Novel Microfluidic Platform for Scalable Transfection of CRISPR/Cas9 RNP in Human T cells

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Introduction

Gene editing of T cells holds great promise for the development of novel CAR and TCR T cell therapies. A notable tool to achieve gene editing in T cells are CRISPR/Cas9 ribonucleoprotein (RNP) complexes, which can be delivered into T cells by means of non-viral transfection techniques. We have developed a novel transfection technology consisting of a microfluidic channel capable of eliciting cell volume exchange for convective transfer (VECT). Here, we sought to characterize how efficient is VECT at eliciting gene editing when transfecting CRISPR/Cas9 RNPs into primary human T cells. Our platform can be readily scaled up by parallelizing the number of microfluidic elements in the system.

Objectives

- Evaluate VECT for the delivery of CRISPR/Cas9 RNP in primary human T cells, as well as cell viability at the end of the microfluidic transfection process.
- Assess the efficiency of VECT when co-transfecting two different RNPs targeting *TRAC* (TCR complex) and *CD5*.
- Characterize the range of RNP concentration, as well as cell density at which VECT can operate consistently.

Experimental Design

Primary human T cells were isolated, activated and cultured before transfection. Cells were washed and placed in fresh native media together with *TRAC* or *CD5* CRISPR/Cas9 RNP at a final concentration of 18μg/ml, immediately before being processed using our microfluidic device. Cells were then collected and placed back into culture. Gene editing efficiency was studied 3+ days after VECT by means of flow cytometry.

Results

VECT delivery of *TRAC* CRISPR/Cas9 RNP (Figure 1) resulted in high gene editing efficiencies of the TCR (up to 90%) in both CD4$^+$ and CD8$^+$ T cells, while maintaining high viability of the cells upon transfection (>70%).

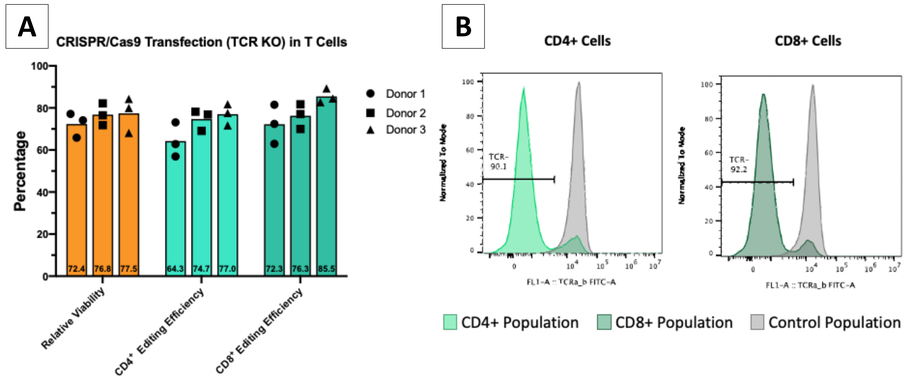

Figure 1. *TRAC* CRISPR/Cas9 RNP transfection using VECT. A) Relative viability (normalized to viability of the cells before transfection) and knockout efficiency of the TCR in CD4$^+$ and CD8$^+$ cells, generated in three independent donors. B) Representative histograms of the TCR knockout efficiency results, as measured by flow cytometry 3+ days after transfection.

Single transfection of *TRAC* and *CD5* CRISPR/Cas9 RNPs (Figure 2) resulted in different efficiencies of knock-out (>70% vs. 40% respectively), likely due to differences in the targeting efficiencies of each gRNA. When RNPs were co-transfected, knock-out efficiencies were similar to those observed when employing the same guides individually, suggesting both payloads are successfully delivered without interference between the two.

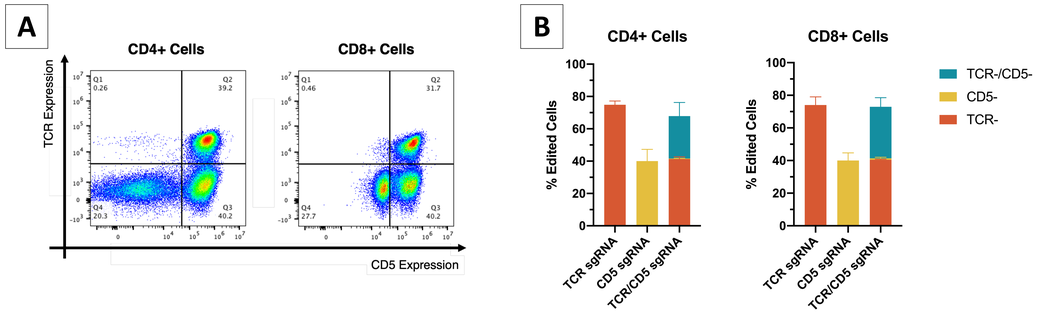

Figure 2. Microfluidic co-transfection of *TRAC* and *CD5* CRISPR/Cas9 RNPs. A) Representative dot plots of cells co-transfected with *TRAC* and *CD5* RNPs, resulting in knockout of TCR complex and CD5 marker on the cell surface, as detected by flow cytometry. B) Bar graphs representing mean knockout efficiency of *TCR* and/or *CD5* RNP transfection (n=3).

We assessed RNP concentration and cell density during VECT in order to better understand the operative range of our microfluidic device. Our prototype could successfully transfect T cells at RNP concentrations as low as 6μg/mL (Figure 3A), and at high density of cells of up to 8 million cells/ml (Figure 3B).

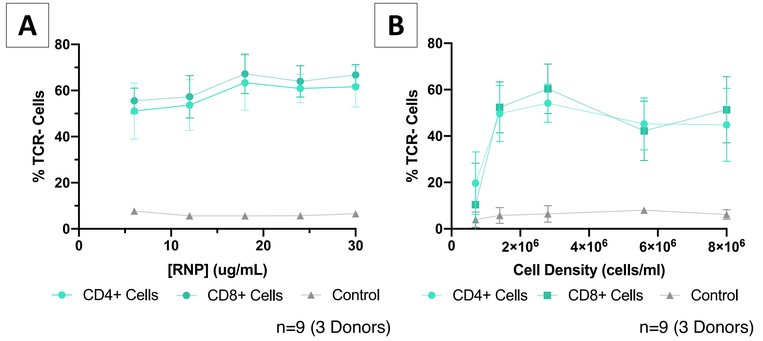

Figure 3. RNP concentration and cell density operative ranges for transfection of CRISPR/Cas9 RNPs. A) Efficient, comparable TCR knockout efficiency is achieved at RNP concentrations spanning from 6 to 30μg/mL. B) Cell concentration does not have an effect on CRISPR/Cas9 RNP knockout efficiency, except for one condition where cells are processed and cultured below 1x10^6 cells/mL.

Conclusions

We have demonstrated that VECT enables highly efficient delivery of CRISPR/Cas9 RNPs into primary human T cells, while retaining high cell viability upon transfection. Co-delivery of two RNPs is as efficient as the delivery of each RNP separately, as measured by the rate of knockout of the TCR complex and CD5 surface marker. Finally, our microfluidic device achieves consistent gene editing efficiencies when operating across a wide range of Cas9 RNP concentration and cell density.

Future Plans

We are developing instrumentation and consumables to be employed across a wide range of applications, from R&D to GMP throughputs. We will also seek to establish microfluidic CAR knock-in gene editing technology by means of VECT.