Microfluidic Facilitated CRISPR-Cas9 RNP Transfection

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Introduction

The gene-editing of T cells has assisted in the creation of therapies such as Chimeric Antigen Receptor (CAR) T and T Cell Receptor (TCR) T-cell therapies. Using CRISPR-Cas9 ribonucleoprotein (RNP) complexes, these innovative therapies offer hope for patients with otherwise untreatable cancers and other devastating diseases. We have developed a microfluidic transfection device which allows volume exchange for convective transfection (VECT). The device uses non-viral means to efficiently deliver CRISPR-Cas9 payload through mechanical transfection, leading to high levels of TCR knock-out, while retaining normal T cell functionality.

Objectives

- Establish operational ranges at which VECT can efficiently deliver *TRAC* CRISPR-Cas9 RNP complexes.
- Determine the effect of CRISPR-Cas9 delivery through VECT on cell functionality.
- Evaluate feasibility and efficiency of gene knockout when RNP components (gRNA and Cas9 nuclease), are delivered separately.
- Assess the efficiency of VECT mediated knockout when co-transfecting multiple RNP complexes.

Experimental Design

Human primary T cells were isolated and activated 12 hours before transfection. Cells were washed and resuspended at a cell density of 3x10^6 cells/mL. They were then combined with *TRAC* or *CD5* CRISPR-Cas9 RNP complexes at a concentration of 18μg/mL and transfected through microfluidic channels. Cells were plated in 1mL media and cultured for 120 hours. Gene-editing, and cell functionality was measured using flow cytometry.

Results

The transfection of *TRAC* CRISPR/Cas9 RNP using VECT (*Figure 1a.)* resulted in a high editing efficiency (>80%) while maintaining high viabilities (up to 80%). We were also able to recover a majority of cells processed through the microfluidic system (80%). Our CRISPR/Cas9 mediated knockout retains a high editing efficiency at varying RNP concentrations (*Figure 1b.* 6μg/mL – 30ug/mL) and cell densities (*Figure 1c.* 1x10^6 – 8x10^6 cells/mL).

Figure 1. Operational Range of TRAC CRISPR/Cas9 RNP mediated knockouts using VECT

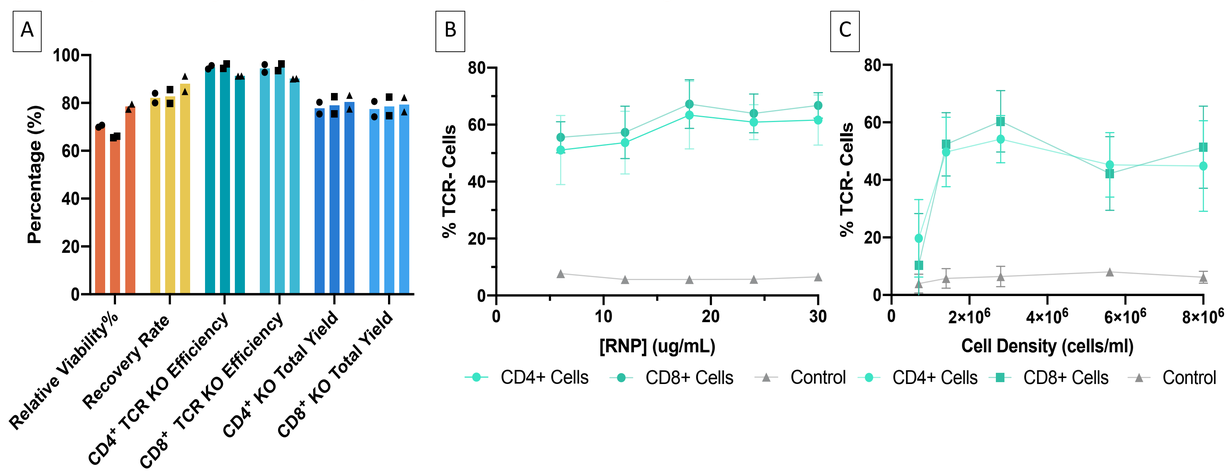
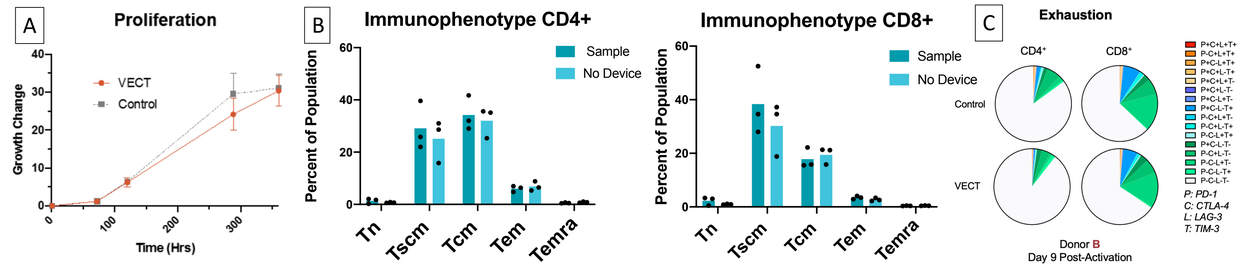


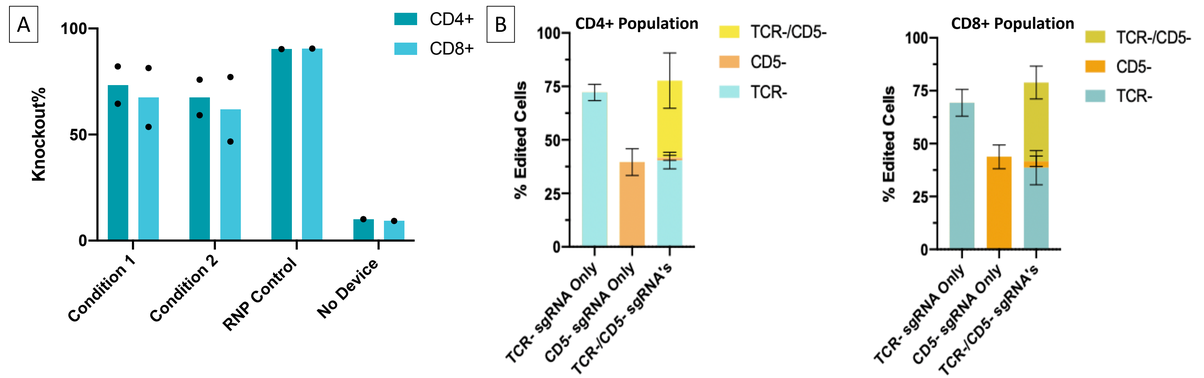
Figure 2. Cell Functionality of TCR KO T cells generated with VECT



The VECT transfection system preserves cell functionality in *TRAC* knockout samples. T cell proliferation is not influenced by microfluidic transfection. Our microfluidic device has no impact on the immunophenotype of either the CD4+ or the CD8+ T cell population *(Figure 2b.).* The profile of four common genes associated with T cell exhaustion also remains unaffected by the VECT system (*Figure 2c.*).

Figure 3. Microfluidic serial processing and co-transfection of CRISPR/Cas9 RNPs in human T Cells

Figure 3a. 5x10^6 T cells were processed with 18μg/mL of *TRAC* single guide RNA in the morning, plated in media and then transfected again with 18μg/mL of cas9 protein. Samples in "Condition 2" were processed at a higher pressure than "Condition 1." *Figure 3b.* 3x10^6 T cells were transfected with our microfluidic device with 18μg/mL of *CD5* and *TRAC* RNP complex at the same time.



The VECT system has the ability to gene-edit T cells at a high efficiency when the components of the RNP complex are delivered separately (*Figure 3a.).* To test this capability, we delivered the *TRAC* gRNA in the morning through a microfluidic device, and then delivered the cas9 nuclease in the in the afternoon. Our transfection rates remained comparable to the RNP control where both components were delivered as a pre-complexed RNP payload. Additionally, when processing the *CD5* and *TRAC* CRISPR-Cas9 RNP complexes we saw a mean knockout of 25% co-transfection. *(Figure 3b.).*

Conclusions

VECT delivery of the *TRAC* knockout shows a large operational range and high editing efficiency in human primary T cells. Our system preserves T cell functionality and can be used to both serially deliver and co-transfect RNP complexes.

Future Plans

In the future we plan on developing the knock-in capabilities of VECT by employing a range of DNA templates for homology-directed repair together with CRISPR/Cas9 RNPs.