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Cell Culture Enters the Third Dimension

Scientists have been culturing mammalian cells in flat petri dishes for decades, but a new generation of tools and techniques is now letting them grow three-dimensional cultures that more closely mimic the biology of real tissues. By Alan Dove

In 1907, Anatomist Ross Harrison removed developing neural tubes from frog embryos, placed them in a solution of frog lymph on a coverslip, and inverted the mixture over a glass slide with a depression in it so the explanted tissue could grow in a hanging drop. Neurons in these first tissue cultures continued their normal three-dimensional growth patterns, allowing Harrison to see how nerve fibers extended themselves during development.

Later generations of cell biologists moved tissue culture into two-dimensional petri dishes, where lines of transformed cells could establish uniform monolayers that were much easier to maintain and subculture than Harrison’s hanging drops. These 2-D cultures revolutionized virology and molecular biology. However, flat cells on a plastic plate are at best mediocre models for complex tissues in the body, and researchers kept trying to develop systems that would combine 3-D physiological relevance with 2-D convenience.

Now, advances in materials engineering, manufacturing, and cell biology are finally starting to turn 3-D cell culture from a finicky, specialized technique into a mainstream tool for biologists. The result is a slew of new platforms and methods, ranging from simple, easy-to-use products for growing tiny cellular spheres to esoteric technologies that recapitulate entire organs in vitro.

A LITTLE MOLD CAN BE A GOOD THING

 “[Some] of the problems through the years have been that the formats for 3-D cell culture have been expensive, cumbersome, [and] difficult to work with,” says Jeff Morgan, president and chief executive officer of Microtissues in Providence, Rhode Island. However, many scientists have been willing to tolerate these problems in order to use 3-D cultures. Indeed, even Harrison’s original hanging-drop method remains a staple technique in some areas of cell biology, despite its limitations.

Hoping to make 3-D systems more convenient and flexible, Morgan and his colleagues found a surprisingly simple solution: agarose. Cells grown on the ubiquitous gelling reagent can’t stick to its surface. That would be a disaster for conventional 2-D cultures, which depend on surface adhesion to form monolayers, but Microtissues turns nonadhesion into an asset. In the company’s system, cells fall into tiny wells molded into the surface of the agarose, where they form spherical structures.

“Microtissues’ 3-D petri dish is a micromolded nonadhesive hydrogel that the cells don’t attach to, which allows them to attach to each other,” says Morgan, adding that in this case “the cells are actually self-assembling.” The resulting spheroids form tight connections between cells, creating gap junctions and other features of intact tissues. So far, investigators have grown more than 40 different cell lines this way, including both primary and transformed cells.

Rather than sell preformed agarose plates, the company sells molds that researchers can autoclave and reuse as many times as necessary, casting new plates whenever they need them. The molds fit into standard 12- or 24-well plastic cell culture dishes. Within each well, a mold can create 96 microwells, so pipetting a
CLIMBING THE SCAFFOLD

No matter how flexible a platform appears to be, though, no 3-D culture system will cover all applications. “We appreciate that there’s no single technology for all needs, there’s horses for courses in many respects, and the investigator will select the tools and technologies most appropriate to enable them to answer their biological question,” says Stefan Przyborski, chief scientific officer of Reinnervate in Sedgefield, United Kingdom.

Reinnervate is one of several companies offering scaffold-based 3-D culture systems. Instead of growing into spheroids of a fixed size, cells in a scaffold can form sheets that mimic many types of animal tissues. In the early 2000s, Przyborski and his colleagues at Durham University were studying xenograft tumors that formed when they transplanted stem cells into animals. “The stem cells were producing all these complex tumors and tissues, and one of the major challenges of stem cell science is to be able to reproduce that level of complexity in vitro,” says Przyborski.

To do that, he created thin, highly porous membranes from polystyrene—the same material used for standard tissue culture plates. Cells can grow inside the porous membrane and form 3-D structures with multiple layers. Researchers can manipulate the system to recreate different types of environments, for example by placing the membrane at the bottom of a culture dish so that it only receives nutrients from one side, or suspending it to expose it to the medium on both sides. Because the scaffold is polystyrene, investigators can also coat it with collagen or other matrix molecules used with traditional 2-D cultures. Reinnervate now sells the membranes under the Alvetex brand name, in versions precut for standard cell culture dishes.

Though product manufacturers are taking pains to make 3-D culture user-friendly, newcomers to the technique should still expect some learning experiences. Technical support from the vendor is crucial. “We have technical services to help [customers] overcome any issues, help them optimize the system, and get the most out of the technology,” says Przyborski.

Besides adapting established cell lines from 2-D to 3-D culture, scientists may find themselves puzzling over other parts of their research routines. “One of the questions we get regularly is ‘how do you visualize cells in 3-D,’ because when you think about it all the imaging technologies are all built around 2-D cell culture,” says Przyborski. Reinnervate offers coaching on 3-D culture visualization as well as other common obstacles new users encounter.

Some problems of scaffold-based cell culture aren’t easy to solve though, at least not yet. One major challenge is that current scaffolding systems aren’t amenable to standard passaging techniques, so researchers have to restart new 3-D cultures from established 2-D lines periodically. Przyborski and his colleagues are working on methods that will address that, and are also developing perfusion techniques to feed scaffold-grown cells continuously. “If we maintain the cell line in 3-D, it adapts and becomes different and actually is more in vivo-like, so then you get more accurate data,” says Przyborski.

AN EXPANDING MATRIX OF CHOICES

Researchers who want to start using 3-D cultures will find themselves in good company; growing more tissue-like structures has become a major focus of both basic and applied research labs. “There have been technologies developed in the past few years that have enabled better 3-D cell culture, and also there’s a movement towards getting more in vivo-like data from cell systems,” says David Welch, associate director of global market development for primary and stem cell systems at Life Technologies in Carlsbad, California.

Don Finley, product manager at Sigma-Aldrich in St. Louis, Missouri, concurs: “3-D cell culture is perceived as a way to better mimic the in vivo situation and what’s in the human without actually having to use humans.” That motivation is especially powerful in the pharmaceutical industry, where recent clinical trial failures have highlighted the need for more

single aliquot of suspended cells into the dish will create 96 individual spheroids. That’s a lot easier than creating an equivalent number of hanging drop cultures. “A hanging drop might dry out, and a 96-well plate for the hanging drop is 96 pipetting steps. In our case we’ve got one pipetting step in a gel that makes 96 spheroids, and they’re in a hydrogel that’s stable for two weeks and beyond for long-term culture,” says Morgan. The number of cells in the aliquot determines the size of the spheroids, making the system easy to modify.

Researchers are already using the molds for a variety of studies. For example, “there’s exciting work with stromal-cancer cell interactions,” says Morgan, adding that by creating spheroids with mixed cell types tumor biologists can manipulate and study the interactions directly. To do this, researchers simply combine the two cell types into the desired ratio, pipet them onto a gel, and after they settle, the cells form a mixed spheroid with the different cell types interacting. In another project, investigators are seeding tumor stem cells into the agarose plates, allowing individual wells to host microtumors derived from single cells.

Algimatrix, which is derived from the tissues of marine sponges, has no growth factors in it. However, it allows researchers to fine-tune their cells’ environment.
predictive preclinical assays for drug efficacy and toxicity.

To help scientists move their cells into the third dimension, Life Technologies now offers three scaffolding products with different features: Get-trex, Cellstart, and Alginatrix. “Get-trex is derived from mouse tumors, and it contains within it a lot of growth factors and other things that can stimulate cell growth,” explains Welch. Cellstart, meanwhile, consists of exclusively human and recombinant materials, for researchers doing clinical research where animal components could cause rejection and other side effects. Alginatrix, which is derived from the tissues of marine sponges, has no growth factors in it. However, it allows researchers to fine-tune their cells’ environment. “You can change the hardness or the rigidity of the structure by adding a supplement, so you can make it softer or harder, and that makes it more or less amenable to different environments and different cell types,” says Welch.

Alginatrix isn’t the only tunable scaffold on the market. Hydromatrix, made by Alphagenix in West Lafayette, Indiana, is composed of synthetic peptide nanofibers. Altering the concentration of the Hydromatrix solution adjusts the 3-D architecture of the self-assembling peptides. The company also makes a scaffold called Maxgel, which includes human extracellular matrix components. Sigma-Aldrich carries both products, and also sells specially designed cell culture bioreactors manufactured by 3-D Biotek in North Brunswick, New Jersey. “I see this as a toolkit, and we’re at a stage where researchers are going to have to do what they do and help us understand what works best with different cell lines,” says Finley.

The diversity of choices underscores the need for tinkering and experimentation in getting a new 3-D cell culture system working. “It probably depends on the cell type and the application,” says Welch, adding that “not all matrices or technologies that have been developed work for all cell types.” Lisa Masterson, a product manager at Sigma-Aldrich, agrees: “I don’t really see any one particular matrix, whether it be synthetic or native, as being a huge winner at this point.”

PATIENT ON A CHIP

As lab suppliers and their early adopter customers work to set up relatively simple 3-D cell cultures, some scientists are pushing the far edge of the field into the realm of science fiction. One of them is Don Ingber, director of the Wyss Institute at Harvard University in Boston, Massachusetts. Flush with $37 million in funding from the Defense Advanced Research Projects Agency, Ingber and his colleagues are now building what amounts to a miniature person: a series of 3-D cell cultures and microfluidic devices that will form 10 interconnected artificial organs, providing a sophisticated in vitro model of human physiology.

Several of the organs are already working, and they’re enabling previously impossible experiments. “If you work on the lung and you want to get access to the lumen, [when] you have epithelial cells from a lung in a matrix gel in 3-D, they form spheroids, and you really can’t get to the center,” says Ingber. Animal models have normal lung lumens, but can’t be studied for long periods or manipulated as extensively as cultured cells. To get around that, Ingber’s team built a “lung-on-a-chip,” with epithelial cells growing on flexible membranes to form 3-D structures that resemble alveoli and microfluidic devices feeding and maintaining the cells. That system revealed that breathing motions may play a crucial role in the pathogenesis of bacterial pneumonia.

In another project, the team built a gut-on-a-chip, which allows human intestinal cells to grow in the presence of peristaltic straining forces. The cells organize themselves into villi, with gene expression patterns that mimic real human intestine. Moreover, the researchers were able to co-culture bacteria on top of the artificial gut without killing the cells. “We can start putting bacteria on the luminal face of the intestinal epithelium, and the human epithelial cells are perfectly happy. Normally in a 2-D culture we’d call that contamination and you’d have to kill the dish,” says Ingber.

Ingber concedes that the organ-chip prototypes have been difficult to build, particularly when it comes to introducing small numbers of cells into the minuscule growth chambers, but once established, the systems can survive for as long as a month. Microperfusion systems keep the cells alive, obviating the need for constant feeding and subculturing.

The investigators are now trying to iron out the bugs in the chips’ production, with an eye toward making them more widely available for research. “I think it’s one of these things where it’s not like any one technical thing is such a hurdle, it’s just putting it all together so people who know nothing about cells can use it,” says Ingber.

Ultimately, the researchers hope to recapitulate enough human biology in chip-based systems to provide reliable preclinical models of pathogenesis and drug activity. Replacing racks of mouse cages with incubators full of automated homunculi may sound far-fetched, but it’s exactly what Ingber intends to do: “I think one by one we’ll replace animal models.”

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

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