Special News Feature

ENTERING THE MATRIX

Animal cells interact with the extracellular matrix to divide, manage shape, and migrate. Amber Dance looks at new techniques aimed at revealing how this happens.

Credit: G. Pluta/University of Illinois

At the University of Illinois at Urbana-Champaign, tissue engineer J.J. Sun Choi is studying blood stem-cell differentiation. Unfortunately for Choi, who is a postdoctoral researcher, scientists still don't have very good markers for the various stages of hematopoietic stem cell (HSC) differentiation. But this has not deterred her—she is now turning to a new technique called photonic crystal enhanced microscopy (PCEM) to advance her research.

PCEM, described recently in the journal *Progress in Quantum Electronics*, measures the very bottom 200 nm of a cell sitting on a slide (1). Although blood cells are only weakly adherent, they do settle a bit, and adhesion levels change as they develop, which means the technique can provide Choi with a biomarker of development. “With PCEM, you get the information right away,” she says.

PCEM and another recently published technique that uses 3-D single-cell traction force microscopy to measure how cells pull on their surroundings (2) are offering cell biologists a new way to explore cell adhesion. During this process, cells grab onto the extracellular matrix (ECM) using molecules such as integrins, linking their cytoskeletons to the collagen, fibronectin, and other materials in their immediate environment. These interactions trigger signaling pathways that manage processes such as cell division and growth. They're also crucial for cell motility. A migrating cell can extend filopodia to probe the ECM in its target direction and grab hold. Then, other adhesions will have to let go in order for the cell to travel.

Cells differ in how they interact with the ECM, and, as Choi found, these interaction can change over time. For example, mature blood cells must detach from the ECM to enter the circulation.

Alterations in cell-matrix adhesion also occur during disease. For example, stiffening of the extracellular matrix, due to

its interactions with cells, underlies liver fibrosis and some tumor phenotypes. That's why a breast tumor manifests as a palpable lump. Cancer cells also rely on cell-matrix interactions to spread around the body.

Crystal-gazing

PCEM started as an idea in the mind of University of Illinois bio-engineer Brian Cunningham, who had wanted to use photonic crystals—materials nanostructured with a grating that propagates light along its surface—for imaging since 2000. It's been a long road, but his team's new microscope, which has been in development for about 6 years, uses photonic crystal sensors to detect the presence or absence of objects such as a cell or virus.

To get more spatial information out of the sensor, Cunningham and his postdoctoral fellow, Yue Zhuo, married it with a microscope. Their imaging approach involves coating slides with a thin layer of photonic crystals and then adding ECM components such as fibronectin and laminin for the cells to adhere to. When light hits the crystal, it generates an enhanced electromagnetic field that extends approximately 200 nm above the slide surface. That's the “sensing zone,” explains Zhuo. If a cell attaches to the crystal, it will interrupt that field, generating a signal.

On its own, the microscope would not be able to differentiate the signal of a probing filopodium from a large cell body or a lightly adhered cell from one that's tightly attached; however, the photonic crystal enhances the contrast between a light or heavy touch on the slide. A spectrophotometer reading the light reflected by the sample will detect a higher wavelength as the cell attaches more tightly.

Zhuo admits that she was initially stumped regarding exactly what the PCEM was measuring. After number crunching, she

figured out that the light signal indicates, to a good approximation, the “effective mass density” of the cell above. “What we measure is a kind of footprint of the cell,” explains Zhuo.

One advantage of this method is that it requires no fluorescent labeling—as does a similar technique, total internal reflection fluorescence (TIRF) microscopy—so there’s no photobleaching with long-term imaging. Another technique, interference reflection microscopy, images the entire cell body, while PCEM only focuses on that bottom-most surface, notes Cunningham. “We can see how cell attachment points at the cell–ECM interface evolve over long periods of time—hours to days,” he says.

Choi’s weakly adherent HSCs were used by Cunningham and Zhuo to show the sensitivity of PCEM. “There are actually quite distinctive changes between undifferentiated cells and cells one day after you induce differentiation, versus a week after,” says Choi.

Tug of War

At the University of Pennsylvania in Philadelphia, computational scientist Vivek Shenoy was initially drawn to the question of cell–ECM interactions by colleague Rebecca Wells, who studies liver fibrosis. After injury, cells deposit ECM and stiffen the tissue to create fibroids. Similarly, as breast cells turn cancerous, they pull on the ECM and stiffen it. “They can actually reorganize the fibers,” says Shenoy.

Shenoy, along with Mingming Wu of Cornell University, wondered how much force the cells exert on the matrix, and vice-versa. They applied 3-D single-cell traction force microscopy, a technique that uses fluorescent beads embedded along with cells in a collagen matrix, to measure that force. While a breast cancer cell pulls on the collagen fibers, the researchers image the positions of those beads. Then, they use the drug cytochalasin D to relax the actin cytoskeleton. The pull on the ECM slackens, and the beads move to a location unaffected by the cells. Shenoy and colleagues designed algorithms to compute the force required by the cell to deform the matrix, based on force measurements taken by Wu’s group plus those bead-displacement images.

As the researchers reported in *Proceedings of the National Academy of Sciences*, they found that the harder the cell pulls, the stiffer the matrix gets. And the stiffer the matrix, the more the cell tugs, creating a positive feedback loop (2).

The result is not entirely a surprise; Clare Waterman of the National Heart, Lung, and Blood Institute calls it a “no-brainer” that matrix stiffness and cell pulling would feed back on each other. It’s known that a stiffer matrix promotes more generation of force by cells in a 2-D matrix, but the relationship in all three dimensions was not so clear-cut, according to Shenoy and Wu. And another study reported that the force provided by cells did not depend on ECM stiffness (3).

Label-free and dynamic detection of stem cell adhesion using the photonic crystal-enhanced microscope. The PCEM images detect how a stem cell adheres to a surface over time. Image courtesy of the University of Illinois.

Building Libraries

“Going forward, it will be exciting for biologists to learn how cell traction force with 3-D biometrics is governed by the cytoskeletal molecules,” says Wu. “This will allow us to gain a handle on controlling cell–ECM cross talk, which will have applications in treating or controlling disease progression.”

Shenoy suspects that more malignant cancer cells may generate the greatest force, a hypothesis he is currently testing. Perhaps if scientists understood how tumors affect the matrix, they could keep them in check. He even suggests that researchers could look at different cell types using this technique to develop a “forceome” from various cells.

At the University of Illinois, Cunningham and his colleagues have similar plans. “We want to build a quantitative, live-cell footprint library,” says Zhuo. Such libraries could help researchers better understand the role of forces on cells and their motility.

In their paper, Zhuo and colleagues actually reported the start of such a library by analyzing the adhesion of four cell types. In addition to Choi’s HSCs, they measured footprints for a brain cancer cell line, a mouse epithelial line, and stem cells derived from pig fat tissue. The neurons and epithelial cells adhered tightly and spread rapidly, while the fat stem cells adopted a spindle shape with mid-level adhesion. Ultimately, these data could even help researchers identify and classify cell types, the authors suggested.

References

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