Supplementary Information

Development of a 3D-printed microfluidic chip for retinal organoid–endothelial co-culture

Rodi Kado Abdalkader*[a], Shigeru Kawakami [b] , Yuuki Takashima [c], and Takuya Fujita [d]

[a.] *Ritsumeikan Global Innovation Research Organization (R-GIRO), Ritsumeikan University, Japan*
[b.] *Graduate School of Biomedical Sciences, Nagasaki University, Japan*
[c.] *Faculty of Pharmacy, Juntendo University*
[d.] *College of Pharmaceutical Sciences, Ritsumeikan University, Japan*
E-mail: rodi@fc.ritsumei.ac.jp (Rodi Kado Abdalkader)
Supplementary Information available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Supplementary Methods

Preparation of rhodamine-labeled liposomes

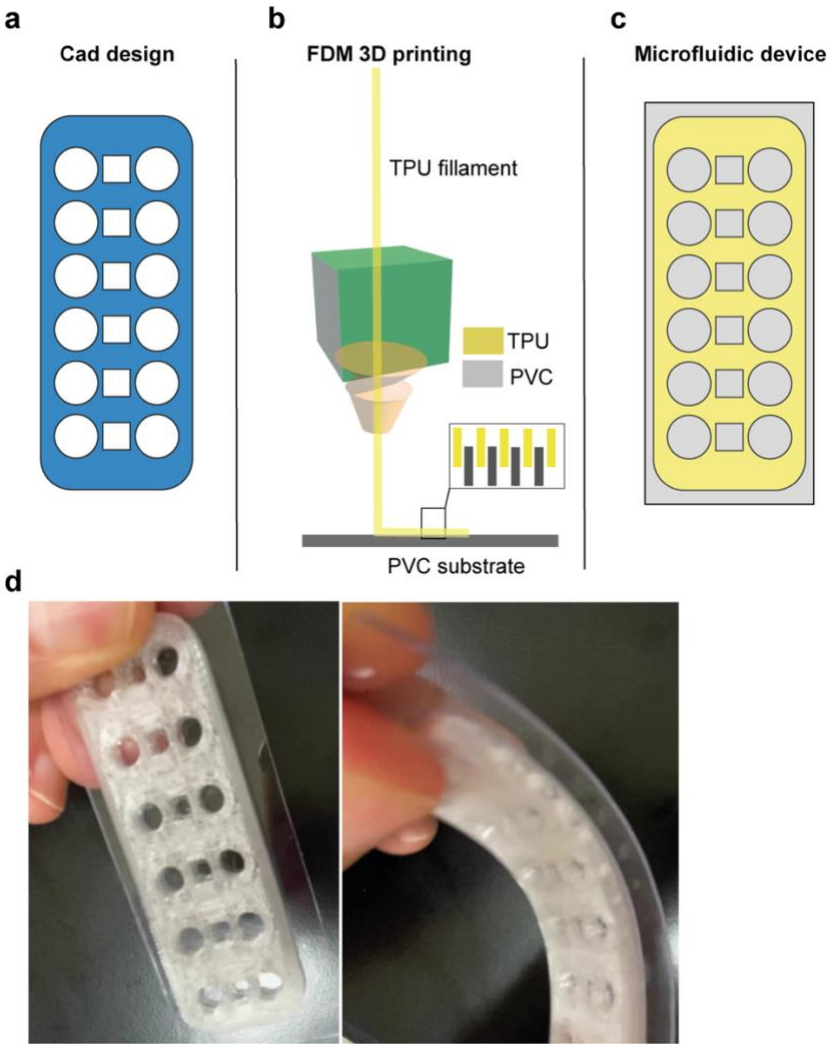
Liposomes were prepared as previously described with minor modifications. A total lipid mass of 20 mg was used, consisting of distearoyl-sn-glycero-3-phosphocholine (DSPC; Avanti Polar Lipids, Alabaster, AL, USA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG2000; NOF Corporation, Tokyo, Japan), and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rhodamine-DHPE; Thermo Fisher Scientific, Waltham, MA, USA) in a 93:6:1 molar ratio. Lipids were dissolved in chloroform, and the solvent was evaporated at 25 °C, followed by vacuum drying overnight at room temperature. The resulting lipid film was hydrated with phosphate-buffered saline (PBS, pH 7.4) pre-warmed to 65 °C for 60 min under mild agitation, giving a final lipid concentration of 8 mg mL^{-1}. The suspension was sonicated in a bath sonicator for 10 min, followed by probe sonication for 3 min, yielding small unilamellar vesicles. Liposomes were stored at 4 °C protected from light until use. Dynamic light scattering (DLS; Zetasizer Nano ZS, Malvern Instruments, UK) was used to measure average hydrodynamic diameter.

Supplementary table 1.

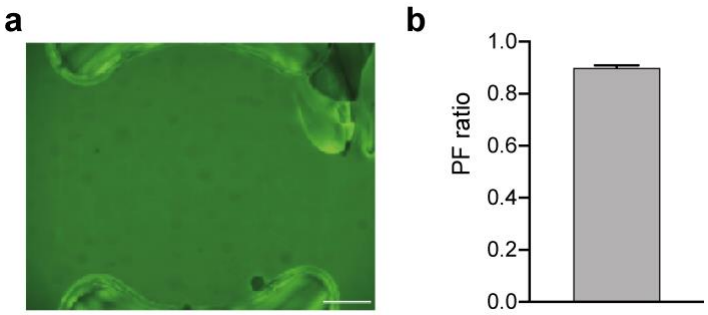
Target protein	Host species	Maker	Catalog #	Working dilution
Beta Tubulin 3/ Tuj1	Mouse	Gene Tex	GTX27751	1:250
VSX2	Rabbit	Proteintech	25825-1-AP	1:100
ZO-1	Rabbit	Proteintech	21773-1-AP	1:100
Recoverin	Rabbit	Sigma-Aldrich	ZRB1107-25UL	1:100
CD31	Mouse	Proteintech	66065-2-Ig	1:50

Supplementary table 2.

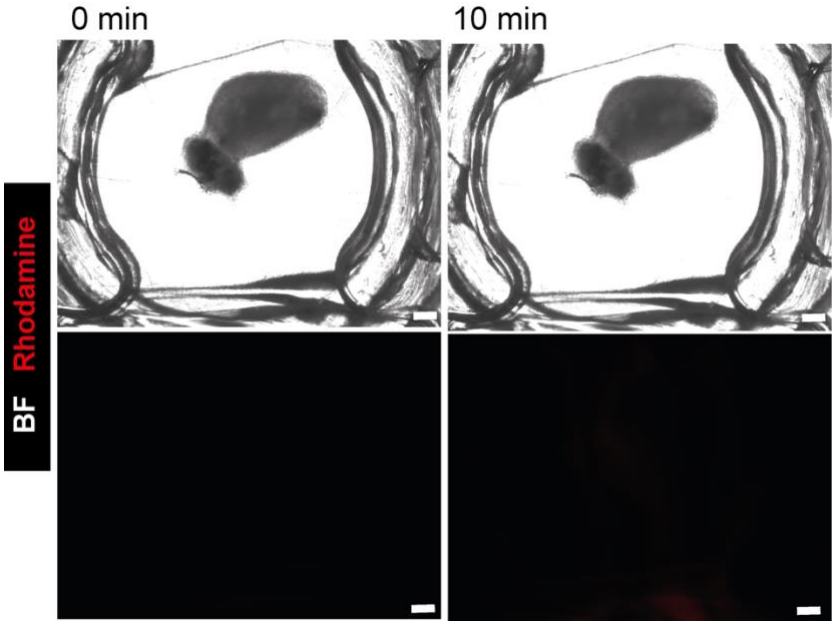
DSPC: $DSPEPEG_{2000}$: Rhodamine-DHPE	
Size	PDI
77.69 ± 2.17	0.27 ± 0.081



Supplementary Fig 1. Design and fabrication of the 3D-printed microfluidic device. (a) CAD layout showing the device design with central circular organoid chambers and side reservoirs. (b) Schematic of fused deposition modeling (FDM) 3D printing process using thermoplastic polyurethane (TPU) filament deposited directly onto a transparent polyvinyl chloride (PVC) substrate. Inset shows layered filament deposition. (c) Final schematic of the assembled TPU–PVC microfluidic device with six parallel culture units. (d) Photographs of the fabricated device showing optical transparency and flexibility of the TPU–PVC structure.



Supplementary Fig 2. Evaluation of paracellular permeability in the microfluidic device. **a** Representative fluorescence image of the culture chamber filled with sodium fluorescein. defined region. Scale bar, 500 μm. **b,** Quantification of the permeability factor (PF) ratio. Data are presented as mean ± SEM (n = 6).



Supplementary Fig 3. Time-lapse assessment of liposomal nanoparticles transport in the organoid-on-chip without HUVECs. Bright-field (BF, top) and rhodamine fluorescence (bottom) images acquired immediately after tracer introduction (0 min) and after 10 min post addition of liposomes. Bright-field images confirm stable positioning and morphology of the retinal organoid within the culture chamber. Scale bars, 200 µm.