

Scientists create 3-D scaffold for growing stem cells

27 December 2006

Stem cells grew, multiplied and differentiated into brain cells on a new three-dimensional scaffold of tiny protein fragments designed to be more like a living body than any other cell culture system.

An MIT engineer and Italian colleagues will report the invention-which may one day replace the ubiquitous Petri dish for growing cells-in the Dec. 27th issue of the *PLoS ONE*. Shuguang Zhang, associate director of MIT's Center for Biomedical Engineering, is a pioneer in coaxing tiny fragments of amino acids called self-assembling peptides to organize themselves into useful structures. Working with visiting graduate student Fabrizio Gelain from Milan, Zhang created a designer scaffold from a network of protein nanofibers, each 5,000 times thinner than a human hair and containing pores up to 20,000 times smaller than the eye of a needle.

The researchers were able to grow a healthy colony of adult mouse stem cells on the three-dimensional scaffold without the drawbacks of two-dimensional systems.

In addition to helping researchers get a more accurate picture of how cells grow and behave in the body, the new synthetic structure can provide a more conducive microenvironment for tissue cell cultures and tissues used in regenerative medicine, such as skin grafts or neurons to replace brain cells lost to injury or disease.

The scaffold itself can be transplanted directly into the body with no ill effects.

"The time has come to move on from two-dimensional dishes to culture systems that better represent the natural context of cells in tissues and organs," said Zhang, whose coauthors on the paper, in addition to Gelain, are from institutes and medical schools in Milan, Italy.

Life in two dimensions

Biomedical researchers have become increasingly aware of the limitations of growing living cells in coated, two-dimensional Petri dishes and glass slides.

In the body, cells are attached to and supported by the cells, other structures and proteins around them. A cell's normal environment is a complex network of tiny fibers, gaps and pores through which oxygen, hormones and nutrients are delivered and waste products filtered away. Cells move within their natural environments in response to chemical signals or other stimuli.

Researchers are aware that cells on flat surfaces have skewed metabolisms, gene expression and growing patterns. But the only choices have been glass labware and a product called Matrigel, a gelatinous protein mixture secreted by mouse tumor cells. While Matrigel does resemble a complex extracellular environment, it also contains growth factors and unknown proteins that limit its desirability for experiments requiring precise conditions.

"Synthetic biopolymer microfiber scaffolds have been studied for more than 30 years to mimic a living 3D microenvironment, but concerns exist about their degradation products and chemicals," the authors wrote in the paper.

Other synthetic polymer biomaterials are simply too big. Getting cells to grow on them is like forcing spiders to build webs on skyscraper girders. Zhang's nanofiber scaffold, around 1,000 times smaller than the existing systems, is much closer in size to the extracellular matrices that living cells manufacture themselves.

Adding motifs

With the addition of defined amino acid fragments called active motifs, the scaffold can be fashioned to coax stem cells to behave in certain desirable

ways-such as differentiating into needed body tissues or migrating toward bone marrow and other natural destinations.

"What makes these designer scaffolds particularly interesting is that cells survive longer and differentiate better without additional soluble growth factors," Zhang said. "This suggests that extracellular microenvironments may play a more important role for cell survival and for carrying out cell functions than previously thought."

The active motif method could be readily adapted to studying cell-to-cell interaction, cell migrations, tumor and cancer cell interaction with normal cells, cell-based drug testing and other diverse applications.

"I believe that in the next 20 years all cell cultures will be in 3D with the designer scaffolds, and most textbooks about cell biology will have to be revised when people obtain results from 3D cell culture studies," Zhang said.

The researchers are now testing the designer scaffold with a variety of cells, including tooth, bone, heart, liver, cartilage, skin, pancreas, blood cells and artery-forming cells.

Source: Public Library of Science

APA citation: Scientists create 3-D scaffold for growing stem cells (2006, December 27) retrieved 19 April 2021 from <https://phys.org/news/2006-12-scientists-d-scaffold-stem-cells.html>

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