SingHealth · Duke-NUS SCIENTIFIC CONGRESS

ABSTRACT SUMMARY	
Event Name:	SingHealth Duke-NUS Scientific Congress 2021 (Abstract)
Abstract Title*:	High-aspect Ratio Nano-structures for Efficient, Minimally Perturbative and Transgene-free Immuno-transfection
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AUTHORS DETAILS				
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ABSTRACT DETAILS	
Aims:	To genetically engineer Chimeric antigen receptor T cell (CAR-T) therapy for cancer immunotherapy [1], biomolecules like DNA must first be delivered successfully into the nuclei of cells, a process known as transfection. Despite several decades of research, it remains a challenge to transfect T cells with high efficiency while preserving critical biological polyfunctionalities. Gold standard viruses and bulk electroporation offer low transfection efficiency [2] while inducing adverse immune responses [3] and delaying cell proliferation [4]. Emerging methods like nanoparticles suffer from poorly controllable intracellular release of cargo while microfluidics run into high operating costs from using concentrated biomolecule in continuous flow.
Methodology:	Here, we describe the magnetic nano-electro-injection (MagNEI) platform which uses localized electric fields to transiently open pores on the membranes of cells magnetically stabilized onto hollow nanochannels before electrophoretically injecting DNA into the T cells. Once DNA enters the cells, magnetic forces are applied *via* magnetic beads to transport them into the nuclei.
Result:	MagNEI provided 50% net transfection efficiency for long-term, stable expression of GFP i.e. 3-4 folds better than gold standard viruses and Lonza bulk electroporation. While viruses and Lonza bulk electroporation adversely reduced T cell proliferation by 20-30% and T cell migration towards chemoattractant IP-10 by as much as 80%, MagNEI did not.
Conclusion:	Our results demonstrate the technical and biological superiority of MagNEI as a transfection method - higher efficiency with minimal perturbations to critical biological attributes. We envision that the use of MagNEI platform can overcome transfection difficulties in laboratories and clinics for genetic engineering of sensitive, primary immune cells to advance immunobiology and cancer immunotherapies.

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2. Tay, A. & Melosh, N. Transfection with Nanostructure Electro-Injection is Minimally Perturbative. *Adv. Ther.* **2**, 1900133 (2019).
3. Cromer, M. K. *et al.* Global Transcriptional Response to CRISPR/Cas9-AAV6-Based Genome Editing in CD34+ Hematopoietic Stem and Progenitor Cells. *Mol. Ther.* **26**, 2431–2442 (2018).
4. DiTommaso, T. *et al.* Cell engineering with microfluidic squeezing preserves functionality of primary immune cells in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E10907–E10914 (2018).