of red blood cells.

David J. Beebe, a bioengineer at the University of Wisconsin, Madison, always tries to make sure that there's a good reason for using microfluidics for a particular application. "If there isn't, it's typically a lot

harder and more expensive than doing things the traditional way," he says. Biology-mimicking microfluidic devices hold most promise for answering important questions about biological systems. But they can also play a role in drug screening.

A number of properties of the flow in microfluidic devices make them attractive as biological mimics. Laminar flow dominates, convective flow is eliminated, and flow can even be stopped, allowing the study of diffusion-controlled processes. In addition, flow can be controlled in such a way as to set up chemical gradients.

The most important of the flow characteristics in microfluidic devices is laminar flow, which allows fluids to flow side by side for long distances without mixing and is a common feature in biological systems. In contrast, petri dishes experience convective flow, even when nothing is being actively added or removed. This convection is generated by temperature differences or by evaporation from the surface.

FOR SOME STUDIES,
scientists can opt to stop the flow in their microfluidic systems altogether. Beebe uses what he calls "inverse microfluidics," channels with no flow where transport occurs purely by diffusion. "It's not necessarily mimicking in vivo, but between the flow and no-flow conditions, you have the bounds on the system. In vivo is clearly somewhere between the two."

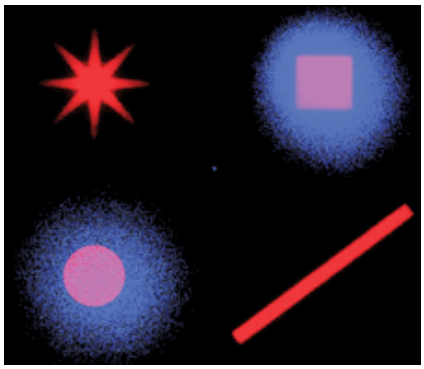
Gordana Vunjak-Novakovic, a bioengineer at Columbia University, is taking advantage of the way microfluidic devices allow her to study biological systems in gradients. "Microfluidics can help study nonuniformities and complex signaling," she says. Nonuniformity "is probably the most biologically relevant issue that conventional systems do not let you study. You're intrinsically limited working in a petri dish, because it has to be uniform."

Scientists are taking advantage of these properties of microfluidics to develop in vitro mimics of natural biological systems and components, including but not limited to the vascular system, the neural system, the liver, cancerous tissue, and stem cells.

"Biological systems are different from chemicals studied in a beaker by virtue of being far from equilibrium," says Rustem Ismagilov, a chemist at the University of Chicago who uses microfluidic devices to study complex biological networks, such as those found in blood clotting and developmental biology. Microfluidic devices allow constant influx of reagents and removal of by-products. "This allows you to overcome the curse of the second law of thermodynamics," he says, referring to the tendency of closed systems to become more disordered.

In addition, "many biological systems have fascinating properties of compartmentalization that are hard to mimic in a stirred beaker," Ismagilov says. "If we're trying to understand how these systems work, it's important to have tools that place molecules where we want them, when we want them."

The vascular system is particularly well-suited to studies based on microfluidic systems. Spence uses microfluidic devices to imitate the environment inside blood vessels. His group lines the device channels with endothelial cells, the same kind of cells that line blood vessels. The researchers can then flow red blood cells or platelets through the channels. Using such devices, Spence is studying nitric oxide stimulation by red blood cells under a variety of conditions.


Adapted from *Angew. Chem. Int. Ed.*

Shape Matters
Clotting is sensitive to both the size and shape of a patch of clotting factors. These patches have the same area, but clotting, shown in blue, occurs only on compact shapes such as the circle and the square.

Spence is also developing, in collaboration with chemist R. Scott Martin at Saint Louis University, a microfluidic device to mimic the blood-brain barrier. The three-dimensional device incorporates a polycarbonate membrane separating two pieces of PDMS. They culture endothelial cells on top of the membrane and flow red blood cells underneath it. "We can get the red cells to release ATP [adenosine triphosphate], and it stimulates NO production in the endothelial cells on top of the membrane," Spence says. ATP is known to stimulate NO production, but recent microfluidic-based work by Spence and others shows that the red blood cells are the source of this ATP.

Spence and Martin have also used vasculature-mimicking microfluidic devices to show how some drugs improve blood flow. Using the drug Iloprost, they found an unexpected mechanism (Lab Chip, in press). This hypertension medicine was thought to improve blood flow by stimulating the production of NO in endothelial cells. It turns out that the drug's efficacy is mediated by red blood cells, Spence says. If either endothelial cells or red blood cells alone are treated with the drug, no nitric oxide is produced. "Such results would not be seen without the enabling power of certain microfluidic devices," Spence says.

In other blood-related work, Ismagilov uses microfluidic systems and chemical models as tools to study blood clotting or thrombosis. He is interested in understanding the mechanism of clotting in the microvasculature and how that clotting can spread. Understanding how clotting initiates locally and then spreads to have larger effects could help in the development of therapies for thrombosis.

Ismagilov's group has previously shown that clotting occurs only when the concentration of clot-activating factors, which form a patch at an injury site, reaches a threshold value. The scientists found that this value depends on the size of the patch. But now the group has shown that the shape of the patches also influences clotting behavior. In their microfliuidic studies, they found that clotting occurs when the patch is a compact shape such as a circle or a square but not on more extended shapes, even with equal area (*Angew. Chem. Int. Ed.* **2007**, *46,*
3660). "The molecules can diffuse faster from these less compact shapes, and the activators never build the concentration up to the threshold," Ismagilov explains.

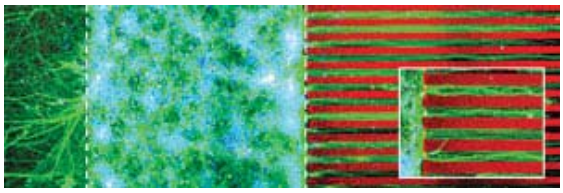
Ismagilov is also using microfluidic devices to investigate the propagation of blood clots from one vessel to another. His group has shown that clotting can spread from small to large channels (*J. Am. Chem. Soc.* **2007**, *129*, 7014). The property that influences this migration is the shear rate, which is linearly related to the rate of blood flow.

Ismagilov is using the results from the microfluidic experiments to make predictions for testing blood clotting mechanisms in animal models. "In vivo experiments are the ultimate tests, but by doing experiments in a microfluidic device, we can make very specific predictions about when things should happen" in a live animal, he says.

"We can test the mechanism in the microfluidic device because we can control all the parameters," he continues. "In the living system, at best we'd be able to measure, but it's very hard to control. If you don't have a quantitative hypothesis by the time you go into rabbits, you end up killing a lot of rabbits, and it's not clear you learned something."

Sangeeta N. Bhatia, a biomedical engineer at Massachusetts Institute of Technology, is using microfluidic devices to study sickle cell disease in collaboration with applied mathematician L. Mahadevan of Harvard. In this disorder, patients have a hemoglobin variant that polymerizes under low-oxygen conditions, causing red blood cells to stiffen and adopt a characteristic crescent shape. These crescent-shaped red blood cells can block blood flow and thus damage surrounding tissue.

The two-layer device, with a capillary network on the bottom and gas flow on the top, controls the oxygen environment around blood from a sickle cell patient. "To understand what causes a vessel to jam, it's not enough to study the oxygen environment around the cells in a beaker," Bahtia says.


Courtesy of Noo Li Jeon

Nerve Growth

Microfluidics and surface micropatterning methods are combined to develop a platform for drug screening to treat spinal cord injury and for axonal regeneration research. Cells (stained blue) are selectively placed on a band coated with polylysine (between dashed white lines). The area to the right is patterned with alternating strips of polylysine and chondroitin sulfate proteoglycan. The area to the left is coated with nonpatterned polylysine. The axons (stained green) grow only on the sections coated with polylysine. The inset is a closeup of the junction between the patterned and nonpatterned regions.

The cells need to move through small- enough channels that their distortion and interactions with each other can block the flow. Many studies of sickle cell disease have focused instead on the kinetics of the polymerization and depolymerization of hemoglobin. "That doesn't tell you how the process maps to blocking a blood vessel that is about the same size as a red blood cell," she says. Bhatia expects that microfluidic devices could be used to screen new drugs for sickle cell disease.

ANOTHER BIOLOGICAL
system that dovetails with microfluidic channels is the lung. Takayama and his colleagues are developing a "lung on a chip" that they hope will reveal fluid mechanical effects on cells in the small airways. In some diseases, such as pneumonia, cystic fibrosis, and asthma, liquid plugs form in the lungs and pop during breathing. Doctors can hear this "crackling" through stethoscopes.

"The cellular-level effects are not well-studied because it's hard to study in vivo. It's in the small parts of your lungs, and it's hard to manipulate the fluidics in a reproducible manner," Takayama says. "You can't really study it in a dish either because the dish doesn't have the lunglike fluid mechanics involved." That's why Takayama is developing a microfluidic device to mimic healthy and diseased states in the lung.

ANOTHER POPULAR
system to mimic with microfluidic devices is the nervous system. The need for alternatives to the petri dish is especially acute in neuroscience, says Noo Li Jeon, a bioengineer at the University of California, Irvine, who uses microfluidic devices to study neurons. "There are no appropriate experimental platforms that recapitulate the complexities of a brain," he says.

His group has designed devices for isolating neurons' cell bodies and axons, the long, thin portion of the cells that carries electrical signals away from the cell body (*Nat. Methods* **2005**, *2*, 599). In the devices, two parallel channels are connected by long, thin grooves. The grooves are thin enough that only the axons and dendrites of fetal rat neurons cultured in one channel can fit; the grooves are long enough that only the axons reach the other channel.

All of the neuritic processes that make it across to the second channel are "100% axon," Jeon says. Because the cell bodies and the axons are segregated, Jeon can cut the axons and observe the cells' response to different drugs. These devices can also be used to model neurodegenerative disease in vitro.

Justin Williams, a bioengineer at UW Madison, also focuses on neuroscience applications. "We're interested in using microfluidics to pulse chemical inputs over a cell and watch how it responds," he says. "We need to do that with very precise timing" because of how quickly neurons respond to their environment.

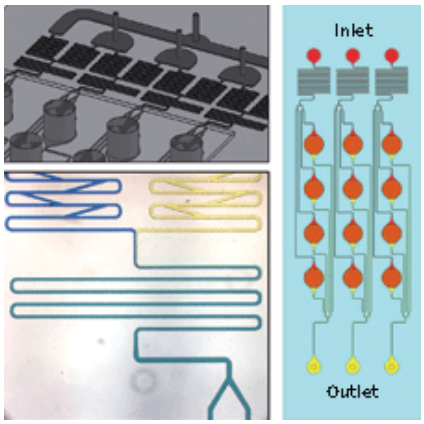
Some of Williams' devices are designed and precisely shaped for work with brain slices rather than individual neurons (*Lab Chip* **2007**, 7, 842). The three-layer device allows him to perfuse the slice from both the top and the bottom.

Laminar flow controls the chemicals that are delivered to particular areas of the brain slice. "We're interested in understanding how a particular drug may affect a very small region rather than the entire brain," Williams says. In addition, he mimics strokes by depriving small regions of the brain slice of oxygen. "Strokes are often caused by a localized vascular event in the brain that deprives oxygen to a very small part of it," he

says. "We're interested in looking at how that affects the part of the brain that is not deprived of oxygen."

Microfluidic devices also give researchers a way to construct 3-D microenvironments for cells and for tissue engineering. Beebe creates 3-D environments to study how cells talk to one another during the formation of breast cancer tumors. No-flow systems allow him to study the effects of soluble signaling molecules. In flowing systems, the signaling molecules are swept away as soon as they are secreted.

He forms the 3-D structures by flowing epithelial cells and the stromal cells of connective tissue in gel streams, stopping the flow, and polymerizing the gels. "You have an epithelial compartment and a stromal compartment right next to each other," he says. If you continue to culture the cells, the stromal cells move over to the epithelial cells and start to form an interface similar to that seen in vivo, he adds.



Courtesy of Gordana Vunjak-Novakovic; adapted from *Lab Chip*

Best Of Both
The microbioreactor array combines the independence of the petri dish with the flow and reagent control of microfluidic devices. Three views of the device are shown: a schematic of the entire device (right), an artist's rendition of the inlet end (top left), and a micrograph of the region where two channels meet.

The no-flow system also works well for Beebe's studies of the mammary gland. "It's clear that in most of the gland, and particularly in the ductal stem cell environment, there's not a continuous flow and that secreted factors are very important," he says. "We have evidence that shows that we can observe interesting cancer biology-related cell behavior in a no-flow microchannel that we can't observe in either flowing systems or conventional culture systems."

MICROFLUIDICS

could make high-throughput 3-D cell-based assays for drug screening practical, Beebe says. Traditional 3-D assays are done in gel blocks on the millimeter to centimeter scale, which makes the diffusion times too long to be useful. "The beauty of microfluidics is that we have diffusion distances of only 100 µm," Beebe says. "We've shown that you can do immunocytochemistry very easily. That's where microfluidics will hopefully be able to make a contribution, where you have a practical advantage" over traditional 3-D assay methodologies.

On the tissue engineering front, Columbia's Vunjak-Novakovic and her coworkers have developed a device that combines the key features from standard cell culture dishes and microfluidics (*Lab Chip* **2007**, *7*, 710). Their microbioreactor array consists of 12 wells that are independently plumbed for flow. Each well boasts not only the independence of a petri dish but also the flow and reagent control of microfluidic devices. She grows embryonic stem cells in the device.

In other studies, Takayama has engineered a way to control the size of the cell clusters known as embryoid bodies, which embryonic stem cells form. A thin thread of cells flows through microchannels. The cells aggregate and break into clusters, the size of which can be controlled by changing the channel geometry (*Lab Chip* **2007**, *7*, 770).

Ismagilov uses microfluidics to control the environment experienced by fly embryos. He would like to figure out how embryos keep their development in sync at various temperatures. One protein, responding to a gradient established by another protein, is always expressed in exactly half of the embryo, even at different

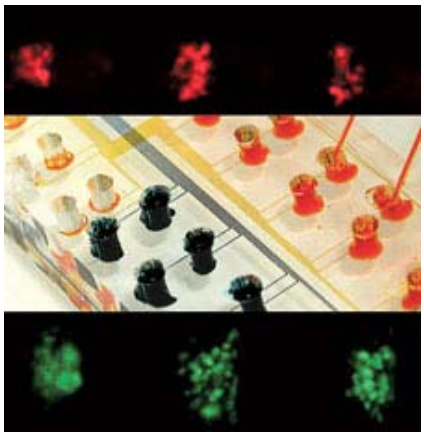
temperatures. "It's like saying I have a balloon of chemicals and the balloon somehow knows exactly where its middle is," Ismagilov says.

He tries to disrupt this process by using laminar flow of fluids at different temperatures to heat and cool different parts of the embryo simultaneously. The warm half always develops faster, so he can tweak the fly embryo to express proteins out of order. No matter how much out of sync the proteins get, however, they end up in the right place, a sign of system robustness. "People understand the basic mechanism by which patterns appear, but it's not clear how this mechanism can compensate for different rates of development," Ismagilov says. "Microfluidic devices allow us to create environments that perturb development very strongly and may pull out which nonessential mechanisms are responsible for robustness."

From the technology side, the drivers for using microfluidic devices as biological mimics are quite powerful. "We can leverage everything that's gone on in the semiconductor revolution quite trivially," Bhatia says. The technology for manipulating tiny silicon features the size of a cell—about 10 µ;m—is decades old, she notes. The soft lithography technology for making polymeric microfluidic devices is also maturing.

ENGINEERS WORRY
that their devices may be too complicated for biologists. "We always struggle with having our engineer hat and designing all these neat features versus finding the ones that the biologists really want and making them as simple as possible," Williams says.

Jeon agrees. "We engineers tend to make things complicated and cool, but nobody in the field of biology would use it," he says. Engineers and chemists need to collaborate with biologists to make sure that their devices do address questions that biologists want to answer. "From the biologists' standpoint, they only want to use microfluidics if they absolutely cannot do what they want to do without microfluidics," Takayama says. "Either we have to find those applications where biologists have no other alternatives, or we have to make microfluidics as simple as pipetting reagents into a well."


Courtesy of Shuichi Takayama
Culture Array
Arrays of cells can be cultured in microfluidic devices. The yellow channels are for cell culture, and the blue channels are for reagent delivery. The channels are connected to reservoirs. The cells grow at the intersections of channels. The upper and lower panels show parts of a channel array where cells have been labeled with different fluorescent stains.

In the push for a broader adoption of microfluidics, there are opportunities for more technology development. "I think there's an opportunity on the instrumentation side to reinvent around these platforms," Bhatia says. "Until now, we all have been leveraging what exists and engineering our systems to fit the measurement technologies." In particular, Bhatia believes that there are opportunities for new detection technologies to be developed for microfluidic systems.

No matter how simple or complicated the devices, biologists will want to see that results from microfluidic devices can somehow be correlated with those from traditional methods. The systems need to be characterized so that biologists can understand their relationship to traditional methods.

Beebe is trying to do some of this basic characterization by comparing cellular stress responses in 96-well plates as well as in microfluidic channels. He realizes that the engineering community may find such studies

boring, but the data developed during such exercises is necessary to increase the biological community's confidence in the devices. "We're at a point where it's important to do these fundamental studies," he says. "Otherwise, we're in danger of the whole field saying it's just a bunch of engineers making a bunch of widgets that are cool but are never going to have any utility."

There's too much opportunity to squander to allow that perspective to take hold, say champions of microfluidic studies. "Being able to study the microenvironment more precisely is a very generalizable thing," Bhatia says. "The low-hanging fruit has been in systems where it's clear that either architecture or dynamics or microscale signaling have been important and have been intractable. Developmental biology and stem cell biology and tissue engineering have been easy first places to look, but I think you'll start to see it in a lot more places."

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