Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2022

Electonic Supplementary material (ESI) for Lab on a chip This journal is ⓒ The Royal Society of Chemistry 2022

Supporting information

Microfluidic outer blood-retinal barrier model for inducing wet age-related macular degeneration by hypoxic stress

Seokhun Lee,^a Seunggyu Kim^a and Jessie S. Jeon^{*a}

^a Department of Mechanical Engineering, KAIST, Daejeon 34141, Korea.

* Co-Corresponding authors

Antibody	Company	Catalog number
ZO-1	Invitrogen	61-7300
Rabbit VE-cadherin	Invitrogen	PA5-17401
Rabbit anti collagen IV	Abcam	Ab6586
Goat anti rabbit-Alexa Fluor 488	Abcam	AB150080
DAPI	Abcam	AB228549
Rhodamine phalloidin	Thermofisher	R415

Supplementary Table 1. Antibody information in immunocytochemistry Information of each antibodies used in immunocytochemistry in this paper is described above.





Supplementary Figure 1. Cross section image of oBRB model Confocal cross-section images of normal oBRB model. (A) GFP and RFP represent HUVEC and ARPE-19 respectively. RPE monolayer is observed in the RPE channel. (Scale bars : 100um) (B) Cross-section image of VEcadherin immunostaining sample. Empty lumen in the gel layer, wrapped with HUVEC is visible. (Scale bar: 50um)

А



Supplementary Figure 2. Tracer perfusing image of oBRB model

Introducing the fluorescent-tagged tracer into the media channel, we demonstrated that the lumen was fully perfusable. (A) Perfusing images of 4 kDa dextran. The shape of the lumen was visible at the beginning, it faded over time, however, due to the high permeability. (Scale bars : 100 um) (B) Perfusing image of 1 um microbeads. Contrary to the dextran setup, lumens were visible after a few minutes, but only the lumen attached to the bottom glass was demonstrated, because of the mass of the microbeads (Scale bar: 200 um) (C) Time-lapse image of 1 um microbeads perfusing. (Scale bar: 500 um)



Supplementary Figure 3. Daily image of HUVEC monoculture system and oBRB coculture system.

In contrast to the organized HUVEC vascularization in the oBRB model, HUVEC, under monoculture condition, shows dispersed shape with the broad, monolayer-like shape. In the coculture model, the lumen of vascularized HUVEC was relatively uniform, and it has about 50 to 100 μ m width. However, in the EC monoculture model, we could easily find the vast lumen, which is broader than 200 μ m. (Scale bars : 100 μ m)



Supplementary Figure 4. Difference in RPE monolayer formation by initial RPE seeding density

Despite these images being acquired from the chip with a different dimension, it shows that the ARPE-19 monolayer conformation speed and uniformity were changed by initial seeding density. In the case of optimized ARPE-19 density $(2 \times 10^6 \text{ cells/ml})$, the ARPE-19 monolayer was uniformly constructed and there was no gap or detached region. Otherwise, if the initial seeding density was low $(5 \times 10^5 \text{ cells/ml})$, even under the same condition except for it, the uniformity of the ARPE-19 monolayer became poor. We could easily observe the gap of the ARPE-19 monolayer which is the region that HUVEC already occupied before ARPE-19 proliferated (White arrows). Once HUVEC penetrated from the gel layer and settled on the surface, ARPE-19 could not retake and extend the monolayer over it. Additionally, since these images were captured before optimizing the chip dimension and conditions, the viability of HUVEC was not secured. (Scale bars : 200 µm)



Supplementary Figure 5. RPE monoculture in hypoxic condition

In contrast to the coculture model, the RPE monolayer was fully maintained even under hypoxic conditions. It supports the hypothesis that the RPE detachment in the hypoxia coculture model was due to the intercellular interaction between HUVEC and ARPE-19. (A) Top view image from day 3 and 6. (Scale bar: $100 \ \mu m$) (B) 3D reconstructed image by confocal microscope. (C) Orthogonal projection image by confocal microscope. (Scale bar: $200 \ \mu m$)



Supplementary Figure 6. 3D printing Mold

(A), (B) Top view and cross section view of the 3D printing Mould. Height and width of gel channel and media channel is 350 µm, 1000 µm and 550 µm, 550 µm, respectively. (Scale bars : 2 mm)



Supplementary Figure 7. Migrated RPE image binarization

(A), (B) RPE migration images binarization process in low gel condition and optimized condition respectively. Each image represents the procedures of image processing, color threshold, ROI crop, and binarization (Scale bars : $100 \mu m$). (1)Images were auto scaled and adjusted to discrete the RPE from HUVEC by Zen blue (Brightness 30 and contrast 1.8 in GFP, Brightness -10 in RFP). (2)Using the color threshold tool in Image J, RFP HUVEC region was erased (Subtract background : 100, Color threshold : Hue 30~200, Enhance contrast : 0.3%). (3) After, adjusted images were cropped as the yellow box in the image. (4) Cropped images were binarized by auto threshold tool, and the noise was discarded. (Auto threshold : intermodes, Remove outliers : 2 pixels, threshold 50)



Supplementary Figure 8. HUVEC image binarization

(A), (B) HUVEC images binarization process in hypoxic condition and normal condition. Upper and lower half of images were acquired 96 hours and 120 hours after incubation respectively. Each image represents the procedures of image processing, image crop, intensity adjust filter, and binarization. (Scale bars : 100μ m) (1),(2)Images were auto scaled by Zen blue and only RFP channel was exported and cropped firstly. (3) Then, to enhance the accuracy, contrast was readjusted (Enhance contrast : 0.3%). (4) Adjusted image was binarized by Image J auto threshold (Auto threshold : RenyiEntrophy)

А