3D vasculature-on-a-chip: a model of perfused human coronary artery endothelial microvessel for studying monocyte to endothelium adhesion under flow

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Abstract

Genetic predispositions and lifestyle can promote atherogenesis that may ultimately lead to cardiovascular adverse events. To study vascular functions and disorders under flow, we envisioned the development of a perfused 3D vasculature model that mimics the *in vivo* situation.

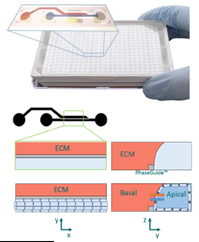
Using the OrganoPlate®, a microfluidic 3D cell culture plate supporting up to 96 tissue models, we have established culture conditions for the formation of microvessels using primary human coronary artery endothelial cells. After 4h-treatment with various concentrations of TNF-Alpha, fluorescently-labeled monocytic cells were perfused into endothelial cell microvessels and adherent monocytes were quantified from captured images, showing a concentration-dependent increase of cell adhesion to endothelial cell microvessels.

In conclusion, the innovation of 3D vasculature models will open new avenues for vascular disease research and applications in pharmacology and toxicology compound screening *in vitro*.

Methods

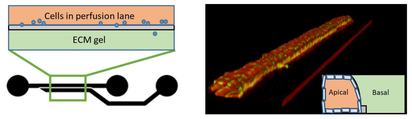
OrganoPlate® is a microfluidics-based culture plate that enables culturing and screening of a wide range of physiologically relevant organ and tissue models.

- 3D micro-vessel model using primary human artery coronary endothelial cells (HCAECs) are developed in the OrganoPlates.
- The steps include the optimization of cell culture conditions in 3D and the quantification of barrier formation and integrity through cell structure staining and imaging, and the perfusion of fluorescent-labeled beads, respectively.



Perfusion channel

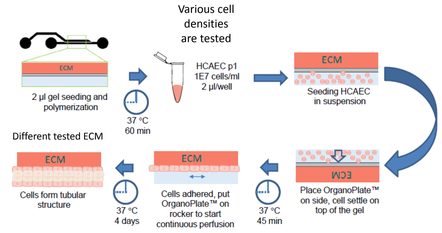
Gel channel



1. HCAEC culture condition optimization for the formation of microvessel

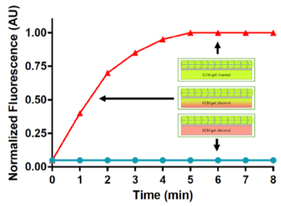
- Cell density.
- Extracellular matrix composition (ECM).

Methods: once ECM is polymerized in the gel channel, HCAECs are seeded into the perfusion channel, and the plate is turned on the side to let them settle on top of ECM. After adherence for 1-2h, the OrganoPlate® is placed on a rocker to start perfusion of cells that will grow and form a micro-vessel within 4-8 days.



2. Barrier formation and integrity evaluation

- Perfuse FITC-dextran (20 kDa) or TRITC-dextran (155 kDa) in the perfusion channel by passive leveling (Rocker 7°, 8 min. interval rocking).
- Image in time-lapse fluorescent signal.
- Calculate the ratio between fluorescent signal in the perfusion and gel channels.



3. Adhesion of monocytic cells to endothelial microvessel under flow

After 4h-treatment with various concentrations of TNF-Alpha, fluorescently-labeled MM6 cells were perfused into nuclear-stained endothelial microvessels for 15 min. After washing with PBS, microvessel images were captured and the number of adherent MM6 cells and HCAECs were counted.

Results

Testing and optimizing conditions for endothelial microvessel formation

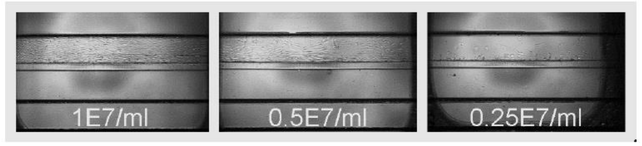


Figure 1: Determination of cell density for the formation of microvessels using primary human coronary artery endothelial cells (HCAECs). Three seeding densities 1E7, 0.5E7 or 0.25E7 cells per milliliter were tested.

Endothelial microvessel barrier formation and integrity over time

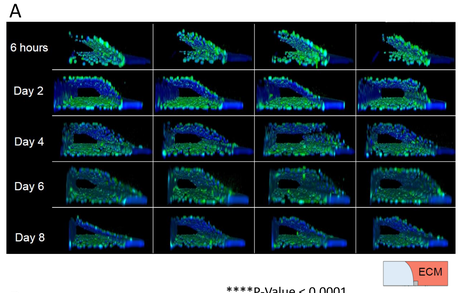
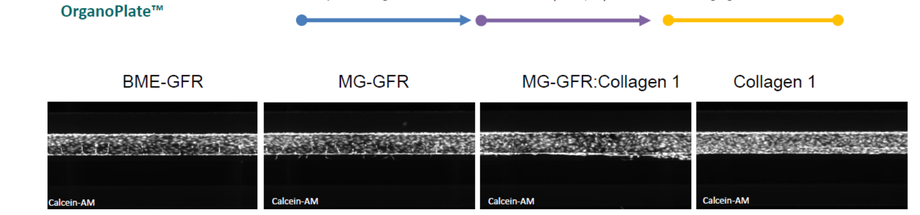
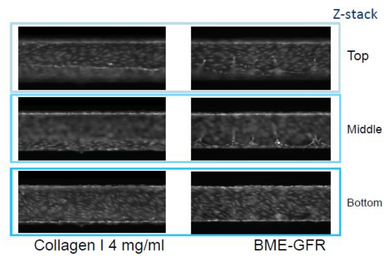


Figure 3: Barrier formation and integrity: (A) HCAEC (10^6 cells/ml) barrier started to form within 6 hours. Images corresponding to 3D reconstruction of perpendicular section of 4 microvessels/condition and permeability assay (B) indicated that optimal barrier was observed already after 2 days and remained stable until 8 days. Blue color: Hoechst (nucleus); Green color: fluorescently-labeled antibody targeting VE-cadherin (cell membrane).





BME-GFR: basement membrane extract growth factor reduced
MG-GFR: matrigel growth factor reduced
MG-GFR:collagen I: 1:1 mixture
Collagen I: rat tail collagen I

Figure 2: Choice of ECM type for the formation of endothelial microvessel. (A) Four types of ECM were evaluated. (B) BME-GFR and MG-GFR both show pattern of perpendicular cells. MG-GFR shows invasion of cells into the ECM. MG-GFR:Collagen I shows barrier formation, but non-confluent. (B) Collagen I 4 mg/mL shows most confluent barrier formation. P2: passage 2.

Adhesion of monocytic cells to endothelial microvessel under flow

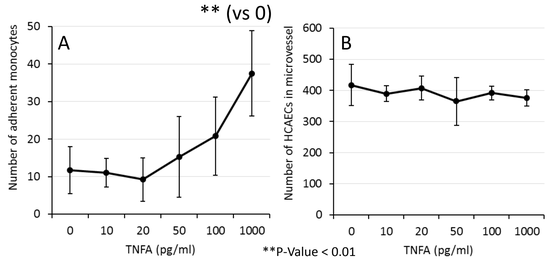


Figure 4: Adhesion of monocytic cells (MM6 cells) to endothelial microvessel (4 days) under flow. (A) After 4h-treatment of HCAEC microvessel with TNF-Alpha, the number of adherent Mono Mac-6 (MM6) cells, a monocytic cell line, enhanced with increased concentrations of TNF-Alpha ranging from 10 to 1000 pg/ml. (B) In parallel, the number of HCAECs in the microvessel remains stable across TNF-Alpha conditions. Results represent the mean ± standard deviation of 4 to 6 microvessels

Conclusions

The formation of endothelial microvessels has been optimized under perfusion considering cell seeding density, ECM type and amount and barrier integrity.

The treatment of endothelial microvessel with TNF-Alpha for 4hr resulted in a concentration-dependent increase of monocytic cell adhesion to perfused endothelial microvessels.

The development of innovative 3D vasculature models on a chip will open new avenues for vascular research and toxicological risk assessment *in vitro*.

Acknowledgments. Project Management Office.

Abbreviations. HCAECs: human coronary arterial endothelial cells; MM6: Mono Mac-6 cells; TRITC: Tetramethylrhodamine; FITC: Fluorescein; ECM: extra-cellular matrix

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Competing Financial Interest

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