Microfluidic-Enabled Delivery of mRNA into PBMCs for Multiplex Transfection of Naïve T Cell, Natural Killer Cell, and Other Lymphocytes

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Introduction

Chimeric Antigen Receptor (CAR) therapy has been pioneered with the use of CD4+ and CD8+ T cells, typically expanded for up to two weeks during manufacturing. This manufacturing process is also being transferred into Natural Killer (NK) Cells and other lymphocytes of interests, such as Natural Killer T cell (NKT) and Gamma Delta (γδ) T cell. Transient mRNA delivery offers a rapid manufacturing alternative to permanent gene modifications generated with integrating viral vectors. In the present work, we study if we can use volume exchange for convective transfection (VECT) to deliver mRNA into multiple cell types within fresh peripheral blood mononuclear cells (PBMCs). Expression of therapeutic genes with mRNA constitutes a faster, more versatile, and safer platform for the potential manufacturing of CAR lymphocytes.

Objectives

- Optimize the VECT microfluidic device dimensions for the processing of PBMCs.
- Optimize process variables to achieve high transfection efficiency (> 60%) , while maintaining high cell viability and cell recovery (> 80%), with emphasis placed on CD4+ T , CD8+ T, and NK cells.

Experimental Design

Fresh PBMCs were washed and resuspended in native media. Immediately before transfection, mRNA payload was added to a final concentration of 60µg/ml. Cells were flowed through the device, collected after transfection. Cell Viability and recovery were assessed 30 minutes post VECT. The mRNA transfection efficiency was studied 24 hours after VECT via flow cytometry.

Results

When we compared three different devices, we found a successful VECT device to process PBMCs as starting material (Figure 1). Device 1 has the optimized VECT microfluidic device dimensions.

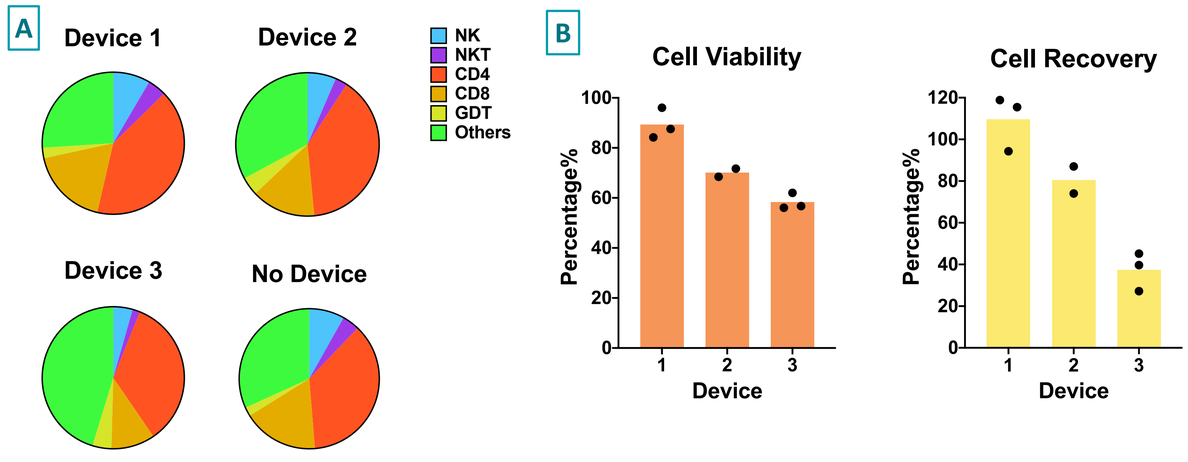

Figure 1. Device comparison demonstrating that Device 1 is the optimal VECT device for PBMCs.
A) Comparison of cell populations in three different VECT devices vs. no device (negative control) conditions. B) Relative viability (normalized to no device condition) and relative recovery (normalized to no device condition) for PBMCs processed with each device.

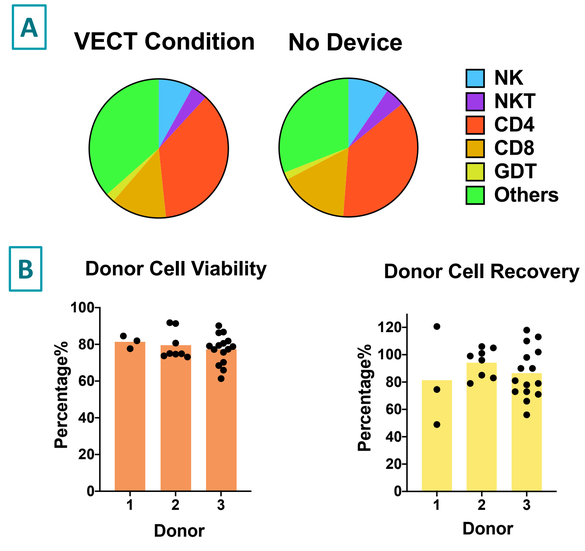

Figure 2. GFP mRNA transfection using VECT.
A) Comparison of VECT and no device (negative control) conditions across three independent donors. B) Relative viability (normalized to no device condition) and relative recovery (normalized to no device condition). C) Representative histograms of GFP mRNA transfection efficiency results across three donors. D) GFP mRNA transfection efficiency of NK, CD4+ T, and CD8+ T cells of each independent donor, as measured by flow cytometry 24 hours after transfection.

Across three independent donors, the average relative viability was 79.35%, relative recovery was 87.42%, and transfection efficiency for NK cells was 62.6%, CD4+T was 71.8%, and CD8+ T was 73.9% (Figure 2).

Conclusions

We have demonstrated that VECT with fresh PBMCs enables highly efficient delivery of GFP mRNA into NK, CD4+ T, and CD8+ T cells while maintaining cell viability and cell recovery above ~ 80%.

Future Plans

We are planning on translating our results into CAR mRNA lymphocytes to show that we can successfully manufacture a functional CAR product. Currently, we are developing our device into a high throughput scale format, with the capacity to transfect 1 billion cells in under a minute.