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Preclinical report

Approach to a multiparametric sensor-chip-based tumor chemosensitivity assay

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Although not widely practiced by oncologists, *in vitro* tumor chemosensitivity assays (TCA) have proved to increase the lifetime of tumor patients in prospective clinical trials. By individualizing cancer therapy, they can support the clinician's decision which is usually based on empirically retrieved data and thereby prevent inadequate chemotherapy. We present the first results of a new sensor-chip-based technology which might be useful for a multiparametric TCA. In particular, the aspect of dynamic on-line data generation on intact cellular specimens is a major difference to alternative assays. A series of experiments has been performed on cell lines and human tumor explants. Cell cultures and tumor tissue explants were placed on miniaturized silicon and glass sensor chips. The sensor data currently analyze metabolic profiles (rates of extracellular acidification and cellular oxygen consumption) and changes in cell morphology (monitoring of electric impedance). With the cell lines, drug-associated cellular signals have been detected with all three parameters, while primary explants so far caused metabolic responses only. In particular, cellular respiration or mitochondrial activity seems to be a most sensitive indicator of acute cytotoxic effects. The experimental results were achieved using different test versions. Besides giving a status report, the theoretical potential and current problems of sensor chip technology in TCA is discussed. [© 2001 Lippincott Williams & Wilkins.]

Key words: Cell adhesion, cell metabolism, chemosensitivity assay, sensor chip, tumor tissue culture.

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Introduction

Rational cancer therapy is based on cyto-pathological and histo-pathological classification of tumor specimens. There is, however, a great variability in the response of patients to therapy, even if tumors have been classified identically. Individualizing non-surgical forms of tumor therapy, particularly chemotherapy, therefore is a challenge in oncology. It was recently shown in first prospective clinical trials that *in vitro* chemosensitivity assays can provide data which can be relevant for an adequate choice of chemotherapy, ultimately improving the mean survival time of tumor patients.[1] Here we report our first experiences with a different methodology, which might be feasible for chemosensitivity tests in a clinical setting. This methodology is based on on-line data acquisition using multiparametric microsensor devices. A viable tumor specimen derived from a surgical specimen is placed immediately on microsensor chips. A fluidic system for the supply of culture media/drug solutions and for the realization of metabolic measurements is connected to the chips. The precise maintenance of *in vitro* microenvironmental conditions is also necessary for the cellular specimen. After connecting the sensor chips to the fluidic system, measurements are started implying a short-term culture lasting for up to several days. The cellular parameters currently amenable by sensor chip readings are metabolic activity (rates of extracellular acidification and cellular respiration) and morphologic properties (particularly cell–cell and cell–matrix adhesion). This combination of microsensors in one system has not been realized previously. We are pursuing this novel kind of chemosensitivity assay, because it offers the following advantages:

(1) By continous data acquisition lasting for up to several days, both acute cytotoxic effects and long-term effects on cell proliferation and cell death should be detectable.
(2) Monitoring an individual specimen before, during and after drug administration allows the determination of relative changes rather than single endpoint data. Thus, exact knowledge of cell numbers is not required.
(3) The fluid system provides a continuous supply of nutrients, growth factors and elimination of metabolic waste products in a way that is close to physiological conditions; periods of drug exposure can be well defined.
(4) Small-scale sensor chip areas require very small amounts of cellular specimen (10^4 cells or less).

McConnel *et al.* developed and described a microsensor-based device, called the Cytosensor Microphysiometer®,[2] that was reported to be useful in the assessment of chemosensitivity of different human tumor cell lines.[3,4] The instrument integrates up to eight channels, and detects sensitively and continuously the rate of extracellular acidification of cellular specimens. As, according to published results, it seems suited for clinical applications, we are encouraged to investigate the possible use of additional parameters accessible by different sensor chips.[5–7]

Materials and methods

Experimental setup

Two microsensor-based test systems for the dynamic analysis of cellular responses have been developed by our group. One of them is equipped with transparent glass chips (GC), the other one with silicon chips (SC). The systems accommodate both adherent cell types and cell suspensions/tissue explants. Figure 1 shows a schematic cross-section of the chip and culture area found in both prototype versions.

The GC version includes two channels of measurement, corresponding to two chips and two cell cultures (Figure 2). It was constructed for use on the stage of inverted light microscopes. The device is thermostatted and connected to a perfusion system, which is driven by a peristaltic pump and controlled by pinch valves. Importantly, the fluidic setup also provides a high ratio between cell number and the volume of the surrounding cell culture medium by adjusting a small height of the cell culture space of 0.2–0.8 mm. The outer dimensions of the glass chips are $24 \times 33.8 \times 0.5$ mm, the active glass chip area (i.e. the area of the sensor chip dedicated to the cell culture) is about 100 mm^2. The glass chip was realized by hybrid integration of a 3.5×7.2 mm silicon chip containing H^+-ion-sensitive field effect transistors (H^+-ISFETs) into the 24×33.8 mm glass chip containing interdigitated electrode structures (IDES), pO_2 sensors and temperature sensors.

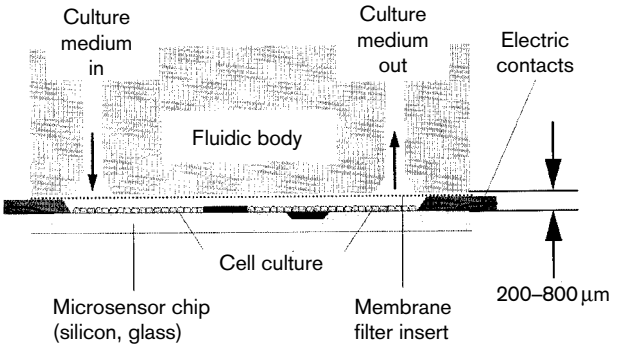

Figure 1. Cross-section of sensor/culture area.

The SC version is a single-channel device. The chip is placed in a ceramic standard 40-pin IC socket. Electric connections are made by bonding wires. The chip is subsequently encapsulated by a biocompatible epoxy resin, thereby creating a containment for cell culture (Figure 3). The SC device is placed inside a cell culture incubator and connected to a fluidic system as the GC version. The outer dimensions of the silicon chip are 7.4×7.4 mm and the active silicon chip area is about 15 mm^2. The chip contains four ISFETs, two IDES and one temperature diode. The inclusion of a planar oxygen sensor within the SC version is in preparation. Based on the single-channel SC version we constructed a six-channel device accommodating six sensor chips and cell cultures, which is now ready for parallel tests (Figure 4).

An additional sensor chip containing merely one IDES sensor was used without any fluidic system for measurements inside the cell culture incubator. The impedance readings of eight sensor chips (corresponding to eight cellular specimens) were acquired in parallel by a signal multiplexer.[8] This multiplexed device is called the IDES version.

Sterilization of both the sensor chips and fluidic system is a prerequisite. Depending on the version used, the sensor chips have been autoclaved (GC and IDES version) or treated with 70% ethanol as disinfectant (SC version). The fluidic system in both the GC and SC version was sterilized for 1 h with a solution containing sodium hypochlorite, followed by washing with deionized, sterile water and culture medium. After sterilization, cells were seeded on the chips, which were cultured in the incubator until used

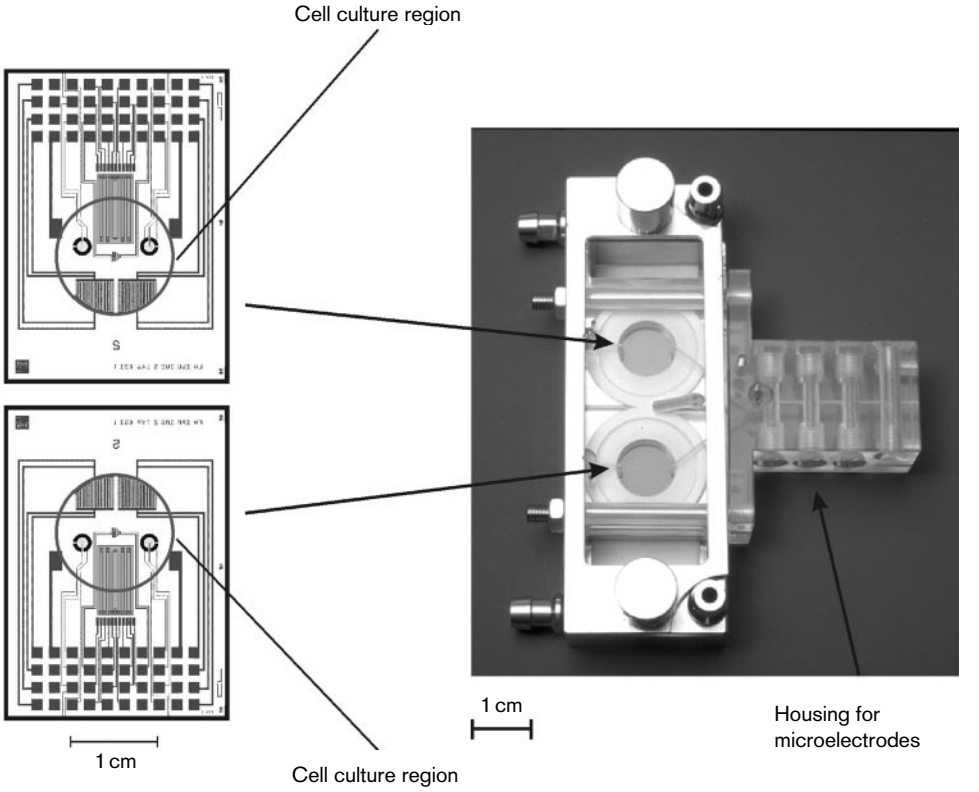

Figure 2. GC version.

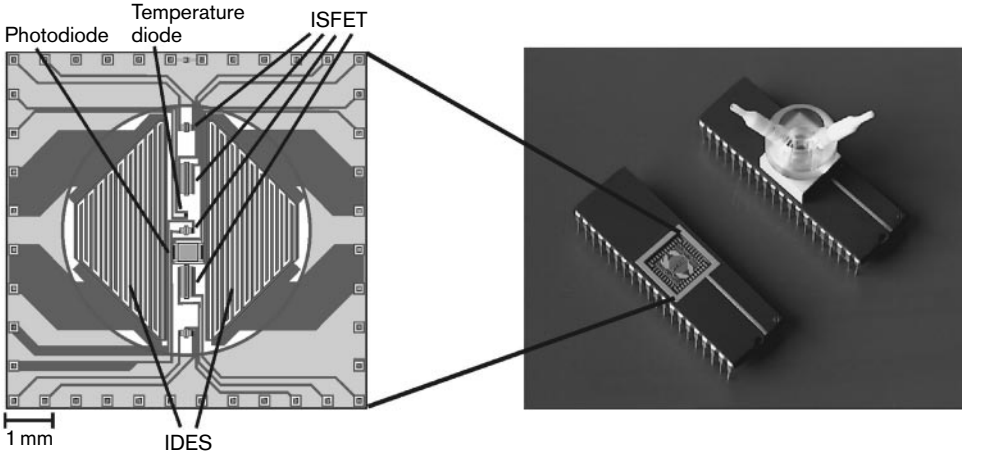

Figure 3. SC version.

for measurements. At the end of each experiment, cells were killed using 0.1% Triton X-100 in order to obtain baseline sensor signals (signals without viable cells).

Sensors

The principles of the function of microsensor structures have been described in detail.[8,9] A brief description follows. Extracellular recording of cellular signals by pH-ISFETs is associated with ion fluxes across cell membranes. pH sensors, by measuring the rate of extracellular acidification, reflect cellular catabolic pathways resulting in a net proton production.[10] Proton selectivity is achieved by the deposition of the amphoteric gate insulator material silicon nitride. The dimensions of the sensitive gate areas can be adapted to specific purposes. For the measure-

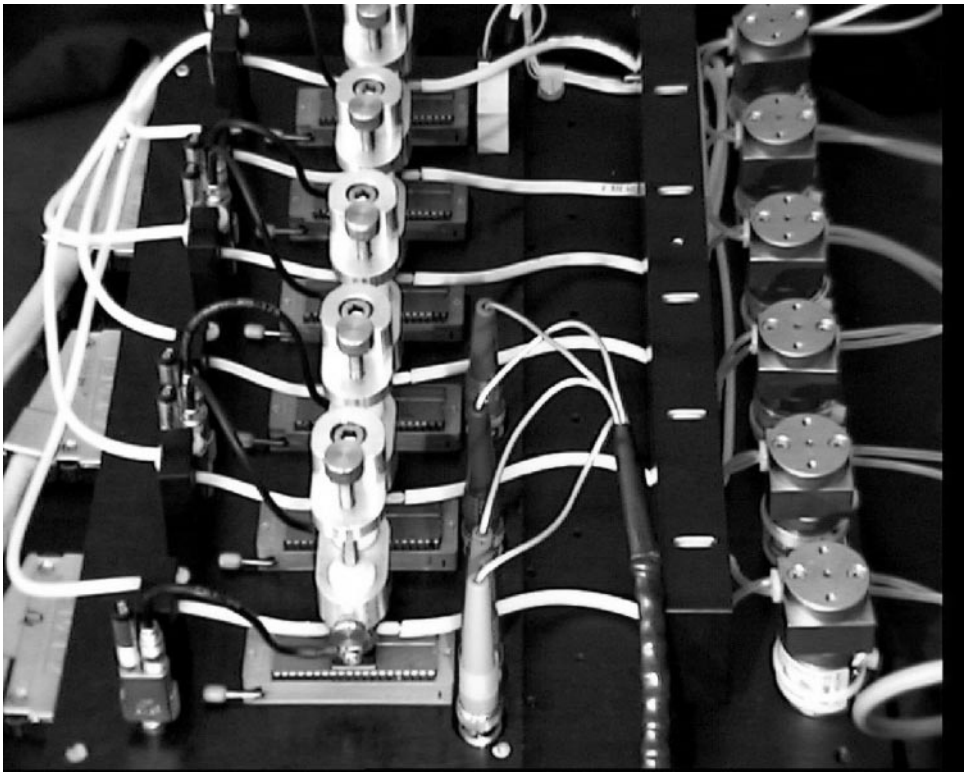

Figure 4. Six channel device for the SC version.

ments experiments described here a gate insulator area of 20×100 μm was used. Metabolic activity is detected by the sensors when the flow of the fluidic system is halted and the cells begin to change the composition of the surrounding cell culture medium. After a time interval of 5–15 min, when the pH has dropped by approximately 0.1–0.2 decades, the flow is started again. The drops during subsequent intervals are graphically displayed and the relative changes can be interpreted.

Records of cellular oxygen consumption (reflecting mitochondrial activity of the cellular specimen) were performed using either planar microelectrode structures or miniaturized Clark-type oxygen electrodes (both developed in cooperation with Kurt-Schwabe-Institut, Ziegra-Knobelsdorf, Germany) positioned in the fluid exit port of the GC device. The same mode of raw data evaluation was used as for pH data (halting the flow of the fluid system and calculation of the resulting change rate of pO_2, with a drop of pO_2 by approximately 10–20% in each interval). The chip area required by one oxygen sensor is about 1 mm^2.

Impedimetric analysis of adherently growing cells by IDES provides information on cell number, cell morphology cell–cell adhesion and cell–matrix adhesion. An alternating current is forced to flow by applying a small alternating voltage to pairs of IDES. Due to the insulating properties of cell membranes, living cells increase the impedance of the system. Any structural change within cellular layers resulting in a change of electric impedance of the system is monitored by the sensor. However, the principle of measurement works well only if cellular membranes are in immediate contact with the electrode structures, particularly if epithelial cell monolayers are formed. The impedance is a complex figure, which can be specified in several, but equivalent ways. We selected an equivalent circuit with a resistance and a capacitance in parallel (R_{par} and C_{par}) and we use these parameters—most often the capacitance for the description of results. However, there is no modeling of the electrode–cell interface behind this equivalent circuit.

Cell culture media and chemicals

The culture medium used for cell lines was RPMI 1640 (Life Technologies, Karlsruhe, Germany), supplemented with 10% FCS (Seromed; Biochrom, Berlin, Germany) and 50 μg/ml gentamycin (Gibco). This medium was buffered with 25 mM HEPES. For S-180

cells and S-180dox cells the medium was additionally supplemented with 2.5 μg/ml amphotericin. As the rates of extracellular acidification are dependent on the buffer capacity of the culture media, we modified the culture medium used for measurements (running medium) by omitting strongly buffering species like bicarbonate and HEPES, and decreasing the FCS supplementation to 5%. Sodium chloride was added at 1.4 g/l to compensate for the osmolarity as a result of the absence of sodium bicarbonate. After all these additions, the pH was adjusted to 7.30 with 1 M NaOH and the medium was filter-sterilized (0.22 μm; Millipore, Bedford, MA). Prior to use, the medium was heated to 37°C for 1 h.

The medium used for the primary explants was also RPMI 1640 (Gibco), supplemented with 15% FCS (Seromed), 1 μg/ml insulin (Roche Molecular Biochemicals, Mannheim, Germany), 2 g/l sodium bicarbonate, 5.96 g/l HEPES and 50 g/ml gentamycin (Gibco).

Doxorubicin (Adriblastin®) was purchased from Farmitalia Carlo-Erba (Freiburg, Germany). Doxorubicin is a DNA-intercalating and -distorting (Topoisomerase II inhibitor) zytostatic antibiotic of the group of anthracyclines with a tetracyline moiety.[11] With microsomal cytochrome P450 reductase, doxorubicine produces semichinon radicals which are responsible for fractures of single and double strands of DNA and for exchanges of sister chromatids. The dose is limited by its cumulative cardiotoxicity and the myelosuppression.

Chloroacetaldehyde (CAA) was purchased from Sigma (München, Germany). CAA is an alkylating agent that originates *in vivo* from the bifunctionally alkylating cytostatic agent ifosfamide by cytochrome P450-mediated enzymatic oxidative dechloroethylation pathways.[12] Ifosfamide is a common agent for the treatment of solid tumors. CAA acts on a broad range of cellular targets, including nucleic acids, proteins and membrane phospholipids.[13]

Cell lines and cell culture

The human colorectal adenocarcinoma cell line LS 174 T (ATCC CL l-188) has been characterized in our laboratory.[14] S-180dox cells were derived from S-180 cells, a mouse muscle sarcoma, by incubation with doxorubicin. Thereby, an overexpresssion of glutathion S-transferase (GST)-π was induced and, compared to the orginal S-180 cells, a 340-fold resistance against doxorubicin was reported.[15] S-180 cells were cultured in HEPES-buffered RPMI 1640; for S-180dox cells the medium was additionally supplemented with 20 μg/ml doxorubicin.

Primary cultures

Surgical specimens from breast and lung cancer were directly delivered from the OR to the pathological laboratory of Professor Niendorf (Medea Forschungs, Hamburg, Germany) for histological diagnosis. The explants were cut into pieces of approximately 3 mm^2 and immersed in cell culture medium (transport medium). Then the explants were sent to our laboratory, where they arrived between 10–15 h after surgical removal. Immediately afterwards the further preparation of the tissue explants was started. Pieces were cut to a size of approximately 0.5–1 mm^2 and then put into collagenase/dispase (100 mg/ml) and DNase (Boehringer) (0.1 mg/ml) for enzymatical dissagregation for 15–30 min. Depending on the histological character of the tissue, this step was repeated several times until cells were washed out. Single cells were isolated by three to five turns of centrifugation at 200 *g* for 5 min. If necessary, tumor cells were separated from blood cells using a Ficoll-Paque gradient (density 1.077 g/ml; Pharmacia, Freiburg, Germany). Subsequently, they were then been placed on the sensor chips, and allowed to settle and accommodate to *in vitro* conditions for another 10–15 h. Measurements were started approximately 24 h after *ex vivo* explantation.

Cell lines were seeded on the sensor chips at a density of 2×10^3 cells/mm^2 and subsequently precultured for approximately 48 h. At this time they formed confluent monolayers. See Figure 5.

Results

The objective of the experiments was to evaluate whether different types of microsensors can meet the expectations outlined in the Introduction. We are aware of the fact that a broader data base will be necessary for a thorough evaluation of the described technique. From this point of view, the results presented here should be regarded as a status report, not a final summary.

LS 174 T

Figure 6 shows the result of two applications of CAA (50 μM) on a LS 174 T cell culture within the SC version. CAA was added twice at 50 μM, allowing for recovery after drug withdrawal. Concentrations in that range are found in plasma of tumor patients under chemotherapy with ifosfamide.[16] Extracellular acidification rates respond by small decreases to drug exposure, while cellular respiration declines pro-

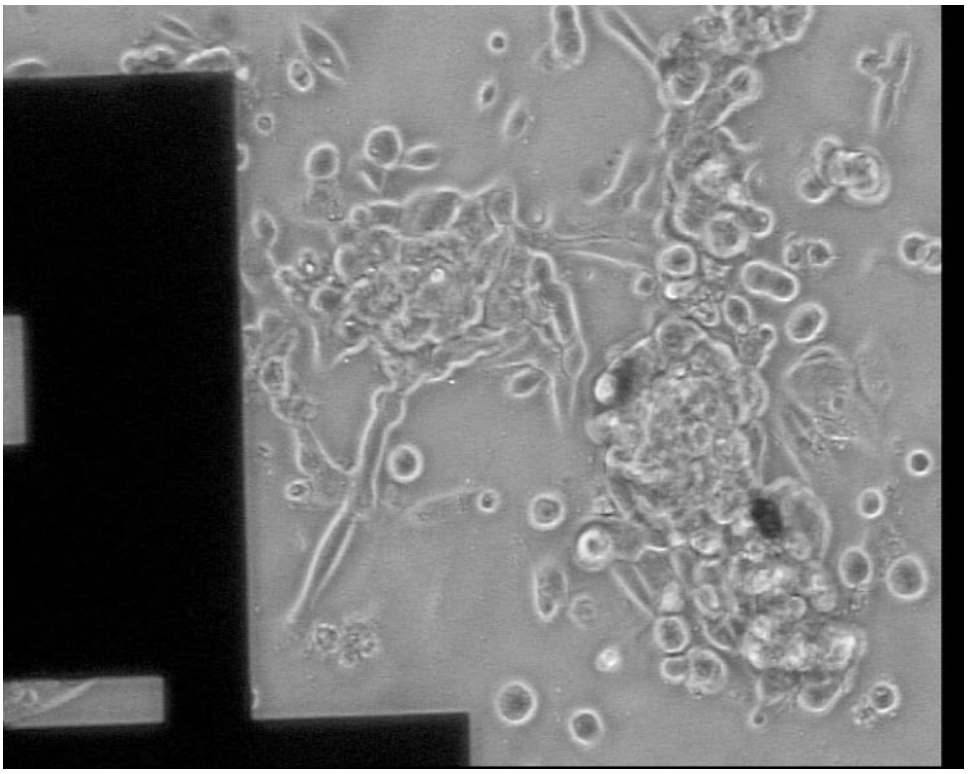

Figure 5. Tumor cells on an IDES GC 5 days after surgical removal and preparation (parts of an electrode structure are visible on the left side).

foundly, particularly after the first drug addition. Mitochondrial functions are obviously impaired heavily, but begin to recover shortly after drug withdrawal. IDES records do not suggest any restoration of cellular adhesion or cellular morphology after drug withdrawal. The acute cytotoxic effect causes sensor signal changes without any detectable delay as soon as the drug is in contact with the cultures. Later in the course of drug action, decreasing numbers of viable cells should contribute to sensor signals as well. At 24 h after applying the drug, cells were killed by 0.1% Triton X-100 in order to obtain the baseline values of sensor signals (values without viable cells).

S-180 and S-180dox

Figure 7(a and c) presents experiments comparing the cellular reactions of two cell lines to doxorubin. The cell lines are sensitive S-180 cells (Figure 7a and c) and resistant S-180dox cells (Figure 7b). The two cell lines served as a model for tumor cells of the same tissue origin but with different chemosensitivities.

Comparing both cell lines, there is a clear difference in cellular responses to doxorubicin. The total metabolic activity (extracellular acidification, cellular oxygen consumption) of sensitive S-180 cells increased with regular, drug-free medium during the experiment, probably reflecting cell proliferation. In contrast, their metabolic activity declined after treatment with doxorubicin (Figure 7a and c). Resistant S-180dox cells showed no response to the drug associated with sensor signal changes. Total metabolic activity even increased after addition of the agent, again probably reflecting cell proliferation (Figure 7b).

Measurements within the IDES version on S-180 cells (Figure 7d and e) generally showed decreasing C_{par} values at day 1 of the experiments, reflecting settlement of the cells and cell proliferation. Daily changes of cell culture medium caused typical temporary cellular responses detected by the impedimetric sensors.[8] Only with doxorubicin-treated, sensitive S-180 cells did C_{par} values began to increase continuously at day 2 of the experiment (Figure 7e), indicating decreasing adherence or cell death.

Primary cultures

Having shown that cellular responses are sensitively detected by all types of microsensors used, the next step was to determine whether the method is equally

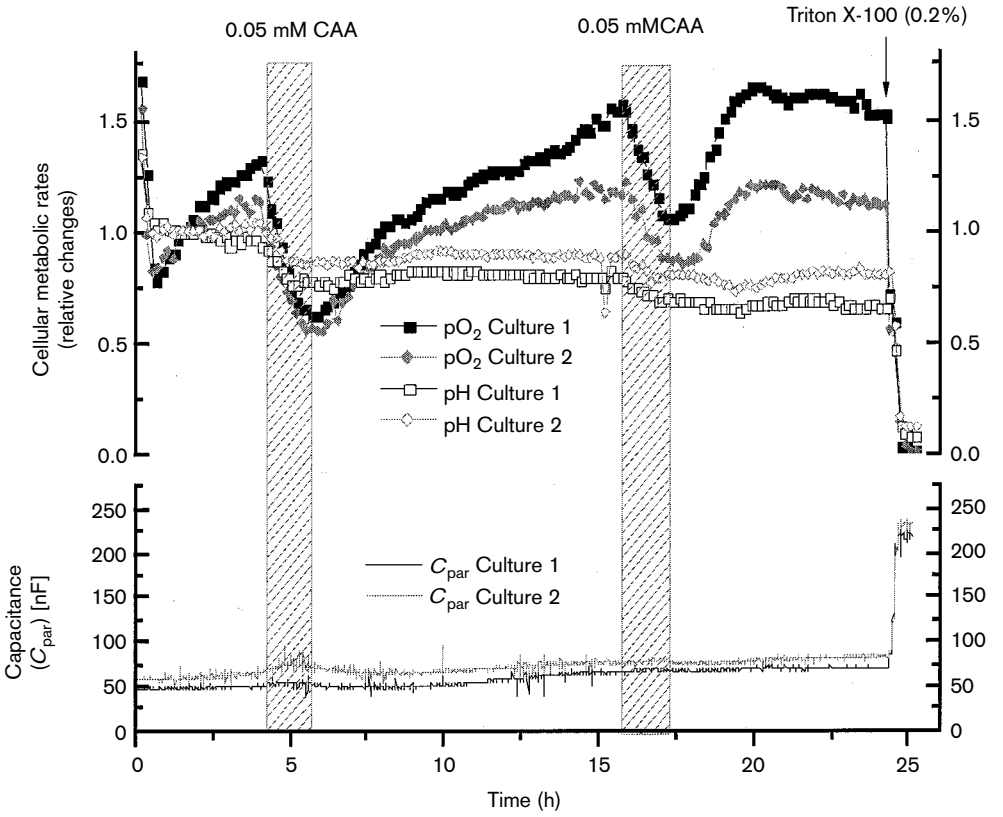

Figure 6. Experiment with the GC version: two cultures of LS 174T cells were treated twice with CAA (hatched intervals).

suited to obtain signals from primary cultures, which are metabolically less active and more prone to irritation. When the glass sensor chips with the prepared cellular specimen were connected to the fluidic system, the cells had settled down on the chip surface but were not yet clearly adherent. Therefore microporous membrane filter inserts were included (GC version) in order to prevent irritation by the moving fluid. As pointed out above, impedimetric analysis is satisfactory only with adherent cells. Since the tumor explants were not allowed enough time to spread on the sensor chips during short-term culture, no significant changes of impedance values have been detected and only metabolic activity could be monitored. Cells started to grow as a monolayer after keeping the primary explants in culture for a longer period of time (Figure 5).

An experiment with the GC version on two primary cultures over 24 h is shown in Figure 8. The cultures were prepared from a lung carcinoma tissue explant. At the moment, we do not have a satisfactory explanation for the observed tendency of decreasing metabolic activity. It may be associated with the frequently observed starving of cells occurring upon adaptation to *in vitro* conditions. In general, it cannot be decided from sensor signals alone whether such signal changes are due to changing cell numbers (caused by cell death or cell proliferation) or changing metabolic rates per cell.

Figure 9 shows an experiment on two primary cell cultures prepared from a breast carcinoma tissue explant and performed in parallel within the GC version. This time, one of the cultures was treated with CAA. We observed rapid impairment of mitochondrial function (decrease of cellular respiration by 90% within 3 h). Extracellular acidification rates declined as well, but not as fast as respiration. Mitochondrial defects are discussed as an important reason of ifosfamide-mediated side effects on a wide range of cell types.[21] Ifosfamide is a precursor of CAA in its metabolic pathway. In the course of further drug action, damage of the nucleic acids followed by cell death should dominate the dynamic course of the sensor signals.

Discussion

The success rate of chemotherapy is still not satisfactory in most solid tumors. Indivdualized therapy is one of the approaches expected to improve the situation.

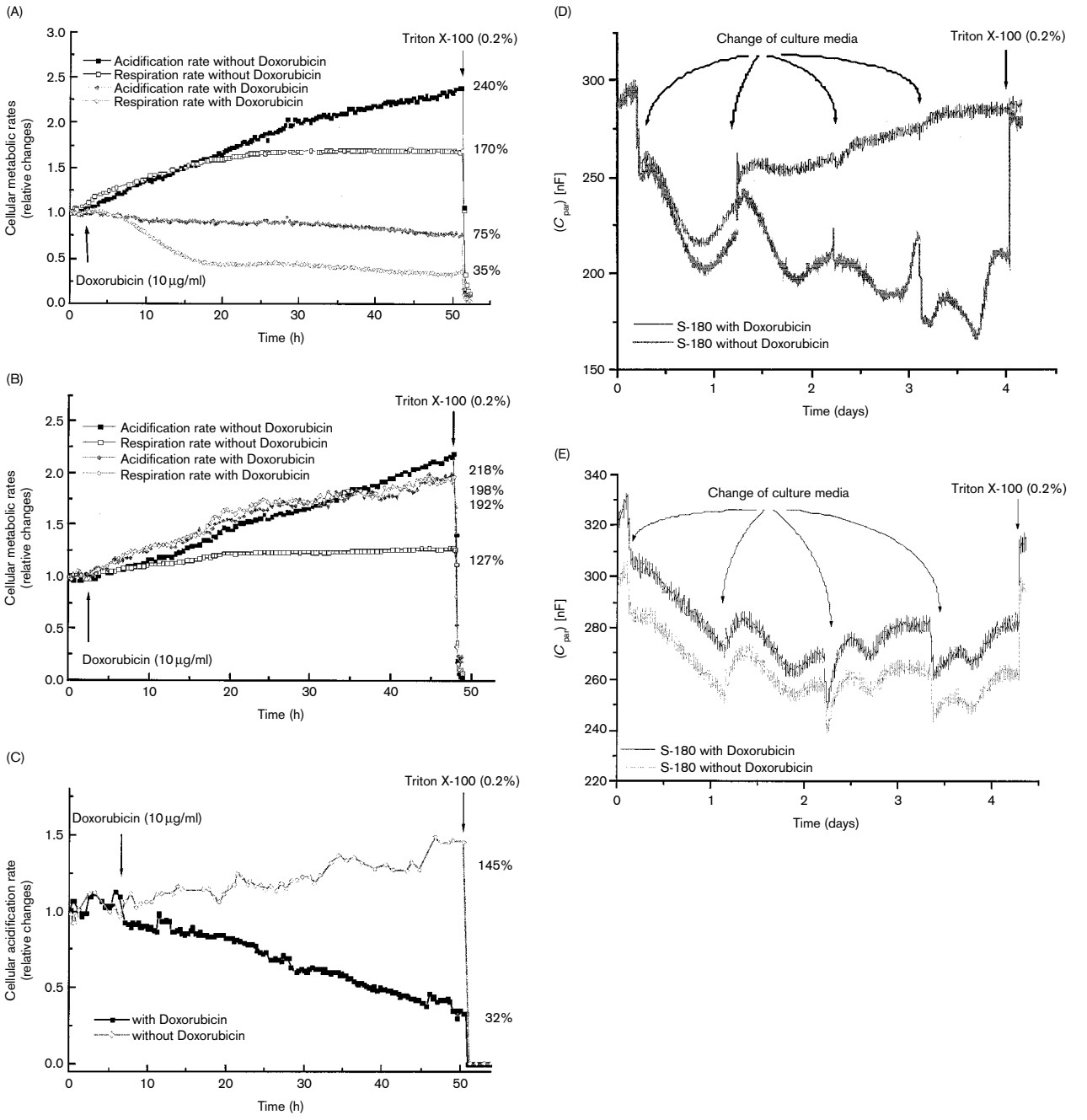

Figure 7. (a and b) Experiment with the GC version: relative changes in cellular metabolic rates of sensitive S-180 (upper graph) and resistant S-180dox cells (lower graph) after application of 10 μg/ml doxorubicin. Each experiment compares a treated and a non-treated culture. The numbers on the right indicate the relative change of metabolic rates throughout the experiment. (c) Experiment with the SC version (ISFET chip): relative changes of the acidification rate of two cultures of sensitive S-180 cells. One culture was treated with doxorubicin (10 μg/ml). (d and e) Experiment with the IDES structures showing the reaction of S-180 (upper graph) and S-180dox (lower graph) cells to treatment with doxorubicin each compared to an untreated culture. The daily changes of culture medium are marked by arrows.

In this context, it is widely assumed that the future development of microarray-based analysis for cancer-associated molecular markers will make feasible assay-directed, individual regimen of drug administration or other forms of non-surgical therapy. Microarrays indeed might become important diagnostic tools in tumor classification, particularly in non-solid tumors.[17] It is less evident, however, whether they can be used to predict

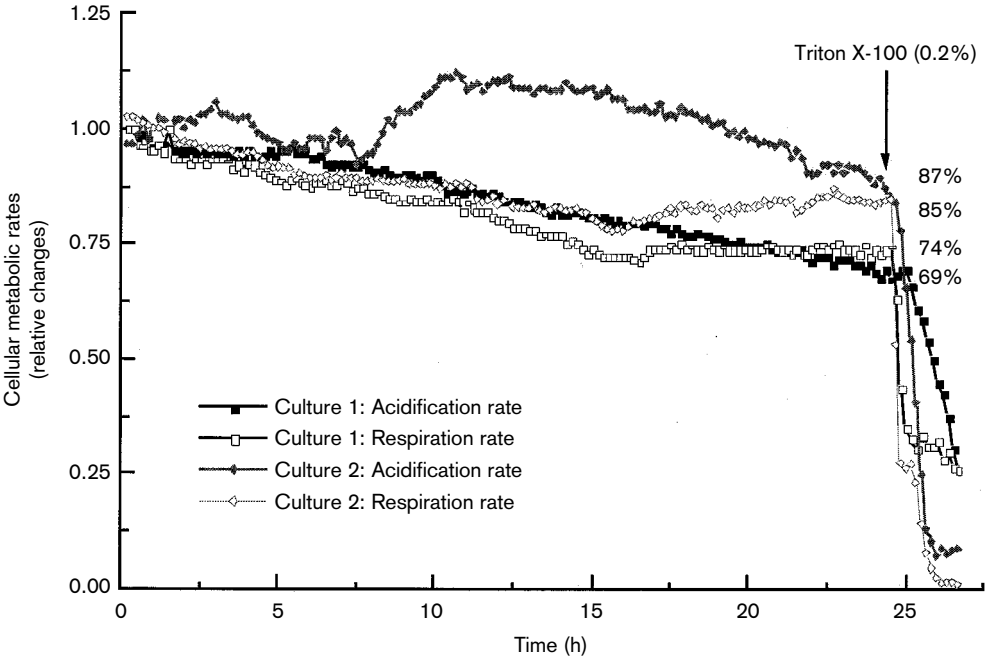

Figure 8. Experiment with the GC version on two primary cultures of lung carcinoma tissue over a time period of 24 h.

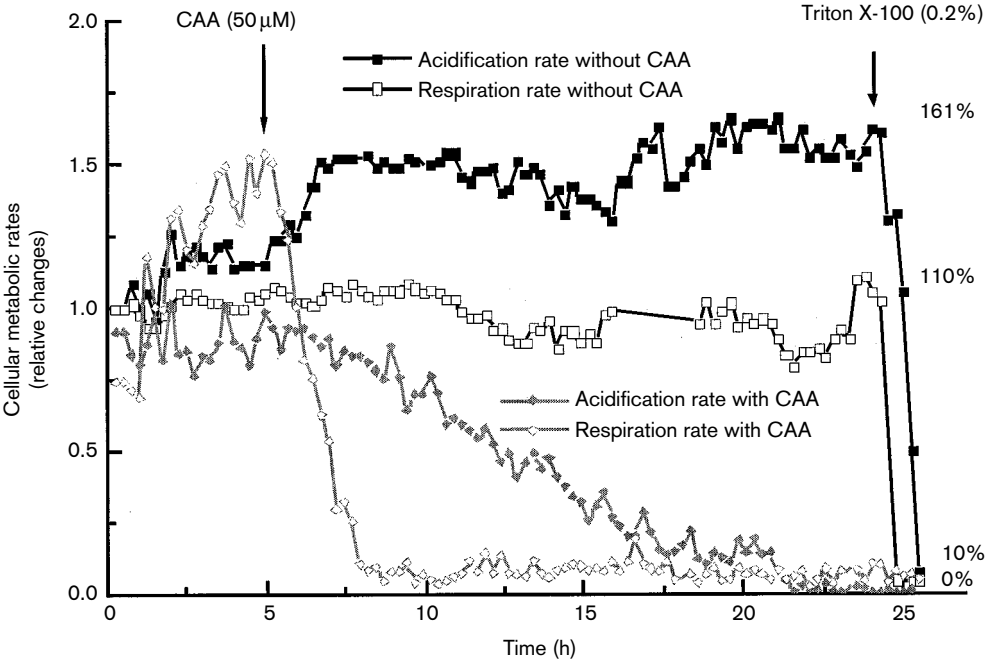

Figure 9. Two cultures of breast carcinoma tissue explant during an experiment with the GC version. The cytotoxic drug CAA was added at a concentration of 50 μM to one culture.

chemosensitivity profiles. The background of these assays is (i) a considerable cellular heterogeneity within individual tumors and (ii) an enormous number of potentially effective gene loci. Therefore, the usefulness of tests for distinct tumor promotor or tumor suppressor genes seems to be limited. The well-known p53 tumor suppressor gene, for example, is reported to be defective in 50% of human malignancies, but clear evidence for a correlation between gene defect and therapeutic behavior is missing in solid tumors.[18]

Another approach, which has been pursued since 1977,[19,20] is *in vitro* chemosensitivity testing. Since these assays are performed on intact, viable tumor cells, the complex cellular network of regulatory mechanisms influencing chemosensitivity is at least partially taken into account. Among the different types of assays which have been published, the use of an bioluminescent ATP-TCA recently proved to increase tumor patient survival in ovarian cancer.[11]

The present work was performed in order to address the question whether a different technological approach using sensor chips for continuous generation of data on cell metabolism and cell morphology could further improve the situation. The most important considerations prompting us to pursue the technology in this context were (i) the dynamic character of data generation, making possible direct comparison of physiological states before and after drug application in a multiparametric, time- and dose-dependent way, and (ii) the potential sensitivity of sensor readings in terms of the cell numbers required. Analyzing tumor cell lines, it could be shown that another sensor-based device (Cytosensor; Molecular Devices, Menlo Park, CA) is clearly superior to the tetrazolium salt in the assessment of drug toxicities.[4] As the first sensor device, our system integrates oxygen and IDES sensors, thereby providing a greater amount of data which can reveal useful information on the dynamics of cellular reactions towards the application of drugs. However, we are aware of the fact that due to the small number of preparations and experiments, and the lack of any clinical correlations, only very careful conclusions can be drawn.

A striking outcome of the experiments with two cytostatic drugs from different classes was the observed rapid and profound impairment of cellular respiration, as compared to extracellular acidification. In one of the primary explants (Figure 9), addition of 50 μM CCA caused a decline of oxygen consumption to below the detection limit within 3 h, while extracellular acidification was still present. In S-180 cells, the application of doxorubicin depresses oxygen consumption more heavily than extracellular acidification. The strong effect on respiratory activity can be explained with the highly specific binding of doxorubicin to cardiolipin, an essential phosphatide for the function of the enzyme cytochrome *c* oxidase.[21] In rats, doxorubicin was reported to strongly inhibit the activities of the enzyme complexes I–III and IV of the mitochondrial respiration chain.[22]

Generally, mitochondria are discussed both as an important target for toxic mechanisms and for drug-mediated side effects of numerous anticancer drugs, including alkylating agents and doxorubicin.[23,24] Mitochondrial susceptibility may be associated with DNA repair mechanisms working less efficiently than in the cell nucleus. Obviously, oxygen sensors are useful for the detection of acute toxic drug action affecting mitochondrial function.

Although drug-related signals from IDES have been obtained with the weakly adherent, non-epithelial sarcoma 180 cell line, freshly prepared tumor explants did not significantly influence impedance values. We attribute this failure to the fact that short-term culture did not leave sufficient time for the fragile primary cultures to create close contact between the cells and sensor. If such a close contact could be achieved in specimens with few connective tissue parts, IDES might be used to qualify cell–substrate adhesion and intercellular adhesion, and thereby support current histo- and cytopathological assessment of tumor specimens prepared, for example, from fine needle aspirates. Intercellular adhesion was shown to be an important parameter for malignancy.[25,26]

Up to now, we have monitored tumor specimens for time periods of 1–2 days. This is certainly sufficient for the determination of short-term cytotoxic drug effects. For the determination of long-term effects on cell proliferation/cell survival and for reversibility studies, however, it might be insufficient, particularly with specimens derived from slow-growing tumors. Therefore, the maintenance of tumor specimens inside the sensor chip system along with continued data acquisition for at least 1 week are of future interest.

The experiments performed on the primary explants from mamma and lung carcinoma indicate the applicability of the sensor-chip-based system for TCA for some of the most frequent solid tumor entities. Since no purified tumor cell population, but a preparation representing the native tumor composition is loaded on the chip, tumor–stroma interactions are (at least partially) taken into account. The importance of environmental, epigenetic mechanisms for the development of drug resistance in cancer is suggested by recent studies.[27] On the other hand, the metabolic activity due to non-tumor cells must be considered, like in other assays based on determinations of cell metabolism.

Although in principle the consideration of tumor–stroma interactions is desirable, in some cases the absolute number of tumor cells required for the assay is more important. This holds true, e.g. in fine needle aspirates with low cell numbers, with purified carcinoma cells derived from peripheral blood, bone marrow or lymphoid tissue or with any small specimens which are to be divided into numerous replicates for tests of various drugs or drug combina-

tions in different doses. In convenient endpoint assays the reduction of cell number seems to be limited by statistical and systematic errors ocurring during subdivision of specimen into replicates, resulting in a variance in cell number and thus in a corresponding variance in single test results. At least theoretically, dynamic assays are not affected by this limitation in specimen size reduction, although the potential for miniaturization has not been evaluated so far.

From the experiments on S-180 cells it could be shown that different degrees of chemosensitivity are detected in a multiparametric, time-dependent manner. In a realistic, clinical setting, however, the degree of chemosensitivity has to be predicted instead of being confirmed. The questions arise how kinetic, multiparametric data are evaluated properly in order to calculate chemosensitivity, how weight factors can be attributed to single parameters and which time points are used to calculate the required index. Again, these bioinformatic questions remain to be addressed in future clinical studies.

The fluid setup for the supply of cell culture media and for drug exposure mimics the *in vivo* situation better than static exposure setups. Aside from monitoring cellular metabolism, sensors can be used to control pH and pO_2 values in the immediate vicinity of the cells. *In vivo*, these parameters are known to affect therapeutic responses, and microenvironmental factors like tumor oxygenation and tumor pH can be critical for tumor progression.[28–30]

In conclusion, sensor chips may be useful tools for the sensitive characterization of cellular sensitivity to non-surgical treatment of cancer. However, it has to be proved by clinical data whether the outlined advantages are realistic and justify the implementation of a new technology.

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