Chapter 12
Nano-Bio Electrochemical Interfacing–Linking Cell Biology and Micro-Electronics

Y. Shacham-Diamand, R. Popovtzer, and Y. Rishpon

Abstract Integration of biological substance within electronic devices is an innovative and challenging area combining recent progress in molecular biology and micro technology. First, we introduce the concept of integrating living cells with Micro Electro Mechanical Systems (MEMS). Following a brief overview on "whole cell based biosensors" we describe the design, fabrication, and process of a biocompatible electrochemical "Lab-on-a-Chip" system. Demonstrating the application of electrochemical interfacing based whole cell bio chips, we present two different configurations: a. integration of prokaryotic cells (bacteria) for water toxicity detection, and b. integration of eukaryotic cells (human colon cancer cells) for rapid evaluation of the effectiveness of drug treatments. Both applications, with either microbes or mammalian cells integrated onto MEMS based biochips with liquid volume in the range of 100 nL–1 μL, function well and yield a detectable signal much higher than noise level after few minutes.

12.1 Introduction

In the last couple of decades, both Micro-electro-mechanical systems (MEMS) and microbiology achieved remarkable progress. MEMS provide very small systems, made of very small electrical and mechanical components. These systems range in size from sub micrometer level to millimeter level, and can include any component number, from a few to millions, in a particular system. MEMS extend the fabrication

Y. Shacham-Diamand (✉)
Faculty of Engineering, Tel Aviv University, Tel Aviv, 69978, Israel
e-mail: YosiSh@tauex.tau.ac.il

R. Popovtzer
School of Engineering, Bar-llan University, Ramat Gan 52900, Israel.
e-mail: rachelap@eng.biu.ac.il

Y. Rishpon
Faculty of Life Sciences, Tel Aviv University, Tel Aviv, 69978, Israel

T. Osaka et al. (eds.), *Electrochemical Nanotechnologies,*
Nanostructure Science and Technology, DOI 10.1007/978-1-4419-1424-8_12,

techniques developed for the integrated circuit industry to many fields like micro-mechanics, micro-fluidics, optoelectronics, and magnetism.

The intensive researches and developments in microbiology, biotechnology, and genetic engineering over the last decade have demonstrated that life science is a central scientific discipline. The introduction of genetic tools such as transposes, coupled with the ability to clone genes and determine DNA sequences, and the subsequent explosion of techniques based upon these methods made it possible to dissect the molecular genetics of any organism. These developments allowed us to answer questions that were previously deemed impossible and that have a major impact on every discipline of biology, with practical applications in medicine, agriculture, bioremediation, and biotechnology.

The integration between the two different disciplines, micro-biology and micro-electronics, poses an exciting and challenging goal for the present decade. The developments related to this area led to the invention of novelty devices and smart biosensors.

A general definition for the term "biosensor" is "the coupling of a biological material with a microelectronic system or a device to enable rapid, accurate, low-level detection of various substances in body fluids, water, and air" [1]. Cell-based biosensors are devices that contain living biological cells that monitor physiological changes induced by exposure to environmental perturbations such as toxicants, pathogens, or other agents [2]. Recent progress in cell culture and micro-fabrication technologies has suggested the development of cell-based sensors for the functional characterization and detection of drugs, pathogens, and toxicants. Unlike other biosensors such as nucleic acid or antibody-based sensors, cell-based biosensors are not specific for certain compounds but are capable of responding to a wide range of biologically active compounds and offer the potential to gather greater information content than bio-molecular-based sensors. Cell-based biosensors measure physiologic, metabolic, or network processes and responses that integrate many biological components [3, 4]. The cell-based biosensors can be used for high-throughput drug discovery and clinical diagnostics, and for the detection of toxic agents and certain odorants. For evaluating toxicology and efficiency of potential pharmaceuticals, the cell-based sensor can substitute for the use of live animals, which is expensive, cumbersome, and is probably going to be restricted in the future.

12.2 Electrochemical "Lab on a Chip" for Biological Applications

Lab-on-a-chip technology characterize in network of channels and wells mainly on silicon substrate that enables faster, better ,and cheaper multi sample handling and detection, on a single integrated system. Basic electrochemical studies and biological researches can be beneficial utilizing electrochemical "lab-on-a-chip" systems, which are easy to use, require low sample and reagent consumption, and enable high reproducibility due to standardization and automation. In the following section

we describe the development of a miniaturized electrochemical "Lab-on-a-Chip" system for multi functional use and for biological based applications.

12.2.1 Device Fabrication

The chip was produced on silicon wafers and includes arrays 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 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 wall of the chambers is constructed from photopolymerized polyimide (SU-8). The silicon chip is wire bonded to a plastic chip which interfaces the electronic circuit (Fig. 12.1).

The device was manufactured as two parts: the first part is a disposable silicon chip – with the electrochemical cells arrays. The silicon chip was wire bonded to a special printed circuit board (PCB) platform, which was directly connected to the data processing units. The second part of the device is reusable, which includes a multiplexer, potentiostat, temperature control and a pocket PC for sensing and data analysis (for more details see [5]). 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 medical applications.

This new design of nano chambers array 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, in order 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.

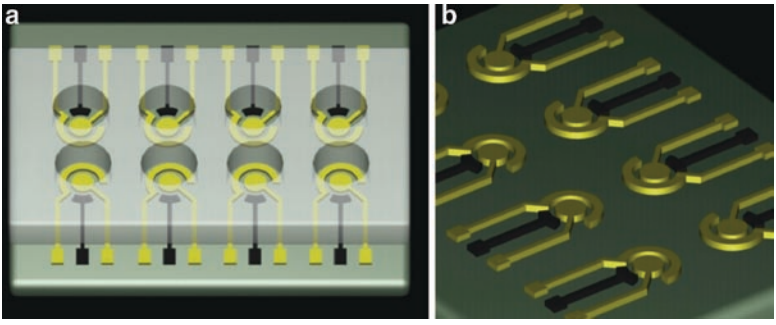

Fig. 12.1 Images of the electrochemical chip. (**a**) Silicon chip contains an array of eight miniaturized electrochemical cells with external pads. (**b**) The electrodes without the top layer (SU-8). Each electrochemical-cell consists of three circular-shaped electrodes: gold working and counter electrodes and Ag/AgCl reference electrode

12.3 Water Toxicity Detection

Here we present a novel method for water toxicity detection by integrating living cells with microelectronic device. Bacteria, which have been genetically engineered to respond to environmental stress, act as a sensor element and trigger a sequence of processes, which lead to generation of electrical current. The specific design and process of the electrochemical "Lab on a chip", which integrates the recombinant bacteria provide highly accurate, sensitive, and rapid detection of acute toxicity in water.

The most important reason for utilizing living cells as biosensors lies in their capability to provide functional information, i.e. information about the effect of a stimulus on living organisms. The aim of the present biochip is to offer the unique "functionality" sensing capability. It answers the question "Is the water toxic?" and it does not intend to perform chemical analysis or identify the nature of the toxicant. These whole-cell sensing systems can be visualized as an environmental switch, which is turned on in the presence of toxins or stressful conditions. In many cases, functional rather than analytical information is ultimately desired. In other applications, it complements and adds a special feature to a system by emulating the behavior of living entities.

The vast development in genetic engineering of living cells enables the use of recombinant cells as cell-based sensing systems [6, 7]. The cascades 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 [8–10].

12.3.1 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 β-galactosidase. This enzyme reacts with the PAPG substrates (molecules that where initial placed inside the chambers) 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 an amperometric technique.

12.3.2 Experimental

Recombinant *E.coli* bacteria bearing plasmid with one of the following promoters: *dnak*, *grpE*, or *fabA* were used. These promoters were fused to the reporter enzyme β-galactosidase [5, 7]. Ethanol [1%] or phenol [1.6 ppm] was introduced to the

bacterial samples in the presence of the substrate PAPG. Immediately after (~1 s), 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 concentration at 3×107 cells/ml was used for all experiments.

12.3.3 Water Toxicity Measurements

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 Figs. 12.2 and 12.3, respectively.

The different *E. coli* reporters are *fabA, dnaK* and *grpE*. Measurement was performed immediately after the ethanol addition (~1 min) at 220 mV working potential vs Ag/AgCl reference electrode. The LB curve represents the bacterial response to the LB medium with the substrate PAPG without ethanol.

The results show that concentration of 1% ethanol could be detected within less than 10 min, and concentration as low as 1.6 ppm phenol could be detected within less than 6 min. Different intensity response of the various bacterial sensors, *dnaK*,

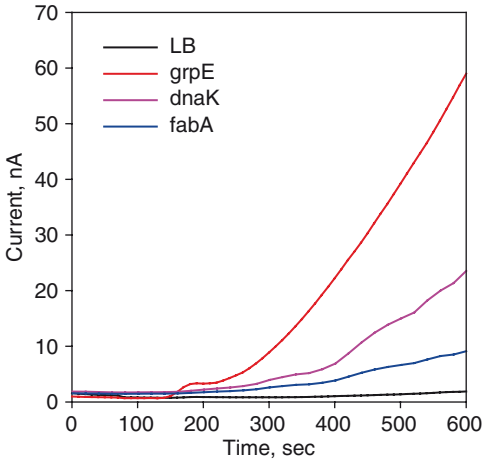

Fig. 12.2 Amperometric response curves for on line monitoring of different *E. coli* reporters in response to the addition of 1% ethanol, using the nano-bio-chip

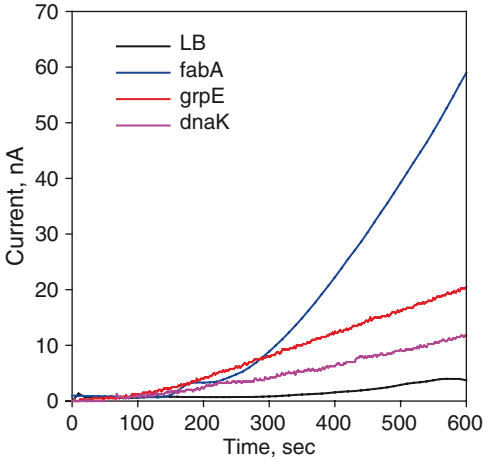

Fig. 12.3 Amperometric response curves for on line monitoring of different *E. coli* reporters in response to the addition of 1.6 ppm phenol, using the nano-bio-chip

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 [6] (Fig. 12.2). *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 a known membrane damage chemical. As expected, *grpE* and *dnaK* promoters were less activated by phenol (Fig. 12.3).

The different *E. coli* reporters are *fabA, dnaK* and *grpE*. Measurement performed immediately after the ethanol addition (~1 min) at 220 mV working potential vs Ag/AgCl reference electrode. The LB curve represents the bacterial response to the LB medium with the substrate PAPG without phenol.

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 min of exposure to the toxic chemical, whilst a recent study [11] which utilized bioluminescent *E.coli* sensor cells, detected 0.4 M (2.35%) ethanol after 220 min. An additional study [1] based on fluorescent reporter system (GFP), enabled detection of 6% ethanol and 295 ppm phenol after more than one hour. Cha et al [12] used optical detection methods of fluorescent GFP proteins, detected 1 g of phenol per liter (1,000 ppm) and 2% ethanol after 6 hours. Other studies [13] 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 result 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 β-galactosidase; thus, current should be generated in all cases. When no current is 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 immense 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 is produced faster, more sensitively, and increasing with time [14]. Combining enzymatic system with electrochemical detection methods enables measurements in turbid solutions and under anaerobic conditions [9].

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 biosensors. Thus, large amount of analytes can be detected simultaneously and independently. Similarly, in experiments aiming to analyze physiological reactions, bacteria harboring different types of promoters can be introduced to the chambers, and thus, this lab on a chip can detect a variety of toxicant types simultaneously.

12.4 High-Throughput Detection of Colon Cancer Cells Response to Drug Treatments

A new method is presented for rapid, sensitive, and high-throughput detection of colon cancer cells (HT-29) response to differentiation therapy, using our novel electrochemical Lab on a Chip system. Differentiation inducing agents such as butyric acid (BA) and its derivatives were introduced to miniature colon cancer samples (few to hundreds cancer cells in each sample) within the chip chambers. The efficacy of each of the differentiation inducing agents to elevate alkaline phosphatase in colon cancer cells was evaluated through electrochemical detection of the cellular enzymatic activity level, while reappearance of normal enzymatic activity denoted effective differentiation therapy drug treatment. The results demonstrate the array based device ability to evaluate simultaneously multiplex drugs effect on miniature tumor sample (~15 cells) rapidly (5 min) and sensitively, with quantitative correlation

between the cancer cell number and the induced current signal. Utilizing nano-volume analytical device is of special interest in clinically relevant samples since it requires less tissue for diagnostics, and enables high-throughput analysis and comparison of various drugs effect on one small tumor sample, while keeping uniform biological and environmental conditions. In addition, this new method can help tailor cancer drugs and treatments to individual patients towards "personalized medicine".

High-throughput detection of nano volume tissue and cell samples offers great potential for increasing the amount and quality of biomedical data and has a wide range of applications. In cancer research, this technology can greatly improve diagnostics and therapy, including basic research, detection of cancer markers, and testing patient's response to different treatment modalities and thereby tailoring the treatment to an individual patient. Currently it is known that tumor treatment response cannot be predicted only from its type and anatomical location, but from its own overall individual parameters [15, 16]. Examination of the particular tumor responses to several drug types simultaneously and in exactly the same conditions, will be invaluable in providing optimal treatment to each patient.

In this study we present a unique high-throughput electrochemical system for the detection of colon cancer cells response to different differentiation inducer agents. Current cancer therapeutic strategies focus predominantly on achieving the removal or death of cancer cells within the patient, through three basic approaches: surgery, chemotherapy [17], and gamma irradiation [18]. These methods are aggressive, highly toxic, and often nonspecific. [19]. "Differentiation therapy" is an alternative less toxic approach for cancer treatment, which employs agents that modify cancer cell differentiation [20]. Upon appropriate treatment, cancer cells restrain their own growth and resume their normal growth rate [21, 22]. In addition, the differentiation therapy is utilized as a complementary treatment to conventional surgery treatment, as the latter can eliminate most, but not completely cancer cells within the patient, leading to remission of the disease.

BA is a potent differentiation agent in a wide variety of cancer cells in-vivo and in-vitro [23, 24]. Butyrate has been shown to specifically affect genes regulation by transcriptional and post transcriptional modifications. It induces dose dependent differentiation and inhibits proliferation of various malignant cells types including erythroleukemia, embryonal carcinoma and colon carcinoma [25, 26]. BA induced the expression of specific differentiation-associated genes when used at concentration between 0.5–10 mM. Differentiated cells are characterized by appearance of regulatory enzymes such as alkaline phosphatase [27].

Butyrate has potential for use in differentiation therapy but is limited by the requirement of millimolar concentrations and short metabolic half-life for efficacy. In order to overcome these problems BA derivatives (prodrugs) have been synthesized and screened. Among them, pivaloyloxymethyl butyrate (named AN-9) has demonstrated impressive anticancer activity in preclinical and clinical studies [23, 24, 28]. AN-9, metabolized intracellularly to acids and aldehyde, affects and penetrates cancer cells about 100-fold faster than BA [25].

In this work we chose HT-29 human colon cancer cells (ATCC) as a model system for high throughput screening of human cancer cells response to differentiation therapy.

HT-29 cells are sensitive to differentiation therapy agents such as BA and its derivatives (AN-7 and AN-9) by enhancement of the alkaline phosphatase activity [25, 29]. According to the enzymatic activity level of the treated cancer cells, the efficiency of the particular drug treatment was evaluated. In general, normal enzymatic activity denotes that the cells differentiate properly as a consequence of the particular drug treatment, whilst lack of enzymatic activity denotes ineffectual drug treatment for the particular cancer tumor and for the particular patient.

12.4.1 Example to Electrochemical Sensing of Cancerous Cells Response to Drug Treatment

Alkaline phosphatase activity measurements: The activity of the enzyme alkaline phosphatase is determined by using the substrate p-aminophenylphosphate (PAPP). The product of the enzymatic reaction, PAP, is oxidized on the working electrode at 220 mV. This oxidation current is monitored.

An array of eight-channeled 100 nL electrochemical chambers was loaded with HT-29 human colon cancer cells that were treated with BA or its derivatives. The HT-29 cells were grown in DMEM medium in the presence of fetal bovine serum for 3 days prior to drugs treatment. The measurements were performed in PBS with the intact cells and without additional treatment of the cancer cells such as lysis.

The treated cells were placed into the electrochemical chambers and the substrate PAPP was added to a 1 mg/ml final concentration at a total volume of 100 nL. Alkaline phosphatase activity was measured by monitored the PAP oxidation current. The electrochemical chips are disposable and were replaced every experiment.

BA at increasing concentrations of 0, 0.078, 0.156, 0.3125, 0.625, 1.25, 2.5, 5, and 10 mM was added to the HT-29 cell cultures and incubated for 72 h for optimization. Optimal butyrate concentration, LC_{50} and viability were calculated accordingly. The optimal BA concentration was found to be 2.5 mM.

12.5 Results and Discussion

The effect of BA, AN-9, and AN-7 on the HT-29 colon cancer cells was examined after incubation for 96 h, by measuring the induced alkaline phosphatase activity. Each electrochemical chamber on the array was loaded with cells exposed to different agent type. BA, AN-7, and AN-9 were tested. The results are shown in Fig. 12.4.

Normal enzymatic activity denotes that the cells differentiate properly as a consequence of the particular drug treatment. As shown in Fig. 12.4, BA and AN-7 induced enzymatic activity of alkaline phosphatase, whilst AN-9 did not induced any enzyme activity. AN-9 showed no induction compared to AN-7 at 50 μM concentration exposure, which may be related to its reduced potency regarding

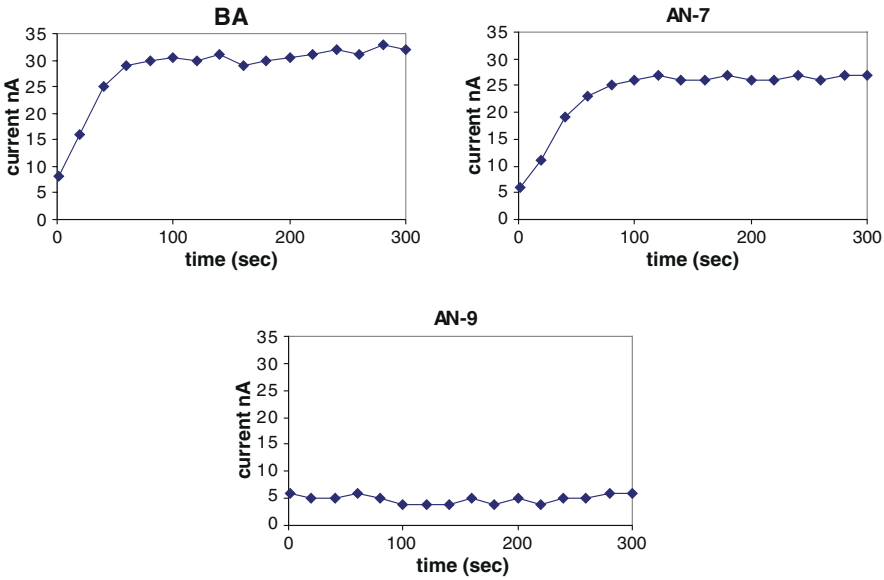

Fig. 12.4 HT-29 colon cancer cells response to BA, AN-7, and AN-9. Amperometric response curves for monitoring of alkaline phosphatase activity using the electrochemical array chip. The HT-29 colon cancer cells were exposed to the differentiation agents: Butyric acid (2.5 mM), AN-7 and AN-9 (50 μM). The HT-29 cells with the substrate PAPP were placed into the 100 nL volume electrochemical chambers on the chip. Current was measured using the amperometric technique at 220 mV

HT-29 cancer cells [30, 31]. In addition, positive and negative controls were performed (data not presented); It is important to note that AN-7 exerted similar differentiation effect on the BA although its concentration was lower by 1.5 orders of magnitude - 50 μM Vs. 2.5 mM for AN-7 and BA, respectively.

To exclude the possibility of false positive and false negative signals, all arrays included controls chambers (data not shown); for false positive control we first amperometrically measured each of the components alone: the untreated HT-29 cells, the treated HT-29 cells, and the drugs. Next we measured the combinations of untreated HT-29 cells with and without PAPP and the different drugs with PAPP. False negative control necessitates current generation in all experiments; we loaded the chamber with purified alkaline phosphatase and PAPP.

12.5.1 Quantification of Cancer Cells by the Biochip

We further examined the correlation between the current density and the number of cells that were presented in the chip chamber. The HT-29 cells were counted under the microscope and the induced current was measured as shown in Fig. 12.5.

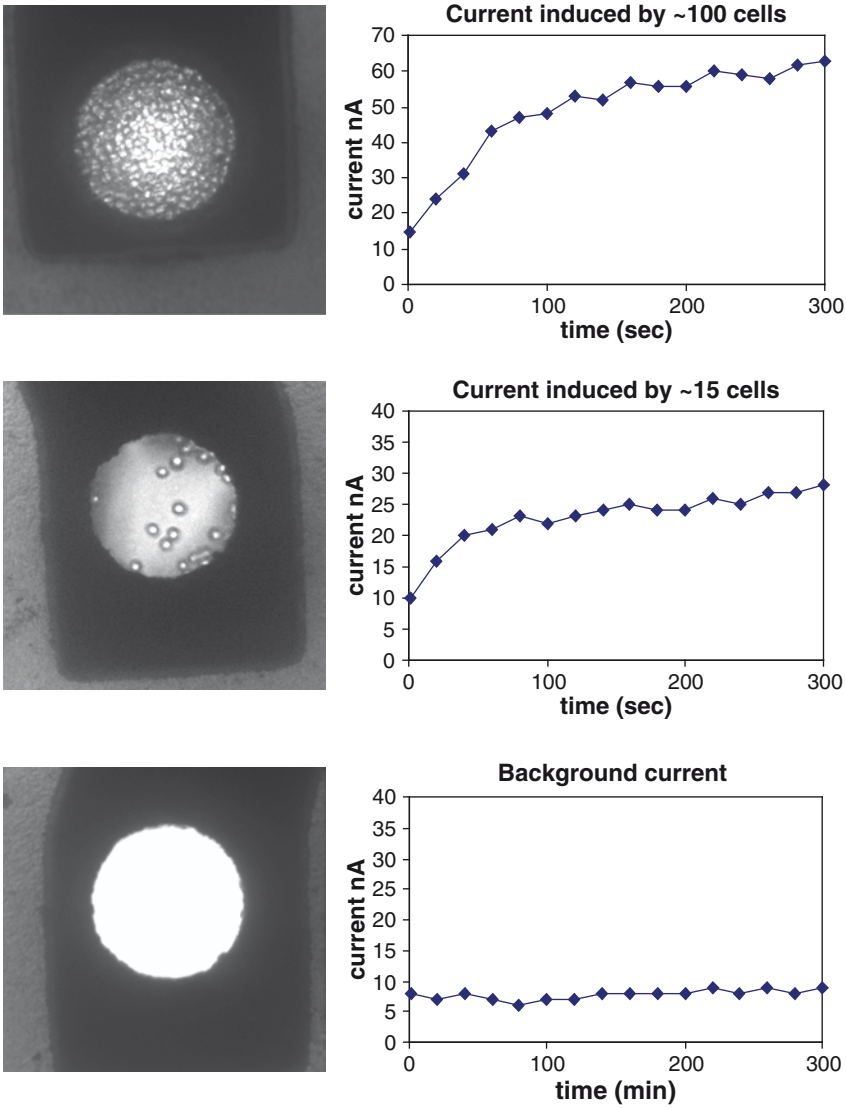

Fig. 12.5 Amperometric response curves for monitoring of alkaline phosphatase activity using the electrochemical array chip. We show the deposited cells with PAPP (*left*) and the output as a function of time on the right correspondingly

That figure shows Amperometric response curves for the monitoring of alkaline phosphatase activity using the electrochemical array chip. HT-29 colon cancer cells were exposed to Butyric acid (2.5 mM). The HT-29 cells with the substrate PAPP were placed into the 100 nL volume electrochemical chambers on the chip (left column). Current was measured using the amperometric technique at 220 mV.

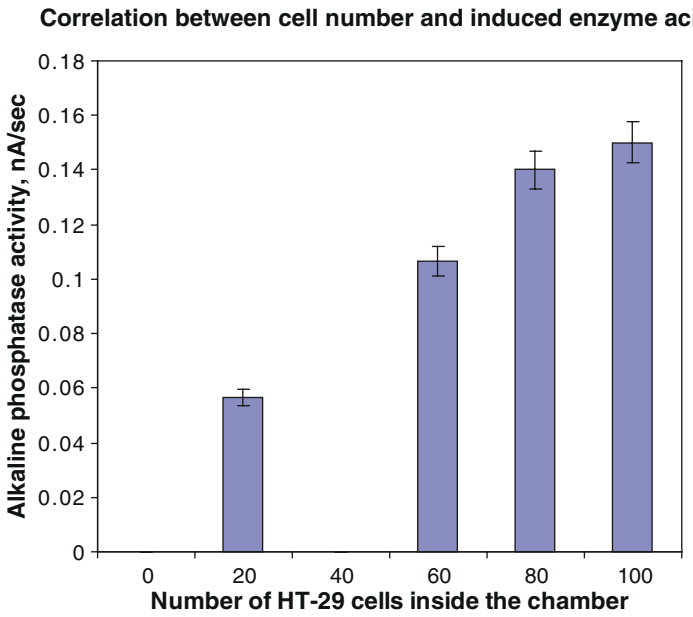

Fig. 12.6 Correlation between HT-29 colon cancer cell number and the induced alkaline phosphatase enzymatic activity. Activity is presented by Δcurrent/Δtime. Each result represents the mean of three measurements. Current was measured using the amperometric technique at 220 mV

Upper middle and lower curves represent the current response of about 100, 15, and 0 cells counted inside the chamber, respectively.

Multiple measurements demonstrated high correlation between cell number counted inside the chamber and alkaline phosphatase activity. The results are shown in Fig. 12.6.

In this study, we have demonstrated a new electrochemical method for highly sensitive, accurate, and rapid multiplex detection of colon cancer cells response to differentiation therapy. The development of an electronic biochip array system, adjusted to biological experiments can help tailor cancer treatment to individual patients. Human colon cancer cells, HT-29, were treated with the differentiation therapy drug agents BA, AN-7, and AN-9, and the cells response was simultaneously measured on line and compared. This microarray technology provides the ability to test on line the affect of multi-drug agents, and to tailor effective therapy to the individual.

We found significant quantitative correlation between the induced current signals and the number of cancer cells counted inside the nano-volume electrochemical chambers. The ability to quantitate the enzymatic reaction of few living cells is due this new geometry, in which electrochemical cell dimensions

were reduced to nano-scale. 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.

12.6 Summary and Conclusion

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. The total weight of the entire system is ~900 g, making it ideal for field environmental monitoring and for medical applications.

A novel integration between microelectronic devices and living organisms for electrochemical detection of toxicity in water has been demonstrated. The electrochemical "Lab on a chip" 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 stress conditions, with a direct correlation between the toxicant concentration and the induced current. During measurement period the bacteria remained active and were capable of performing cellular gene expression and enzymatic activity, which demonstrate the chip biocompatibility.

The use of MEMS technology in the health care arena leads to the developments of indispensable sophisticated intelligent devices. The miniaturization of these analytical devices is critical since it will enable the analysis of large number of drugs per sample and may be used directly with small biopsy's samples or small volumes of body fluids. This in turn leads to fast response time, sensitive and cost-effective analysis.

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 multicultural organisms, and may lead to interesting pharmaceutical and clinical developments.

The power of the microelectronic technology, and its usefulness to extreme and precise miniaturization enable the construction of highly sensitive, fast, and robust devices, which can provide exciting opportunities in the biosensors field as well as in the basic research on microorganisms.

References

1. Belkin S (2003) Microbial whole-cell sensing systems of environmental pollutants. Curr Opin Microbiol 6(3):206–212
2. Stenger DA et al (2001) Detection of physiologically active compounds using cell-based biosensors. Trends Biotechnol 19(8):304–309
3. Rudolph AS, Reasor J (2001) Cell and tissue based technologies for environmental detection and medical diagnostics. Biosens Bioelectron 16(7–8):429–431
4. Pancrazio JJ et al (1999) Development and application of cell-based biosensors. Ann Biomed Eng 27(6):697–711
5. Popovtzer R, Neufeld T, Ron EZ, Rishpon J, Shacham-Diamand Y (2006) Electrochemical detection of biological reactions using a novel nano-bio-chip array. Sensor Actuator B-Chem 119(2):664–672
6. Daunert S et al (2000) Genetically engineered whale-cell sensing systems: coupling biological recognition with reporter genes. Chem Rev 100(7):2705–2738
7. Kohler S et al (2000) Detection of 4-chlorobenzoate using immobilized recombinant Escherichia coli reporter strains. Sensor Actuator B-Chem 70(1–3):139–144
8. Biran I et al (1999) On-line monitoring of gene expression. Microbiology-Sgm 145:2129–2133
9. Paitan Y et al (2003) On-line and in situ biosensors for monitoring environmental pollution. Biotechnol Adv 22(1–2):27–33
10. Paitan Y et al (2004) Monitoring aromatic hydrocarbons by whole cell electrochemical biosensors. Anal Biochem 335(2):175–183
11. Premkumar JR et al (2001) Antibody-based immobilization of bioluminescent bacterial sensor cells. Talanta 55(5):1029–1038
12. Cha HJ et al (1999) Green fluorescent protein as a noninvasive stress probe in resting Escherichia coli cells. Appl Environ Microbiol 65(2):409–414
13. Nivens DE et al (2004) Bioluminescent bioreporter integrated circuits: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring. J Appl Microbiol 96(1):33–46
14. Sagi E et al (2003) Fluorescence and bioluminescence reporter functions in genetically modified bacterial sensor strains. Sensor Actuator B-Chem 90(1–3):2–8
15. Grigsby PW, Corn BW (1992) Localized urethral tumors in women–Indications for conservative versus exenterative therapies. J Urol 147(6):1516–1520
16. McLeod H (2007) Graying anatomy? Toward molecular tumor characterization. Pharmacogenomics 8(1):15–16
17. Guillemard V, Saragovi HU (2004) Novel approaches for targeted cancer therapy. Current Cancer Drug Targets 4(4):313–326
18. Konski A, Feigenberg S, Chow E (2005) Palliative radiation therapy. Semin Oncol 32(2):156–164
19. Cao T, Heng BC (2005) Differentiation therapy of cancer. Potential advantages over conventional therapeutic approaches targeting death of cancer/tumor cells. Med Hypotheses 65(6):1202–1203
20. Miller SJ (2004) Cellular and physiological effects of short-chain fatty acids. Mini-Reviews in Medicinal Chemistry 4(8):839–845
21. Sell S, Pierce GB (1994) Maturation arrest of stem-cell differentiation is a common pathway for the cellular-origin of teratocarcinomas and epithelial cancers. Lab Invest 70(1):6–22
22. Bell DR, Van Zant G (2004) Stem cells, aging, and cancer: inevitabilities and outcomes. Oncogene 23(43):7290–7296
23. Patnaik A et al (2002) A phase I study of pivaloyloxymethyl butyrate, a prodrug of the differentiating agent butyric acid, in patients with advanced solid malignancies. Clin Canc Res 8(7):2142–2148
24. Rephaeli A, Zhuk R, Nudelman A (2000) Prodrugs of butyric acid from bench to bedside: Synthetic design, mechanisms of action, and clinical applications. Drug Dev Res 50(3–4):379–391

25. Boren J et al (2003) The stable isotope-based dynamic metabolic profile of butyrate-induced HT29 cell differentiation. J Biol Chem 278(31):28395–28402
26. Newmark H, Lipkin M (1994) Omega-3-fatty-acids and rectal epithelial-cell proliferation. Gastroenterology 107(6):1892–1894
27. Comalada M et al (2007) The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype (vol 132, pg 487, 2006). J Cancer Res Clin Oncol 133(3):211–211
28. Reid T et al (2004) Phase II trial of the histone deacetylase inhibitor pivaloyloxymethyl butyrate (Pivanex, AN-9) in advanced non-small cell lung cancer. Lung Cancer 45(3):381–386
29. Pawitan Y et al (2005) Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. Breast Cancer Res 7(6):R953–R964
30. Entin-Meer M et al (2005) Butyric acid prodrugs are histone deacetylase inhibitors that show antineoplastic activity and radiosensitizing capacity in the treatment of malignant gliomas. Mol Canc Therapeut 4(12):1952–1961
31. Engel D et al (2005) Novel prodrugs of tegafur with antiangiogenic properties and improved anticancer activity. Clin Canc Res 11(24):9052S–9052S