VIDEO LENSFREE MICROSCOPY FOR THE QUANTIFICATION OF ENDOTHELIAL CELLULAR NETWORK FORMATION

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Introduction

We assessed the use of the Iprasense Cytonote lensfree video microscope in combination with a customized version of the "Angiogenesis Analyzer" image plug-in. This turns into a unique platform to exhaustively and precisely study angiogenesis and cellular network formation over large field of view (29.4 mm²) and extended period of acquisition (days to weeks). As a first case study, we have analyzed and quantified the spontaneous network formation of HUVEC endothelial cells on three-dimensional (3-D) extracellular matrices.

Lensfree microscopy for large field of view time lapse Imaging

Experimental setup of the lensfree videomicroscope (2014 IPRASENSE Cytonote). (Left) A CMOS image sensor is used for image acquisition and an LED and a 330 µm pinhole for illumination (5 cm above the image sensor). (Right) Acquisition and phase reconstruction of a culture of primary human umbilical vein endothelial cells (HUVECs) (scale bar 1000 µm). The field of view is 29.4 mm² and contains about 4000 cells.

Acquisition of the network formation of HUVEC endothelial cells

Network formation with HUVEC cells observed by means of lensfree video microscopy over 24 h. The cells are cultured on 75 µl of liquid Matrigel. 5.10⁴ HUVEC cells were then seeded on Matrigel (BD Biosciences) and incubated at 37°C. Scale bar is 1 mm.

Automated analysis of the HUVEC network formation

Temporal analysis of the HUVEC network formation. The plot of the different devised metrics as a function of time clearly define three different steps for the network formation: initiation, a stabilization period and then the fusion of meshes. During the first 4 hours, the network is in formation: the number of meshes increases, and so do the number of segments and junctions (see figure 3d for the definitions). Following this, for 6 hours, the network remains stable. Towards the end, the meshes merge to form larger meshes. After 24 hours, the networks present the following architecture: total meshes area of 9 mm², with 60 meshes, with an average size of 0.15 mm².

Conclusion

Lensfree video microscope in combination with the Angiogenesis Analyzer image plugin provides a unique mean to quantify angiogenesis and to screen ex vivo anti-angiogenic therapies.

The methods is label-free, high-throughput, ease of use, working directly inside the incubator and relatively low-cost.

We expect that it will irreversibly change the quantification of cell culture, and angiogenesis quantification in particular.