TECHNOLOGY FEATURE

A LIVING SYSTEM ON A CHIP

For years, scientists have struggled to reconstruct tissues and organs by combining cells and nanotechnology. These devices are now edging from cool concept to practical application.

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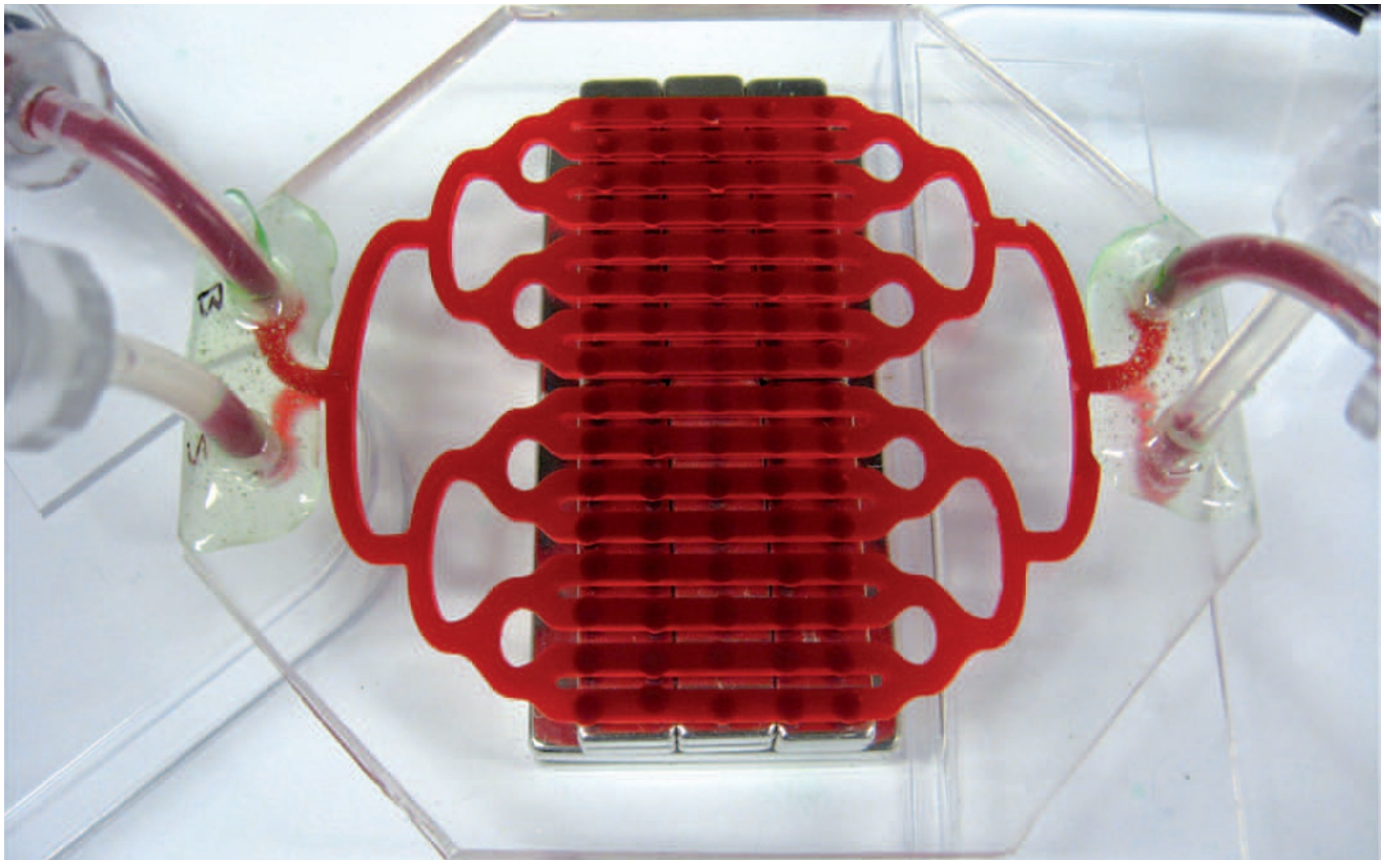


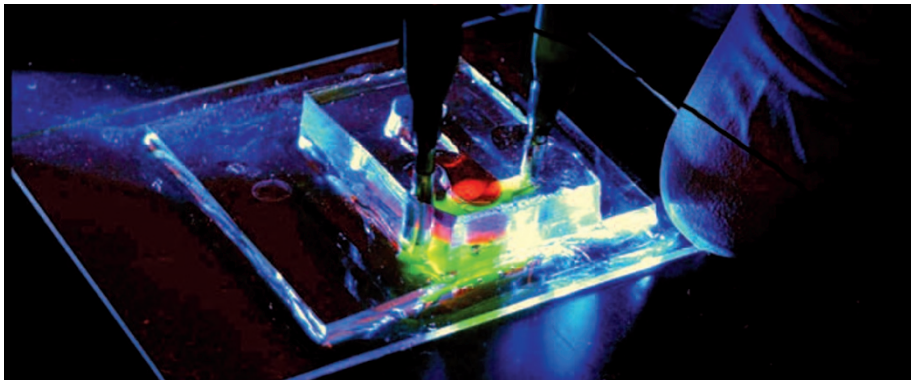
Researchers are making miniature versions of organs such as the spleen (above). Such organs on chips could speed tests of drugs and toxicity.

BY MONYA BAKER

For more than a decade, researchers have been etching grooves into silicon and plastic wafers, filling the spaces with living cells, and hoping that the resulting devices will mimic biological systems such as the liver or gut. Scientists at the Wyss Institute for Biologically Inspired Engineering at Harvard University in Boston, Massachusetts, have created one of the most sophisticated devices so far: a lung on a chip that represents several types of tissue[1]. "We started with the simplest embodiment of human airway and capillary cells, and then introduced immune cells," says Donald Ingber, head of the institute. The chip holds a pair of microchannels separated by a flexible, porous 10-micrometre membrane. One channel contains air and a layer of epithelial cells such as those lining the tiniest air sacs in the lung; the other holds the type of cell that lines capillaries, along with flowing liquid to simulate blood. The set-up even models breathing: vacuum chambers attached to the channels simulate the mechanical forces that cells encounter as a person's chest expands and contracts.

The chip showed that the cells' behaviour changes when they are stretched. To model the effects of air pollution on the lungs, Ingber's team placed toxic nanoparticles on the surface of the air-sac cells. More particles moved across the membrane from the air channel to the blood channel when the vacuum-controlled 'breathing' apparatus was operating than when the 'lung' was at rest, indicating that toxicity tests on static cells underestimate the detrimental effects of airborne particulates. More-complex behaviours could also be monitored: when substances known to provoke an immune response were introduced into the air channel, white blood cells migrated across the membrane, simulating what occurs in actual inflamed lungs.

The goal, says Ingber, is not to make replacement organs for transplant, but to replicate enough of a lung's functions to make the chips



N. DOUVILLE/Y. TUNG/S. TAKAYAMA LAB, UNIV. MICHIGAN

An air sac on a chip can mimic the mechanical stress on cells caused by fluid in the lungs.

useful in testing substances for therapeutic and toxic effects. "We are not making a lung," he says. "We are inspired by design principles of what makes a lung relevant physiologically."

Although researchers have many ways to study isolated proteins and cultured cells, experimenting on tissues generally requires whole animals or freshly dissected body parts. Such experiments are costly and often unreliable, and can raise ethical issues. Organs on chips are still very much a work in progress, but advances in culturing cells and manufacturing nanomaterials mean that they could eventually supplement or supplant animal studies.

A little-appreciated advantage is that the chips are more consistent than whole mice, says Judith Swain, executive director of the Singapore Institute for Clinical Sciences. "People may say it's halfway between *in vitro* models and animal models," she says, "but it goes past that. It endeavours to create the smallest functional unit so that you can control things and you're not confounded by variability."

Chips need further validation before they can move from research project to research tool. "We think this is tremendously exciting, but it has a good way to go before it can substitute for some of these animal tests," says Jesse Goodman, chief scientist at the US Food and Drug Administration (FDA). Nonetheless, the agency is preparing guidelines on how to replace animal tests with chips or related technologies, including computational and cell-based screening. Even if the FDA does not end up considering experiments on chips to make its decisions, says Goodman, the technology can still make drug discovery more efficient by helping companies to decide which drug candidates to prioritize for animal studies. The chips will be especially useful, he says, if they can be used to study toxicity over several days of repeated exposure, or if they can be seeded with cells from different patient groups to reflect varying responses to drugs.

The simulated lung will need to be even more complex than it is at the moment, says Alan Ezekowitz, an immunologist at Merck Research Laboratories in Rahway, New Jersey. "The lung on a chip is the beginning; it's a very simple prototype," he says. Modelling how absorption changes as cells stretch is impressive, but Ezekowitz would like a way to model the lungs' muscles too, so that screens can assess what might cause effects such as spasms in the bronchial tubes. And the Wyss Institute's current chip includes only one kind of white blood cell — neutrophils — when in fact the lung is monitored by several types, including dendritic cells, lymphocytes and macrophages, all modulating each other's effects.

Ingber is adding more types of cell. He foresees the lung on a chip eventually being seeded with cells derived from people with conditions such as asthma, being customized for different assays, or being used to gauge rates of pulmonary scarring or absorption of inhaled drugs. Chips won't replace animal testing, says Ingber, but they could reduce it and provide options for diseases for which no good animal models exist.

TACKLING THE WHOLE ANIMAL

Given the difficulty of recreating a single organ, representing the entire body on a chip sounds impossible — but it was actually one of the first biology-on-a-chip projects to be tackled. Michael Shuler, a bioengineer at Cornell University in Ithaca, New York, is credited with coining the phrase 'animal on a chip' in the late 1990s, after he and a colleague, Gregory Baxter, began etching silicon wafers to form tiny compartments that would hold gut, liver and fat cells, all linked by microfluidic channels. The approach, which Shuler calls a "microscale cell-culture analogue", is a physical manifestation of mathematical models used to predict how drugs move through and accumulate in various organs[2].

Frank Sonntag, a biosystems technologist at the Fraunhofer Institute for Material and Beam Technology in Dresden, Germany, leads a group that is trying to predict systemic toxicity using what Sonntag calls a chip-based multi-micro-organoid culture system[3]. His chips hold six identical micro-bioreactors, each containing cells chosen to mimic the liver,

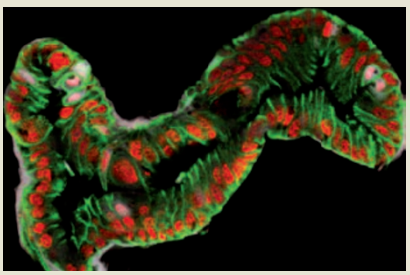
Just cells

The development of simulated tissues on chips has been hindered because researchers couldn't grow cells that functioned as they would in the body. That may be changing, says Alan Ezekowitz, an immunologist at Merck Research Laboratories in Rahway, New Jersey. "The birth of embryonic stem cells and induced pluripotent stem (iPS) cells has provided a biological opportunity to mesh into bioengineering expertise." Such cells could be used without chips, because they can develop into complex tissues on their own.

James Wells, a molecular biologist at the Cincinnati Children's Hospital Medical Center in Ohio, this year showed that iPS cells can form hollow clumps called spheroids. These are made of multiple types of cell, including mucin-secreting goblet cells and nutrient-absorbing enterocytes[8]. Hans Clevers, a molecular geneticist at the Hubrecht Institute in Utrecht, the Netherlands, and his colleagues had previously made similar structures using intestinal stem cells[9].

Most drugs are swallowed, and enter the bloodstream through the intestines, so scientists hope that reconstructed gut tissue will be useful in medical research. "You can envision a lot of primary drug screens that are looking not just for toxicity but also absorption and bioavailablity," says Wells. The gut also makes several hormones that influence appetite and obesity, he says, and the structures could be used to hunt for compounds that modulate the secretion of these hormones. But there are several challenges: the cells need to be produced in larger quantities, and they must be adapted to culture conditions amenable to monitoring.

Meanwhile, Wells and others are trying to create structures to represent other organs. "That really is the next step for a lot of tissues, to generate three-dimensionality," says Wells. Upcoming tissues on chips might not be on chips at all. **M.B.**



J. WELLS LAB

Pluripotent stem cells form gut tissue *in vitro*.

brain and bone marrow. A third team, led by Kiichi Sato, a bioanalytical chemist at the University of Tokyo, has created a chip[4] to test how cell lines representing breast cancer, liver and intestine interact with drugs.

One difficulty with the chips is the complexity of modelling the proportion and sequence of blood flow to each 'organ'. Shuler says that some devices capture blood distribution at least as well as mathematical models, but they do not model other aspects, such as how blood flows within an organ.

The greater issue, however, is that current devices rely on cell lines that grow readily in culture, rather than the more-finicky cells that better represent organ function. Chips will become more predictive in the next few years as researchers learn to cultivate "more authentic" cells, says Shuler.

FROM ANIMALS TO ORGANS

Shuler is now working on reconstructing better models of the organs through which drugs move. The intestines, a barrier that must be passed by all swallowed drugs, seem surprisingly easy to model (see 'Just cells'): using cell lines representing only the gut epithelium, mucin-secreting cells and lymphocytes, Shuler and his colleagues have been able to recreate the mucoid layer in the gut[5]. With the help of an absorbent polymer gel that can be used to build microscale scaffolding, the team has even crafted a collagen structure to represent the villi that line the intestinal wall[6]. Meanwhile, the Wyss team is developing a model of the gut that mimics peristalsis using vacuum chambers similar to those in the lung chip. This model allows researchers to observe molecules passing from the gut chamber into the blood chamber, says Ingber.

"More complex cultures are needed for more complex questions."
Linda Griffith

Several companies are developing chips that can be used as miniature testing systems. Myomics in Providence, Rhode Island, for example, grows models of skeletal muscle in multi-well plates. It is collaborating with pharmaceutical partners to screen drugs that might harm muscles, as well as one that could be used to treat muscle disorders.

It can be difficult to create systems that are robust enough to be shipped and simple enough for most scientists to use, says Robert Freedman, chief executive of Hurel in New Brunswick, New Jersey. The company was co-founded by Baxter in 2005 and is developing chips to investigate liver toxicity and skin allergies. Part of the product-development process, says Freedman, was switching from opaque silicon chips to transparent plastic ones, to enable microscopy studies. Company researchers also had to put chips packed with living cells on an aeroplane to make sure that they could withstand pressure changes during shipping.

The company's most important task is picking systems that scientists want to buy. For example, a European Union directive to phase out animal testing for cosmetics from 2009 has created a market for *in vitro* evaluation of skin irritants, so Hurel is working with the world's largest cosmetics company, L'Oréal in Paris, to develop a replacement for a test in which a potential allergen is rubbed behind a mouse's ear. The 'allergy test on a chip' holds skin and immune cells. "Once you work out all the kinks, it will be better than the animal test because you'll use all-human materials," says Martin Yarmush, chief scientific adviser at Hurel.

LEARNING ABOUT THE LIVER

Liver toxicity is among the most common biological reasons for drug candidates to be pulled from clinical development, so it is important to be able to predict it. Even if a molecule does not harm the liver, that organ's detoxifying

The real McCoy

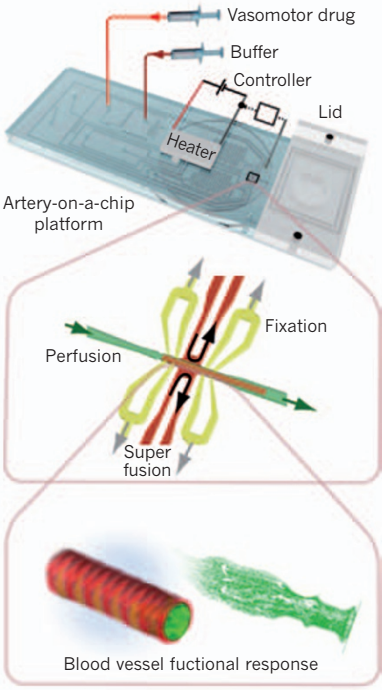
Rather than trying to build an organ from the cells up, some technologists are now turning to microfluidics platforms to help them study actual organs. One example published last year is the artery on a chip[10], engineered by Axel Günther, a bioengineer at the University of Toronto in Canada, and his colleagues. The device is set to be commercialized later this year by Quorum Technologies in Guelph, Canada. It provides a way to study 'resistance arteries', tiny vessels that keep blood from rushing into and damaging capillaries, and so help to regulate blood pressure.

The standard technique for studying such a vessel is to remove one from a mouse and suture it at both ends, manipulating its fluid environment so that the pressure in and around the vessel mimics *in vivo* conditions. The procedure is time-consuming and requires considerable training to master. "It's a very tedious and manual approach," says Günther. Even the most skilled technicians often damage arteries beyond use, and the areas around the sutures become so damaged that they cannot be studied at all.

Günther got the idea for the chip after visiting a collaborator's lab and seeing a similar technique first-hand. "If you had a more scalable approach" than the one he had seen, he says, "perhaps you could introduce that into drug development, sacrifice fewer animals per screen, and still get better data".

ARTERY ON A CHIP

A microfluidics chip positions blood vessels for multiple tests.

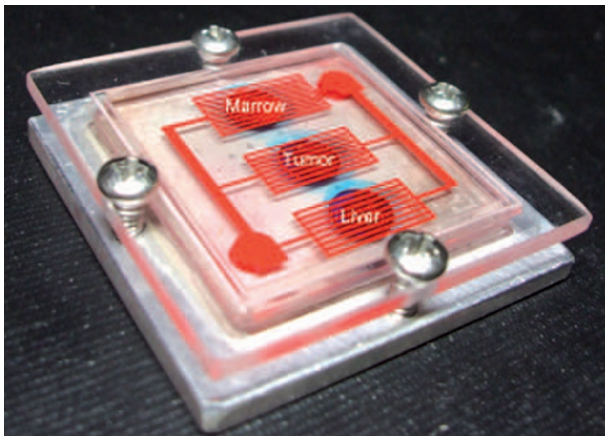


A. GÜNTHER

The chip that Günther and his colleagues designed lets researchers place a blood vessel on a microfluidic chip, where a specially designed chamber holds it in place and feeds liquid through it continuously. Not only is it significantly faster to mount vessels onto chips than to suture them for conventional study, says Günther, but imaging and reproducibility are also improved; the same artery can be exposed to different doses of drugs over time, using a computer-controlled system. It could show, for example, whether the artery wall responded when a chemical irritant was applied to the other side.

The next step, says Günther, is making chips that can hold more than one artery at a time, so researchers can collect statistical data. Such a set-up would let them compare vessels collected from different parts of the body, from different types of mouse or from clinical biopsies. If the mounting and isolation procedures can be sufficiently simplified, he says, chips could be used with vessels taken from individual patients to help doctors tailor blood-pressure medication regimes. M.B.

J. H. SUNG/SHULER LAB, CORNELL UNIV.



Channels can simulate blood flow to 'organ chambers' on the chip.

actions may harm the molecule, rendering potential drugs ineffective. Compounds can be tested in 'primary' cultures of liver cells, which have been gathered from cadavers, but these are in short supply. Moreover, the cells behave differently and die quickly when grown flat in a dish.

Consequently, several companies and academic labs are developing liver platforms with an eye to drug screening. Hurel plans to launch its liver-cell chips later this year. In 2007, RegeneMed in San Diego, California, began selling three-dimensional liver co-culture plates and screening services as an outgrowth of previous efforts to develop artificial organs for transplantation. Each of the 96 wells in a co-culture plate is set up with what Dawn Applegate, RegeneMed's chief executive, calls a "jungle gym": nylon scaffolding with openings the right size for cells to pass through. The cells grow over the scaffolding to simulate tissue. "Cells need a third plane to express the extracellular-matrix proteins and growth factors that they would express in the body," says Applegate. Reconstituted tissue can live for up to six months, and the technology supports liver cells from several species, so it can help to resolve conflicting results obtained in different animal models. The plates contain not only hepatocytes, the most common type of cell in the liver, but all the other types as well, says Applegate. Although hepatocytes carry out most drug metabolism, chips must model interaction between different cell types to provide an idea of full liver function.

"Anything that requires dynamic control rather than just static control is a challenge."
Shuichi Takayama

In March, Hepregen of Medford, Massachusetts, launched HepatoPac: a liver platform based on microfabrication technology developed by Sangeeta Bhatia, a bioengineer at the Massachusetts Institute of Technology (MIT) in Cambridge. A substrate is dotted with collagen, which keeps different types of liver cell in their places and holds colonies of hepatocytes surrounded by supportive cells; the cells can remain functional for 4–6 weeks, says Bhatia. The platform is being developed through a partnership with companies including Boehringer Ingelheim Pharmaceuticals in Ridgefield, Connecticut. At a toxicology meeting this month, scientists from Hepregen and Alnylam Pharmaceuticals in Cambridge, Massachusetts, presented results showing that HepatoPac predicted liver damage from repeated doses of fialuridine, a potential treatment for hepatitis B that failed clinical trials in the early 1990s because it was found to cause severe toxicity in humans — an effect that had not been predicted in animal studies.

In January, CellASIC in Hayward, California, began selling a 96-well sample plate riddled with channels that provide oxygen and a continuous flow of media to hepatocytes in the wells, simulating how blood delivers drugs and toxins to the liver. The cells are assembled in 60-micrometre tubes imprinted with an artificial structure that mimics the effects of cell–cell interactions, and the hepatocytes retain a suite of liver-specific activities for more than four weeks, says Philip Lee, who co-founded CellASIC with Paul Hung in 2005. The two had developed the technology while working in the laboratory of Luke Lee, a bioengineer at the University of California, Berkeley.

The microfluidics technology in the plates relies on gravity rather than a pump system to pull media and test compounds from an inlet well, past the cells and into an outlet well, where the liquid can be collected and analysed for metabolites and other cell products. The goal, says Lee, was to create a robust product that can run on an automated system, minimizing operator-to-operator variability. Researchers can study cells directly by imaging, or collect them and break them up to study gene expression or the induction and inhibition of drug-metabolizing enzymes.

Other systems are still in academic laboratories. Linda Griffith, a bioengineer at MIT, has built silicon scaffolds less than 2 centimetres across and filled them with wells that allow liver cells to grow in three dimensions[7]. These structures are placed inside multiwell plates. Micropumps maintain oxygen and nutrient gradients — similar to those found in the body — between the wells in the silicon scaffolds. Currently, Griffith is comparing how three-dimensional liver tissue containing several cell types compares with flat hepatocyte cultures in predicting drug toxicity. The goal is to get the most information possible from the simplest culture possible, she says. "You may be able to use the simple cultures as an early screen. More complex cultures are needed for more complex questions."

PUTTING IT TOGETHER

Creating more complex cultures is getting easier, says Shuichi Takayama, a bioengineer at the University of Michigan, Ann Arbor, who has constructed chips that represent bone, liver and lung. The cell types needed for such devices are becoming more accessible, as are the growth factors and extracellular-matrix proteins needed to keep the cells healthy. But for tissues more than 3 millimetres thick, the chips also need to provide a circulatory system, and many will need to supply some sort of mechanical perturbation: tension on skin and muscles, flow in blood vessels, compression on bone and so on. "Anything that requires dynamic control rather than just static control is a challenge," says Takayama. And of course, each organ represents its own set of challenges: to simulate beating heart tissue, for example, muscle fibres must be aligned on a chip that does not interfere with the mechanical and electrical activity of cells (see 'The real McCoy').

There are other challenges associated with the logistics of the chips, says Shuler: for example, the effects of polymers and microfluidics on cell behaviour are still poorly understood. The very small sample volumes involved make collecting and analysing drug metabolites difficult, and some materials used to build the devices may actually absorb drugs. Not surprisingly, many chips require considerable expertise to operate and troubleshoot, limiting the ease with which they can be adopted by inexperienced labs.

Still, progress is real, says Ali Khademhosseini, a bioengineer at MIT, who is developing ways to create artificial circulatory systems that can keep engineered tissue alive. "The perception of chips being just cute little things is changing, and there is now more of the view that they can make a significant impact," he says ■

Monya Baker *is technology editor for* Nature *and* Nature Methods.

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