3D-µFLUIDICS—Automated Long-term Culturing of Organs-on-a-chip

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Organ-on-Chip (OoC) is by nature a very diverse field covering topics that range from developmental biology, microfabrication, tissue engineering, organoids, and organ-specific models to regulatory aspects and disease modelling. The development of OoC follows six steps: (1) organ-level functions to be mimicked, (2) design & bioengineering, (3) qualification & bio validation, (4) standardization, (5) production, upscaling & high throughput, and (6) adoption by pharma community. Nowadays, the main challenge is to develop technologies allowing to standardize as well as parallelize and increase the robustness of OoC. A first response to this challenge is our maintenance system which automatically exchange culture media over long periods. We have successfully demonstrated its potential on mouse neurons during a 3-week differentiation period.

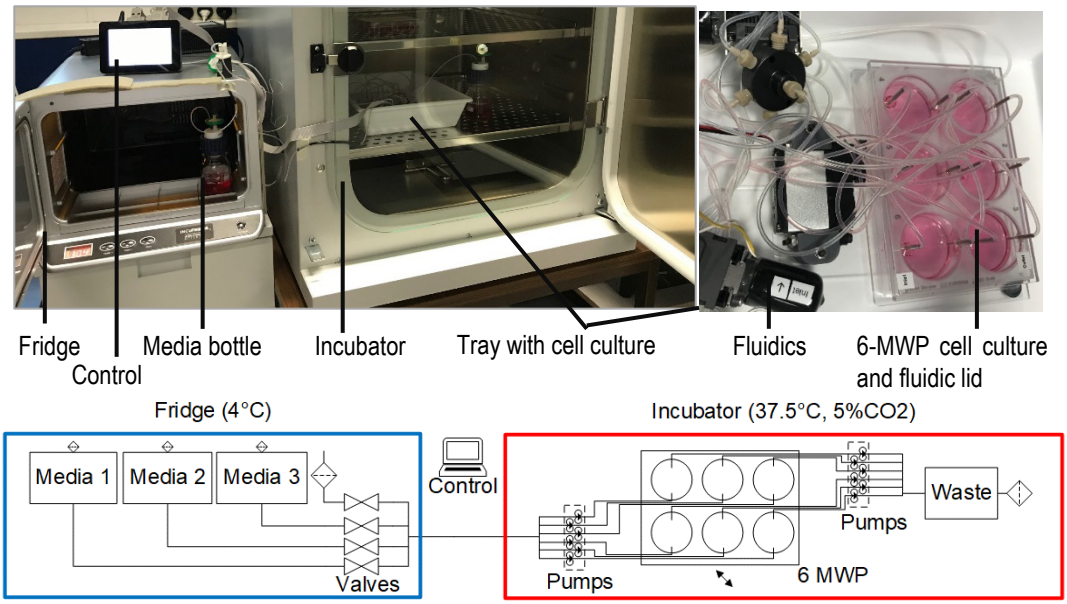

Figure 1: Automated culturing system with the buffer stored in the fridge and the cell culture in the incubator.

Micro-well plates ranging from 6 to 1536 assays have become a standardized platform in analytical research and clinical diagnostic laboratories including handling by robots. This international standard is increasingly adopted by Organ-on-Chip (OoC) systems for high throughput screening. There are two common denominators: base-plate and lid. All OoC systems, regardless of the biological model, require continuous maintenance as well as bio-sensing for analysis. For this purpose, we have designed a maintenance system and successfully demonstrated it with mouse neurons over the 3-week differentiation period.

Our maintenance system (Figure 1) pumps fresh media directly from a bottle stored in the fridge while the cells are kept in the incubator. The fluidic lid sitting on the well plate helps to refresh culture media. The frequency of media exchange can be chosen by the operator and is then performed fully automatically. The setup is designed, such that the operator still has the freedom to check the cell health on a microscope.

To validate our setup we have cultured mouse neurons over the 3 week differentiation period in a 6-well plate and performed an automated media exchange every second day. In a first step, the well plate is automatically tilted and about 2 ml of the 4 ml-working volume is removed. In a second step, the well plate is moved back into the horizontal position and 2 ml of fresh buffer is added. From time to time, the operator also moved the setup on to a microscope to check the cell health. Finally, Figure 2 shows the successfully cultured mouse neurons and its control. Both images show the presence of neurons (red), astrocytes (green) and cell's nucleus (blue) as it would be expected. The initial sterility has been achieved by autoclaving and ethanol sterilization. Furthermore, bottles have been equipped with filters. In a mass product, these sterilization steps will be replaced by gamma sterilization.

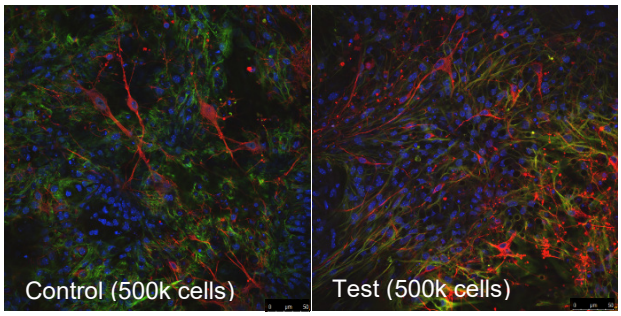

Figure 2: Immunocytochemistry stainings of mESC-derived neuronal cultures on glass. Neurons are stained with β-III Tubuline antibody (red). Astrocytes are stained by GFAP antibody (green). DAPI stains the nucleus of the cells (blue). Images were acquired on an Axiovert microscope with 40x oil objective.

After the successful test with mouse neurons, we will test our system with human neurons. Furthermore, we are going to implement an electro-chemical sensor to monitor the cell behavior with, *e.g.*, glucose consumption. The operator will then receive a direct feedback of the cell health without moving the cell culture on a microscope.

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