TECHNOLOGY FEATURE

ORGANS FROM THE LAB

The body's organs are more complex than any factory. Attempts to mirror their physiology in the laboratory are getting closer to capturing their intricacies.

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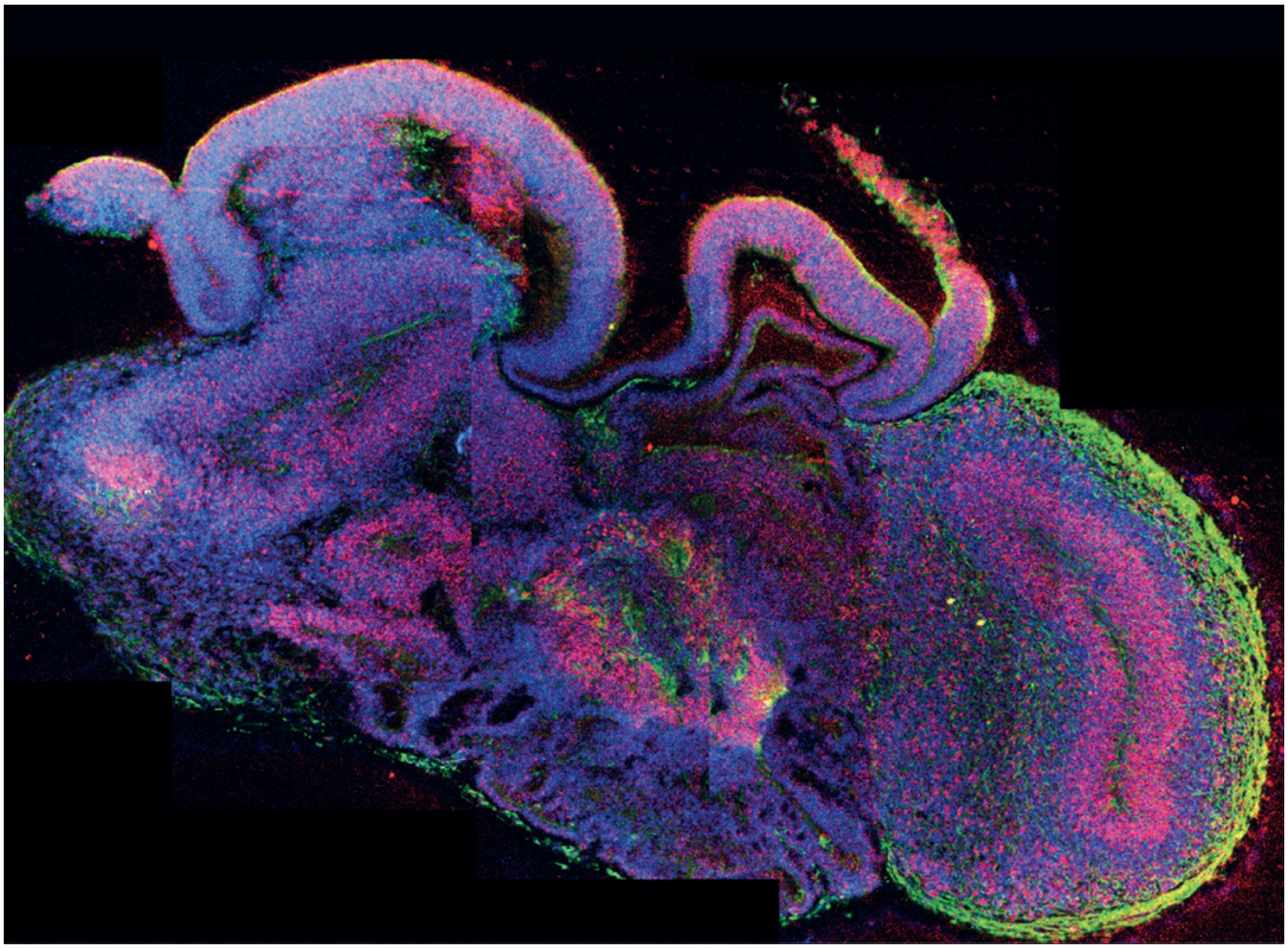

Stem cells can be coaxed into forming organized clusters called organoids, such as this brain model.

BY VIVIEN MARX

In their quest to create organs in the laboratory, researchers have come a long way. Engineered tissues are already used in medical research and have even entered clinical trials. But they are much simpler than the real thing. To make a stomach, a lab might use 3D printing to create a mould that could be seeded with the appropriate cells. But without cues provided by blood flow and interactions with other tissues, the result would be simply a stomach-shaped statue, unable to digest or growl. An organ is much more than a mass of cells arranged in a particular configuration: it also has support scaffolds, blood vessels to deliver nutrients and signal molecules, and a hierarchy of intricate control functions that can respond to internal and external cues.

All this makes it tough to build a functional, physiologically relevant organ in the lab, says Rosemarie Hunziker at the US National Institutes of Health, who manages the funding of programmes devoted to designing and building artificial organ systems.

But tissue engineers are making inroads into the problem. To try to tackle the biological complexity of organs, they can choose from various fabrication approaches. One method is to place cells into elaborate, but still simplified models of an organ the size of a microscope slide, which can then be connected together to probe how organs interact. These miniature 'organs-on-chips' provide a unique vantage into organ function and disease, and for applications such as toxicity tests of drug candidates. An alternative approach is to foster the ability of cells to self-assemble, in the hope that they will recapitulate actual organ development and reveal insights into the process.

Whatever the strategy, researchers can start with biologically simple approaches, and ▶

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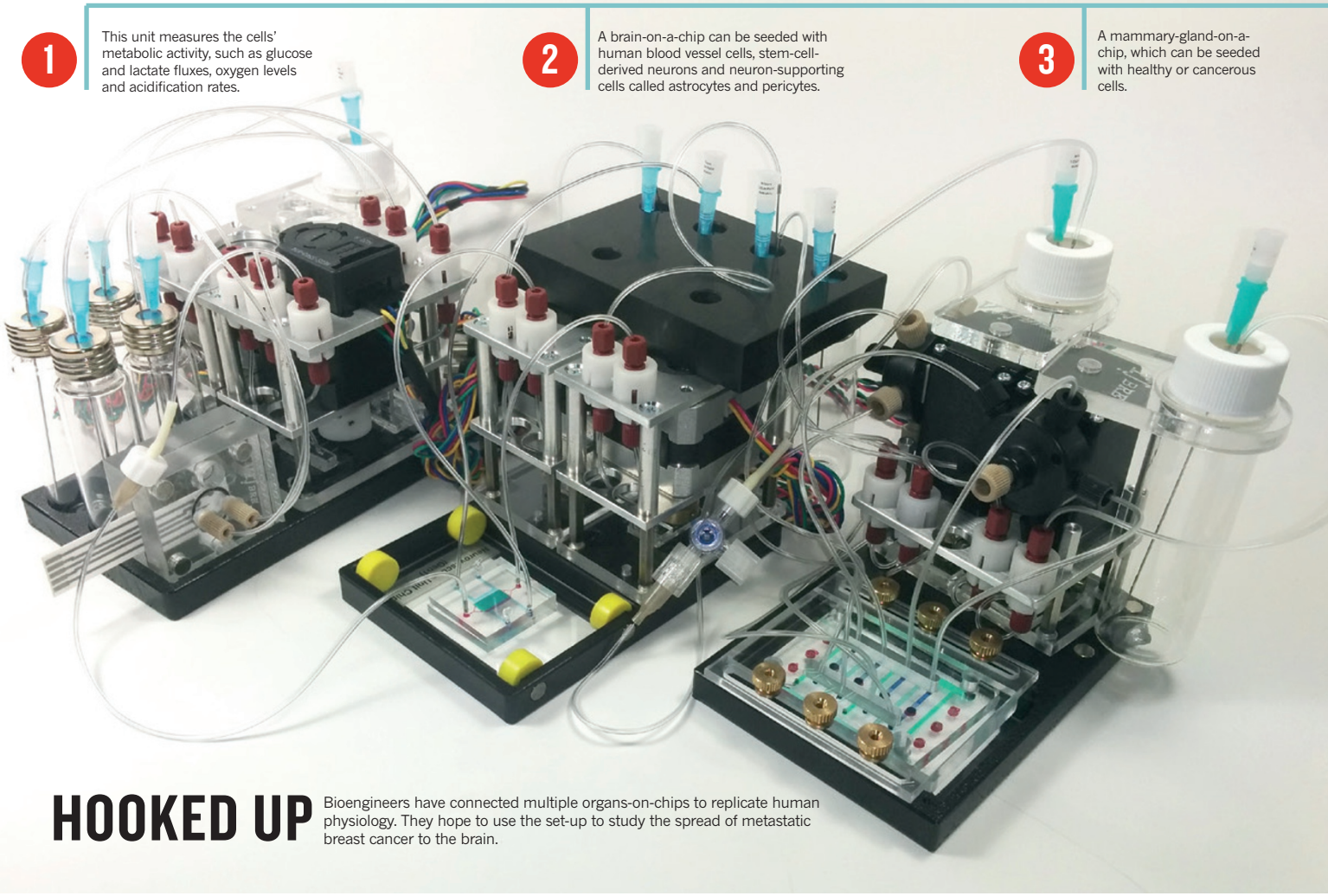

HOOKED UP

Bioengineers have connected multiple organs-on-chips to replicate human physiology. They hope to use the set-up to study the spread of metastatic breast cancer to the brain.

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then add complexity to the model a little at a time. Just how similar an artificial version of an organ needs to be to its original depends on the questions that are being asked of it, Hunziker says. Artificial organs may look very different from their *in vivo* counterparts but nonetheless be useful for drug testing and basic research. Whether the goal is to understand an organ or to replace it, the eventual aim is an engineered system that functions as reliably as the real thing, Hunziker adds.

Researchers across the world are using these systems to address a wealth of important questions. They can, for example, help to reveal how cancer cells detach from a tumour to invade other tissues, and allow scientists to recapitulate processes in disease and development, such as what might go awry in neurodevelopmental disorders.

SYSTEMS THINKING

The most highly engineered organ models are the organs-on-chips that look the least like organs in the body. They are made using similar manufacturing techniques to those for silicon microchips in computers. First, a photosensitive material is layered onto silicon, and ultraviolet light is used to etch grooves in a desired pattern into silicone rubber. This guides the production of a 3D network of hollow tubes inside a rubbery rectangle the size of a computer memory stick. The tubes are seeded with cells of the desired types and hooked up to pumps and an external fluid source, providing inlets and outlets through which scientists can mimic blood flow and deliver nutrients and environmental signals. Perfusion by continuously flowing liquid mirrors the dynamic environment in organs. The set-up also lets bioengineers modulate a tissue's stiffness as well as mechanical, chemical and electrical cues to reproduce the signals that cells might receive in healthy or diseased states, says John Wikswo of Vanderbilt University in Nashville, Tennessee (see 'Hooked up'). Researchers can replicate inflammation, for example, by adding the molecular messengers known as cytokines and even living immune cells into the chips' channels — they then watch the inflammatory response that is characteristic of most tissues when damaged or infected[1].

> *"The aim is an engineered system that functions as reliably as the real thing."*

The chips are usually transparent to allow high-resolution, real-time imaging of cells, says Donald Ingber director of Harvard University's Wyss Institute for Biologically Inspired Engineering in Boston, Massachusetts. The liver, kidney, lung, intestine, fat, muscle and the blood–brain barrier have all been rendered into chip form[2].

Now researchers are combining chips into multi-organ systems that can replicate some of the body's physiology. Gordana Vunjak-Novakovic and her team at Columbia University in New York City are building a model of the heart–liver–blood system with which to probe drug toxicity and disease. Wikswo at Vanderbilt, and his colleagues at the University of Pittsburgh, Pennsylvania, are linking organ chips together to predict the effects of potentially toxic chemicals and drugs. He believes that a liver–kidney model could identify safety problems before a drug reaches testing in humans, because these are the organs in which toxicity first becomes apparent. To emulate *in vivo* situations of health or disease, researchers can grow the appropriate cells in 3D support structures and explore their reactions to cues delivered into the system, says Wikswo.

The key to successful mimicry is attention to microstructure, says Hunziker. Careful placement of liver cells across a chip can better replicate real liver tissue, which has different zones close to and away from the main blood supply. These zones differ in the genes that are active, which results in differences in cell development and behaviour, and different responses to chemical stresses, she says.

Microscale organ systems allow experiments that cannot be done in cell cultures,

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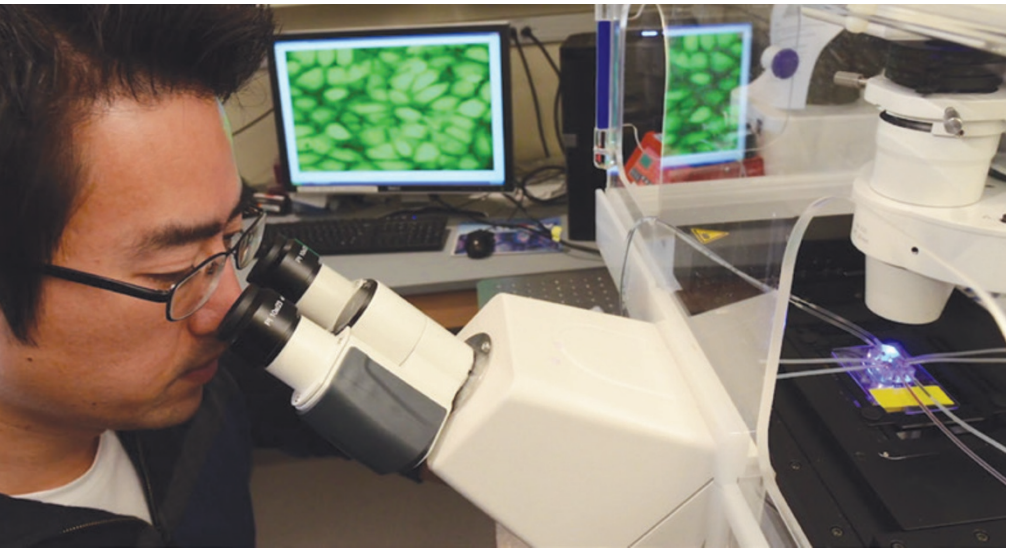

WYSS INST. HARVARD UNIV.

Dan Dongeun Huh at Harvard University's Wyss Institute tracks cellular events in a lung-on-a-chip.

animals or people, says Ingber. Organ chips lined with cells from individual patients enable the assessment of physiological differences between health and disease, and between people, in more detail and over a longer period than would be practical in people or in animal models, he says. Ingber's team has kept multi-organ chips going for more than a month. With an organ chip, it is also possible to adjust parameters to see what happens in a way that is not possible in a patient.

Several labs have formed spin-out companies to commercialize their model tissues. Emulate, in Cambridge, Massachusetts, founded by Ingber, is developing organ-on-chip systems for high-throughput drug screening and toxicity testing. The company Hepregen in Medford, Massachusetts, co-founded by bioengineer Sangeeta Bhatia at the Massachusetts Institute of Technology in Cambridge, uses the technique of 'micropatterning' to develop liver models in which different cell types are precisely placed to produce a platform that more closely mimics the complexity of the liver. These are being developed as drug-screening assays. HemoShear Therapeutics in Charlottesville, Virginia, founded by two University of Virginia scientists, has developed several organ modelling systems, including one that specifically mimics blood flow in tissues. In January, HemoShear began a collaboration with pharmaceutical company Pfizer of New York to find better ways to predict injuries to blood vessels, such as inflammation, that drug candidates might cause.

Right now, microfabrication is out of reach for many labs. However, there are some companies that offer services to make chips for labs that do not have the necessary equipment or expertise. And many universities offer microfabrication capabilities through core service centres. Meanwhile, labs at the cutting edge are working to make engineered chips better homes for living cells. One challenge is seeding cells evenly throughout the devices and maintaining their growth within the tiny channels, says Ingber. Another is that bubbles in the system can injure the cells.

THREE-DIMENSIONAL HELP

In contrast to organs on chips, soft scaffolds seeded with cells can result in artificial organs that look much more like the real thing. This approach blends a variety of synthetic materials to make a support system. It is then seeded with cells that grow and develop throughout the scaffold and thus become arranged in the desired configuration. In one well-known example from the early days of the field, Linda Griffith at Massachusetts Institute of Technology and Charles Vacanti at Massachusetts General Hospital in Boston and their colleagues used such a scaffold implanted under the skin of a mouse to guide bovine cartilage-forming cells to grow tissue in the form of a human outer ear[3,4]. The polymers in the scaffold degraded as the tissue formed, leaving behind the structure made of cartilage.

Today, Griffith and her team use a custom-built 3D printer to create highly intricate tissue scaffolds. A stream of photoreactive polymer spurts out of the instrument's nozzle and one layer at a time is exposed to ultraviolet light to stabilize the structure. Material is removed in an iterative process to a build micrometre-scale substructure.

Scientists have also developed ways of mimicking the mechanical stimuli that seem crucial to tissue development. For example, the early development of teeth in a mammalian embryo involves embryonic cells packing closely together. To mimic this process, Ingber's lab has developed a polymer that acts like shrink-wrap at certain temperatures[5]. When the polymer is warmed to body temperature, it shrinks and compacts the cells it encloses, which activates genes responsible for tooth development. Bioengineers could potentially use this material to induce tissue development for a variety of therapies, Ingber says, because cartilage and other internal organs (such as the lungs and kidneys) also undergo cellular compaction as they develop.

Incorporating blood flow into a model organ is particularly challenging, especially when trying to mimic the heart, which pumps rhythmically for a lifetime. Nevertheless, tissue engineers are well under way in their search for therapies to help heal injured hearts, and eventually perhaps, to find alternatives to heart transplants. Starting with a cell-sheet technology that does not incorporate a scaffold, Teruo Okano, a biomedical engineer at Tokyo Women's Medical University and his colleagues have made vascularized heart-tissue patches. The experiments start with thin layers of cells, which they can grow from a variety of cell types, including rat neonatal cardiac cells, human muscle cells and induced pluripotent stem (iPS) cells. These sheets are grown in dishes coated with a temperature-sensitive polymer. When the temperature is lowered, researchers can harvest sheets of cells that remain connected to each other without any kind of scaffold, says Okano's colleague Tatsuya Shimizu. In ongoing clinical trials, the team is evaluating 30 patients with heart problems who have received implanted tissue patches made from muscle-cell-derived sheets. These sheets secrete several types of cytokine, which promote blood-vessel formation and inhibit cell death in the patient's heart tissue. In the future, Shimizu and his colleagues hope to transplant tissues with beating cells.

But these sheets are not yet optimal. The ideal grafts need to be thick, especially because events such as heart attacks lead to thin heart tissue. The team has returned to the lab to engineer thicker patches that will be infiltrated with even more blood vessels and should remain viable for longer than the previous versions. They have grown cell sheets from human iPS cells and transplanted them into rats just under the skin on their backs, building up a patch 1 millimetre thick made of 30 cell sheets[6]. After implantation, small blood vessels from the rat sprouted through the layers. By moving smaller stacks into more vascularized areas, the researchers were able to cause more and more blood vessels to grow and eventually to connect the stack directly to larger blood vessels, such as the jugular vein. The heart-muscle cells continued to beat during six months of observation.

"Engineered tissues are starting to allow incisive experiments and even replacement therapies."

However, similar multiple surgical interventions could not be carried out in people. So the researchers have developed a technique that relies on a gel on which they can grow multiple layers of rat-cell sheets in the lab.

One day, Okano and his team hope, it will be possible to engineer such grafts for use in

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M. LEVIN/J. MOROKUMA/A. WESTFALL/TUFTS UNIV.

humans with severe heart failure. The general approach could also be applied to engineer tissue to mimic the liver or kidneys.

SELF-ASSEMBLY

Other teams rely even more heavily on the intrinsic ability of cells to assemble into complex structures. Stem cells grown in suspension can be coaxed to form organized clusters called organoids, and these have been made for diverse tissues, including intestine, kidney and retina. Organoids are usually much smaller than the actual organ, just a few millimetres across, and with a much simpler assortment of cells, but some teams are now making organoids with more cell types and more complex structures, and even attempting to model the most daunting organ — the brain.

In 2013, Madeline Lancaster and Juergen Knoblich at the Institute of Molecular Biotechnology of the Austrian Academy of Sciences in Vienna generated human brain-tissue organoids about the size of a lentil[7]. They started from groups of human pluripotent stem cells, which are differentiated into neural tissue. Part of the protocol is to let the biology unfold.

Under the right conditions, the differentiating cells self-organize into a tight swirl of neural tissue with multiple cell types, including radial glial stem cells that give rise to cells in the brain such as neurons. The swirls even include rudimentary brain structures such as the beginning of a forebrain and retina. "We pretty much recapitulate the formation of neural tissue in a dish, letting it develop as it does in the embryo," says Knoblich.

These cerebral organoids have helped them to address questions that are hard to answer when growing neurons flat on the surface of a culture dish. The team studies the human neurodevelopmental disorder microcephaly, in which infants have markedly small brains. Although mice can be used to model the disorder, the animals do not show the extreme difference in brain size. But when the team reprogrammed skin cells from a patient with microcephaly into iPS cells that developed into cerebral organoids, the resulting structures bore clear characteristics of the disease. In these organoids, the radial glial cells proliferated less and, in some regions, differentiated into neurons prematurely. Even under normal conditions, radial glial cells do not proliferate in developing mice the way they do in humans, and so human organoids are a promising way to study how these neural precursor cells might be involved in the disorder.

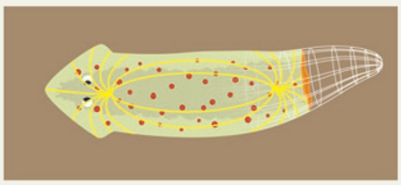
Lancaster and Knoblich also used organoids to assess the effects of a gene called *CDK5RAP2* that helps to guide cell division. The patient with microcephaly had a mutation in this gene that probably results in an aberrant protein. When the team introduced an undamaged protein into the organoid, some cells developed into types akin to radial glial cells, indicating that the loss of function of this gene contributes to microcephaly[7].

ORGAN BUILDING

Instead of replacing damaged or diseased organs, some labs have attempted to stimulate organ regeneration. Researchers have tapped into the innate chemical and bioelectric signalling of flatworms to induce the development of a second head. The regeneration occurs because the altered pattern is stored across the animal's bioelectric network.

1 An adult normal flatworm.

2 Manipulation of the worm's bioelectric network.



3 The worm is permanently altered by rewriting the memory of what should be regenerated.

There are still plenty of challenges for organoid technology. Lancaster and Knoblich point out that their organoids lack a blood supply and the interaction that neural tissue normally has with surrounding tissue. Over time, the organoids begin to die and lose resemblance to early brain tissue. The team has managed to keep them alive for as long as a year, but how useful late-stage organoids are for disease modelling remains to be seen, says Knoblich.

Another challenge is consistency, because the organoids take on different shapes from one batch to the next, he says. The lab is continuing to tinker with the growth conditions in the hope of overcoming these problems and being able to model more complex neurodevelopmental disorders.

LIKE AN EMBRYO

Bioengineers intent on building organ models can tap into the complexity of intercellular signals — the wealth of biochemical and bioelectrical messages that tell cells to differentiate, migrate, change shape or clump together to form an organ. This approach has already been used to regenerate legs on frogs that are normally too old to naturally regrow an amputated limb. That work, led by Michael Levin, a biomedical engineer at Tufts University in Medford, Massachusetts, might translate more readily to humans than many expect: children can regenerate fingertips, but adults cannot.

To get the frogs' legs to regrow, Levin and his team chemically tinkered with the pattern of electrical charge in the limb so that it matched the bioelectrical gradient found in the limbs of young animals[8], and this induced the cells at the tip of the amputated limb to grow. They also induced the formation of a two-headed flatworm (see 'Organ building'). The alterations the team had made caused a permanent change in the memory of what to form, which was encoded in an electrical circuit just like memories in our brains. Levin describes the process as "manipulating information" within the tissues in ways that cause predictable, large-scale changes in growth and form. That, he says, "makes the job of growing anything much easier".

Instead of trying to micromanage organ building, Levin believes in leveraging the body's own processes. He and his team are developing a physiological 'phrase book' of mathematical models and software. Scientists can use these software tools to search for factors to manipulate in their experiments, and so find ways to tell cells what tissues to build. The goal is to link data sets about genes, proteins and signalling pathways to knowledge about how organ shape and function is regulated. "These are the kinds of tools that will be indispensable as bioengineers confront the complexity barrier facing the creation of even simple organs," says Levin.

Ultimately, the usefulness of the tool is what is important, not the specific approach that is chosen. Engineered tissues are starting to allow incisive experiments and even replacement therapies. And perfectly mirroring nature may not, in all cases, be what is needed. "What is critical is that the organ has enough complexity to accomplish its function," says Hunziker.

Whether it be a patch for damaged hearts, a better toxicity test or an insight into a devastating brain disease, tissue engineering delivers what scientists crave: more understanding, and the potential to help people. ■

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