Efficient gene editing for cell-based therapy using a novel microfluidic droplet squeezing platform

You-Jeong Kim[1,2*], Ha-Sung Lee[1], and Aram Chung[1,2]

[1]Department of Biomedical Engineering, Korea University, Korea

[2]Interdisciplinary Program in Precision Public Health (PPH), Korea University, South Korea

*kyouj0125@korea.ac.kr

Abstract

We present a novel droplet-based intracellular delivery platform leveraging droplet microfluidics with cell mechanoporation. The efficient intracellular delivery, transfection, and gene editing were achieved by passing droplets co-encapsulating a cell and target molecules through a single constriction at a high speed. The platform enables transfection using mRNA, and plasmid DNA, and gene editing using CRISPR Cas9 RNP with high efficiency over conventional bench-top technics including electroporation and lipofection. Notably, this strategy presented here can find potential application in cell-based therapy.

1. Background

Intracellular delivery of external cargoes, including DNAs, RNAs, proteins, Ribonucleoproteins (RNPs), and synthetic nanoparticles is the first steps for cell-based therapy. Traditionally, intracellular delivery tools, as shown as Fig. 1, have been norm for internalizing biomolecules into living cells. However, they are limited due to their genotoxicity, inconsistency, low scalability, high preparation cost, and/or time and labor consuming process [1]. To address these drawbacks, several microfluidic approaches including cell squeezing, which is using series of constrictions smaller than cells, [2]. is already effective enough to approval for clinical applications (NCT04084951). However, the approach is limited because of failure of delivering plasmid DNA and large nanoparticles, wastefulness of delivery molecules, and clogging of constrictions. To overcome these limitations, a novel droplet squeezer platform is introduced that can be considered as a next-generation intracellular delivery platform.

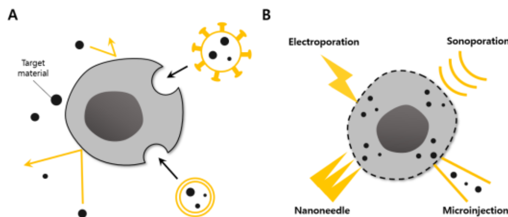

Figure 1. Conventional intracellular delivery strategies (A) viral transduction and lipid nano particles (B, clockwise from top left) electroporation, sonoporation, microinjection, nanoneedle.

2. Methods

This novel microfluidic droplet squeezer platform mold was prepared by etching a silicon wafer using a deep reactive ion etching process. K562 cells were cultured according to standard protocols. The cells suspended with the target molecules and recyclable oil were pumped into a microchannel by the syringe pump. Additionally, FITC-dextran, 996 nt EGFP-mRNA, plasmid DNA, or CRISPR Cas9 RNP was resuspended in 2×10^7cells/mL delivery buffer. The delivery efficiency of the cells was analyzed using flow cytometry (Luminex, USA), and cell viability was measured using a standard trypan blue assay.

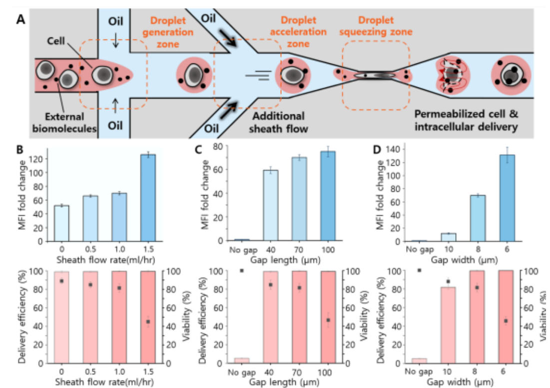

Figure 2. (A) Schematic image of our platform. (B-D) 2000kDa FITC-dextran delivery into K562 cells at different operational conditions. Mean fluorescence intensity (MFI), Delivery efficiency, and viability for different (B) sheath flow rate, (C) gap length and (D) width.

3. Results and Discussion

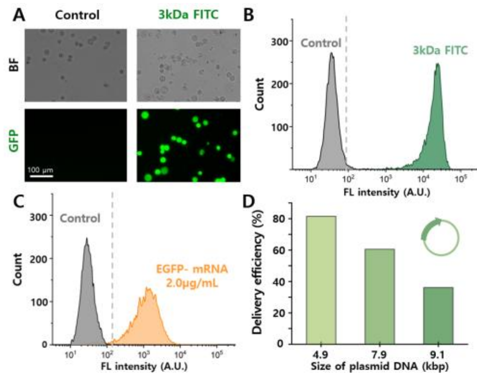

Figure 3 : (A) Bright field and fluorescence image of 3kDa FITC-dextran. Flow cytometry histogram of (B) 3kDa FITC dextran, (C) 2.0μg/mL of EGFP-mRNA, and 50μg/mL of plasmid DNA with different size.

This droplet squeezer comprises three functional units: a droplet generation zone, droplet acceleration zone and droplet and cell squeezing zone for membrane permeabilization (Fig. 2A). Cells and biomolecules are first co-encapsulated into droplets; additional sheath oil flow accelerates the droplets for maintaining stable and efficient intracellular delivery when droplets pass through a single constriction at a high speed. During droplet squeezing, the cell membrane is permeabilized and the cargos dispersed in the vicinity are transported into cells. To characterize this droplet squeezer performance, large polysaccharides (2000 kDa) was delivered using different operational conditions (Fig. 2 B-D). As shown in Fig. 2B-D, nearly 100% delivery efficiency was achieved with a minimum of 80% cell viability.

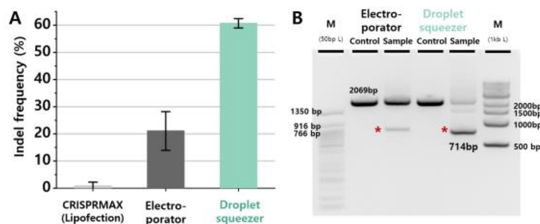

Figure 4 : (A) indel frequency from lipofection, electroporation, and our platform. (B) 1.5% TAE agarose gel analysis of EMX1 gene multiplexing of K562 cells transfected with CRISPR Cas9 RNPs using electroporator and our platform.

Using the identified optimal channel geometry and flow conditions, approximately 98% of EGFR mRNA (Fig. 3C), more than 80% of 4.9 kbp, 60% of 7.9kbp, and 36% of 9.1kbp of plasmid DNA (Fig. 4D) were delivered into K562 cells. Compared to Vect platform, which is similar to cell squeezing, 43% of 5.8kbp plasmid DNA delivery efficiency was reported.[3]. As aforementioned, cell squeezing method has critical disadvantages in that it cannot deliver plasmid DNA [4].

To demonstrate that our approach can edit the gene for cell-based therapy, we internalized CRISPR Cas9 RNPs into K562 cells. We compared the indel frequency using our platform with those processed using CRISPRMAX, and electroporation, (Fig. 4A) and platform showed advanced gene editing efficiency. Recently, K562 cells were tested by internalizing two kinds of CRISPR Cas9 which are targeting EMX1 sites. As shown in Figure 4B, note that presented platform showed higher gene editing efficiency compared to electroporation (Fig. 4B).

4. Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (No. 2021R1A2C2006224).

5. References

[1] Stewart, M., Sharei, A., Ding, X. *et al. In vitro* and *ex vivo* strategies for intracellular delivery. *Nature* **538,** 183–192 (2016)

[2] Jeongsoo Hur and Aram. J. Chung, Microfluidic and nanofluidic intracellular delivery, *Advanced Science*, 2021, pp. 2004595

[3] Anna Liu, Muhymin Islam, Nicholas Stone, Vikram Varadarajan, Jenny Jeong, Samuel Bowie, Peng Qiu, Edmund K. Waller, Alexander Alexeev, Todd Sulchek, Microfluidic generation of transient cell volume exchange for convectively driven intracellular delivery of large macromolecules, Materials Today, 2018, Volume 21, Issue 7, Pages 703-712

[4] Armon Sharei, Janet Zoldan, Andrea Adamo, WooYoung Sim, Nahyun Cho, Emily Jackson, Shirley Mao, Sabine Schneider, Min-Joon Han, Abigail Lytton-Jean, Pamela A. Basto, Siddharth Jhunjhunwala, Jungmin Lee, Daniel A. Heller, Jeon Woong Kang, George C. Hartoularos, Kwang-Soo Kim, Daniel G. Anderson, Robert Langer, Klavs F. Jensen, Vector-free microfluidic delivery platform, Proceedings of the National Academy of Sciences Feb, 2013, 110 (6) 2082-2087; DOI: 10.1073/pnas.1218705110