Scientists develop new way to grow adult stem cells in culture

July 15 2010

Researchers at the Stanford University School of Medicine have developed a technique they believe will help scientists overcome a major hurdle to the use of adult stem cells for treating muscular dystrophy and other muscle-wasting disorders that accompany aging or disease: They've found that growing muscle stem cells on a specially developed synthetic matrix that mimics the elasticity of real muscle allows them to maintain their self-renewing properties.

"Cells don't normally exist in contact with a rigid cell culture dish," said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor and member of Stanford's Institute for Stem Cell Biology and Regenerative Medicine. "They sit on soft tissue. By mimicking this environment we can really influence their function and allow them to self-renew in ways we've never been able to achieve before."

Adult stem cells already exist in the body, and are important in regenerating tissues like blood, muscles and neurons in the brain. But scientists have struggled to produce them in quantities needed for therapies because the cells differentiate and lose their "stemness" as soon as they're placed in a tissue culture dish. This new method of growing the cells creates a way to study the behavior of many types of adult stem cells in culture and may revolutionize the ability to produce these cells for future therapies, say the researchers.

Blau is the senior author of the research, which will be published online July 15 in Science Express. Postdoctoral scholar Penney Gilbert, PhD,

and graduate student Karen Havenstrite share first authorship of the work.

Self-renewal, or the ability to become both another stem cell and a differentiating daughter cell, is a defining trait of stem cells. This ability is necessary for a small number of cells to, for example, fully reconstitute the pantheon of blood cell types necessary to regenerate a patient's immune system after chemotherapy or to successfully contribute to the long-term generation of new, healthy muscle tissue. Until now, however, all attempts to grow these and some other adult stem cells, like blood stem cells, in culture have resulted in the cells differentiating into more specialized — but less therapeutically useful — progenitor cells. This differentiation constitutes a major obstacle to treating muscle-wasting diseases, for using cord blood or for treating blood cancers.

The researchers wondered if the way the cells are normally grown in culture could be the problem. After all, as Blau pointed out, cells are used to rubbing shoulders comfortably with their neighbors on all sides rather than being splayed out and anchored on a rigid plastic culture dish that is 100,000-fold less elastic than true muscle.

To find out if the cells would be happier on a softer, more giving surface, they had to develop an entirely new culture system. They turned to a material called hydrogel, which is made up of a latticework of polyethylene glycol polymers filled with water. Decreasing the percentage of polymer molecules in the mix makes the resulting matrix more elastic and wobbly; increasing it makes it more dense and rigid.

Hydrogel is often used as scaffolding to grow cells in two- and three-dimensional arrays useful in tissue engineering. But because it can swell over time, it was not possible to accurately calibrate the amount of proteins and other components needed to maintain the cells in this type

of experiment. Gilbert and Havenstrite tinkered with the system until they came up with a version that maintains a constant volume, making it possible to test the effects of gels of different elasticity that all contained the same amount of protein. They then patterned the gel into hundreds of tiny wells and added one freshly isolated muscle stem cell per well.

After letting the cells grow for one week, the researchers found that the softer, or more pliant, gels mimicking the elasticity of muscle tissue had many more cells than the less-elastic gels. Closer investigation using an algorithm they developed for automated cell tracking showed that it wasn't because the cells were dividing more quickly, but because not as many were dying during the culture period. The computer program, which they have called the Baxter Algorithm to honor the Baxter Foundation that funded this portion of the work, reduced the time needed to analyze the cell division data by more than 90 percent.

"This in itself is a huge advance," Blau said of the software. "Until now it's been pretty impossible to do these studies without spending half a year or more manually scoring pictures or movies of cells in culture. Now we can figure out exactly how the cells divide and move, who begets who. As a result we can begin to study all types of variables."

After studying the dynamics of the muscle stem cells' division and dying, the researchers began to study specific aspects of their biology. They found that the cells grown on the softer surface were less likely than those grown on the harder surfaces to express a gene associated with differentiation called myogenin. They were also as able as freshly isolated muscle stem cells to contribute to the development of leg muscles when transplanted into recipient mice.

"Testing their function in animals like this is extremely important," said Blau. "It's really the only way to confirm their 'stemness.'"

To prove definitively that the stem cells were self-renewing, Gilbert and Blau turned to a "doublet" experiment. In this test, Gilbert allowed just one cell to divide just one time, resulting in two daughter cells. There are three potential combinations for these resulting doublets: two stem cells, one stem cell and one progenitor cell, or two progenitor cells. The first two represent self-renewal; the last does not. (A progenitor cell is one that can go on to differentiate into more specialized cells.)

Gilbert found that one-third of cells grown on the muscle-mimicking substrate expressed a muscle-stem-cell-specific gene (indicating that at least one of the two cells was a stem cell) but that only 6 percent of those grown on the plastic surface did so. Furthermore, when Gilbert transplanted five doublets (for a total of 10 cells) into mice from the muscle-mimicking substrate, the cells made themselves at home and began to contribute to muscle fiber development in three of 12 recipient animals. When she repeated the experiment with doublets grown on hard surfaces, none of the animals demonstrated similar engraftment.

"Clearly the cells grown on the more-elastic surfaces have better survival and self-renewing properties than those grown on standard tissue culture dishes," said Blau. "We conducted our experiments with muscle stem cells, but I expect this will be true for other types of adult stem cells as well."

In addition to exploring this possibility in the future, the researchers will also investigate how their findings may help advance therapies for conditions like muscular dystrophy. "Researchers really had no way to grow these cells in the laboratory before," said Blau. "These findings may allow us one day to replenish the muscles of patients with muscular dystrophy and other muscle-wasting diseases with healthy stem cells."

Provided by Stanford University Medical Center