Tech News

Go Cell, Go!

The maze is ridiculously simple — something you might find on a child's placemat in a family-friendly restaurant. Yet this maze isn't intended for Crayon-wielding toddlers; it's has a slightly smaller audience.

For one thing, the maze has more than one solution, with two bifurcated paths of differing lengths. For another, it only measures 1,600- μm^2 . While that's a tad small for chubby fingers, it's perfect for microscale objects — which, it turns out, is convenient: The maze is a proving ground for epithelial cells.

To the naked eye the maze is perfectly transparent. Under phase-contrast illumination, it appears charcoal gray, with sharply defined passages edged in black and white hinting at a three-dimensional architecture. Fluorescent green cells enter at the bottom and exit at the top. The question is, what route will they take to get there? Some 70% of human mammary epithelial cells chose the short route, and about 5% take the long way 'round; the rest, well they got lost in the maze. Yet all made their choice without any apparent chemical or physical guidance, a feat you can watch on YouTube (www.youtube.com/watch?v=yzW5GGYk5Co).

These findings demonstrate something interesting about epithelial cells, says Daniel Irimia, the researcher who designed this challenge and an assistant professor in the Surgery Department at Massachusetts General Hospital: In the absence of external cues these cells can navigate via a self-generated gradient, an observation that could help explain how normal epithelial cells move during wound repair, and how cancer cells metastasize.

Irimia is interested in the mechanics and metrics of how cells move: their morphology, speed, directionality, and persistence. The objective, he explains, is to move the science of cell motion from the qualitative to the quantifiable, taking advantage of microfluidic ("lab-on-a-chip") devices to make precise measurements. After all, only by understanding the

normal range of cell behavior — the average speed of an epithelial cell, for instance — can researchers begin to understand how disease impacts that behavior.

"If you think about it, every cell in the body moves or has moved at some point in the lifetime of the individual. There is no cell that is static, and motility is essential for many health and disease processes."

Profiling Neutrophils

One of those processes governs the body's natural defenses. Take, for instance, the

chemokine-filled side channels each 1-mm long, like a comb. The goal is to drive the cells into the narrow channels; by forcing the cells to constrict themselves as if they are worming their way through a loose matrix, the chip approximates what happens when neutrophils leave a blood vessel and move towards infected or damaged tissues.

This contrasts the classical method of studying chemotaxis on flat plastic dishes where the cells are free to move in any direction. Under those conditions, Irimia says, cells following a chemotactic gradient tend to engage in a "biased random walk," like a drunkard staggering home.

"The neutrophil's natural environment is a confined space, where it has to move within physical constraints from every side and still find its way to the target," he says. Using neutrophils from healthy donors, the team found that the cells confined in narrow channels tend to move at a consistent rate of around 18 microns — about two cell diameters — per minute. When they repeated the experiment using cells from individuals who had been burned over from 10% to 60% of their body, the cells moved more slowly — between 1 and 10 microns per minute — though that speed often recovered over time, correlating with a favorable clinical outcome.

Why the cells move more slowly isn't clear to Irimia — perhaps they are overstimulated and exhausted, he suggests. They may also be a bit punch-drunk: In a separate set of (ongoing) experiments, Irimia and his team designed a microfluidic device that gives neutrophils a choice of routes towards a chemotactic reward, one long and one short. Ninety percent of cells from healthy donors opt for the short route. But cells from burn victims are less sure of themselves sometimes looping through the maze without direction, implying they could have problems reaching damaged tissues in vivo.

"Their directionality is still broken," Irimia says, "their 'steering wheel' is still broken, and they cannot make it to the target."

PC9 lung cancer cells can spontaneously migrate through mazes in the absence of pre-existent chemical gradients. This composite image shows GFP-tubulin cells at 1, 3, 4.5, 6, 7, 8.5 and 10 hours. Courtesy of Daniel Irimia, reproduced from Scherber et al. *Integrative Biology* (Cambridge), 2012, 4, 259-269.

systemic immune compromise that often follows severe burns. Patients who suffer burns over significant portions of their body surface area are at heightened risk for infection. Irimia wanted to know understand how that trauma influences a patient's white blood cells.

He and his team designed a microfluidic device from a spongy elastomeric material called PDMS to measure the speed of neutrophils. The device consists of a spacious main channel through which cells move, and an array of tight (3 x 6 micron)

CHIP-Move

Lab-on-a-chip devices are finding wider use in the cell motility field. Yu Huang, a postdoctoral fellow in John Kuo's lab at the University of Wisconsin, Madison, is using microfluidics to study migration of glioblastoma stem cells, while Eric Balzer, a postdoctoral fellow with Konstantinos Konstantopoulos at Johns Hopkins University, uses them to study chemotaxis.

The classic techniques to address migration and chemotaxis are the wound-healing and Boyden chamber assays, respectively. In the former, a monolayer of cells is gouged with a pipette tip to create a "wound," which the cells then move in to "repair." In the latter approach, cells are seeded in small membrane-bottomed cups immersed in a bath of chemoattractant; the assay is read by measuring how many cells follow the attractant and cross the membrane. Trouble is, the wound assay is lengthy, and cells can fill the gash by both moving and dividing, while the Boyden chamber is an endpoint assay: it isn't possible to know what the cells are doing during the assay, and how they get into the receiving chamber. It also isn't possible to image the cells during the experiment, to watch the behavior of individual cells, or to change experimental conditions.

Balzer and his coworkers have constructed a special microfluidic device that could overcome those issues. Made from PDMS, Balzer's device uses three fluid inlets — one for chemokine and two for buffer — to establish a chemical gradient by laminar flow. A series of 100-micron-long "microchannels" of varying width couple that gradient to a "cell-seeding" channel. Over a period of 8 hours or more, cells in the seeding channel work their way into and through the microchannels, moving

On the go, in vivo: Intravital imaging of macrophages labeled with Texas Red dextran (red) within a tumor formed by GFP-expressing MTLn3 mammary adenocarcinoma cells (green). Courtesy of Chengsen Xue

up the gradient as they go.

According to Konstantopoulos, this design allows researchers to study cell movement in a confined three-dimensional space without all the variability, such as matrix porosity, rigidity, and so on, normally encountered with such assays. "In our device we have one parameter that we can change," he says: "We can change the physical constriction."

In their proof of concept paper, the team tested channels as wide as 50 microns and as narrow as 3 microns.

The former is akin to a 2D planar surface whereas the latter mirrors the in vivo condition, or at least a more three-dimensional environment. Balzer refers to it as "two-and-a-half-D."

"The beauty of our device lies in two things," Konstantopoulos says, "the fact that you can directly compare set to set the 2D migration versus migration in confined spaces ... [and] that this migration can be dictated by a chemotactic gradient."

Already, his team is learning new things about cell migration. For instance, the classic protrusion-and-retraction amoebic motion of cells on planar surfaces does not occur when they are confined in narrow spaces; instead, they appear to maintain a relatively constant shape and size. "What we have learned so far on a 2D surface appears to be very different from what is happening in confined spaces," says Konstantopoulos.

Now, says Balzer, the goal is to use his 2-1/2D device to reconcile how cells behave under both sets of conditions. "That could help to unify the field."

In Vivo Motility

Of course, another way to unify the field is to observe cells "in the wild", an approach being adopted by Jeffrey Segall.

Segall, a professor of anatomy and structural biology at the Albert Einstein College of Medicine, studies the role of macrophages in promoting cancer metas-

On the scent: Over 16 hours these MDA MB 231 cells migrated towards (and beneath) an agarose drop laced with epidermal growth factor, following the indicated tracks. Courtesy of Josh Rappoport

tasis. To do that, he uses two different methods, both in vivo.

First, there is an in vivo invasion assay. Here a 33-gauge needle, filled with a mixture of Matrigel (a 3D growth matrix) and growth factors such as EGF or CSF1, is inserted into an anesthetized animal's tumor, establishing a chemical gradient. That gradient attracts both tumor cells and macrophages, just as a warm pie inevitably attracts a cat in Saturday morning cartoons. The cells invade the needle, and when removed, those cells can be counted, providing a quantifiable measure of invasion. Segall and his colleagues have used this approach to document a positive-feedback paracrine loop between macrophages and tumor cells in both breast and brain cancers.

Segall also employs intravital imaging, specifically multiphoton fluorescence microscopy, to directly witness migration as it happens. Multiphoton microscopy is well suited to intravital imaging because it uses relatively low power excitation and is able to penetrate deeper into tissue than standard confocal microscopy.

In this case, Segall and his collaborators image the tumor surface through a surgical flap or glass "window," visualizing motion with the help of genetically encoded fluorescent tags — for instance, collecting time-lapse images as red macrophages enter and leave a cyan fluorescent protein-labeled tumor. More recently, they have

adopted photoconvertible proteins, fluorescent proteins (such as Dendra) that fluoresce at one wavelength until excited by light of a specific energy, causing them then to emit a different color.

With that tool, Segall and his team can effectively time-stamp proteins to distinguish those that are newly synthesized from those that are not. He hopes to use them to study the trafficking patterns of tumor macrophages in transgenic animals — where they come from, how long they stay, and where they go.

“Now that we have photoconvertible proteins, we’ll be able to track the macrophages over days and see what they do,” he says.

Imaging Etc.

Time-lapse imaging is key for researchers studying cell migration in vitro, too. Josh Rappoport, co-director of the Birmingham Advanced Light Microscopy facility at the University of Birmingham, UK, uses the technique to study the impact of endocytosis on cell migration in cancer cell lines in the lab.

Several years ago, Rappoport, unsatisfied with the in vitro migration and chemotaxis assays available at the time developed a low-tech solution. Published in *BioTechniques* in 2010, his approach first spots chemoattractant-laced low-melting-point agarose into tissue culture dishes, after which cells are plated and watched as they “invade” the droplets — or, more accurately, burrow underneath them.

Rappoport describes the event in terms reminiscent of a college student with a bad case of the munchies. “Imagine if there was a giant pile of snow in your backyard, and somebody put a pizza underneath the pile of snow,” he says. “So you lay down on the ground and you crawl underneath the pile of snow to get to the pizza.”

He images that process with a mixture of techniques including brightfield, epifluorescence, confocal, and total internal reflection fluorescence microscopy, as well as scanning electron microscopy, quanti-

Cells-on-a-chip: MDA-MB-231 cells migrating (from bottom to top) through 10- or 20 micron-wide channels. Blue: nuclei; yellow: F-actin. Courtesy of Eric Balzer

fying both the number of cells to penetrate a chemoattractant-filled droplet, and the distance they travel.

The assay does have one significant drawback, Rappoport says: It isn’t easy to quantify the chemical gradient at any location in the culture dish. Still, he hopes to use it to understand vesicle trafficking in migrating cells. Preliminary data suggest that blocking trafficking pathways can inhibit chemotaxis, an observation with potential therapeutic implications.

Indeed, studies of cell migration can often yield unexpected insights into fundamental biological processes. In February, Lawrence Berkeley National Laboratory investigator Mina Bissell, an expert in tumor microenvironments, used time-lapse confocal microscopy to demonstrate that mammary gland epithelial cells organize themselves into acini by spinning, a “unique cellular movement” they called “coherent angular motion,” which appears to be disrupted in cancerous cells.

That observation could never have been made without the proper tools, but someone also had to be watching. And with more and more researchers doing exactly that, expect other exciting — and unexpected — findings to follow.

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