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News

NEWS

Using Sound to Separate Circulating Tumor Cells

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Since 2010, Tony Huang, Ph.D., from the Acoustofluidics laboratory at Penn State University in University Park, Penn.; Ming Dao, Ph.D., from the Massachusetts Institute of Technology in Cambridge; and Subra Suresh, Ph.D., now president of Carnegie Mellon University in Pittsburgh, came up with the idea of using sound waves to tweeze out pathological cells from normal blood cells. Four years later, they showed that their sound-based device, named Acoustic Tweezers (AT), could separate MCF-7 breast cancer cells from normal white blood cells. This first-generation, dime-sized cell sorting device recovered about 71% of the MCF-7 cells from the white blood cells with 84% purity (*Proc. Natl. Acad. Sci. USA* 2014;111:12992–7). Now their second-generation AT has been scaled up for performance, and it efficiently tweezed out circulating tumor cells (CTCs) from normal blood cells in patient samples with a higher throughput and recovery rate, recovering more than 83% of the CTCs (*Proc. Natl. Acad. Sci. USA* 2015;112:4970–5).

CTCs are shed by a tumor, which then may lead to metastasis. They are rare cells: A 7.5 mL sample of blood may contain only 1–100 cells. "Looking for them in a blood sample is like looking for a needle in a haystack," Huang said. But separating and culturing CTCs for analysis may serve as a "liquid biopsy," allowing clinicians to screen for various biological signatures of cancer, such as probing mutations in tumor cells or examining their drug susceptibility.

Label-Free Approach

More than 30 competing CTC-separating technologies are in development, and according to Huang, all are being polished for higher efficiency, sensitivity, and throughput, as well as lower cost (*see* Box). However, a fair number of them, like the U.S. Food and Drug Administration–approved CellSearch, rely on prior knowledge of tumor-specific biomarkers and require an additional step using antibodies or fluorescence markers to correctly label those biomarkers. The AT, however, collects CTCs as a "label-free liquid biopsy."

> *"With an integrated experimental [and] modeling approach, [this] new generation of the device has improved cell sorting throughput 10–20 times higher than previously achieved and made it possible for us to work with patient samples."*

To Huang, the inspiration for using sound waves to separate CTCs came from doctors who use gentle, harmless, low-power ultrasound to monitor the development of a fetus. "When we realized that cells can be manipulated in a touchless, label-free manner by the acoustic waves that share similar frequencies and energy densities as ultrasonic imaging, we knew that it will be a powerful, unique tool for cell manipulation," Huang said. Though not for cell manipulation, the idea of using sound waves in labs is not new. Companies such as Labcyte and EDC Biosystems already use them to handle small quantities of liquid reagents.

Next to sound, Huang's team realized that the difference in the biophysical properties of normal cells from the pathological ones, such as the cell size, density, and compressibility, is the driving factor that helps them tease out the CTCs. Of all, Huang's team thinks that size is the major differentiation factor in separating cells. For example, in their first-generation device they found that the MCF-7 cells were larger (20 μm) than the normal white blood cells (12 μm), which made the sound-assisted separation easy.

Ming Dao, Ph.D.

The physical properties of CTCs for different cancers are highly heterogeneous, and no centralized database lists the numbers attributed to each property. "The knowledge of CTCs is still very limited," Huang said. But his team has already started developing such a database. Nevertheless, their results from testing this second-generation device hold true for its success in tweezing out CTCs of certain cancers, if not all, Huang said.

Beads to Cells With a Tilt

According to Huang, the approach works under the principle of AT is acoustic levitation. Some magicians levitate water

droplets or particles without touching them, using an electronic transducer and a reflector. "It looks like an antigravity phenomenon, but it is [this] force that holds the objects in the air," Huang said. AT uses a similar electronic component that can create a sound-induced force, called the acoustic radiation force, to levitate the CTCs from other cells in the liquid medium of cancer cells or blood. While the particle (and cell) dynamics in the medium creates a drag force in the movement of the cells, the viscosity of it creates a unique flow profile. Acting together, these effects orchestrate the trajectory of the levitation. And this effect of sound is greater for CTCs than for normal cells. Thus, the device would ultimately separate CTCs with its unique sound field, just like how a coin sorter in a bank would sift coins by their distinct weights, Huang said.

Working with device experts, the team initially modeled eight to 10 designs for the AT. For effective CTC separation, they tried creating fields of different sound strengths, altering the speeds at which the blood sample flows through the device (flow rate), and the angle at which the sound field can be oriented. Finally, the researchers found that a slight physical tilt of about 5° in the angle at which the sound is supplied would create the right sound field for a high-throughput and accurate separation of CTCs.

The researchers first tested the device by sorting out polystyrene beads in solution, before trying to pull out MCF-7 and HeLa cells from white blood cells. They found the device to have about a 90% removal rate. But they did have to remove the red blood cells from the blood, because those cells crowd out and negatively affect the separation sensitivity, accuracy, and throughput. Going further, the researchers tweezed out CTCs from the drawn blood of three patients with metastatic breast cancers. The results were comparable to those of other label-dependent separation procedures.

"With an integrated experimental [and] modeling approach, [this] new generation of the device has improved cell sorting throughput 10–20 times higher than previously achieved and made it possible for us to work with patient samples," said coauthor Dao. This work led to the design and development of a platform that could preserve the integrity of the cells during separation, said Suresh, another coauthor. He also added, "[the study] promises to offer new avenues for basic research into the pathology and metastasis and for clinical diagnosis of rare tumor cells."

Room for Improvement

Many CTC-separating devices are in various stages of development, improvement, trial, approval, and commercial use. Huang said that his team too is working to improve the throughput and sensitivity of their device for patients' samples.

Mehmet Toner, Ph.D., at Boston's Massachusetts General Hospital Cancer Center, said this new approach is definitely a positive contribution to the field. Toner is part of the team that developed the CTC-iChip that has uses beyond just collecting and counting of CTCs (*J. Natl. Cancer Inst.* 2014;106:4–6). To Taher Saif, Ph.D., at the University of Illinois at Urbana-Champaign, AT could be useful in clinics. However, he said, the device needs improvement in batch fabrication for industrial production and cost reduction.

Huang and team make it clear that they have accomplished a proof of principle by testing the device with three patient samples. So Peter Kuhn, Ph.D., from the University of Southern California, who works to understand the dynamics of CTCs in cancer prognosis, said, "the audience should not interpret [the results] beyond this initial proof." He added that at this point, any trajectory could be possible for the future, and it is hard to predict the device's clinical impact or its regulatory trajectory.

Noting the few patients that the Huang team tested, Daniel Hayes, M.D., at the University of Michigan Comprehensive Cancer Center in Ann Arbor, said, "this paper is long on technology . . . but very short on clinical science." He added, "We wait more extensive clinical studies of [this] new platform to see if it is really an advance."

Stefanie Jeffrey, M.D., at Stanford University School of Medicine, added another point regarding the clinical reliability of the device. That is, the device's "future use will require analytical and clinical validation, including determination of threshold values in healthy controls without cancer." The blood draw could bring in, for example, epithelial cells from skin in addition to the CTCs, or other normal cells that circulate in blood may have similar biophysical properties to those of CTCs that could confound acoustic separation. Hence, setting this threshold value could boost the reliability of the device. She also added that "evidence of clinical utility must be proven before any CTC technology will be used for clinical decision making. However, a technology such as this that provides gentle separation of live CTCs warrants further clinical investigation."

François-Clément Bidard, Ph.D., at the Institut Curie in Paris, France used the FDA-approved CellSearch to collect CTCs for one of his recent studies (*Lancet Oncol.* 2014;15:406–14). He highlighted the AT's advantage of being independent of antibodies to capture the CTCs, unlike CellSearch. But the current flow rate at which the AT separates CTCs (20 μL/min) could be a major limitation in clinics, as it would take nearly 4–8 hours to isolate them from a single blood sample (5–10 mL), Bidard said. He also added that, before AT hits the clinics, the authors should consider testing it to separate CTCs from other types of cancers, like pancreatic cancer, the CTCs of which are further lower than the ones they have tested for other cancers this study. In addition, he noted that the authors shall consider separating CTCs from more patient samples, than from cell lines, as the cells' physical properties would differ significantly.

Joshua Lang, M.D., at the University of Wisconsin School of Medicine and Public Health in Madison said, CTC separation technologies move into using them for genomic and proteomic studies, beyond just collecting and counting them. So, he said that in the future, the authors shall consider a CTC recovery of much higher purity. "Removal of ~90% of white blood cells still infers that thousands of contaminating cells remain. This issue must be further explored in this technology for ultimate clinical utility," Lang said. Lang and colleagues have used both positive and negative selection methodologies, of the 100 or more CTC separating technologies that are in the pipeline.

Huang said the team is testing and gaining better understanding of CTCs from patient samples, which would in the future address the questions of the device's utility and robustness in clinics. Moreover, he said, his team is also looking into miniaturizing and optimizing the device to make it amenable for clinicians. "In the future, we will integrate everything, including disposable chips, electronics, and fluid delivery unit into a small box, about the size of a laptop computer," Huang said. He is optimistic that within 5 years, their device could hit the clinics as a fully integrated device that takes the sample in and gives the answer out within 24 hours.

At the same time, he also admits that no perfect solution exists for CTCs out in the market. "In the following years, it will be interesting to see which technology gains more traction than the others," Huang said.

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