Detection and quantification of ATP and heavy metal with electronical micro-circuits

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Abstract

In order to use whole eukaryotic cells as an active element in the detection and amplification of biological signals, for both *in vitro* and *in vivo* applications, we have undertaken a first approach to interface live cells and integrated circuit, and evaluate the possibility to develop a microbioreactor. An amplified photodiode system was designed and built as an electronical circuit in a way that it could easily be miniaturised. In parallel micro-chips with silicium chambers were used as microbioreactors to adhere cells. We showed here that this etched silicon chamber allows endothelial and CHO cells spreading, permitting determination of a number of cell properties *on line* providing appropriate integrated circuits are designed to perform the desired functions. The photodiode system reacting to the luminescent luciferase system permitted, through the use of appropriate software from a personal computer (PC) connected on line *in vitro*, the determination of ATP concentration, and using different luciferase transfected bacteria permitted the detection of constitutive or induced luminescence.

Introduction

The recent development of microelectronics allows emergence of smart microsensors, permitting detection, amplification and signals processing on the same micro integrated circuit (Bousse, 1995), as already generalized for physical parameters, exemplified by numerous applications in the car industry. More direct strategies involving integration of biosignals processing, and design of microbioreactor (Son et al., 1995) are under development. The construction of a smart Whole-cell/integrated circuit device implies that cells can be seen as another component (analogous to a transistor, capacitor or resistor) fully integrated in the designed circuit and that those cells, if used as a smart microsensor, could be engineered to reach the expected target.

In a previous report, using a less sensitive electronical system, we have shortly reported the use

of such microbioreactors etched into a silicium microchip to grow recombinant t-PA producing CHO cells and compare their productivity at 32 and 37 °C (Charlier et al., 1999).

In this article, we demonstrated that animal cells adhered on silicon chamber of the micro-chips and that ATP and heavy metal synthesised by bacteria cells have been detected by our biochips.

Materials and methods

Electronical circuit interface

An electronical circuit was designed where:

$$V_{\text{Out}} = V_{\text{Ref}} - R_2/R_1 (V_0 - \int (i_d + i_{ph})/C_i dt - V_{\text{Ref}}),$$

where V_{Out} = output voltage of the amplifier and VDD: supply voltage (5 V). V_0 is the reset voltage,

a)

b)

Figure 1. A precharged photodiode integrates the generated photocurrent. The signal is buffered and amplified before analog to digital conversion. The reset period and the gain are programmable as is the reference voltage for the voltage amplifier. Data are processed on the PC (personal computer). VDD: supply voltage (5 V); V_{Out} : output voltage of the amplifier; R_1 and R_2 : resistances.

that is the voltage across the photodiode right after a reset pulse. The voltage across the photodiode is a function of time. It decreases as the dark current (i_d) and the photocurrent (i_{ph}) are integrated by the photodiode capacitance C_i . V_{Ref} is generated by a programmable digital to analog (DA) converter and is chosen equal to V_0 . The gain of the amplifier is determined by the ratio of resistors R_2 and R_1 . Resistor R_2 is selected through a set of analog switches that allow to change the gain of the amplifier (gains of 1, 10 and 100 were achievable with our experimental set-up) (Figure 1a).

When the reset signal is high, the voltage across the photodiode is the full voltage supply VDD. As soon as the reset signal returns to its low value, the voltage across the photodiode drops to V_0 (the value of V_0 depends on the dimensions of the reset switch and the capacitance of the photodiode). From V_0 , the voltage across the photodiodes decreases further as both the dark current and the photo-current are integrated. The derivative of the voltage across the photodiode is proportional to the sum of the dark current and the photo-current. The photodiode is connected to a CMOS unity gain buffer (characterized by a very high input impedance) (Figure 1b).

The buffered signal is then further amplified before being converted by an analog to digital converter. The contribution of the dark current is evaluated with a blank and is subtracted from the acquired data's to isolate the contribution of the photo-current alone.

It was connected to a photodiode in order to analyse and measure emission of light. Microchips with silicium chambers were used as microbioreactors to grow cells. The behaviour of the cells on the chips is observed either with a simple binocular or by scanning electron microscopy.

Cell culture

The Chinese Hamster Ovary (CHO) and endothelial cells (either immortalized or not) were cultivated as reported elsewhere (Marique et al., 1997; Blankaert et al., 1998). The same medium conditions are used in presence of the microchip. All cells were usually grown in 80 cm² T.C. Flasks at 37 °C in a 5% CO₂ and 95% humidity atmosphere.

The cells were grown in a DMEM-HAMf-12 (50% v/v) supplemented with 5% Fœtal calf serum, 4 mM glutamine and 20 mM glucose, 2% of amino acid essential (Gibco) and 1% of amino acid non-essential (Gibco) for CHO-k1 cells and RPMI 1640 for pSV1

cells supplemented with 20% foetal calf serum, 25 µg ml⁻¹ endothelial growth factor. The cell concentration determination was carried out manually with a haemocytometer and viability by trypan blue dye exclusion. The cells were inoculated at 37 °C at 1.5×10^5 cells ml⁻¹.

Transfected bacteria were also tested. They were provided to us by Dr. P. Corbisier from the VITO. Both CM813 (constitutive luminescent *E. coli*) and CM 2624 (luminescent under Hg induction) were grown as described elsewhere (Corbisier et al., 1999).

ATP determination

Before to determine the intracellular ATP of different cell line (bacteria and mammalian cell cultures), we have realised a standard curve to calibrate the signal of the micro-chips. 20 µl of different concentrations of ATP (Boehringer Mannheim) solution is added to 20 µl luciferin–luciferase complex (40 mg ml⁻¹) on a 96 multi well plate.

After this preliminary results, we have tested the intracellular ATP in CHO-K1 cells and in *E. coli* cells.

The overnight culture of bacteria and confluent CHO-k1 cells are extracted with 10% TCA (1 hr treatment). The extract is centrifuged and washed twice with 5% trichloric acid (TCA). The pooled supernatants were neutralized with NaOH before ATP determination using luciferase from SIGMA prepared in 25 mM glycine buffer pH 7.8. A 5 fold dilution of luciferin in 10 mM dithiothreitol (DTT) is used (200 mM luciferin for 20 µl of lysed cells are measured on the photodiode).

Results

Spreading of cells on microchips

We showed that CHO and endothelial cells adhere, spread on the silicium microcircuit device used. Adhesion on oxide and nitrite silicium is confirmed for both CHO (Figure 2) and endothelial cells (data not shown). Further analysis must be done to study the growth in prolonged culture of different cell line (mammalian and bacteria cells) on the microbioreactor.

Figure 2. Spreading of CHO-K1 cells on nitrate silicium microcircuit (inoculation at 10^6 cells) after 4 hr of incubation at 37 °C. Photo is realised by electronical microscopy.

Figure 3. Determination of ATP concentration in function of the signal. (a) For a standard curve with different concentration of commercial ATP; (b) for ATP amount determination in CHO cell line and bacterial CM 2624 line.

Figure 4. Detection of bacteria growth by luminescence with the micro-chips signal. For inductible bacteria (○) and for constitutive bacteria (◆). Different volumes are taken from the culture with a D.O. = 1.320. The error of each value is 0.2 mV s^{-1} .

Figure 5. Determination of Hg concentration in function of signal for inductible bacteria. Each point is the average of four measurements. The error of each value is 0.2 mV s^{-1} .

Figure 6. Different dilutions of luminescent bacteria (CM2624) on multi well plate.

Quantitative determinations by luminescence

ATP measurements

By using an electronical circuit allowing the measure of bioluminescence through a photodiode system, we have developed a sensitive way to measure ATP through the luciferase complex activity determination. By injecting 20 μl of luciferin–luciferase complex (40 mg ml^{-1}) from Sigma and 20 μl of different ATP concentrations, we were able to device an ATP standard curve. The values were the average of 2 successive measurements in the same conditions. The error on each measurement is 2 mV s^{-1} . The limiting concentration of ATP for which we received a significant signal is 1×10^{-8} M. The detection limit is of 100 femto-mole of ATP.

These data confirmed that the sensitivity is 5 to 10 fold higher than the limit of the ATP detection from the Sigma firm (Figure 3a). The results were confirmed by biological analysis. The ATP concentration determination and luminescence from either prokaryote or eukaryotic systems can be measured with our electronical system (Figure 3b). The ATP is extracted from CHO-K1 (2×10^6 cells) and bacterial CM2624 cells (20×10^7 cells). Twenty μl of extracted ATP solution is added to 20 μl of luciferin–luciferase complex (40 mg ml^{-1}). The experiment has been done twice. The error on each measurement is 0.2 mV s^{-1} . We observed differences in both experiments probably due to the extraction method of ATP and the different ATP amounts present in cells.

The ATP calibration appeared thus to be a method which permits to detect the presence of life cells (bacteria or animal cells).

Growth of bacteria

Different volumes of an overnight cell culture are used to test the growth of constitutive and inducible bacteria whose emit luminescence (induction with 10^{-5} M HgCl for 2 hr). The D.O. of the two bacteria suspension is 1.320, we have taken 4 measurements by injected volume with a gain of 10 (Figure 4). The relation between the culture volume and the signal of luminescence is linear. It seems clear that we are able to detect the quantity of the different bacteria from sample.

Hg analysis

By using bacterial cells transfected with Hg inducible luciferase complex system, quantitative determinations can be made (Figure 5). In microtiter plates

(Figure 6), 2-fold dilutions in individual wells (right to left) of the induced bacterial suspension presents a gradient of luminescence detectable by the naked eye. The incorporation of a photodiode microchips appropriately designed in each well could be easily recorded a quantitative signal.

Conclusions

Biocompatibility of microchips was demonstrated for endothelial and CHO cells and microbioreactor could be developed, permitting on line analysis on the basis of the design of an appropriate sensor system and the development of a tool allowing on line analysis. The microreactor could be connected directly to an appropriate sensor for different kinds of measurements and/or control (the possible signals to measure could result for example from enzyme-substrate, receptor-binder, antibody-antigen interactions, ... for which appropriate sensors should be designed). The same concept as the present one based on luminescence detection through a photoelectronic device that could easily be miniaturised, is of course valid for any other biomolecular signal. A number of interesting applications may therefore be soon at reach. Our system is however still at present just at the limit of the sensitivity of the luminescence emitted by the available luminescent transfected eukaryote cells tested. Further improvements should however soon permit applications in this field of interest for high throughput diagnostic screening.

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References

- Blankaert D, Simonart T, Van Vooren JP, Parent D, Liesnard C, Farber CM, Marique T & Wérenne J (1998) Quantitative release of metalloprotease-9 (92 kDa type IV collagenase) by Kaposi's sarcoma cells. *J AIDS Human Retrovir* 18: 203–209.
- Bousse L (1995) Whole cell biosensors. The 8th International Conference on Solid State Sensors and Actuators, and Eurosensors IX (pp. 483–486), Stockholm, Sweden.
- Charlier O, Goffaux MJ, Marique T, Hendrick V, De Vos L & Wérenne J (1999) Whole cell smart microsensor: A first approach towards interfacing live cells and electronics. In: A

- Bernard, B Griffiths, W Noé & F Wurm (eds.) *Animal Cell Technology: Products from Cells, Cells as Products* (pp. 219–221), Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Corbisier P, Van der Lelie D, Borremans B, Provoost A, Delorenzo V, Brown NL, Lloyd JR, Hobman JL, Csoregi E, Johansson B & Mattiason B (1999) Whole cell and protein based biosensors for the detection of bioavailable heavy metal in environmental samples. *Analyst Chim Acta* 387: 235–244.
- Marique T, Blankaert V, Hendrick V, Raschella A, Declerck B, Alloin C, Teixeira-Guerra I, Sandron D, Cherlet M, Parent D, Kirkpatrick C, Van Vooren JP & Wérenne J (1997) Biological response of endothelial cells and its modulation by cytokines: Prospects for therapy and bioprocess. *Cytotechnol* 25: 183–189.
- Son M, Peddie F, Yeow T, & Haskard M (1995) Microbioreactor created by interfacial membrane polymerization. The 8th International Conference on Solid State Sensors and Actuators, and Eurosensors IX (pp. 894–897), Stockholm, Sweden.