MAGNETO-NANO-STRUCTURES FOR CENTRIFUGE-FREE AND EFFICIENT IMMUNE T-CELL TRANSFECTION

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Background: The World Health Organization estimates that one in every six deaths worldwide is attributed to cancer, a disease with an annual economic impact of ~ US$ 1.16 trillion. A subclass of cancer immunotherapy known as chimeric antigen receptor T cell (CAR-T) therapy is a promising treatment using genetically reprogrammed T cells to treat cancer. However, for this to happen successfully, DNA must be delivered efficiently and safely into the nuclei of cells through a process known as transfection. Despite several decades of research, it remains a challenge to transfect human primary T cells with high efficiency while preserving high cell viability and critical cellular polyfunctionalities. Limitations of existing technologies: Gold-standard, FDA-approved viruses and bulk electroporation which are clinically used to transfect T cells offer low transfection efficiency (~10-15%). They also induce aberrant cytokine productions (*DiTommaso et al., PNAS, 2018*), which can compromise patients' safety by eliciting cytokine release syndrome and neurotoxicity which have led to deaths and halts of clinical trials. There is also growing evidence suggesting that viruses and bulk electroporation can slow down cell proliferation (*Tay & Melosh, Advanced Therapeutics, 2019*), leading to delayed, less efficacious, and more costly treatments as large cell numbers, typically 10^{8-10}, are used as a single clinical dose for CAR-T therapy. On the other hand, emerging methods using nanoparticles and microfluidics suffer from limitations such as poorly controllable intracellular cargo release and high operating costs due to use of high DNA concentrations in micro-channels respectively (*Tay, ACS Nano, 2020*). Results & Discussions: Here, we describe the magnetic nano-electro-injection (MagNEI) technology for transgene-free, highly efficient, and minimally perturbative T cell transfection (Fig. 1a). During MagNEI transfection, localized electric fields transiently open pores on cell membrane and electrophoretically draw DNA into T cells which are magnetically stabilized onto high aspect-ratio nano-structures without using bulky centrifuges. Once DNA enters the cells, magnetic forces are applied via FDA-approved Dynabeads to bias their intracellular transport into the nuclei. MagNEI offers 50% net transfection efficiency in delivering GFP plasmid for stable gene expressions for >2 weeks i.e. 3-4 folds better than viruses and Lonza electroporator (Fig. 1b). MagNEI-treated T cells proliferated up to 40% faster than T cells treated with viruses and bulk electroporation (Fig. 1c). Additionally, unlike viruses and bulk electroporation, MagNEI induces minimal perturbations to critical biological attributes including cytokine production, trafficking, and gene expressions (data not shown here). Conclusions: MagNEI enables transgene-free, highly efficient delivery of DNA for CAR-T cell engineering. Unlike viruses, it is transgene-free. MagNEI is also much less perturbative to healthy functions of transfected cells than bulk electroporation. In addition to these advantages, MagNEI transfection can also occur without the use of bulky centrifuges, making it a valuable tool for end-to-end CAR-T manufacturing.

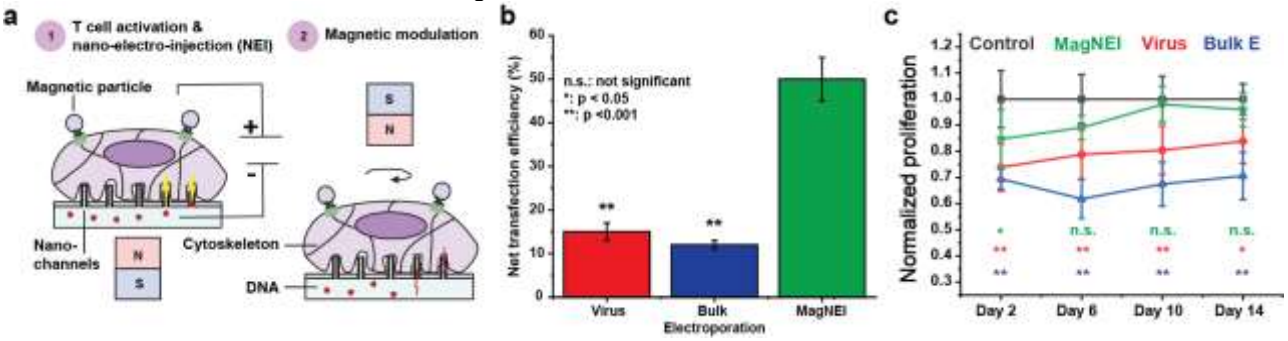

Fig. 1 MagNEI for transgene-free, highly efficient, and minimally perturbative T cell transfection. (a) Mechanism of MagNEI involving nano-structure-localized electroporation and magnetic modulation. (b) MagNEI achieved ~50% net transfection efficiency in delivering GFP plasmid for stable gene expressions for > 2 weeks i.e. 3-4 folds higher than that by gold standard FDA-approved viruses (15%) and Lonza electroporator (12%). (c) T cells transfected with viruses and bulk electroporation proliferated 20-30% slower than control and MagNEI-treated cells after 2 weeks of expansion. Error bars shown are ± standard mean error.