

# Electrochemical 'Lab on a chip' for Toxicity Detection in Water

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## ABSTRACT

An electrochemical 'Lab-on-a-chip' for water toxicity detection is presented. This miniaturized device containing an array of nano liter electrochemical cells, which integrates bacteria and can emulate physiological reactions in response to different chemicals. Bacteria, which have been genetically engineered to respond to environmental stress, act as a sensor element and trigger a sequence of processes, which leads to generation of electrical current.

The silicon chip contains an array of nano-volume electrochemical cells that house the bacteria, connected to a sensing and data analysis unit. Each of the electrochemical cells in the array can be monitored independently and simultaneously with the others.

A measurable current signal, well above the noise level, was produced within less than 10 minutes of exposure to representative toxicants. This miniature device provides high throughput rapid and sensitive real-time detection of acute toxicity in water.

**Keywords:** Electrochemical sensor, bio-chip, whole cell biosensors, lab on a chip, BioMEMS.

## 1. INTRODUCTION

The use of living organisms as active components in electronic devices is an innovative and challenging area combining recent progress in molecular biology and micro technology. The aim of this study is to develop a system, composed of new design and process of MEMS that integrates genetically engineered living organisms serving as physiological sensors, and an electrochemical system functioning as an electrical sensor.

Nowadays, there is a tendency to limit the use of animals in laboratory experiments and to prefer technologies that uses microorganisms as experimental models. Although microorganisms differ from highly developed organism, at the molecular and physiological levels there are many shared features by both species including response to toxicants, drugs and stressful conditions[1].

The vast development in genetic engineering of live cells, enables the use of recombinant cells as cell-based sensing systems [2, 3]. The cascade of mechanisms by which *E.coli* bacterial reactions to toxic chemicals or to stressful condition are electrochemically converted into electronic signals have been previously reported[4-6].

The potential uses for microorganisms-based systems could have important applications in pharmacology, medicine, cosmetics, and environmental monitoring.

## 2. CHIP DESIGN AND FABRICATION

The chip has been designed and fabricated using micro-system-technology (MST) methods. The materials for chip construction have been selected with special considerations on their biocompatibility characterization, since they are aimed to be in direct contact with living cells. The chip was produced on silicon wafers and includes an array of eight independent electrochemical cells, which are temperature-controlled. Each electrochemical cell can hold 100 nL of solution and consists of three embedded electrodes: 1) Gold working electrode, 2) Gold counter electrode and 3) Ag/AgCl reference electrode. The wall of the chambers constructed from photopolymerized polyimide (SU-8). The shape and size of the nano chambers as well as of the microelectrodes were designed so it could be easily modified, and their size could be scaled up and down for any specific applications. The device was manufactured as two parts: the first part is a disposable silicon chip - with an array of electrochemical cells. The silicon chip was wire bonded to a special printed circuit board (PCB) platform, which was connected to the data processing units. The PCB manufacturing process consists of selective deposition of gold which creates conducting bands. The bands width in the center were designed to be the same width as the external gold pads of the silicon chip in order to wire bond between them, while the bands width in the external region of the PCB fitted precisely to the socket of the electronic sensing unit (Figure 1). The second part of the device is reusable, which includes a multiplexer, potentiostat, temperature control and a pocket PC for sensing and data analysis. This setting allows continuous reusing for multiple measurements.

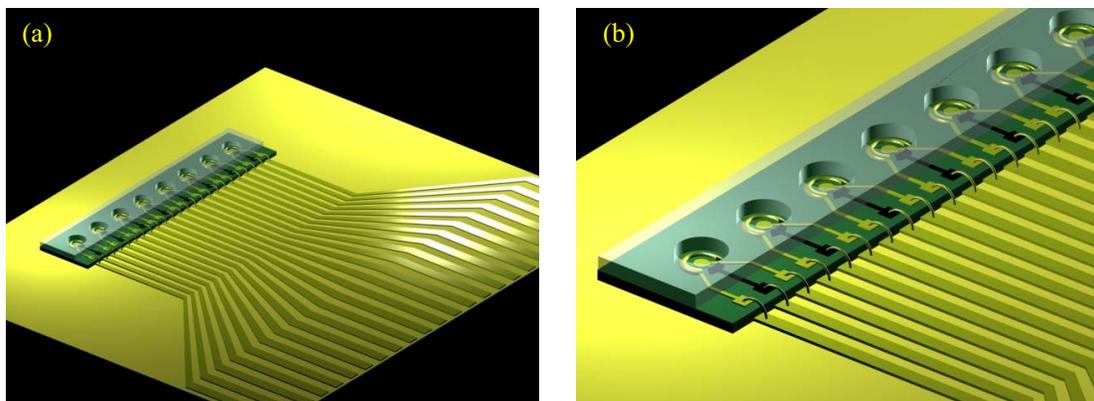


Figure 1: Image of the electrochemical silicon chip wire bonded to the PCB platform. (a) Array of eight 100nL electrochemical cells on a silicon chip. The chip is glued to the tailored PCB platform, and the chip's gold pads are wire bonded to the gold PCB's electrodes. The PCB board enters directly to the socket of an external sensing circuit. (b) Electrochemical-cells on chip consist of three embedded electrodes: gold working electrode, gold counter electrode, and Ag/AgCl reference electrodes.

### 3. RECOMBINANT BACTERIA

Genetically engineered bacteria were used as whole cell sensors for acute toxicity in water. The recombinant bacteria react to the presence of toxin by activating specific promoter (regulatory DNA sequence). This promoter induces the production of the reporter enzyme  $\beta$ -galactosidase. This enzyme reacts with the PAPG substrates (molecules that were initially placed inside the chambers) to produce two different products: electrochemically active product *p*-aminophenol (PAP), and inactive product  $\beta$ -D-galactopyranoside. The PAP molecules are oxidized on the working electrode at 220mV. This oxidation is converted to a current signal using an Amperometric technique.

### 4. EXPERIMENTAL

In the present work, we used recombinant *E. coli* bacteria bearing plasmid with one of the following promoters: *Dnak*, *grpE* or *fabA*. These promoters were fused to the reporter enzyme  $\beta$ -galactosidase [5, 7]. Ethanol [1%] or phenol [1.6ppm] were introduced to the bacterial samples in the presence of the substrate PAPG. Immediately after (~1second), the suspensions were placed in the electrochemical cells. The response of the bacteria to the toxic chemicals was measured on-line by applying a potential of 220 mV. The substrate, PAPG, was added to a final concentration of 0.8 mg/ml (100 nL total volume). The product of the enzymatic reaction (PAP) was monitored by its oxidation current. Additional measurements in the absence of the bacteria were performed to exclude the

possibility of electroactive species in the LB medium, in the substrate, or in the substrate and the LB medium mixture, which can contribute to the current response.

Bacteria at  $3 \times 10^7$  cells/ml were used for all experiments.

### 5. RESULTS AND DISCUSSION

Real time detection of the response of recombinant *E. coli* bacteria, with one of the promoters *Dnak*, *grpE* or *fabA*, to ethanol and phenol are shown in figure 2 and 3 respectively. The results show that concentration of 1% ethanol could be detected within less than 10 minutes, and concentration as low as 1.6ppm phenol could be detected within less than 6 minutes. Different intensity response of the various bacterial sensors, *dnaK*, *grpE* and *fabA*, to ethanol and phenol is due to the specific activation of each promoter to the type of the toxicant. The promoters *dnaK* and *grpE* are sensitive to protein damage (SOS system), thus, they were induced in response to ethanol which is known as protein damage agent [2] (figure 2). *grpE* showed high induction activity in response to ethanol, *dnaK* showed reduced enzyme activity and *fabA* was only slightly induced above the background level. *fabA* promoter is sensitive to membrane damage, and thus, reacts to phenol exposure, which is known as membrane damage chemical [ ]. As expected, *grpE* and *dnaK* promoters were less activated by phenol (figure 3).

In comparison to equivalent optical detection methods using whole cell biosensors for water toxicity detection, these results proved to be more sensitive and produce faster response time. Concentrations as low as 1% of ethanol and 1.6 ppm of phenol could be detected in less than 10 minutes of exposure to the toxic chemical, whilst a recent study [8], which utilized bioluminescent *E. coli* sensor cells, detected

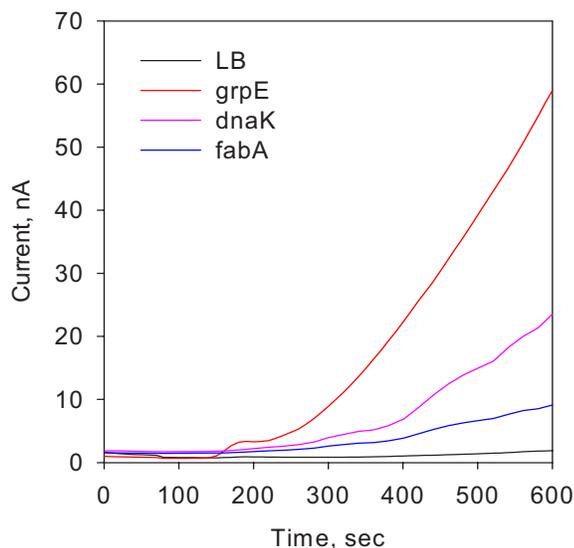


Figure 2: Amperometric response curves for on line monitoring of different *E. coli* reporters in response to the addition of 1% ethanol, using the nano-bio-chip. The different *E. coli* reporters are *fabA* *dnaK* and *grpE*. Measurement performed immediately after the ethanol addition (~1min) at 220mV working potential vs Ag/AgCl reference electrode. The LB curve represents the bacterial response to the LB medium with the substrate PAPG without ethanol.

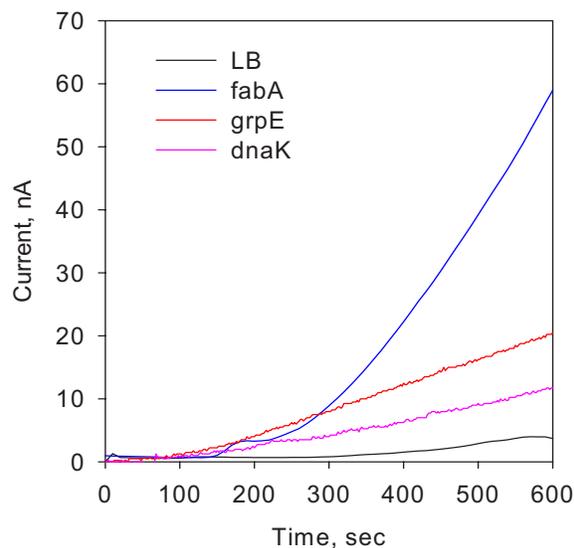


Figure 3: Amperometric response curves for on line monitoring of different *E. coli* reporters in response to the addition of 1.6ppm phenol, using the nano-bio-chip. The different *E. coli* reporters are *fabA* *dnaK* and *grpE*. Measurement performed immediately after the ethanol addition (~1min) at 220mV working potential vs Ag/AgCl reference electrode. The LB curve represents the bacterial response to the LB medium with the substrate PAPG without phenol.

0.4M (2.35%) ethanol after 220 minutes. An additional study[9] based on fluorescent reporter system (GFP), enabled detection of 6% ethanol and 295 ppm phenol after more than one hour. Cha et al [10] used optical detection methods of fluorescent GFP proteins, detected 1g of phenol per liter (1000ppm) and 2% ethanol, after 6 hours. Other studies[11], could not be directly compared due to different material used, however their time scale for chemicals identification is hours.

These results emphasize the advantages of merging electrochemical detection methods with adjusted design and process of MEMS, which resulting in fast response time and low detection limit. Enhanced sensitivity and high signal to noise ratio is achieved by optimizing the ratio between working electrode area and cell volume. The larger the ratio the higher the signal.

In order to prevent false alarms, all arrays include positive and negative controls chambers. In the positive control chamber other than adding the tested sample with the unknown chemicals to the bacterial solution, pure water was added. In case a current signal was generated, it is a false alarm. A negative control chamber includes w.t. (MG1655) *E. coli* bacteria that constitutively expresses  $\beta$ -galactosidase, thus, current should be generated in all cases. When no current generated, measurement is incorrect, due

to bacterial death from highly toxic chemicals added or from other unknown reason. However, chemicals can produce only constant DC current signal, while the enzymatic reaction act as an intrinsic amplifier, and generates increasing current signal.

Biochemical process, which intends to produce a measurable signal, has a great benefit while utilizing enzymatic activity. Since enzymes form continuously, and each enzyme reacts with many substrate molecules successively, this enzymatic mechanism serves as an intrinsic amplifier; consequently the signal produces faster, more sensitively and increasing with time [12]. Combining enzymatic system with electrochemical detection methods enables measurements in turbid solutions and under anaerobic conditions[5].

In addition to the aforementioned capabilities, this 'lab on a chip' system could be easily adapted to different applications, including specific identification of chemicals by using binding techniques, i.e., each electrochemical cell in the array can incorporate different biosensor. Thus, large amount of analytes can be detected simultaneously and independently. Similarly, in experiments aiming to analysis physiological reactions, bacteria harboring different types of promoters can be introduced to the chambers, and thus, this lab on a chip can detect simultaneously a variety of toxicant types.

## 6. CONCLUSIONS

Integrating recombinant microorganisms tailored to respond to specific biochemical events, together with unique and adjusted nanoscale electronic systems, provides exciting opportunities in the biosensors field as well as in the basic research on microorganisms. In this study, a miniaturized and portable electrochemical analytical device was fabricated, studied and characterized. The benefit of this new geometry, in which electrochemical cell dimensions were reduced to nano-scale, and the ratio between the working electrode area and cell volume was optimized, is demonstrated by the results presented here, which showed high sensitivity and extremely fast response time. The construction of an array of nano chambers on one silicon chip leads to high throughput in addition to the capability of performing multi experiment simultaneously and independently.

This design enables performance of multi experiments simultaneously and each electrochemical cell can be measured independently. The total weight of the entire

system is ~900 grams, making it ideal for field environmental monitoring and for medical applications. Further size reduction is achievable using identical process and can provide MEMS for single cell measurements as well as for ensemble, but should be considered by the specific application requirements. In addition, better packaging can reduce the size and weight, keeping all the electrical parameters intact. Miniaturization of the electrochemical cell and favourable area to volume ratio, leads to short diffusion distance of the analytes towards electrode surface, therefore, providing improved signal to noise ratio, faster response time, enhanced analytical performance and increased sensitivity

The main features of the proposed "lab on chip" include small sampling requirements, high throughput, low false positive and false negative rates, high robustness and reliability and most probably very low cost in mass production. The proposed platform can be used for various applications that require 'electrochemical cell-based lab on a chip'.

## REFERENCES

- [1] L. H. Hansen and S. J. Sorensen, "The use of whole-cell biosensors to detect and quantify compounds or conditions affecting biological systems," *Microbial Ecology*, vol. 42, pp. 483-494, 2001.
- [2] S. Daunert, G. Barrett, J. S. Feliciano, R. S. Shetty, S. Shrestha, and W. Smith-Spencer, "Genetically engineered whole-cell sensing systems: Coupling biological recognition with reporter genes," *Chemical Reviews*, vol. 100, pp. 2705-2738, 2000.
- [3] S. Kohler, T. T. Bachmann, J. Schmitt, S. Belkin, and R. D. Schmid, "Detection of 4-chlorobenzoate using immobilized recombinant Escherichia coli reporter strains," *Sensors and Actuators B-Chemical*, vol. 70, pp. 139-144, 2000.
- [4] I. Biran, L. Klimentiy, R. Hengge-Aronis, E. Z. Ron, and J. Rishpon, "On-line monitoring of gene expression," *Microbiology-Sgm*, vol. 145, pp. 2129-2133, 1999.
- [5] Y. Paitan, D. Biran, I. Biran, N. Shechter, R. Babai, J. Rishpon, and E. Z. Ron, "On-line and in situ biosensors for monitoring environmental pollution," *Biotechnology Advances*, vol. 22, pp. 27-33, 2003.
- [6] Y. Paitan, I. Biran, N. Shechter, D. Biran, J. Rishpon, and E. Z. Ron, "Monitoring aromatic hydrocarbons by whole cell electrochemical biosensors," *Analytical Biochemistry*, vol. 335, pp. 175-183, 2004.
- [7] R. Babai and E. Z. Ron, "An Escherichia coli gene responsive to heavy metals," *Fems Microbiology Letters*, vol. 167, pp. 107-111, 1998.
- [8] J. R. Premkumar, O. Lev, R. S. Marks, B. Polyak, R. Rosen, and S. Belkin, "Antibody-based immobilization of bioluminescent bacterial sensor cells," *Talanta*, vol. 55, pp. 1029-1038, 2001.
- [9] S. Belkin, "Microbial whole-cell sensing systems of environmental pollutants," *Current Opinion in Microbiology*, vol. 6, pp. 206-212, 2003.
- [10] H. J. Cha, R. Srivastava, V. M. Vakharia, G. Rao, and W. E. Bentley, "Green fluorescent protein as a noninvasive stress probe in resting Escherichia coli cells," *Applied and Environmental Microbiology*, vol. 65, pp. 409-414, 1999.
- [11] D. E. Nivens, T. E. McKnight, S. A. Moser, S. J. Osbourn, M. L. Simpson, and G. S. Saylor, "Bioluminescent bioreporter integrated circuits: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring," *Journal of Applied Microbiology*, vol. 96, pp. 33-46, 2004.
- [12] E. Sagi, N. Hever, R. Rosen, A. J. Bartolome, J. R. Premkumar, R. Ulber, O. Lev, T. Scheper, and S. Belkin, "Fluorescence and bioluminescence reporter functions in genetically modified bacterial sensor strains," *Sensors and Actuators B-Chemical*, vol. 90, pp. 2-8, 2003.