1. Technology

1.1. Device Description:

This is a beta prototype of a biotransport-mediated vector maximizer meant to enhance transduction of many target cells with any viral vector. Use of this device to transduce cells ensures that viral vector-cell interactions can occur without diffusion limitations of conventional systems that would otherwise result in wasted viral vector and lengthy transduction times since high vector concentration can be maintained without having to use large quantities.

Physically constraining the viral vector-cell interactions using this technology allows:

- Label-free, highly efficient transduction
- Reduction of vector usage
- Accelerated transduction times
- Combination of other transduction-enhancing technologies
- Optimization of transduction protocols with minimal cell and reagent use
- Reliable scale up due to fixed spatial parameters

1.2. Warnings:

Follow all applicable safety protocols and use appropriate personal protective equipment (PPE) when handling cells and viral vector with this device. This device is meant for single use. To ensure the quality and purity of transduced cells, please use a new device for every transduction. Please dispose of this device according to your institution's biosafety policies for any material that has been in contact with recombinant viral vector and cells.

1.3. Contents:

- 1 beta prototype of the biotransport-mediated vector maximizer
- 1 disposable syringe (5 mL)
- 1 blunt tip needle with luer adapter (18 gauge, 0.5")

Stability and Storage
These devices can be stored at room temperature, and are stable for at least one year at ambient conditions.

2. Applications

2.1. Cell Types

The physical mechanism of this platform is generally suitable for use on numerous cell types. This technology has primarily been tested on a variety of suspension cell lines and primary cells. We are continuing to test various cell types. Please fill out our survey after trying this device to help us better characterize which cell types are compatible with this technology: forms.gle/7H9YeYwrRzUhkDw97

Cell Line	Cell Type	Source
Jurkat	Acute T cell leukemia T lymphocyte	Human
K-562	Myelogenous leukemia	Human
Primary hematopoietic stem cells		Human, Mouse
Primary T cells		Human

2.2. Viral Vector Types

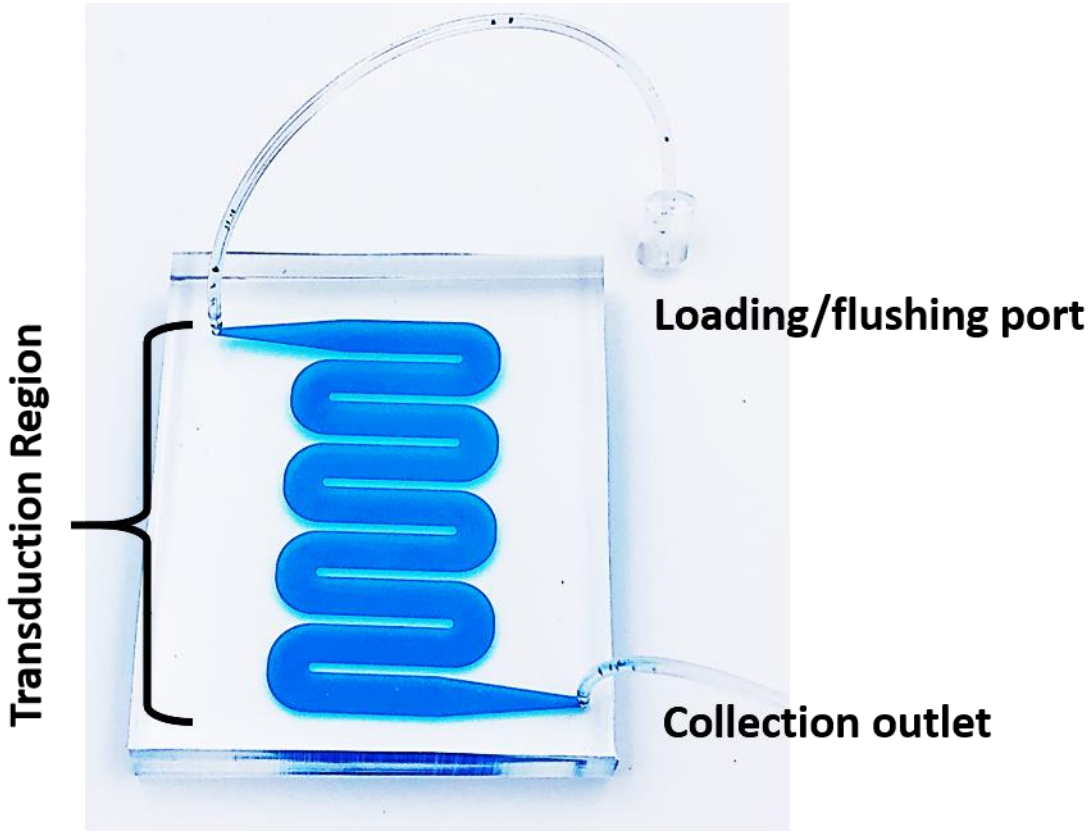
This physical mechanism of this platform is generally suitable for use with any viral vector. We have primarily tested this platform with lentiviral vectors. We are continuing to test various viral vectors. Please fill out our survey after trying this device to help us better characterize which viral vectors are compatible with this technology: forms.gle/7H9YeYwrRzUhkDw97

Viral Vector	Type	Application
Adeno-Associated Virus	AAV2, AAV6	Increase transduction
Lentivirus	VSV-G	Increase transduction
Primary T cells	VSV-G	Increase transduction

3. Biotransport-mediated Vector Optimizer Components

Operation of the device is designed to be simple. Cells and viral vector are loaded together into the transduction channel via the inlet port. Once the cells have been transduced for the desired amount of time, they can be flushed out using a buffer or media-filled syringe directly into a collection tube. The optimized channel height enables high concentrations of viral vector to more readily come in contact with target cells before degrading.

Device Specification	
Channel Height	110µm
Channel Volume	100µL (10µL minimum, 110µL maximum capacity)
Transduction Region Surface Area	10.0cm^2
Maximum Cell Capacity	4x10^6 cells



4. Suggested Procedure

4.1. General Considerations

The instructions presented below are a generalized protocol that enabled successful gene transfer into target cells. For best results, we recommend that you start with this generalized protocol, then adapt and optimize as necessary. Results may vary due to considerations such as viral vector titer and culture conditions. The amount of viral vector used, transduction time, and number of cells may have to be adjusted for optimal results. Since the device volume is relatively low compared to traditional cell culture vessels, do not exceed 50% viral vector concentration unless the vector is specifically formulated in the media used for the target cell or leave cells in the device for longer than 24 hours without media exchange to preserve cell viability.

4.2. Cell Culture

Suspension cells should be cultured in a standard culture vessel until ready for transduction using suitable media composition and cell density to promote healthy cell growth.

4.3. Device Preparation

Sterilization of the devices can be quickly achieved by flushing with 70% ethanol diluted in deionized water. Flushing can be achieved by directly pipetting into the inlet port or connecting to a vacuum line and aspirating through the device. Before loading cells, flush the device in a similar manner with phosphate buffered saline (PBS) or the cell culture media to wash away any residual ethanol. Aspirate as much fluid from the device as possible. We recommend using a micropipette tip attached to an aspirator to achieve this. Once the device has been evacuated of fluid, the device is ready for use. Some droplets may remain within the channel, but these will not disturb the loading.

4.4. Suggested Transduction Protocol

Prepare cell/viral vector mixture

1. Calculate the volume of viral vector necessary for desired multiplicity of infection (MOI) based on titer and number of cells.
    i. MOI = # viral particles / # target cells
2. Centrifuge the desired number of cells at a speed that is suitable for the target cell type (Suggested: 300 x *g* for 5-10 minutes at room temperature).
3. Prepare a mixture of the cell culture media and viral vector stock to achieve desired MOI or viral vector concentration. (Note: Do not exceed 30% viral vector concentration [30µL volume] unless the viral vector is specifically formulated in the final culture medium used for cell transduction). The cell density should be between $5.0x10^6$ – $4.0x10^7$ cells/mL. The higher the cell density, the shorter the transduction should be.
4. Remove cells from centrifuge and aspirate supernatant. Re-suspend cells in the viral vector mixture.

Load cells for transduction

5. Load the desired volume of cell/viral vector mixture directly into the device inlet adapter port with a micropipette.
    i. 100µL or less should be loaded into the device. Do not attempt to load more than 110µL.
    ii. Partial loading of the device is fine if lower cell numbers are being transduced. Adjust the volume accordingly and refer to the Appendix for alternative loading techniques if loading 50µL or less.
    iii. Maintain a steady pressure on the plunger while pushing the mixture from the pipette tip into the device.
    iv. Watch carefully as the cell/vector mixture enters the channel, and gently continue applying pressure on the plunger until the mixture has been pushed entirely through the inlet tubing.
    v. Do not remove pressure from the plunger until the pipette tip has been removed from the device inlet adapter port.
6. Place the loaded device into a petri dish.
7. Add a conical tube cap filled with sterile water or PBS to the petri dish to maintain humidity. Alternatively, place a moistened delicate task wiper in the petri dish.
8. Place the petri dish with the loaded device into an incubator set to 37°C/5% CO_2.
9. Transduction time may need to be optimized depending on cell type and vector, but 6-12 hours is generally sufficient. Do not exceed 24 hours without replenishing media.

Collect cells from the device and wash

10. To retrieve cells from the device, load a 5 mL syringe with sterile PBS or cell culture media and attach the included blunt tip needle.
11. Place the device on a vortexer set to a high setting and vortex for 3-5 seconds to release cells from the bottom of the channel.
12. Position the outlet collection tubing into a conical tube to collect cells.
13. Insert the blunt tip needle into the device inlet adapter port.
14. Gently flush the channel with PBS or media at a rate of ~5 mL/min.
15. Centrifuge the collected cells at 300 x *g* for 5-10 minutes to wash away excess viral vector.
16. Aspirate the supernatant and re-suspend the cells in warmed cell culture media to the appropriate culture density.

Culture cells until stable gene expression

17. Transfer the cells to a well plate or flask at the desired culture conditions.
18. Culture the cells in an incubator set at 37°C/5% CO_2 until ready to assess transduction efficiency (typically 72hrs or greater for stable gene expression).

5. Appendix

5.1. Troubleshooting

Bubbles may form from dissolved gas expansion within the channel after placing the device in an incubator. However, unlike other microscale systems, formation of bubbles in this platform does not negatively impact cells, and instead simply displaces them slightly within the channel. For more troubleshooting tips, please send inquiries to rtran4@emory.edu.

5.2. Alternative Loading Technique

For small volumes that would not allow for sufficient force to push the fluid into the device (10-50µL), use the following technique:

1. Withdraw the desired volume into a micropipette.
2. Place the loaded tip into the inlet adapter port.
3. Gently eject the micropipette tip so that it serves as a reservoir for the cell/vector mixture.
4. Take a P1000 micropipette with an appropriate tip and depress so that air is pushed out.
5. From the other end of the device, place the empty P1000 micropipette tip into the outlet adapter port while maintaining pressure on the plunger.
6. With the pipette tip placed firmly in the outlet adapter port, gently release pressure from the plunger as if withdrawing fluid. This will cause fluid from the loaded pipette tip at the inlet to be withdrawn into the channel.
7. Once the fluid has completely moved from the inlet tubing into the channel, remove the pipet tip from the inlet adapter port.

For all inquiries or additional information, please contact Reginald Tran, PhD at rtran4@emory.edu.

Additional information may be found in our 2017 Molecular Therapy publication:
https://www.sciencedirect.com/science/article/pii/S1525001617303167