Light-controlled cellular surgery

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Pathologist Michael Teitell likes to think of the invention as a microscopic cat door. He and colleagues have developed a laser-powered nanoblade that makes small slits on the surface of cells, through which DNA, proteins, and larger material, up to a few microns in size, can enter. One can imagine pushing cargo through the cell membrane flap much like a cat pokes its head through a pet door, explains Teitell, who is based at the University of California, Los Angeles (UCLA). The hope is to one day use the technology to quickly and reliably deliver antibodies, proteins, or even life-saving drugs into cells.

The technology draws from the interdisciplinary expertise of Teitell and UCLA colleague Pei-Yu "Eric" Chiou. Teitell earned his medical degree and doctorate doing immunology research, then completed residency in clinical pathology. His laboratory studies mechanisms of cancer metastasis. Chiou earned an electrical engineering doctorate designing "optoelectronic tweezers," a light-induced electrode for manipulating cells and molecules. When Chiou arrived at UCLA as an assistant professor in 2006, he and Teitell brainstormed new ways to use engineering for biomedical purposes.

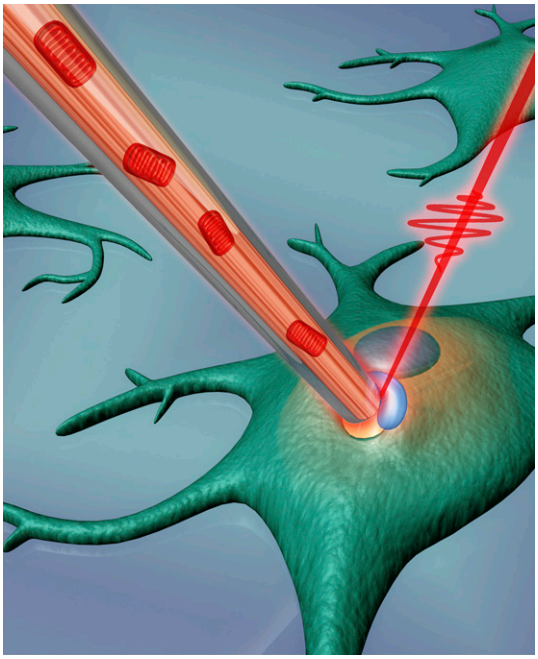
At the time, researchers were generating pluripotent cells by creating embryos with somatic cell nuclear transfer. The procedure—which involves removing the nucleus from an egg cell and inserting it into a donor nucleus from a body cell—was laborious and inefficient, Teitell says. "There weren't many ways to put big things into cells."

Pondering how to get large cargo into cells and seeking to leverage Chiou's expertise in photonics, Teitell hatched a plan. It involved tweaking a method from his own realm, cancer research. The approach, photothermal therapy, attacks tumors with antibodies that target proteins found only on the surface of cancer cells but not on normal cells. If the antibody is linked to a metal, heating the sample with a laser pulse produces explosions that rupture the antibody-coated tumor cells while leaving normal cells untouched.

The researchers reasoned that if the explosions could be made smaller and more localized, maybe the light-based approach could open the membrane just enough to deposit drugs, genes, or proteins without destroying the cell. Light propagated on metal generates a lot of heat, says Chiou, so it should be possible to heat a tightly focused area really quickly. But at the time "we had no idea what would happen," he says.

Laser Focused

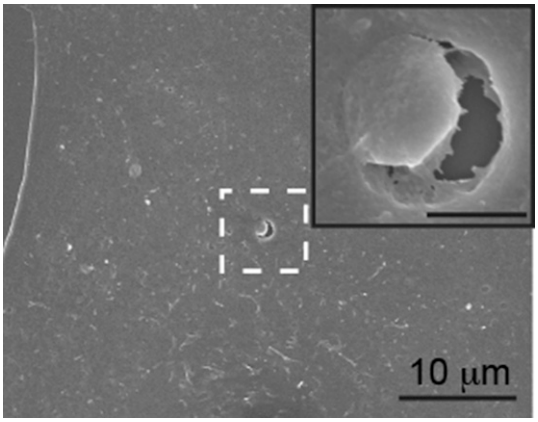
Still, Chiou figured it was worth a try, even more so after discovering a 2003 paper by a team of researchers in Boston. The paper described a method for creating localized cell damage using gold nanoparticles heated by short laser pulses (1). By controlling the concentration of gold particles in the sample, the researchers showed they could use a laser to create explosive vapor bubbles at the tip of the pipette. The collapse of the bubbles poked holes in the membrane without killing the cells themselves. "That gave us the confidence that this idea could really work," says Chiou.



The nanoblade creates a cell membrane opening through which researchers can deliver DNA, proteins, and other large cargo. Image courtesy of Ting-Li Wu (graphic designer).

Chiou bought an inverted microscope, took a pipette from the microinjection laboratory, and asked his graduate student, Ting-Hsiang Wu, to cover the pipette's tip with a thin layer of gold. Wu placed a sample of cells under the microscope, touched the pipette tip to the surface of a cell, and zapped it with a green laser. The procedure clearly did something to the cells—there was visible membrane damage—but "we didn't have concrete data to prove what happened," Chiou says. "It all occurs within a microsecond."

No camera on the market can capture such a fast event, so the researchers had to build their own. Taking cues from a time-resolved imaging system described in a 2006 paper by researchers at the University of California, Irvine (2), the UCLA team spent $100,000 for another microscope and camera to construct a system



To create the nanoblade's "cat door" opening, researchers angle the micropipette tip on the cell's surface, fashioning a flap that the cell can easily repair. Reproduced with permission from ref. 3.

"to see what's really going on 100 to 200 nanoseconds after we pulse the laser," Chiou says.

But there was another problem: cell death. In their initial experiments, a single laser pulse instantly melted the gold on the micropipette tip. The gold then dispersed as particles, bombarding nearby cells and killing them. Fortunately, the researchers found a fix. Instead of coating the pipette tips with gold, they switched to titanium, which adheres well to glass and better withstands the laser because it has a higher melting temperature.

Special Delivery

The approach appeared sound, but the group still had to clearly demonstrate the nanoblade's utility. Tinkering a bit more with the physical set-up and cargo delivery conditions, the team published its first working nanoblade in 2010 (3). By angling the pipette tip at the cell surface, the researchers created a crescent-shaped flap the cell could easily repair. When the "surgery" was timed with pressurized cargo delivery, the researchers could transfer material of various sizes—from DNA to 200-nanometer beads and even two-micrometer bacteria—before the membrane flap resealed (4). Experiments using the nanoblade to deliver live bacteria directly into the cytoplasm of cells helped UCLA microbiologist Jeff Miller discover a surprising mechanism for how an intracellular pathogen spreads (5). And in May of this year, Chiou and Teitell reported they could use the technology to transfer mitochondria, a one- to two-micrometer organelle about as wide as a bacterium, from one cell to another, paving the way for basic research that could lead to new therapies for mitochondrial disease (6).

Although the technique works more reliably than other methods—such as microinjection—for transferring large cargo into cells, it is still slow and laborious: about 100 cells per hour. But Teitell and Chiou have scaled up the nanoblade concept to create a high-throughput platform that enables delivery of large cargo into 100,000 cells in just one minute (7). Called BLAST (biophotonic laser-assisted surgery tool), the system is "like 10,000 to 100,000 nanoblades being operated more or less simultaneously," Teitell says. Cells are loaded onto a one-centimeter by one-centimeter chip containing many three-micrometer holes coated with titanium. When the chip is scanned with a pulse laser, vapor bubbles form and explode in the holes, disrupting the membrane of the cell underneath. The team has founded a start-up company, NanoCav, to commercialize the BLAST platform for researchers in academia and industry. "We are working mostly with BLAST since the higher throughput is a big bonus," says Teitell, However, he says the nanoblade may prove more useful for precision cargo transfer into cells. (Teitell declined to elaborate further because of ongoing intellectual property pursuits.)

The possibility of light-controlled cellular surgery has piqued the interest of other researchers. Andrew Belmont, a biologist at the University of Illinois at Urbana–Champaign, saw the UCLA team's paper on BLAST last year and wondered if the high-throughput technology could help with his research on chromatin: complexes of proteins and genetic material that form inside the nucleus of eukaryotic cells. To map chromatin domains, Belmont and coworkers have long relied on a difficult procedure that involves microinjecting gold-labeled antibodies into cells and visualizing them with electron microscopy. Only a fraction of cells successfully receive antibody. Then, after a week or two spent finding those few cells and processing them for electron microscopy, once you reach the final step "if you screw up the sectioning, you lose all your work," says Belmont.

This spring Belmont sent the UCLA team some of his cells and antibodies to see if BLAST could boost his microinjection efficiency. Preliminary results are promising. BLAST handles 100,000 cells in a minute and has succeeded in getting antibodies into about 80% of them.

"We want to make a platform that's easy to use. . .and allows researchers to devise anything they can think of a few microns or smaller that would be helpful for their research," Teitell says, "whether that's inserting antibodies, pathogens, synthetic materials, or something else we haven't imagined."

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