Chapter 9
Future Trends of Micro/Nano Cell and Molecule-Based Biosensors

Ping Wang, Ning Hu, Chunsheng Wu, and K. Jimmy Hsia

Abstract The novel micro/nano cell- and molecule-based biosensors are powerful tools in cell and molecule study, ranging from the traditional microbiosensors and novel nanotechnology. In this chapter, intelligent micro/nano cell- and molecule-based biosensor biosystems, including intelligent multiparameter biosensors, intelligent high-throughput biosensor array, and intelligent multifunction biosensors, are introduced. Moreover, application perspective in biomimetic devices, health care, and rehabilitation was further discussed. These micro/nano cell and molecular biosensors have demonstrated excellent performance in cell and molecule applications and will pave a wide way in biomedicine and environmental applications.

Keywords Intelligent biosensors biosystem • Nano-micropatterned cell culture • Nano-sensors

9.1 Introduction

Biosensors will develop rapidly in the future and will become more powerful and versatile by using bioactive elements such as tissues, cells and molecules, and even organoid as sensing elements combined with microfabrication and nanotechnology to produce biomimetic sensors. These cell- and molecule-based biosensors have superior performance such as high sensitivity and selectivity to environment and other objects, which may be similar as or even superior to sensing performance of human and animals. Cell- and molecule-based biosensors employ living cells and bioactive molecule components to qualitatively and quantitatively detect and

P. Wang (✉) • N. Hu
Biosensor National Special Laboratory, Department of Biomedical Engineering, Zhejiang University, Hangzhou, China
e-mail: cnpwang@zju.edu.cn

C. Wu
Institute of Medical Engineering, School of Basic Medical Sciences, Health Science Center, Xi'an Jiaotong University, Xi'an, China

K.J. Hsia
Department of Biomedical Engineering, Carnegie Mellon University, Pittsburg, PA, USA

P. Wang et al. (eds.), *Micro/Nano Cell and Molecular Sensors*,
DOI 10.1007/978-981-10-1658-5_9

measure the presence of substances and their concentrations. These cell- and molecule-based biosensors can sense much functional information from external physical or chemical stimulations. Besides, cell- and molecule-based biosensors as biomimetic sensors will also be used in biomedical practical application. Significantly, the performance of these biosensors can be enhanced to very high levels by nanotechnology and nanomaterial, which dramatically improved the sensitivity and selectivity of cell- and molecule-based biosensors.

Future trends of cell- and molecule-based biosensors will be tightly combined with the state-of-the-art technologies, e.g., microfabrication, nanotechnology, and molecular biology to build the multifunctional, integrated, and intelligent biosensor. In this chapter, we will discuss the novel trends in micro/nano cell- and molecule-based biosensors in the near future.

9.2 Intelligent Micro/Nano Cell- and Molecule-Based Biosensors and Biosystems

Integrated cell- and molecule-based biosensors can be divided into three types: integrated multiparameter biosensors, high-throughput biosensor arrays, and multifunctional biosensors. Both integrated and multifunctional cell-based biosensors can provide either high-throughput information or different functional parameters of living cells and bioactive molecules. Nowadays, micro/nano sensors integrated with cells and molecules for biomedical applications have attracted broad attention of researchers in a variety of fields.

For compound assessment, it is important to select the suitable model to evaluate the compound effect, so the integrated multifunctional sensors can be employed in these cases to determine the right compound analysis models. After these bioassays, multiparameter data can be collected for further study. Thus, the suitable models or screening methods can be determined for the subsequent high-throughput analysis with sensor array. These intelligent biosensors could significantly reduce the cost for compound analysis. For example, in vitro cellular bioassay can be performed prior to using the drug candidates on animal models, which is time-consuming and expensive. Consequently, nowadays researches are focused to integrate different sensors into miniaturized biochips for a multiparameter online analysis of living cells and bioactive molecules for physiological and environmental monitoring. The throughput of extracellular parameters can be greatly enhanced, and molecule concentration can be measured by these intelligent sensors. It is a different approach in functional online analysis of living cells and bioactive molecule in physiologically controlled environments for extended periods of time.

9.2.1 Intelligent Multiparameter Biosensors

Different biosensors and detection technologies are used to obtain more information about living cells and bioactive molecules. Bernhard Wolf et al. [1, 2]

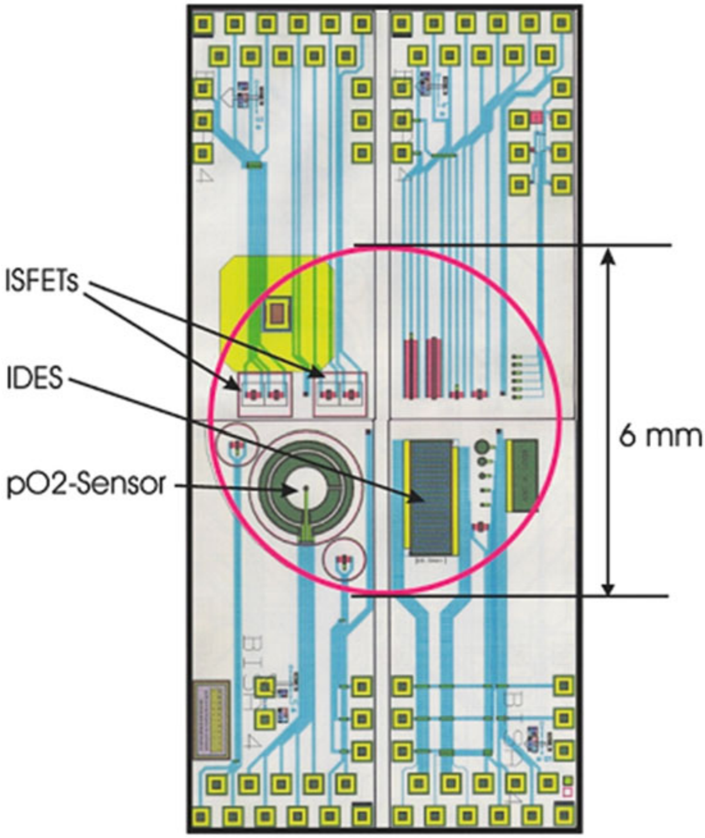

Fig. 9.1 Intelligent sensor chips based on integration of potentiometric, amperometric, and impedance sensor (Reproduced with permission from Ref. [5]. Copyright 2003 Royal Society of Chemistry)

fabricated several types of integrated chips, which contain interdigitated electrodes (IDES), ISFET, O_2 sensor, and temperature sensors as shown in Fig. 9.1. This intelligent miniaturized biosystem can obtain sufficient information from cells and their metabolic molecules. These various cell- and molecule-based biosystems allowed online and noninvasive measurements of different parameters of signaling based on cells and molecular detection [3, 4].

These multiparameter integrated sensors are used for investigations of cells by real-time recording extracellular physiological status and molecular changes in the microenvironment. Potentiometric, amperometric, and impedance sensors are designed on the single chip for multiparameter monitoring [5]. Extracellular acidification rates (by ion-sensitive field-effect transistors), oxygen consumption rates (by Pt electrode), and cell morphology (by impedance electrode) can be simultaneously monitored on single chips for days.

Integrated cell- and molecule-based biosensors with multiparameter biosensors play a significant role in recent decade. Multiparameter biosensors can provide

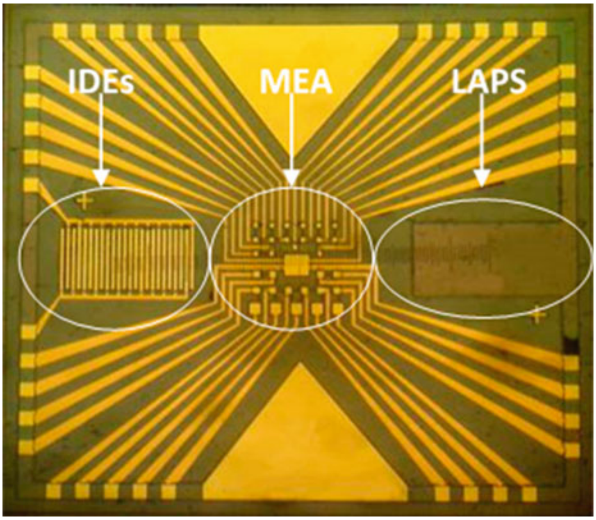

Fig. 9.2 Integrated cell- and molecule-based biosensors including ECIS, MEA, and LAPS

more information on the status of cells and molecules. As shown in Fig. 9.2, the integrated chip is integrated by interdigitated electrodes (IDEs) that measure the impedance of cells for growth evaluation, microelectrode array (MEA) which detects the extracellular potential of cells, and light-addressable potentiometric sensor (LAPS) which monitors the cellular metabolites in microenvironment. In a multiparameter integrated chip, these three biosensors are incorporated into a single chip. After living cells are seeded on this chip, both chemical and electrical parameters can be recorded. Living conditions of the target cells, including the cell growth and metabolism, can be analyzed from the data of IDA and LAPS part. The extracellular potential can be detected by the MEA part.

9.2.2 Intelligent High-Throughput Biosensor Array

Intelligent cell- and molecule-based biosensors array is utility for high-throughput bioassays after the model determination, and biosensor array can dramatically improve the efficiency of bioassays. MEA, FET, LAPS, and ECIS array are commonly used in cell and molecule detection and measurement. These high-throughput cell- and molecule-based biosensor arrays can provide extracellular information of living cells and bioactive molecules.

Thomas Geisler et al. integrated multiparameter, bioelectrical, and biochemical sensors for the analytical monitoring of intra- and extracellular parameters, as shown in Fig. 9.3 [6, 7]. The intelligent multiparameter biosensor array can provide more information on cells and molecules and has the high-throughput analysis data, and thus more information can be derived for further analysis. The extracellular potential, growth, and metabolism status can be monitored in real time, which

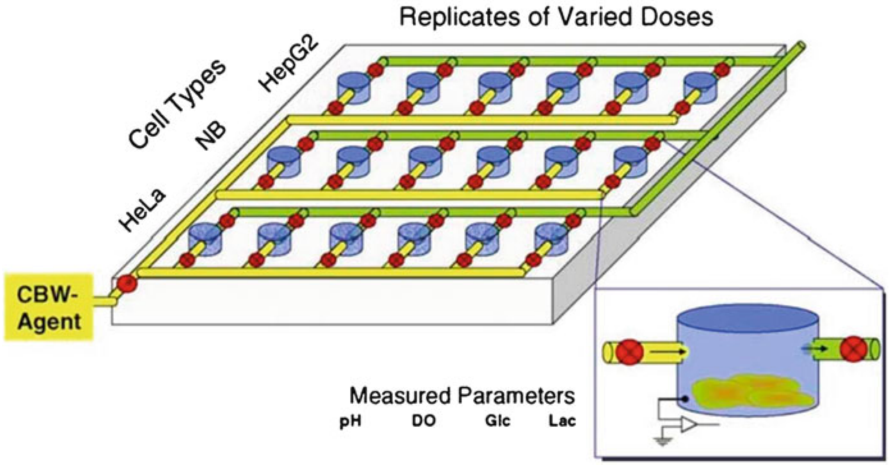

Fig. 9.3 High-throughput multiparameter biosensor array (Reproduced with permission from Ref. [8]. Copyright 2006 Elsevier B.V.)

reflected extracellular status. These high-throughput cell- and molecule-based biosensor arrays can not only improve the efficiency of bioassays but enrich the high-content analysis as well.

Multifunction cell- and molecule-based biosensors are another important advancement, which is different from the multiparameter sensors. They can perform different functions through different working modes. ECIS can monitor the cell growth status and record the cardiomyocyte beating status under the high-speed mode. LAPS is another typical multifunction sensor. Usually, LAPS is used to monitor the cellular metabolic molecules, while it also can be used to detect the cardiomyocyte beating status. Higher and higher levels of integration are the most important feature of the requirement of integrated cell- and molecule-based biosensors. Most integrated cell-based biosensors involve all three types of intelligent cell- and molecule-based sensors at the same time. High level integration can provide more comprehensive information to give precise determination of cell and molecular functions. Therefore, more precise and rigorous preselection of identified compounds can be achieved with these integrated cell- and molecule-based biosensors.

9.2.3 Devices Through Integrating MEMS/Micro/Nano Technologies and Live Cells for Cell Behavior Measurements and Manipulation

Hybrid systems and devices through integrating MEMS/micro/nano technologies and live cells have been actively developed in recent years. Although many studies focus on biological and biochemical properties of cells, several recent studies utilize mechanical principles of such devices to perform certain functions [9–11], including measuring cell properties and manipulating cell responses. One such device is a novel vibrational platform developed using MEMS technology for cell mass measurements as shown in Fig. 9.4 [9]. Cell growth and cell division are highly complex biological processes that are critical in all living systems. However, there were no reliable techniques to accurately measure the mass of individual live cells as they grow and thus to determine the cell growth rate. The vibrational platform developed by Park et al. makes use of the mechanical principle of resonance frequency to measure the mass of individual live cells. The method took into account cell elasticity and cell viscosity and was able to follow the cell

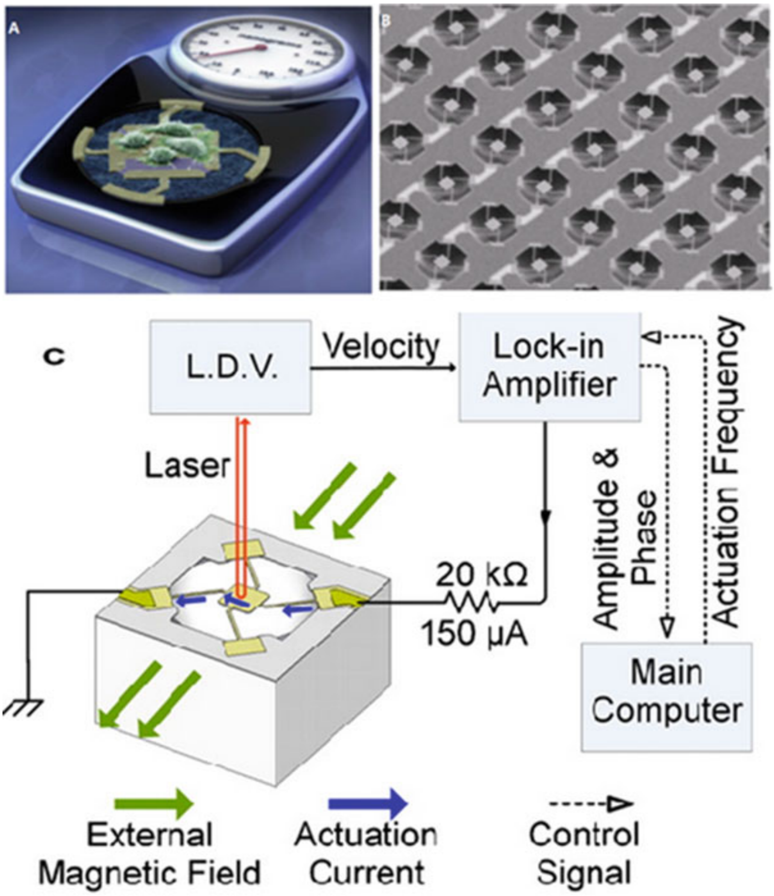

Fig. 9.4 A novel vibrational platform developed using MEMS technology for cell mass measurements: (**a**) scheme of cell mass measurement, (**b**) SEM of the array chip for cell mass measurements, and (**c**) schematic diagram of an integrated chip for cell mass measurements (Reproduced with permission from Ref. [9]. Copyright 2010 National Academy of Sciences)

growth → cell division → daughter cell growth cycles in real time. Mechanics modeling was carried out to interpret the measurement results. Understanding of what control cell growth and division processes in mammalian cells will have tremendous impact on issues like cancer cell growth and proliferation.

9.3 Application Perspective in Biomimetic Devices, Health Care, and Rehabilitation

In recent decades, nanotechnology and nanomaterials have been driving the development in science and technology. A nanometer (nm) is a billionth of a meter ($1\ nm = 10^{-9}\ m$). However, nanostructure dramatically enhances the performance of the cell- and molecule-based biosensors. Nanotechnology contains the investigation, manipulation, fabrication, and employment of materials, devices, and systems under 100 nm in one dimension. Nanomaterials, e.g., nanotubes, nanopillars, nanowire, nanorods, nanocapsules, nanoparticles, and nanoporous membrane, are widely used in design and fabrication of sensors. Besides, nanotechnology also plays an increasingly significant role in the progress of cell- and molecule-based biosensors. Performance of these biosensors will be wonderfully improved by using nanomaterials or nanostructures. Nanomaterials and nanostructures present superior physical and chemical characteristics due to size effects like the quantum size effect, minisize effect, surface effect, and macro-quantum tunnel effect.

Many studies have reviewed the various nanomaterial and nanostructure-based biosensors, even at the molecular level [12]. Moreover, the nano-based biosensors also revolutionize research, analysis, and application at the cellular level. The convergence of MEMS, nanotechnology, and biology leads a new way to manipulate nanomaterial for a wide variety of biomedical applications for living cells and bioactive molecules. Nano-based biosensors will be promising tools for monitoring in vivo and in vitro biological processes, which will greatly improve our understanding of cellular function, thereby promoting cell biology.

9.3.1 Nano-micropatterned Cell Cultures

The nano-micropatterned biosensors become popular for cell cultures due to their good biocompatibility based on the characteristics for study of sensor arrays, tissue engineering, and cellular physiology in recent years. Nanomaterials and nanostructure-based biosensors have been employed in vitro and in vivo experiments, which construct the bioactive tissues and organs, improve biocompatible characteristics, and perform the relative biomedical researches. Properties of nanomaterials and nanostructures are extremely important to the design, fabrication, and use of nano-based devices. Some researches [13–15] have reported the perspective of integrated

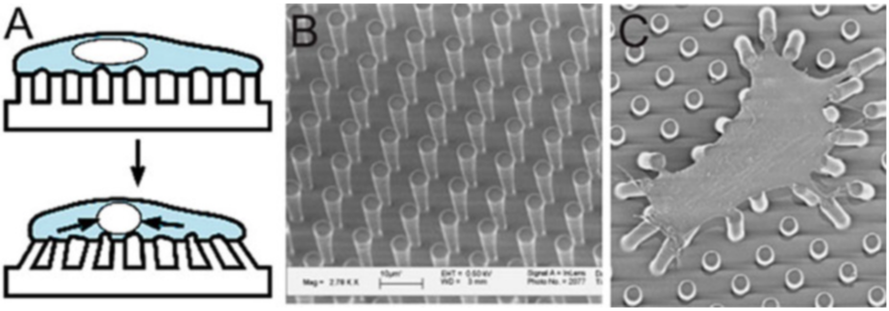

Fig. 9.5 Smooth muscle cell culture on micropillars: (**a**) cell behavior on the micropillars; (**b**) a uniform array of micropillars; and (**c**) SEM of the cell deforming the micropillar after adhesion (Reproduced with permission from Ref. [16]. Copyright 2003 National Academy of Sciences)

nanomaterials and nanostructures to sense living cells. In many researches, the nanoparticles are assembled to pattern and fabricate the substrate for cell study. The nanomaterials and nanostructures can significantly increase the surface area which can enhance the performance of biosensors for detection and measurement. Furthermore, a nanomaterial or nanostructure substrate can affect the conformation and functionality of proteins which attach onto its surface. Moreover, cell status in terms of morphology, adhesion, proliferation, differentiation, etc. can be influenced by micro- and nano-modified surfaces.

Researchers [16] described a method to measure mechanical interactions of cell-cell attachment and cell-substrate attachment by using microfabricated arrays of elastic PDMS micropillars (Fig. 9.5). Cells cultured on the surface can attach and spread on the micropillars. The deformation of the micropillars occurred independently of neighboring micropillars; therefore, the deformation of micropillars directly reflects the subcellular distribution of interaction forces. By controlling cell attachment on these micromechanical sensors, cell morphology regulates the magnitude of interaction force generated by cells. These results demonstrate a coordination of biochemical and mechanical signals to regulate cell attachment, and micropillar array is introduced to fabricate mechanically isolated sensors to measure the mechanical interactions of cells.

9.3.2 Nanoporous-Based Biosensor

The high surface area-to-volume ratio is one of the most important characteristics of nanomaterials. The increased nanomaterial or nanostructure surface can act as the adsorbents to embed small molecular particles into the pores. The properties of nanoporous, e.g., pore size, morphology, structure, and specific distribution which directly interact with cells or molecule, enhance absorption and ultimately result in

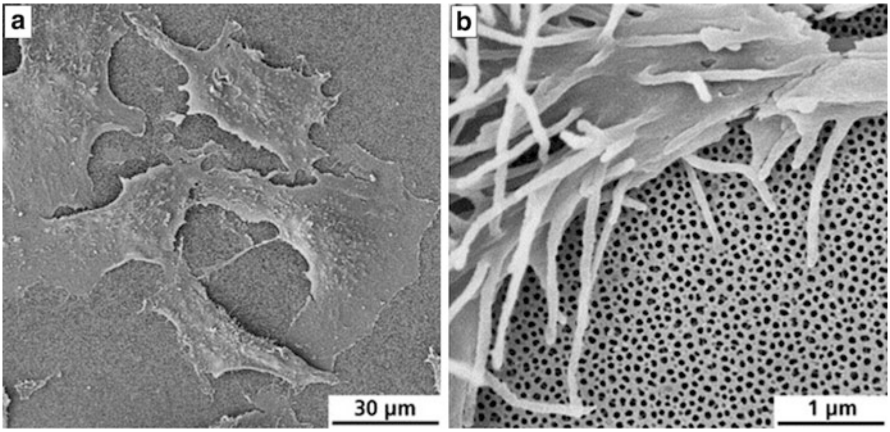

Fig. 9.6 HepG2 cells on self-supporting aluminum oxide membrane 1 (pore diameter is about 75 nm). Overview (**a**) and magnification of a cell border (**b**) (Reproduced with permission from Ref. [18]. Copyright 2007 Elsevier B.V.)

substantial improvements on functional applications such as reaction catalysis, membrane attachment, electrode sensitivity, and so on.

Nanoporous materials are not only used in biomedical fields but other research fields as well. Nanoporous anodic aluminum oxide (AAO), or known as alumina, attracted more attention due to excellent biocompatibility and fabrication process, as shown in Fig. 9.6 [11, 17, 18]. Alumina has already been extensively employed as a sensor substrate for cell and tissue culture. To achieve applications of cell or tissue engineering, the biocompatibility of the nanostructure surface is one of the most important factors for cell study. For example, osteoblast has been used in the study of alumina surfaces in different labs. In some study, a two-step anodization process was optimized with uniform pore dimension and distribution in the fabrication of nanoporous alumina membranes. The impact of the nanopores was studied by evaluating osteoblast adhesion, morphology, and proliferation via different methods. The results show that alumina surfaces have significant biointegration for cell growth and the measurement of cell response can be greatly improved with nanoscale structure.

Our research group has successfully fabricated AAO using a two-step anodization procedure, with pores sized between 50 and 120 nm. A cell molecule-based biosystem was established with PDMS chamber to perform the cell culture and molecule measurement [19]. Different cells were cultured on the AAO surface of sensor, and cell responses to specific compounds have been observed. In our new analytical biosystem based on this, the cancer cells' response to anticancer drug can be found in multifrequency electrical impedance spectra. The new nanostructure biosensor will be promising to enhance the sensor performance and replace the traditional time-consuming cell bioassays or microscopic techniques in anticancer drug screening in the future.

9.3.3 Nanoprobes to Intracellular Nano-sensors

With the advancement of nanotechnology, nanoscale devices can be used to probe the intracellular environmental status of single living cells, leading to more true information of the whole cell. It can greatly improve our understanding of cellular function as a novel method. Those nano-sensors could be fabricated to nanoscale size, which makes them suitable for sensing and measuring intracellular physiological parameters of single cell.

Zinc oxide (ZnO) is popular in the sensor fabrication due to its semiconducting, unique optical, piezoelectric, and magnetic properties. ZnO nanostructures present good characteristic including high catalytic efficiency and strong adsorption ability. In the recent years, the interest has been focused toward the application of ZnO in biosensing based on its high isoelectric point, biocompatibility, and fast electron-transfer kinetics, indicating the potential applications of ZnO as one of the promising materials for biosensor applications [20].

Nanostructure ZnO nanorods are suitable for intracellular pH measurement. Some authors have reported ZnO nanorods as an intracellular sensor for pH measurements [21]. Main effort has been made to construct nanorod tips, which are capable of penetrating the cell membrane as well as optimization of the electrode properties. ZnO nanorods are grown on the tip of a borosilicate glass capillary (0.7 μm in diameter), and a highly sensitive ZnO nanorod pH sensor is created for monitoring intracellular environment of single cell. The ZnO nanorods, functionalized by proton H^+ and hydroxyl OH^- groups, present a pH-dependent behavior versus an Ag/AgCl reference electrode (Fig. 9.7). The pH potential difference is linear over a large dynamic range. Therefore, these nanoelectrode devices can carry out analytical measurements in single living cells and sense individual chemical species in specific locations within a cell.

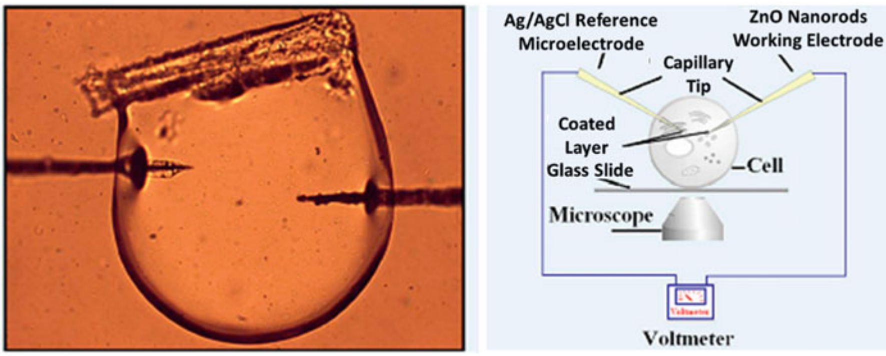

Fig. 9.7 Optical image and schematic diagram illustrating intracellular pH measurements performed in a single human fat cell using ZnO nanorods (Reproduced with permission from Ref. [21]. Copyright 2007 American Institute of Physics)

9.3.4 Nano- and Micro-patterned Surface for Cell Behavior Manipulation

Micro/nano technologies have also been used to control cell behavior. By seeding C2C12 cells on a surface with submicron-/micron-scale waviness, Grigola et al. [10] succeeded in aligning the migrating cells along the wavy directions. More importantly, they were able to identify the critical conditions governing cell alignment. C2C12 are skeletal muscle cells. Being able to align them allows us to form precursor tissues that can be differentiated into muscle strips. This technology is potentially very useful to constructing integrated cell-nanostructure machines capable of performing mechanical functions. Another technique to form elongated cell clusters with MCF10a cells (breast cells) was developed by Joaquin et al. [11]. Instead of submicron waviness, they produced a surface with submillimeter-scale waviness. They showed that, in a 3D in vitro culture environment, these breast cells migrate following mechanical stiffness gradient to form elongated cell clusters, a precursor to ductal shaped tissues. MCF10a cells cluster into acini, i.e., hollow spheres in common 3D culture environment in vitro. However, they form hollow ducts in an in vivo culture environment, through which milk is transported in female mammals. The technique developed by Joaquin et al. [11] not only helps us understand the ECM microenvironment governing duct formation in breast cells but also has potential for tissue engineering to reconstruct breast tissues. These hybrid bio-micro/nano structured systems and devices have opened up doors for vast varieties of different functionalities to be achieved, including sensing, actuation, and shape and form manipulations.

9.4 Summary

The novel micro/nano cell and molecule-based biosensors are discussed in this book, ranging from the traditional microbiosensors to novel nanotechnology. Traditional biosensors, such as microelectode array (MEA), impedance sensor, field-effect transistor (FET), and light-addressable potentiometric sensor (LAPS), are useful tools in cell and molecule analysis, and the nanostructures and nanomaterials-based biosensors emerge gradually with the advance of nanotechnology. These nanostructure and nanomaterial-based biosensors have demonstrated excellent performance in cell and molecule applications. With a combination of sensor technology and nanotechnology, the novel micro/nano cell and molecule biosensors can explore a wide way in biomedicine and environment monitoring applications.

References

1. Baumann W, Lehmann M, Schwinde A, Ehret R, Brischwein M, Wolf B. Microelectronic sensor system for microphysiological application on living cells. Sens Actuators B. 1999;55 (1):77–89.
2. Wolf B, Brischwein M, Lob V, Ressler J, Wiest J. Cellular signaling: aspects for tumor diagnosis and therapy. Biomed Tech. 2007;52(1):164–8.
3. Henning T, Brischwein M, Baumann W, Ehret R, Freund I, Kammerer R, Lehmann M, Schwinde A, Wolf B. Approach to a multiparametric sensor-chip-based tumor chemosensitivity assay. Anticancer Drugs. 2001;12(1):21–32.
4. Otto AM, Brischwein M, Motrescu E, Wolf B. Analysis of drug action on tumor cell metabolism using electronic sensor chips. Arch Pharm. 2004;337(12):682–6.
5. Brischwein M, Motrescu E, Cabala E, Otto A, Grothe H, Wolf B. Functional cellular assays with multiparametric silicon sensor chips. Lab Chip. 2003;3(4):234–40.
6. Geisler T, Ressler J, Harz H, Wolf B, Uhl R. Automated multiparametric platform for high-content and high-throughput analytical screening on living cells. Autom Sci Eng IEEE Trans. 2006;3(2):169–76.
7. Becker D-IB, Lob V, Janzen N, Grundl D, Ilchmann F, Wolf B. Automated multi-parametric label free 24 channel real-time screening system. In Editor (Ed.)^(Eds.) Book automated multi-parametric label free 24 channel real-time screening system. Springer; 2008, edn, p. 186–189.
8. Eklund SE, Snider RM, Wikswo J, Baudenbacher F, Prokop A, Cliffel DE. Multianalyte microphysiometry as a tool in metabolomics and systems biology. J Electroanal Chem. 2006;587(2):333–9.
9. Park K, Millet LJ, Kim N, Li H, Jin X, Popescu G, Aluru N, Hsia KJ, Bashir R. Measurement of adherent cell mass and growth. Proc Natl Acad Sci. 2010;107(48):20691–6.
10. Grigola MS, Dyck CL, Babacan DS, Joaquin DN, Hsia KJ. Myoblast alignment on 2D wavy patterns: dependence on feature characteristics and cell-cell interaction. Biotechnol Bioeng. 2014;111(8):1617–26.
11. Joaquin D, Grigola M, Kwon G, Blasius C, Han Y, Perlitz D, Jiang J, Ziegler Y, Nardulli A, Hsia KJ. Cell migration and organization in three-dimensional in vitro culture driven by stiffness gradient. Biotechnol Bioeng. 2016;9999:1–11.
12. Jianrong C, Yuqing M, Nongyue H, Xiaohua W, Sijiao L. Nanotechnology and biosensors. Biotechnol Adv. 2004;22(7):505–18.
13. Helmke BP, Minerick AR. Designing a nano-interface in a microfluidic chip to probe living cells: challenges and perspectives. Proc Natl Acad Sci. 2006;103(17):6419–24.
14. Kriparamanan R, Aswath P, Zhou A, Tang L, Nguyen KT. Nanotopography: cellular responses to nanostructured materials. J Nanosci Nanotechnol. 2006;6(7):1905–19.
15. Yap F, Zhang Y. Protein and cell micropatterning and its integration with micro/nanoparticles assembly. Biosens Bioelectron. 2007;22(6):775–88.
16. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS. Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc Natl Acad Sci. 2003;100 (4):1484–9.
17. Swan EEL, Popat KC, Grimes CA, Desai TA. Fabrication and evaluation of nanoporous alumina membranes for osteoblast culture. J Biomed Mater Res A. 2005;72(3):288–95.
18. Hoess A, Teuscher N, Thormann A, Aurich H, Heilmann A. Cultivation of hepatoma cell line HepG2 on nanoporous aluminum oxide membranes. Acta Biomater. 2007;3(1):43–50.
19. Yu J, Liu Z, Liu Q, Yuen KT, Mak AF, Yang M, Leung P. A polyethylene glycol (PEG) microfluidic chip with nanostructures for bacteria rapid patterning and detection. Sensors Actuators A Phys. 2009;154(2):288–94.
20. Kumar SA, Chen SM. Nanostructured zinc oxide particles in chemically modified electrodes for biosensor applications. Anal Lett. 2008;41(2):141–58.
21. Al-Hilli SM, Willander M, Öst A, Stralfors P. ZnO nanorods as an intracellular sensor for pH measurements. J Appl Phys. 2007;102(8):4304.