3D HUMAN MICROVESSEL-ON-A-CHIP MODEL FOR STUDYING MONOCYTE-ENDOTHELIAL ADHESION UNDER FLOW – APPLICATION IN SYSTEMS TOXICOLOGY

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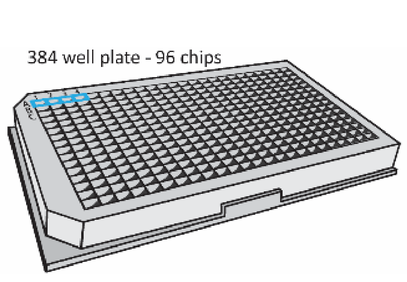
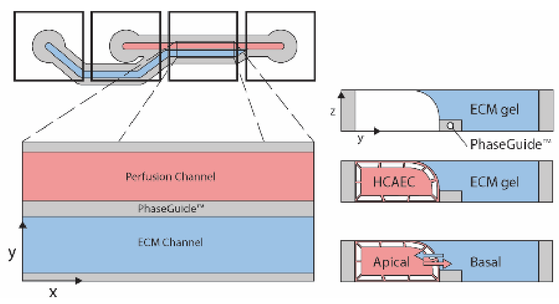
Background - The endothelium is a single layer of cells at the interface between circulating blood and organ tissues that plays critical roles in vascular processes, such as barrier permeability, leukocyte adhesiveness and extravasation, blood clotting, and angiogenesis. Endothelial dysfunction increases permeability, adhesiveness, transmigration of leukocytes, and accumulation of fatty streaks in subendothelial compartments and is a hallmark of atherosclerosis, characterized by the development of plaques that can become unstable and rupture, resulting in cardiovascular adverse events. Two-dimensional (2D) and static endothelial in vitro models have been used extensively, but more physiologically relevant models are required that integrate the three-dimensional (3D) geometry of vessels and hemodynamic flow. Lifestyle and genetic predisposition affect the development of vascular disorders that result in atherogenesis. Smoking, for instance, is a recognized major risk factor for the development of cardiovascular diseases. Over time, smokers develop low-grade inflammation and oxidative properties in the systemic compartment, which can alter endothelial function, resulting in the initiation and progression of atherosclerosis. We set out to develop a vasculature model that could be used to characterize this initial development.

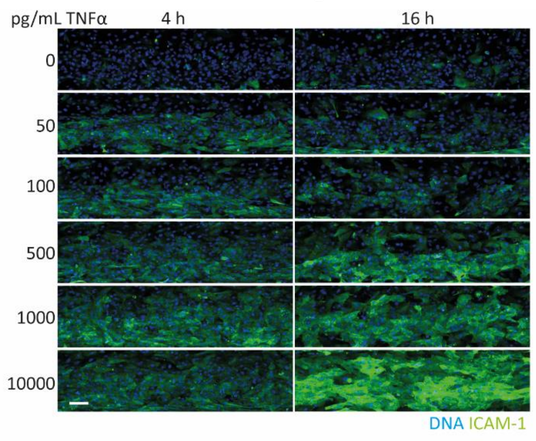
OrganoPlate® technology - The culture platform is based on a 384-well plate, consisting of 96 microfluidic chips. A gel channel (blue) holds an extracellular matrix (ECM) in place through the PhaseGuide's pressure barrier function. Human coronary artery endothelial cells (HCAEC) are seeded in the adjacent medium perfusion channel (red). Perfusion flow can be achieved through passive leveling of the medium by placing the OrganoPlate on a rocking platform.



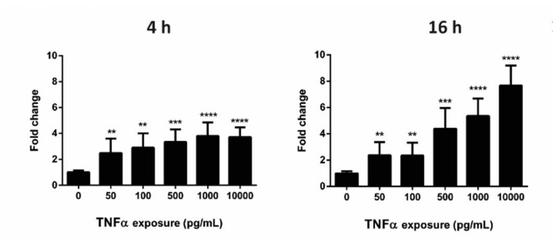


Functional assays - HCAEC vessels were exposed to several concentrations of TNFα for 4 h or 16 h. MM6 monocytes were labeled green and perfused for 15 min through the vessel, in which the nuclei were labeled blue. The number of monocytes adhering per nucleus was counted, and monocyte adhesion relative to the vehicle control (VC) was determined. The vessels were also fixed after TNFα exposure and stained for DNA and adhesion marker ICAM-1, which was quantified as intensity per nucleus, and relative change to the VC was determined.

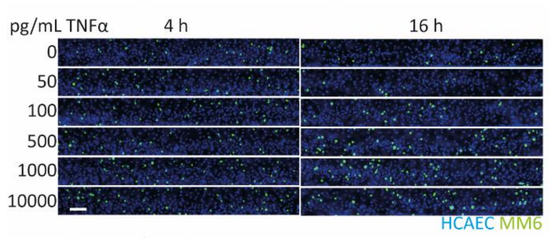
ICAM-1 immunostaining



Quantification



Monocyte adhesion assay



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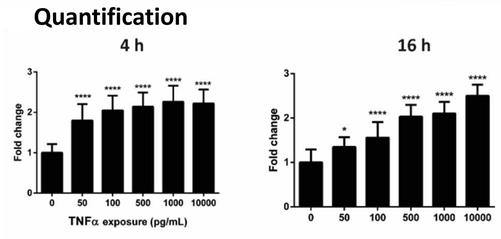
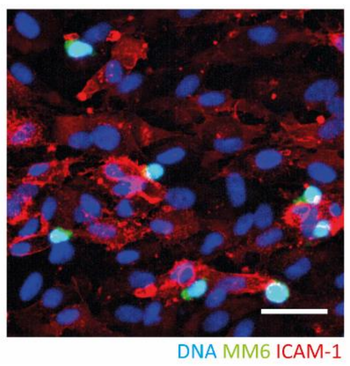




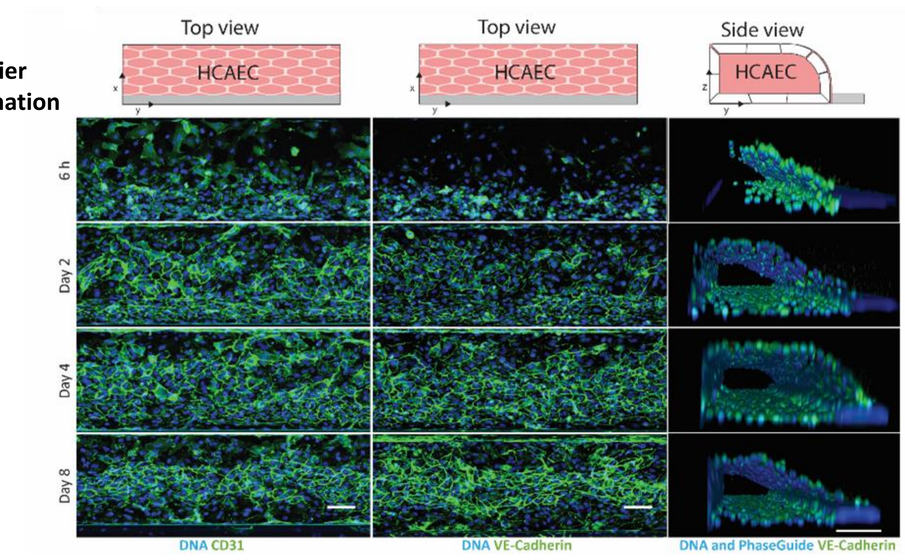
Conclusion and future directions

We developed and optimized a 3D vasculature-on-a-chip model using the microfluidic system in the OrganoPlate to investigate the process of monocyte adhesion to the lumen of HCAEC microvessels under flow. As functional readout, we have set up an assay to measure the adhesion of monocytes to the lumen of perfused microvessels. Furthermore, we have established the staining and quantification of a protein marker of inflammation, ICAM-1. To demonstrate the usefulness of the developed vasculature-on-a-chip model in systems toxicology, we assessed the impact of the aerosol from a heat-not-burn product (THS 2.2) compared to the smoke from a reference cigarette (3R4F) on the adhesion of monocytes to the lumen of the vessels. The results show that THS 2.2 aerosol-conditioned medium had a reduced effect on the monocyte-to-endothelium adhesion relative to 3R4F smoke-conditioned medium.

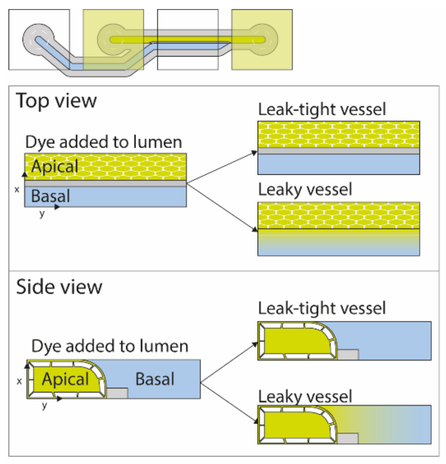
Literature The data in this poster were published in: Poussin, Carine et al. "3D human microvessel-on-a-chip model for studying monocyte-to-endothelium adhesion under flow–application in systems toxicology." ALTEX-Alternatives to animal experimentation (2019).

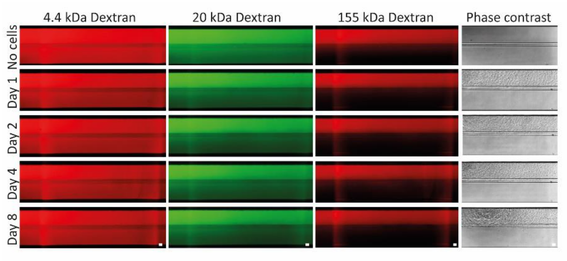
Characterization of vasculature model HCAEC vessels were cultured up to 8 days and stained for DNA, CD31, and VE-cadherin to visualize barrier formation and stable tubular morphology. Barrier formation can also be quantified by perfusing a fluorescent dye through the lumen of the vessel and measuring the intensity of this fluorescent dye diffusing into the adjacent gel channel. The vessels maintained their barrier function from day 1 to day 8 after seeding.

Barrier formation

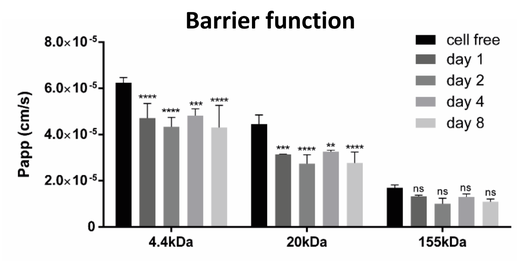


Barrier integrity assay



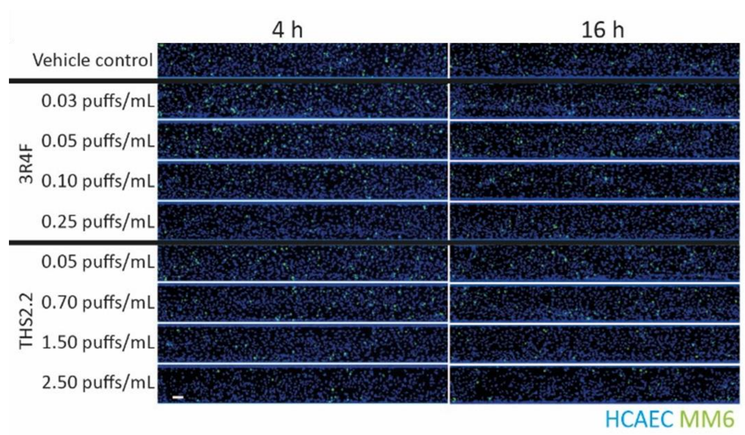


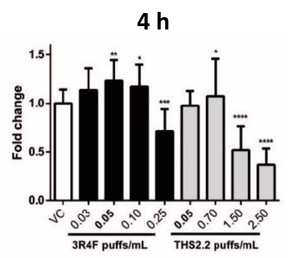
Barrier function



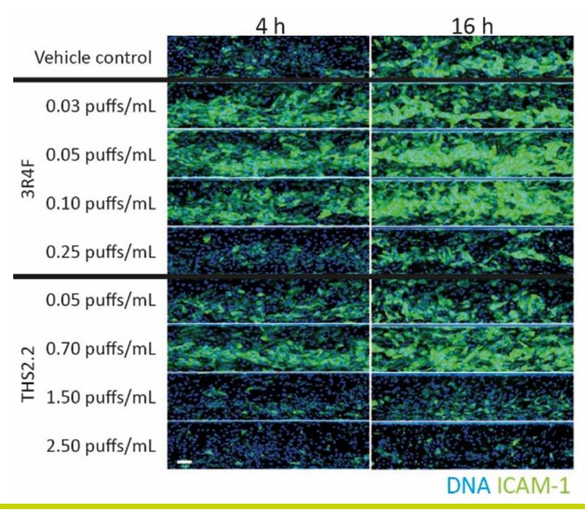
Systems toxicology - As a user case, the biological impact of the aerosol from THS 2.2, a heat-not-burn product, was compared to that of smoke from 3R4F, a reference cigarette, by using the optimized vasculature model. Monocytes were exposed to culture medium through which smoke from 3R4F cigarettes or aerosol from THS 2.2 heatsticks was bubbled. After 2 h, the monocytes were pelleted and conditioned medium (CM) was collected. Vessels were exposed to various concentrations of CM or TNFα (used as positive control) for 4 h or 16 h. After exposure, the monocyte adhesion assay and ICAM-1 quantification were performed as optimized before. THS 2.2-aerosol CM showed no significant effect on ICAM-1 or monocyte-to-endothelium adhesion at the concentration at which 3R4F-smoke CM showed the maximum effect. Increasing the concentration of THS 2.2-aerosol CM by a factor ~14 resulted in a similar effect to that of 3R4F-smoke CM. Overall, these results indicate that THS 2.2 aerosol has a reduced impact on the inflammation-driven monocyte-to-endothelium adhesion process relative to 3R4F smoke.

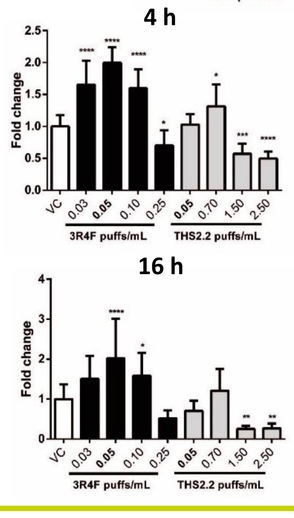
Monocyte adhesion assay





ICAM-1 immunostaining





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