LAB-ON-A-CHIP

Receptive cells feel the squeeze

Forcing cells through a small opening in a microfluidic channel lets foreign particles enter.

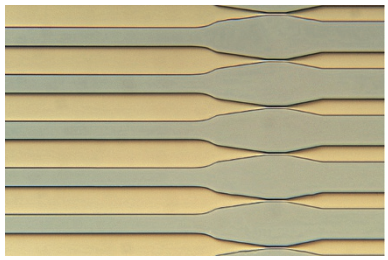
Countless schemes have aimed to conquer the cell's membrane defenses to fill it with a payload. Some use electricity or chemicals to poke holes; others use deception, letting molecules hitchhike with carrier proteins or viruses that hold an entry ticket. The limitations of these methods still leave many molecules out in the cold, and some cell types are unassailable. At the Massachusetts Institute of Technology, scientists are trying something different, using a giant bear hug to coax cells to open up.

Klavs Jensen and his team sought to be free of chemical and viral delivery methods, which can be efficient but often toxic or limited to nucleic acid cargos. Electroporation—using voltage to perforate the membrane—can work for a broader range of molecules but tends to perform poorly for nanoparticles and uncharged proteins. They chose to focus on direct injection, a "very slow and very precise way," according to Jensen, of getting virtually any material into any cell.

Jensen's group first tried to automate the arduous microinjection process by introducing cells, one by one, to fixed needle at the end of a microfluidic channel. When this proved too slow, the idea took a more forceful turn, and blasts of fluid were used to gain entry. But, as Jensen explains, "viscous damping in microfluidic channels is enormous." The weak jets led to flaccid failures—but they also generated a curious observation that took the work in yet another direction.

Armon Sharei, a graduate student in the lab, noticed that some cells took up foreign material despite the underperforming water guns. Reasoning that cells were more receptive when squeezed inside the device, he began testing channels with different constriction designs. The outcome of this work was a silicon chip with many straight, parallel channels set over Pyrex. Cells flow through each channel until they hit a narrow point, which causes them to deform and take up molecules from the surrounding fluid.

The observations are consistent with the explanation that mechanical stress opens holes in the cell membrane, exposing the cytoplasm. By following the diffusion of fluorescent payloads, the researchers saw cells repair their holes within 5 minutes.



Pinching cells in channels helps deliver material to the cytoplasm. Image courtesy of F. Frankel.

Mechanical methods have been used in the past to gain access to the cell, but they were far less efficient. The chip can clock 20,000–100,000 cells every second, allowing over 1 million cells to be processed quickly before it clogs.

In a molecular free-for-all, the team sent small RNAs, DNA, proteins, antibodies and nanoparticles into cells. Delivery efficiencies depended on speed, cargo and cell type but generally tended to improve on existing methods. A major triumph was their delivery into previously recalcitrant primary cells, such as immune cells, without loss of viability. Even mouse embryonic stem cells, which "tend to die or differentiate," according to Sharei, behave well in their chip.

Despite having invented a cheap, efficient and flexible device that scales up easily, Jensen and Sharei are modest, noting that certain methods will be better suited for a particular cargo. For example, Sharei muses that the chip has a less obvious advantage for mRNA or small DNA because it may not promote nuclear entry, which the commonly used chemical Lipofectamine does.

The chip has proven itself on tough applications, reprogramming skin cells with four proteins that it delivers more efficiently than can cell-penetrating peptides. Other exciting uses are being developed, including the previously impossible therapeutic goal of delivering molecules to blood cells before returning them to the patient. "Our current efforts range from developing methods to regenerate damaged tissue to training the immune system to combat cancer," says Sharei.

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Sharei, A. *et al.* A vector-free microfluidic platform for intracellular delivery. *Proc. Natl. Acad. Sci. USA* **110**, 2082–2087 (2013).