The microbial metabolite p-cresol compromises the vascular barrier and induces endothelial cytotoxicity and inflammation in a 3D human vessel-on-a-chip

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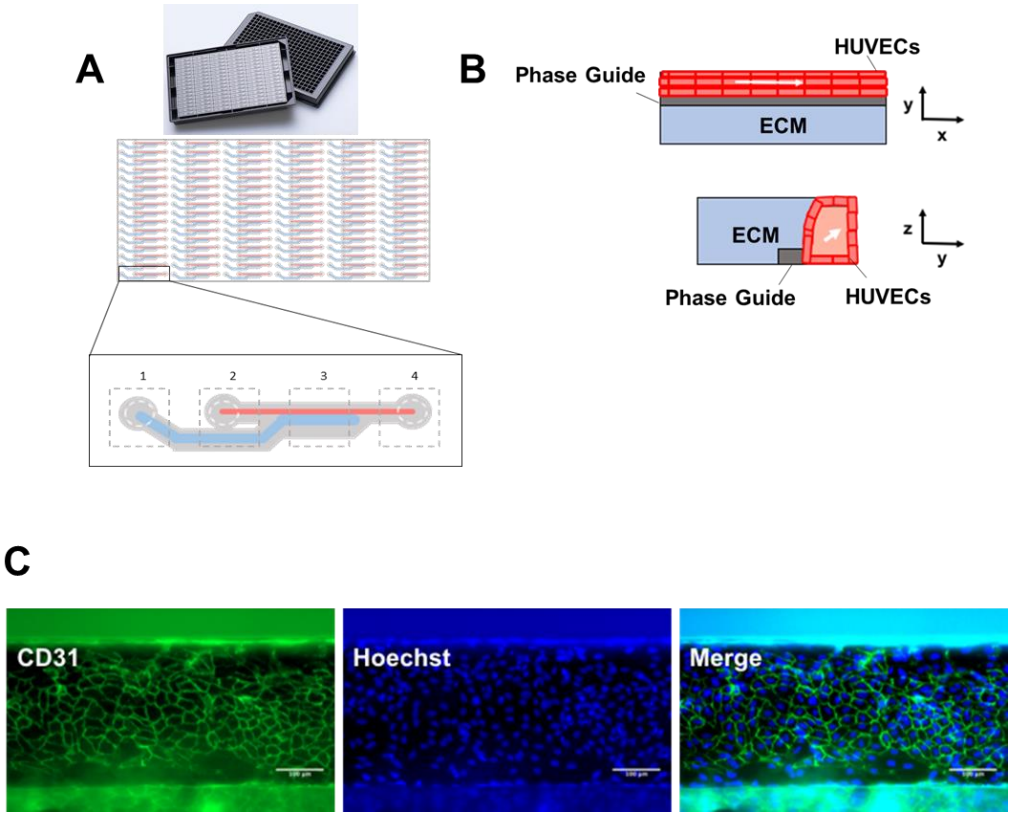
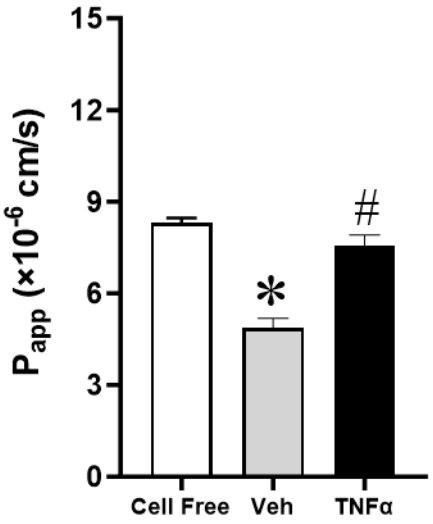
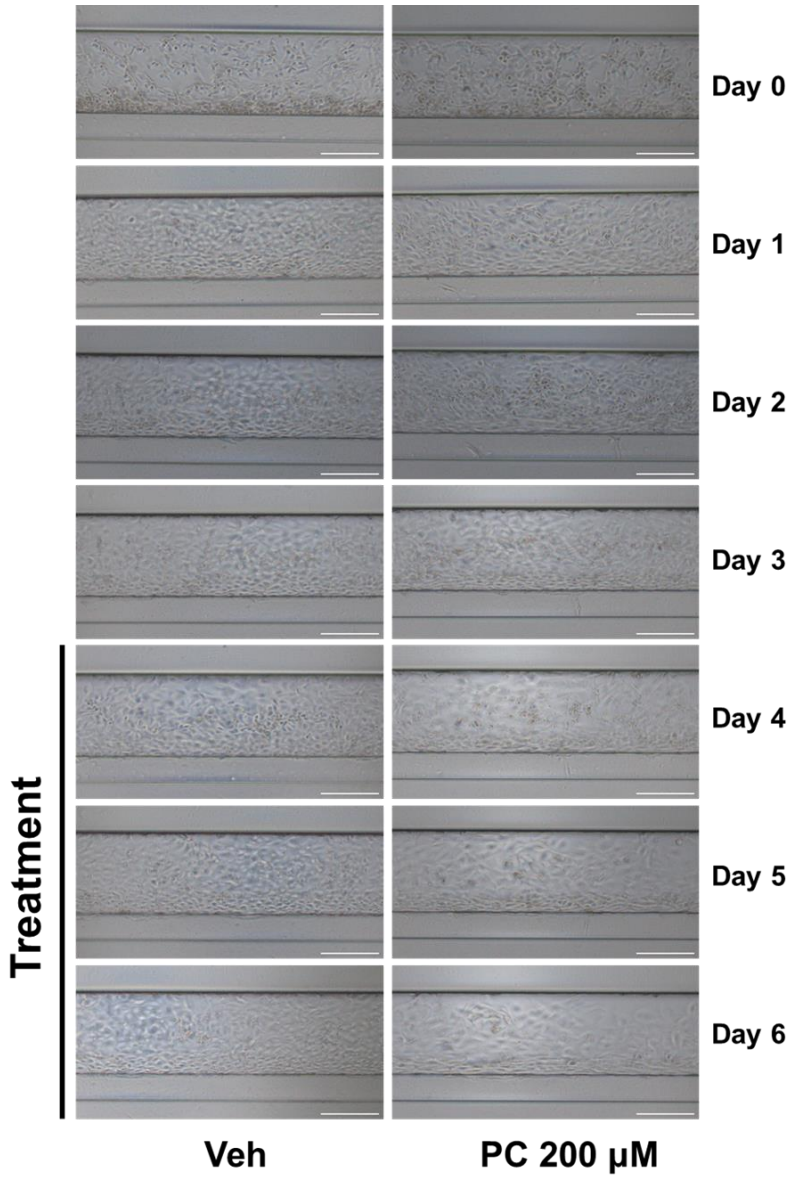
Supplementary Information



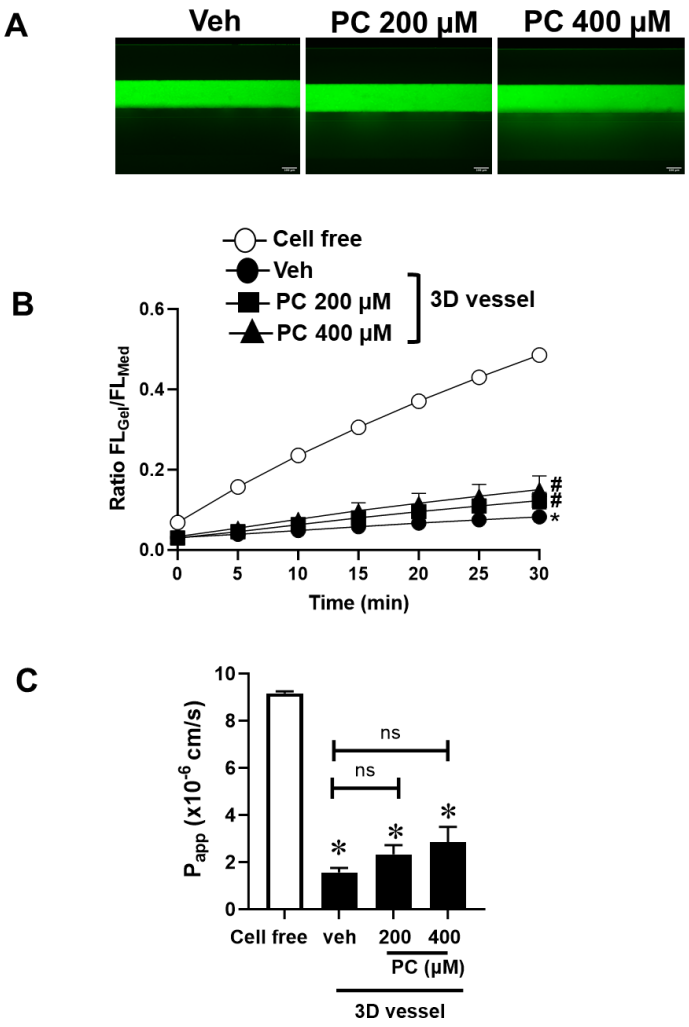
Supplementary Figure 1. Schematic diagram of a vessel-on-a-chip model. (A) Schematic illustration of the OrganoPlate® 2-lane (Mimetas, the Netherlands) and its layout based on a 384-well plate microfluidic device. A chip is composed of four compartments with a (1) gel inlet, (2) medium inlet, (3) observation window, and (4) medium outlet. (B) The arrangement of human umbilical vein endothelial cells (HUVECs) forming 3D vessel next to the extracellular matrix (ECM) in the microfluidic device with PhaseGuide™ separating the two compartments. (C) Immunofluorescence images showing CD31 (green), an endothelial cell marker, counterstained with DAPI (blue); magnification: 20X; scale bar: 100 µm.



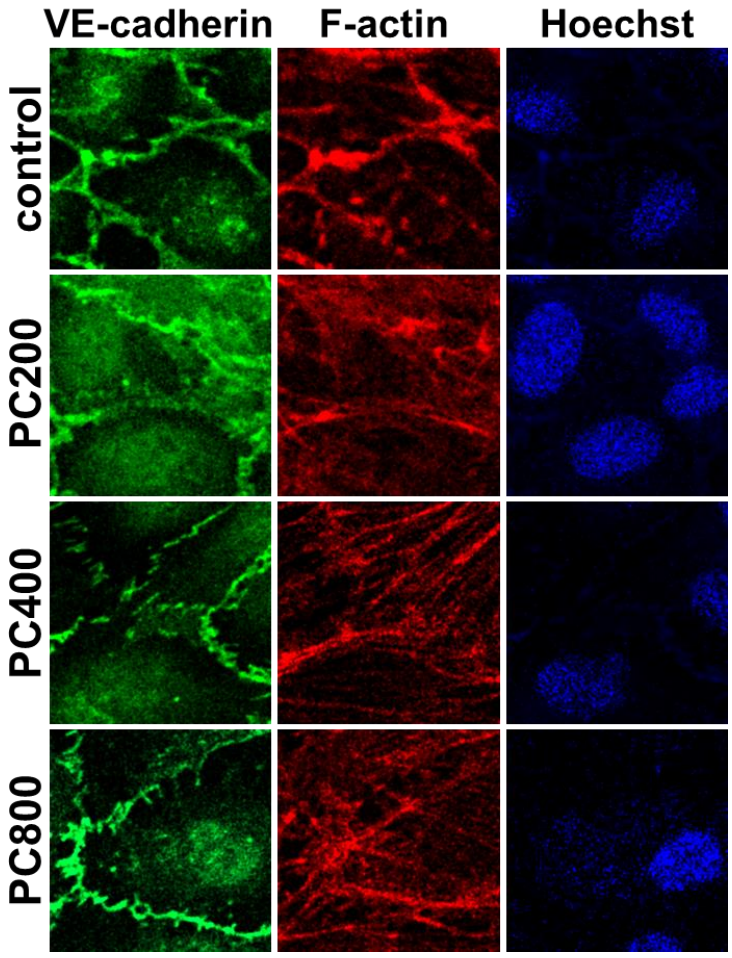
Supplementary Figure 2. The apparent permeability (Papp) of 70 kDa dextran in 3D vessels treated with vehicle (Veh) or 10 ng/mL TNFα for 24 hours. Data are mean ± SEM, n = 15-19. *p < 0.05 vs. cell-free, #p < 0.05 vs. Veh, one-way ANOVA multiple comparisons.



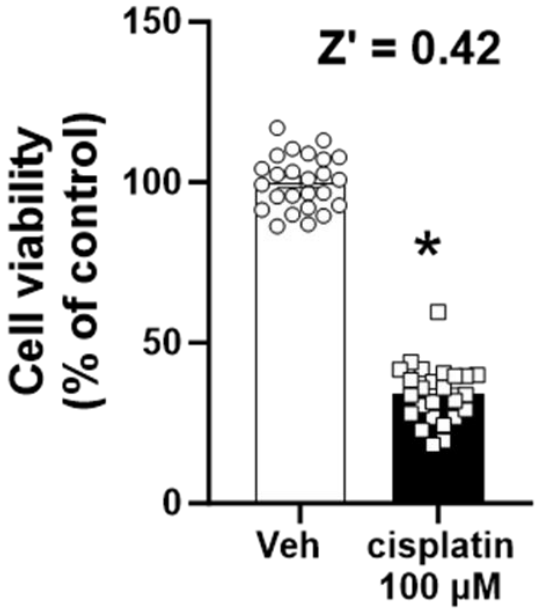
Supplementary Figure 3. Representative cropped images to show the morphology of endothelial cells in 3D vessels from days 1 to 6 with either vehicle (Veh) or 200 µM p-cresol (PC) treatment for 3 days (72 hours). Magnification: 5X; Scale bar = 1000 µm



Supplementary Figure 4. Effects of 24 hours of p-cresol (PC) treatment on 3D vessel permeability. EC vessels were stimulated with either vehicle (Veh) or different concentrations of PC (200 and 400 µM) for 24 hours before vascular permeability was determined. (A) Representative time-lapse images at 30 minutes of 70 kDa FITC-dextran 70, (B) the ratio of fluorescence intensity between the ECM and luminal channels, and (C) apparent permeability (Papp) of 70 kDa dextran. Magnification: 4X; scale bar: 1000 µm. Data are mean ± SEM, n = 7-8. Two-way repeated measures ANOVA followed by Bonferroni's test was used for the ratio of fluorescence intensity while one-way ANOVA multiple comparisons were used for Papp; *$p < 0.05$ vs. cell-free, $^{\#}p < 0.05$ vs. Veh, ns = not significant.



Supplementary Figure 5. Confocal images of 3D vessels immunostained for VE-cadherin (green), F-actin (red), and Hoechst nuclei (blue) after treatment with either vehicle control or p-cresol (PS) at different concentrations (200, 400, and 800 μM) for 72 hours. Cropped images of 420 x 420 pixel ROIs from 20X magnification images; scale bar: 20 μm.



Supplementary Figure 6. Cell viability in 3D vessels after stimulation with 100 µM cisplatin compared with vehicle treatment (Veh). Cell viability assay in 3D vessels was validated by a robust Z' factor (0.42), which is considered good for compound screening. N = 7-8, n = 25 for each group. * $p < 0.0001$ vs. Veh, t-test.

Supplementary Video legends

Supplementary Video 1. 3D view of a human vessel-on-a-chip showing the luminal and basolateral surfaces. HUVEC cells were cultured in a microfluidic device (2-lane Organoplate, Mimetas) for 4 days. The red fluorescence indicates the F-actin cytoskeleton of endothelial cells and blue indicates Hoechst nuclei staining.

Supplementary Video 2. Time-lapse movies demonstrating the vascular leakage of 70 kDa (A) and 10 kDa (B) FITC-dextran diffusing from the luminal surface to adjacent ECM after treatment with 200 µM of p-cresol (PC) for 72 hours. Quantification data are shown in Figure 2.