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Building a Better Cell Culture System

Howard Hughes Medical Institute (HHMI) researchers have created a new type of “intelligent scaffold” for growing human cells that mimics conditions inside the body better than the standard, flat Petri dishes often used in laboratories.

This scaffold is a step toward developing new kinds of cell culture systems that can be used to grow cells to reconstruct damaged tissues, says chemical engineer Kristi S. Anseth, an HHMI investigator at the University of Colorado, Boulder.

Anseth’s team constructed their scaffold from hydrogels, which are polymer-based compounds that absorb water. The scientists built the hydrogel using a light-sensitive molecule that permitted them to snip apart the scaffolding with the flick of a laser beam. Anseth’s group showed that they could use the laser to alter the gel’s stiffness, sculpt out chambers and channels, or release signaling molecules inside the gel matrix.

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- Kristi S. Anseth

“What we are trying to do is create a material that gives an ultimate level of control of a cell’s environment—presenting a molecule at the right time, in the right place, at the right dose, in the right context. These properties are important whether you are trying to control cell function, regenerate tissues, or deliver drugs,” Anseth said.

Anseth and her colleagues described their photodegradable hydrogels in an April 2, 2009, article in *Science Express*, which provides electronic publication of selected *Science* articles in advance of print. April Kloxin, a postdoctoral fellow in Anseth’s laboratory was the first author of the study.

The researchers have already used their light-sensitive hydrogels to study the behavior of cancer cells and cartilage-forming stem cells.

Anseth says hydrogels are useful for studying cell behavior because they mimic the natural environment in the body better than flat Petri dishes, which are typically used to grow cells in culture. “For example, cancer cells migrate very differently when they are on a two-dimensional surface than in three-dimensions,” Anseth explained.

To build a hydrogel that could be manipulated with light, Anseth and her colleagues incorporated nitrobenzyl ether, a molecule that can be cleaved with wavelengths of light that do not damage cells. Attaching this molecule was a critical step, Anseth said, because it gave the scientists the ability to remodel the hydrogel even after cells had begun growing in it. “Also, it responds in seconds to minutes, which enables us to alter that microenvironment on the same time-scale as cellular events,” Kloxin explained.

The researchers demonstrated several ways that they could take advantage of this light-sensitive property of their hydrogels. In one experiment, they embedded mesenchymal stem cells -- immature cells that can differentiate into bone, cartilage, fat, muscle, and pancreatic cells -- in the hydrogel. The mesenchymal cells were compact and round when packed inside the hydrogel. But when the researchers used light to partially degrade the gel structure -- making it more pliable -- the cells quickly spread into a new shape.

“This experiment showed that we have the ability to control the local structure of the gel surrounding the cell,” said Kloxin. “Imagine, then, that we could use focused light to selectively degrade the material between two cells and then study their interaction.”

In more elaborate experiments, the researchers focused their laser to selectively create channels within the gel. “Since these structures can control where cells will move, one can now begin to think about building three-dimensional structures that mimic how cells are organized in the hierarchy of tissues,” said Anseth. “For example, one could build vessel-like structures by forming tubules in a gel, or structures that could direct and control the connectivity of neurons.”

In another set of experiments, the researchers selectively eroded material around living cells to “herd” cells within the gel without affecting their viability. Cell migration is of major interest to cancer researchers, and the team used fibrosarcoma cells, which cause tumors of connective tissue, in these experiments.

Anseth and her colleagues also demonstrated that they could control biochemical signaling inside the hydrogel. They constructed a hydrogel in which they used the photodegradable molecule to tether a short sequence of amino acids to the polymer. That sequence promotes the survival of mesenchymal stem cells – but it also inhibits them from differentiating into the more specialized cartilage-forming cells known as chondrocytes. Anseth’s team wanted to see what would happen to the cells if they suddenly lost that signal, so after the cells had grown in the hydrogel for ten days, they released the amino acid sequence with a laser beam. The biochemical signal rapidly diffused out of the hydrogel, and the mesenchymal stem cells were able to differentiate into chondrocytes.

The ability to control the space in which a cell grows and the signals that it receives should make the new hydrogel a powerful tool for designing vehicles for drug delivery and engineering new tissues in the laboratory, Anseth says. “Ultimately, I want to design materials that I can use to culture cells”—cells for use in reconstructing damaged tissues, such as knees and hearts.