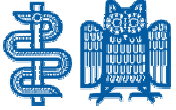




# Applications of Silicon-Sensor-Chip Apparatus to Determine Cell and Tumour Chemosensitivity *ex vivo*.



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## Introduction

In the last few decades numerous methods of assessing the chemosensitivity and chemoresistance of tumour tissue and for drug screening have been developed. Cell viability tests and clonogenic assays are important examples (Bellamy, 1992). In recent years the introduction of silicon-sensor technologies have opened new test possibilities. Sensor chips are able to detect metabolic changes in living cells by transforming chemical signals into electrical signals that are measurable in intensity and time. Silicon technology has made considerable progress, leading to the construction of arrays with several sensors placed together on one chip (Wolf et al., 1998). Such a multisensor array permits the simultaneous online measurement of metabolic parameters such as 1) pericellular acidification, 2) oxygen consumption and 3) cell adhesion (Ehret et al., 2001). The first parameter is connected with the extrusion of protons derived from metabolic (mostly anaerobic) activity. The second gives information about the metabolic pathways largely connected with mitochondria and therefore with cell respiration (Fig. 1). The third helps to clarify phenomena associated with the attachment of cells to substrate and with cell motility (changes of cell shape) and adhesion. For these purposes the array was constructed to include a sensor composed of interdigitated electrodes (IDES) that measure impedance (Ehret et al., 1998). In this study results obtained with a prototype of the "Bionas 2500"® system (Bionas, Inc.), a sensor-chip apparatus which is able to measure online reactions of permanent cell lines under the influence of metabolic inhibitors and cytostatic agents, are presented and discussed in the face of medical applications (Fig. 2).

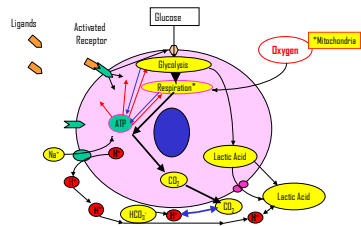


Figure 1

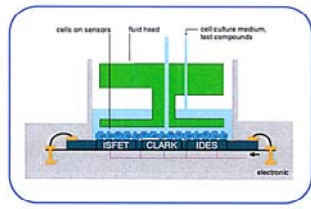


Figure 2

- ISFET - Acidification (glycolysis, respiration)
- CLARK - Oxygen consumption (respiration)
- IDES - Adhesion (confluence)

**Material and Methods. Reagents:** The medium F12 (Gibco) was used as a standard medium to which was added 10 % FCS (PAA) and Glutamine (Gibco), Penicillin-Streptomycin (Gibco), Geneticin (G418; Sigma). For taking measurements with the sensor-chip apparatus a low-buffered medium such as un-buffered F12 (Gibco) is required. Sodium fluoride and cyanide, both purchased from Merck, were used as metabolic inhibitors. As an example of anti-cancer drugs Taxol® (Pachitaxel, Bristol-Myers Squibb) was chosen. **Cell lines:** Several permanent cell lines were used for these studies. However, the results presented here refer only to the line CHO purchased from ATCC (cat. n° CRL-1984). **Measurement of cellular metabolic activity:** The cells were placed in suspension either directly on the chip or on an insert (Costar) and cultured at 37°C for approximately 24 hours outside of the sensor-chip apparatus. After this period, the cells were transferred to the micro-chamber for further experiments and measurement. The fluidic system provides the micro-chamber with a medium, removes waste, and works in a stop-and-go rhythm. In the present experiments the average length of the stop phase was 7 minutes and that of the go phase 3 minutes. This schedule can of course be adapted according to the necessities of object, established empirically in each case. Measurements were taken during the stop phase (Fig. 5). The experiments were finished with the application of Triton X-100, a detergent that killed the cells and thus served as a final vitality check. The function principle of the sensor-chip apparatus is explained in detail elsewhere (Mestres et al., 2005). **Microscopy:** Cell arrangement and growth patterns were studied with DAPI and fluorescence microscopy. For scanning electron microscopy [standard and environmental (wet mode) conditions] an ESEM FEG 30 (Fei/Philips) was used. Cells growing on inserts were prepared for electron microscopy and semi-thin sections (0.5–1 µm thick) were stained with methyl blue and Azur II.

## Results

### Morphology of CHO cells growing on sensor-chip surfaces and on insert membranes

Suspensions with a cell concentration of 5x10<sup>4</sup> attach to the free chip surface (Fig. 3a, b), covering it almost completely. The cells attach throughout, without preferences for special areas such as the sensors. Mitotic figures were observed in all stages of culture. No differences in growth patterns were observed between cultures in the sensor apparatus or in an oven for cultures. With SEM shape and attachment of cells can be seen (Fig. 3c, d). Fine surface profiles of cells cannot be seen in wet mode. Cells growing on inserts show a pattern of attachment and growth similar to that observed with the chips. Fine filopodia and sometimes the whole cell body can move through the holes of the insert membrane (Fig. 4).

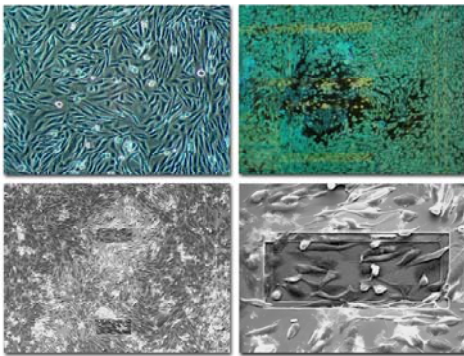


Figure 3

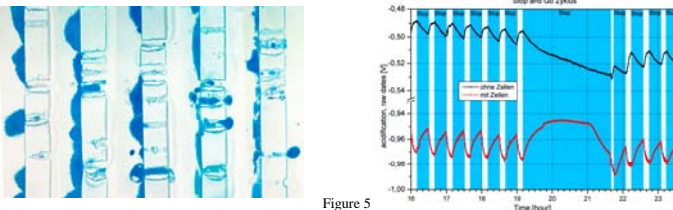


Figure 4

Figure 5

### Metabolic activity of cells under control conditions

CRL cells incubated in the micro-chamber with a standard medium show relatively stable metabolic activity (Fig. 5).

## Acknowledgements

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## Effects of NaF

The cells respond to NaF immediately, and recover quickly when the medium is replaced by a standard medium, i.e. without NaF. The response pattern is different for acidification (metabolism) and oxygen consumption. While acidification rates fall to a considerably low level, the respiration rates do too but to a lesser degree (Fig. 6). A remarkable point is the slight increase of oxygen consumption at the beginning of NaF treatment. After that the respiratory activity decreases progressively, reaching its lowest level during the phase with the standard medium. Long term measurements show that NaF effects are only partially reversible: after repeated NaF applications the cells still recover but less and less completely (Fig. 6).

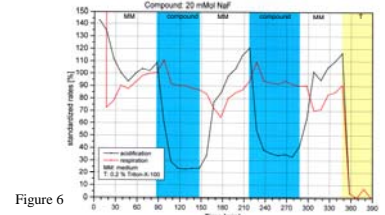


Figure 6

## Effects of cyanide (KCN)

As was expected, responses to cyanide show a different pattern. At a concentration of 5 mM cyanide causes a substantial fall in respiration. With the application of fresh medium (cyanide-free) the cells recover and are able to respond again. However, it seems that cells cannot regain the initial rates measured before treatment with cyanide. In contrast, the curves of acidification sporadically show discrete increases during the stop phase (Fig. 7a). At a concentration of 20 mM respiration rates diminished considerably, but in this case no recovery was observed when fresh medium was applied. The acidification rates can increase slightly (15-20%) after cyanide application (Fig. 7b).

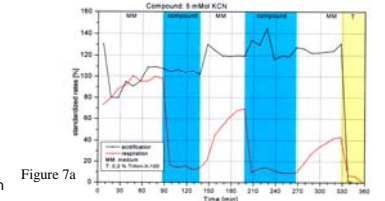


Figure 7a

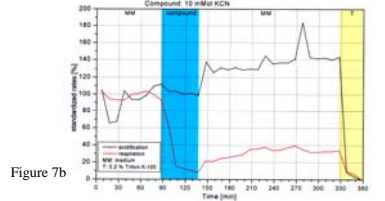


Figure 7b

## Effects of Taxol

The CHO cells respond to Taxol after a certain period of time (Fig. 8). For 1-2 hours controls and cells treated with Taxol show similar rates of acidification. But after this interval acidification diminishes steeply in accordance with the dose applied (Fig. 8a). Respiration diminishes immediately at the beginning of Taxol application and then remains stable for approximately two hours. After that the decrease continues more rapidly (Fig. 8b). In both cases, after approximately 20 hours of Taxol application the level of metabolic activity was extremely low but still detectable. After the Triton-X 100 application the curve falls to zero, confirming the fact that until then vital cells had still remained (Fig. 8).

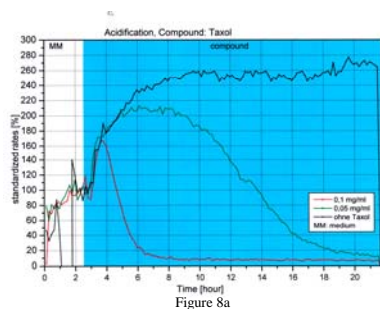


Figure 8a

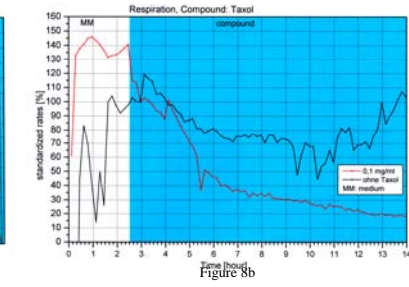


Figure 8b

## Conclusions

The experiments presented here show that the cells can be placed directly on the chip or, using a transfer aid, on the inserts. In both situations consistent measurements can be performed. The inserts allow the cells to be re-cultivated after a stage in the sensor-chip device and to be prepared for further studies or repeated sensor-chip analysis. The sensor chips can be cleaned after use, but very often it is difficult to eliminate organic products (medium proteins, cell debris, etc.), particularly when the cells grow directly on the chip because of conditioning of the surfaces, so sensor-chips should be considered as one-way material. In the case of cell cultures on inserts, however, conditioning is practically negligible and multiple use of chips can be recommended. Nevertheless, careful examination of such chips before an experiment is necessary.

Our results show that our sensor-chip system is able to detect changes in cellular activity after the application of metabolic inhibitors. Sodium fluoride is a well-established inhibitor of the enzyme enolase, influencing in particular anaerobic metabolic pathways (Hafner, 2000, Eklund et al., 2004, Mestres et al., 2006). Our results are consistent with this biochemical background. Cyanide was included in our experimental design because of its ability to inhibit cell respiration, reducing aerobic activity with a corresponding depletion of cellular ATP (Eklund et al., 2004). The oxygen sensor after Clark yields solid results without interference with other sensors included in the chip.

Taxol was selected in view of studies in progress in our laboratory on the chemosensitivity of human tumours. Taxol is an anti-cancer drug that, in contrast with colchicine or Vinca alkaloids, stabilizes the microtubules and acts directly on the centrosomes (Abal et al., 2003). The response to Taxol is dependent on cell cycle, however, in our non-synchronized cultures important effects on cell metabolism were observed. The patterns of response were different in metabolism (glycolysis) and cell respiration. A precise explanation of such responses is not yet possible. It is probable that the cytoskeletal damage due to Taxol can alter the course of many enzymatic processes located in the cytosol. The diminution in respiration rates observed *ab initio* suggests that not only the cytoskeleton but also the mitochondria might be altered.

The question of reversibility of drug effects as seen with a sensor-chip device has not yet been directly addressed in the literature. Our preliminary observations show that reversibility is an important criterion to be included in future measurement schedules, particularly regarding the assessment of the chemosensitivity or chemoresistance of tumours. It is a question that is also faced by studies in progress in our laboratory that aim to develop a useful test for configuring individualized chemotherapy on the basis of silicon-based technologies (Mestres et al., 2005, 2006). Finally, it should be mentioned that this technology can find a broad spectrum of applications in the field of ligand-receptor interactions because of the metabolic implications of the cascade of enzymatic reactions connected with receptor activation (Hafner, 2000, Mestres, 2001).