Microfluidic platform for continuous perfusion of transwell-based barrier models

Amanzhol Kurmashev[1], Julia A. Boos[1], Mario Modena[1] and Andreas Hierlemann[1]

[1]*ETH Zürich, Department of Biosystems Science and Engineering, Basel, Switzerland*

We present a microfluidic platform for establishing continuous perfusion in transwell-based *in-vitro* barrier models. We developed a poly(methylmethacrylate) (PMMA)-based microfluidic device, which allows for dynamic control of culture microenvironments in both, apical and basolateral compartments of cell-covered transwell inserts.

Transwell inserts are membrane-based cell-culture devices, which are widely used for transport studies across *in-vitro* barrier models. Transwells are easy to use configuration and offer direct access to both the apical and basolateral sides of the cell layer, however, the static nature of conventional transwell cultures poses major limitations in recapitulating physiologically-relevant microenvironments, which more closely resemble the *in vivo* situation. Moreover, the use of high-resolution imaging methods is difficult, as long-distance oculars are needed, and as there is a significant risk that the cell-covered porous membranes detach. The operation of transwell based barrier models in a dynamic microenvironment under continuous monitoring of the cellular responses at high resolution will significantly expand the experimental options of conventional transwell cultures.

We propose a platform that consists of a PMMA-based microfluidic chip, fabricated by a CNC milling process. The bottom surface of the chip contains a micro-milled channel of 200 µm height, in which the cell-coated transwells are inserted for high resolution imaging. The stable and leakage-free insertion of the transwell into the micro-milled PMMA-chip is realized with an elastic silicon gasket, which tightly seals the transwell insert. The basolateral side of the transwell is closed with a custom-designed polylactic-acid (PLA)-based lid. Perfusion control in the apical and basolateral compartments of the transwells is realized by using fluidic connections at the inlet and outlet ports of the PMMA chip and PLA lid. The chip features standard microscope slide dimensions (72 x 25 mm^2) and contains four individual fluidic units for transwell insertion and culturing.

In a proof-of-concept application of our device, we demonstrated its usability for high-resolution confocal imaging of a placental-trophoblast barrier. We performed immunofluorescent stainings of cell nuclei, the tight junction protein ZO-1, and the cytoskeleton marker F-actin, all of which confirmed the formation of a tight and confluent placental-trophoblast barrier on the apical side of the transwell insert.

Acknowledgements
This work was financially supported by the Swiss National Science Foundation (SNSF) within the framework of the National Competence Center in Research (NCCR) "*AntiResist", New approaches to combat antibiotic-resistant bacteria* under contract number 51NF40_180541.