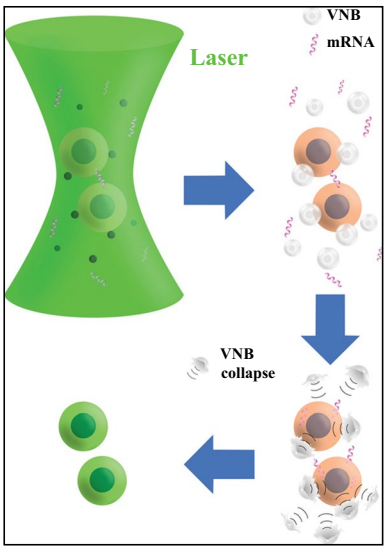
Photoporation developed as an effective nonviral gene delivery system

The cell and gene therapy market was valued at USD$2.6 billion in 2020 and is estimated to reach USD$25 billion by 2027. Delivering exogenous cargo material ranging from mRNA, plasmid DNA, or proteins into cells (i.e., transfection) has been a crucial bottleneck in designing clinical cell therapies as it directly determines the number of modified cells, and thus the treatment efficacy. Although viral vectors have been successful in the clinical manufacturing landscape of US Food and Drug Administration-approved CAR-T (chimeric antigen receptor) cell immunotherapies, there has been an exponential interest in nonviral transfection systems, owing to the immunogenicity and toxicity concerns with viruses. Despite having a high transfection delivery, cells transfected using standard methods such as viral vectors and electroporation (for which an electrical pulse is used to create temporary pores in cell membranes) are unimpressive as both suffer from *ex vivo* transfection-mediated cytotoxicity (*Advanced Therapeutics* https://doi.org/10.1002/adtp.201900133).



Schematic representation of polydopamine-sensitized photoporation for the delivery of mRNA into cells. VNB is water vapor nanobubbles. Credit: *Advanced Functional Materials*.

Now, research work led by Kevin Braeckmans from Ghent University shows the use of photoporation as a nonviral transfection method capable of transfecting immune cells for cell-based cancer immunotherapies. As reported in a recent issue of *Advanced Functional Materials* (https://doi.org/10.1002/adfm.202102472), nanoparticle-mediated photoporation is based on the principle that small nanoparticle-photothermal sensitizers attached to cell surfaces can create vapor nanobubbles when excited by shining a laser pulse over them. The rapid expansion and collapse of the vapor nanobubbles induce transient pores on the cell membranes, which allow extracellular biomolecule cargo to pass into the cells (see **Figure**). The first author of the publication, Aranit Harizaj, tells *MRS Bulletin*, "With an eye on using photoporation in a clinical setting, we explored the potential of biocompatible and biodegradable polymeric nanoparticles as a replacement for inorganic photosensitizing nanoparticles." Previously reported light-induced cargo delivery have typically used inorganic and nonbiodegradable gold or iron-oxide nanoparticles as photosensitizers.

Using polydopamine nanoparticles as a biodegradable photosensitizer, the research group demonstrated efficient delivery of mRNA into different cell types, including hard-to-transfect human T cells. In fact, polydopamine-induced photoporation yielded higher transfection efficiency of functional molecules like proteins and nucleic acids as compared to bulk electroporation. The photoporation technique was able to deliver cargo at a high throughput rate of 100,000 cells per second and, in addition, conserved their cellular viability after transfection, resulting in around 2.5 times more viable immune T-cells when compared to bulk electroporation.

Furthermore, Harizaj says that "photoporation as an intracellular delivery method could be used in the engineering of therapeutic relevant immune cells for cancer immunotherapy (T cells, macrophages, dendritic cells, NK cells) and stem cells for regenerative medicine. Both functional bioactive molecules as well as imaging agents could be delivered into cells via photoporation, making it useful for [therapeutic and diagnostic] applications." Harizaj also notes that "photoporation is a very gentle method which provides a good balance between the delivery efficiency and viability of the treated cells."

Ciro Chiappini, a senior lecturer at King's College London researching nonviral transfection using nanoneedles (who was not part of the study) is excited about the exploration of light-induced delivery, stating, "The optoporation approach [Harizaj et al.] propose is simple, fast and effective, showing low toxicity compared to established methods, which makes it a promising candidate for bringing T-cell therapies to the clinic." Chiappini also notes that the complexity of optical systems and the cost of the technique need to be addressed for clinical translation.

Nicolas Voelcker, a professor at Monash University working on *ex vivo* nonviral engineering of immune cells, and who was also not part of the study group, tells *MRS Bulletin*, "There is no doubt in my mind that nonviral transfection techniques will soon take over as the dominant gene delivery systems in cellular therapy including for CAR-T production. The big question is which 'new kid on the block' will be favored in the market?"

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