Automation of the organ-on a chip assay: automated culture, imaging and analysis of angiogenesis

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Introduction

There is a critical need for biological model systems that better resemble human biology. Three-dimensional (3D) cell models and organ-on-a-chip (OoC) structures representing various tissues were successfully used for studying complex biological effects, tissue architecture, and functionality. The OrganoPlate® was developed as an OoC platform allowing the formation of 3D microfluidic-based, long-term cultures of live cells. However, the complexity of 3D models remains a hurdle for its wide adoption in research and drug screening. Automation of the cell culture, assays, and analysis can provide the necessary tools to facilitate and scale up the use of OoC systems.

Here we describe a workflow for automation of OoC culture, as well as monitoring, and automated cell analysis. The automated method utilizes an integrated work-cell comprising several instruments that allow the automation and monitoring of cell culture. The high-content imaging system also enables the characterization of 3D cell model development, as well as testing the effects of compounds. The integrated system includes the ImageXpress® Micro Confocal High-Content Imaging System, an automated CO_2 incubator and liquid handler (Biomek i7), and collaborative robot. We developed methods for automation of cell seeding, media exchange, and for monitoring the development and growth of 3D vasculature. In addition, the method facilitates automated compound testing and evaluation of toxicity effects. We have used the angiogenesis assay as an example for the automation process.

Methods

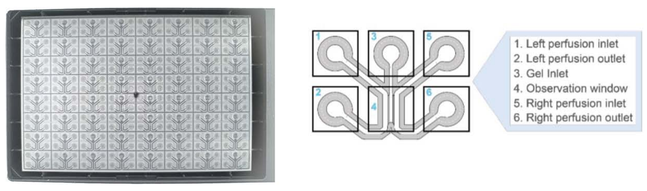


Figure 1. The 3-lane OrganoPlate comprise of 64 or 40 culture chips. Shown here (left) is the view from the bottom side of the OrganoPlate® 3-lane 64. Middle: Schematic representation of an OrganoPlate 3-lane 64 tissue chip. Right: Illustration of a tubule of cells grown against an ECM gel.

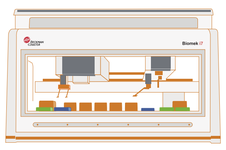
3D angiogenesis model in the 3-lane OrganoPlate

A high-throughput microfluidic platform, called the OrganoPlate, was employed to establish the angiogenesis model. The OrganoPlate 3-lane comprises 64 or 40 tissue culture chips, with each consisting of three channels (Figure 1). A collagen-I extracellular matrix (ECM) gel was seeded in the middle channel of each chip and was patterned by phase-guides. Endothelial cells (primary, cell line, or iPSC-derived) were grown in the right channel of each chip and forms an endothelial vessel under perfusion by placing the OrganoPlate in the LiCONiC Wave incubator programmed to rock the plates at regular intervals to allow flow of media by gravity, simulating perfusion. Addition of a cocktail of angiogenic factors in the left channel induces the directed formation of angiogenic sprouts from the parent vessel. Angiogenic sprouts were allowed to form for 1–5 days and then were fixed and stained for quantitative comparison. Vascular cells and sprouts were fixed with 4% formaldehyde and stained with a primary antibody against VE-cadherin, followed by a secondary Alexa488 antibody (green) and nuclei were stained with Hoechst (blue).

Workcell setup for automation

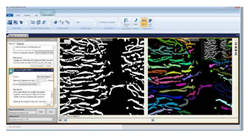
The workcell consisted of the following primary components: Hotel for storage of microtiter plates, LiCONiC STX44 automated incubator with wave function, AquaMax® Microplate Washer (Molecular Devices), HiGTM4 automated centrifuge (BioNex Solutions Inc), SpectraMax iD5® Multi-Mode Microplate Reader, ImageXpress Confocal HT.ai system, ImageXpress® Pico Automated Cell Imaging System (Molecular Devices), and Biomek i7 Automated Workstation (Beckman Coulter Life Sciences). The plate handling robot is a PreciseFlex400 robot on a 2 meter rail to access all plate nests. The Green Button Go® scheduling software is used to create the automation workflow and schedule setup. This software provides single user interface for control of all devices in the workcell.

For automation of the OrganoPlate, the Biomek i7 automated workstation was used for plate preparation (ECM seeding) and cell seeding. The Biomek i7 can also be used for addition of growth factors, media exchange, and cell fixing and staining. Transfer of the OrganoPlate can be done with the PreciseFlex400 robot to the Biomek i7 for media changes. Transfers from the incubator to the imager can also be set up for long term discontinuous monitoring of



Automated liquid handler (Beckman i7)

ImageXpress Micro Confocal High-Content Imaging System with water immersion option



Custom Module Editor in MetaXpress High-Content Image Acquisition and Analysis Software

Image acquisition and analysis

Images were acquired with the ImageXpress Micro Confocal System (Molecular Devices). Acquisition settings were: confocal mode (60 µm pinhole spinning disc) with the 10X, or 20X water immersion objectives. For 20X magnification, z-stacks of 45-58 image planes were acquired at 2–4 µm intervals. For 10X objective, z-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.

Images were analyzed using the Custom Module Editor (CME) in MetaXpress® High-Content Image Acquisition and Analysis Software. Images were analyzed using a 3D custom module within the MetaXpress environment.

Results

Automation workcell layout and software control

The cell culture process is typically a manual procedure that requires user intervention at multiple points. These processes can be automated with an integrated workcell (Figure 2). For example, microplates can be transferred from the incubator to the sterile liquid handler for media exchange. In addition, compound treatment and end-point assays can similarly be automated. The process for cell culture maintenance (monitoring and feeding), and end-point assays can be automated which gives reproducible results, saves on resources, and is highly scalable.

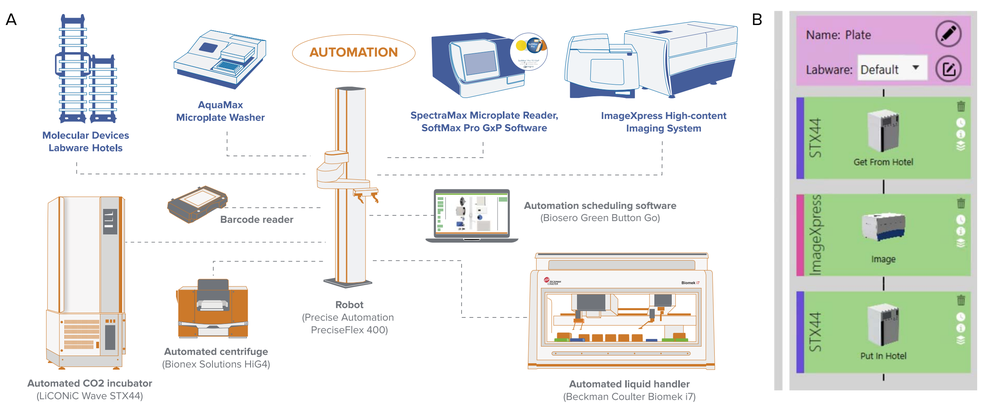


Figure 2. Layout of the individual instruments in the workcell is illustrated in (A). The instruments are controlled by an integrated software (Green Button Go) that allows for set up of processes. An example of the process to monitor cells in culture is shown in (B). Here, the plates are moved from the incubator to the ImageXpress Confocal HT.ai for imaging in brightfield and then back to the incubator. The process can also be scheduled, and plates that need to be imaged can be entered as a list to enable easier batch processing. More complex routines that include the liquid handler for media exchanges (feeding) can also be implemented.

Results

Automation for an angiogenesis model using a scalable microfluidic platform

OoC technology has been successfully used to mimic the physiological organ and its environment. OoC models allow for regulation of key physiological conditions related to the tissue microenvironment such as tissue-organ interactions, cell patterning, tissue boundaries, and concentration gradients.

Here, the MIMETAS OrganoPlate 3-lane 64 is used to model angiogenic sprouting *in vitro* on an automated platform (Figure 3). The OrganoPlate integrates perfusion and a concentration gradient and can be used to study angiogenic sprouting and microvascular stabilization. Automated liquid handling improves assay reproducibility and increases throughput.

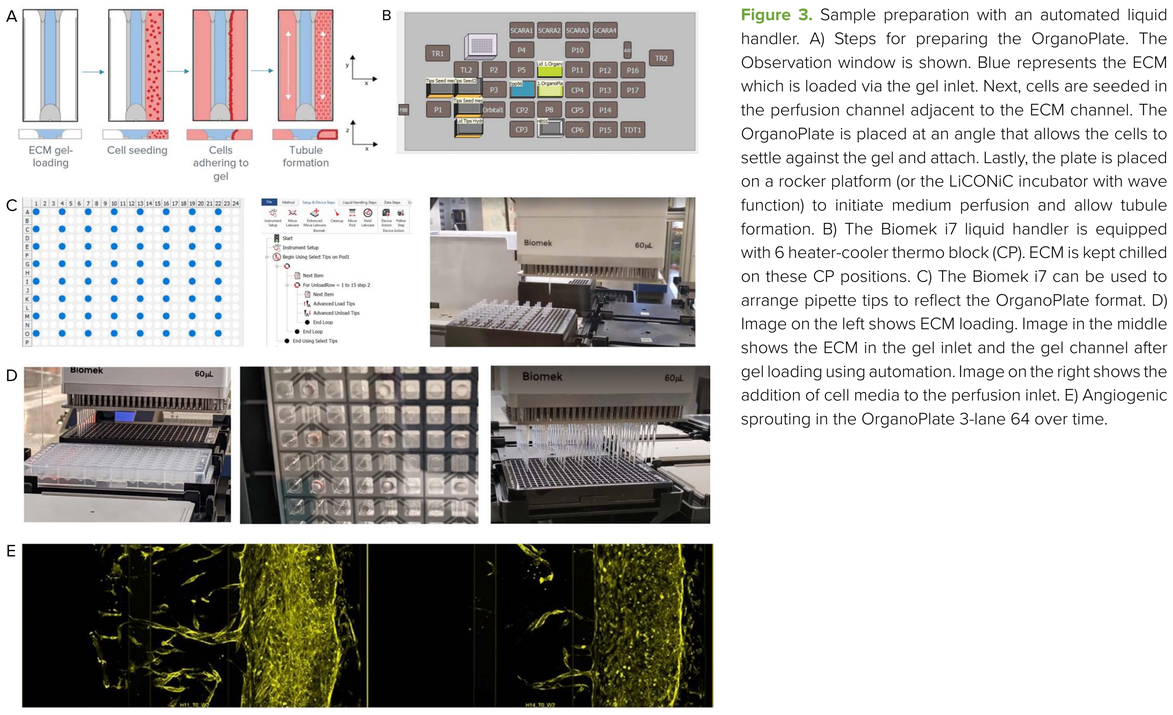


Figure 3. Sample preparation with an automated liquid handler. A) Steps for preparing the OrganoPlate. The Observation window is shown. Blue represents the ECM which is loaded via the gel inlet. Next, cells are seeded in the perfusion channel adjacent to the ECM channel. The OrganoPlate is placed at an angle that allows the cells to settle against the gel and attach. Lastly, the plate is placed on a rocker platform (or the LiCONiC incubator with wave function) to initiate medium perfusion and allow tubule formation. B) The Biomek i7 liquid handler is equipped with 6 heater-cooler thermo block (CP). ECM is kept chilled on these CP positions. C) The Biomek i7 can be used to arrange pipette tips to reflect the OrganoPlate format. D) Image on the left shows ECM loading. Image in the middle shows the ECM in the gel inlet and the gel channel after gel loading using automation. Image on the right shows the addition of cell media to the perfusion inlet. E) Angiogenic sprouting in the OrganoPlate 3-lane 64 over time.

Formation of angiogenic sprouts over time

The OrganoPlate 3-lane was used to model angiogenesis. Endothelial cells were seeded to form a tubule. Growth factors were added to the opposite channel to create a concentration gradient across the ECM. Angiogenic sprouts were observed growing towards the growth factor-containing channel (bottom) (Figure 4).

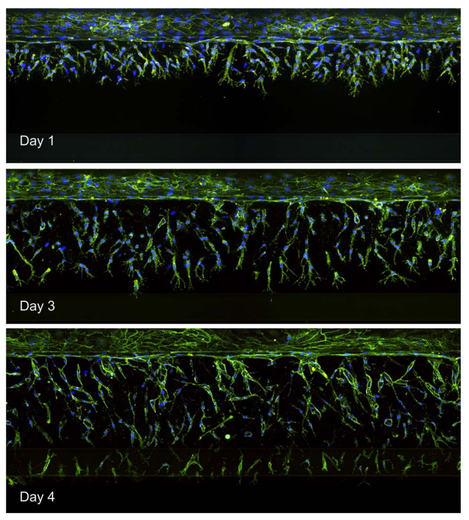


Figure 4. Images of endothelial cells (top channel) growing towards the bottom channel. Maximum projection images are shown representing angiogenic sprouts over time.

3D analysis to quantify angiogenic sprouts over time

Time dependence of angiogenesis was modeled over five 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 and the growth of angiogenic sprouts were characterized by multiple readouts including, total numbers of sprouts and nuclei, their intensities, volumes, distances between objects. The number (or average) of nuclei per individual sprout can also be determined (Figure 5). Secondary analysis was completed using Microsoft Excel.

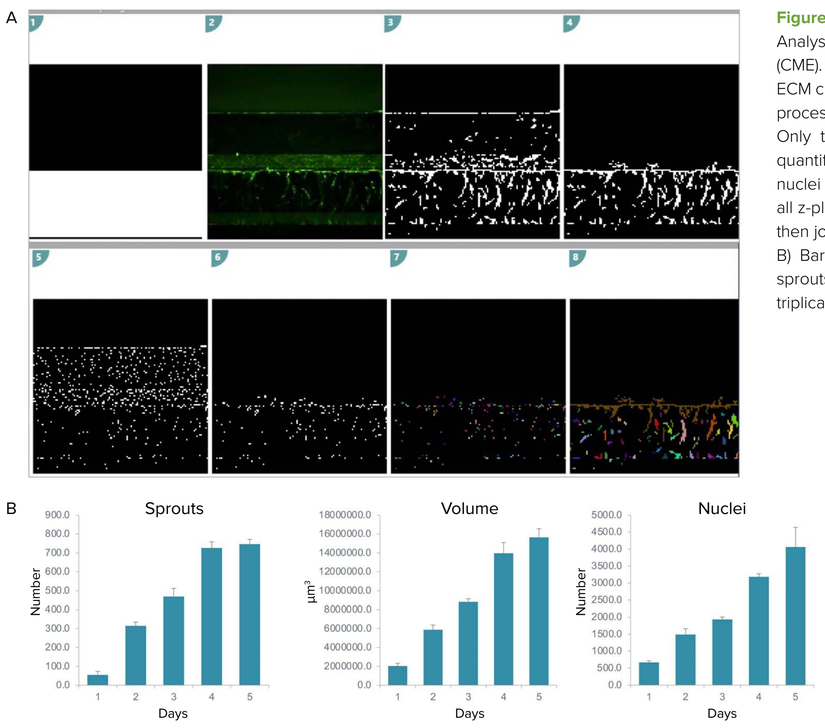


Figure 5. 3D analysis of angiogenic sprouts. A) Analysis set up in MetaXpress Custom Module editor (CME). Briefly, the analysis region which represents the ECM channel was created (white overlay). Image pre-processing was done to improve sprout segmentation. Only the sprouts within the analysis region were quantified (1–4). A similar set up was used to quantify nuclei (5–6). This segmentation was reiterated over all z-planes. The resulting segmentation masks were then joined to create volumetric measurements (7–8). B) Bar graphs representing quantitative growth of sprouts in 3D over time. The assay was performed in triplicates, error bars represent STDEV.

Conclusions

- We have developed an automated workflow for an OoC model of angiogenesis.
- Benefits of automation include the ability to increase assay throughput and improved data reproducibility.
- 3D imaging and analysis of angiogenic sprouts offers quantitative measures to describe complex biological processes.