

Credit: J. Munson.

etri dishes pervade cell biology, providing flat surfaces on which cells can attach and grow. But researchers are increasingly recognizing that introducing a third dimension into cell culture changes cell behavior and more closely matches the natural environments of living cells.

However, that's not the sole advantage of 3-D culture systems. They also allow researchers to study the effects of physical and mechanical stress on cells and even provide a look at physiologically relevant processes such as fluid flow.

Aside from informing basic science, more complex 3-D cell culture systems are also paving the way for realistic in vitro systems to study normal organ function as well as disease pathology. Organs-on-a-chip, which integrate cells in 3-D culture within microfluidic systems, could soon play a vital role in drug discovery.

### Cultural tools

The majority of cells in our bodies reside in 3-D environments. Compared with cultures atop a 2-D surface, these 3-D microenvironments are more complex in terms of interactions between cells and the extracellular matrix, cell-cell communication, and transport of soluble factors.

For decades, researchers have realized that changes in the cell microenvironment can lead to profound differences in cell behavior and pathology. Even relatively simple differences, such as whether a cell is mounted on glass or plastic, can change its response to drugs, notes chemical engineer Steven Caliari of the University of Virginia. Twenty-five years ago, Mina Bissell's group at Lawrence Berkeley National Laboratory demonstrated that normal breast epithelial cells plated in two dimensions exhibited tumor-like characteristics. But when cultured in a 3-D environment that closely

resembled glandular structures, these same cells adopted a "healthier" phenotype (1).

"That's a good example of a healthy cell going down a disease path when you culture them in the wrong environment," Caliari explains.

Caliari's group is focused on designing new materials that researchers can use to probe fundamental questions in cell biology. Today, there are an array of options for culturing cells in 3-D, many of which are commercially available (2). Collagen, the primary protein constituent of many tissues, is a good model material for 3-D culture. "You can buy sources of collagen where you can simply adjust the pH and the temperature to a physiological range, and that collagen will self-assemble into a hydrogel," Caliari says. A downside of collagen hydrogels, however, is that they're often very soft, so they aren't always the best choice when a test system needs to be mechanically robust.

In some cases, a research question dictates hydrogel choice. Also at the University of Virginia, Jennifer Munson studies brain cells and the environmental factors that affect their behavior. Since hyaluronic acid (HA) is abundant in the brain, it's the basis for many of her experiments rather than collagen, which doesn't occur in that tissue type.

Because one of 3-D cell culture's selling points is the ability to test the effects of physical stresses on cell behavior, tuning the mechanical properties of 3-D culture hydrogels is often of great interest to researchers. But this feature can be hard to control using natural biopolymers. That's a primary reason for choosing synthetic hydrogels, Caliari says, where researchers can adjust the mechanical properties and also change ligand presentation, adhesive motifs, or growth factor availability. Although polyacrylamide is ubiquitous in 2-D culture, its precursors are toxic, so it can't be used to completely

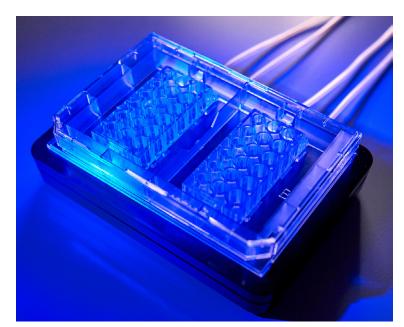
encapsulate cells. Polyethylene glycol (PEG) hydrogels, on the other hand, are popular for 3-D culture because the material has been widely used with many available protocols.

PEG is also a simple platform for introducing chemical modifications that can allow researchers to create formulations that gel when mixed or when exposed to light. One downside to the use of synthetic polymers can be cell recovery. To address this, some PEG kits include peptide-based degradation sequences that can be cleaved with matrix metalloprotease (MMP). With a natural polymer hydrogel, a variety of enzymes can provide a relatively gentle way to recover cells.

Harvard University researcher Ali Khademhosseini is trying for the best of both worlds by combining gelatin with photocrosslinkers to exploit the biocompatibility of natural polymers with a programmable chemistry (3). With 3-D printing or other layering strategies, it is possible to build structures using patterns of light to lock in the gel architecture. According to Khademhosseini, the combination of tunable mechanics and hydrogel degradation provides the opportunity to modify the environment with growth factors or other molecules. His team has now extended this idea to elastins, which they use to create more rubbery gels that have better mechanics for epithelial cells or that might even be suitable for use as surgical sealants (4).

Although many scientists equate 3-D culture with spheroids, simple molding strategies can work, too, Caliari says. Hydrogel precursors—polymer, crosslinker, and initiator—can be put in any sort of mold. Something as simple as the cut-off top of a syringe can create a small cylindrical plug. For fibrous morphologies, electrospinning can provide a way to mimic those architectures.

To make their 3-D cultures, Munson's group resuspends a single cell type within a liquid form of their HA hydrogels and then places that solution in the reservoirs of a 96-well plate. The resulting structures are up to 75 microliters in volume and roughly 1 millimeter tall. More complex cultures that include



Olivier Guenat's team has created a prototype lung-on-a-chip. Credit: O. Guenat.



Ali Khademhosseini of Harvard Medical School designs biomaterials for tissue engineering and to control cellular behavior. Credit: A. Khademhosseini.

a range of cells—astrocytes, microglia, and more—might be larger, up to 150 microliters in volume. Using a resected tumor, the researchers can isolate patient cells and reconstruct the cellular makeup.

## 3-D insights

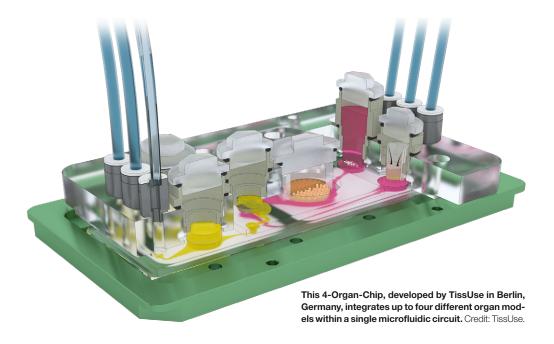
Munson has a keen interest in studying fluid flow within tissues, particularly the role of fluid in normal brain tissue and brain cancers. Clinically, the flow of fluids shapes both the pathology and invasion of cancers, and it is crucial for drug delivery. Because

2-D culture has no barriers to fluid movement or drug uptake, 3-D culture is necessary for all of Munson's experiments.

Munson's team has screened small molecules against 3-D cultures with various combinations of healthy glia and brain cancer cells. In a simple culture containing only malignant cells, small molecule inhibitors of cancer spurred the cells toward invasion. However, when the researchers applied fluid flow with the same small molecules in a culture with a more complete tumor microenvironment—including cancer cells, microglia, immune cells, and astrocytes, the inhibitors worked, stopping the cancer cells in their tracks. The group has now seen this effect in three different patient populations.

"It really shows that it's important to put everything in context when you're trying to find new therapies," Munson says.

Bioprinting, covered extensively in last month's Tech News article, is also making its mark in 3-D cell culture. At Drexel University, Wei Sun and his team have developed a proprietary bioprinting strategy that they're



using to print 3-D models of cancer. They first demonstrated the process with HeLa cells (5). More than 90% of the cells survive the printing process, Sun says, and the proliferation rates, protein expression, and resistance to anti-cancer drugs correspond more closely to tumor tissue than do existing 2-D models.

Sun's team is working on building this tumor model into a microfluidic system, which he notes could be useful for pharmaceutical drug discovery. Eventually, this device could give clinicians a platform for building personalized models of patient tumors to help choose the most appropriate therapy for each case.

#### From culture to models

Perhaps the most exciting area for 3-D culture right now is the "organ-on-a-chip." These microfluidic devices include a subset of healthy or diseased cells subjected to in vitro environments that mimic the physical, mechanical, and chemical inputs experienced within the human body.

"The field is just opening up," says Khademhosseini. If you consider all of the work invested in studying physiology and diseases and predicting toxicity and drug efficacy, "now is the prime time to actually make those kinds of predictive systems outside the body," he adds.

Currently, researchers are taking two different approaches with their organ-on-a-chip devices: either modeling the cells and tissues of a single organ or combining cells from multiple organs on the same chip. While both strategies can answer important questions, focusing on a one organ, such as the lung, allows researchers to work out the complexities within that single system first, says Olivier Guenat of the University of Bern.

Guenat has scientific training in microfluidics and microfabrication. But it was during graduate school, when a rare dis-

ease required him to get a liver transplant, that he got interested in organ-on-a-chip technologies. After finishing his PhD, Guenat pursued postdoctoral work at Massachusetts General Hospital and Harvard Medical School, developing liver-on-a-chip devices. In 2009, he moved his independent laboratory to Bern, Switzerland, where pulmonary physicians were looking for engineers to help them explore questions about lung cancer and lung fibrosis. That partnership led to the world's second lung-on-a-chip device in 2015 (pictured on Page 94, bottom-left). (The Wyss Institute at Harvard announced the first such device in 2010.)

Clinicians don't currently know the exact causes of idiopathic pulmonary fibrosis. But repeated microinjuries to the lung epithelium appear to be the disease's starting point. Abnormal wound healing then leads to thicker, stiffer scar tissue. Treatment options are often limited to a lung transplant, without which the disease is fatal.

Guenat's lung-on-a-chip device makes it possible to tease apart many interacting parameters within a tissue and test their effects individually. One important design feature is the ability to look at mechanobiology and directly measure the connection between mechanical stress on the tissue and the physiological responses of the cells. The researcher has accurate control over the system's components, including cell

composition, growth factors, and the addition of pressure or fluid flow.



## Increasing the complexity

Other research teams are taking organs-on-a-chip a step further. Emulate (http://emulatebio.com), a company that came out of the Wyss Institute, has developed a technology to link multiple organs-on-a-chip. And TissUse in Berlin, Germany, has developed a platform for integrating several organs within a single chip.



Jennifer Munson of the University of Virginia combines chemical engineering and neuroscience to study fluid movement in the healthy brain and in brain cancers. Credit: J. Munson.

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According to Reyk Horland, TissUse's Vice-president for Business Development, this means researchers can study the interactions of different organs and cytokines in the human body, but in an in-vitro environment. With a platform that can integrate up to four different model organs at once, the company has developed several tests for the cosmetics and pharmaceutical industries.

In cosmetics, a typical combination of organs might include skin and liver, to screen for chemicals that penetrate the skin and determine what their liver toxicity might be downstream. The pharmaceutical industry, on the other hand, might be more interested in testing oral uptake and liver toxicity using a combination of an intestinal barrier model along with liver tissue. Multi-organ chips can also be useful for testing secondary metabolite toxicity, Horland says, where an initial drug isn't toxic but its uptake produces toxic byproducts. In addition, researchers are interested in constructing multi-organ chips as disease models to test the safety and efficacy of potential therapies.

Challenges remain in moving organ-on-a-chip approaches into routine use. Even though there are many opportunities for pharmaceutical research, adoption questions loom, Khademhosseini says. "Pharma has been spending for decades building up their assays, so they really need to see solid reproducible validation before they adopt a new kind of completely different tool set."

But validation processes are currently too slow, Horland says. For example, he notes that a basic alternative for testing skin tissue took more than a decade to validate. If you multiply that by a 10-organ chip, that would be 100 years, an untenable time line. One additional challenge is different testing standards and validation processes in different parts of the world. (For example, the European Union banned animal testing for new cosmetic ingredients in 2013, but such testing continues in other parts of the world.) Horland would like to see international regulatory agencies work together on validation strategies so that organ-on-a-chip developers can design tools that could be easily adopted worldwide.

#### References

- Peterson, O.W. et al. 1992. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proc Natl Acad Sci USA. 89:9064-9068.
- Caliari, S.R. and J.A. Burdick. 2016. A practical guide to hydrogels for cell culture. Nat Methods. 13:405-414.
- Nichol, J.W. et al. 2010. Cell-laden microengineered gelatin methacrylate hydrogels. Biomaterials. 31:5536-5544.
- Zhang, Y.-N. et al. 2015. A highly elastic and rapidly crosslinkable elastin-like polypeptide-based hydrogel for biomedical applications. Adv Funct Mater. 25:4814-4826.
- Zhao, Y. et al. 2014. Three-dimensional printing of Hela cells for cervical tumor model in vitro. Biofabrication. 6:035001.

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