

Supplementary Information for
Redirecting the Route: Monocyte-Mediated Delivery of oHSV-1 Across a
Human BBB-on-chip Model

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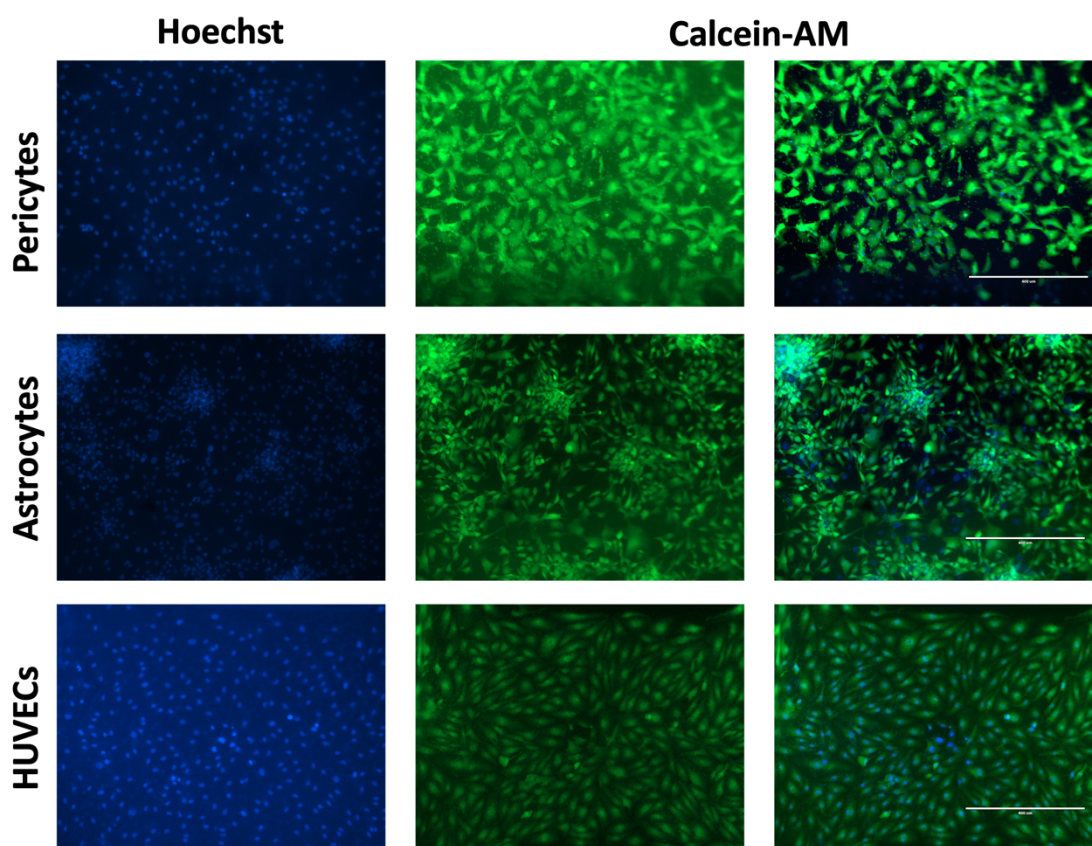


Figure S1. Representative Live/Dead assay results. Fluorescence microscopy images showing the results of the Live/Dead assay for pericytes, astrocytes, and HUVECs seeded in the BBB-on-chip at day 7. Live cells cytoplasm are stained in green with Calcein-AM, nuclei of cells appear blue stained with Hoechst. The images confirm successful cell attachment and high viability of all three cell types, indicating the stability and integrity of the BBB-on-chip model. Scale bars: 400 μ m.

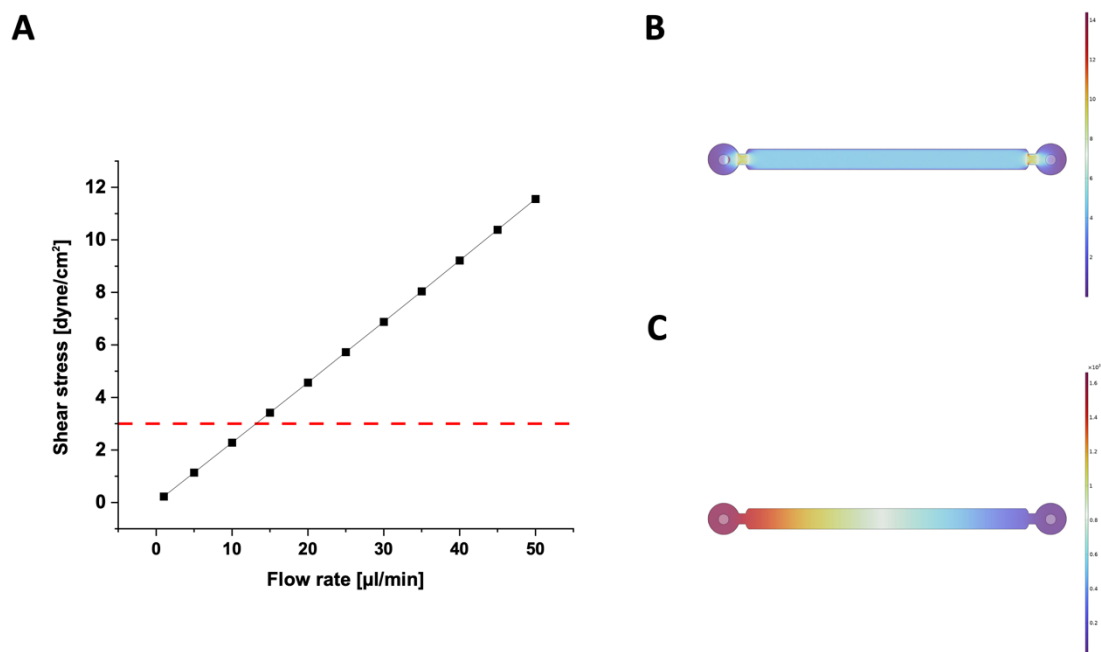


Figure S2. Consol Multiphysics simulations of flow parameters. **A.** Result of the parametric sweep to estimate the value of wall shear stress by varying the flow rate. **B.** Top view of the shear stress profile at the walls of the vascularized channel for 30 $\mu\text{l/min}$ flow rate, selected to simulate the physiological condition within the blood side channel. **C.** Top view of the pressure profile at the walls of the vascularized channel for the same 30 $\mu\text{l/min}$ flow rate.

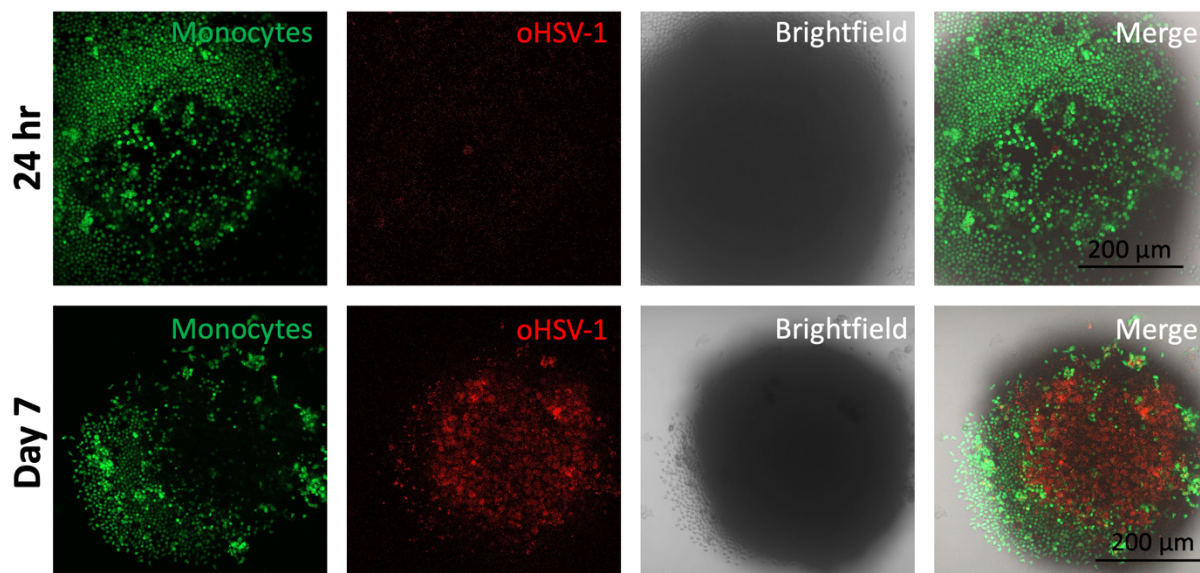


Figure S3. Human primary monocytes deliver their OV's cargo to GBM spheroids. Human primary monocytes (green) loaded with oHSV-1 mCherry (red) with U87-GBM spheroids in microwell cultures. At 24 hours, monocytes successfully migrate and infiltrate the tumor spheroids, while limited viral signal is detected. By day 7, a substantial increase in oHSV-1 signal within the spheroid is observed, indicating effective viral transfer. Brightfield images and merged channels confirm spatial localization. Scale bar: 200 μm .

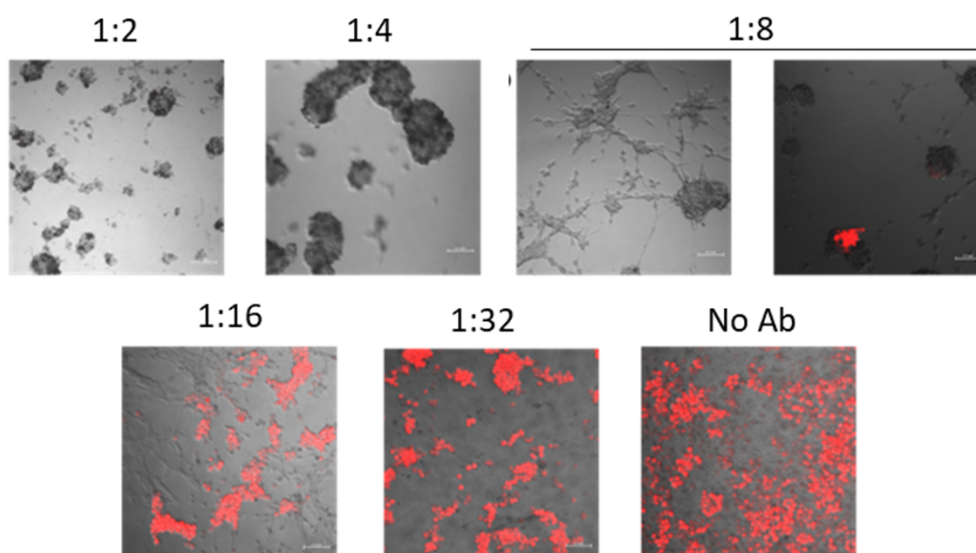


Figure S4. Human hyperimmune gamma globulins (IgGs) titration study. At 48 hours, human hyperimmune IgGs dilutions equal to or lower than 1:8 effectively neutralize oHSV-1 mCherry replication in U87-MG cells. U87-MG cells, seeded in 96-well plates (10,000 cells/well), are infected after one day for one hour with oHSV-1 mCherry (MOI = 3 PFU/cell), properly resuspended in DPBS and serial dilutions of IgGs (ranging from 1:2 to 1:32). After infection, DMEM medium with 2% v/v FBS containing the corresponding antibody (Ab) dilutions are added to each well. Confocal microscopy analysis at 48 hours (10x; scale bar 100 μ m) reveals an absence or low level of viral replication for Ab dilutions of 1:2, 1:4, 1:8. In contrast, viral replication is observed at dilutions of 1:16, 1:32, and in the absence of antibodies.

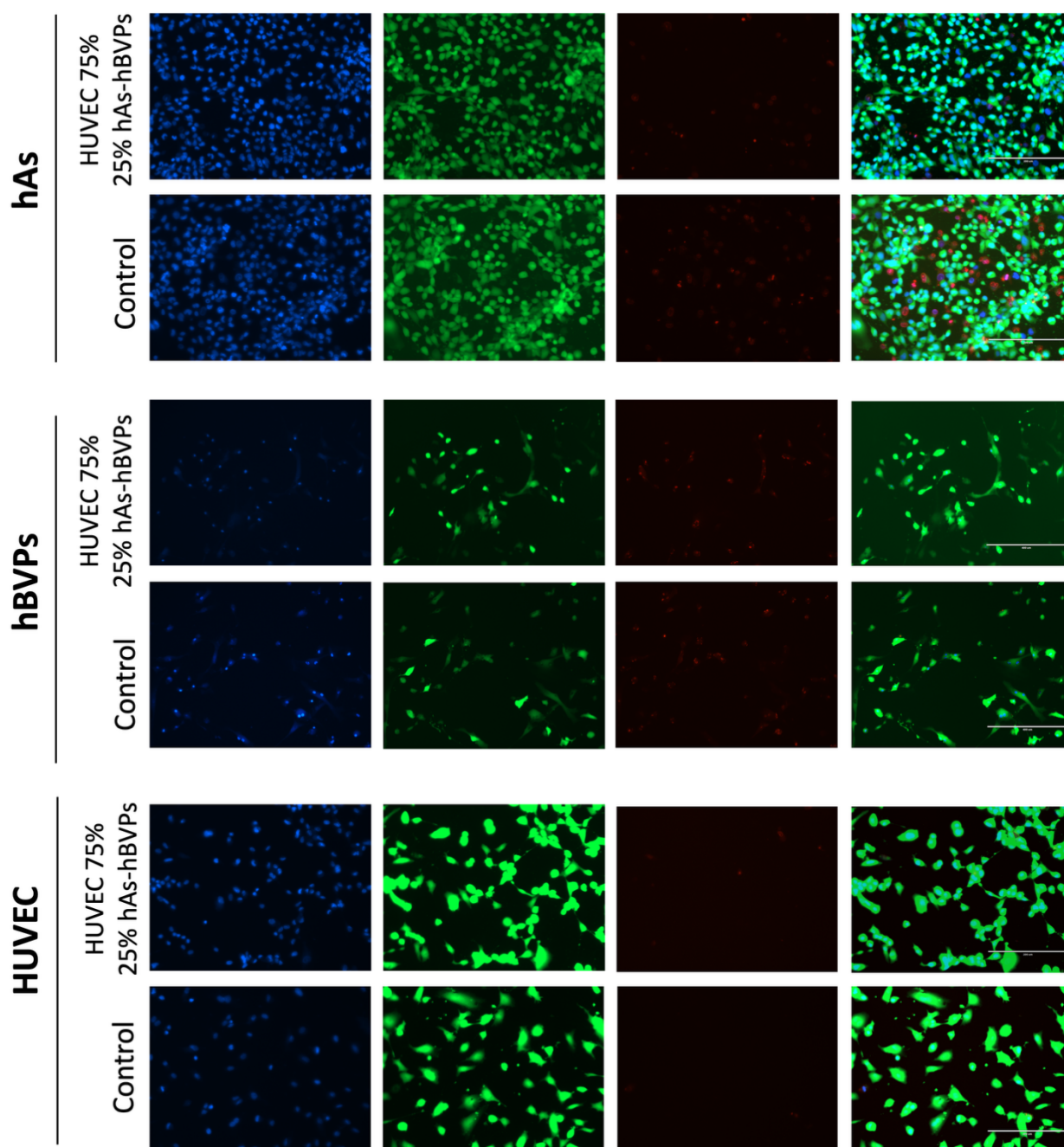


Figure S5. Media composition optimization. Live/Dead assay of individual 2D cell cultures (hAs, hBVPs, and HUVECs) seeded in multiwell plates to evaluate the effect of medium composition on cell viability. Each cell type is cultured either in its standard control medium or in a mixed medium composed of 75% HUVEC medium and 25% (1:1) hAs/hBVPs medium. This condition is tested to identify a shared perfusion medium capable of supporting all three cell types. Staining includes Hoechst (blue) for nuclei, Calcein-AM (green) for live cells, and Propidium Iodide (red) for dead cells. The optimized medium maintains high viability across all cell types. Scale bars: 400 and 200 μm .