ENCOURAGING AND PREDICTING THE GROWTH OF STEM CELLS

Stem cells have the unique ability to differentiate into numerous types of cells. Mesenchymal stem cells, or MSCs, for example, can grow into fat, cartilage or bone cells. Scientists have long known that cells' behavior, including differentiation, is influenced by the microenvironment around them, and biologists today have the ability to induce differentiation into desired cell types using genetic and biochemical methods. But very few researchers have looked at the use of mechanical means to influence the behavior of MSCs.

"Increasingly over the last few years, biologists and engineering researchers alike have been realizing how important mechanics are in regulating cell functions," said Assistant Professor **Jianping Fu**, who studies how the mechanical and physical signals in cell microenvironments affect cell behaviors.

"We know that cell functions are regulated mainly by extracellular stimuli, and recent evidence suggests that the mechanical properties of the scaffolding that surrounds a cell—the extracellular matrix—and particularly the rigidity of the matrix, can influence a number of cellular functions," he added.

In work published in the journal *Nature Methods,* Fu recently invented, fabricated and demonstrated an array of microstructures that can encourage MSCs down different differentiation pathways. Using micromolding and microfabrication techniques used in the semiconductor industry, Fu fabricated an array of post-like microstructures out of a type of silicone. By changing the height of the tiny structures, he is able to control their rigidity.

Fu has shown that MSCs placed on top of the more rigid (shorter) posts prefer to differentiate toward bone cells, while MSCs in the same chemical microenvironment but placed on top of the more flexible substrate (the longer posts) show a tendency toward fat cells. "All other factors being equal, the mechanical environment is critical," he said.

As the cells attach to the micropost substrate, they exert force, which bends the tops of the posts. Because the mechanical properties of the post are known, investigators can calculate the traction force the cell is exerting. "In that way we can use the posts as sensors to monitor one of the mechanical traits of the cell," said Fu.

In addition, Fu has found that the traction force of a cell also can be used as a nondestructive predictor of MSC differentiation. Stem cells that commit toward bone cells, or osteoblasts, show much higher contractility at the earliest stages than cells that will become fat cells, he and his team found. By contrast, undifferentiated MSCs that are treated with chemical growth factors—the conventional way of encouraging differentiation *in vitro*—typically have a heterogeneous response.

"Some cells go down one path, some go down another, and some show no response at all—and there's no way to predict their behavior," he said.

When conventionally cultured cells differentiate, it can take days if not weeks for scientists to learn what type of cell they will become. In Fu's system, however, cells show a differential contractility response within 24 hours. His findings have tremendous implications for developing novel high-throughput drug screening platforms to test molecules related to the encouragement or inhibition of differentiation into different cell types. The platform also can serve as an efficient testbed to explore other rigidity-dependent stem cell functions.

While the current substrate system is passive, the research team also is exploring active environments, such as adding a stretching force where the cell membrane adheres to the post. Stretching the membrane in effect stretches the cell, introducing an active force control.

"Now we can modulate two independent controls," explained Fu, who plans to use both passive and active techniques to improve the survival rate of embryonic stem cells. Currently about 99% of human embryonic stem cells die in culture if they are plated as single cells, significantly hampering research progress. Scientists add chemicals to improve their survival, but that only raises the rate to between 20 and 30%. "The chemicals aren't ideal for cells," said Fu, "and we're hoping our mechanical cure can replace them one day soon."

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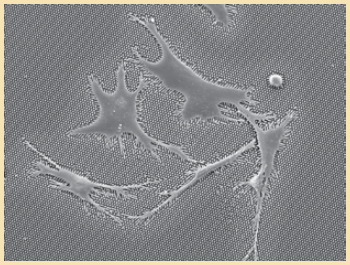

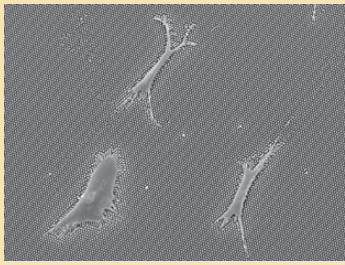

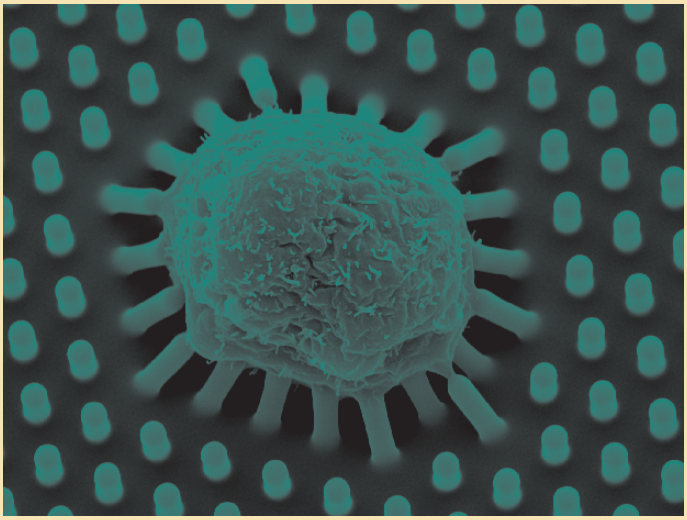

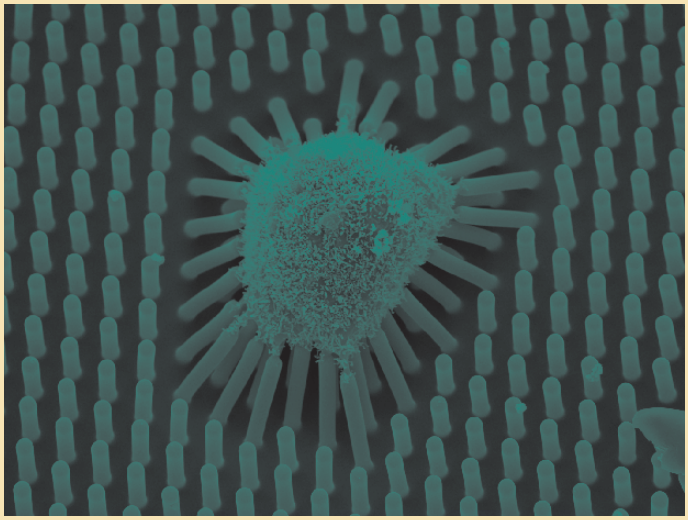

Representative immunofluorescence image of human mesenchymal stem cells (hMSCs) plated on the PDMS micropost arrays. hMSCs were stained with fluorophore-labelled phalloidin, anti-vinculin and DAPI to visualize actin filaments, focal adhesions and the nuclei, respectively.