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 atherosclerosis which 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 OrganonPlate®, 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 monocyte 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

OrganonPlate® 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 coronary artery endothelial cells (HCAECs) are developed in the OrganonPlates.
• 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 OrganonPlate® 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 77, 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 monocyctes 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

A

Endothelial microvessel barrier formation and integrity over time

B

Adhesion of monocyctes to endothelial microvessel under flow

A

B

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 4h resulted in a concentration-dependent increase of monocyte 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.

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