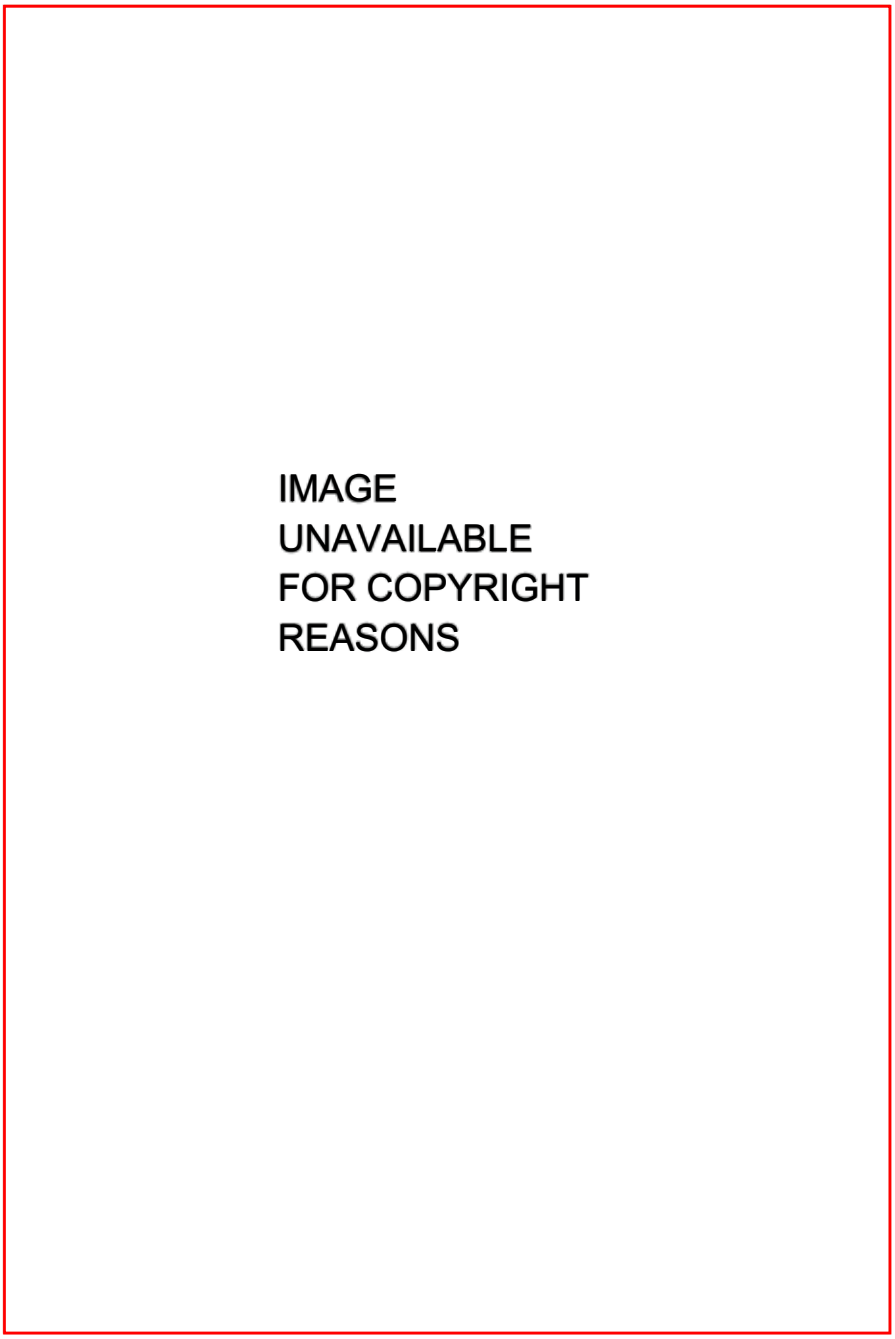
Meet the stripped down rat

Are wafers of silicon that support cells cultured from vital organs the future of drug testing and toxicology? Roxanne Khamsi talks to the pioneers creating model animals — and humans — on a chip.



Bare essentials: the 'rat on a chip' developed by Michael Shuler and Gregory Baxter.

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For Michael Shuler, downsizing is not a dirty word. In the mid-1990s, his lab bench was cluttered with flasks connected by surgical tubing. Each flask contained cells from a different organ, suspended in nourishing fluids. Shuler's goal was to model how chemicals entering the body can be metabolized into more toxic forms.

Today, the apparatus has been shrunk to a tiny device that looks like something from the insides of your mobile phone. Shuler's lab is tidier, and his chips are more adept at simulating the way in which minute quantities of fluid carry chemicals between cells in our bodies. They may also point toxicologists towards a more humane future — reducing the number of animals required to establish the safety of a drug or other chemical. "We're not using this to replace animal studies," Shuler stresses. "We're trying to have more intelligent use of animals."

Shuler, a chemical engineer at Cornell University in Ithaca, New York, was converted to the chip-based approach in 1997, after Gregory Baxter arrived on the campus to take up a position developing nanofabrication technologies. "We got talking and saw that there were a lot of advantages of adapting the system to fit on a silicon chip," Shuler recalls. Within a year, the pair had created a prototype 'animal on a chip' — a postage- stamp-sized silicon wafer that held cells from a rat's brain, heart and liver in different trenches linked by tiny fluid channels.

Channel hopping

Since then, Shuler has continued to refine his chips[1–4]. The latest versions consist of two sheets of acrylic sandwiching a piece of silicon about 25 millimetres square containing fine custom-etched channels and troughs. The devices typically store around 100,000 liver cells, about 10,000 from another organ such as the lung, plus smaller numbers from tissues such as fat. "We can grow and keep cells on the chip for weeks," says Shuler.

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Liver cells are crucial because they are the body's workhorses for metabolizing drugs and other chemicals — in particular, they produce enzymes of the cytochrome P450 family, which oxidize a wide range of drugs. It is also important to mimic the way in which some chemicals are absorbed by the body's fat reserves — Shuler's team is experimenting with lipid gels[5] in addition to fat cells.

Controlled by a micro-pump, pinkish fluid circulates from one chamber of cells to another. The nutrient liquid travels down channels about the size of a human hair, carrying drugs or other chemicals from one compartment to another. In these compartments, cells can cosy up to one another

much as they would in a living tissue, bathed in small quantities of fluid in which biologically active molecules can exert much greater effects than they would swilling around in a flask.

But Baxter and Shuler had to think carefully about the architecture of their chips, to simulate the ways in which cells are exposed to body fluids and dissolved chemicals in different organs. To mimic the rapid blood flow in the lung, for instance, the researchers initially made chambers containing multiple parallel channels. But this required more pressure, and disrupted the flow. So the researchers have since opted for larger chambers incorporating pillars on which lung cells can grow.

Proof-of-concept studies have highlighted the chips' value. Typical lab tests on single cell types, for example, struggle to reveal the lung toxicity of naphthalene, the main ingredient in mothballs. But Shuler's chips have no problem: the liver cells convert naphthalene into its toxic product naphthoquinone, a reactive compound which can then move to the 'lung' compartment where it damages the lung cells[6].

On the move

In 2001, Baxter left Cornell to commercialize the technology at Hμrel in Beverly Hills, California, a company he co-founded. One of Hμrel's studies has used the technology to demonstrate the known action of tegafur, an anticancer drug that must be converted into an active form by enzymes in the liver. As expected, the drug had no effect on its target — colon cancer cells — unless it first ran through the liver-cell compartment to get switched on.

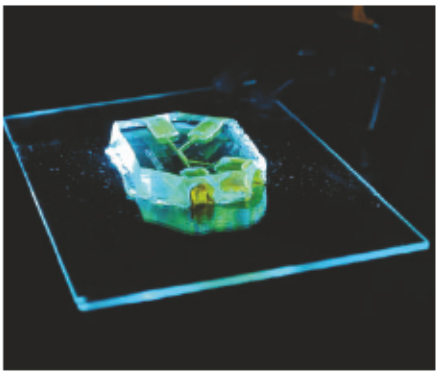
Shuler's group has now started using the chips to investigate multidrug-resistant cancer. Doctors have found that mixing medicines offers new hope for some patients, but the number of possible combinations is too great to be investigated in expensive clinical studies. With the chips, this is not a problem. "What we hope to do is screen treatments for disease that would not be screenable with animal or human studies," says Shuler.

An extra bit of tweaking has even enabled Shuler's team to mimic the blood–brain barrier, which prevents many chemicals, including harmful ones, from entering the central nervous system. The endothelial cells that form this tight barrier quickly lose their special characteristics when cultured on their own. But by growing them alongside nerve-supporting cells called astrocytes on an ultrathin membrane, the researchers have managed to restore their protective function[7].

The Cornell devices can run experiments for a couple of days, but problems then arise as bubbles of air start to form in the fluid. To try and get round this difficulty, Shuichi Takayama, a biomedical engineer at the University of Michigan, Ann Arbor, is developing chips made from gas-permeable silicone gel. The trade-off is that the chips' contents slowly evaporate.

The pattern of flow from one compartment to another in Shuler's devices is predetermined for each application by the custom-etched pattern of channels, but in the Michigan version the fluid can be moved in different ways on the same chip. Takayama's group also has an unusual and novel approach to steering the fluids around.

The idea came from Wei Gu, an undergraduate student in Takayama's lab, who happened to read about a device called a



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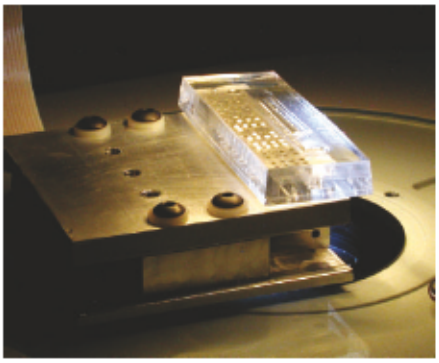

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Shuichi Takayama's chips have been used to sort sperm (above). In the latest versions, fluid flow is controlled by an adapted Braille device (below).

refreshable Braille display while browsing in the university library. This hardware features a moving arrangement of pins that converts text from a computer screen into a form the blind can read by touch. Gu realized that he could apply the grid of vertically moving pins to open and close the fine channels on the pliable silicone chip. Following computer controls, the system pinches off flow much like a person stepping on a water hose — doing away with the need for more intricate pumps and valves[8].

"Although many labs are working on microfluidic pumps, valves and even complete organ subsystems, devices that can be integrated onto a chip and be remotely controlled by a computer are rare," says Takayama. "Our goal is to not have to worry about microfluidics and worry about what to do with cells."

So far, Takayama's team has been culturing just one cell type on the chips, and has also been using them for tasks such as separating healthy from non-motile sperm. But like Shuler and Baxter, he aims to incorporate cells from different tissues and organs.

In addition to using samples from established cell lines for toxicological studies, Takayama and his colleagues plan to place undifferentiated stem cells on their chips. They then hope to mature these into nerve, bone and muscle cells, for example, by flowing the right signalling compounds through different paths, to study aspects of developmental biology.

Another dimension

Other researchers are trying to represent the three-dimensional structure of tissues more accurately, which can be important if cultured cells are to behave as they would in the body. Linda Griffith, a tissue engineer at the Massachusetts Institute of Technology, has focused her efforts on liver cells. At her lab in Cambridge, Massachusetts, researchers etch silicon to produce microscopic honeycomb-like scaffolds containing a multitude of channels and walls that cells can stick to[9]. These structures more accurately simulate the uptake of fluids in the liver, according to Griffith.

Hμrel might also experiment with similar chips in future. "It would be easy to modify the compartments to accommodate any three-dimensional tissue constructs," says Baxter. But for now, the company is concentrating on commercializing its current designs, the first of which could reach the market in about a year.

Either animal or human cells can be put on the chips. Shuler and Baxter began to incorporate the latter into some of their chips back in 2000. Toxicologists and drug developers typically work with rats because they need a living system that roughly matches human physiology. But in the end, notes Robert Freedman, chief executive of Hμrel, "a rat liver is not a human liver".

The new chips may not look much like rats or people. But if they can ensure that drugs reaching clinical trials have a better chance of being safe and effective than at present, they're likely to have a busy future. ■

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