# Highly effective intracellular delivery via non-linear microfluidic cell stretching platform for cancer immunotherapy

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## Abstract

A novel non-linear microfluidic cell stretching platform is presented, enabling highly effective intracellular delivery of external biomolecules into various immune cell types. A viscoelastic fluid, methylcellulose (MC) solution, was utilized to attain significant increased intracellular delivery. In the proposed platform, cells suspended in the MC solution were injected into a linear channel, where cells rapidly pass through a single constriction with a dimension slightly smaller than the cell diameter. Due to the using of the MC solution, a high shear force was applied to the cells, effectively generating transient nanopores; therefore, exogenous cargos can be internalized into cells through the created membrane discontinuities. With this platform, a high delivery efficiency (>97%), high throughput (>7x10$^5$ cells/min), low-cost operation (<$1), nearly clogging-free performance, and rapid delivery of various biomolecules into different immune cells were demonstrated for cancer immunotherapy applications.

## Background

Cancer immunotherapy is a new form of cancer treatment approach utilizing patient's own immune system to eradicate cancer. For example, chimeric antigen receptor (CAR)-T cell therapy is a representative therapy which have demonstrated remarkable clinical advantage in treating non-Hodgkin lymphoma (NHL) subtypes, such as diffuse large B-cell lymphoma (DLBCL) [1]. To manufacture CAR-T cells, CAR transgene needs to be delivered into patient's T cell.

Traditionally, viral transduction, electroporation, and lipofections have been used for cellular engineering; however, these methods are limited due to its high cost, low scalability, and/or inconsistent delivery efficiency. Recently, to address these problems, microfluidic platforms have shown high potential as alternative solutions [2]. Among them, the cell squeezing platform attract attention because of its simplicity and versatility. But the platform has limitation such as serious channel clogging due to excessively narrow constriction compared to cell diameter [3].

Here, we present a novel non-linear microfluidic cell stretching platform, enabling highly effective intracellular delivery of external biomolecules into various immune cells using viscoelastic fluids, methylcellulose (MC) solution. The cells suspended in the MC solution are injected into a single constriction with a dimension slightly smaller than the cell diameter. Due to the high shear force given by the MC solution, significantly higher level of deformation can be achieved, generating membrane nanopores. Therefore, external biomolecules can be effectively internalized through the created membrane discontinuities without clogging. Utilizing this platform, a high delivery efficiency (>97%), high throughput (>7x10$^5$ cells/min), low cost (<$1), and nearly clogging-free operation are shown for various immune cell lines for cancer immunotherapy.

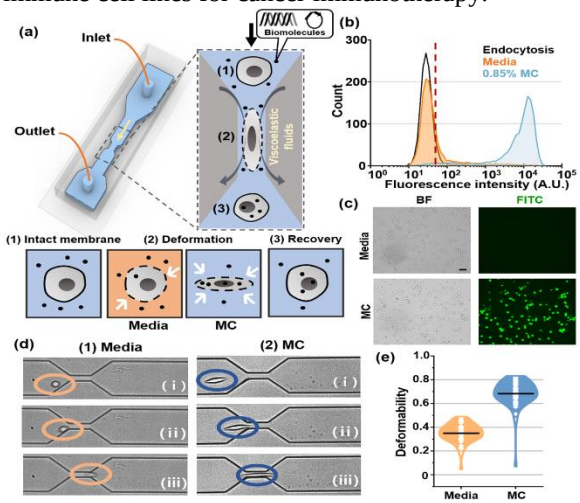


Figure.1 (a) Operating principles of intracellular delivery, (b)-(c) fluorescence intensity histograms and fluorescence microscopy images for internalization of 3-kDa FITC-dextran into K562 cells, (d)-(e) high-speed camera images and cell deformability of media and MC solution (scale bar: 50 μm).

## Results and Discussion

The cells suspended in the MC solution were injected into a linear microchannel with a single narrow constriction as one-step operation (Figure. 1(a)). As presented in Figure 1(b), close to 100% delivery efficiency for 3-5 kDa FITC-Dextran into K562 cells with a cell density of $10^6$ cells/ml was demonstrated in the use of MC solution whereas nearly-zero delivery was showed for pure media. A higher level of deformation can be achieved in MC solution due to enhanced shear force (Figure. 1(d)-(e)).

The delivery of external biomolecules into cells was affected by the flow rate, MC concentration, and channel configuration. By changing such parameters, an optimal operational condition was identified that yielded the highest delivery efficiency and cell viability (Figure. 2(a)). Under the identified optimal operation condition, a trial was executed to deliver different dextran sizes (3-2,000 kDa) and test various cell density and cell types (Figure. 2(b)-(d)). As shown, the presented method was insensitive to the evaluated cargo materials, cell density, and cell types.

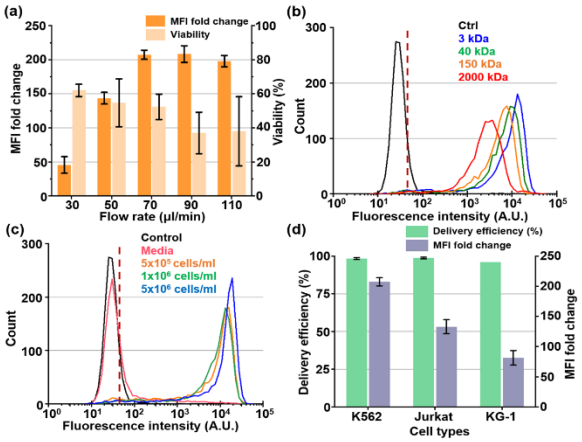


Figure.2 (a) Optimization process: MFI fold change and viability for flow rate, (b)-(c) Fluorescence intensity histograms for various FITC-dextran sizes and different cell density, (d) Delivery efficiency and MFI fold change for various cell types.

To further evaluate the possibility of the platform for functional nanomaterial delivery, EGFP mRNA was internalized into K562 cells. As shown in Figure. 3(a), approximately 97% mRNA transfection efficiency (mean fluorescence intensity fold change of close to 40) was obtained, demonstrating the potential of the platform for cellular engineering. Furthermore, non-linear microfluidic cell stretching platform showed advanced transfection performance in mRNA delivery compared to lipofection (Figure. 3(c)-(d)).

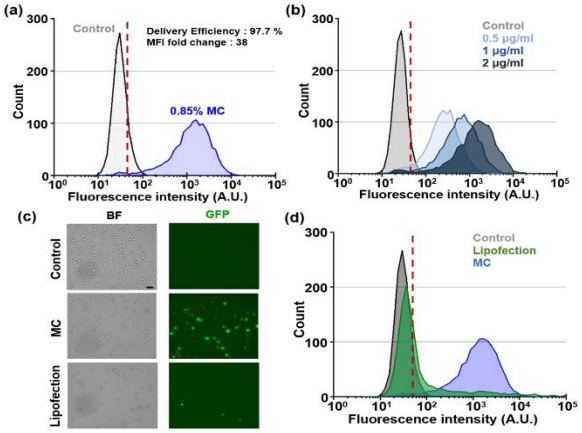


Figure.3 mRNA delivery: (a) Fluorescence intensity histograms for internalization of EGFP mRNA into K562 cells, (b) Fluorescence intensity histograms for various mRNA concentration, (c)-(d) fluorescence microscopy images and fluorescence intensity histograms showing mRNA expression via control, MC, and lipofection (scale bar: 50 μm).

## Conclusion

We present a novel non-linear microfluidic cell stretching platform using high viscoelastic fluids, methylcellulose (MC) solution, which is characterized by high delivery efficiency, high throughput, low-cost operation, nearly clogging-free, and simple operation. Therefore, the proposed platform demonstrates a practical, robust, and cost-effective approach that is expected to influence cancer immunotherapy, cell-based research, and genome engineering.

## Acknowledgements

This work is supported by the Samsung Research Funding and Incubation Center for Future Technology (Grant No. SRFCIT1802-03) and the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (No. 2021R1A2C2006224).

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