A Humanized Microfluidics BBB Model With Recirculation for CNS ARV Distribution Evaluation

Johid R Malik[1], Ukamaka O Modebelu[1], Courtney V Fletcher[1], Sean N Avedissian[1]
[1] University of Nebraska Medical Center, Omaha, NE

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BACKGROUND

Antiretroviral (ARV) efficacy in the central nervous system (CNS) is hindered by poor penetration across the blood–brain barrier (BBB). Current in vitro BBB models are limited by non-physiologically relevant cell lines and/or static conditions that restrict formal pharmacokinetic/pharmacodynamic (PK/PD) sampling and analysis. As such, we developed a microfluidic BBB model with primary human brain cells that includes physiologic blood flow, offering a more clinically relevant platform for preclinical drug development and CNS pharmacology assessment in HIV.

METHODS

A master mold was generated using an in-house 3D printer, fabricated with Polydimethylsiloxane (PDMS) to construct a functional microfluidic BBB chip. The system was seeded with primary human BBB component cells, including brain microvascular endothelial cells (hBMECs), astrocytes, pericytes, and neurons, and integrated with a pressure-controlled recirculating circuit connected to inlet and outlet ports to better replicate the anatomical and physiological properties of the human BBB. The device was optimized for repeated sampling under a constant-flow regimen of dolutegravir (DTG), emtricitabine (FTC), and tenofovir disoproxil fumarate (TDF), each maintained at clinically relevant maximum plasma concentrations. Media from the outlet tubing was collected at multiple time points over 48 hours, and drug concentrations were quantified using our established liquid chromatography with tandem mass spectrometry methods.

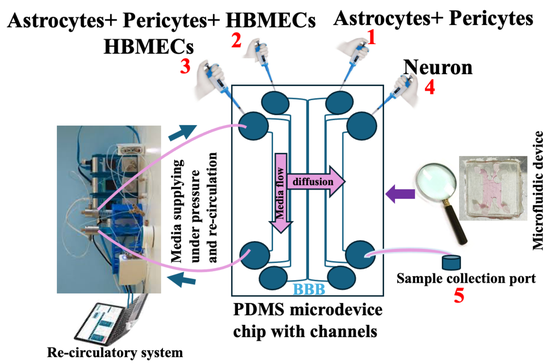


Fig. 1. Schematic presentation of our in-house BBB model showing the strategy of workflow, with the right side showing a real microdevice BBB image post-experiment. Numbers indicate the steps in BBB development and experimentation.

Key Findings: Our microfluidic human BBB "organ-on-a-chip" model results, using DTG+FTC+TDF as a test regimen, support the notion of multiple PK/PD sampling under physiological blood flow and shear stress conditions to optimize ARV distribution across the BBB.

RESULTS

As shown in **Figure 3**, all three ARVs exhibited distinct distribution profiles across the BBB. DTG and FTC demonstrated sustained distribution, which peaked after ~30 hours. In contrast, TDF displayed consistently lower distribution compared to DTG and FTC, with only modest increases over time and no clear peak in distribution.

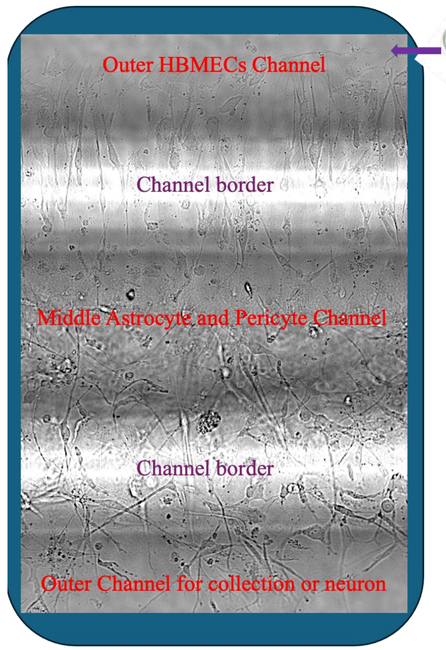


Fig. 2. Enlarged and phase contrast view of microfluidic BBB device under Evos microscope showing cells growing in the microfluidic BBB device. The outer channel on the top shows a monolayer of human brain endothelial cells (HBMECs) followed by the bright light of the border separating it from the middle channel of astrocyte and pericyte microvasculature growing in 3D orientation, followed by the bright color of the border separating it from the outer channel for sample collection

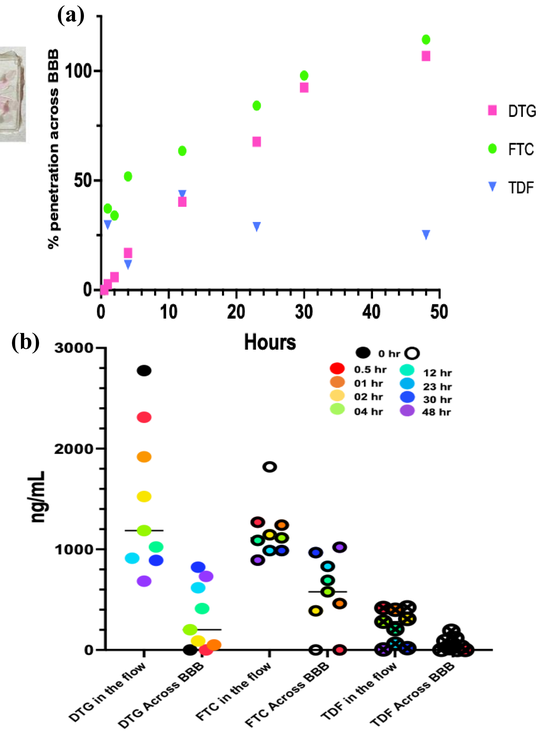


Fig. 3. The top panel (**3a**) represents a scatter plot showing the multi-time distribution estimation in the microfluidic BBB for DTG, FTC, and TDF, recirculated as a regimen, maintaining a constant Cmax value of the drugs. The bottom panel plot (**3b**) shows the real-time concentration of drugs in the flow versus across the barrier. The colored circles indicate the time points when corresponding samples were collected over a 48-hour span.

CONCLUSIONS

Clinical assessment of ARV CNS distribution is limited by reliance on lumbar punctures, typically restricted to single-time-point CSF collections that do not capture the dynamic processes of BBB drug transport. Our microfluidic human BBB "organ-on-a-chip" model overcomes these barriers by enabling repeated PK/PD sampling under physiologic flow conditions, providing a more accurate and mechanistic understanding of ARV distribution across the BBB. By bridging the gap between preclinical models and human physiology, this platform offers a clinically translatable tool for optimizing CNS-targeted ARV.

FUTURE DIRECTIONS

Future plans involve assessing other combination ARV regimens and including infection to the cells via microglia incorporating to evaluate if we can predict and assess different ARV combinations for the potential treatment of NeuroHIV.

SPECIAL THANKS/AKNOWLEDGMENTS

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PLAIN LANGUAGE SUMMARY

Lab models that mimic the human blood–brain barrier conditions can be used to screen drugs designed for the brain. Measuring how HIV drugs reach the brain clinically is difficult because it ~~usually~~ relies on a spinal fluid sample, usually one at most. Multiple samples are not possible, and thus, how drugs behave over time is unknown. Our human blood–brain barrier microdevice chip allows repeated tracking of drug movement under realistic flow conditions. This provides a more human-relevant way to understand and optimize HIV drug delivery to the brain.