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PAPER IN FOREFRONT

Patrizia Pasini · Nilesh Powar
Ricardo Gutierrez-Osuna · Sylvia Daunert · Aldo Roda

Use of a gas-sensor array for detecting volatile organic compounds (VOC) in chemically induced cells

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Abstract An application of gas sensors for rapid bioanalysis is presented. An array of temperature-modulated semiconductor sensors was used to characterize the headspace above a cell culture. Recombinant *Saccharomyces cerevisiae* yeast cells, able to respond to 17β-estradiol by producing a reporter protein, were used as a model system. Yeast cells had the DNA sequence of the human estrogen receptor stably integrated into the genome, and contained expression plasmids carrying estrogen-responsive sequences and the reporter gene *lac-Z*, encoding the enzyme β-galactosidase. The sensor-response profiles showed small but noticeable discrimination between cell samples induced with 17β-estradiol and non-induced cell samples. The sensor array was capable of detecting changes in the volatile organic compound composition of the headspace above the cultured cells, which can be associated with metabolic changes induced by a chemical compound. This finding suggests the possibility of using cross-selective gas-sensor arrays for analysis of drugs or bioactive molecules through their interaction with cell systems, with the advantage of providing information on their bioavailability.

Keywords Metal oxide sensors · Volatile organic compounds · Recombinant yeast cells · Chemically induced cells · 17β-Estradiol · Drug analysis

P. Pasini (✉) · A. Roda
Department of Pharmaceutical Sciences, University of Bologna,
Via Belmeloro 6, 40126 Bologna, Italy
e-mail: patrizia.pasini@unibo.it

N. Powar
Department of Computer Science and Engineering,
Wright State University,
45435 Dayton, OH, USA

R. Gutierrez-Osuna
Department of Computer Science, Texas A&M University,
College Station, TX 77843, USA

S. Daunert
Department of Chemistry, University of Kentucky,
Lexington, KY 40506, USA

Introduction

Volatile compounds emitted by living cells can provide valuable information about the metabolic and physiological conditions of the cells. Electronic nose systems, which rely on a combination of cross-selective gas sensors to produce specific response patterns for different analytes, have been shown to be suitable for monitoring changes in the volatile organic compounds produced by living cells [1]. In addition, electronic nose technology is currently being investigated for a number of applications, including quality control in the food, beverage, and fragrance industries, environmental monitoring for assessment of odor sources (e.g. wastewater, livestock, and landfill), industrial bioprocessing, and medical diagnostics [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13].

A variety of different signal-transduction mechanisms has been employed for electronic noses, including metal oxide sensors, metal-oxide-silicon field-effect transistors (MOSFET), conducting polymers, surface acoustic wave devices, quartz microbalances and fiber optic chemical sensors [14]. Different pattern-recognition methods have been used for evaluation and interpretation of datasets derived both from simple and complex odors. Linear pattern-analysis methods including partial least squares, principal-component regression, and discriminant function analysis have been applied to simple problems [2]. However, when quantification or multicomponent mixture classification have to be performed more powerful non-linear prediction tools such as artificial neural networks are commonly required [15].

It has been reported that the detection and identification of bacterial, yeast, and animal cells can be achieved by measurement of the VOC produced in the headspace of cultured cells, by use of sensor arrays combined with neural network classifiers [1, 16, 17]. Living cells produce a large variety of metabolites as a result of different metabolic pathways, and some of these are known to be semi-volatile or volatile organic compounds whose qualitative and quantitative composition can provide information about the

metabolic and physiological state of the cells [1, 18, 19]. The introduction of chemical compounds can influence the balance of metabolites, and this can be revealed as characteristic VOC emissions. The sensor array can be placed close to the cell culture such that continuous changes in the VOC from the culture can be sensed, as is usually performed in the monitoring of bioreactor cultures [1].

In the work discussed in this paper we explored the possibility of utilizing a semiconductor gas-sensor array to detect compounds interacting with a cell system. The hypothesis is that changes in cell metabolism, in response to the presence of a chemical compound (e.g. a drug), result in changes in the cell VOC profiles. Different VOC profiles have previously been detected by electronic noses in different strains of the same yeast species [18], thus rendering these cells a suitable model for our purpose. Electronic noses have also been shown to be appropriate for online monitoring of yeast cultivation, because they are able to separate the phases of glucose growth, ethanol growth, and stationary condition, and estimate physiological variables such as growth rate and glucose uptake rate [1, 20]. Yeasts are also well-established cell models for study of biologically active compounds by using receptors linked to reporter genes and detecting gene activation. These systems are widely used to evaluate the biological effects of natural and synthetic compounds [21]. In addition, yeast cells are easy to culture, grow rapidly, and do not require strict sterility conditions.

Experimental

Instrumentation

Four different commercial semiconductor tin oxide gas sensors were used: TGS 2602 for detection of air contaminants, TGS 2610 for detection of combustible gases, TGS 2611 for detection of methane, and TGS 2620 for detection of solvent vapor (Figaro Engineering, Glenview, IL, USA). The sensors were inserted in a screw cap fitting 30-mL glass vials, in which samples were placed for analysis. The sensors were connected to a voltage divider, as suggested by the manufacturer. A laptop PC provided with Lab View 5.0.1 software (National Instruments, Austin, TX, USA) and a DAQcard-1200 (National Instruments) was used for data acquisition.

Metal-oxide semiconductors can be used as sensors by measuring the electrical resistance changes that occur when vapor is adsorbed by the semiconductor surface. When sensors are exposed to air, oxygen is adsorbed by the sensor surface, removing electrons from the conduction band of the semiconductor, thereby increasing its electrical resistance. The interaction of reducing volatile compounds with the surface-adsorbed oxygen reduces this electron trapping, leading to characteristic increases in the electrical conductance (decreases in electrical resistance) of the sensor [22].

Metal-oxide sensors are typically run at elevated temperatures (up to 400 °C) to detect VOC. There are two major techniques for excitation of the sensors – isothermal excitation and temperature modulation. Isothermal excitation is based on application of a constant DC voltage to the heater terminal, and subsequent measurement of the resistance of the sensing terminal with a voltage-divider circuit [22]. In temperature modulation the heater voltage is gradually changed, typically following a sinusoidal waveform [23]. As a result, the temperature of the sensor changes gradually to follow the oscillation of the heater voltage. Because different VOC have unique reaction rates at each temperature, the response of a temperature-modulated sensor to a particular VOC also has a unique signature pattern. These temperature-modulated patterns have been shown to provide information that is more discriminatory than that of isothermal excitation methods [24, 25, 26].

In this study the sensors were excited with a 1–7 V sinusoidal temperature profile. The same heater voltage was used for the four different sensors. A voltage divider circuit was used to convert the change in sensor conductivity into an electrical output signal, which was then acquired by the computer. An instrumentation circuit was used to control the heater voltage and acquire the temperature-modulated responses. The heater voltage set point was generated with the aforementioned Lab View-controlled multi-function data-acquisition card and subsequently current-boosted by a Bipolar Junction Transistor (BJT).

Yeast strains

A recombinant *Saccharomyces cerevisiae* yeast strain able to respond to estrogen-like chemicals was provided, with permission, by Professor John Sumpter (Brunel University, UK). The DNA sequence of the human estrogen receptor α (hERα) was stably integrated into the main chromosome. The yeast cells also contained expression plasmids carrying estrogen-responsive sequences controlling the expression of the reporter gene *lac-Z*, encoding the enzyme β-galactosidase [27]. On binding an active ligand the estrogen-occupied receptor interacted with the estrogen-responsive elements to modulate gene transcription. This caused expression of the reporter gene *lac-Z* and production of β-galactosidase, which was secreted into the culture medium and detected by means of a chemiluminescent (CL) substrate, as described below.

A different *S. cerevisiae* strain, common baker's yeast, was used as control strain in validation experiments.

Yeast cell cultures

Both recombinant and baker's yeast strains were cultured as previously reported [27]. Briefly, growth medium (5 mL) was inoculated with a single yeast colony from an Agar plate and incubated at 30 °C for approximately 20 h on an orbital shaker (250 rpm) until an optical density at 620 nm ($OD_{620\,nm}$) of 1.0 was achieved. Fresh growth medium (50 mL) was seeded with yeast cell suspension (2 mL) from the 20-h culture, thus furnishing a dilute suspension containing approximately 1×10^6 yeast cells per mL. Aliquots (5 mL) of diluted recombinant or baker's yeast cell suspension were used for induction or validation experiments, respectively.

Yeast cell induction

In the induction experiments the natural receptor ligand 17β-estradiol (Sigma, St Louis, MO, USA) was used as the inducer chemical, because it is known to have the highest affinity for the estrogen receptor contained in the recombinant yeast cells. 17β-Estradiol was dissolved in ethanol to obtain a 2×10^{-3} mol L^{-1} stock solution, which was serially diluted with growth medium to obtain a set of standard solutions of concentration 2×10^{-6}, 2×10^{-7}, and 2×10^{-8} mol L^{-1}. These standard solutions contained different concentrations of ethanol, 0.1, 0.01, and 0.001% (*v*/*v*), respectively. Although the net amount of ethanol present in each standard solution was very small, a set of blanks (one for each estradiol concentration) containing 0.1, 0.01 and 0.001% (*v*/*v*) ethanol in culture medium, respectively, was prepared, because the sensors used were particularly sensitive to ethanol.

Standard solutions or blanks (250 µL) were incubated with 5 mL diluted recombinant cell suspension (1×10^6 cells mL^{-1}) in sterile culture tubes at 30 °C for 24 h on an orbital shaker (250 rpm). Aliquots (5 mL) of cell suspension alone (without estradiol and ethanol) were also incubated as controls. At the end of the incubation time the cell suspensions were transferred to the analysis glass vials and these were capped tightly before exposure to the sensor array.

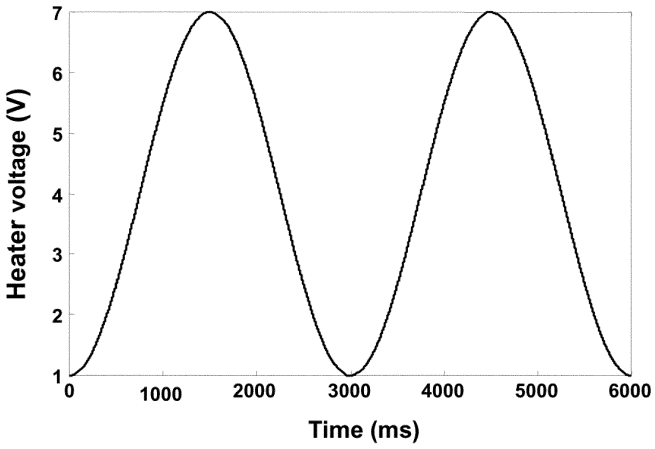

Fig. 1 Heater voltage profile used for temperature modulation of the sensors

To increase the number of cells in the measurement vials and increase the headspace VOC concentration, two approaches were explored. In one the concentration of the diluted cell suspension was increased by seeding 50 mL fresh growth medium with 4 mL (rather than 2 mL) recombinant yeast cell suspension from the 20-h culture, thus furnishing a suspension containing approximately 2×10^6 yeast cells per mL. The subsequent induction step proceeded as previously described. Alternatively, the volume of cell suspension analyzed was increased. Standard solutions or blanks (500 µL rather than 250 µL) were incubated with 10 mL (rather than 5 mL) seeded growth medium containing approximately 1×10^6 cells per mL. Similarly, the corresponding control consisted of 10 mL cell suspension alone.

Validation of the sensor array set-up

Qualitative and quantitative validation experiments were carried out. The ability of the sensor array to discriminate single volatile compounds was evaluated using acetone and ethanol, both at 0.1% (*v*/*v*) in aqueous solutions. The sensor response was also assessed in terms of the ability to separate complex volatile mixtures produced by two yeast strains – common baker's yeast and the recombinant yeast strain described above.

The ability to discriminate different analyte concentrations was also assessed for both a single volatile compound and a complex volatile headspace produced by a cell culture. Ethanol was analyzed at 1, 0.1, and 0.01% (*v*/*v*) in aqueous solutions. The possibility of performing quantitative analysis was further evaluated by exposing the sensor array to a complex VOC headspace produced by a yeast cell suspension in total (1:1), diluted 1:2, and diluted 1:4 with culture medium.

The analyzed sample volume was 5 mL in all validation experiments.

Data acquisition

Sensors were pre-heated for 1 h at a constant heater voltage of 5 V every day before each analytical run.

Cell suspensions and single volatile aqueous solutions were exposed to the sensors inside glass vials tightly closed with the cap on to which the sensors had been mounted.

Total data collection for each specimen included two phases, a sample phase and a recovery phase. The sample phase involved

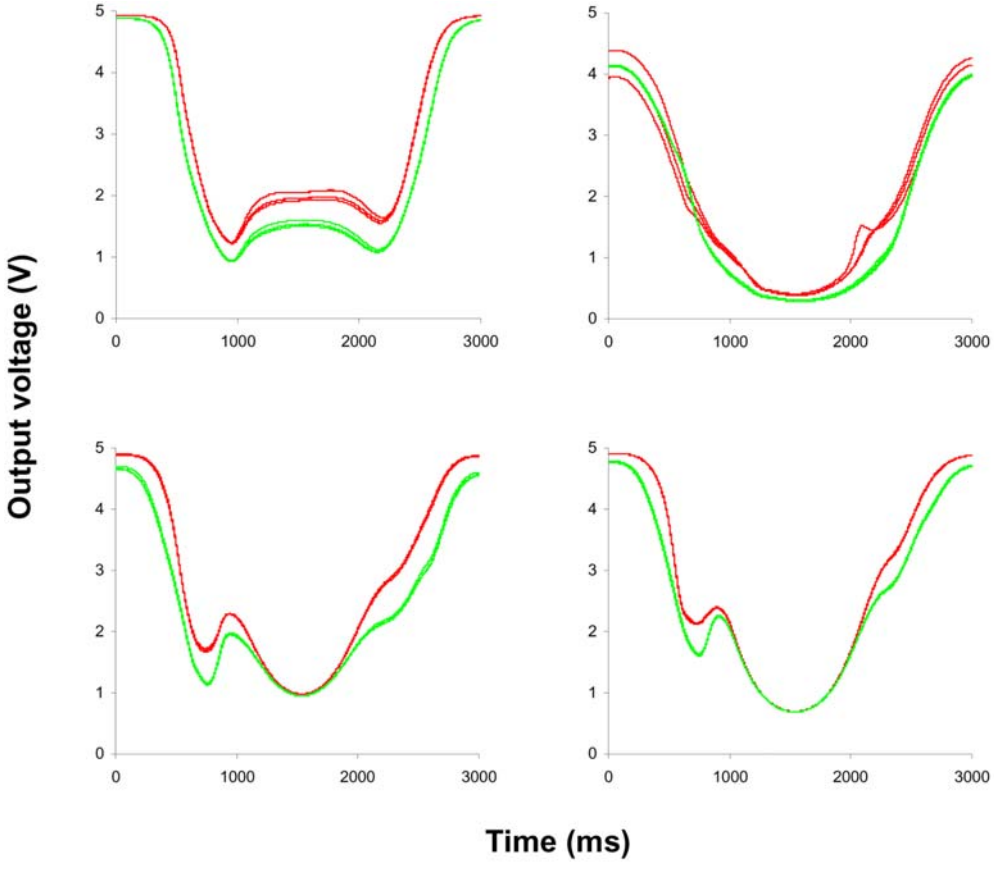

Fig. 2 Sensor array response to single volatile compounds in aqueous solutions (0.1% *v*/*v*): acetone (*red lines*), ethanol (*green lines*). Sensors: 2602 (*top left*), 2620 (*top right*), 2611 (*bottom left*), 2610 (*bottom right*). Three replicates for each sample are shown in the figure

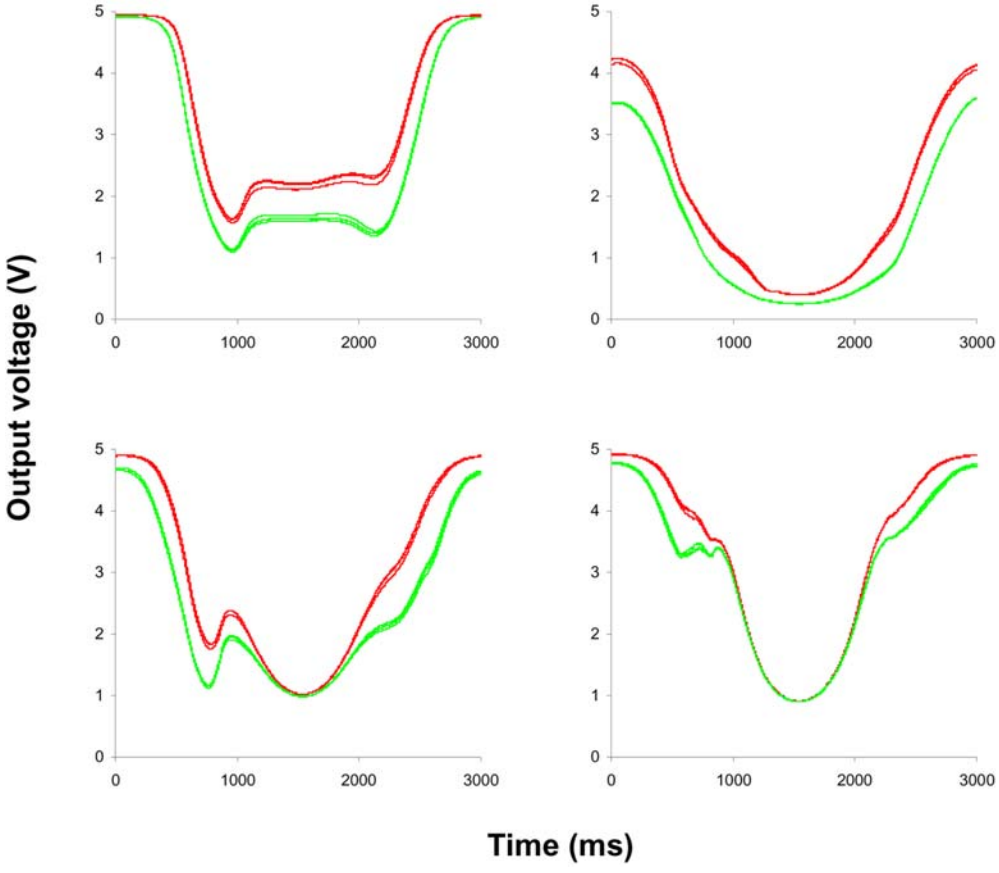

Fig. 3 Sensor array response to different *Saccharomyces cerevisiae* yeast strains: baker's yeast cells (*red lines*), recombinant yeast cells (*green lines*). Sensors: 2602 (*top left*), 2620 (*top right*), 2611 (*bottom left*), 2610 (*bottom right*). Three replicates for each sample are shown in the figure

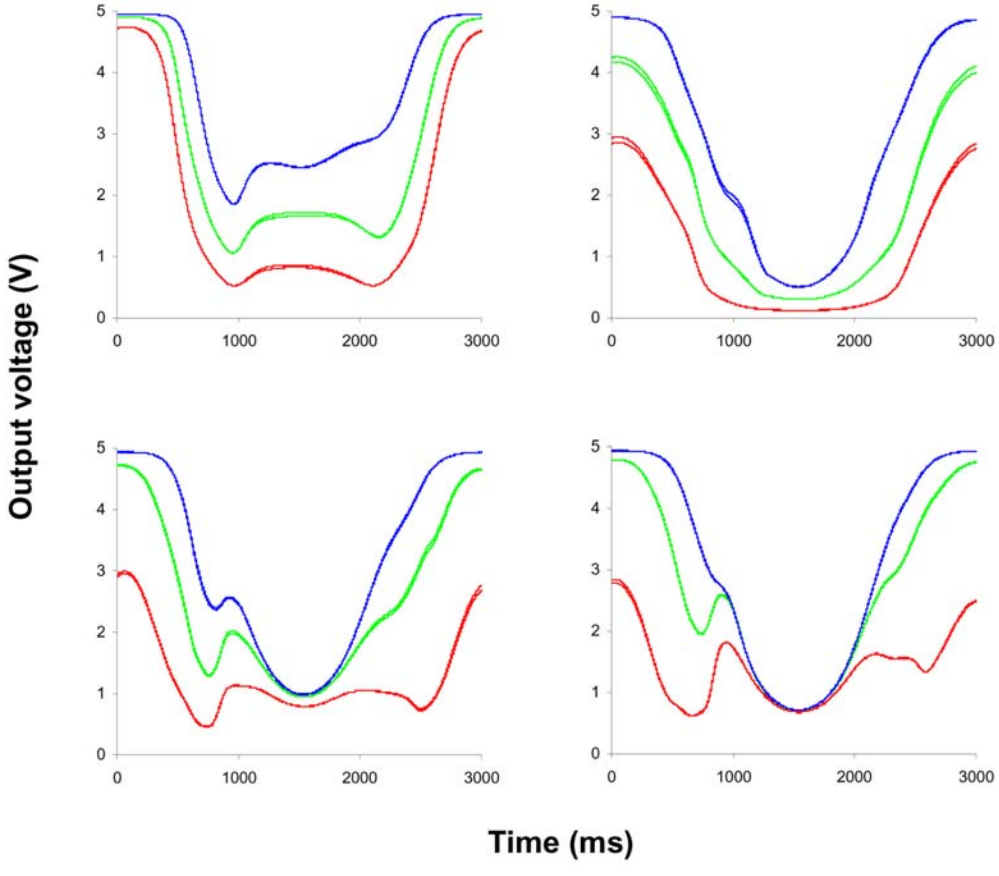

Fig. 4 Sensor array response to different concentrations of ethanol in aqueous solutions (*v/v*): 1% (*red lines*), 0.1% (*green lines*), 0.01% (*blue lines*). Sensors: 2602 (*top left*), 2620 (*top right*), 2611 (*bottom left*), 2610 (*bottom right*). Three replicates for each sample are shown in the figure

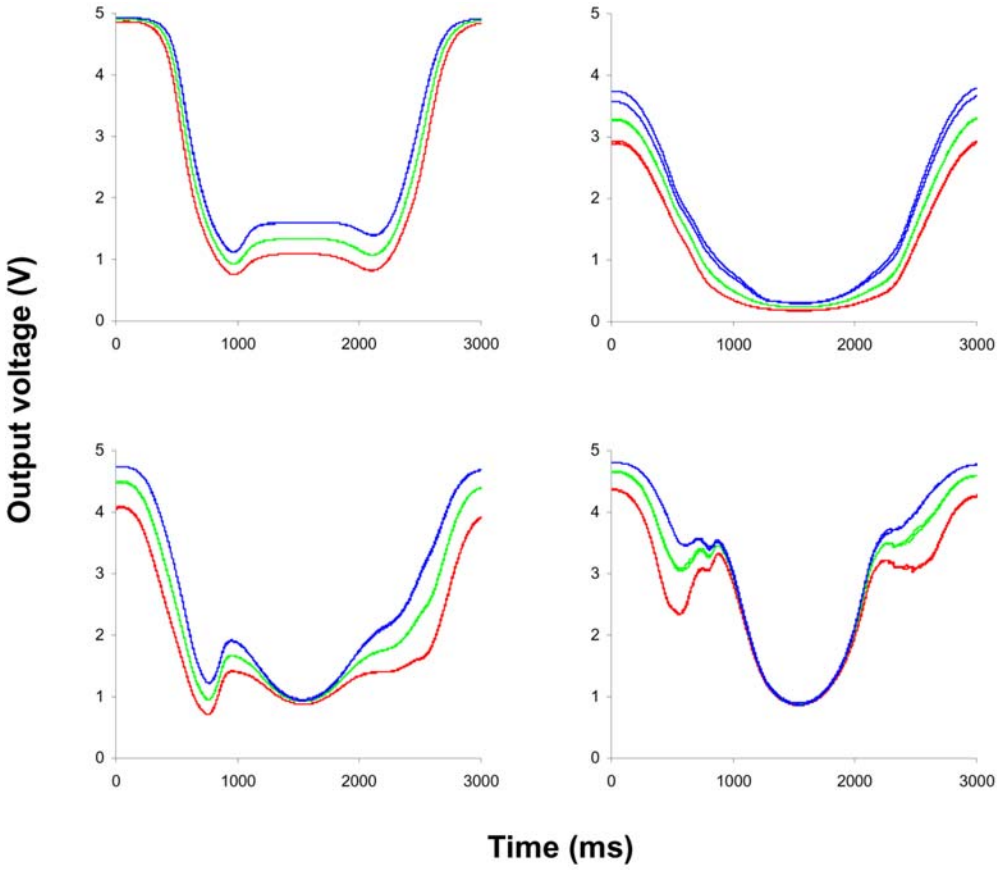

Fig. 5 Sensor array response to different dilutions of yeast cell suspension: 1:1 (*red lines*), 1:2 (*green lines*), 1:4 (*blue lines*). Sensors: 2602 (*top left*), 2620 (*top right*), 2611 (*bottom left*), 2610 (*bottom right*). Three replicates for each sample are shown in the figure

two sinusoidal temperature modulation cycles (1–7 V), each lasting 5 min (Fig. 1). The first cycle served as an equilibration stage and the second cycle was used for data collection and analysis. The recovery phase involved the same heater profile (two 5-min sine waves), but the sensors were exposed to air to facilitate desorption of VOC.

In the induction experiments, induced-cell samples, blanks, and controls were sniffed in replicate, each replicate being sniffed two or three times. In the validation experiments, cell suspensions and single volatile aqueous solutions were sniffed in the same manner. To minimize memory effects the measurement sequence was randomized.

The sensors' electrical resistance, measured as the output voltage of a divider circuit, was monitored during the complete temperature-modulation cycle, resulting in a response as a function of time. The response of a sensor in the array during the second 5-min sinusoidal cycle of the sample phase is shown in Figs. 2, 3, 4, 5, 6.

Chemiluminescent detection of β-galactosidase activity

The recombinant yeast cells expressed and secreted the reporter enzyme β-galactosidase as a response to the inducer chemical 17β-estradiol. At the end of the 24-h induction time the enzyme activity was measured by a chemiluminescent assay [28] using a 1,2 dioxetane-β-D-galactopyranoside CL substrate (Galacto-Light Plus, PE-Tropix, Bedford, MA, USA). Culture medium (20 µL) containing the enzyme was added to 70 µL CL substrate in 96-well microtiter plates and incubated at room temperature for 1 h. Light emission accelerator (100 µL) was then added and the CL signal was measured using a photomultiplier-tube-based microplate luminometer (MLX, Dynex Technologies, Chantilly, VA, USA). Enzyme activity was then normalized to the number of cells, estimated by measuring the turbidity ($OD_{620\,nm}$) of the cell suspensions.

β-Galactosidase activity and turbidity were measured in all induced-cell samples, blanks, and controls before and after the sniffing process.

Results and discussion

The sensors used for the experiments were metal oxide chemoresistors, which are commonly used to detect organic vapor. The sensors were directly exposed to the headspace of the cell cultures in a vial. This static headspace procedure eliminated possible cooling effects caused by the effluent flow, which could disturb the temperature-modulated sensor patterns.

Pre-heating the sensors before each analytical run allowed us to reduce drift and improve the stability of the response, thus improving the repeatability of subsequent measurements.

Validation of the sensor array set-up

All four sensors could discriminate between acetone and ethanol (Fig. 2), and between the recombinant and baker's yeast strains (Fig. 3). The responses of the sensors were consistent for three different replicate measurements of the same sample, thus showing good repeatability of the sensor system in response to single volatile compounds or complex VOC mixtures produced by cultured cells. Repli-

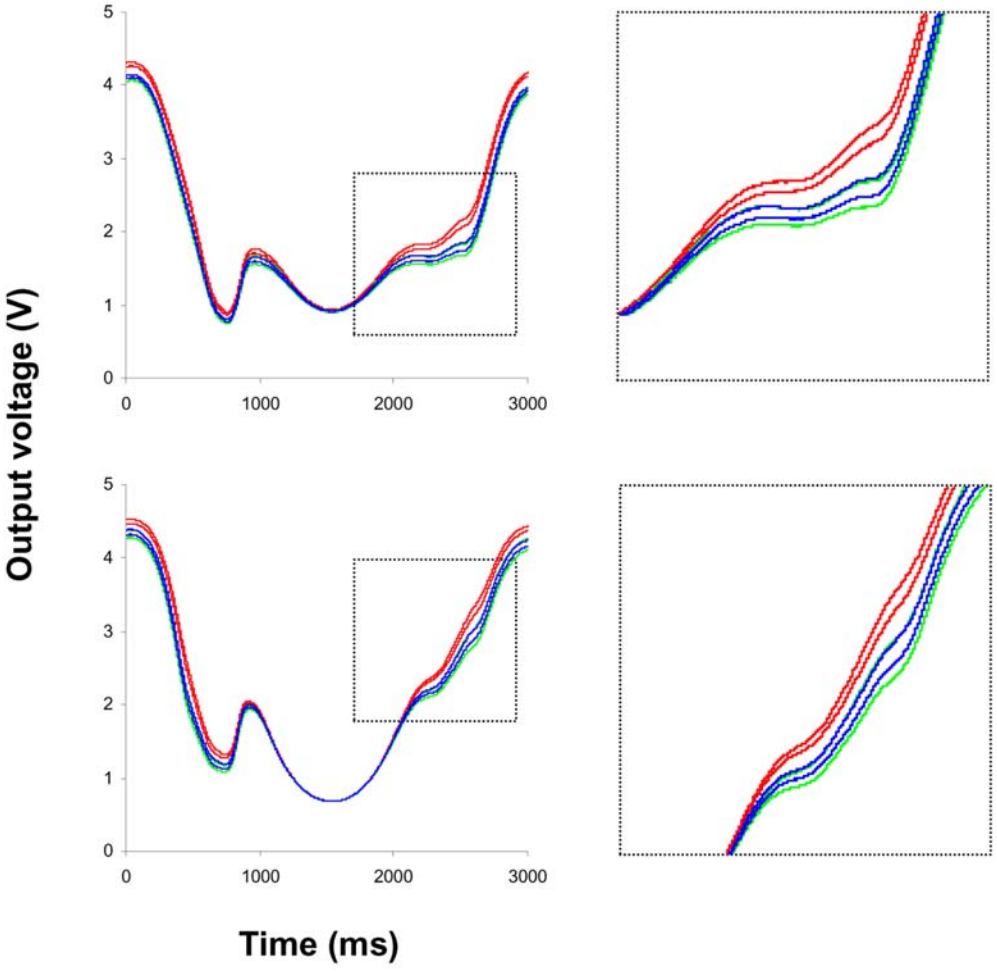

Fig. 6 Sensor array response to chemically induced cells (*red lines*), blanks (*green lines*), and controls (*blue lines*). Sensors: 2611 (*top*), 2610 (*bottom*). Two replicates for each sample are shown in the figure. Zoomed portions of the response curves are shown (*right panels*) to emphasize the differences between signals from induced and non-induced cell samples

cates of each sample in the same analytical run also provided consistent response profiles (data not shown).

The sensor array was also able to respond quantitatively, as demonstrated by the separation between the sensor responses to different ethanol concentrations in aqueous solutions, with the highest ethanol concentration producing the lowest output voltage, i.e. the lowest sensor resistance (Fig. 4). The resistance of the sensors increased monotonically with the decrease in ethanol concentration, as predicted by the power-law:

$$Rs = [C]^{-\alpha}$$

where Rs is the sensor resistance, $[C]$ is the gas concentration, and α is the slope of the Rs curve on a logarithmic scale.

The response profiles obtained with different dilutions of a cell suspension were also distinguishable, with the undiluted (1:1) cell suspension producing the lowest output voltage (Fig. 5).

Note that the clear separations achieved in these experiments demonstrate only that the sensor circuitry and sample presentation procedure are appropriate for measuring possible changes in VOC produced by cell cultures.

Analysis of induced-cell samples

Recombinant yeast cells were induced with 17β-estradiol at different concentrations. When induced cells were analyzed, small but noticeable discrimination between the cell sample induced with the highest 17β-estradiol concentration (2×10^{-6} mol L^{-1}) and the corresponding blank and control was observed for two out of four sensors (Fig. 6). These results indicate that cells induced with the chemical compound produced VOC that changed the response of two of the sensors compared with that for non-induced cells. The higher signal generated by induced cells could be because the exogenous compound affects cell metabolism by reducing ethanol production, which in turn increases sensor resistance. In fact, ethanol is known to be both one of the main yeast cell metabolites and a component to which metal oxide sensors have high sensitivity. Alternatively, chemical induction could have caused a change in the VOC qualitative composition, with the formation of metabolites to which the sensors are less sensitive. Fig. 6 also shows that blanks and controls were not distinguishable, demonstrating that the initial amount of ethanol present in induced-cell samples and blanks, as the 17β-estradiol solvent, did not affect the sensor response.

The cell samples induced with 2×10^{-7} and 2×10^{-8} mol L^{-1} 17β-estradiol were not separable from each other or from the corresponding blanks and control, indicating that the sensor array could not detect changes in VOC composition induced by lower chemical concentrations. This might be because low 17β-estradiol concentrations generated variations in VOC production too small to be detected by the sensor array. To separate the cell samples induced with 2×10^{-7} and 2×10^{-8} mol L^{-1} 17β-estradiol from the corresponding blanks we increased the concentration of VOC present in the headspace of the measurement vials by increasing the number of cells in the vials. Neither increasing the concentration (number of cells per mL) nor the volume of cell samples improved discrimination between induced-cell samples and blanks.

A final experiment was performed to rule out the possibility that the separation between blanks and cell samples induced with the highest dose was because of the presence of the drug itself rather than its effect on cell metabolism. Cell samples were analyzed immediately after addition of 2×10^{-6} mol L^{-1} 17β-estradiol. No separation was observed between such samples and blanks, confirming that discrimination between blanks and cell samples induced with the same dose for 24 h was actually because of metabolites produced as a response to the drug, and not to the presence of the drug itself.

Chemiluminescent detection of β-galactosidase activity

The expression of β-galactosidase was used as a control to confirm the cellular response to 17β-estradiol and assess the stability of the cell system during exposure to the sensor array. The number of cells was also estimated by measuring the turbidity ($OD_{620\,nm}$) of the cell suspensions. β-Galactosidase activity and turbidity were measured in all induced-cell samples, blanks, and controls before and after the sniffing process. Non-statistically significant differences were observed between the pre-sniffing and post-sniffing values for both properties, showing that the cell system was in a steady state during the complete VOC detection process.

The β-galactosidase assay showed that the cell system was able to respond to the inducer in a dose-dependent manner in terms of reporter enzyme production. When the VOC production was measured with the sensor array we observed that only one chemical concentration (the highest) induced a detectable variation in VOC profile. This could be because of either a lower response of the cell system in terms of VOC production compared to the reporter protein synthesis, or insufficient sensitivity of the sensor array for detecting small qualitative or quantitative variations in VOC composition.

Conclusion

A metal oxide sensor array was used to detect the volatile organic compounds produced by cultured recombinant yeast cells expressing the human estrogen receptor α, which were induced with the natural receptor ligand 17β-estradiol. The system proved suitable for separation of cells induced with a given ligand concentration from non-induced cells. These results demonstrated that the sensor array detected changes in the cells VOC composition caused by an exogenous chemical compound. This finding suggests the possibility of using cross-selective gas sensor arrays for analysis of drugs or bioactive molecules by monitoring their interaction with cell systems, with the advantage of providing information on their bioavailability. The system could also be used to check the cell response to different types of stimuli, for example variations of temperature, pH, nutrient concentrations, etc.

Although the results achieved were encouraging, further experiments are required to optimize the system, analyze its long-term repeatability and reproducibility, and extend its applicability. In addition, the use of a pattern recognition method to process the data might enable better characterization of the differences between induced and non-induced cells.

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