Transepithelial permeation of bioactive molecules determined online by impedance-based monitoring in a co-culture setup

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Standardized assays to determine the permeability of epithelial and endothelial cell layers are commonly based on labeled tracer molecules [1,2]. Fluorescent-labeling of tracer compounds is preferred over radio-labeling to reduce laboratory safety requirements and more complicated waste management. However, covalent attachment of a fluorophore to a compound of interest might alter the physicochemical properties of the tracer significantly. Hence, a label-free permeation assay would overcome all the limitation described above.
This study describes a new assay strategy to measure the permeability of unlabeled, bioactive molecules that is based on impedance monitoring in a co-culture setup. Similar to the existing assays, the barrier-forming cell layer is grown to confluence on a porous polymer support so that the cell layer separates an apical and a basal fluid compartment. The unlabeled, bioactive tracer compound is added to the apical compartment at the beginning of the assay. Its time-dependent permeation into the basal compartment is followed with the help of a second cell population (sensor cells) that is grown on indium-tin-oxide (ITO) electrodes deposited on the bottom of the lower compartment like in regular impedance-based monitoring setups (cf. figure 1). The sensor cells are selected for their ability to respond to the presence of the bioactive tracer compound by cell shape changes that are sensitively reported by impedance readings of the ITO-electrodes. Thus, the arrival of the tracer in the basal compartment is reported by changes in impedance of the cell-covered electrodes in the basal compartment. In *proof-of-concept* experiments as presented here we have made use of sensor cells that endogenously express G-protein coupled receptors (GPCRs) and we used agonists to these receptors as tracer molecules to probe their transepithelial permeability. After the agonist (e.g. histamine), added to the apical compartment at the beginning, has permeated across the barrier forming cell layer into the basal compartment, it is detected by the response of the sensor cells to the activation of the corresponding GPCR. Dependent on the type of sensor cells and their individual signal transduction that is triggered by receptor activation, a characteristic time course of impedance is observed and analyzed with respect to the permeation rate of the tracer.
The new impedance-based readout detects the permeation of unlabeled receptor ligands and offers semi-quantitative information about the permeability of the probed ligands. The described label-free permeation assay combines GPCR target screening assays with ligand permeability studies.

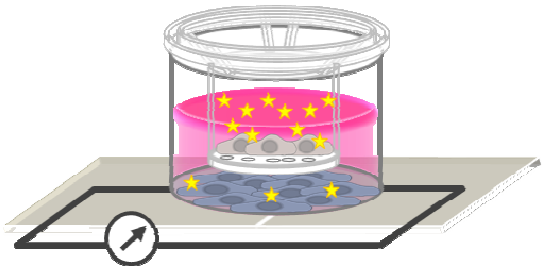

Figure 1: Experimental setup to probe the permeation of bioactive tracers across endothelial or epithelial cell layers by impedance readings of sensor cells in the basal compartment.

References:

[1] S.E. Sanders; J.L. Madara and D. McGuirk, *Epithelial Cell Biology*, 4 (1995), 25-34.
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