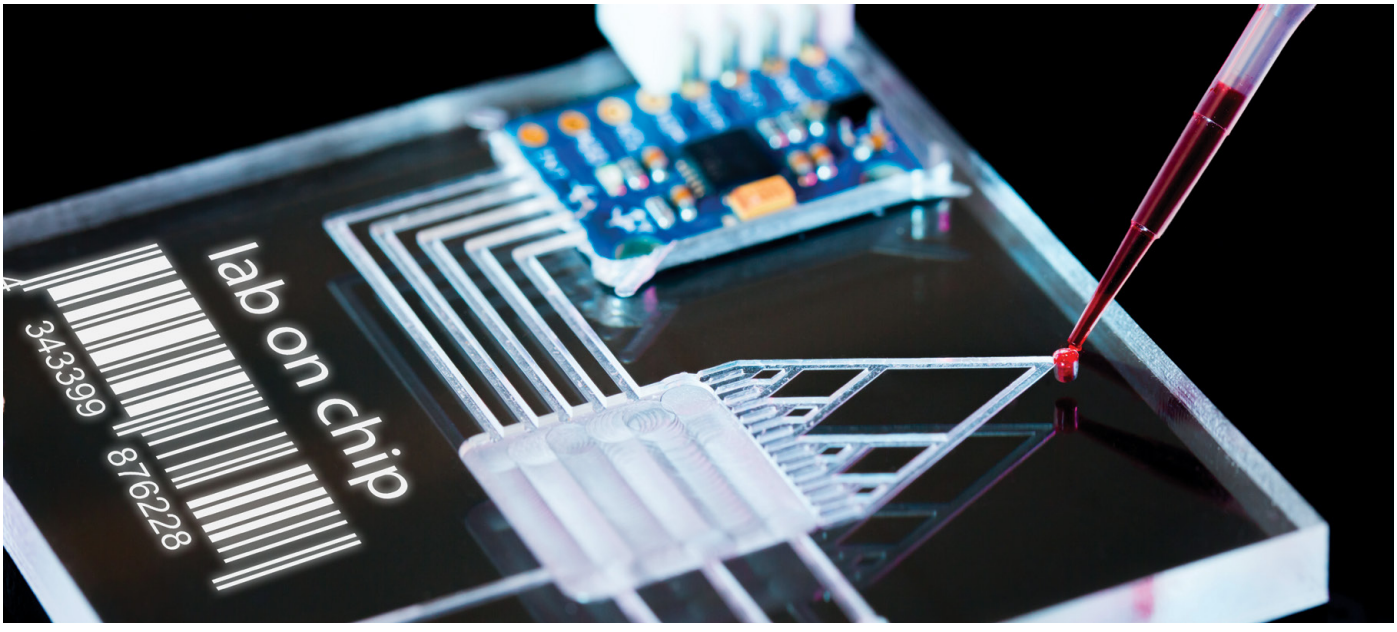

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In vitro veritas:
Biosensors and microarrays come to life

Advances in biosensors and microfluidic devices are driving a quiet revolution in biomedical research, which could lead to the reduction or elimination of animal use in many experiments. **By Alan Dove**

For decades, laboratory biologists have regarded animal models as a necessary evil. While some activists decry their use on moral grounds, even the most practical-minded researchers acknowledge fundamental problems with them. Animals are expensive, provide only imperfect replicas of human biology, and introduce numerous variables into experiments that can be difficult or impossible to control.

These flaws aren't purely academic. Pharmaceutical researchers have struggled for years with late-stage development failures, in which drugs that look promising in multiple animal systems turn out to be useless or even toxic in humans. Nonhuman models have simply been the least bad tool for detailed studies on human biology.

Thanks to advances in completely unrelated fields, though, that grim situation is starting to change. Using improved biosensors that can monitor microscopic compartments, and microfluidic devices that combine the miniaturized features of computing chips with living cells and tissues, researchers are now building systems that can reduce or even eliminate the need for laboratory animals while simultaneously yielding better data.

Diamonds are for sensors

Pharmacokinetics researchers have been among the most enthusiastic proponents of improving on animal models. Ideally, researchers would like to know exactly where a drug goes in the body and how and when it's processed, generating a detailed history from dosing to metabolism to excretion. In practice, that has entailed cumbersome techniques such as medicating numerous animals and sacrificing them for analysis at different times. Besides being laborious and expensive, these experiments give only coarse measures of drug metabolism across time and body compartments.

In recent years, investigators have implanted tiny, electrochemical carbon-based sensors into animals, which provide measurements of metabolic changes in real time in a single animal. That's worked for natural signals such as dopamine and serotonin levels in the brain, but the high background noise and low dynamic range of carbon probes make them poor choices for studying drug metabolism.

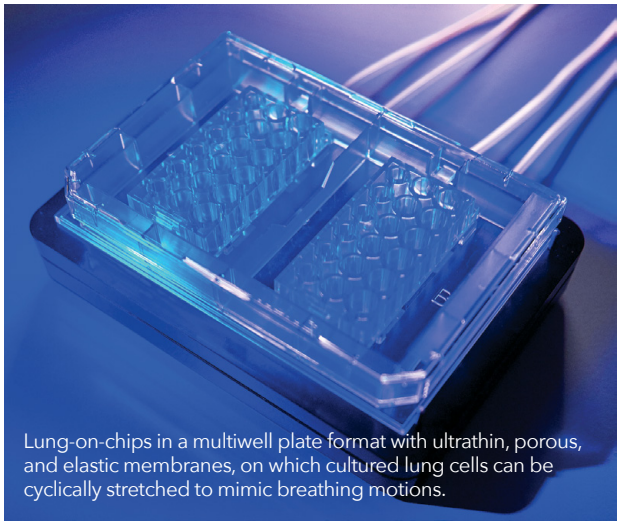
Chemists and biologists are collaborating to overcome those limitations. Yasuaki Einaga, professor of chemistry at **Keio University** in Yokohama, Japan, has worked on electrochemical sensors for decades. Einaga's group has found that boron-doped diamond probes are particularly good at detecting electrochemical changes in solutions. His researchers have tested these probes in systems ranging from wastewater treatment to chemical synthesis.

"To further explore their biological applications, in 2007 [we] succeeded in downsizing the ... probes to micro scale," says Einaga. Hiroshi Hibino, professor of molecular physiology at **Niigata University School of Medicine** in Niigata, Japan, saw the system's potential for pharmacokinetics applications, and began collaborating with Einaga in 2011.

The two labs have since found that boron-doped diamond probes can detect electrochemical changes caused by several classes of drugs in live animals and explanted organs, cont.>

providing real-time data on drug concentrations. "The number of drugs measurable by these sensors is much more than that of any conventional electrode," says Einaga. In one recent paper, for example, the team accurately measured concentrations of a diuretic, an anticonvulsant, and a chemotherapeutic agent in guinea pigs' inner ears.

"[We] have a plan to test the kidney, [and] both groups are discussing a further collaboration to develop an implantable microsensing system to track [a drug] and its effects longitudinally in organs such as the brain," says Hibino. He adds that the two labs are now in discussions with several other researchers who want to use the technology in fields ranging from oncology to psychopharmacology.



Lung-on-chips in a multiwell plate format with ultrathin, porous, and elastic membranes, on which cultured lung cells can be cyclically stretched to mimic breathing motions.

"We want to be able to take cells from a patient and test them ... and see which therapy is going to be best for that patient."
– Olivier Guenat, CEO of AlveoliX.

Plumbing codes

While improved biosensors can extract more and better data from each animal, they can also be combined with microfluidic devices to replace an animal outright, at least for some types of experiments. Microfluidic device makers borrow techniques from the electronics industry to manufacture miniature laboratories on chip-like wafers. The small size of the chips' channels and chambers means that fluids can flow through them rapidly. Microfluidic devices can also include complex structures that mimic biological compartments, making cultured cells behave more naturally. Finally, the chips can be mass-produced on semiconductor manufacturing equipment, making them relatively cheap.

Over the past few years, biologists have built a series of progressively more complex microfluidic devices, which have essentially evolved into artificial, miniaturized human organs. Biosensors built into these systems allow researchers to watch in real time as, for example, a tiny human-like liver or kidney reacts to an incoming dose of a drug.

The power of these new systems can be intimidating, though. "The biggest challenge for us instrument providers is to educate people," says Fabien Crespo, head of marketing and sales at **Elveflow** in Paris, France. Crespo adds that "people are kind of afraid of microfluidics, which is a lot about plumbing."

Elveflow and a few other companies have made this microscopic "plumbing" their main focus. "Researchers can now find microchips suited to their applications, and we are providing everything to control liquid flow inside those microchips," says Crespo. Because the microfluidics field has standardized the different types of fittings that go into and out of chips, a single liquid-handling system can adapt to changing laboratory needs. In Elveflow's setup, for example, a point-and-click computer interface allows researchers to build multistep protocols controlling liquid flows. More advanced users can use a scripting interface to drive the system programmatically.

Such flexible, modular systems are undoubtedly one reason microfluidics are becoming so popular. "We are seeing a lot of expansion in the field, especially over the last two years," says Crespo. He adds that while the first users of microfluidics were mostly in academic research laboratories, he's seen increasing demand from industrial scientists. That's likely driven by interest in developing new preclinical drug screening assays, but Crespo also expects microfluidics to start showing up in point-of-care diagnostic tests within the next few years.

The government chips in

The groundwork for the new boom in microfluidics began nearly a decade ago, through a combination of basic research and farsighted government assistance. When researchers in a few academic laboratories began building "organ-on-a-chip" systems, administrators at the **U.S. National Institutes of Health** (NIH), the U.S. Food and Drug Administration, and the Defense Advanced Research Projects Agency took notice. "It became apparent ... that this was going to be a promising tool and technology," says Danilo Tagle, associate director for special initiatives in the Office of the Director at the National Center for Advancing Translational Sciences (NCATS), one of 27 Institutes and Centers at NIH.

Tagle and his counterparts at other agencies convened a meeting with researchers in 2011 to discuss turning the new tissue and organ chips into practical models for drug testing and regulatory approval. "Numerous studies in the past few years have indicated that as much as 90% of the attrition in drug development is caused by failure to predict safety as well as efficacy when using [cell] culture systems and in vivo animal models," says Tagle. "What we're hoping [is that] these tissues on chips or organs on chips can fill in the missing information we need in order to have better success in drug development."

To help make that happen, NIH created the **Tissue Chip for Drug Screening** program in 2012, which NCATS administers. Collaborating with several academic labs, the initiative's five-year goal was to build organs on chips that could yield accurate predictions about drug responses in humans. Researchers funded by the program had to build devices that could keep cells alive in a setting that would mimic specific organs or tissues, and incorporate biosensors to measure the cells' physiology. The program has now established several independent testing centers for organ chips, using over 100 drugs that cleared traditional preclinical testing only to fail in clinical trials. "We're asking the question, 'Would [a given chip] have predicted the adverse event that the 2D culture systems and the animal models were unable

PHOTO: COURTESY ALVEOLIX AND UNIVERSITY OF BERN, SWITZERLAND

Featured participants

AlveoliX
www.alveolix.com

ARTORG Center for Biomedical Engineering Research
www.artorg.unibe.ch

Elveflow
www.elveflow.com

Emulate
emulatebio.com

Hesperos
www.hesperosinc.com

Keio University
www.keio.ac.jp/en

Niigata University School of Medicine
www.med.niigata-u.ac.jp/eng/top.html

Tissue Chip for Drug Screening Program, U.S. National Institutes of Health
ncats.nih.gov/tissuechip

Wyss Institute
wyss.harvard.edu

to predict?'" says Tagle. Organ chips that do well in these tests could be used to supplement or even replace animal data in future regulatory filings by drug makers.

Having concluded its first phase in July, with several promising systems being tested for assaying drug toxicity, the Tissue Chip program is now focusing on models for drug efficacy. These single- and multiorgan systems will be built to mimic particular diseases, including Parkinson's disease, amyotrophic lateral sclerosis, and osteoarthritis.

Deep breaths

The main advantage organ chips have over traditional cell culture systems is their ability to simulate the complex structures and dynamics of intact organs. Instead of being restricted to flat, solid surfaces, chip makers can incorporate microscopic channels, curves, pores, and layers for the cells to populate. The carefully controlled, programmable fluid flows of a microfluidic device add to the realism of the technology, recreating the forces those same cell types would encounter in living humans.

Lungs provide a good example of both the challenges and the potential of this approach. In the human lung, cells maintain a semipermeable barrier with two distinct sides, allowing gas exchange between air and blood, while keeping the two fluids separate and withstanding regular cycles of flexing with each breath. Researchers at Harvard University's **Wyss Institute** first mimicked this system with a lung chip in 2010. **Emulate** in Boston, Massachusetts, is continuing development on that and other organ chips.

The Boston team isn't alone, though. "We saw this very interesting paper from the Wyss Institute, [and] we wanted to go further [with the concept]," says Olivier Guenat, CEO of **AlveoliX** in Bern, Switzerland. Guenat is also a group head at the University of Bern's **ARTORG Center for Biomedical Engineering Research,** where his lab collaborates with clinical teams treating patients with lung diseases.

To simulate the structure and three-dimensional deformation of a lung, Guenat's team developed a platform that seeds cells onto thin layers of silicone containing regularly spaced holes. Only 3 micrometers thick, the layers are nonetheless strong enough to withstand repeated, breath-like flexing. Having accomplished that, "the second biggest challenge was that we wanted ... to develop something that is very easy to use," says Guenat.

After several design iterations, AlveoliX now has a prototype system that maintains 12 lung chips on a standard-size multiwell plate. This arrangement enables users to handle and test the chips with existing microscopes, plate readers, and other common laboratory equipment. Ultimately, "we want to be able to take cells from a patient and test them ... and see which therapy is going to be best for that patient," says Guenat.

The chips should also prove useful for preclinical studies, allowing scientists to control fluid flows, mechanical stresses, and other parameters with far greater precision than they can in living models, while simultaneously eliminating the challenges of animal handling. "I don't know any biologist who likes to sacrifice animals, and with organs on chips [now available], we really want to reduce animal testing," says Guenat.

Concerto for organs

As organ chips become more established, scientists in the field are already pushing microfluidics and biosensor technology to the next logical step: multiorgan systems. In theory, one could simply pump culture medium through different chips in series, circulating drugs and metabolites within a high-tech homunculus. The reality turns out to be considerably more challenging.

"There's just a lot of things that can go wrong when somebody tries to do this," says Mike Shuler, president and CEO of **Hesperos** in Orlando, Florida. The company specializes in multiorgan chip systems. Shuler, who is also a professor of engineering at Cornell University in Ithaca, New York, says even people intimately familiar with multiorgan chip systems can have trouble getting them running. "When I'm transferring technology from my [academic] lab down to the company, sometimes it takes a few iterations for us to get it to work right," he says.

As a result, Hesperos has built its business on offering multiorgan models as a service, rather than trying to sell and support them as stand-alone products. To date, the company has collaborated with several pharmaceutical companies interested in testing drug leads on chips. Shuler says Hesperos typically builds systems with four or five interconnected organs. "The liver is almost always the critical one, then cardiac and neuromuscular junctions ... are probably the [next] most popular," he says, adding that the company has also built systems incorporating artificial skin, gastrointestinal tracts, and blood–brain barriers.

One of the biggest challenges has been keeping the systems running long enough for extended metabolic testing. "We try to operate out to 28 days," says Shuler. At that timescale, the difference in solubility between oxygen and carbon dioxide in the cells' medium can cause gas bubbles to accumulate, disrupting the system's tightly controlled fluid flows. Hesperos has addressed that problem by eliminating the pumps normally used to control microfluidic devices, and using a carefully designed gravity flow system instead.

As the technology continues to develop, proponents of microfluidic systems expect their popularity to skyrocket in the next few years. "We're already being put into workflows for large and small pharma, [and] at this point we've been able to meet just about every single milestone that people have set out for us," says James Hickman, Hesperos' chief scientific officer. Shuler projects that human organ chips "eventually will, I think, replace animals [or] so greatly reduce animal use that it's a much less significant part of the drug development process, because you are getting data on human systems."

Alan Dove is a science writer and editor based in Massachusetts.