Novel method to enhance T-lymphocyte viral transduction by acoustophoresis

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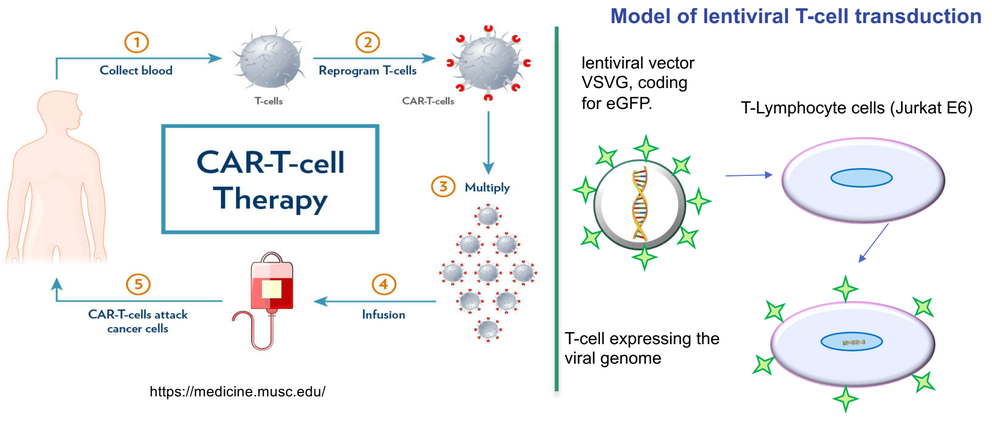
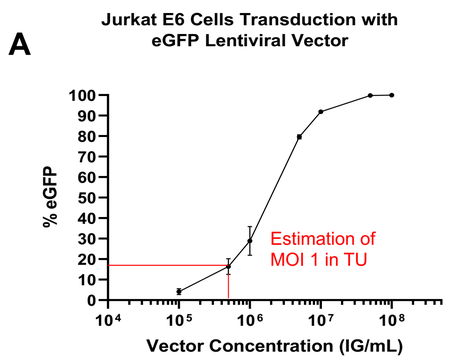
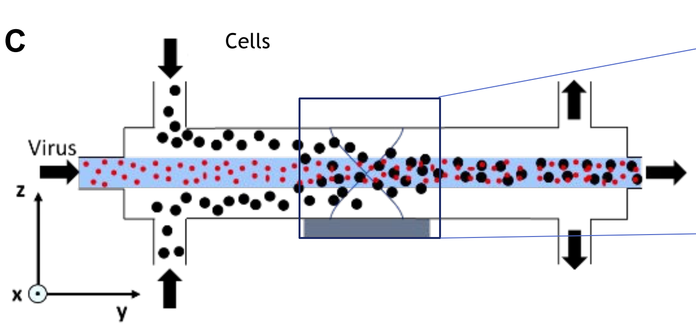
Background

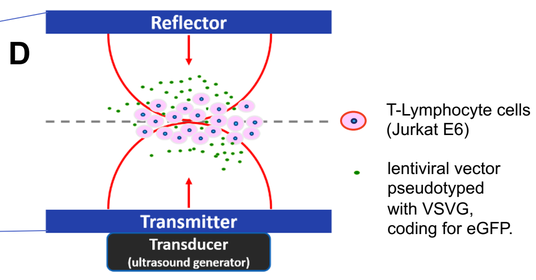
CAR-T cell therapy has shown remarkable success in the treatment of various cancers. However, producing these cells for clinical use is still challenging. One of the significant challenges in CAR-T cell production is transducing the cells with lentivirus vectors efficiently. Low transduction efficiency results in lower CAR expression and reduced cytotoxicity, ultimately affecting the efficacy of the therapy. This poster presentation discusses the use of acoustic technology to enhance the transduction efficiency of CAR-T cells. Benefits of using acoustic to enhance transduction: Acoustic technology has recently emerged as a promising approach for enhancing the transduction efficiency of CAR-T cells. Acoustic technology uses sound waves to create a membrane rearrangement and permeability in the target cells. Cell membrane transient alteration allowing efficient vector delivery without causing cell damage or toxicity [1]. Furthermore, the acoustic method does not require any additional reagents or steps, making it a cost-effective and time-efficient approach.



Methods





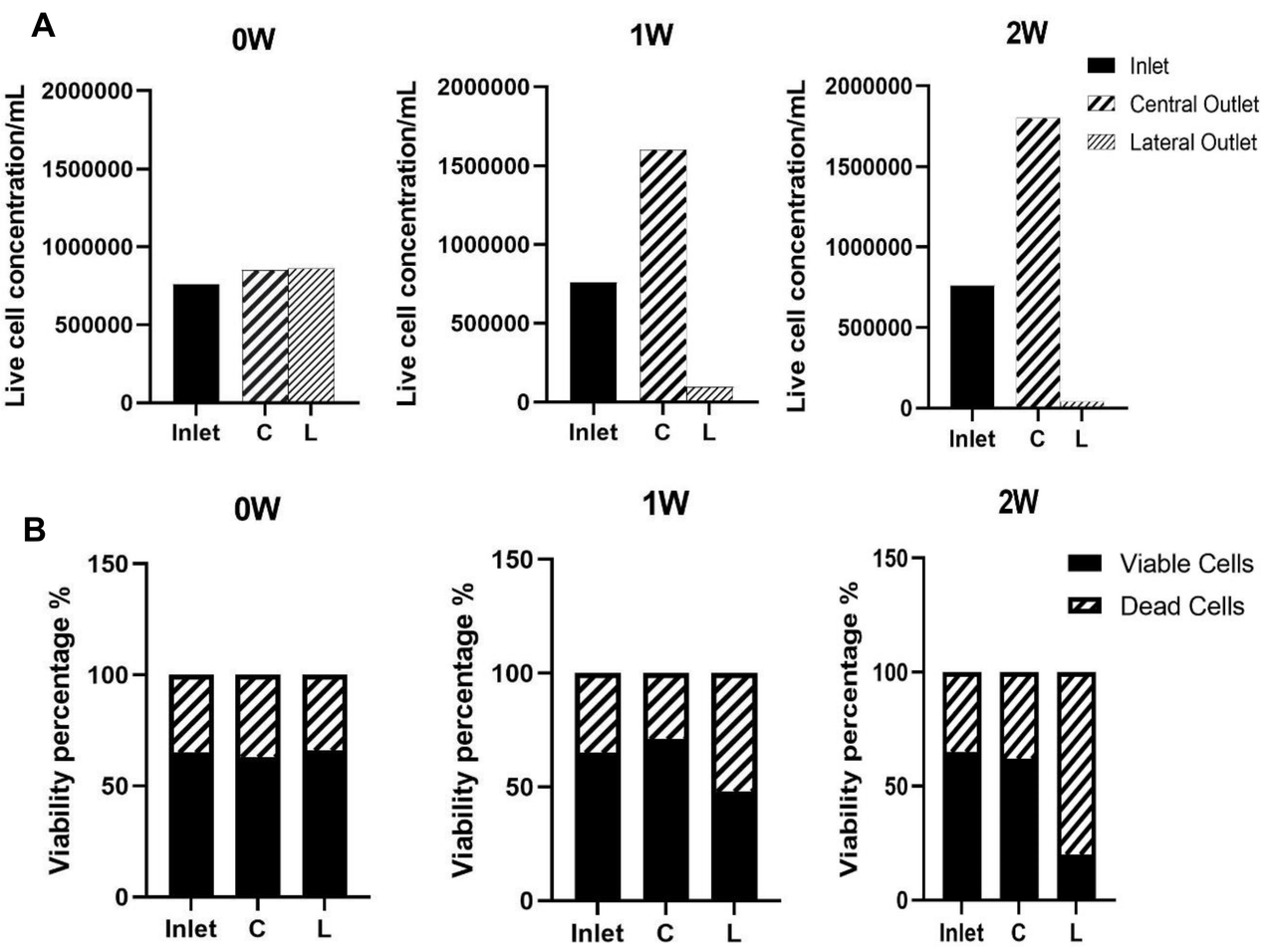


schematic demonstration of acoustophoresis channel A. Lentiviral vector (LV) transduction of Jurkat cells in static conditions. Jurkat E6 cells were transduced with a LV-eGFP/VSVG vector, the LV infectious titer (infectious genome (IG)/mL) was measured by PCR 6 hours post transduction at 37° C 5% CO2. After a wash cells were cultured for 8 days. Results are average +/- SD % of eGFP quantified by FACS (n=4) B. Picture of fabricated acoustic channel PMMA channel used for the experiment. C schematic demonstration of the experimental setup using PMMA channel in with cells and virus in flow. D Schematic of a zoom of acoustic node with cells and virus in the channel.

Design of the channel allows to:

- ✓ Cells concentration at the acoustic node
- ✓ Increase the cell-virus encounter.
- ✓ Change the mechanical properties of the cell membrane that could enhance entrance of the viral particle to the cells.

Cell recovery and concentration using acoustic

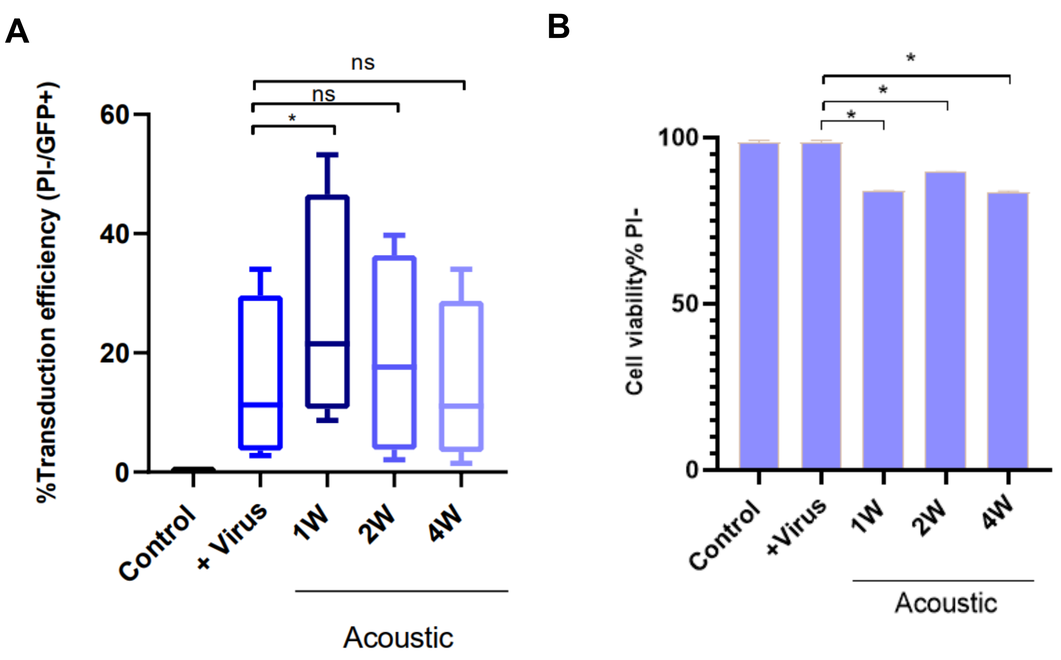


Acoustophoresis concentration studies using Jurkat T-cells. The volume and the number of cells which were collected through the central (C) or a lateral (L) outlets were measured. Live and dead cells were counted by microscopy using Trypan blue exclusion dye A. Live cell concentration B. Percentage of viable cell

Conclusion

This result demonstrated that the acoustic waves could be harnessed to re-concentrate the cells at the central channel. These results supports the concept of cell-virus encounter. The cell viability data under the two different acoustic powers comparing to the control condition confirmed no negative effect of acoustic on cell functionality.

Promising preliminary data on Transduction efficiency using acoustic channel 3



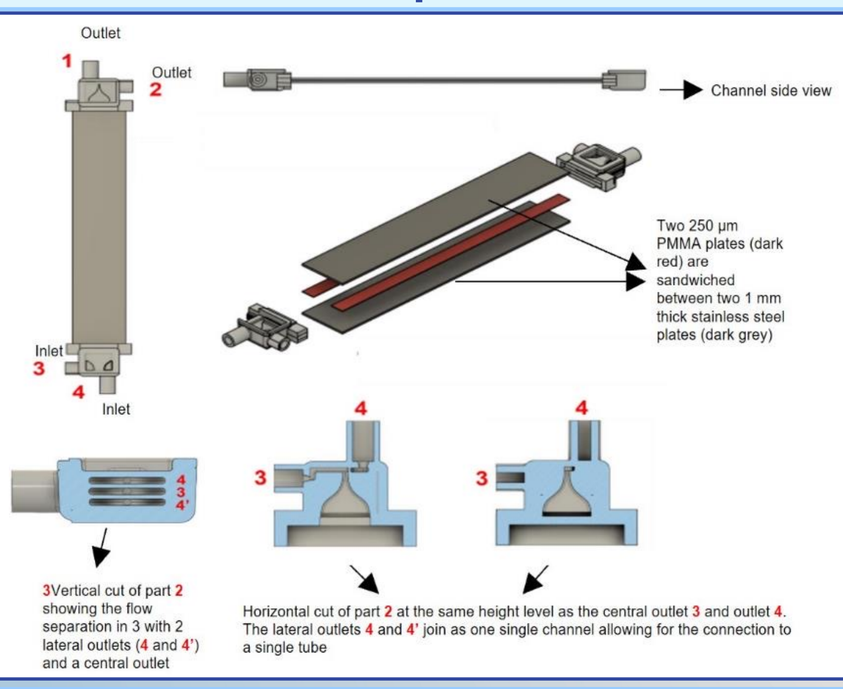
Transduction efficiency result using Aenitis acoustophoresis system

A. Lentivirus transduction efficiency of Jurkat E6 cells quantified using CANTO II flow cytometry gated for PI (Propidium iodide) negative cells (viable cells) and cells positive for GFP B. Cell viability analysis using PI. Results are expressed as mean ± SEM. *P<0.05; **P<0,01; ***P<0,001

Conclusion: Preliminary results show that applying a 1W acoustic power to the cells preserves cell viability (> 70% by propidium iodide), enables a cellular concentration in the central outlet of the system, and can double the average transduction efficiency compared to cells not subjected to acoustophoresis (from 14% to 26% average positive cells using an MOI of 1.

This result suggests that acoustic technology can effectively facilitate the delivery of lentiviral vectors into cells, leading to higher transduction efficiency. Overall, the positive effect of acoustic technology on transduction efficiency can outweigh any potential negative impact on cell viability when the appropriate acoustic parameters are used. By optimizing the acoustic setup, it is possible to achieve high transduction efficiency while maintaining high cell viability.

Developing the acoustic channel for transduction process automation



- ✓ We are developing the acoustic channel with medical grade stainless steel, this approach would be compatible with the T-cell processing in sterile conditions.
- ✓ Stainless steel transmits ultrasounds better than plastic which would allow for higher flow rates and better cell recovery yields. These channels can be use in batches to enhance the sample volume capacity.

Conclusions

The use of acoustic technology with a low MOI of 1, may provide a promising approach for improving the production of CAR-T cells for clinical use. However, it is important to note that further studies are still needed to confirm and optimize these results for different cell types, vectors, and acoustic parameters in order to develop a standard protocol for T-Cells transduction using acoustophoresis technique.

Perspectives

We are going to validate our results on PBMC driven primary T-cells. In the next step we plan to develop a high throughput system using the parallel acoustic stainless steel channels with an objective of preserving the cell viability while enhancing the transduction efficiency. These channel would be integrated into the instrument that makes the process automated and favorable to optimize for industrialization.

References

1. Peng, C. H., Woung, L. C., Lu, K. H., Tsai, C. Y., Lee, S. D., Huang, C. S.& Hwang, D. K. (2018). Acoustic waves improves retroviral transduction in human retinal stem cells. *Journal of the Chinese Medical Association*, *81*(9), 830-836.

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