Mechanoporation delivers cargo molecules into cells efficiently

Intracellular delivery of biomolecules across the impermeable cell membrane has been a key step in designing clinical therapeutics. Because introducing cargo into cells, referred to as "transfection" in biology jargon, has the capacity to genetically reprogram them, it is often the limiting step in manufacturing gene therapies and adoptive cell-based therapies. In a recent issue of *Lab on a Chip* (https://doi.org/10.1039/D0LC01224F), an international research group from the Technische Universität Dresden, Max Planck Institute for the Science of Light in Erlangen, Germany, and The Institute of Cancer Research in London, UK, has shown a novel approach that can deliver relevant biological cargo such as plasmids, proteins, or drugs into living cells without compromising their viability and transfection performance.

The technique called "progressive mechanoporation" involves the passage of cells through a poly(dimethylsiloxane) (PDMS)-based microfluidic device consisting of multiple microchannels to progressively deform the cells with surrounding hydrodynamic forces. Under the controlled pressure, transient pores form on the cell surface that allow extracellular material containing biological cargo to diffuse into them before the membrane recovers and reseals (see **Figure b**). By focusing on crucial factors such as cell elasticity, the research team believes that the "pre-deformation step" allows cells to adjust to the constricted flow avoiding an immediate mechanical shock, but at a controlled capacity to maintain their viability. This is critically unmet by existing physical and chemical intracellular delivery methods, which suffer from low cell viability despite a high transfection yield. The cells under constricted flow are only temporarily deformed. The cell membrane is resealed and repaired soon after, without any adverse long-term effects or loss in viability. To visualize the path followed by cells in the microchannels, the researchers used fluorescent beads, which in this case, mimic cells and can be seen to progressively squeeze through the microchannels (see **Figure a**).

Alena Uvizl from Technische Universität Dresden and Ruchi Goswami from Max Planck Institute for the Science of Light in Erlangen, the lead authors of the article, told *MRS Bulletin*, "[Progressive mechanoporation] is suitable for a broad range of biomedical, biological, and clinical applications including advanced analysis of the relationship between cell deformation and intracellular delivery, testing novel drugs, and intracellular delivery into hard-to-transfect cells such as immune cells and stem cells."

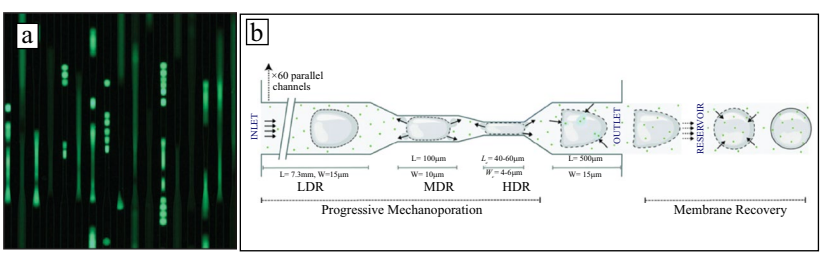
Delivering cargo into cells is not news to scientists working in transfection research. Many FDA-approved therapies available in the clinical market use viral vector or lipid carrier-based approaches to deliver genetic information into the cells. However, the novelty of progressive mechanoporation arises from the progressive multistage cell deformation to ensure high throughput and reproducibility.

The PDMS microfluidic chip is capable of gently squeezing and delivering cargo at a high-throughput rate of 10,000 cells/second with low cell injury. The lead authors say that, "the main goals and challenges in the development of nonviral [cargo delivery] systems are to reach a comparable delivery efficiency like with viral vectors while keeping minimal adverse effects on cells."

The progressive mechanoporation technique has shown to perform better than previously reported mechanoporation modalities in maintaining more than 80% cell viability, greater than 90% delivery efficiency, and also preserving proliferative capacity post-transfection. In addition, by eliminating the use for viruses and electroporation, the progressive mechanoporation method is free of transfection-associated biological toxicities (*Molecular Therapy,* https://doi.org/10.1016/j.ymthe.2018.06.002) and altered gene expression profiles (*PNAS,* https://doi.org/10.1073/pnas.1809671115). The researchers hope that "in the future, progressive mechanoporation could be employed in gene therapies based on *ex vivo* manipulation of patient cells," eventually revolutionizing the global gene therapy market.

Masaru P. Rao from the University of California, Riverside, who was not part of the research work, is delighted about the new mechanoporation technique, which combines the advantages of both shear- and contact-based cellular deformation. Rao told *MRS Bulletin* that "nonviral delivery alternatives, particularly those based on microfluidics, […] address critical challenges in the discovery, development, and manufacturing of engineered cellular therapeutics."

Arun Kumar



(a) Fluorescent beads mimicking cells passing through the parallel channels of the progressive mechanoporation device. Credit: Salvatore Girardo. (b) Schematic design of the progressive mechanoporation microchannels in the device. LDR, low deformation region; MDR, medium deformation region; HDR, high deformation region. Credit: *Lab on a Chip.*