Soft substrate promotes pluripotent stem cell culture

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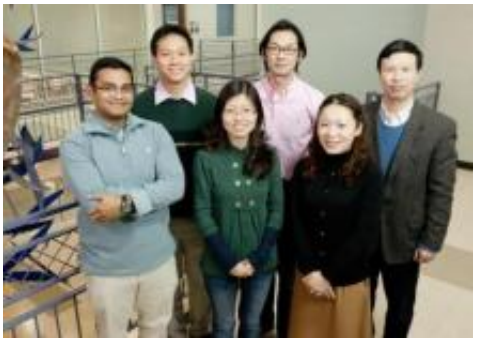

Illinois researchers developed a soft growth medium for stem cells that allows them to culture homogenious pluripotent colonies, without expensive growth factor chemicals. The team: front row from left, graduate students Farhan Chowdhury and Yanzhen Li and visiting scholar Tamaki Yokohama-Tamaki; back row from left, graduate student Yeh Chuin Poh; Tetsuya Tanaka, professor of animal sciences; and Ning Wang, professor of mechanical science and engineering. Credit: L. Brian Stauffer

University of Illinois researchers have found a key to keeping stem cells in their neutral state: It takes a soft touch.

In a paper published in the journal PLoS One, the researchers demonstrated that culturing mouse embryonic stem cells (mESCs) on a soft gel rather than on a hard plate or dish keeps them in their pluripotent state, a ground state with the ability to become any type of tissue. The soft substrate maintains homogeneous pluripotent colonies

over long periods of time – without the need for expensive growth chemicals.

"This has huge applications in the future of regenerative medicine," said mechanical science and engineering professor Ning Wang, who co-led the group with animal sciences professor Tetsuya Tanaka. "It's an exciting area. There's still a lot of work to do, but our work is a step toward understanding the basic biology of stem cells."

The difficulty of maintaining mESC colonies that are homogeneously pluripotent has been one of the main obstacles in stem cell research. Pluripotent stem cells spontaneously differentiate, beginning to turn into specialized tissue types such as skin or muscle. Scientists use chemicals called growth factors to keep mESCs in their unchanged state, but even then it's not long before the culture is a mixture of cells in various stages of differentiation, with diverse gene expression and morphologies.

Such diversity in a sample makes it very difficult for researchers to induce a culture of stem cells to become a particular type of tissue – the ultimate goal of stem cell research.

"If we start from a homogenous population of undifferentiated cells, differentiation toward the tissue of our interest might become much more homogenous than we've been able to achieve," said Tanaka, who also is affiliated with the U. of I. Institute for Genomic Biology. "So then, in generating a specific cell type – the main application of pluripotent stem cells – I think that there is an advantage to having a homogeneous culture to start with."

After noticing that pluripotent mESCs tend to stick together in round colonies while cells on the colony edges in contact with the rigid growth plate tend to differentiate more quickly, the team decided to focus on mESC mechanics rather than chemistry. Since stem cells are 10 times

softer than mature cells, the researchers wondered if the mechanical forces between the plate and the cells were spurring differentiation. Wang and Tanaka's earlier research found that even small mechanical forces could be used to direct cell differentiation; could mechanics also hamper differentiation?

The team did side-by-side comparisons of mESCs grown on a traditional medium with growth factor and mESCs grown on a soft gel with the same stiffness as the cells, both with and without growth factor. They found that cells grown on the soft gel had greater homogeneity and pluripotency, even without growth factor, and even more than three months and 20 passages later.

"It's two sides of the coin: Mechanical force can induce differentiation, and here we said if you can lower the forces between the substrate and the cells, they stay pluripotent. They are complementary processes," Wang said. "Our paper shows that mechanical environment plays at least as important a role as chemical growth factors, if not greater. In vivo, cells produce growth factors for a short time and then they stop. On the other hand, mechanical forces bear on every cell all the time."

Next, the researchers want to try their soft-substrate method with induced pluripotent stem cells (iPSCs), mature cells that have been genetically reprogrammed to a pluripotent state. These cells hold a lot of promise for medical applications, but are notoriously hard to culture and not as well understood as embryonic cells.

"We can try culturing mouse iPSCs on the same soft substrate and see if the same benefit applies to achieve homogenous stem cell cultures," Tanaka said. "If that's the case, the impact would be significant."

More information: The paper, "Soft Substrates Promote Homogeneous Self-renewal of Embryonic Stem Cells via

Downregulating Cell-matrix Tractions," is available online at www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0015655

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