APPLICATION NOTE

AX/AX R Confocal microscope

Organ-on-a-Chip

Time-lapse imaging analysis of angiogenesis induction using a 3D model

It is known that a decrease in the homeostasis function of blood vessels is involved in the onset and progression of various diseases and pathological conditions such as cancer, arteriosclerosis, chronic inflammation, and ischemia. Against this background, it is very important to understand the detailed mechanisms relating to new vascular structure formation by angiogenesis etc. and microvessel damage. This application note introduces an example of detailed observation and analysis of a 3D angiogenesis structure (*in vitro* perfusion angiogenesis model) with an AX/ AX R confocal microscope, using the Mimetas' OrganoPlate® 3D tissue culture platform.

Overview

The OrganoPlate® 3D tissue culture platform (manufactured by Mimetas) is a system that enables co-culturing, preparation of tissues having tubular structures, and reproduction of microenvironment in the vicinity of organisms, by means of perfusion utilizing microchannels. OrganoPlate® 3-lane 40 consists of 40 tissue culture chips, each consisting of one lane for gel introduction and two lanes for perfusion (Fig. 1).
After injecting gel into the central channel, HUVEC was seeded in the upper channel and perfused in a CO_2 incubator using OrganoFlow® to form a vascular structure. After that, angiogenesis growth factors were added from the lower channel and the course of change over time was observed (Fig. 2).

Fig. 1

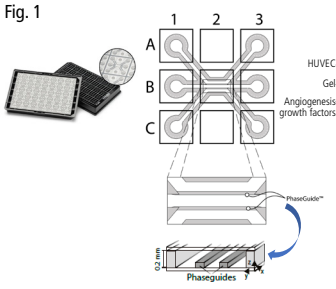
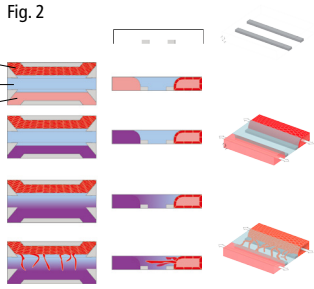


Fig. 2



https://www.mimetas.com/en/organoplate-3-lane-40/

Results

To observe the morphology of the tip of blood vessels and localization of constituent cells, actin and CD31, which is a marker for vascular endothelial cells, were stained. Images obtained with a water-immersed objective suitable for samples in cultures and gels were so bright and clear that slight differences in actin and CD31 localization were detected (Fig. 3b). This suggests that this method can be applied to the study of cells involved in angiogenesis such as tip cells, stalk cells, and phalanx cells, by using an objective suitable for observation purposes.

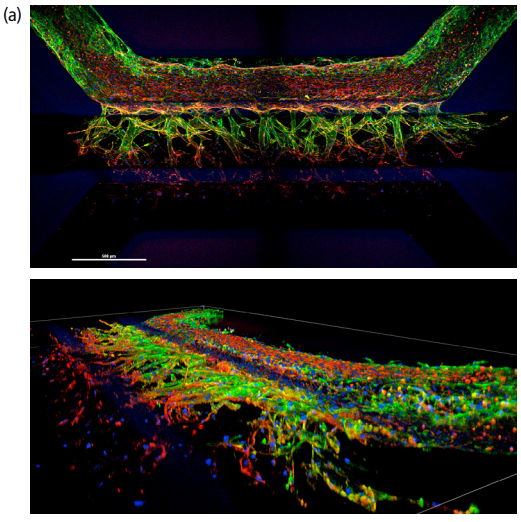


Fig. 3: Vascular 3D structures created in OrganoPlate®
(a) Image of an entire blood vessel in a chip, acquired using a CFI Plan Apochromat Lambda 10X objective (NA 0.45)
(b) Image of one blood vessel acquired using a CFI Apochromat Lambda S 40XC WI objective (NA 1.25)

Throughput is important for detecting the presence or absence of angiogenesis on a chip-by-chip basis. With a 4x objective, the whole chip can be observed in one shot (Fig. 4). In addition, since low-noise XY-Z images can be acquired at the high speed of the resonant scanner by using the microscope AI module Denoise.ai, these can be expanded for quantitative evaluation by image analysis.

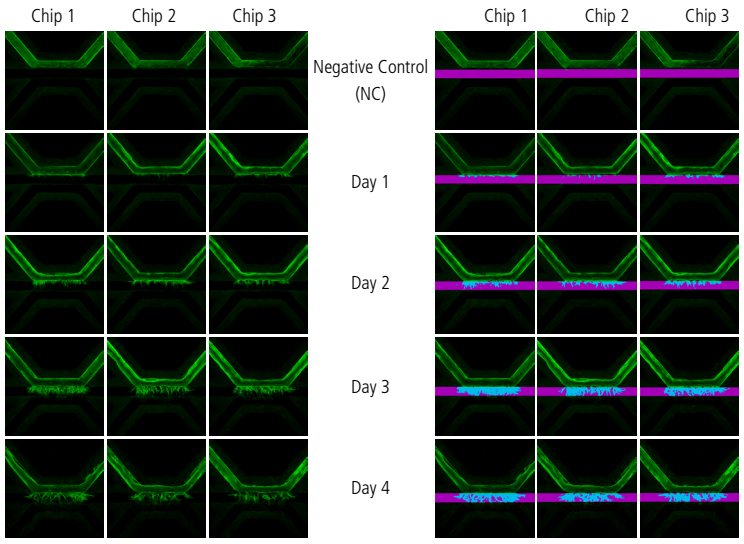


Fig. 4: Time-lapse imaging analysis of 3D angiogenesis induction on multiple chips

Samples were collected without stimulation, and on days 1, 2, 3, and 4 after stimulation.
Left: Original image (MIP image)
Right: Analysis result image
The analysis area is shown in purple, and the blood vessels in the analysis area are shown in light blue.

Imaging conditions:
Resolution: 512 x 512 pixels
Z stack (Range: 20 µm, Steps: 25)
Resonant scanner and Denoise.ai

Objective: CFI Plan Apochromat Lambda 4X (NA0.20)

In addition, quantitative analysis is possible not only for 2D images but also for 3D images. In Fig. 5, angiogenesis three-dimensionally distributed in the gel region was analyzed using the number of cell nuclei, the volume of the actin region, and the volume of the CD31 region.

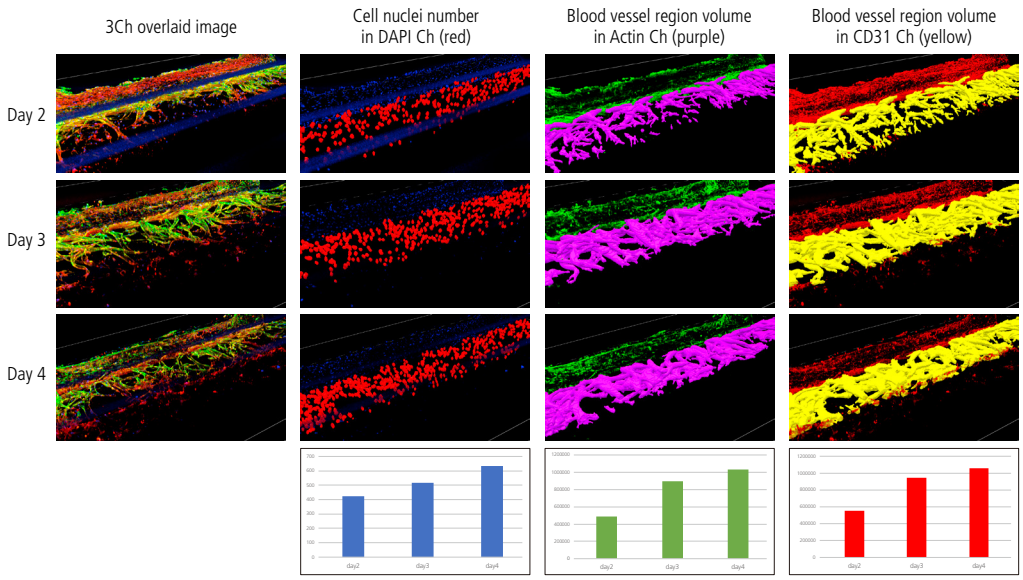


Fig. 5: 3D analysis of vascular structure of each chip in OrganoPlate® 3-lane 40 using GA3 (general analysis 3) function

Objective: CFI Plan Apochromat Lambda 10X (NA 0.45)

Summary

The AX/AX R confocal microscope has the widest FOV in the industry (diagonal 25 mm) and can capture a large area in one image. Also, high-speed scanning using Denoise.ai can reduce the acquisition time for multiple chips (Fig. 4).
By using an appropriate objective, confirmation was obtained that detailed observation is possible of the morphology of each blood vessel and 3D localization of the cells that compose them (Fig. 3b).
In addition, the GA3 image analysis tool enables quantitative evaluation of angiogenesis (Fig. 5).
This suggests that this system can be applied to toxicity evaluation and drug efficacy evaluation using a 3D angiogenesis model that requires high throughput.