



INTO THE THIRD DIMEN- SION

*To overcome the shortcomings of standard
2-D cell culture, scientists are adding another
dimension to make cells happy.*

by Richard Saltus | illustration by Steve Wilson



IRST SHE DOTTED GLASS SLIDES WITH liver cells to study infection. Now her larger assemblies of cells, in a disc about the diameter of a soda can, are being implanted into rodents to carry out most of the liver's 500 different tasks. Thinking big but starting small, Sangeeta Bhatia is closing in on her ambitious goal: growing human livers in the lab from scratch.

"We hope we're on the way to growing big pieces of liver for people," says Bhatia, an HHMI investigator who has pioneered sophisticated methods of removing cells and keeping them happy and behaving much as they would in their natural habitats. It's been a formidable challenge. "Primary liver cells are difficult to culture," she says. "When you take them out of the body, they lose all their functions."

Since the 1950s, scientists have probed the molecular secrets of cells plucked from the body and grown in the laboratory on flat plates or Petri dishes. These standard cultures have taught us about normal cell biology, cancer, and other diseases.

From a cell's point of view, however, these two-dimensional (2-D) habitats—dubbed "plastic palaces" by one researcher—are a poor substitute for real life. Scientists have come to realize that a cell's surrounding microenvironment plays a much larger role in directing its growth and shaping its behavior than anyone understood 10 or 20 years ago.

In the body, cells are accustomed to living large, in three dimensions, embedded within an extracellular matrix (ECM). Through the pores of the fibrous ECM, a cell is bathed in nutrients and signaling molecules. A thin basement membrane anchors the cell to surrounding connective tissues and emits chemical signals that regulate some cell processes. In addition, physical forces push and pull the cell from all directions.

Without this extracellular community, cells grown in single layers on standard flat cultures will proliferate, but they usually don't differentiate into specialized cells forming structures such as capillaries. They can be inadequate models yielding misleading results. For example, researchers testing drugs against cancer

cells in 2-D cultures may overestimate their potency, because the cells are more vulnerable to being killed.

So scientists in a wide range of fields are turning to more complex and realistic three-dimensional (3-D) culture methods to provide cells with a more familiar home away from home. Generally, these microenvironments are gel-like materials with components of the basement membrane and the ECM.

The differences are spectacular. Now, cells are being coaxed to form blood vessels or potential implantable repair tissues, including miniature livers and other organs; they can form tiny spheres that mimic breast tissue for use in breast cancer research; and the methods are showing great promise for stem cell research.

REALISTIC REACTIONS

"It is remarkable how much we can learn from these three-dimensional systems," says Shahin Rafii, an HHMI investigator at Weill Cornell Medical College who recently reported a major advance in sustaining adult stem cells in the lab (see Web Extra, Nurturing Stem Cells). For one thing, he explains, "When you introduce a third dimension, cells turn on a whole slew of new physiologically relevant genes." For example, in 3-D cocultures with vascular cells, stem cells start to behave like stem cells in their natural niche, initiating preset programs for self-renewal and differentiation.

A 3-D culture also recapitulates what happens in development, Rafii adds. Compared with stem cells grown on flat cultures, cells nurtured in Rafii's 3-D environments experience a more normal physiological, biochemical, metabolic, and physical milieu *in vivo*, enhancing their viability for transplant.

HHMI investigators Kristi Anseth at the University of Colorado at Boulder and Sangeeta Bhatia at the Massachusetts Institute of Technology have created 3-D systems in ambitious tissue engineering projects. Bhatia's functioning miniature livers implanted in mice bring her closer to her goal of helping patients with liver disease (see Web Extra, "Livers in the Lab"). Anseth is tinkering with the composition of 3-D gel matrix materials that someday might allow doctors to repair cartilage and bone defects with cell implants activated by light from outside the body. With HHMI investigator Natalie Ahn, Anseth is using 3-D cultures to learn just how cancer cells move around—and finding more surprises (see Web Extra, "Smart Scaffolds").

"I think everybody now is considering whether their experimental questions might be better answered in a 3-D setting," says Joan Brugge at Harvard Medical School.

Since the early 1990s, Brugge, a former HHMI investigator, has been studying the development of breast cancer by inserting oncogenes into "hollow cyst-like structures that resemble the milk-secreting glandular structures found in the human

breast,” she says. “In 3-D, different oncogenes induced distinct architectural changes that resembled structural variations seen in different types of breast cancer.”

Noting that the microenvironment surrounding cancer cells helps determine their invasiveness—a process difficult to model in 2-D systems—oncogene pioneer Robert Weinberg at the Whitehead Institute wrote in a 2002 review, “Suddenly, the study of cancer cells in two dimensions seems quaint, if not archaic.”

An early multiuse 3-D cell culture technique came from research on the basement membrane, which underlies epithelial cells that line hollow organs. Made up of collagen and large proteins called laminins, it is a protective barrier, an anchor, and a source of signals that regulate processes such as blood vessel formation and wound healing.

In the mid-1980s, Hynda Kleinman at the National Institutes of Health (NIH) had been studying an extract of mouse tumors that produces a basement membrane. Kleinman’s lab group was analyzing basement membrane chemistry, unrelated to cell biology. Some of her colleagues had suggested seeing what effect the extract would have on cells.

In 1983, in anticipation of a site visit by Harvard cell biologist Elizabeth Hay, Kleinman (who was traveling) told a postdoc to place some endothelial cells—the building blocks of blood vessels—on a sample of the matrix extract. “I had no clue as to what would happen,” recalls Kleinman.

“He threw some endothelial cells on it, and they went crazy. Within hours they had formed capillary-like vessels with hollow lumens,” says Kleinman. “The reviewers and Dr. Hay loved it! They took some home with them. Then we published on this and on many other cell types, and a lot of people wanted to use it in their research.”

The extract was eventually sold commercially under the name Matrigel, the first and still widely used basement membrane substrate for 3-D cultures. A common application is to study metastasis: cancer cells placed on a thick slab of the gel will migrate to the interior, modeling a process that can’t be replicated in two dimensions.


A 3-D BRIDGE

The rise of 3-D cultures does not spell the demise of standard cultures, by any means. Even in cancer research, says Brugge, 2-D methods are still useful for studying cell cycle progression pathways, apoptosis, protein interactions in signaling pathways, and some aspects of drug sensitivity.

Scientists say that 3-D culture is establishing itself as a bridge between traditional cell culture and animal models. Cells grown in the new systems can replicate the features of some diseases better than costly animal models do. Functions of genes and proteins can be studied first in these cultures before going on to more laborious experiments in animals. And, of course, conditions can be controlled much more easily in a 3-D culture experiment than in a living animal.

The list of applications of 3-D studies goes on and on: new assays for blood vessel formation, drug discovery, and cancer invasiveness; studies of the effectiveness of drug delivery methods; tissue engineering experiments of all kinds.

Surveying the leaps that 3-D methods have made in a little more than 20 years, NIH’s Kleinman says, “I’m just blown away by the very creative ways people are using it.” ■

 **WEB EXTRA:** For more depth on efforts to use 3-D cell cultures to grow livers in the lab, regenerate joints, and extend the lives of stem cells, see www.hhmi.org/bulletin/aug2010.



NATALIE AHN, KRISTI ANSETH, AND SANGEETA BHATIA ARE LEARNING THAT PHYSICAL FORCES AND CHEMICAL SIGNALS FROM ITS NEIGHBORS MAKE A CELL BEHAVE MORE LIKE A CELL.