Electrochemical detection of biological reactions using a novel nano-bio-chip array

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Abstract

In this study we developed an innovative electrochemical 'lab on a chip' system that contains an array of nano-volume electrochemical cells on a silicon chip. Each of the electrochemical cells can be monitored simultaneously and independently, and each cell contains three embedded electrodes, which enable performance of all types of electrochemical measurements. The integration of living organisms on an electrochemical array chip that can emulates reactions of living organisms and senses essential biological functions have never been demonstrated before. In order to show the wide range of applications that can be benefited from this device, biological components including chemicals, enzymes, bacteria and bio-films were integrated within the nano-chambers for various applications. During the measurement period the bacteria remained active, enabling cellular gene expression and enzymatic activity to be monitored on line.

The miniaturized device was designed in two parts to enable multiple measurements: a disposable silicon chip containing an array of nano-volume electrochemical cells that are housing the biological material, and a reusable unit that includes a multiplexer and a potentiostat connected to a pocket PC for sensing and data analysis.

This electrochemical 'lab on a chip' was evaluated by measuring various biological reactions including the microbial current response to toxic chemicals. These bacteria were genetically engineered to respond to toxic chemicals by activating cascade of mechanisms, which leads to the generation of electrical current. A measurable current signal, well above the noise level, was produced within 5 min of exposure to phenol, a representative toxicant. Our work shows faster and more sensitive functional physiological detection due to the unique concept demonstrated here.

1. Introduction

Integration of biological substance 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 biocompatible electrochemical 'lab on a chip' system, composed of new design and process of MEMS that can integrates biological substance as well as living organisms and enables accurate, sensitive and rapid electrochemical measurements, using nano-volumes of materials.

Miniaturization of electrochemical devices is highly important because of portability, convenient handling and reduce amount of reagents required. Moreover, there are several additional fundamental advantages. The 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 [1]. These characteristics result in more sensitive and rapid detection of biochemical and physiological processes that are essential for basic research as well as for medical applications.

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Recently several studies reported experiments in which biological materials were integrated with microelectronic devices. Some works rely on structural recognition and specific interactions within the biological material such as enzymes [2–5] and DNA biosensors [6,7], which enables identification and quantification of known chemicals, but useless when the target chemical is unknown. Other studies used recombinant microorganisms [8–10], neurons [11–14] and tissues [15] in order to achieve physiological reactions to drugs and toxicants. The present challenge is to improve whole cell electrochemical biosensors in order to exploit the 'lab on a chip' concept for real-life applications such as in medical diagnostics and on site environmental pollution detection.

The vast development in genetic engineering of live cells, enables the use of recombinant cells as cell-based sensing systems [16,17]. Mammalian cells [18,19] and animal tissues [20] provide physiologically and medically relevant information regarding drugs and toxin detection. However, these types of biosensors are generally less stable, and therefore, their applicability as biosensors is limited. Bacteria are the most convenient and commonly used tools for genetic engineering, since they are readily amenable to genetic manipulation, have large population sizes, and are stable in a variety of environmental conditions [21,22]. The cascade of mechanisms by which *Escherichia coli* bacterial reactions to toxic chemicals or to stressful condition are electrochemically converted into electronic signals have been previously reported [23–25].

Previously, we reported the specific application of this "lab on a chip" system for water toxicity detection [26]. The work we present here focuses on the design, process and fabrication of the device and demonstrates its use for a few general biological applications. To the best of our knowledge, this is the first demonstration of a device containing an array of nano-volume electrochemical cells, which integrates bacteria and can emulate physiological reactions in response to different chemicals.

The portable device includes two parts: a disposable unit composed of a silicon chip which contains an array of nano-volume electrochemical cells. Each electrochemical cell can hold 100 nL, and includes three embedded electrodes, which permit performance of all types of electrochemical measurement. The second reusable part contains a multiplexer, potensiostat and pocket PC. 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 g, making it ideal for field environmental monitoring and for medical applications. Further size reduction is possible using special application specific integrated circuits (ASIC) design. Also, better packaging can reduce the size and weight, keeping all the electrical parameters intact. The main features of the proposed "lab on chip" include small sampling requirements, high signal to noise ratio, 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 biocompatible 'electrochemical lab on a chip'.

2. Experimental

2.1. Bio-chip design

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. The nano-volume electrochemical cell was specially designed for high sensitivity, by altering the ratio between working electrode area and chamber volume. Each electrochemical cell can hold 100 nL of solution and consists of three embedded electrodes: (1) gold working electrode; (2) gold counter electrode; (3) Ag/AgCl reference electrode. 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 chamber constructed from photopolymerized polyimide (SU-8). The major fabrication steps are illustrated in Fig. 1.

The device was manufactured as two parts. (a) A disposable chip—with an array of chambers which are used as electrochemical cells. The chip is interfacing the external electronic circuitry. (b) A reusable part, which includes a multiplexer, potentiostat, temperature control and a pocket PC for sensing and data analysis. This setting allows continuous reusing for multiple measurements.

2.1.1. Chip process
2.1.1.1. Gold electrodes.
The bio-chip was fabricated on (1 0 0) 4 in. diameter p-type silicon wafer coated with thermally grown Si/SiO$_2$ (500 nm). The front side of the wafers (with the oxide) was coated with a thin film of tungsten (20 nm) on titanium (20 nm). The W/Ti film was deposited using an RF sputtering and it was designed to be an adhesion layer; providing good adhesion of the following gold layer to the silicon-dioxide/silicon
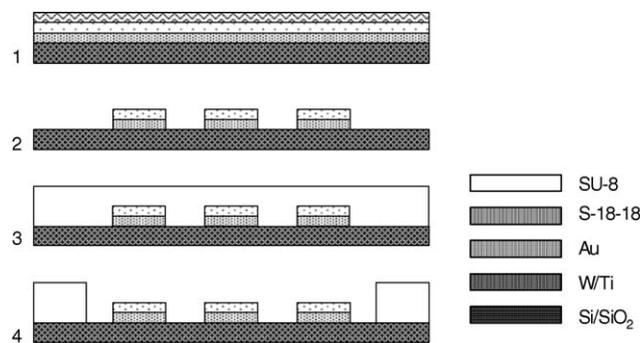

Fig. 1. Schematic diagram of the major fabrication steps (in transverse section). Key: The cleaned silicon wafer was isolated (SiO$_2$), coated with W/Ti film. A thin film of gold (Au) was deposited by rf sputtering and than coated with positive S-18-18 photoresist (1). The photoresist was patterned and than developed, leaving three microelectrodes patterned (2). The sample was coated with a positive photoresist SU-8 (3). The SU-8 was patterned and developed, leaving three microelectrodes at bottom of a chamber (4).
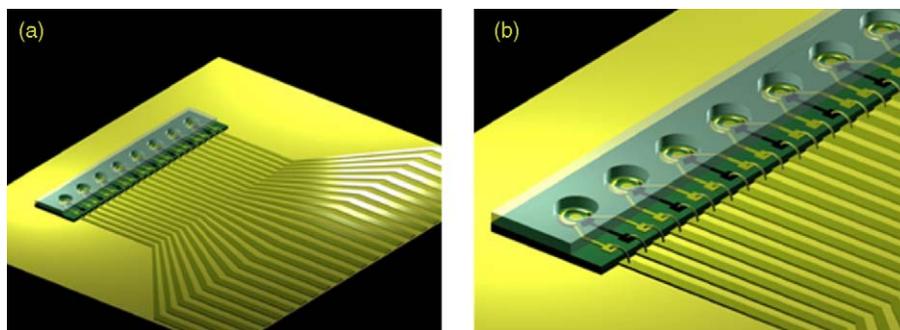
Fig. 2. Images of the electrochemical silicon chip wire bonded to the PCB platform. (a) Array of eight 100 nL electrochemical cells on a silicon chip. The chip is glued to the tailored PCB platform (4 cm × 4.8 cm), and the chip's gold pads (500 μm × 500 μm) are wire bonded to the gold PCB's electrodes. The PCB board enters directly to the socket of an external sensing circuit. (b) Electrochemical cells ($r = 800$ μm) on chip consist of three embedded electrodes: gold working electrode, gold counter electrode, and Ag/AgCl reference electrode.

substrate. Since the adhesion of gold to most dielectric films is typically poor, the W/Ti layer was important to achieve good reliability and electrochemical stability. On top of the adhesion layer, a thin film of gold (Au) was deposited by RF sputtering with a thickness of 200 nm. Next, a 1 μm thick positive photo resist (Shipley^{TM} type S-1818) was spin-coated, at 2800 rpm for 40 s. Next the wafer with the photo resist was soft baked for 90 s at 95 °C. The photo resist was patterned by UV exposure (360 nm) through a mask which defined electrodes shape for 7 s, using a Karl-Suss MA6 mask aligner. After the exposure the wafer was developed using a metal-ion free developer type MF-319 for 40 s at room temperature. After that, the chip was hard-baked 2 min at 100 °C to stabilize the resist. Au layer was etched by I_2/KJ/H_2O 1 g:4 g:40 mL (2.5 min) and the layer of W/Ti was etched by 30% H_2O_2 (2 min) without heating. After the successful removal of the W/Ti/Au layers the rest of the photoresist was removed by NMP, followed by cleaning the wafer with DI water and iso-propanol (C_3H_7OH) and dried with nitrogen. The result of the process included an array of gold electrodes on the silicon wafer surface.

2.1.1.2. Wall formation. The samples were baked at 180 °C for 10 min to remove surface moisture and were spin-coated (5000 rpm for 40 s) with a negative SU-8 photoresist to form a thickness of 50 μm. The SU-8 film was soft-baked at 100 °C for 15 min, and then patterned by UV exposure through a new tailored mask for three times, each time for 10 s, using a Karl-Suss MA6 mask aligner and then the chip was soft baked for 90 s at 95 °C. Next the SU-8 was developed by EC solvent for 5 min to form circular micrometer-scale chambers over the electrode area, leaving the bonding pads uncovered (Fig. 2b). To stabilize the process the chip was hard backed at 200 °C for 25 min cleaning the wafer was formed with DI water and iso-propanol (C_3H_7OH) and was dried with nitrogen. Process and cleaning were inspected visually under a microscope.

2.1.1.3. Reference electrode. A layer of silver was electrodeposited on the designated reference gold electrodes from an aqueous plating solution of 0.2 M $AgNO_3$/2 M KI/0.5 mM $Na_2S_2O_3$, containing the complex ion $[AgI_2]$- K+. A current

of −0.5 mA was passed for 1 min through the electrode, using a coil of platinum wire as a counter electrode. Although the deposition time was not critical, a special care had to be taken to limit the charge. This is important as in case of longer times the silver layer could touch the working electrode and cause a short, hence a failure of the device. Finally, the silver halide reference electrode was completed by passing +0.5 μA through the pre-designed electrode in a solution of 0.1 HCl and allowing the anodic current to flow for 10 s, using the same platinum wire as a counter. During this period the current decayed slowly as the halide layer was deposited on the silver surface. The wafers were designed in a way that all the reference electrodes on the wafer were connected together; hence, the coating of the reference electrodes was achieved by passing current through one external pad. Finally, the wafers were diced to a set of bio-chips and each one was wire-bonded and packaged separately.

2.1.1.4. Chip packaging. Electronic packaging provides the interconnection between the silicon chip and the printed circuit board (PCB). The PCB served as a carrier that was connected to the interfacing electronics and data processing units. The PCB provided the desired mechanical and environmental protection to ensure reliability and performance of the device. The PCB manufacturing process consists of selective deposition of gold on the PCB surface, in order to act as 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 (Fig. 2a). Next, each chip was spread with biocompatible metal glue on his backside and assembled on the printed circuit board in a precise setting. The PCB pieces were cleaned prior the assemble of the silicon chips, in ultrasonic bath with iso-propanol (C_3H_7OH) for 1 h and than rinsed in acetone ((CH_3)$_2$CO) and DI water.

2.1.1.5. Bonding. Thermosonic bonding is used with gold wire (1 mil). A combination of temperature and ultrasonic energy forms a reliable connection between the silicon chip and the

PCB. Bonding temperature was 150 °C. Visual inspection used for ensuring the reliability of the wire bond process.

2.2. Experimental set-up

The measurement set-up includes two units: The silicon chip unit attached to the PCB which was replaced every experiment, and the reusable unit which contains multiplexer, potentiostat, temperature control and a pocket PC (Palm Instruments BV-2004) for sensing and data analysis. A fixed potential was applied between the working and the reference electrode in each electrochemical cell in the array on the chip by the potentiostat, and the output signal was measured.

The chip was cleaned after fabrication in ultrasonic bath with iso-propanol (C_3H_7OH) for 1 h and maintained in C_3H_7OH until use. Prior to the experiments the chip was rinsed with acetone ((CH_3)$_2$CO) and DI water.

2.2.1. Electrochemical measurements

Chronoamperometric detection methods were performed with successive aliquots additions of redox compound $K_4Fe(CN)_6$ in 0.1 M KCl to 100 nL electrochemical final cell volume. The generated current was detected at fixed 350 mV working potential.

2.2.2. Enzymatic measurements

Enzymatic measurements were performed with alkaline phosphatase. Different concentrations of the enzyme ranging from 36 to 690 pg were placed into the 100 nL volume electrochemical cells on the chip. Then, the substrate PAPP (*para*-amino-phenyl-phosphate) was added to final concentration of 1 mg/mL. The resultant enzymatic activity was monitored by measuring the product of the enzymatic reaction, *p*-aminophenol (*P*-AP), at 220 mV working potential.

2.2.3. Bacterial based functional measurements

In order to present device ability to integrate and monitor living organisms, genetically engineered bacteria were used as whole cell sensors to detect toxic chemicals. Recombinant bacteria act as physiological sensors, and react to a presence of a toxin by activating specific promoter (regulatory DNA sequence). This promoter induces the production of the reporter enzyme β-galactosidase. This enzyme reacts with the PAPG substrates (placed inside the chambers to allow the enzymatic reaction) to produce two different products: electrochemical active product *p*-aminophenol (PAP), and inactive product β-D-galactopyranoside. The PAP molecules are oxidized on the working electrode at 220 mV. This oxidation is converted to a current signal using the amperometric technique.

In these experiments we used recombinant *E. coli* bacteria bearing plasmid with one of the following promoters: *fabA*, *Dnak*, or *grpE*. These promoters were fused to the reporter enzyme β-galactosidase [24,27]. Phenol, a representative toxicant, was added in increasing concentrations (1.6, 8.3 and 16.6 ppm) to the bacterial samples, in the presence of the substrate PAPG. The PAPG was added to a final concentration of 0.8 mg/mL (100 nL total volume). Immediately after (~1 s), the

suspensions were placed in the electrochemical cells. The bacterial response to phenol was measured on-line by applying a potential of 220 mV. 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 electro-active species in the LB medium (bacterial nutrients), in the substrate, or in the substrate and the LB medium mixture, which can contributes to the current response. Also, control measurement including bacteria, substrate and LB medium without Phenol were performed.

The *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.

2.2.4. On-chip bacterial bio-film response to phenol

Bacterial entrapment into Agar-gel measurements: LB medium containing 0.7% Agar was prepared for bacterial entrapment. The soft agar was heated to 50 °C and allowed to cool down at room temperature. When it reached 40 °C, bacteria and the substrate PAPG were added to 3×10^7 cells/mL final concentrations. Then the agar containing bacteria and the PAPG was added to the electrochemical chambers and allowed to solidify at room temperature. Recombinant *E. coli* bacteria producing β-galactosidase where entrapped into the agar media, to construct on-chip biofilm-like structure. The entrapped bacteria were inserted into the 100 nL volume chambers on the chip and were exposed to 10 ppm of phenol. The enzymatic activity was monitored by amperometric technique ($V = 220$ mV).

3. Results and discussion

3.1. Bio-chip fabrication

Using silicon as a substrate for biochips is a commonly used technology with many advantages, including architectural and process flexibility, performance stability and reproducibility. In addition, it is compatible with the widely used CMOS integrated circuit technology. Therefore, it is relatively simple to integrate the current bio-chip with conventional chips using standard packaging methods. Silicon, gold and SU-8, which were used in the fabrication process, are recognized as biocompatible materials [1,28]. The chambers shapes and sizes were optimized for the current particular experiments, by considering the need for sufficient amount of enzymes or bacteria to generate a measurable current signal, and on the other hand, the necessity to reduce features dimensions for miniaturization, enhanced throughput and portability. The short diffusion length of the analytes to the electrode surface in the nano-volume electrochemical cell, yields significant advantages in analytical speed, higher signal to noise ratio and reduced sample/reagent consumption as well as cost reduction. Furthermore, the 100 nL chambers enables droplet to be inserted into the chambers and overcome surface tension. However, chamber sizes can be easily reduced for different applications with an identical process protocol. The construction of an array of nano-chambers on one silicon chip enables

performance of multi experiment simultaneously and independently and leads to high throughput. However, extending the present research into multi arrays requires additional considerations on the effects that can disrupt high signal integrity like noise, cross-talk and other sources for electronics interference. At this experimental stage, we simplified the electronics by integrating eight electrochemical cells into a vector. In our current bio chip design the signal time scale is in the range of seconds. Therefore, we assume that we have a slow-changing current signals and the voltage on the device remains constant. Therefore, readout of the various channels can be done in relatively low-bandwidth that reduces noise and with minimal cross talk. Using analog to digital conversion may introduce some cross talk due to capacitive coupling. However, in this paper, we discuss mostly the array technology and the current signal extraction is done sequentially from the eight channels. Extending the number of channels will require in the future more thorough analysis of the noise and cross talk that may affect the system detection limits.

3.1.1. Electrochemical behavior of the device

Ferro-cyanide amperometric measurements: To explore the electrochemical characteristics and performance of the device, amperometric measurements were first recorded for the redox compound $K_4Fe(CN)_6$ as a model system. Fig. 3 shows the amperometric response of a representative electrochemical cell to successive nL additions of $K_4Fe(CN)_6$ in 0.1 M KCl. All chambers exhibited reproducible and reliable electrochemical signals. The working electrode was held at 350 mV versus embedded micro-Ag/AgCl reference electrode. Each step represents the increased current oxidation signal, due to serial additions of different concentrations of the Ferro-cyanide compound. The serial additions caused cell dilution, resulting in decreasing current signal steps. There is a good linear correlation between the signal response and the chemical concentration that

emphasize the good performance of these nano-volume electrochemical cells on chip. Moreover, repeated experiments showed similar result, which emphasize the stability of the reference electrode.

The applicability of the chip can be utilized to identify other specific chemicals by direct electrochemical measurements for screening of drugs having redox electrochemically characterization (reduction–oxidation) such as acetaminophen, vitamins and several antibiotics, which is especially important in human disease. The array enables simultaneous multiplex measurements of different chemicals, and the nano-litter chambers require nano-liter of the tested sample which leads to high throughput and cost reduction.

3.1.2. Physiological applications of the electrochemical nano-bio-chip

Enzymatic measurements: Different concentrations of alkaline phosphatase activity were simultaneously and independently measured by the nano-volume electrochemical cells array. The results are shown in Fig. 4. Calibration curve exhibit high sensitivity and linearity within various enzyme concentrations. This nano-bio-chip presents rapid detection, within 1 min, of low concentrations of alkaline phosphatase such as 36 pg/cell volume. Additionally, the high sensitivity expressed by achieving high resolution between adjacent enzymes concentrations, as shown in Fig. 4. These results accomplish the same range of sensitivity compared to chemilumunisecent assay methods [16]. However, the electrochemical lab-on-a-chip system is favorable due to its rapid detection, portability and miniaturization.

An important area that can benefit from this chip is identification of human diseases via electrochemical measurements
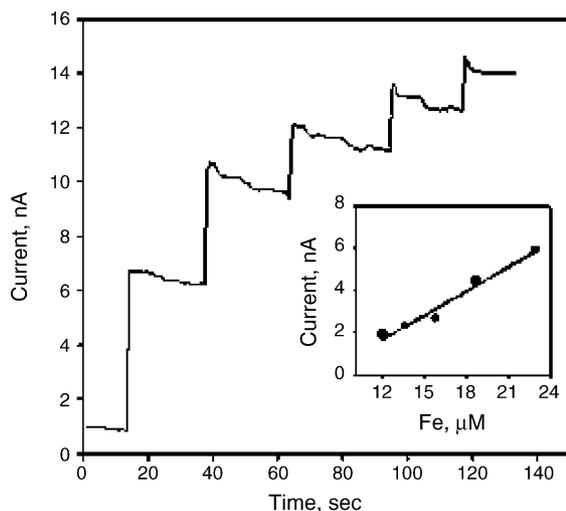
Fig. 3. Amperometric response to successive aliquots additions of redox compound $K_4Fe(CN)_6$ in 0.1 M KCl to 100 nL electrochemical final cell volume. Measurements were performed at fixed 350 mV working potential vs. Ag/AgCl. Inset: Calibration curve of Fe final concentrations (μM) vs. current signal (nA).
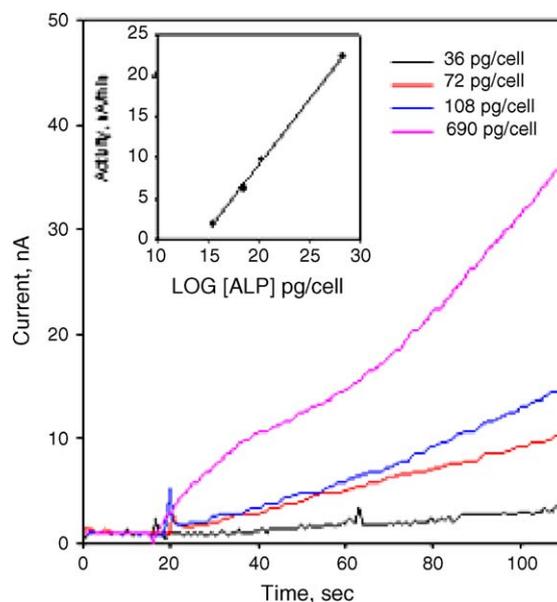
Fig. 4. Amperometric response of the activity of Alkaline Phosphatase. Different concentrations of the enzyme ranging from 36 to 690 pg/cell were placed in the nano-bio-chip. The substrate PAPP was added to final concentration of 1 mg/ml. The enzymatic product, p-aminophenol (P-AP) was measured at 220 mV vs. Ag/AgCl. Inset: Calibration curve of alkaline phosphatase activity shown as Δcurrent/Δtime.

of enzymatic reactions. Different human diseases are identified by the presence or absence of specific enzymes. For example, benign or malignant cells can be diagnosed by their specific enzymatic activity. Differentiation therapy of human colon cancer is associated with reappearance of alkaline phosphatase normal activity [29].

High throughput screening, sensitive detection and fast response time are especially important to improve existing identification techniques. Chip technology combined with the advantage of electrochemical systems provides a powerful tool for such demands. Furthermore, this silicon-based biochip can be future employed to identify specifically and analytically various chemicals by immobilized specific enzymes on the working electrode. The enzymes can be chemically bound to the gold working electrode on the silicon chip by using same MEMS techniques.

3.1.3. Bacterial based functional measurements

Genetically engineered bacteria can be tailored to generate a current signal in response to toxic chemicals. These whole-cell sensing systems can be visualized as a toxicant switch, which is turned on only in the presence of toxic chemicals. These recombinant bacteria were integrated into the electrochemical cells array on the chip. During the measurement period the bacteria remained active and capable to perform cellular gene expression and enzymatic activity.

Amperometic detection of the response of three types of recombinant *E. coli* bacteria to phenol is shown in Fig. 5. The results show that the physiological reaction of the bacteria to phenol exposure can be detected rapidly with high sensitivity. For example for the *fabA* promoter, after 300 s, a current signal of 10 nA is produced in response to 1.6 ppm phenol, and a current of 14 nA is produced in response to 3.3 ppm phenol. These current signals are well above the background signal, which is defined as the measured current without phenol. The current signal increases significantly with time, since the current results from an enzymatic reaction [26]. The enzymes are continuously generated due to a sustained exposure of the bacteria to the toxicant. Therefore, after 600 s, a current signal of 60 nA is

produced in response to 1.6 ppm phenol, and 80 nA in response to 3.3 ppm phenol. Fig. 5 shows that current intensity is linearly proportional to the toxicant concentration, whilst the toxicant concentration is above the detection limit. The dashed line represents a linear interpolation between the minimal detection concentration (1.6 ppm) and zero phenol concentration. When the phenol concentration was lower than 1.6 ppm, no significant reaction was measured; the current was approximately zero during the measurement time.

Different intensity response of the bacterial sensors *fabA*, *grpE*, and *dnaK* to phenol, is due to the specific activation response of the promoter to the type of the toxicant. *fabA* promoter is sensitive to membrane damage, while the promoters *dnaK* and *grpE* are more sensitive to protein damage [16]. Therefore, *E. coli* bacteria harboring the *fabA* promoter showed high induction activity in response to phenol exposure, which is a known membrane damage chemical. As expected, *grpE* and *dnaK* promoters were less activated by phenol. In comparison to equivalent optical detection methods using whole cell biosensors for detection of toxic chemicals, these results proved to be more sensitive and produce faster response time. Concentrations as low as 1.6 ppm phenol could be detected within 5 min of exposure to phenol, whilst recent study [21], based on fluorescent reporter system (GFP), enabled detection of 295 ppm phenol after more than one hour. Cha et al. [30] used optical detection methods of fluorescent GFP proteins, detected 1 g of phenol per liter (1000 ppm) after 6 h. Other studies [8], 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 nano-volume electrochemical cells array on silicon chip, which results in small sampling requirement and fast response time. Enhanced sensitivity and high signal to noise ratio are 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 instead of introducing toxic chemicals to the bacterial solution,
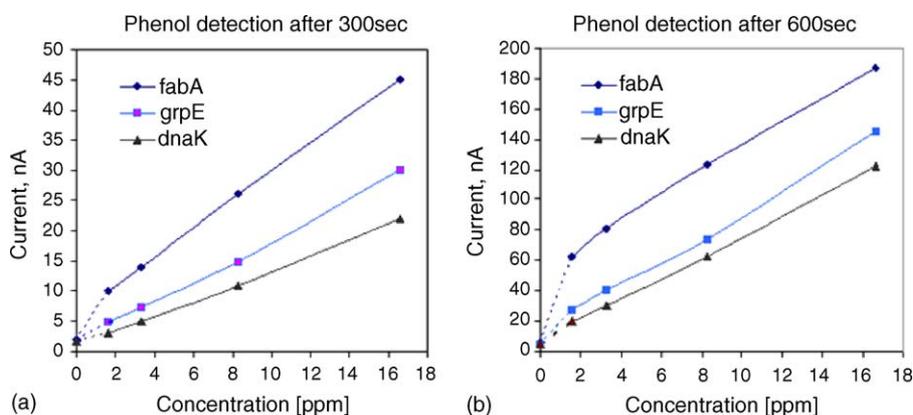

Fig. 5. Recombinant bacterial current response on chip to increasing concentrations of phenol: (a) after 300 s and (b) after 600 s. The *E. coli* reporters are *fab*A, *grp*E and *dna*K. Amperometric measurements were performed immediately after phenol addition (~1 min) at 220 mV working potential vs. Ag/AgCl reference electrode. The dashed line represents a linear interpolation between the minimal detection concentration (1.6 ppm) and zero phenol concentration.
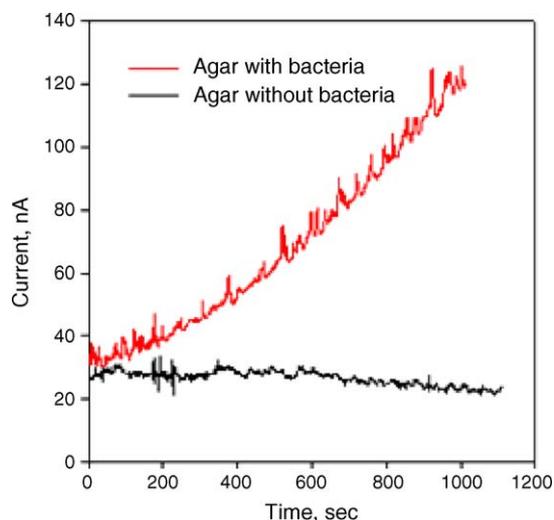
Fig. 6. Amperometric response curves of biosensors agar film to phenol exposure. Agar containing bacteria and PAPG substrate was added to the electrochemical 100 nL volume chambers on the chip, and was exposed to 10 ppm of phenol. The enzymatic activity was monitored on line by amperometric technique ($V = 220$ mV).

pure water was added. In this case the generation of a current signal represents a false alarm. A negative control chamber includes w.t. (MG1655) *E. coli* bacteria that constitutively expresses β-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. Moreover, direct electrochemical reaction (without the biological reaction) can produce only constant DC current signal, while enzymatic reaction act as an intrinsic amplifier, and generates increasing current signal.

A 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 [31]. Combining enzymatic system with electrochemical detection methods enables measurements in turbid solutions and under anaerobic conditions [24].

3.1.4. Bacterial entrapped into Agar-gel measurements

Efficient immobilization of the living cells inside the biochip is an important issue for practice means and permits improved preservation possibilities. Modified *E. coli* bacteria immobilized in an LB agar gel were used as a model structure of biofilm layer. This technique is biocompatible and maintains cells viability by supplying all vital nutrients. The activity of the induced enzyme reporter, β-galactosidase, as a response to phenol, a representative toxicant, is shown in Fig. 6. The results demonstrate the ability to maintain as well as to measure the recombinant bacteria response to toxicants, while they are immobilized in LB agar film, and deposited inside the nano-volume chambers on the silicon chip. No current was generated in the control system (without bacteria), which contained agar, PAPG enzyme sub-

strate and phenol, which indicates that the signal is due to physiological reaction of the bacteria to the toxicant. Moreover, these results emphasize the advantages of fabricating nano-volume electrochemical cells that permit high detection sensitivity, especially when integrating biofilm layer that reduce diffusion coefficient.

In addition to the aforementioned capabilities, this 'lab on a chip' system could be easily adapted for 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.

4. Conclusions

In this study, a miniaturized and portable electrochemical "lab on a chip" was fabricated, studied and characterized for multi function use, mainly for biological based applications. The benefit of this new geometry, in which electrochemical cell volumes were reduced to nano-liter 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. Integration of living cells can emulate the behaviors of humans and sense functions that have never been demonstrated before. Together with adjusted nano-scale electro analytical system, the device can provide exciting opportunities in the biosensors field as well as in the basic research on microorganisms.

Further reduction of chambers dimensions is achievable using identical process and can provide MEMS for single cell measurements as well as for ensemble. However, this should be considered by the specific application requirements.

Future extension of the present research project includes in vivo and clinical applications. Interfacing biological materials with microelectronics may make it possible for chips to be inserted as reporter elements within humans. It may be utilized as nano-laboratories, on-chip data acquisition and partial processing as well as communicating with bio-systems. These technologies will change the scope of our abilities to monitor biochemical reactions in multi-cellular organisms, and may lead to interesting pharmaceutical and clinical developments.

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