Supplementary Information for

Microfluidic Organ-on-a-Chip Model of the Outer Blood-Retinal Barrier with Clinically Relevant Read-outs for Tissue Permeability and Vascular Structure

Yusuf B. Arık[a,b]† · Wesley Buijsman[a,b] · Joshua Loessberg-Zahl[b] · Carlos Cuartas-Vélez[c] · Colin Veenstra[c] · Sander Logtenberg[a,c] · Anne M. Grobbink[a] · Piet Bergveld[b] · Giuliana Gagliardi[d] · Anneke I. den Hollander[d,e] · Nienke Bosschaart[c] · Albert van den Berg[b] · Robert Passier[a,f] · Andries D. van der Meer[a]

Supplementary Information Text:

Materials and Methods:

Effect of Reactive Oxygen Species and Permeability Assay

For oxidative stress experiments, HUVECs were seeded at a density of $0.75 \cdot 10^5$ cells/cm^2 on a culture wells plate (Gibco) which was coated with 0.1 mg/ml collagen I. HUVECs were incubated for a day in endothelial growth medium (EGM-2: EBM-2 with EGM-2 SingleQuots, Lonza) followed by changing media to EBM-2 (supplemented with 2% FBS and 1% P/S). The next day, cells were incubated in various concentrations of H_2O_2 (50-1000 µM) dissolved in EBM-2 (with 2% FBS and 1% P/S) for 1, 2 or 5 days. Every day, the medium was refreshed with freshly made H_2O_2 solutions.

Following treatment, cells were counted using images of nuclei which were stained with DAPI. First, images were converted to 8-bit and applied threshold in order to develop a contrast between images and background using Fiji software.[38] After that, each image was converted to binary to apply watershed on the particles, where multiple nuclei were divided into single nuclei. Then, particles (pixel size: 10- infinity, circularity: 0.00-1.00, excluded on edges) were counted.

For permeability experiments, HUVECs were seeded at a density of $1 \cdot 10^5$ cells/cm^2 on a Transwell insert (Corning) which was coated with 0.1 mg/ml collagen I. HUVECs were incubated for a day in EGM-2. After that, media was changed to EBM-2 (with 2% FBS and 1% P/S). The following day, cells were incubated in various concentrations of H_2O_2 (Sigma Aldrich, Germany) dissolved in EBM-2 (with 2% FBS, 1% P/S). (50-1000 µM) for 1, 2 or 5 days. The levels of medium in both Transwell compartments were equalized to avoid pressure-driven fluid flow. After exposure to H_2O_2, the medium in the insert was replaced with EBM-2 containing fluorescently labelled dextran (FITC-Dextran, 40 kDa, fluorescein label (ex/em: 494/521), ThermoFisher) at a concentration of 15 µg/ml, whereas the medium in the bottom well was replaced with fresh EBM-2. After that, from the beginning of the experiment, a sample of 50 µL was collected from the bottom well every 30 min and the levels were normalized by adding EBM-2 to the bottom compartment. These samples were read by a plate reader (Victor3, Perkin Elmer). Using a standard curve, fluorescence values were matched with concentrations. The permeability (P_{cell}) of HUVEC was calculated by:

$$P_{total} = \frac{dC Volume_{well}}{dt\, C_i\, Area_{well}}$$

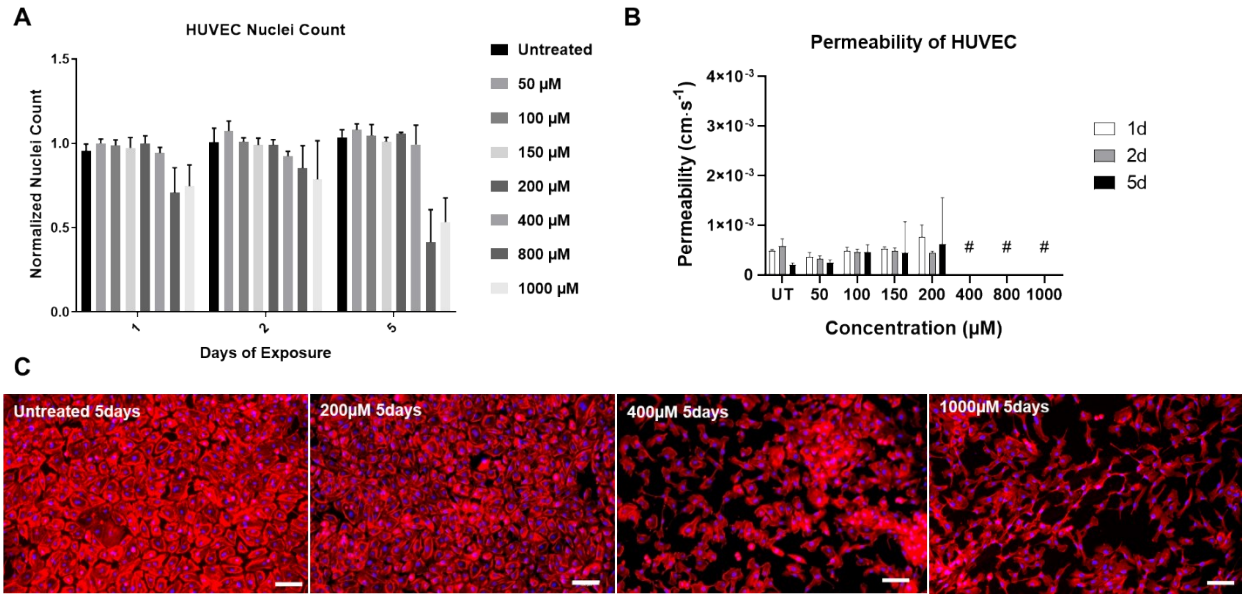
$$\frac{1}{P_{cell}} = \frac{1}{P_{total}} - \frac{1}{P_{empty}}$$

With P_{total} the permeability in cm/s, C_i the initial dextran concentration in µg/ml. $Area_{well}$ and $Volume_{well}$ are the dimensions of the bottom compartment in cm^2 and dC/dt was the change in the concentration (µg/ml·s) (Equation 1). [39] To calculate the permeability coefficient of the cells, the coefficient of an empty insert was subtracted from the cell containing insert (Equation 2).

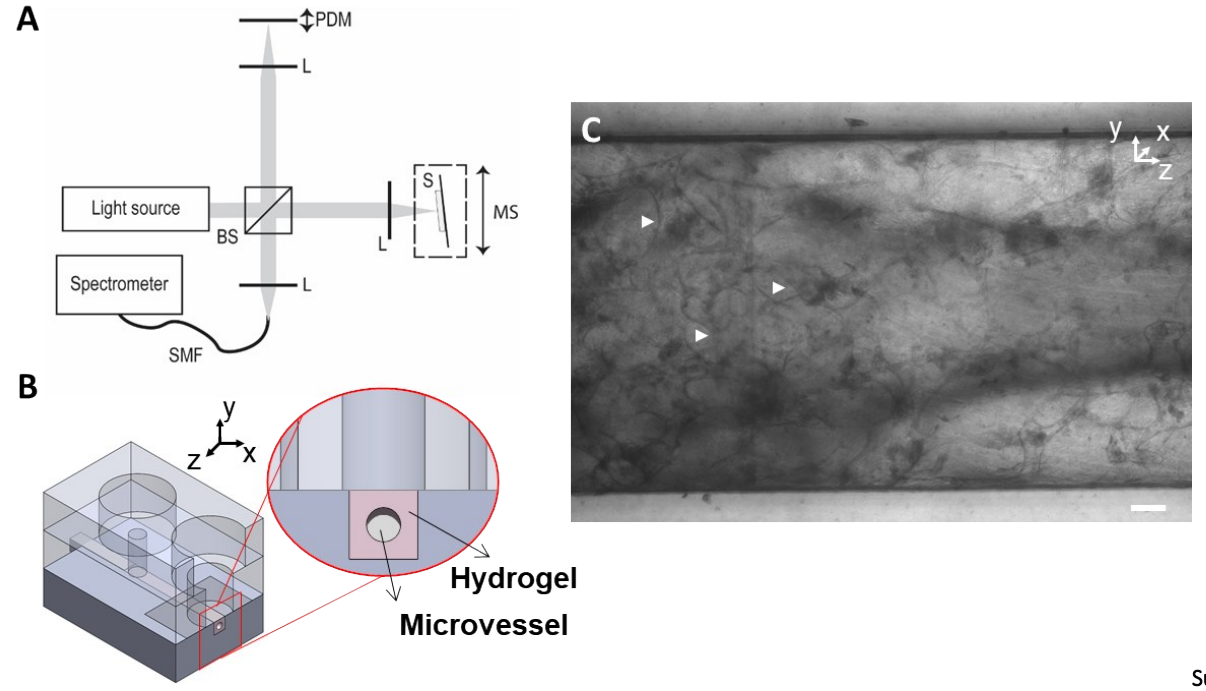
Result and Discussion:

HUVEC Morphology and Permeability under H_2O_2 Exposure

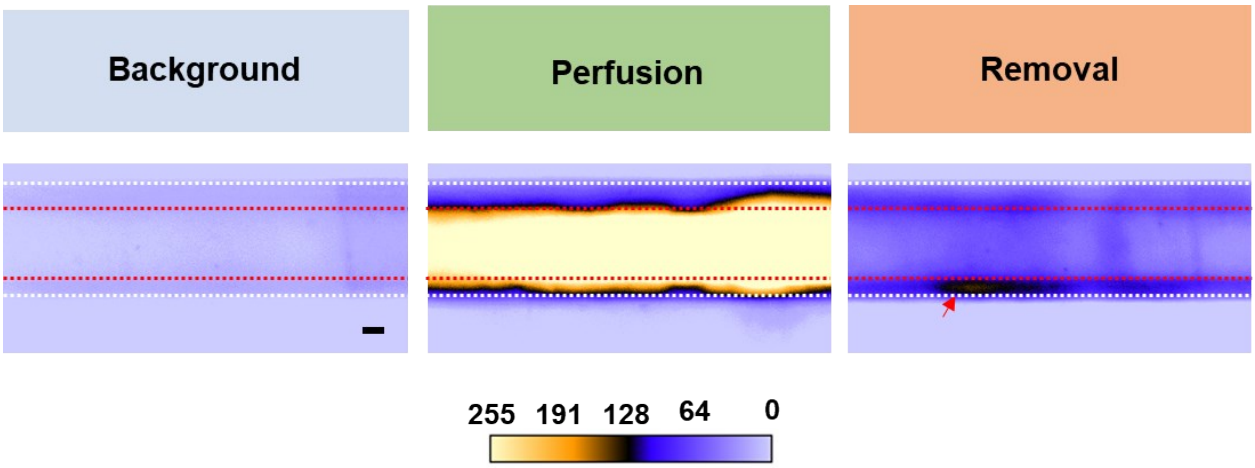
HUVECs in culture well plates were exposed to various concentrations of H_2O_2 for 1, 2 and 5 days. While low concentrations did not result in considerable cell loss as compared to untreated cells, higher concentrations significantly decreased the cell number (Suppl.Fig.1A). Using these concentrations, changes in barrier integrity of HUVECs were tested in a Transwell system as a mean of dextran diffusion. Here it is worth noting that passage of dextran was only possible through the intercellular spacing of the cells, and the Transwell membrane was not a limiting factor in the diffusion of the tracer. Upon quantification of permeability, we could observe a dose and exposure dependent response to H_2O_2 (Suppl.Fig.1B) which was consistent with the morphology of HUVECs (Suppl.Fig.1C). Continuous exposure with high concentrations of H_2O_2 resulted in a barrier close to an empty insert, thus not represented.



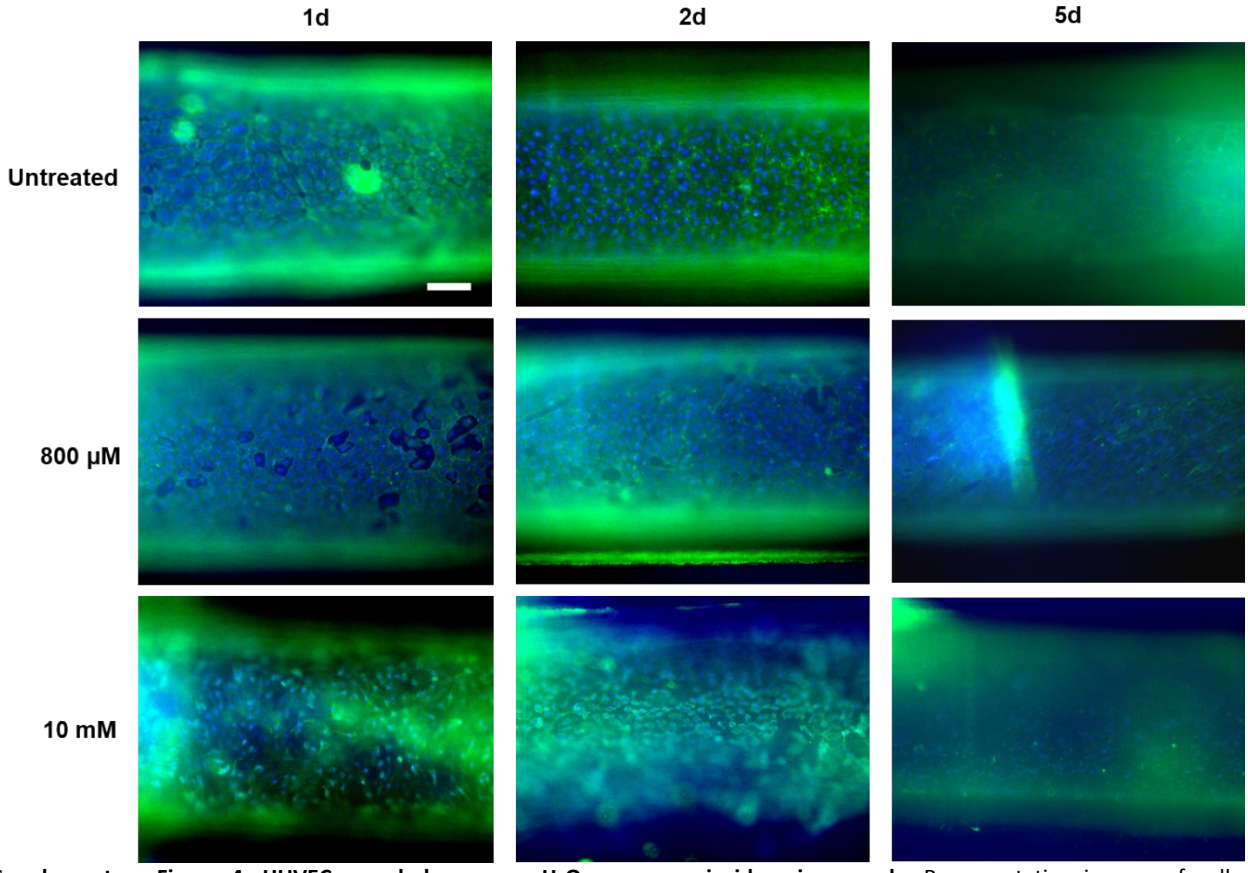
Supplementary Figure 1: Assessment of HUVEC morphology and barrier function under H_2O_2 exposure. **(A)** Cell number in response to H_2O_2 were calculated (results were normalized to untreated). Error bars indicate standard error of the mean. **(B)** In addition, permeability of HUVEC was assessed using a Transwell system. Compared to untreated cells (UT), a dose dependent response (UT vs condition) in permeability was detected in higher H_2O_2 concentrations and exposure. Values equal or worse than an empty insert were not represented (Denoted by hashes). **(C)** Dose dependent response to H_2O_2 was evident in morphology of HUVEC. Red: Phalloidin (actin filaments), blue: DAPI (nuclei). n=3. Scale bar: 400µm.



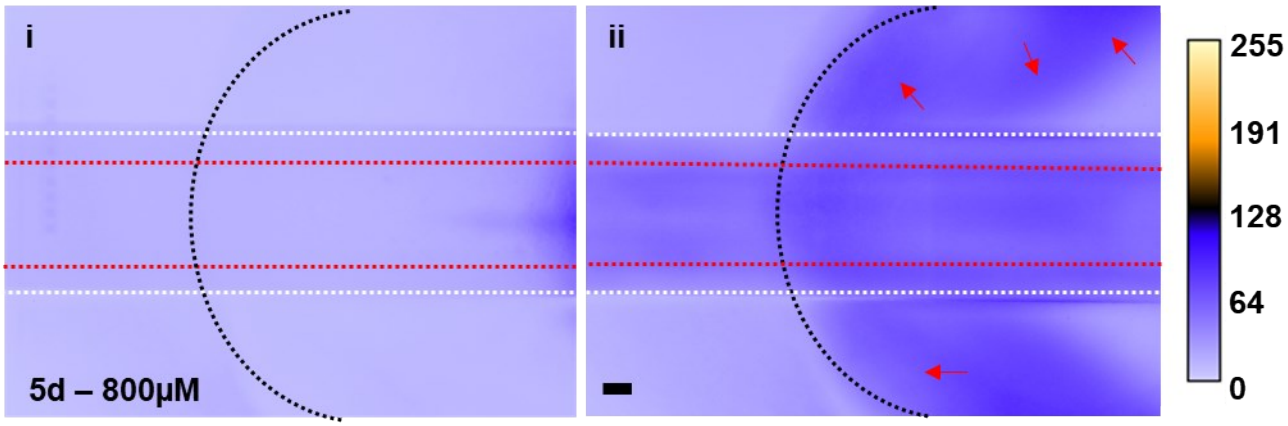
Supplementary Figure 2: Overview of the OCT setup. **(A)** Schematic overview of the OCT setup. Light travels from the source to the sample. Spectrometer calculates the distance based on the comparison with piezo driven mirror. BS: Beam splitter, L: Lens, PDM: piezo driven mirror, S: Sample, MS: motorized stage, SMF: single mode fiber. **(B)** Schematic overview of the organ-on-a-chip device with inset showing the cross-section view of the channel. **(C)** Phase contrast image of the chip with a fibrin matrix, with arrow heads showing the formation of vascular network branches. Scale bar: 100 µm.



Supplementary Figure 3: Illustration of localized enhanced dye accumulation in the microvessel. Exemplary image represents a device with HUVEC and ARPE-19 co-culture. Speculative dye accumulation after removal of labeled dextran was shown by red arrows. Contrast in raw images were enhanced using a lookup table. Microvessel and channel borders are indicated by red and white lines respectively. Scale bar: 200 µm



Supplementary Figure 4: HUVEC morphology upon H_2O_2 exposure inside microvessels. Representative images of cells fluorescently stained for nuclei (DAPI; blue) and actin filaments (green). Scale bar: 100 µm.



Supplementary Figure 5: Dye accumulation in the epithelial culture chamber in devices with endothelial monocultures. Representative image of a device with HUVEC mono-cultures inside the microvessel after treatment with 800 µM H_2O_2 for 5 days. Dye accumulation in the epithelial culture chamber (black line) before (i) and following FITC-dextran perfusion (ii) is shown. Red arrows indicate the dye accumulation in the culture chamber. Contrast in raw image is enhanced using the lookup table on the right. Microvessel and channel borders are indicated by red and white lines respectively. Scale bar: 200 µm.