

# Cell Profiling Assays

## Multiparametric, Label-Free and Non-Invasive

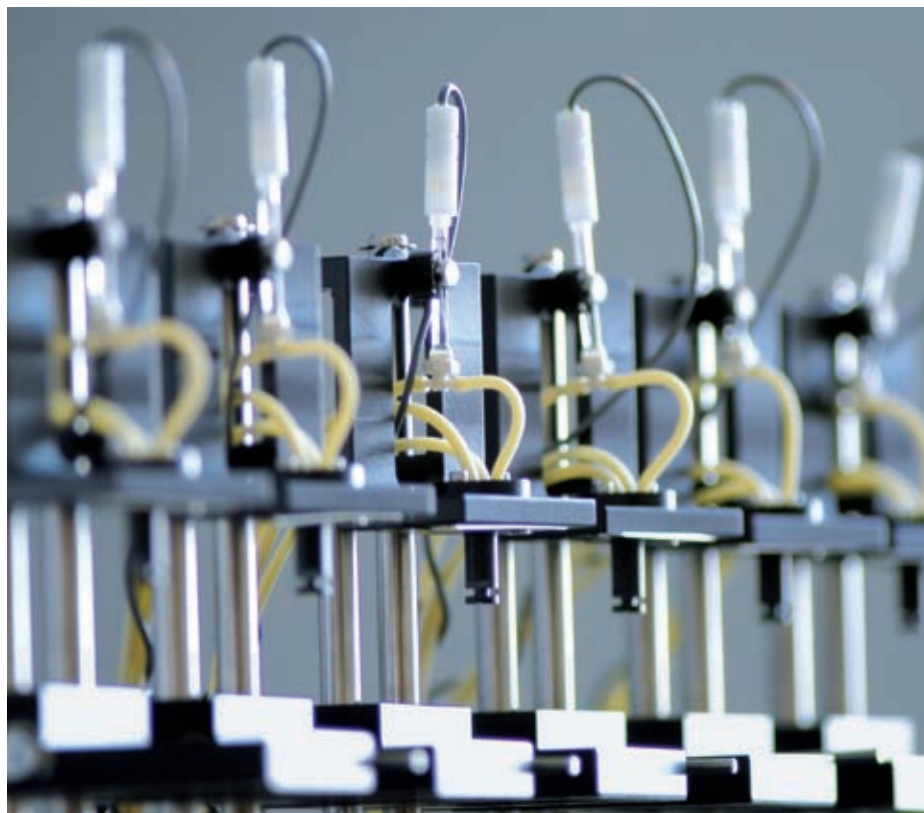


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**C**ell-based biosensors utilize the physiological responses of living cells to detect biologically active agents. They exploit the naturally evolved sensitivity of cells to a wide range of biochemical stimuli. We describe a biosensor system that simultaneously measures three metabolically relevant parameters, such as oxygen consumption of test cells, the extracellular acidification rate as well as cell adhesion, for cytological profiling of living cells.

Cytological profiling systems that are both multiparametric and high throughput still need to be developed. While pooling compounds can increase throughput, multiparametric data analysis is imperative.

We have developed a biosensor system that simultaneously measures three metabolically relevant parameters such as oxygen consumption of test cells, the extracellular acidification rate as well as cell adhesion. The readout is continuous for up to several days. The technology, which can be used with many different cell types, including primary cell cultures, is easy to use and allows non-invasive and continuous observation of the effect of a test substance on the cells' metabolism. The sensor system is label-free, meaning cells are not affected by the actual measurements since no cell staining is involved. Concentration dependence or regeneration effects can also be investigated. Besides phenotypic screening and target validation, other areas of application include research in cell science and drug discovery.



The analyzing system

### Cytological Profiling

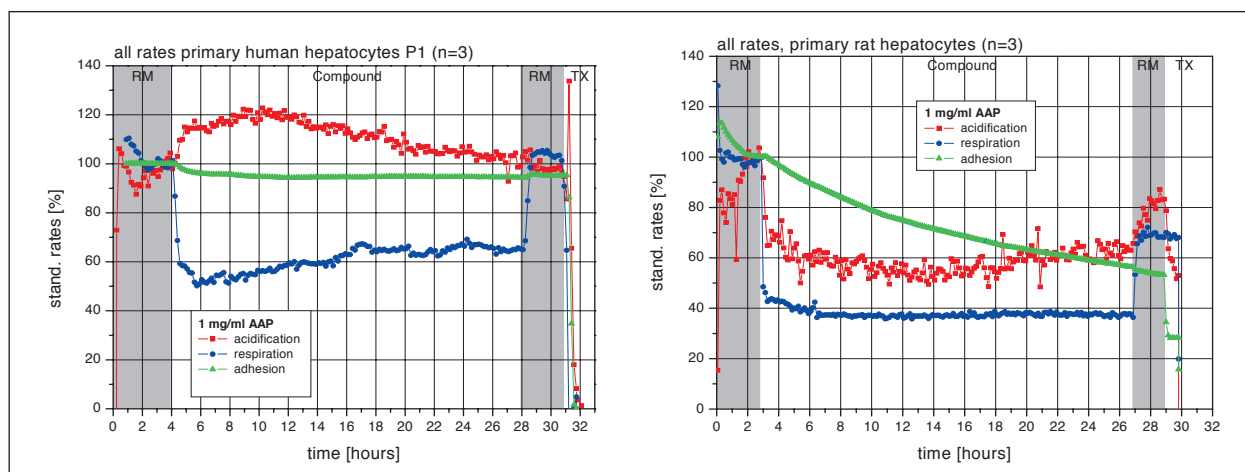
Drug candidates can activate or inhibit metabolism. Activation is associated with increased glucose turnover and oxygen consumption, and with increased excretion of acidic breakdown products. Reactions in the cell such as signal transduction and the stimulation of membrane-bound receptors (G-protein-coupled receptors, tyrosine-bound receptors or ion channels) require energy. The steps involved in the underlying reaction cascades are directly or indirectly dependent on ATP. Similarly, inhibition of metabolism is accompanied by a reduction in the excretion of acidic breakdown products and usually a reduction in oxygen consumption. In addition, inhibition with adhering cells is mostly associated with a change in attachment behavior or membrane stability. The sensor system can detect a drugs' influence on cellular metabolism by measuring the acidification rate, the depletion of oxygen in the medium and cell adhesion.

### Phenotypic Screening

The sensor system is useful for phenotypic screening of compounds in cells without knowing their target and assaying for varying effects such as proliferation, apoptosis and altered cellular metabolism to identify anticancer compounds and compounds against metabolic diseases or diseases that affect immune functions. Following the selection of candidate targets a crucial prioritization process takes place during which the sensor system helps select those targets that warrant more in-depth analysis. Besides target validation, the biosensor helps identify compounds that enhance or inhibit the abnormal glycolytic bias of tumor cells, or that modulate fatty acid oxidation (obesity) and glycolysis and respiration (diabetes).

### Prediction of Human Hepatotoxicity

Predicting liver toxicity earlier in lead optimization is a major concern. Standardized HepG2 cells and non-immortalized



**Fig. 1: Primary hepatocytes from humans (left) and rats (right) were incubated with paracetamol (Compound). Shown here are the acidification rate (red), respiratory rate (blue) and adhesion (green) of the primary hepatocytes. RM (running medium, medium without test substance), stand. rates (standardized rates), TX (0.2% Triton X-100 in RM, to kill the cells and used as a test for the sensors, showing that the recorded data is valid and represent no artefact).**

primary human hepatocytes have been shown to be ideal biosensors for the analysis of lead compounds. Hepatotoxicity liabilities can also be studied with conventional endpoint-based methods, but online and real-time observation has distinct advantages, which become apparent when comparing primary human hepatocytes and primary rat hepatocytes.



**Fig. 2: Silicon sensor chip with sensors for measuring pH and oxygen changes as well as monitoring adhesion.**

Rat and human test cell lines were cultivated in special medium without carbonate buffer on collagen coated sensor chips. Paracetamol (acetaminophen (AAP): IC50 19mM), which is known for its liver toxicity, served as the test substance. Paracetamol belongs to the group of non-steroid anti-inflammatory and antiphlogistic agents. The mechanism of action takes place via inhibition of cyclooxygenases (COX). Figure 1 first shows an initial run in medium without paracetamol (Running medium, RM). After about three hours (3h) 1 mg/ml of paracetamol (compound) is added. Shown here are

the acidification (red), respiratory rate (blue) and adhesion (green) of primary hepatocytes from rats and humans. Whereas only the respiratory activity is reduced in human hepatocytes, the rat hepatocytes clearly show stronger effects. Not only is the respiratory rate faster and more strongly reduced, but also the acidification rate is significantly inhibited even during the first 30 minutes of exposing rat hepatocytes to paracetamol. In addition, adhesion of the cells to the surface slowly but continuously declines.

The continuous process monitoring clearly shows the sequential influence on the cells' metabolism, which cannot be observed with end-point analysis methods. To investigate regeneration, only medium instead of medium + paracetamol is directed over the cells (from ca. 27h, grey field). Whereas the human hepatocytes show complete regeneration, the acidification rate in the rat cells reaches only 85% and the oxygen rate only 70% of the original activity. The adhesion shows no regeneration at all, which indicates irreversible effects on the cells. While the human cells could be used for further tests, the rat hepatocytes have undergone irreversible damage due to paracetamol.

### Multiple Applications in Research

Besides phenotypic screening, target validation and drug discovery, the sensor system has multiple applications in research. One example is stem cell research. Stem cell differentiation is characterized by a change in the ratio of mitochondrial respiration and glycolysis.

By measuring oxygen consumption and extra cellular acidification, the sensor system permits predictions about the degree of stem cell differentiation, and thanks to the non-invasive and label-free analysis one is able to categorize the stem cells.

The sensor system can also be used to monitor receptor-specific activation from most GPCR's by measuring the impedance upon their stimulation. Contributors to the impedance measurements are changes in cell adherence, changes in cell shape and volume, and changes in cell-cell interactions. These will affect the flow of extra cellular and transcellular current and hence the magnitude and characteristics of the signal measured. Each of these physiological changes can be linked to receptor stimulation through classical signaling pathways and can be monitored with the sensor system. Apoptosis is a strictly regulated, genetically encoded cell suicide that is morphologically characterized by severe alterations in cell shape, such as cell shrinkage and disintegration of cell-cell contacts. The sensor system monitors apoptosis-induced changes in cell shape in an integral and quantitative fashion with a high time resolution. Conversely, the sensor technology can also be used for proliferation assays.

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