SCIENTIFIC AMERICAN.COM

December 28, 2006

A 3-D Replacement for the Petri Dish

For modeling cell growth and behavior in the body, scientists are turning to three-dimensional culture systems that create more lifelike environs

Since its first use in 1877, the petri dish has become an icon of the laboratory, sharing the same rarified space as the microscope, Bunsen burner and lab coat. But now that research has moved away from relatively simple work in, say, antibiotic resistance to more sophisticated processes such as tissue regeneration, a more robust system for cell cultures is necessary--one that more closely approximates the environment that cells inhabit in the body.

Shuguang Zhang, associate director of the Center for Biomedical Engineering at the Massachusetts Institute of Technology, believes he's found a way to take these systems to three dimensions. His method involves a tube filled with a self-assembling peptide--a short molecule of amino acids called RADA16--that spontaneously organizes itself into 3-D scaffolding where cells can grow like they do on extracellular matrices inside the body.

In their natural surroundings, cells attach to other cells and binding molecules such as the protein collagen, which is the main ingredient in connective tissue. Enmeshed in this porous webbing, the cell is able to exchange nutrients and receive oxygen necessary for metabolism and hormone-signaling cues.

"Tissue cells are normally grown in a 3-D environment, not 2-D," explains Zhang. "As we all know, 3-D is drastically different from 2-D. For example, 2-D only provides the surface, but 3-D also provides the space. So cells on a 2-D environment do not behave normally. Their morphology changes to fit the 2-D flat environment."

In a petri dish, cell clusters spread out on the surface of a medium. This causes all the cell's adhesion receptors to migrate to one side of the cell, which dramatically changes its metabolic functions and growth patterns.

Over the past 30 years, previous attempts to create artificial microfiber environments made of synthetic polymers have run into two main obstacles. Most create microfiber scaffolds with diameters that are several orders of magnitude larger than cells, essentially recreating the same effect as the petri dish. A biomaterial that creates appropriate fiber sizes called Matrigel has proven to be useful in mimicking extracellular environments, but the substance, secreted from tumor cells in mice, cannot be used inside the body and comes with numerous growth factors and ingredients that are uncharacterized and inconsistent from sample to sample. As Zhang puts it, "we don't know what's in there."

"Decades of experience with two-dimensional culture have taught us that relatively minor changes in matrix or growth conditions can dramatically affect cell phenotype, survival and differentiation," says Richard T. Lee, associate professor of medicine at Harvard Medical School. "Cell responses in three-dimensional scaffolds are likely to be just as affected by the scaffold design and by growth factors delivered by the scaffold."

Zhang's self-assembling peptide system creates scaffolds made of fibers with diameters that are about 0.2 percent the size of most cells--about 5,000 times thinner than human hair. To a base of RADA16, Zhang added motifs--amino acid fragments known to influence stem cells to differentiate into a certain type of cell--such as collagen and bone marrow homing peptides. He then tested these motifs to see how well neural stem cells from mice would differentiate in their presence. He found that RADA16 augmented with bone marrow homing peptides performed much better than RADA16 itself in providing an environment where progenitor cells could mature into neurons. Furthermore, the elongated peptide performed on par with the mysterious mixture that is Matrigel.

"Before this work, it was an open question whether chimeric peptides composed of self-assembly sequences and biologically active sequences would work together," said Todd C. Holmes, a neurobiologist at New York University. "The present results prove that they do."

Zhang asserts that systems such as the one created here can be tweaked for other uses, including tissue repair for regenerative medicine, such as skin grafts. Michael E. Davis, a biomedical engineer at Emory University, agrees that self-assembling peptides are a versatile class of polymer. "Ideally, once the optimal sequence is found for a particular application," he notes, "it would probably be rather inexpensive to bring it to the bedside."

According to Zhang, the shift toward 3-D scaffolding systems is essential to keep researchers from "barking up the wrong tree" by chasing targets that reflect a cell's mutated behavior under two-dimensional systems. "I tell the people," Zhang says, "that probably in 20 years, all the textbooks concerning tissue culture will have to be revised because of these 3-D cell culture systems."