Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting information

Manufactured tissue-to-tissue barrier chip for modeling the human blood-brain barrier and regulation of cellular trafficking

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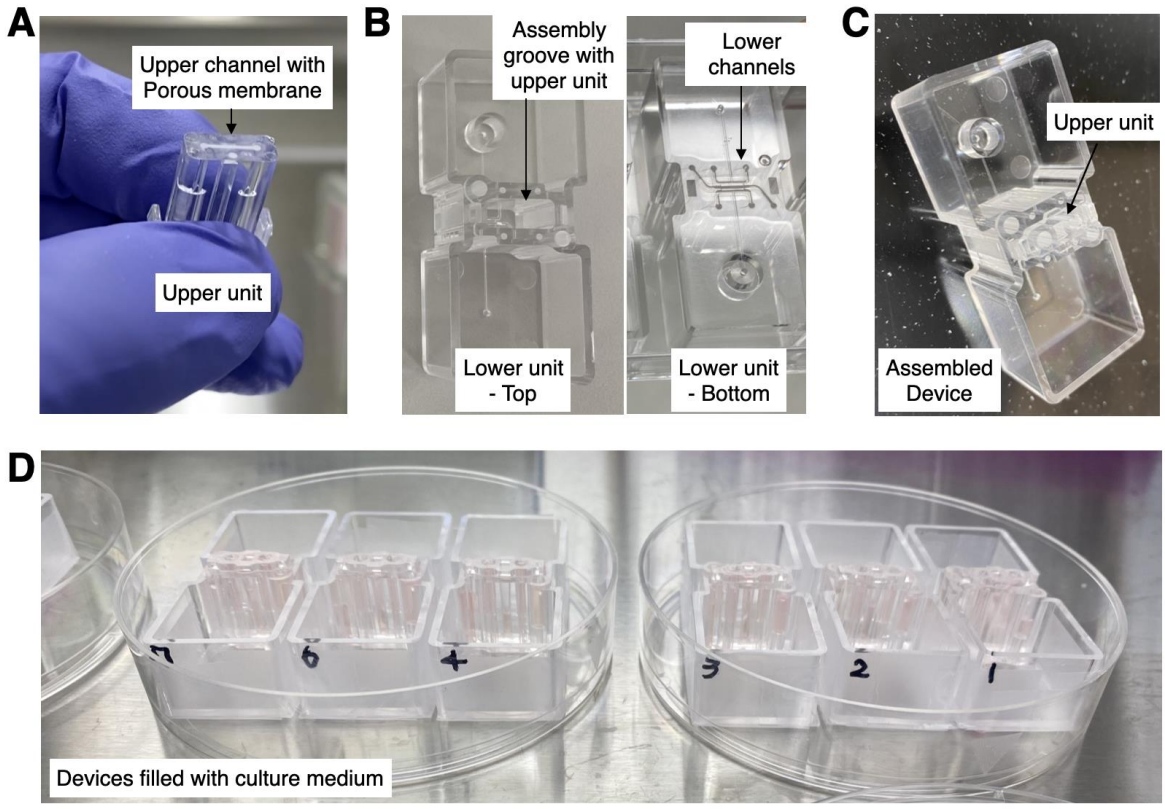

Figure S1. Pictures of the micro-engineered physiological system-tissue barrier chip (MEPS-TBC). (A) Upper unit has upper channel with a porous membrane at the bottom. (B) Lower unit has an assembly groove with the upper unit and lower unit has lower channels at the bottom. (C) Assembled device. The upper unit was inserted into the assembly groove and mechanically assembled. (D) Manufactured chips filled with culture medium for in vitro BBB model culture.

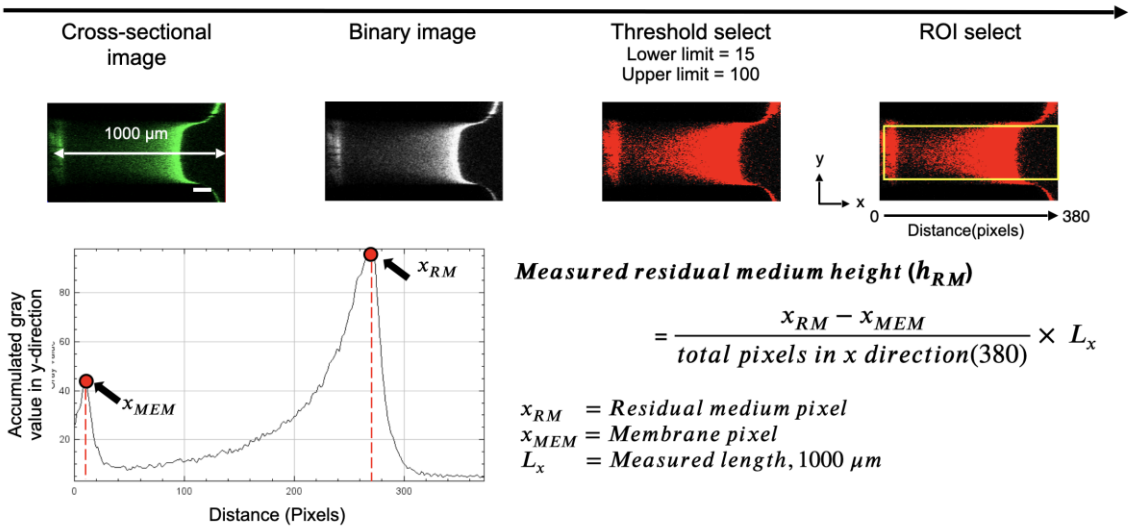

Figure S2. Residual medium height (h_{RM}) measurement. Cross-sectional images of hydrogel stained with immunofluorescence were captured using confocal microscopy. A gel boundary was then confined, and the accumulated gray value in the y direction was measured using the ImageJ tool. The total pixels of the lower channel in the x direction were measured 380. The highest gray value intensity of gel was set to the porous membrane and the residual medium region, respectively. (Scale bar = 100 μm)

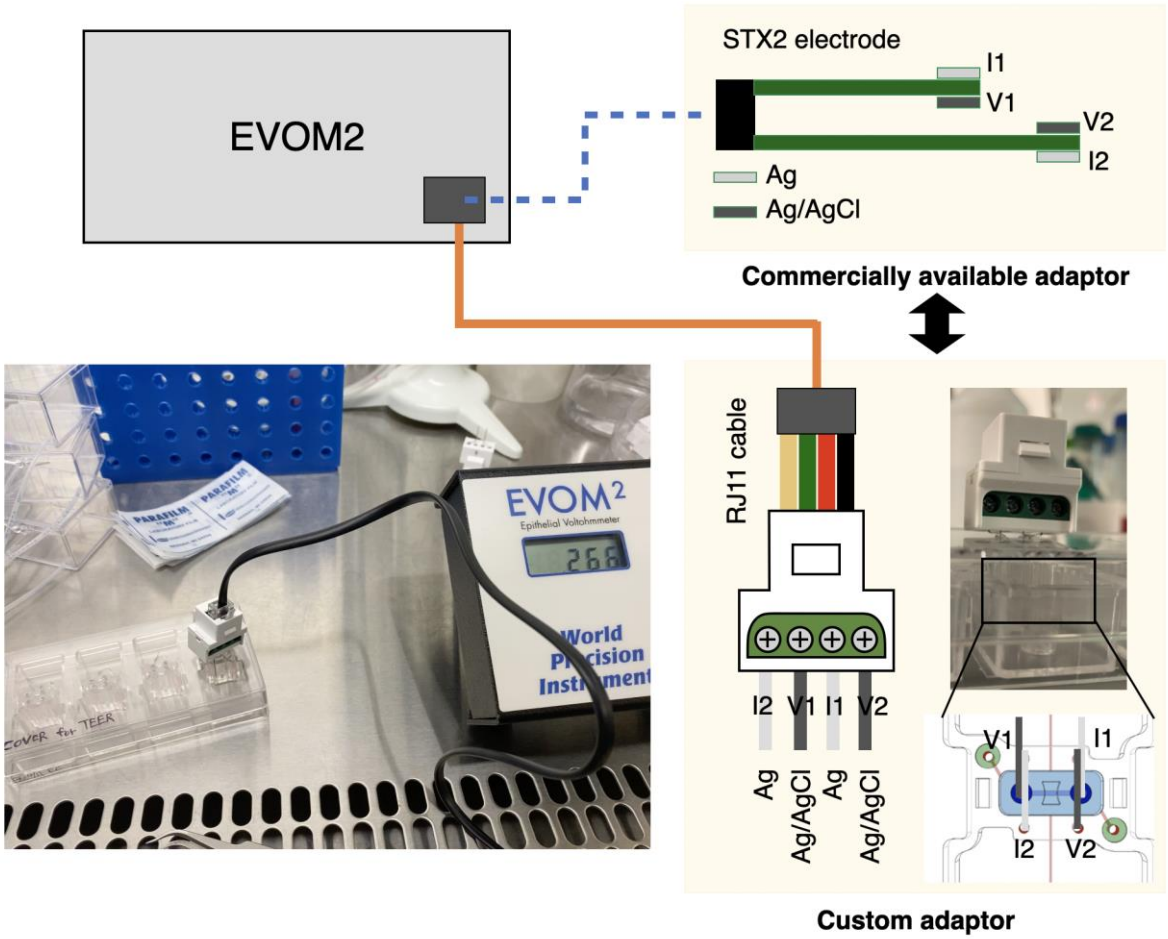

Figure S3. Trans-endothelial electrical resistance (TEER) measurement in MEPS-TBC. TEER was measured using a commercially available volt-ohmmeter (EVOM2) with a custom electrode adaptor made with Rj11 cable, RJ11 female connector and Ag, Ag/AgCl electrode wires. Each of the Ag and Ag/AgCl wire is inserted into the upper (indicated by blue circles) and the lower side channel (indicated by red circles).

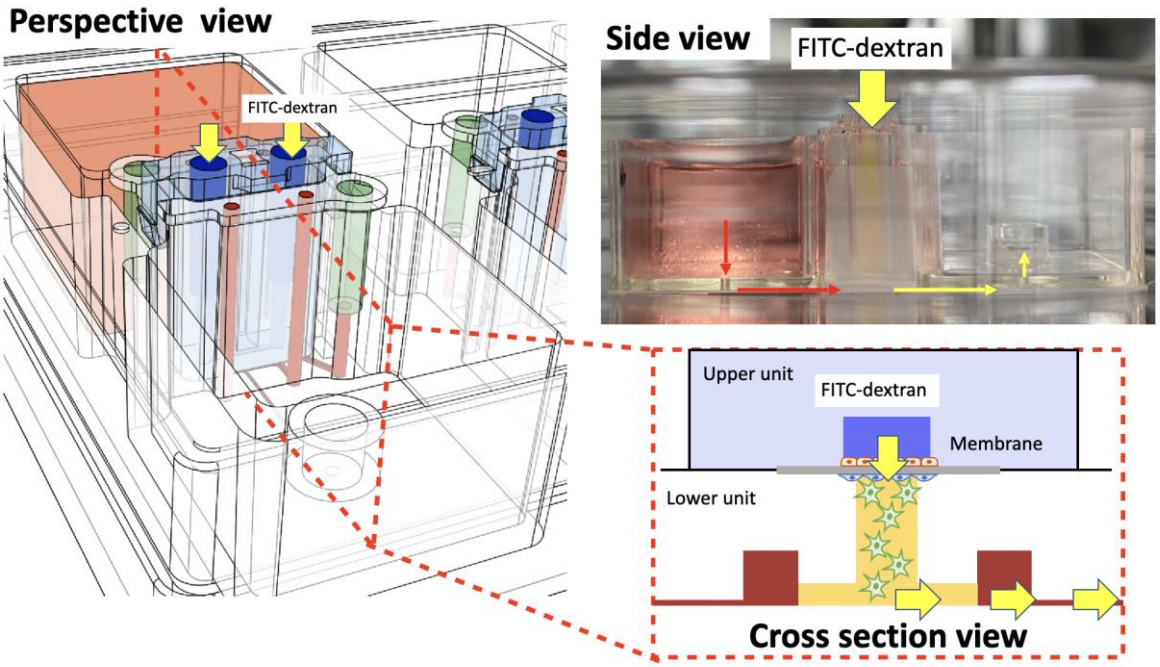

Figure S4. Permeability measurement in MEPS-TBC. Culture medium containing 500 µg/ml of 4kDa or 40kDa FITC-dextran was dosed through the vascular channels for 1h. Before measuring a permeability, 3 ml of media was filled in only one side of the well reservoir connected to the side channel, allowing the interstitial flow in the low-center channel through gravity-driven flow. Red arrows indicate input medium flow and yellow arrows indicate dextran containing out medium which collected in the sampling reservoir. The media flowing out from the low-center channel were collected twice at 30 min intervals for 1 h.

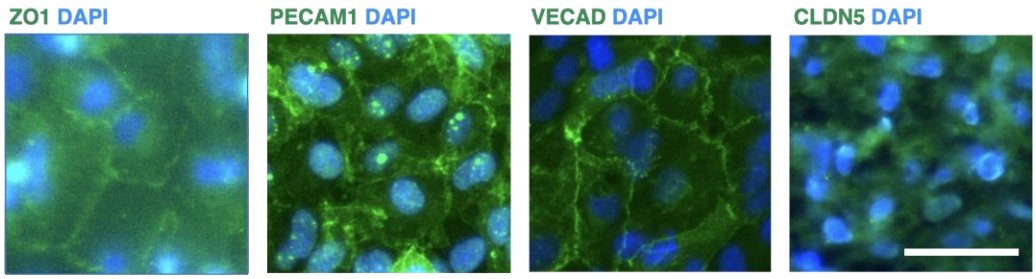

Figure S5. Tight junction related protein expression (ZO1, PECAM, VECAD and CLDN5, Green) in hBMECs. (Scale Bar = 50 μm)

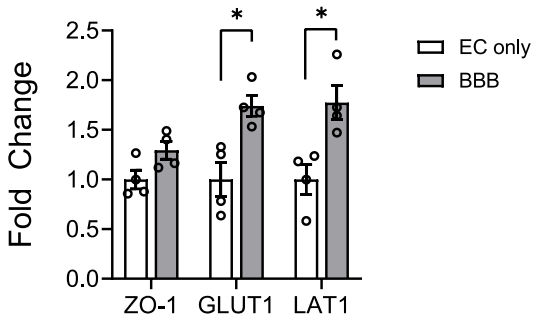

Figure S6. ZO-1, GLUT1, and LAT1 gene expression of hBMEC only and BBB (tri-culture) model by qRT-PCR (n =4 for each condition, *p < 0.05 by student t-test).

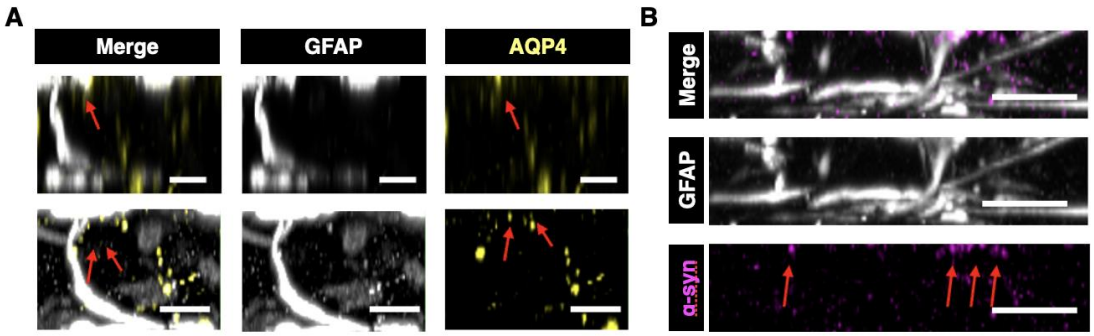

Figure S7. Polarization of hAs end feet toward hBMEC layer. (A) Side view of hAs (GFAP, White, and AQP4, Yellow). Red arrows indicate AQP4 expression in the end feet at the interface of hBMECs and hAs (Scale Bar = 20 μm). (B) Side view of hAs (GFAP, White, and α-Syn, Magenta). Red arrows indicate α-Syn expression in the end feet at the interface of hBMECs and hAs (Scale Bar = 20 μm).

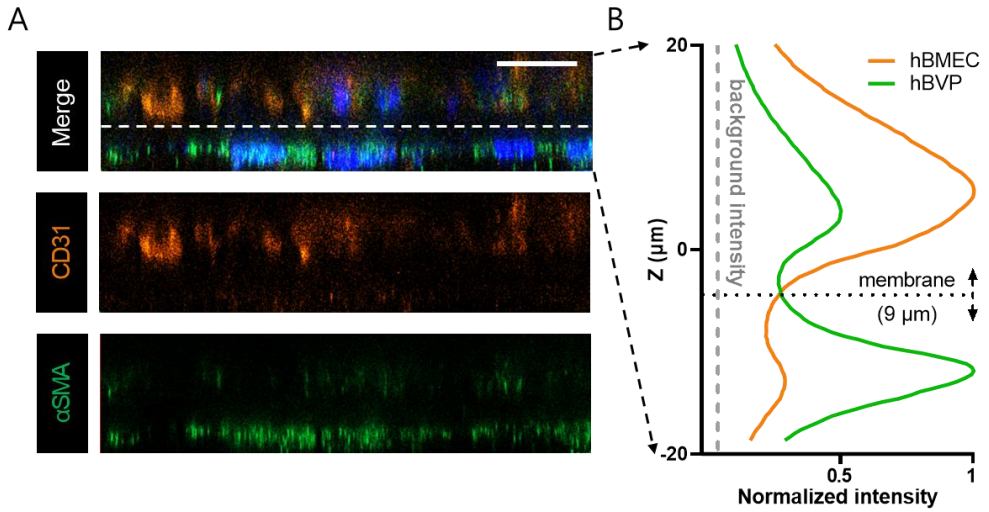

Figure S8. Endothelial-pericyte proximity in MEPS-TBC. (A)The side view of immunofluorescence confocal images of hBMEC (CD31, orange) and hBVPs (αSMA, green) between the porous membrane. (Scale Bar = 20 μm) (B) the normalized fluorescence intensity profiles along the z-direction indicate the distribution of hBMEC (CD31, orange) and hBVPs (αSMA, green) in between the vascular and perivascular channel.

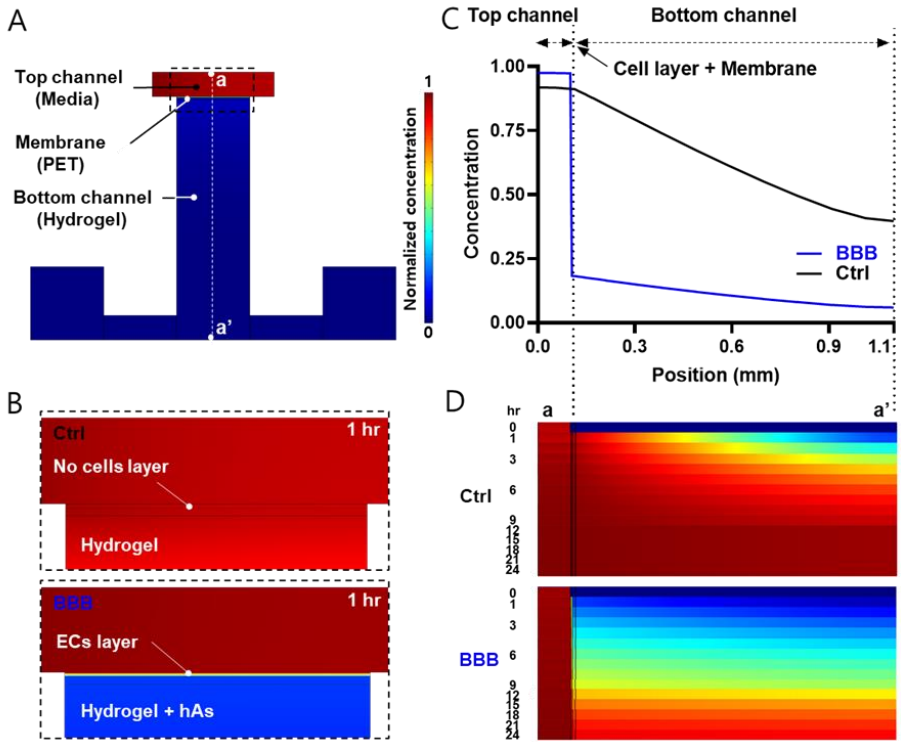

Figure S9. Computational simulation of the diffusive transport of 4kDa FITC-dextran in the MEPS-TBC system. **(A)** The initial concentration distribution of dextran in a 2D cross-sectioned MEPS-TBC and **(B)** The diffusion gradient of the 4kDa FITC-dextran in black dotted line box in (A) after 1 hour. The BBB case constituted the upper channel (Source of the 4kDa FITC-dextran), ECs layer, porous membrane and hydrogel. The Control (Ctrl) case includes the porous membrane and hydrogel but no ECs layer. **(C)** Simulated diffusion profiles of cross section a-a' in (A) at 1 hour after the injection of 4kDa FITC-dextran to the upper channel. **(D)** Simulated gradient changes of the dextran concentration for 24 hours. The dextran concentration was normalized when calculating the diffusion gradient.