# Editorial: Injecting new life into intracellular delivery methods

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Effective methods for intracellular delivery of nucleic acid, protein or other cargo presents an ongoing challenge for both basic research needs and clinical applications. With the expansion of CRISPR/Cas9 gene editing for research and therapeutic purposes (Fellmann, Gowen et al. 2017) and with recent clinical successes of genetically modified cell therapies for cancer treatment (Brudno and Kochenderfer 2018), improvements to existing gene delivery technologies have been highly sought after (Singh, Shi et al. 2017). The main approaches can be generally categorized as viral, chemical, or physical techniques. In their review, Tay and Melosh describe these different approaches for cellular transduction in mammalian systems with a focus on technological improvements made within the last year to each (Tay and Melosh, 2018).

## Types of intracellular delivery

Approaches for intracellular delivery of molecules (DNA, RNA, proteins) have been crucial for studying fundamental biological processes, developing *in vitro* and *in vivo* disease models, for modification of cells to be transplanted into an animal and for direct therapeutic purposes (e.g. gene therapy) in humans. Of the methods, viral carriers including lentivirus, retrovirus, or adeno associated virus (AAV) are highly advantageous for their specificity and efficiency. These viruses are modified to remove pathogenic factors while maintaining the infectious components to gain access to intracellular compartments (Thomas, Ehrhardt et al. 2003). Chemical approaches on the other hand, including polymers or lipid-based carriers, use electrostatic properties to facilitate transfer of nucleic acid to the cell membrane where upon they are internalized by endocytosis. These methods may be less immunogenic compared to viral approaches, and typically do not require a specialized skillset to manufacture. However, the efficiency and targeting capabilities are often lacking in comparison (Lai and Wong 2018). In contrast to most viral and non-viral carriers, physical methods offer the advantage of introducing not only nucleic acids but proteins and other cargo, and can be designed for high efficiency transfer into cells of interest. Physical methods, including electroporation, introduce a specific stimulus, e.g. electrical pulses, to transiently disrupt the cell membrane and permit uptake of any proximal materials (Stewart, Sharei et al. 2016). Advances in nanotechnology have enabled precision control of intracellular delivery and also offer the versatility of being used in combination with viral and non-viral carriers. Such physical methods, however, may require more involved set up and are typically limited to *ex vivo* preparations.

## New technological advances in gene delivery

Despite the variety of techniques available for intracellular delivery, challenges remain for efficient targeting of specific types of cells, e.g. stem cells, immune cells, or neurons, while maintaining cell health and viability. Additionally, the need to deliver specific materials may limit the approach (e.g. nucleic acids versus proteins or small molecules). With these challenges in focus, Tay and Melosh describe new technology to improve AAV targeting of neurons as well as the use of Zika virus to target glioblastoma (Chan, Jang et al. 2017, Zhu, Gorman et al. 2017). They go on to highlight studies working to improve both the

efficiency and targeting of polymers, including the combined use with nanoparticles (Cheng, Sellers et al. 2017, Wang, Li et al. 2017). Finally, the review touches on recent advances in physical techniques for cell transduction, many of which make use of combined approaches. These include the use of microfluidics along with electrical stimuli and disruption of cell membrane with changes in osmolarity (Ding, Stewart et al. 2017), or the combined use of magnetic nanoparticles with viral carriers (Schubert, Trenholm et al. 2018). These approaches offer unique benefits including rapid DNA delivery (within an hour), or the specificity of single cell targeting, respectively. Another significant improvement to physical methods includes the ability to not only introduce cellular materials, but to repeatedly collect intracellular contents over time from the same sample. This is performed using the "nanostraw" technique, which employs the use of nanomaterials that protrude from a polycarbonate membrane to facilitate electroporation of cells in culture. The platform on which the cells grow is itself designed to both introduce and collect intracellular materials, while maintaining high levels of cell viability (Xie, Xu et al. 2013, Cao, Hjort et al. 2017).

As Tay and Melosh note, the approach used by the researcher will ultimately hinge on the unique constraints of the system combined with the desired outcome. Fortunately, the drive for improved intracellular delivery has spawned numerous options to choose from, and will continue to do so as new challenges arise.

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