3D time lapse imaging and quantitative analysis of the active migration of human vascular endothelial cells into a multilayered cell sheet with Yokogawa's confocal image cytometer CQ1

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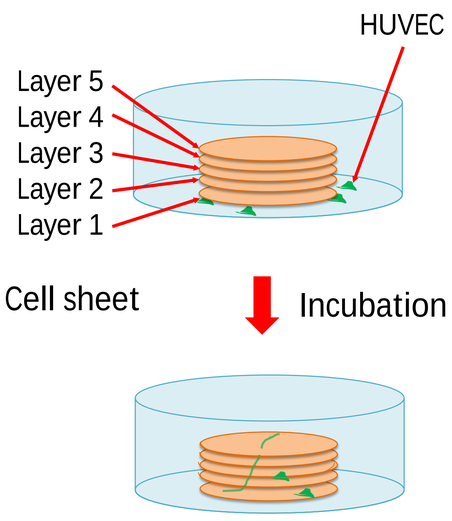
Introduction

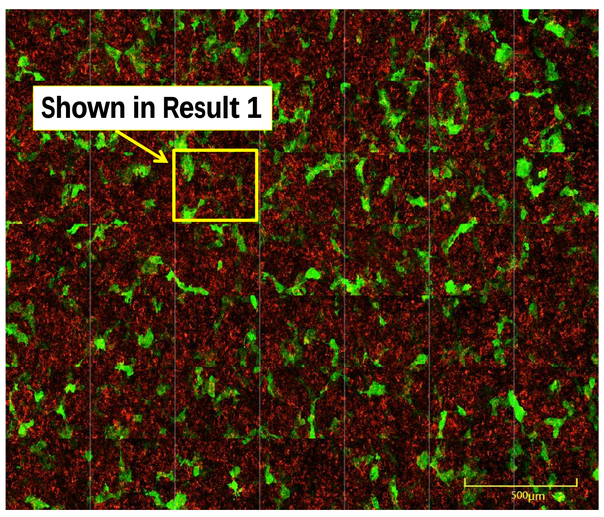
Time lapse confocal imaging has been an essential method to investigate the 3D dynamic behaviors of cells in tissue cultures. For long-term live cell imaging, it is critical to reduce phototoxic damage to the cells caused by repeated laser scanning. Yokogawa CSU (confocal scanner unit) is a confocal unit using a microlens-enhanced dual Nipkow disk confocal optical system, which has been shown to be less harmful to living cells compared to conventional single beam scanning devices. The CQ1 is an all-in-one confocal quantitative imaging cytometer based on the CSU. Here we report the 3D time lapse live cell imaging in a multilayered cell sheet using CQ1.

Methods

Five-layered myoblast cell sheets were constructed from human skeletal muscle myoblasts (HSMM) and human skeletal muscle fibroblasts (HSMF) by using a thermo-responsive surface and stamp system [(1)(2)]. HSMMs and HSMFs were labeled with CellTracker™ Orange. Human umbilical vein endothelial cells (HUVEC) expressing GFP (GFP-HUVEC) were overlaid by the cell sheet and co-cultured. Time lapse imaging (67 hours, 30 min interval, 40x objective lens , 49 fields) was performed by CQ1 equipped with an internal incubation chamber to regulate culture environment.



Method fig. 1. The five-layered cell sheet



Method fig. 2. Large field stitched 3D image acquired by CQ1
7 x 7 fields,
Green; HUVEC (GFP),
Red; Cell Sheet (CellTracker™ Orange)

Results

1. Dynamic migration and network formation of GFP-HUVECs captured by 3D time lapse imaging

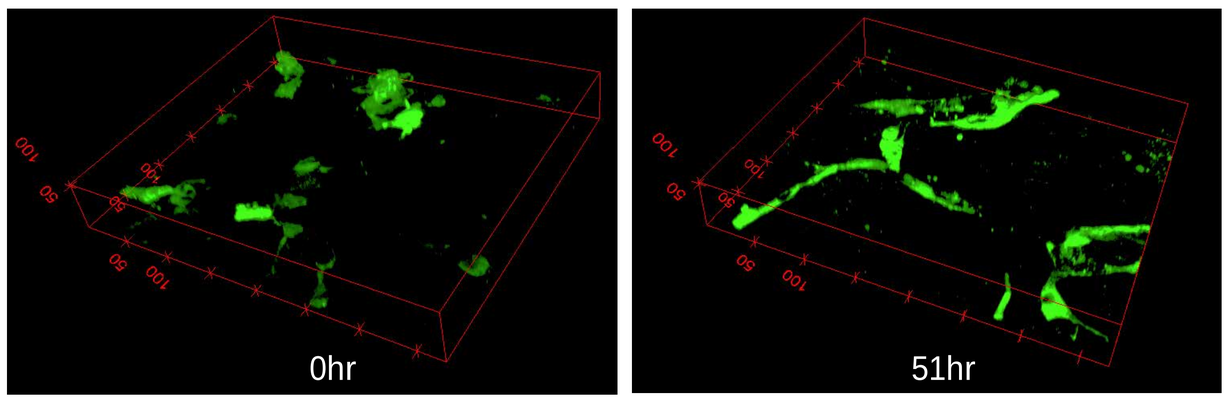


Fig. 1-1. 3D images of the cell sheet at the beginning of and after 51 hours incubation.
Images were reconstructed from Z stack images of the field indicated by the yellow frame in the large field stitched image in **Method fig.2**.

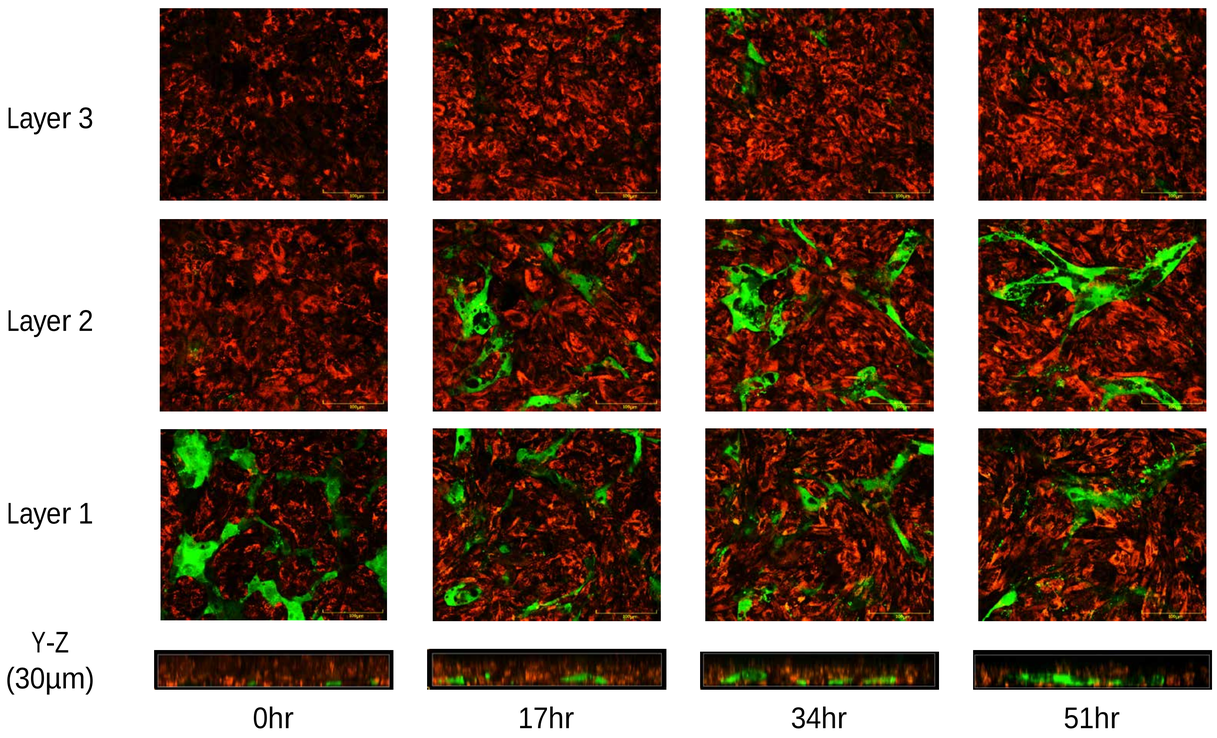


Fig. 1-2. Migration of the GFP-HUVECs into the cell sheet.
Single slice images showing the migration of HUVECs into upper layers. (*Rows, from top to bottom*) Single slice images of layers 3, 2, 1 and corresponding Y-Z plane images of the cell sheet. (*Columns, from left to right*) Images acquired at 0, 17, 34 and 51 hr incubation. The image filed is the same as fig. 1-1.

2. Quantification of the migration of GFP-HUVECs into the five-layered cell sheet

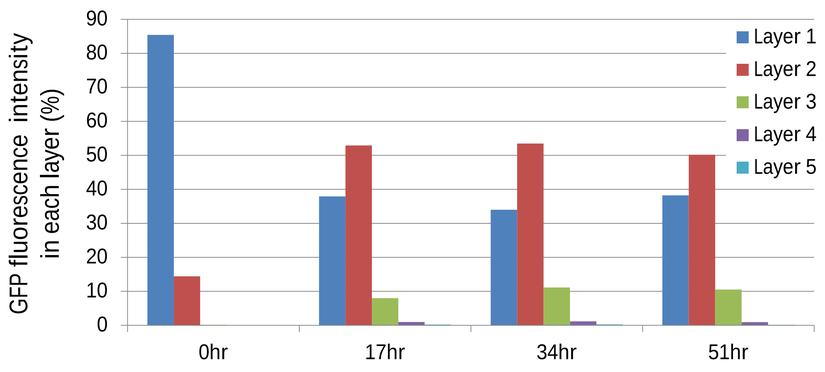


Fig. 2-1. Temporal change of the distribution GFP-HUVECs in the cell sheet.
GFP fluorescence intensity in each layer was indicated as the ratio against the total GFP intensity in the cell sheet.

Summary & Conclusions

- Ø GFP-HUVECs dynamically migrated upward into the five-layered cell sheet constructed from HSMMs and HSMFs.
- Ø The GFP-HUVECs formed a reticulate network in the horizontal plane in the middle layers.
- Ø Long-term 3D time lapse imaging by CQ1 revealed a dynamic process of the active migration and the formation of the cellular network in the multilayered cell sheet.
- Ø CQ1 would be a powerful research tool in tissue engineering as well as regenerative medicine and drug screening.

Acknowledgements
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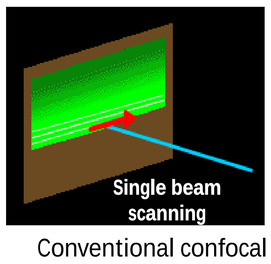
Reference
(1) Kino-oka et al., J. Biosci. Bioeng., 113, 128-131 (2012)
(2) Nagamori et al., Biomaterials, 34, 662-668 (2013)

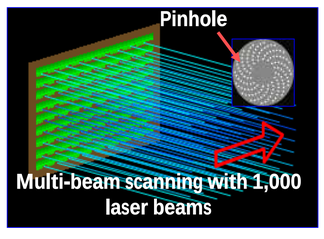
YOKOGAWA Core Competence

Microlens enhanced dual Nipkow disk scanning
→ high-speed, low photo toxicity and low photo bleaching

A Nipkow spinning disk and a second spinning disk with microlens rapidly scan the field of view with about 1,000 laser beams when rotated.
Multi-beam scanning not only increases scanning speed, but also results in significant lower photo toxicity and photo bleaching, because multiple excitation needs only a low level of laser power at the specimen to fully excite fluorescence.



Conventional confocal



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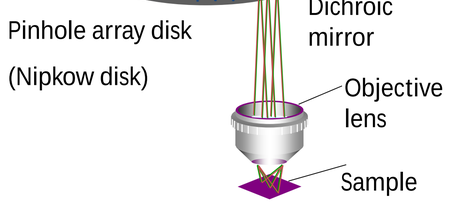

Confocal Quantitative Image Cytometer CQ1

- ü Easy to use to measure the feature data of cells
- ü Cell clusters are directly measured by 3D imaging
- ü Gently 3D image acquisition
- ü Bench-top and no need for darkroom