Application Note # 137

Drug interaction with Cell monolayer measured with MP-SPR

Cell monolayer was deposited on a SPR sensor slide. Multi-Parametric Surface Plasmon Resonance (MP-SPR) was used to measure drugs (propranolol and D-mannitol) interaction with the cell monolayer in real time and in controlled flow conditions. It was possible to distinguish between paracellular and transcellular drug absorption routes.

Introduction

In vitro cell assays are widely used during drug discovery. Traditionally these assays needs labelled materials and the analysis is based on post-detection with e.g. UV-, fluorescence or mass spectroscopy. MP-SPR enables real time measurements of interaction, in a constant and controlled flow conditions and without any labels. MP-SPR measures wide angle range and whole SPR curve is monitored which enables observing not only SPR peak minimum position but also other parameters as peak minimum intensity and total internal reflection (TIR). TIR region is sensitive to the optical properties of the media outside the evanescent field (bulk), whereas the main SPR peak angular position and intensity are highly sensitive to the optical properties of the media within the evanescent field (Fig.1). These additional parameters can be utilized to understand more about measured interaction.

Madin-Darby canine kidney (MDCKII) cells have low expression of drug transporters and little metabolic activity, which makes it a valuable cell line for studying passive drug transport processes. D-mannitol and propranolol are passively absorbed drugs but propranolol uses transcellular and D-mannitol paracellular absorption route.

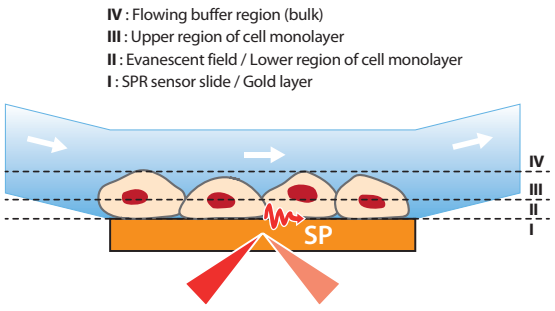

Figure 1. MDCKII cell monolayer was deposited on a gold sensor slide. Drug interaction with the cell monolayer was measured in constant flow conditions.

Materials and methods

MDCKII cells were cultured on the cleaned gold sensor slide (Fig.1). The trypan blue test was performed on cells cultured directly on the SPR sensor slides as well as treated polystyrene wells (used as reference surface). Optimization of the cell immobilization protocol was important, because the surface coverage of cells on the SPR sensor slide has influence on the shape of the full SPR angular spectra and this also negates non-specific interactions of the drug with pure gold.

Small molecule weight drugs propranolol HCl (259g/mol) and D-mannitol (182g/mol) interaction with cell monolayer was measured. Each test compound was diluted in a buffer composed of Hank's Balanced Salt Solution (HBSS) supplemented with 10 mM Hepes and adjusted to pH 7.4 (running buffer). For more details, please see the original publication [1].

Measurement was done with BioNavis SPR Navi™ 200-L instrument. At the beginning sensor slide was quickly inserted into the instrument so that the cell layer could not dry. The experiments were performed under a constant flow rate of 10 µl/min in 20˚C and using Angular Scan mode. MP-SPR measurements were compared to simulated full SPR angular spectra.

Results and discussion

Intermediate cell seeding density ($7x10^4$ cells/cm^2) and 3-4 days seeding time was found to be optimal for immobilizing a uniform, almost cluster-free and fully confluent cell monolayer. Morphology of the cells seeded directly on sensor slides and reference surface was found to be the same. Cell monolayers also remained confluent with hardly any changes in morphology after being exposed to the MP-SPR measurement and the flow conditions.

Full MP-SPR angular spectra was measured from pure gold coated SPR sensor slide and slide with immobilized MDCKII cell monolayer. Cell monolayer caused clear change in SPR peak angular position, peak intensity and total internal reflection (TIR) region when compared pure gold surface (Fig.2).

Measured drugs caused rather big change in SPR peak angular position even if they were small molecule weight drugs (Fig.3). SPR peak angular position remained at higher values after stimulating the cells with propranolol, whereas it returned to the baseline level after stimulation with D-mannitol (Fig.3). This suggest that part of the propranolol remains in the cell monolayer whereas D-mannitol is removed from the cell monolayer after stimulation

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despite of the concentration used. Propranolol response showed clear concentration dependency (Fig.3). Control measurement with both of the drugs and gold layer showed that measured interaction was drug-cell interaction and not drug-gold interaction.

SPR peak angular position versus main SPR peak minimum intensity from all repetitions of stimulation measurements with same concentration showed clear difference between plots of propranolol and D-mannitol (Fig. 4). Propranolol shows large changes in both angle and intensity (Fig.4A) whereas D-mannitol shows very small changes in intensity, leading to curves with a more horizontal appearance in these plots (Fig.4B). The same trend in the intensity versus angle plots was seen for all the concentrations tested [1].

The theoretical results as well as the measurements show that SPR signals measured with cells mostly from indirect response of the cells to the stimulus with drugs. The measurement and theoretical model results indicate that change in the SPR peak angular position reflects both drug accumulation and morphological changes in the cell monolayer, and that the change in the main SPR peak minimum intensity is mainly due to mass redistribution within the cells. The experiments also demonstrated that the MP-SPR method can yield much more information than a simple single-parameter SPR assay.

Conclusions

MP-SPR enables new type of cell based measurements for life science research. Drugs or other molecules interaction with the cell monolayer can be measured in real time and label free. MP-SPR measures whole SPR peak and much more information from interaction is get compared to single parameter SPR measurements. With MP-SPR measurement it was even possible to see difference in drug stimulation response due to different drug absorption routes.

References:

[1] Viitala et.al., PLOS One, 2013, 8 (8)

Recommended instrumentation for reference assay experiments
SPR Navi™ 200, 210A or 220A with additional wavelength (L)
Sensor surfaces: Au or other metal
Software: SPR Navi™ Control, SPR Navi™ DataViewer, SPR Navi™ LayerSolver, Additionally TraceDrawer™ for SPR Navi™

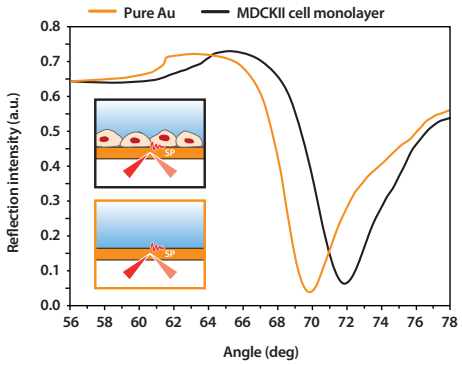

Figure 2. MP-SPR full angular spectra from pure gold sensor slide and sensor slide with MDCKII monolayer.

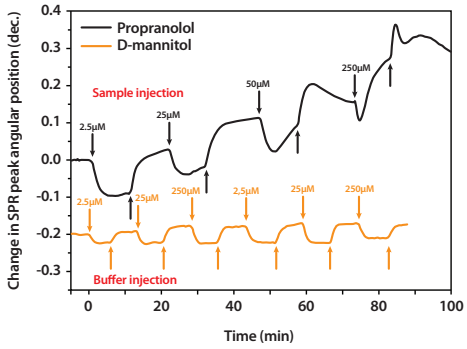

Figure 3. Change in SPR peak minimum angular position as a function of time during cell monolayer stimulation with drug molecules in different concentrations.

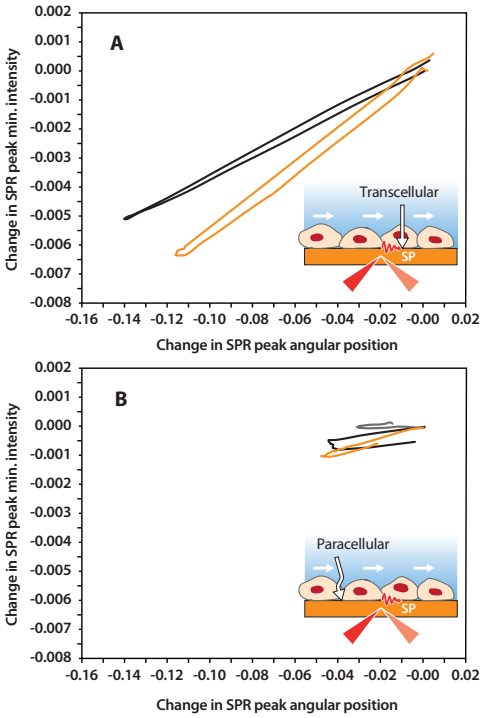

Figure 4. SPR peak minimum intensity versus SPR peak angular position during drug stimulation A) two replicates of propranolol 250 nm B) three replicates with D-mannitol 250 nm.

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