

## Destiny written in the cytoskeleton



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Vincent Shen

*A stem cell's destiny may be written in its cytoskeleton. Vincent Shen looks at two cytoanatomists using semiconductors and atomic force microscopy to decipher the mysteries of the cytoskeleton.*

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In 1978 at Harvard Medical School, Judah Folkman—the father of anti-angiogenesis therapy research—was watching cells slip and slide. Then a professor of pediatric surgery and cell biology, he had an idea that cell shape somehow influenced tumor angiogenesis.

To investigate, he developed slippery culture plates by coating their surface with poly(2-hydroxyethyl methacrylate). By varying the chemical concentration, he could control the surface's stickiness, which in turn affected cell shape. In typical cell cultures, an adhesive surface is desirable because it provides cells with a stable environment to grow in. But Folkman's sliding and constantly unstable cells did not grow (1).

"It wasn't a perfect nonadhesive," says Christopher Chen, professor of innovation in the University of Pennsylvania's Tissue Microfabrication Lab. "But that was the publication that first implicated cell shape as a potential regulator of cell fate."

Since then, cell biologists have continued to look at how mechanical forces on the cell's cytoskeleton affect DNA synthesis and gene expression. Stem cell researchers have discovered that cell shape influences a cell's differentiation. Flat cells differentiate into bone cells; round cells differentiate into fat cells. If researchers could uncover the cell shape mechanisms that control these cell's destinies, then stem cells could be easily differentiated into the specific cell types necessary for regenerative medicine applications. But the tools to understand these mechanisms remain limited.

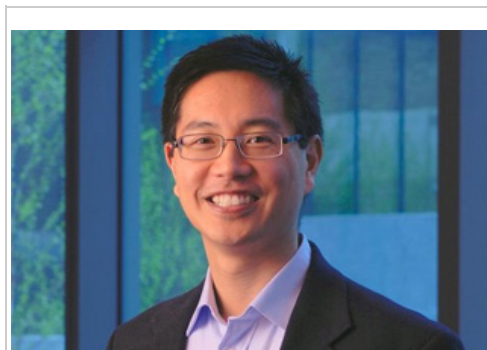
So cell biologists are developing techniques to better understand how the cytoskeleton is linked with cell fate, a task that is more important than ever, since, arguably, the future of regenerative medicine hinges on it. Two North American researchers—one American, one Canadian—are providing some particularly insightful findings using different methods. One is continuing in Folkman's footsteps, patterning cell adhesion on semiconductor chips; the other is taking a new approach, using atomic force microscopy to poke at a cell's cytoskeleton.

### Stamp collecting and microneedles

Chen is an international stamp collector, exchanging them with different collaborators and laboratories from around the world. But these are not the type of stamps that you lick and place in the corner of an envelope for postage. These are used to print cell culture micropatterns on semiconductor chips. Each stamp has a different pattern: squares, circles, stars, and flowers. Every pattern has a unique effect on cell behavior.

To create these stamps, Chen's team uses a soft lithography approach. They build a patterned stamp template by exposing a light-sensitive substrate to ultraviolet rays through a stencil-like mask. Liquid polydimethylsiloxane is then poured onto this mold and cured into a hard rubber. This stamp is then inked with hexadecanethiolate and stamped onto the semiconductor chip. A cell-adhesion protein is then applied to the stamped semiconductor chip, sticking to the hexadecanethiolate micropattern. The cells seeded on the semiconductor take on the geometric shape of the stamped pattern.

"The patterns can be replicated," says Chen. "If someone in another lab has a pattern that I want to use, I don't need to



**Christopher Chen has improved the cell culture micropatterning techniques and has introduced the microneedle bed to measure cell traction forces.**  
Source: NIH

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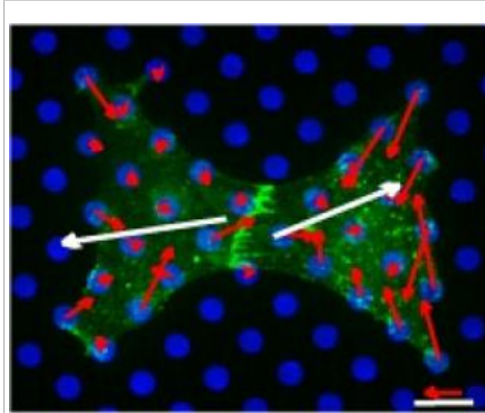


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start from the beginning because I can just have it copied by molding a negative replica of that lab's stamps."

Using his micropatterning cell culture technique, Chen published a paper in *Developmental Cell* in 2004 that showed that cell shape determined whether human stem cells became fat cells or bone cells. When Chen's group forced human mesenchymal stem cells (hMSCs) to stay round, the cell produced less RhoA G protein—a regulator of actin polymerization and differentiation—leading cells down the path to fat cells. In contrast, flat hMSCs upregulated activated RhoA, formed intact cytoskeletons, and became bone cells (2).

And there are new collectors with whom Chen can exchange stamps, thanks to new techniques and methods to produce these semiconductor chips. Martin Bastmeyer, an investigator at the Paul Scherrer Institute in Switzerland, used micropatterning to show how fibronectin density affects cell spreading (3). Linda Griffith, an investigator at the Massachusetts Institute of Technology, combined micropatterning with time-lapse microscopy to track cell migration patterns in substrates with various ligand density patterns (4). "The technique has become progressively less difficult to reproduce," said Chen. "Cost of production and the time for a student or person to produce those patterns has decreased."



**Chen and his team replaced the semiconductor chip with a bed of cast rubber microneedles to record the mechanical forces exerted by a single cell. Source: University of Pennsylvania**

While semiconductor micropatterning provided new insight into how cell shape regulates gene expression, it could not measure the push or pull of these shaped cells. So Chen and his team replaced the semiconductor chip with a bed of cast rubber microneedles to record the mechanical forces exerted by a single cell. The microneedles can be designed with various specifications depending on the experiment; they can be anywhere from 3 to 50  $\mu\text{m}$  tall, from 2 to 10  $\mu\text{m}$  thick, and from 2.7 to 1600  $\text{nN}/\mu\text{m}$  in stiffness.

Similar to the semiconductor chips, the researchers stamp the cell-adhesion proteins onto the microneedle bed in specific shapes. When a cell is placed onto this pattern, it becomes stuck to a series of needles, pulling them as it struggles with the shape-shifting adhesive. The deflection of the needles, which will be equal to the cell's traction forces, can then be measured.

Using the microneedle bed system, Chen and his lab published a paper in the *Proceedings of the National Academy of Science* in 2010 that demonstrated that when mechanical forces try to pull two neighboring endothelial cells apart, the cells respond by reinforcing their adherens junction, which connects cells in a tissue (5). While the microneedle bed is providing some insights, Chen believes that more systems are needed to investigate intercellular forces and the role of the cytoskeleton. "We certainly have examples where mechanical forces are important," said Chen. "I think it's not clear when they play a dominant role in biology."

### **Poking at butterflies and stem cells**

It's not hard to imagine Andrew Pelling as a young boy poking a dead animal with a stick, curious about life and death. Now, Pelling leads the Laboratory for Biophysical Manipulation at the University of Ottawa, using confocal microscopy and atomic force microscopy (AFM) to probe a wide variety of life's mechanisms, from cell development to apoptosis.

"A large part of my work is collaborative," said Pelling. "I'm good at doing biophysics and mechanical measurements, and I like to work with experts in different fields." In short, Pelling is willing to attempt to measure anything that grabs his attention.

In 2009, Pelling published an article in the *Journal of the Royal Society Interface* that introduced an ultrasensitive detection approach—called optical beam deflection—to measure the heartbeat of a monarch butterfly during metamorphosis. The optical beam deflection technique developed for this study was actually from an AFM device. Normally used to measure the mechanics of molecules or cells, it was adapted to measure an entire organism.



**Andrew Pelling reinvented the use of atomic force microscopy to study stem cell architecture. Source: Flickr, PellingLab**

In AFM, researchers usually tap or drag a probe along the surface of a molecule or cell. This probe is attached to a cantilever that bounces up and down as the probe moves along the microscale surface. A laser source is reflected off the back of this cantilever and into a detector. As the cantilever moves, the deflection angle changes. By measuring this deflection, researchers can begin to extract a picture of the surface.

To measure the butterfly's heartbeat, Pelling and his colleagues took hardened chrysalides and glued a 1-mm<sup>2</sup> silicon nitride micromirror next to the pupa's ventral side over the heart. He reflected the laser off the micromirror while the detector captured the frequency of the deflections. With these heartbeat measurements, Pelling and colleagues were able to identify distinct stages of development within the larvae when the heartbeat became more regular during the final stages of development (6).

After the butterfly work, Pelling began collaborating with stem

cell researcher Farlan Veraitch, a lecturer of biochemical engineering at the University College London, where Pelling did his postdoctoral work. Veraitch wanted to understand how mouse embryonic stem cells responded to nanomechanical forces; Pelling suggested an AFM cantilever.

The cantilever was used to push down on the stem cell's surface with forces ranging 5–10 nN, and the cell's response was observed using laser-scanning confocal microscopy. Pelling and Veraitch decided to measure two types of mouse embryonic stem cells (mESCs): round, undifferentiated stem cells and flatted cells that had already begun to differentiate (7).

What they found was that only the round cells formed mechanically induced blebs; the flat cells had a more developed cytoskeleton. From these blebs, Pelling and Veraitch reasoned that cytoskeleton development must begin early in stem cell differentiation. That is, pluripotent cells have underdeveloped cytoskeletons, but when they differentiate, the cytoskeleton matures and forms stronger links to the cell membrane.

“That was a total fluke,” said Pelling. “That’s actually the nice part about science sometimes. It actually led us down a road we didn’t predict, and we learned something pretty fundamental about cytoskeleton here.”

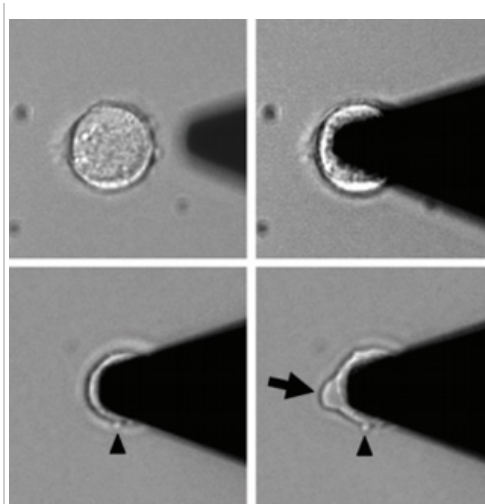
Now, Pelling is shifting his focus to cell population mechanics and developing devices to poke and mechanically strain stem cell populations in a different way. And he believes that studying population morphology may be the key to producing the numbers of stem cells necessary for regenerative medicine to really take off.

“Can we use mechanical cues to dictate differentiation?” says Pelling. “There are applications here because you would want to reproducibly recreate large numbers of cells of a certain type.”

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**Keywords:** cell biology AFM micropatterning



**When the AFM cantilever was used to push down on the stem cells, only the round cells formed mechanically induced blebs; the flat cells had a more developed cytoskeleton. Source: Cell Health and Cytoskeleton**



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