DESIGNING A MICROSCALE LAB

It can take significant time to design and fabricate a microfluidic solution for your experiments. But as Jeffrey Perkel finds out, all that effort can lead to novel payoffs.

A multiplexed system containing eight separate assays on a single chip. (Kim, et al., *Lab Chip*, 15:301, 2015); Credit: Roger Kamm.

It's not easy to find circulating tumor cells (CTCs). Émigrés from distant tumors, CTCs are incredibly rare—as few as one in a billion cells in human blood in fact. At that density, it takes extraordinary luck to glimpse CTCs under a microscope—unless you enrich for them.

In 2007, researchers in Mehmet Toner's lab at the Massachusetts General Hospital and Harvard Medical School developed a microfluidic device for enriching CTCs. Microfluidics, the manipulation and application of fluid properties and dynamics on the microliter or nanoliter scale, increasingly popular in the life sciences, is being used to automate and downsize reactions, boost reproducibility, and conduct experiments at the single-cell stage. By forcing blood cells to slowly flow through a vast forest of 78,000 “microposts,” each coated with antibodies to the tumor cell antigen EpCAM, Toner's “CTC-chip” maximized cell-antibody interactions, capturing CTCs with ~65% efficiency.

Measuring 25 mm × 66 mm, the CTC-chip was effective but challenging, according Shannon Stott, a former postdoc of Toner's who was not involved in the first CTC-chip project. Made of silicon, CTC-chip was expensive and difficult to produce—a problem as the device became popular in the lab. Working with fellow postdoc Chia-Hsien Hsu, Stott set about trying to develop an alternative design.

In 2010, Stott and Hsu's modified fluidic circuit, which displays a subtle Art Deco-esque beauty, was published in the *Proceedings of the National Academy of Sciences*. A single inlet on one end of the glass slide-sized circuit feeds

into eight parallel channels, which then rejoin at the other end and exit through a single outlet. In between, samples run a gauntlet of engineered fluid chaos. The channels are long rectangular paths studded with antibodies—think of a Pez refill turned on its side. But the channel “ceiling” isn't smooth. Instead, it is decorated with parallel, zig-zagging arches directed upwards—a motif known as “herringbone.” This design is crucial since it causes the fluid stream to tumble as it moves forward, thus maximizing contact between cells and antibodies.

“These herringbones create anisotropic flow,” Stott explains. “They pull the flow up, and it causes it to fold over on top of one another, and that's the chaotic flow that's resulting.”

Stott and Hsu's final design is both aesthetically pleasing and technically effective—capture efficiency is on par with the original CTC-chip. Yet being made of rubbery polydimethylsiloxane (PDMS) bonded to a glass slide, it is considerably easier to fabricate. But how did Stott and Hsu hit on the design, and just how difficult is it to design microfluidic solutions for your experiments?

Mixers and herringbones

“We had to go through a bunch of iterations,” recalls Stott regarding the design process—iterative design is common in microfluidics.

Stott first analyzed the fluid mechanics of the original CTC-chip, finding relatively little interaction between the traveling cells and the antibody-coated microposts. If an-

tibody and antigen don't meet, capture is impossible. So she began searching for alternative designs that could incorporate mixing. Hsu, meanwhile, was working on a device that would align particles as they traveled. It was based on a "staggered herringbone mixer" design first described by George Whitesides' lab in 2002 for mixing parallel flowing streams.

Stott and Hsu quickly realized the same design might make for a more efficient (and more easily fabricated) capture device. But that is easier said than done. Everything had to be tested: the thickness of the herringbone "chevrons," their height, their spacing, and more. Stott would typically fabricate 10 small-scale chips at a time and test them in parallel using healthy blood samples spiked with known numbers of fluorescently labeled cancerous cells, selecting the designs that proved most efficient.

To produce PDMS chips, researchers first have to etch a negative of the design into a silicon wafer. To do that, researchers draw the desired design using software such as Adobe Illustrator or AutoCAD. They then use that design to create a photomask—a transparency with light and dark regions indicating where the microfluidic features will go. That photomask is placed above the wafer, which has been coated with a photoreactive polymer to a desired thickness—say, 50 μm —and the chip is then hit with UV light, causing a reaction in the polymer in those areas that were illuminated. The wafer is then chemically treated to digest unreacted polymer, completing the process. According to Stott, the whole process takes about eight hours in an environmentally controlled cleanroom, complete with "bunny suits" to avoid contamination of the wafer with dust, skin cells, and fibers. "You basically plan your day so that you go to the bathroom and eat a good meal before you go in there," she says.

To make the microfluidic device itself, those "masters" are used as molds for unpolymerized PDMS. "I equate it to a Jell-O mold," Stott says. Theoretically, a single master can

Herringbone chip co-developer Shannon Stott. Image courtesy of D. Stott.

A "Jell-O mold" of a microfluidic chip, etched on a silicon wafer.
Credit: Roger Kamm.

be used indefinitely, and she does have one herringbone master that's been in service for five years. More typically, though, they break over time, so Stott tries to always to have several working masters available for any design.

As Stott tells it, she and Hsu were working in a darkened microscope room (to protect the fluorescence of the labeled cells) "where we had 12 different syringe pumps set up with 8 syringes on each pump. So we would run up to 48 devices at once, quickly running down the assembly line, hooking up the tubing, and then hitting the 'go' button so that we could quickly go through all of these iterations.... It was quite an assembly line that we had going."

The most important variables, it turns out, were the flow rate and the height of the chevrons in the channel ceiling. "If we made them too shallow they did not work as well, and if we made them too tall, the shear stresses were too high and they would tear the cells apart."

Stott and Hsu prototyped on small-scale chips that handled only 200 μl of blood. Patient samples measure in the milliliters, so the team had to scale their designs up. That also was easier said than done. Among other things, the full-size device lacked rigidity along the long reaction channel—it would tend to "bow" over time, confounding the mixing. The team tried stabilizing "pillars" to hold up the roof, but those didn't work. What did work was splitting one large channel into eight smaller, parallel channels separated by thin walls down the length of the device. That, in turn, complicated the chip's "header design"—the piping that shunts one sample evenly into each of the eight subchannels, which required still more optimization.

Stott estimates the team went through 40 iterations before the device was finalized. Yet today, the herringbone-CTC chip isn't being used to isolate human CTCs at all—the lab developed a new, more efficient design based on negative selection. But Stott, who now runs her own lab at Massachusetts General Hospital, uses the herringbone chip to isolate exosomes and microvesicles from blood—part of her research on cancer biomarkers. "There's still a life left for the herringbone," she says.

Sorters and viruses

With their tailored designs, microfluidic devices offer unique opportunities for researchers. Harvard University physicist David Weitz says microfluidics provide a way to “have [an] impact” in medicine and biomedical research. Weitz’s lab pioneered and perfected the art of producing reproducible lipid droplets in aqueous solution, using them as picoliter-scale reaction vessels for everything from single-cell transcriptomics to metagenomics.

In 2010, a Weitz lab postdoc named Jeremy Agresti described in *Proceedings of the National Academy of Sciences* a novel microfluidic design for screening the results of a directed evolution experiment. Each droplet contained a single yeast cell expressing a plasmid with a mutant copy of horseradish peroxidase, as well as a fluorogenic HRP substrate. The chip funneled the droplets down a long pipe at about 2000 drops per second, then into the path of laser. Highly fluorescent droplets—those with particularly high HRP activity—were shunted to one side of a Y-junction by an electric field applied with on-chip electrodes; the rest of the droplets went the other way. By growing the sorted yeast cells, mutating them, and repeating the process, Agresti produced HRP variants an order of magnitude more efficient than the starting enzyme.

If Agresti’s design sounds suspiciously like a cell sorter, that’s precisely what it is. But it is more than that, too. For instance, John Heyman, in collaboration with other members of the Weitz lab, demonstrated it was possible to use the system to sort for high-producing hybridoma lines, screening that isn’t possible with conventional cell sorting since FACS cannot normally screen for secreted proteins.

According to Weitz, the Agresti chip took nearly four years to perfect. In part, that’s because Agresti, a biologist, “had to learn a lot of little things, a lot of little teeny-weeny details.” For instance, the chip features four narrow chevron-shaped channels linking the two arms of the Y, which serve to equalize the pressures in the two sorter branches. “A very, very trivial thing,

but it took us probably six months to figure out that that’s what we needed,” recalls Weitz.

Weitz, who describes himself as “a very simple-minded physicist,” leads a team of more than 30 researchers, including physicists, chemists, biologists, and mechanical and chemical engineers. “I want as broad a diversity as we can possibly have, because that just makes our group stronger and lets us address many different problems.”

One recent hire, postdoctoral fellow Hee Sun Han, came to Weitz’s lab with a chemistry PhD from the Massachusetts Institute of Technology (MIT), where she studied quantum dot imaging in whole animals. Now Han is adapting Agresti’s design for detecting and sequencing individual viral genomes from environmental samples.

Han hopes to use the fluorescence produced by fluorophores binding to PCR amplicons as a selection method, and she has to optimize both the molecular biology and chip design to make the system work across multiple purification rounds. Among other things, she says, she is tweaking the sorter geometry to account for the anticipated heterogeneity of her samples. “If you have a very, very low purity sample, then you might need a very high-throughput screening,” she says. Han also has tweaked the fluidic circuitry to allow for better droplet spacing and faster sorting, drawing inspiration from Weitz lab alumnus Adam Abate, who recently described a novel design that can sort 30,000 droplets per second.

But as always, the process isn’t as easy as simply adding something new. “Making the device itself can take either a day or two,” she says. Typically, Han tests “four or five” designs at a time, evaluates the results, and repeats and refines. “If none of them is working, or if I find that there’s a trend that I can see, and there might be another design that might work better, then I create another set of those.”

Tumors and chemotaxis

According to Roger Kamm, the Cecil and Ida Green Distinguished Professor of Biological and Mechanical Engineering

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at MIT, one particularly useful application of microfluidics is to make cell culture more representative of in vivo conditions.

Kamm designs relatively simple microfluidic devices to recapitulate tumor metastasis in vitro. In a recent study, his team developed a circuit with three parallel channels to study the migration of breast tumor cells across vascular boundaries, a model of tumor extravasation.

The center channel of this particular device is filled with endothelial cells, osteoblasts-like cells, and mesenchymal stem cells in a gel of bone-like extracellular matrix, while the flanking channels provide fresh nutrients and remove waste. Within days, the cells in the center channel self-assemble into a primitive vascular network, like a capillary bed. Then, the team adds breast cancer cells, which get trapped in the vascular network, and measures the frequency at which they escape.

Breast cancer, Kamm explains, frequently metastasizes to bone but not to muscle. So, by allowing the matrix outside the vascular network to form into bone (or alternatively, muscle), the team can study those processes. Among other things, they found that adenosine influences the extravasation rate, seemingly confirming a hypothesis that had been floated in the literature that the reason so few metastases form in muscle is because of adenosine. “If you block the adenosine receptor in the cancer cells introduced to the muscle model, you increase the extravasation rate,” Kamm says. Conversely, by introducing adenosine in the bone model, extravasation rates dropped 4-fold, from about 60% to 15%.

Kamm says his lab strives for relatively simple microfluidic designs, a concession to the biologists who are the ultimate consumers of these devices. “A lot of the complexity and gadgetry in microfluidics, they have no interest in,” he says. “In fact, in some ways it turns them off.” As a result, most of his circuits follow the same basic structure. One of the most significant structural features, Kamm says, is a series of trapezoidal teeth that line the gel-filled channels. These provide surface tension to contain the gel, and their design has been carefully calibrated by running simulations to optimize the spacing, cross-section, and shape of the teeth.

Beyond circuitry, Kamm says microfluidics engineers need to consider the material in which those circuits are built. The most popular option, PDMS, is easy and inexpensive to use, but is also gas-permeable, hydrophobic, and has the potential to leach unpolymerized PDMS into a reaction, which can cause toxicity during long-term culture. Silicon is sturdier, but also more expensive and difficult to work with. Kamm founded a company in Singapore commercializing microfluidic devices built from hard plastics for use in both research and drug discovery. But to help researchers interested in making their own devices, his team typically includes instructional videos as supplemental data in published papers, he says.

Researchers also need to optimize a circuit's inter-

An early version of a microfluidic device Kamm's lab used to study angiogenesis. Copyright 2009, Volker Steger/Science Photo Library.

action with the macro-scale world. For instance, Alex Groisman, associate professor of physics at the University of California, San Diego, recently developed a circuit that exposes *Dictyostelium* to traveling waves of chemoattractant—a model system for studying chemotaxis.

The team set up a pair of pneumatic pumps off-chip, which controlled two flowing streams of buffer. In between those streams is a flowing stream of chemoattractant, cyclic AMP. By adjusting the flow rates of the two buffer streams (but keeping the total flow constant) the system caused the cAMP to move laterally across the channel, producing, from the point of view of a stationary cell, a periodic wave with a well-defined bell-shaped profile moving over it. The team could then ask how the cell behaves as the wave period changes.

According to Groisman, the design of this circuit was relatively simple, requiring just one or two iterations. “It was fairly clear for us what to do, and we had enough experience to actually build something,” he says. Other designs, though, can be considerably trickier. One device, still in development, attempts to capture a single *Caenorhabditis elegans* nematode and keep it immobile throughout its lifetime, during which the worm grows about a thousand-fold in volume. With dozens of designs tested, the chip still isn't perfect, Groisman says. “It's a genuinely difficult problem. We have to mechanically clamp this worm without inflicting serious damage.”

Microfluidics, Groisman says, is both a trade and an art—balancing book knowledge with practical experience. “Good solutions require creativity.”

But ultimately, it doesn't matter if a design is creative or pedestrian, merely that it works. “The greatest thing we can do as engineers is create something and then give it to all our friends,” says Stott, quoting Toner. Given the increasing penetrance of microfluidics in the life sciences, the gift is being well received.

Written by Jeffrey Perkel, Ph.D.

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