



Keywords or phrases

Angiogenesis, HUVEC, label-free analysis, cell culture, cell tube formation

Quantification Of Endothelial Cellular Network Formation For In-Vitro Angiogenesis Analysis

Abstract

Angiogenesis, vital for tissue growth and repair, also fuels cancer progression. Inhibiting it is a strategy for treating cancer. **CYTONOTE**, with its "angiogenesis" module, provides a unique platform for the study of angiogenesis. It enables analysis of endothelial cell networks and tube formation. **CYTONOTE** enables label-free, high-throughput, user-friendly, incubator-based analysis, a valuable tool for cell-tube assays and anti-angiogenic screening.

Introduction

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels in the earlier stage of vasculogenesis. Angiogenesis is a normal and vital process in growth and development, as well as, in wound healing and in the formation of granulation tissue. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer.

The **CYTONOTE** allows to study angiogenesis and cellular network formation over large field of view (29.4 mm²) and extended period of acquisition (days to weeks). The **CYTONOTE** is able to analyse the meshes of endothelial cell network, the number of junctions, the number of





branching intervals, the number of master segments, and the steps of analysing vascular endothelial cells into tubes. As a case study, we have analyzed and quantified the spontaneous network formation of HUVEC endothelial cells on 3D extracellular matrices.

Material & Methods

The **CYTONOTE** is able to perform measurements inside the incubator and it recognizes cells without any labelling. In standard, the **HORUS** software automatically calculates cell number, cell saturation, cell area, cell concentration, cell morphology... Unlike a conventional microscope, the absence of focus in the **CYTONOTE** allows an extremely wide field of view. The **CYTONOTE** is used in combination with the “Angiogenesis Analyzer” module from **HORUS** software.

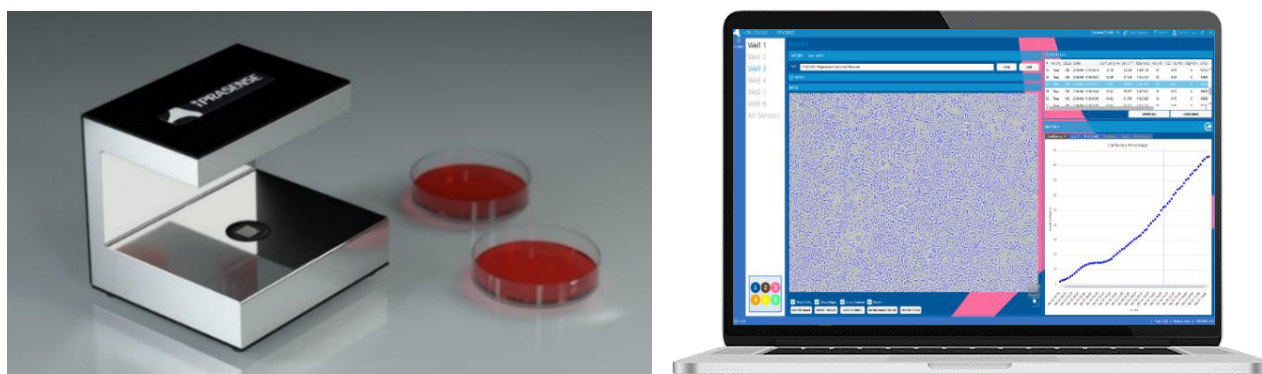


Figure 1 : **CYTONOTE 1W** on the left and **HORUS** software on the right.

For the experiment, primary human umbilical vein endothelial cells (HUVEC) are cultivated on liquid Matrigel (BD Biosciences) in Petri dish of 35 mm² and incubated at 37°C.

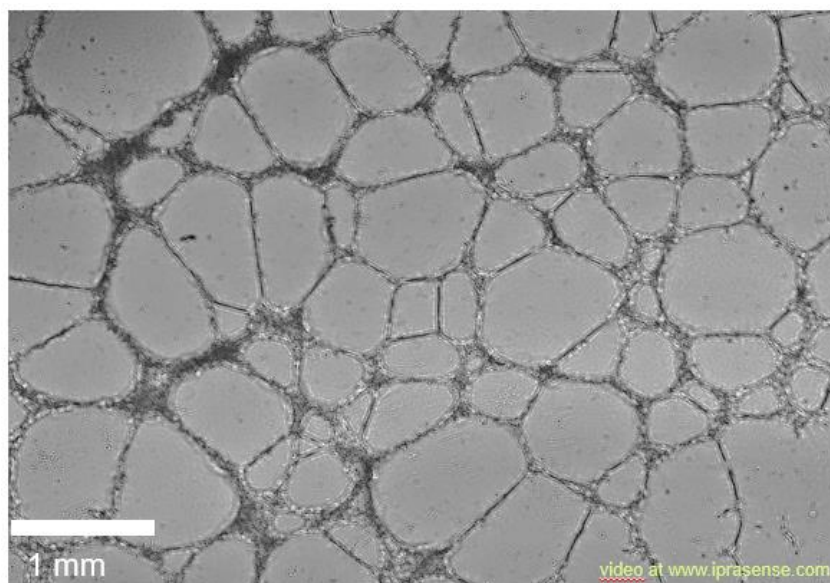


Figure 2 : Network formation with HUVEC cells observed by means of **CYTONOTE** over 24h

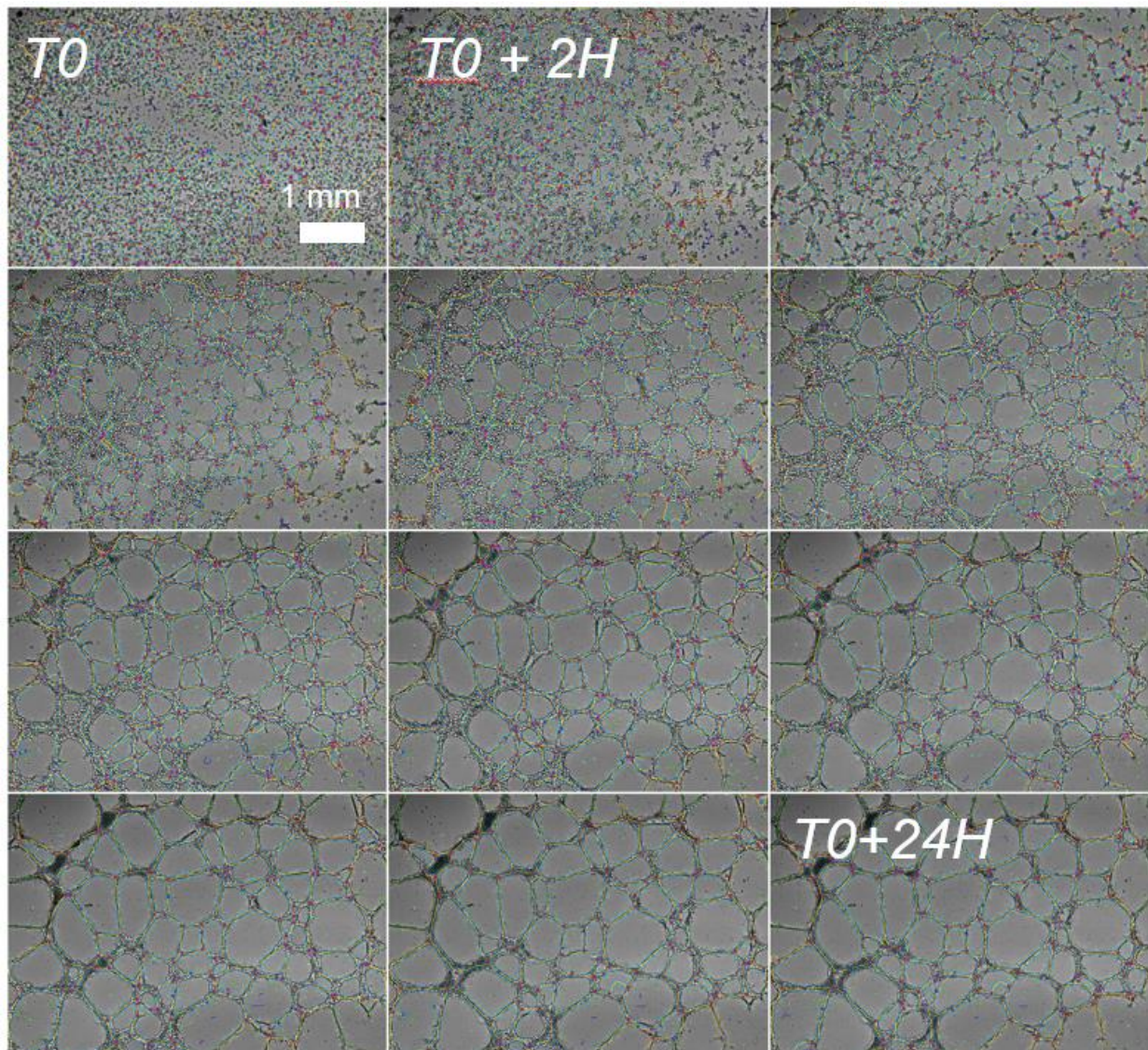


Figure 3 : Analysis is performed with the “Angiogenesis Analyzer” module from **HORUS** software

Segments are in yellow, branches in green, red points are nodes, meshes (closed structures, also called tube-like-structures) are in cyan, and isolated structures are in blue. After 24h, the networks tend towards a stable architecture: total meshes area is around 9 mm², the number of meshes is approximately 60, and their mean size is 0,15 mm².



Results

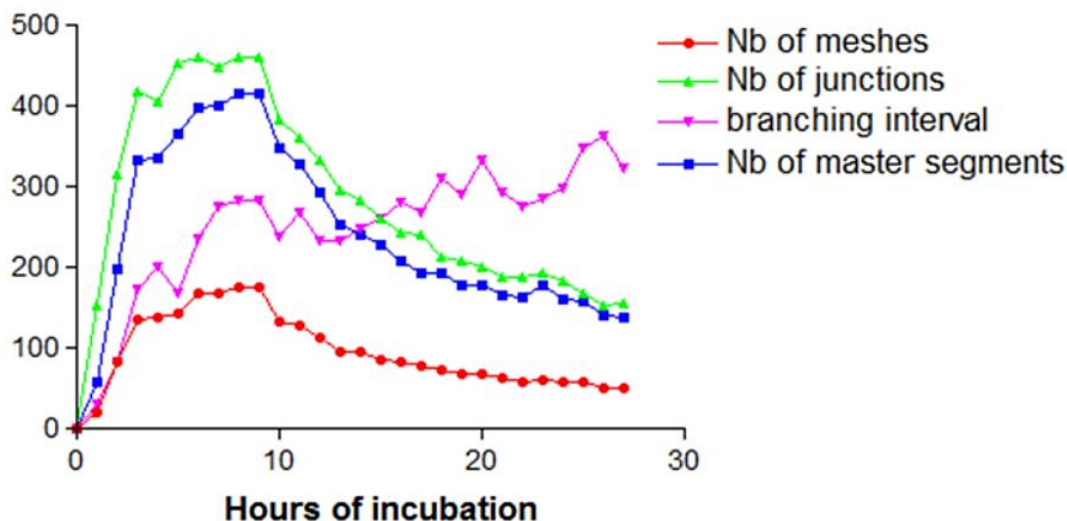


Figure 4 : Temporal analysis of the HUVEC network formation

The plot of the different devised metrics as a function of time clearly defines 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. 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

The **CYTONOTE** combined with the “Angiogenesis Analyzer” module from **HORUS** software provides a unique mean to perform cell tube formation assay, to quantify angiogenesis and to screen ex-vivo anti-angiogenic conditions. This method is label-free, high-throughput, ease of use, working directly inside the incubator and relatively low-cost.



References

- (1) Online version available at <https://www.iprasense.com/angiogenesis/>
- (2) Allier C, et al. *Video lensfree microscopy of 2D and 3D cultures of cells*. Proc. of SPIE 2014 Vol. 8947 89471H-8
https://www.researchgate.net/publication/283044865_Video_lensfree_microscopy_for_the_quantification_of_endothelial_cellular_network_formation

OTHER PRODUCTS



CYTONOTE 6W

Monitoring cell culture in 6-well plates allows you to obtain real-time images of



CYTONOTE SCAN

Monitoring cell culture in multi-well plates allows you to obtain real-time images of your cells from your incubator.

See Our Products : <https://www.iprasense.com/products/>

Contact Us : <https://www.iprasense.com/contact-us/>