Introduction

Angiogenesis is the physiological process of formation and remodeling of new blood vessels and capillaries from pre-existing blood vessels. This can be achieved through endothelial sprouting or splitting of the vessels and capillary. Vascular cells respond to appropriate stimuli by degradation of the extracellular matrix, then proliferation and migration of endothelial cells. Cells undergo these processes to create a tube, containing a lumen—a dynamic space that facilitates blood flow and exchange of oxygen, carbon dioxide, nitric oxide and nutrients.1

Angiogenesis is a vital process in growth and development, as well as in wound healing. Angiogenesis also supports the invasion of tumor cells and is commonly measured in cancer research. Many anti-angiogenic drugs have been developed to use in cancer therapy, while pro-angiogenic molecules may hold potential in regenerative applications. In vitro experiments to date have modeled only some aspects of angiogenic mechanisms including scratch assays, Boyden chambers, and tube formation assays. MIMETAS developed advanced and more physiologically relevant models that mimic the actual growth and sprouting of vessels from a main perfused vessel into a collagen extracellular matrix as directed by pro- or anti-angiogenic factors.

High-content imaging allows visualization of angiogenic sprouts and complex imaging analysis of angiogenesis. Multiple quantitative descriptors of angiogenesis were characterized that could be used for comparative research for studying disease phenotypes and compound effects. We describe the methods for 3D imaging and analysis of the angiogenic growth in the OrganoPlate® platform that allows visualization and quantitation of the number, volume, and length of angiogenic sprouts, as well as quantitation of cell proliferation and expression or angiogenic markers.

Cell models

3D angiogenesis model in OrganoPlate® 3-lane

A high-throughput multiphase platform, called the OrganoPlate 3-lane, was employed to establish the angiogenesis model. The OrganoPlate 3-lane comprises 40 tissue culture chips, which each consist of three channels (Figure 1). A collagen-I extracellular matrix (ECM) gel was seeded in the middle channel of each chip and form an endothelial vessel under perfusion by placing the OrganoPlate on a rocker platform. Addition of a cocktail of angiogenic factors in the bottom channel promoted formation of angiogenic sprouts from the parent vessel. Angiogenic sprouts were allowed to form for 0–4 days and then were fixed for quantitative comparison.

Confocal imaging and analysis of angiogenic sprouts in 3D

Vascular cells and sprouts were fixed with 4% formaldehyde and stained with a primary antibody against VC-cathem, followed by a secondary Alexa488 antibody (green). Actin filaments were stained with ActinRed (RedProbes™ reagent kit) and nuclei were stained with Hoechst Blue. Cells were imaged with the ImageXpress® Micro Confocal High-Content Imaging System (Molecular Devices). Images of cells were taken using confocal mode (60 μm pinhole spinning disk) and 10X, or 20X water immersion objectives. For 20X magnification, 2 stacks of 45-58 image planes were acquired at 2–4 μm intervals. For 10X objective, 2 stacks of 15–25 images were acquired using 4–6 μm intervals. Nuclei were imaged with the DAPI channel and angiogenic sprouts with the FITC channel. At 100 ms and 400 ms exposures respectively.

Results

Modeling angiogenesis

Time dependence of the angiogenesis process was modeled in OrganoPlate 3-lane. Endothelial cells seeded in the upper channel formed a tube in three days. The model includes a tube of endothelial cells formed in the top channel line, or in both top and bottom channels, with collagen in the middle gel channel (Figure 1). Addition of growth factors to the bottom channel promoted formation of angiogenic sprouts through the collagen that could be imaged and analyzed (Figure 2).

3D analysis protocol in CME

The CME and 3D image analysis capabilities are needed for the described analysis method. The custom module contained several steps. First, angiogenic sprouts were defined and segmented in each image using the Neuron Outgrowth module, then objects in different z-planes were connected in 3D space using the “connect by best match” option. Then the number of angiogenic sprouts, as well as their volumes and intensities were defined during analysis.

Cell nuclei were defined as an optional step and either total number of nuclei, or number of nuclei per sprout was calculated. The region of interest mask was used during the analysis to include only objects that are located in the gel channel, but not in the endothelial tube channel. This way only angiogenic sprouts, not cells in the upper channel were counted during analysis. The developed custom module was able to be used with both 20X and 10X images. Alternatively, image analysis can be performed in 2D by using a maximum projection image.

3D analysis of angiogenesis

Images were analyzed using the Custom Module Editor (CME) in MetaXpress High-Content Image Acquisition and Analysis Software. The Neuron Outgrowth module was used to identify sprout extensors, and the Count Nuclei module for nuclei characterization. Then the objects were connected between 2 planes in 3D space using “connect by best match” function. Images were analyzed using a 3D custom module within the MetaXpress environment.

Quantitative characterization of time-dependent growth

Time dependence of the angiogenesis process was modeled over four days. A time-dependent increase in the number and volume of sprouts was observed, as well as an increased number of cells or nuclei. Images were analyzed in 3D volume and defined sprouts and nuclei can be characterized by multiple readouts including total number of sprouts and nuclei, their intensities, volumes, distances between objects, also defining number of nuclei or cells per individual sprout, or average values. Secondary analysis was completed using Microsoft Excel.

Conclusion

It is essential to derive quantitative data from phenotypic changes of complex biological processes like angiogenesis. While 3D biological models offer a better representation of the complexity of human biology, image analysis of convoluted 3D structures can be challenging.

We developed and optimized imaging and analysis protocols that allow capturing, visualization, and quantitative analysis of angiogenic sprouts in MIMETAS assay. The imaging protocols were developed for ImageXpress® Micro Confocal system and MetaXpress software to offer an integrated workflow for imaging and analysis. Combining the system with the strength of a scalable organ-on-a-chip platform unlock quantitative characterization of phenotypic effects for disease modeling and compound screening.

References


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