Letter

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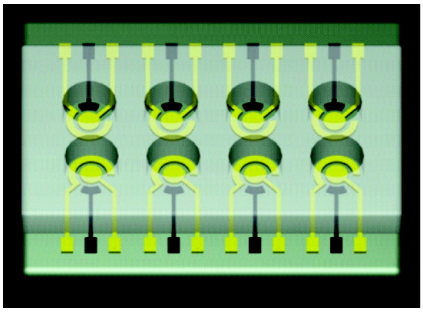

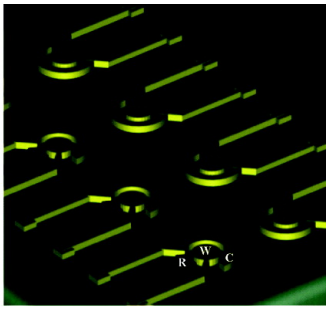

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Novel Integrated Electrochemical Nano-Biochip for Toxicity Detection in Water

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ABSTRACT

An electrochemical nano-biochip for water toxicity detection is presented. We describe chip design, fabrication, and performance. 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. This novel, portable and miniature device provides rapid and sensitive real-time electrochemical detection of acute toxicity in water. A clear signal is produced within less than 10 min of exposure to various concentrations of toxicants, or to stress conditions, with a direct correlation between the toxicant concentration and the induced current.

Integrating living cells with microelectronics is a novel approach that takes advantage of both worlds: biology and engineering. Potential uses for cell-based systems have a wide range of applications in the fields of pharmacology, medicine, cell biology, toxicology, basic neuroscience, and environmental monitoring. The most important reason for using living cells is to obtain functional information, i.e., information about the effect of a stimulus on living systems. In many cases, functional rather than analytical information is ultimately desired. In other applications, functional information complements and adds a special feature to a system by emulating the behavior of living entities.

The vast development in genetic engineering of live cells enables the use of recombinant cells as cell-based sensing systems.[1,2] These whole cell-sensing systems employ sensor cells that express a reporter gene upon exposure to toxic substances. The reporter produces a readily measurable signal. These whole-cell sensing systems can be visualized as an environmental switch that is turned on in the presence of toxins or stressful conditions.

In the present era, the increased threat of chemical warfare terrorism and the pollution of groundwater due to rapid industrialization have stimulated investigations of methods to detect water toxicity. Previously published papers report the detection of only specific toxicants, using binding components such as antibodies, nucleic acids, or protein ligand.[3] Other investigations demonstrated the use of biosensors based on living cells in detecting water toxicity.[4,5] Most current bacterial biosensors demonstrate the ability of bioluminescence-based bacterial biosensors to detect water toxicity.[6,7] These optical detection-based devices lack the advantages offered by electrochemical detection,[8,9] such as sensitivity, fast response time (within minutes), simplicity, compactness, and mobility. We offer the advantages of microchip technology, which makes it feasible to detect toxicants in the field, in real-time, and at low cost, with electrochemical monitoring that produces electrical output, which is convenient to handle and analyze. Thus, the measurement is reliable, useful for nanoliter water samples, and overcomes practical problems such as water turbidity and the presence of particles.

Here we present an innovative nano-biochip, which contains an array of nanovolume electrochemical cells, based on silicon microsystem technology (MST). *Escherichia coli* bacteria (MC1061), genetically engineered to express electrochemical detectable signals in the presence of toxicants,[10] were integrated into the chip. The electrical current generated by the cells was detected and analyzed. We first present our experimental setup, our device, and processes. Next, we show data on testing the device on contaminated water. Finally, we present a discussion and conclusions.

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The device has been designed and fabricated using standard microsystem technology methods. Its architecture includes an array of miniaturized electrochemical cells. The nanovolume chambers (i.e., the electrochemical cells) contain the bacteria, which are brought into contact with the water to be examined. The cylindrical chambers hold 100 nL volume each. All arrays include positive and negative controls chambers.

The device is manufactured in two parts: (a) a disposable chip, with the nanochambers containing the bacteria, and (b) a reusable chip, with an interface to electronic circuitry which includes a multiplexer, potentiostat, temperature control, and a pocket PC for sensing and data analysis. This setting allows continuous reuse for multiple measurements.

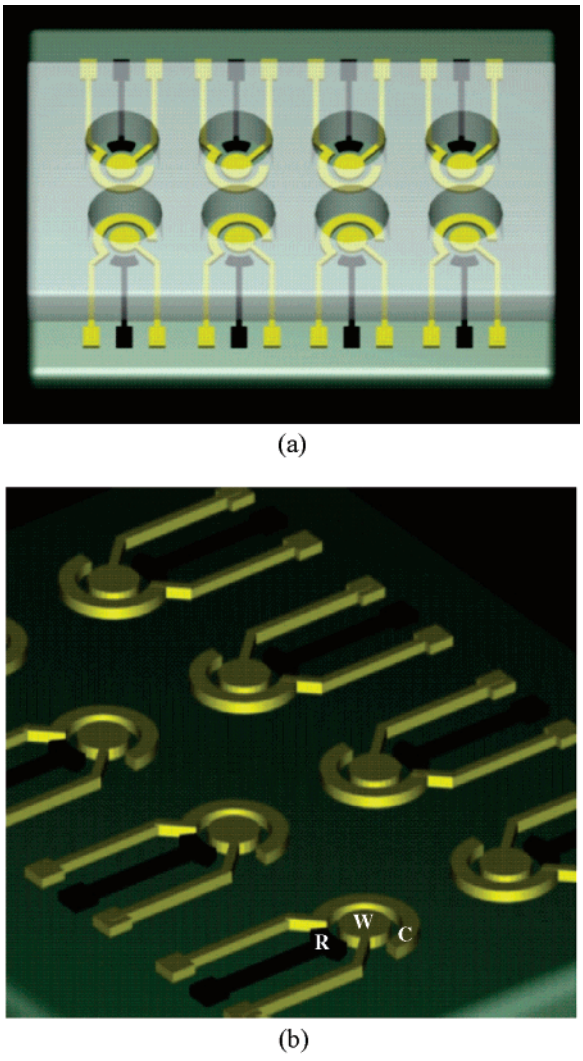
The chip was produced from silicon and contains an array of eight miniaturized electrochemical cells. Each electrochemical cell consists of three circular-shaped electrodes, surrounded by an insulating silicon nitride layer: (1) gold working electrode, (2) gold counter electrode, and (3) Ag/AgCl reference electrode. The electrodes are made by gold sputtering, microlithography, and by selectively depositing Ag and anodizing it in a chloride-containing solution for the reference electrode. The chamber walls were constructed from photopolymerized polyimide (SU-8) (Figure 1). The silicon chip was wire bonded to a plastic chip, which was interfacing the electronic circuit.

This new design of nanochamber arrays on chips allows a broad band of measurements. We can simultaneously test eight different toxicant types with the general stress responsive promoter by introducing to each chamber a different toxicant, or, to specify unknown aqueous sample, we can test the sample with eight different stress responsive promoters, thus we obtain an indication of the toxicant type. In addition, the array configuration enables the addition of positive and negative control chambers for each experiment.

The signal sensing is performed by the handheld palm-potentiostat with an interface to electronic circuitry for electrode signal regulation and detection (Palm Instruments BV-2004). The electronics consists of eight independent duplicate circuits of electrochemical cells, which are temperature-controlled. A potential is applied between a working and a reference electrode in each electrochemical cell, and the output current is measured.

The work was carried out using *E. coli*, which is the most convenient tool for genetic engineering, and a suitable host cell for carrying a variety of promoter−reporter fusions. We used *E. coli* (MC1061), which have a deletion in the chromosomal *lacZ* gene and carry recombinant plasmids that contain fusions of a promoterless *lacZ* gene to promoters of heat shock genes, coding for the GrpE, and DnaK heat shock proteins (*grpE::lacZ* and *dnaK::lacZ*, respectively). The *grpE* and *dnaK* promotors respond to a variety of stresses, mainly those which cause protein denaturation, such as elevation in temperature and exposure to a broad spectrum of chemicals including ethanol or heavy metals.[11,12]

This recombinant *E. coli* strain, containing a promoterless *lacZ* gene fused to promoters of choice, is used for electrochemical monitoring of the enzyme activity of β-galactosidase, which reports on the activity of the promoter. These bacteria, which are genetically engineered to respond to environmental stress, act as a sensing element and trigger a cascade mechanism: (a) in the presence of a toxin, this promoter is activated and induces the production of the reporter enzyme β-galactosidase; (b) as the enzyme is produced, the activity of β-galactosidase is determined by using the substrate *p*-aminophenyl β-D-galactopyranoside (PAPG); (c) the product of the enzymatic reaction, *p*-aminophenol (PAP), is oxidized at an electrode at 220 mV.[13,14] This oxidation current is monitored and visualized on the PC screen.



(a)

(b)

Figure 1. Image of the electrochemical chip. (a) Silicon chip contains an array of eight miniaturized electrochemical cells with external pads. (b) The electrodes without the top layer (SU-8). Each electrochemical cell consists of three circular-shaped electrodes: gold working (W), gold counter (C), and Ag/AgCl reference (R) electrodes. Dimensions of the electrodes: working electrode area 30 nm^2, counter electrode area ~300 nm^2. Total volume of each electrochemical cell is 100 nL.

Bacterial Cultures. *E. coli* strains were grown to early log phase at 30 °C in 100 mL of Luria broth (LB) medium with aeration by shaking. Ampicillin, at a final concentration of 100 μg/mL, was added to ensure plasmid maintenance. Cultures at 3×10^7 cells/mL were used for all experiments.

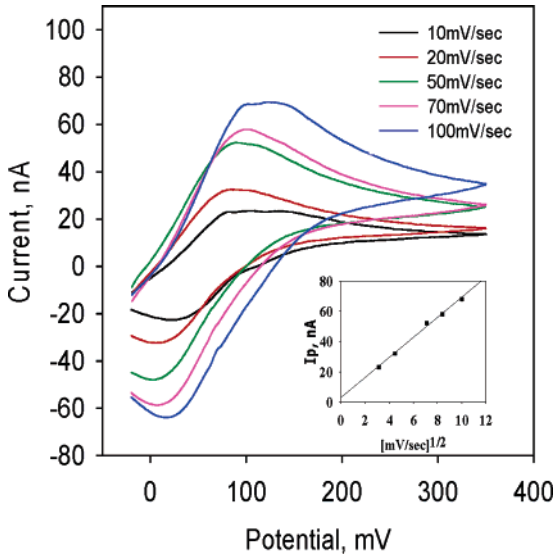


Figure 2. Representative cyclic voltammetric responses of 5 μM PAP in 0.1 M phosphate buffer containing 0.1 M KCl pH = 7.5, in one of the 100 nL volume on chip electrochemical cells. The inset represents the relation between [scan rate]$^{1/2}$ versus Ip (anodic peak current).

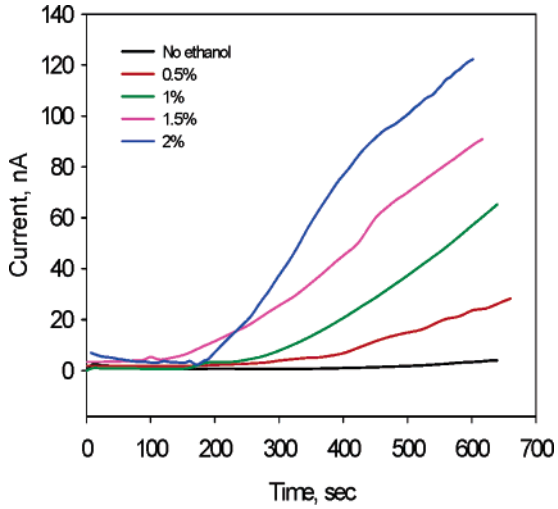


Figure 3. Amperometric response curves for real-time monitoring of ethanol using the nano-biochip. The recombinant *E. coli* containing a promoterless *lacZ* gene fused to promoter *grpE* exposed to 0.5−2% concentration of ethanol. The bacteria cultures with the substrate PAPG and the ethanol were placed into the 100 nL volume electrochemical cells on the chip immediately after the ethanol addition (∼1 min) and were measured at 220 mV.

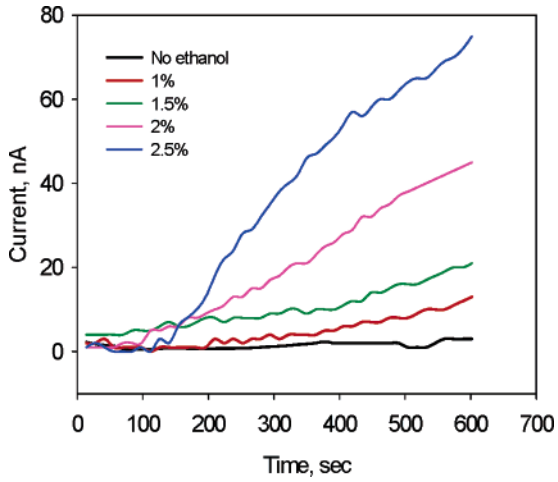


Figure 4. Amperometric response curves for real-time monitoring of ethanol using the nano-biochip. The recombinant *E. coli* containing a promoterless *lacZ* gene fused to promoter *dnaK* exposed to 0.5−2% concentration of ethanol. The bacteria cultures with the substrate PAPG and the ethanol were placed into the 100 nL volume electrochemical cells on the chip immediately after the ethanol addition (∼1 min) and were measured at 220 mV.

Electrochemical Measurements. Several toxic chemicals were introduced to the bacterial samples at increasing concentrations, together with the substrate PAPG. Immediately after exposing the bacteria to the toxic chemicals, the suspensions were placed on the electrochemical cells. β-galactosidase activity was measured in real-time by applying 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 and the resultant current was monitored.

The electrochemical performance and characterization of the device was investigated with the redox model compound, *p*-aminophenol (PAP), in 0.1 M phosphate buffer containing 0.1 M KCl pH = 7.5. Typical results of steady-state cyclic voltammetry with different scan rates are shown in Figure 2. The linear correlation between the square root of the scan rates and the Ip (anodic peak current) shows diffusion-controlled process (Figure 2 inset).

The analytical performance of the nano-biochip was determined with various chemicals to exemplify its ability to detect water toxicity. The advantage of our system is that the cells are genetically engineered to amplify their activity under stressful conditions, rather than wait until the cells start to lose viability and die. The enzyme is generated continuously due to the toxicant exposure, thereby the signal is increasing with time. Ethanol and phenol, efficient inducers of the heat shock proteins, were introduced to *E. coli* cultures harboring the promoters *grpE* and *dnaK*. *E. coli* cultures (3×10^7 cell/mL) with the PAPG substrate were brought into contact with samples containing increasing concentrations of ethanol or phenol and were immediately (∼after 1 min) placed into the electrochemical chambers. The induced β-galactosidase activity was monitored electrochemically and the results are shown in Figures 3, 4, and 6. The results indicate that there was a direct correlation between the currents signals and the toxicant concentrations. Moreover, concentrations as low as 0.5% of ethanol and 1.6 ppm of phenol could be detected in less than 10 min of exposure to the toxic chemical, while others,[4,15] using the fluorescent reporter system, detected 6% ethanol and 295 ppm phenol after more than 1 h. Cha et al.[16] were able to detect 2% ethanol after 6 h.

The obtained results, which are with agreement to the results of previous studies,[16,17] illustrate that the response current signals that were generated by the *dnaK* promoter were typically lower than that of the *grpE* promoter (Figure

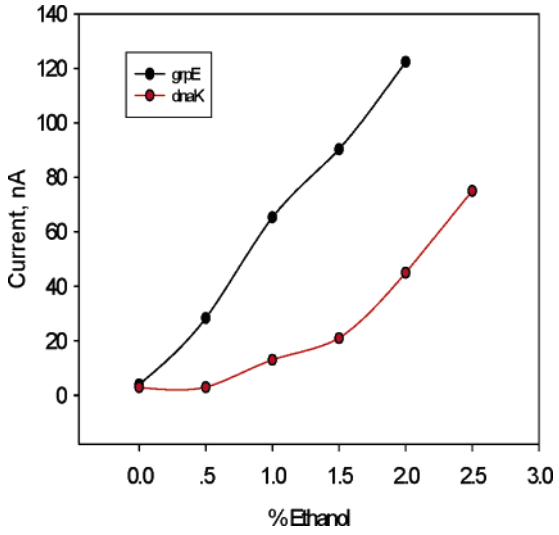


Figure 5. Comparison between the grpE and dnaK promoters response to ethanol detection. The two experiments were performed under the same conditions. Current points were sampled after 600 s.

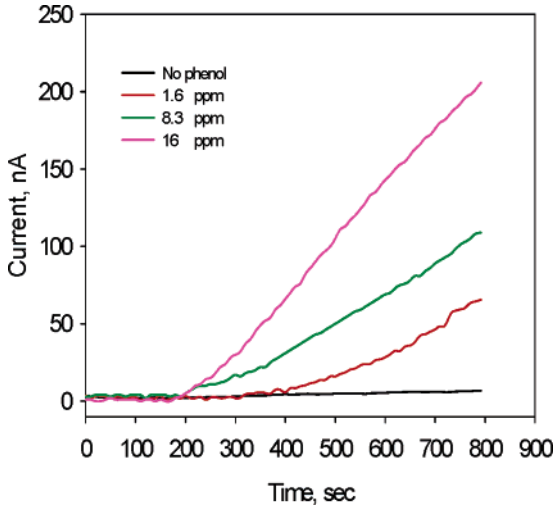


Figure 6. Amperometric response curves for real-time monitoring of phenol using the nano-biochip. The recombinant *E. coli* containing a promoterless *lacZ* gene fused to promoter grpE exposed to 1.6 ppm-16 ppm concentration of phenol. The bacteria cultures with the substrate PAPG and the phenol were placed into the 100 nL volume electrochemical cells on the chip immediately after the phenol addition (~1 min) and were measured at 220 mV.

5). Moreover, an induction period appears before the current start to emerge since there is a response time at the genetic level.

There are fundamental differences between nonenzymatic microbial sensing systems (for example GFP), in which the concentration of the reporter protein is determined, and these systems, which are based on measuring enzyme activity (e.g., β-galactosidase and alkaline phosphatase). The latter methods are more sensitive and rapid, as enzymes work catalytically, converting many substrate molecules and resulting in signal amplification. Electrochemical detection methods are also highly sensitive, since the measured current signal, i.e., the number of transferred electrons that result from an enzymatic reaction, can be accurately quantified. Furthermore, electrochemical monitoring can be carried out in turbid solutions or even under anaerobic conditions, as it does not require oxygen, in contrast to the light-emitting biosensors.[14] Our nano-biochip electrochemical system based on the enzymatic activity of β-galactosidase provides all these aforementioned advantages.

The results presented here demonstrate the ability of the electrochemical nano-biochip to detect toxic cellular stress and to determine the direct correlation between the toxicant concentration in water samples and the induced current. Moreover, the results emphasize the main advantages of this electrochemical device: (1) real-time response and a clear signal, (2) fast response time within minutes, (3) highly sensitive measurements and the ability to detect miniaturize levels of toxic chemicals in water.

A novel integration between a microelectronic device and living organisms for electrochemical detection of toxicity in water has been successfully developed and tested. The bacterial-based device was found to be sensitive and promises significant advantages in analytical speed, reduced sample/reagent consumption, and cost reduction. However, there are still many question marks regarding electrochemical biochips. In this paper we demonstrated one approach where the Au/Ag/AgCl electrodes were made by a combination of sputtering, plating, and anodization. Other methods are possible and it is yet to be determined which is the best method. Also, we use a silicon substrate, which is compatible with CMOS integrated circuit technology. However, Si substrates are expensive, and in the future we anticipate developing similar processes on plastic substrates.

The electrochemical detection method has been shown to be sensitive, simple, and can be performed even in turbid solutions and under anaerobic conditions. By using a compact and portable device we are capable of measuring water toxicity in the field. The nL volume array chambers enable simultaneous measurement of eight samples, and it can be extended to more samples in the future. The small volume (100 nL) of the electrochemical cells allows minimum interaction with the water flow, resulting in increased speed and sensitivity due to the small diffusion length of the analytes to the electrode surface.

The novel technology described here, which combines biology and engineering, enables multianalyte detection, high throughput screening, miniaturization, portability, and real-time detection. It is expected that this technology can be most beneficial to the healthcare and medical industries, for environmental monitoring, and for control of chemical or pharmaceutical industry processing.

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