

Review

Review on Impedance Detection of Cellular Responses in Micro/Nano Environment

Kin Fong Lei

Graduate Institute of Medical Mechatronics, Department of Mechanical Engineering, Chang Gung University, 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, 333 Taiwan;

E-Mail: kflel@mail.cgu.edu.tw; Tel.: +886-3-211-8800 (ext. 5345); Fax: +886-3-211-8050.

Received: 4 September 2013; in revised form: 30 October 2013 / Accepted: 28 December 2013 /

Published: 7 January 2014

Abstract: In general, cell culture-based assays, investigations of cell number, viability, and metabolic activities during culture periods, are commonly performed to study the cellular responses under various culture conditions explored. Quantification of cell numbers can provide the information of cell proliferation. Cell viability study can understand the percentage of cell death under a specific tested substance. Monitoring of the metabolic activities is an important index for the study of cell physiology. Based on the development of microfluidic technology, microfluidic systems incorporated with impedance measurement technique, have been reported as a new analytical approach for cell culture-based assays. The aim of this article is to review recent developments on the impedance detection of cellular responses in micro/nano environment. These techniques provide an effective and efficient technique for cell culture-based assays.

Keywords: impedance measurement; cell culture; cellular responses; microfluidics

1. Introduction

Cell culture, which cultures cells as a monolayer on a surface of a cell culture vessel (e.g., Petri dish or multi-well microplate) is widely used in life science research for the investigation of cellular behavior. It has the advantage of simplicity in terms of operations and observations. In general cell culture-based assays, monitoring of cell number, viability, and metabolic activity are commonly performed to provide information of cellular responses under a specific culture condition studied. Conventionally, counting cells microscopically, quantifying indicative cellular components (e.g., DNA), live/dead fluorescent dye staining, and analysis of indicative metabolites synthesized by the cultured

cells are adopted. These analytical methods have become standard protocols for the cell culture-based assays. However, these approaches are normally labor-intensive and time-consuming, limiting the throughput of the cell culture-based assay works like drug screening or toxin testing. In addition, analysis of the indicative cellular components and fluorescent dye staining normally need to sacrifice the cultured cells and thus hamper the observation of the subsequent cellular responses. Therefore, alternative analytical methods are crucial in need for achieving both effective and efficient detections.

In the past decade, microfluidic system, also called “lab-on-chip (LOC)”, “bio-chip”, or “micro-total-analysis-system (μ TAS)”, has attracted attention because of its capability of combining engineering and life science [1–3]. Therefore, it is often interpreted as a miniaturized and automatic version of a conventional laboratory. Due to their miniaturization and automation, there are a number of advantages of using microfluidic systems, such as less sample/reagent consumption, reduced risk of contamination, less cost per analysis, lower power consumption, enhanced sensitivity and specificity, and higher reliability. Microfluidic systems have been developed for various biological analytical applications, such as DNA analysis [4–8], immunoassay [9–13], and cell analysis [14–18]. Moreover, a number of demonstrations showed that cell culture can be performed on the microfluidic systems to achieve higher throughput and more reliable results [19,20]. For example, a microfluidic device for culturing cells inside an array of microchambers with continuous perfusion of medium was reported to provide a cost-effective and automated cell culture [21]. Each circular microchamber was 40 μ m in height and surrounded by multiple narrow perfusion channels of 2 μ m in height. The high aspect ratio between the microchamber and the perfusion channels offered a stable and homogenous microenvironment for cell growth. Human carcinoma (HeLa) cells were cultured in 10 \times 10 microfluidic cell culture array and able to grow to confluency after eight days. Moreover, a fully automated cell culture screening system was developed and demonstrated on maintaining cell viability for weeks [22]. Individual culture conditions in 96 independent culture chambers can be customized in terms of cell seeding density, composition of culture medium, and feeding schedule. Each chamber was imaged with time-lapse microscopy to perform quantitative measurements of the influence of transient stimulation schedules on cellular activities. In these excellent demonstrations, optical imaging was utilized to quantify cellular activities. However, this measurement technique is time-consuming and may induce large tolerance. Alternatively, impedance measurement was proposed to be one of the promising techniques to quantify cellular responses during culture on the microfluidic systems. The detection results are represented by electrical signals, which can easily interface with miniaturized devices. Typically, a pair of electrodes as an electrical transducer is utilized to measure the impedance change caused by the existence of the biological substances. Literature has demonstrated the use of the similar principle for the detection of various biological substances such as enzymes [23], antibodies and antigens [10,24–26], DNA [27,28], and cells [17,29–33]. This technique provides a non-invasive and label-free measurement, and is found practically useful for the detection of substances in miniaturized analytical devices like microfluidic systems.

The aim of this article is to review recent developments on the impedance detection of cellular responses in micro/nano environment. Cell number and cell viability are the important characteristics during cell culture, and can be monitored by various impedance measurement techniques. Moreover, as a microfluidic system is an integrated system for multi-purposes, monitoring of metabolic activities of cells with cell stimulation is also significant for cell culture-based studies. Literature review and in-depth discussion of the impedance measurement will be presented. Microfluidic systems

incorporated with impedance measurement technique provide an effective and efficient technique for cell culture-based assays.

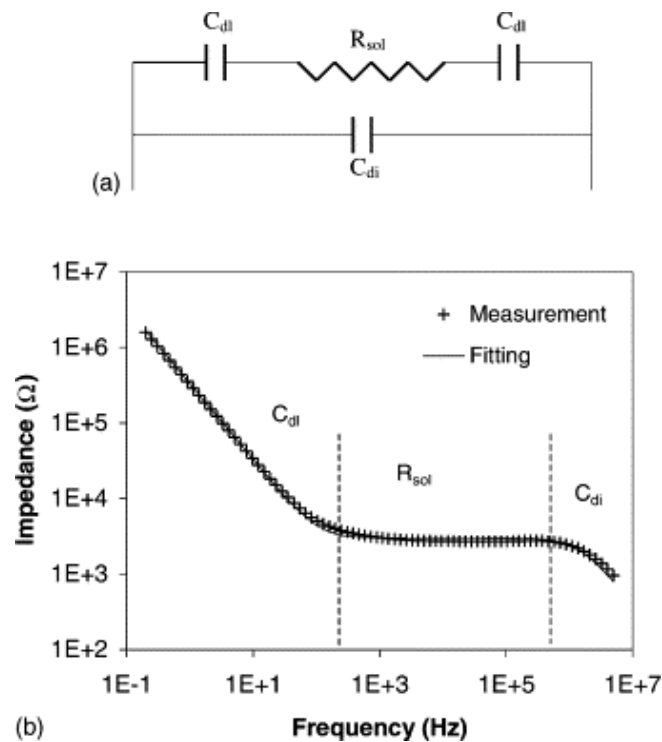
2. Electrical Equivalent Circuit

Generally, an electrical equivalent circuit is used to curve fit the experimental data for the explanation of the characteristics of the impedance detection system. A number of electrical equivalent circuits were proposed to describe the cellular detection [34]. In order to have an easier understanding, a simplified electrical equivalent circuit and its impedance spectrum were reported and are shown in Figure 1 [31]. It is generally suggested that two identical double layer capacitances at each electrode (C_{dl}) are connected to the medium resistance (R_{sol}) in series, and the dielectric capacitance of the medium (C_{di}) is introduced in parallel with these series elements. In the equivalent circuit, there are two parallel branches, which are C_{di} and $C_{dl} + R_{sol} + C_{dl}$. The impedance of each branch could be expressed with the following equations:

$$|Z_1| = \sqrt{R_{sol}^2 + \frac{1}{(\pi f C_{dl})^2}} \quad (\text{for branch } C_{dl} + R_{sol} + C_{dl}) \quad (1)$$

$$|Z_2| = \sqrt{\frac{1}{(2\pi f C_{di})^2}} \quad (\text{for branch } C_{di}) \quad (2)$$

Figure 1. (a) Electrical equivalent circuit of impedance measurement system with interdigitated electrode. (b) Typical impedance spectrum. C_{dl} is the double layer capacitance at each electrode. R_{sol} is the resistance of the medium. C_{di} is the dielectric capacitance of the medium. (Copyright 2004. Reprinted from [22] with permission from Elsevier).



At a frequency below 1 MHz, the C_{dl} is inactive and is modeled as an open circuit. Current could not pass through the branch of dielectric capacitance and the total impedance is expressed as Z_I . Both C_{dl} and R_{sol} are included in this frequency region, and they dominate at different frequencies, as shown in the impedance spectrum. At a low frequency range, the spectrum shows capacitive characteristics, which is contributed by the C_{dl} . The impedance decreases with increasing frequencies. Up to a certain frequency (depending on the electrode dimensions, and the conductivity and permittivity of the medium), the C_{dl} offer no impedance. The total impedance is contributed by the R_{sol} and is frequency-independent (resistive characteristics). When cells are present in the system, the presence of the electrically insulated cell membranes influences the C_{dl} as biological cells are very poor conductors at frequencies below 10 kHz [32]. The conductivity of the cell membrane is around 10^{-7} S/m, whereas the conductivity of the interior of a cell can be as high as 1 S/m [35]. Therefore, cell proliferation can be estimated by the total impedance at low frequency region.

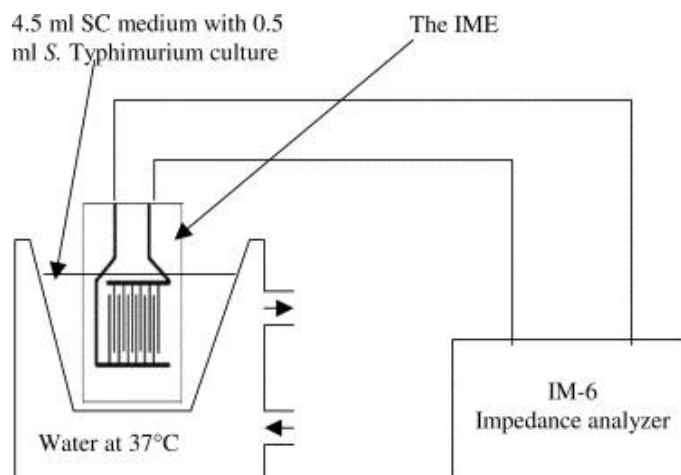
3. Monitoring of Cell Number

3.1. Detection of Cells Adhered on the Electrode Surface

If cells adhere and proliferate on the surface of the measurement electrodes, the electrode surface area is effectively reduced and the total impedance across the electrodes is, hence, increased for the detection of the presence of cells. Most of the impedance biosensors are based on this principle. A pioneer work of cellular monitoring with an applied electric field was reported in 1984 [36]. Later, impedance measurement of cell concentration, growth, and the physiological state of cells was demonstrated [32]. An interdigitated electrode was utilized to demonstrate on-line and real-time cellular monitoring. Long-term cellular behavior was clearly shown by the impedance change of the electrodes. This detection principle was also applied to detect *Salmonella typhimurium* in milk samples [31]. An interdigitated microelectrode was utilized as impedance sensors to measure the bacterial growth curve at four frequencies (10 Hz, 100 Hz, 1 kHz, and 10 kHz). Illustration of the experimental setup is shown in Figure 2. The most significant change in impedance was observed at 10 Hz. The biosensor can detect the bacterial concentration of 10^5 – 10^6 CFU/mL. Moreover, in order to detect cells specifically, antibodies are utilized to capture cells and provide selectivity to the sensor. Microelectrode array biosensors, with surface functionalization, were reported for the detection of *Escherichia coli* O157:H7 [37] and *Legionella pneumophila* [17]. The sensor surface was functionalized for bacterial detection using immobilized antibodies to create a biological sensing surface. The bacteria suspended in liquid samples were captured on the sensor surface and the impedance change was measured over a frequency range of 100 Hz–10 MHz. The sensors were able to determine cellular concentrations of 10^4 – 10^7 CFU/mL and 10^5 – 10^8 CFU/mL, respectively. Another approach was to use magnetic nanoparticle-antibody conjugates (MNAC) to capture the specific cells. A microfluidic flow cell with embedded gold interdigitated array microelectrode was developed for rapid detection of *Escherichia coli* O157:H7 in ground beef samples [38]. MNAC were used to separate and concentrate the target bacteria from the samples. The cells of *E. coli* O157:H7 inoculated in a food sample were first captured by the MNAC, separated and concentrated by applying a magnetic field, washed and suspended in solution, injected through the microfluidic flow cell, and attracted by magnetic field on the active layer for impedance

measurement. This impedance biosensor was able to detect as low as 1.6×10^2 and 1.2×10^3 cells of *E. coli* O157:H7 cells present in pure culture and ground beef samples, respectively.

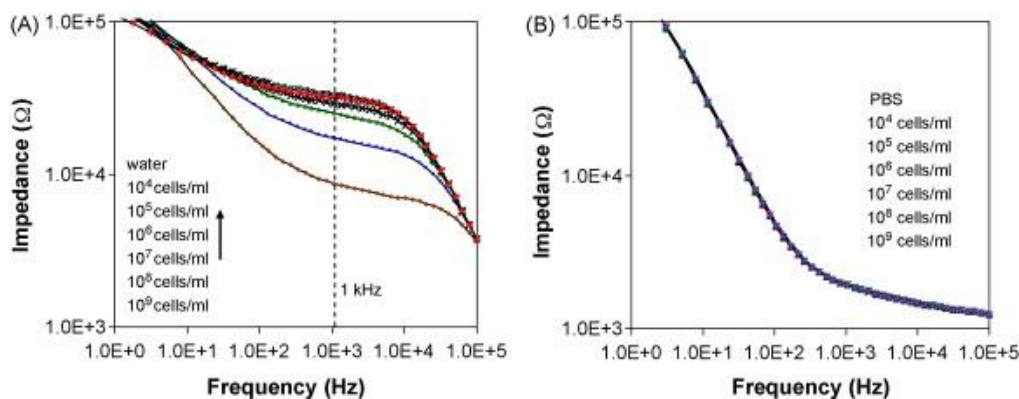
Figure 2. Experimental setup of the impedance measurement with the interdigitated electrodes for the detection of cells. (Copyright 2004. Reprinted from [22] with permission from Elsevier).



3.2. Detection of Suspended Cells

When cells suspend in the liquid buffer, impedance measurement can also be used to determine cell number in the buffer. However, the impedance spectroscopic responses are very dependent on the conductivity of the buffer used in the systems. The detection of *Salmonella* cell suspensions was demonstrated in deionized (DI) water and phosphate buffered saline (PBS), respectively [39]. It showed that bacterial cell suspensions in DI water with different concentrations can result in different electrical impedance spectral responses; conversely, cell suspensions in PBS cannot. The impedance spectra are shown in Figure 3. It was reported that the impedance of the cell suspensions in DI water decreased with the increasing cell concentration. It was suggested that the cell wall charges and the release of ions or other osmolytes from the cells caused the proportional impedance change.

Figure 3. Impedance spectra of *Salmonella* suspensions in (A) DI water and (B) PBS with the cell concentrations in the range of 10^4 to 10^9 cfu/mL, along with water and PBS as controls. Frequency range: 1 Hz–100 kHz. Amplitude: ± 50 mV. (Copyright 2008. Reprinted from [27] with permission from Elsevier).



4. Monitoring of Cellular Viability

Cell death leads to the release of cells from the surface of the measurement electrode. That induces the decrease of the impedance measured across the electrodes. Real-time evaluation of targeted tumor cells treated with a combination of targeted toxin and particular plant glycosides was demonstrated [40]. HeLa cells were seeded onto interdigitated electrode and treated with targeted toxin. The impedance was directly correlated with the cell viability and able to trace the temporal changes of cell death during treatment. The above demonstration utilized a two-electrode system (*i.e.*, interdigitated electrode) for the measurement. A three-electrode system was also demonstrated for the monitoring of cell growth with the treatment of potentially cytotoxic agents [41]. It has the advantage of better reproducibility than traditional two-electrode impedance measurement. The cell chip consisted of an eight-well cell culture chamber incorporated with a three-electrode system on each well, as shown in Figure 4. Human hepatocellular carcinoma cells (HepG2) were cultured in the chamber and toxic effects on the HepG2 cells was monitored. The impedance was decreased after treatments with several toxicants, such as tamoxifen and menadione, indicating the detachment of dead cells. Moreover, a 10×10 micro-electrode array was used to monitor the culture behavior of mammalian cancer cells and evaluate the chemosensitivity of anti-cancer drugs using impedance spectroscopy [42]. Human oesophageal cancer cells were cultured on the surface of the electrodes and then treated with anti-cancer drug. Morphology changes during cells adhesion, spreading, proliferation, and chemosensitivity effects on cells can be monitored by impedimetric analysis in a real-time and non-invasive way. Recently, commercial cell analyzers are available to monitor the cellular responses. Although they are not designed for microfluidic environment, but impedance measurement shows a promising tool for cellular analyses. Real-time detection of cell death in a neuronal cell line of immortalized hippocampal neurons (HT-22 cells), neuronal progenitor cells (NPC), and differentiated primary cortical neurons was demonstrated using the system [43]. Schematic overview of the measurement principle is shown in Figure 5. These excellent demonstrations showed that impedance measurement is a convenient and reliable technique for real-time monitoring of cellular responses.

Figure 4. (A) Configuration of the microfabricated cell chip: RE, reference electrode; WE, working electrode; CE, counter electrode. (B) Fabricated cell chip. (Copyright 2005. Reprinted from [29] with permission from Elsevier).

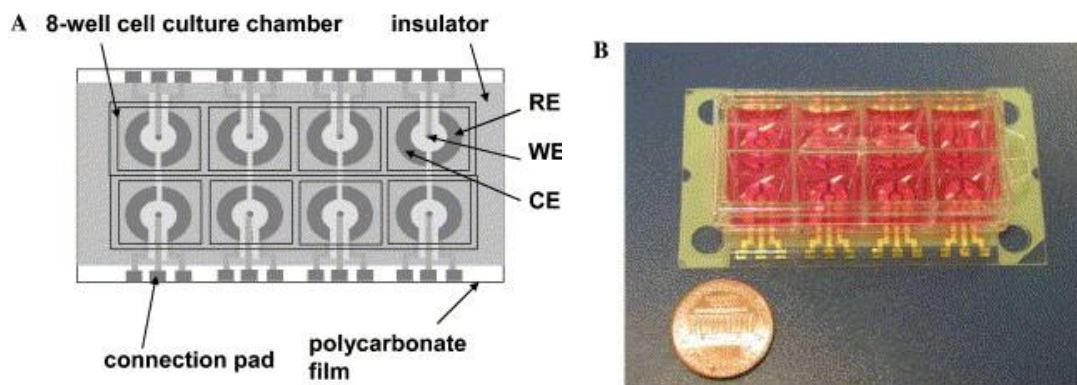
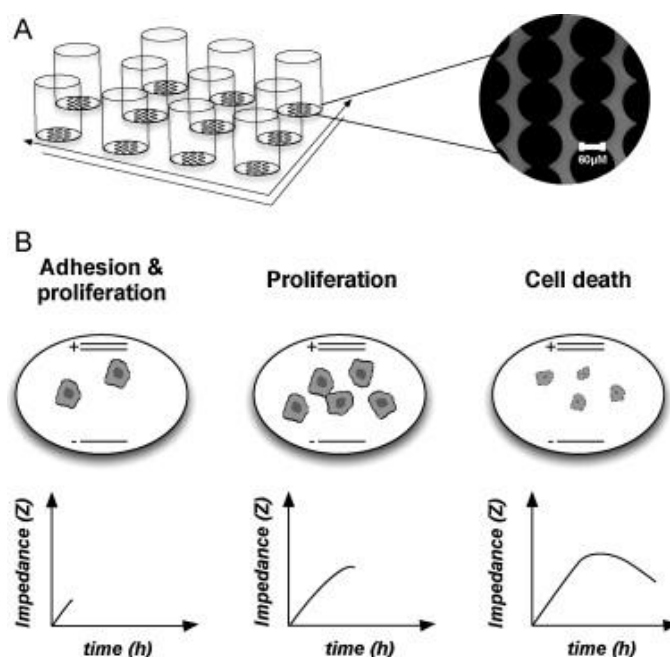


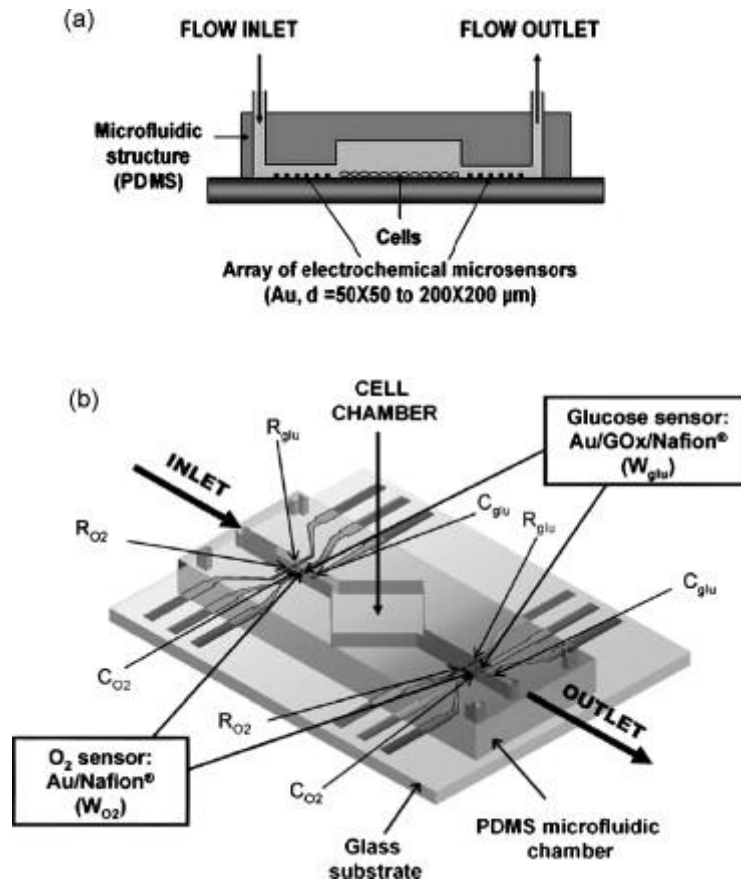
Figure 5. Schematic overview of the measurement principle of cellular impedance. (A) Each well of the culture dish features a bottom with embedded gold-electrodes. The electrode array has a minimal distance of 30 μm between the electrodes. The right picture shows an upright view of the electrode array. (B) Cells were seeded on top of the electrode-covered surface of the culture dish. After attaching to the bottom of the well, the cells partially insulate the electrodes, causing a rise in impedance. With an increasing cell density, the cells have a greater overall insulating capacity, showing in a further increase in impedance. Inflicting cellular damage and cell death causes changes in membrane morphology, cellular shrinkage, and detachment, resulting in a decrease of the cellular impedance. (Copyright 2012. Reprinted from [31] with permission from Elsevier).



5. Monitoring of the Metabolic Activity of Cells

Monitoring of the metabolic activity during cell culture is very important for the study of cell physiology. A microfluidic chamber was reported to enable the real-time measurement of extracellular lactate of single heart cell under simultaneous electrical stimulation [44]. This device is comprised of one pair of pacing microelectrodes, used for field-stimulation of the cell, and three other microelectrodes configured as an electrochemical lactate micro-biosensor. Single heart cell was stimulated at pre-determined rates and its metabolic conditions were explored under the "working" situation. Moreover, monitoring of cell medium by comparing the rates of glucose and oxygen before and after contact with cells was demonstrated [45]. Two arrays of glucose and oxygen electrochemical sensors were fabricated at the inlet and outlet microchannels of the microfluidic cell culture chip, as shown in Figure 6. Real-time monitoring of glucose and oxygen was shown and the chip was utilized to the study of transient effluxes of these species during cell culture.

Figure 6. (a) Cross-section and (b) general schematic view of the developed biochip composed of two arrays of glucose and oxygen electrochemical microsensors integrated at the inlet and outlet microchannels of a PDMS microfluidic chamber. (Copyright 2008. Reprinted from [33] with permission from Elsevier).



6. Cell Monitoring from 2D to 3D Cell Culture Format

Impedimetric cell monitoring in 2D cell culture format in microfluidic systems has been discussed and showed an effective and efficient technique for cell culture-based assays. 2D cell culture is widely adopted because of its simplicity in terms of operations and observations of cellular behavior. More recently, 3D culture format was proposed to provide a better approximation of the *in vivo* conditions in some cases [46,47]. Three-dimensional cell culture is that cells are encapsulated in a 3D polymeric scaffold material and can mimic the native cellular microenvironment since animal cells inhabit environments with very 3D features [46]. Thus, that might provide a more physiologically meaningful culture condition for cell-based assays. However, since cells are encapsulated in the scaffold, direct observation of cellular behavior cannot be practically performed. Destructive methods, such as detection of indicative cellular components and fluorescent dye staining are commonly used for the cell analysis. Alternatively, impedance measurement technique was reported to provide a real-time and non-invasive way to monitor cellular response in the 3D scaffold [33]. A microfluidic chip integrated with a pair of vertical electrodes in the 3D culture chamber was developed for quantifying cell number in the 3D scaffold. The impedance change was directly proportional to the cell number from 10^3 to 10^7 cells/mL in the 3D scaffold. This demonstration showed that the impedance measurement can be extended to

monitor cellular responses from 2D to 3D cell culture format. It is expected that more demonstrations for real-time and non-invasive cellular monitoring will be reported.

7. Conclusions

With the rapid development of impedance measurement technique, commercial cell analyzers have been launched recently to provide convenient and reliable equipment for life science research and pharmaceutical development. In this article, impedance detection of cellular response in micro/nano environment has been discussed. The microfluidic systems incorporated with impedance measurement technique provide non-invasive and label-free monitoring of cellular responses in 2D and 3D culture format. More importantly, these systems are miniaturized and automatic. A sterile and homogenous microenvironment for cell culture can be created for precise monitoring. It is believed that more cell culture-based assays will be reported using the microfluidic cell culture systems.

Acknowledgment

Author would like to thank the National Science Council, Taiwan for the financial support (project no. NSC101-2221-E-182-003-MY3).

Conflicts of Interest

The author declares no conflict of interest.

References

1. Lei, K.F. Microfluidic systems for diagnostic applications: A review. *J. Lab. Autom.* **2012**, *17*, 330–347.
2. Andersson, H.; van den Berg, A. Microfluidic devices for cellomics: A review. *Sens. Actuators B* **2003**, *92*, 315–325.
3. Zhang, C.; Xu, J.; Ma, W.; Zheng, W. PCR microfluidic devices for DNA amplification. *Biotech. Advances* **2006**, *24*, 243–284.
4. Erickson, D.; Liu, X.; Krull, U.; Li, D. Electrokinetically controlled DNA hybridization microfluidic chip enabling rapid target analysis. *Anal. Chem.* **2004**, *76*, 7269–7277.
5. Wang, L.; Li, P.C.H. Microfluidic DNA microarray analysis: A review. *Anal. Chim. Acta.* **2011**, *687*, 12–27.
6. Weng, X.; Jiang, H.; Li, D. Microfluidic DNA hybridization assays. *Microfluid. Nanofluid.* **2011**, *11*, 367–383.
7. Lei, K.F.; Cheng, H.; Choy, K.Y.; Chow, L.M.C. Electrokinetic DNA concentration in micro systems. *Sens. Actuators A* **2009**, *156*, 381–387.
8. He, Y.; Tsutsui, M.; Fan, C.; Taniguchi, M.; Kawai, T. Gate manipulation of DNA capture into nanopores. *ACS Nano.* **2011**, *5*, 8391–8397.
9. Diercks, A.H.; Ozinsky, A.; Hansen, C.L.; Spotts, J.M.; Rodriguez, D.J.; Aderem, A. A microfluidic device for multiplexed protein detection in nano-liter volumes. *Anal. Biochem.* **2009**, *386*, 30–35.

10. Lei, K.F. Quantitative electrical detection of immobilized protein using gold nanoparticles and gold enhancement on a biochip. *Meas. Sci. Technol.* **2011**, *22*, doi:10.1088/0957-0233/22/10/105802.
11. Hervas, M.; Lopez, M.A.; Escarpa, A. Electrochemical immunosensing on board microfluidic chip platforms. *TrAC Trends Anal. Chem.* **2012**, *31*, 109–128.
12. Ng, A.H.C.; Uddayasankar, U.; Wheeler, A.R. Immunoassays in microfluidic systems. *Anal. Bioanal. Chem.* **2010**, *397*, 991–1007.
13. Bhattacharyya, A.; Klapperich, C.M. Design and testing of a disposable microfluidic chemiluminescent immunoassay for disease biomarkers in human serum samples. *Biomed. Microdevices* **2007**, *9*, 245–251.
14. Van den Brink, F.T.G.; Gool, E.; Frimat, J.P.; Borner, J.; van den Berg, A.; Le Gac, S. Parallel single-cell analysis microfluidic platform. *Electrophoresis* **2011**, *32*, 3094–3100.
15. Zare, R.N.; Kim, S. Microfluidic platforms for single-cell analysis. *Annu. Rev. Biomed. Eng.* **2010**, *12*, 187–201.
16. Wu, M.H.; Huang, S.B.; Lee, G.B. Microfluidic cell culture systems for drug research. *Lab Chip* **2010**, *10*, 939–956.
17. Lei, K.F.; Leung, P.H.M. Microelectrode array biosensor for the detection of *Legionellapneumophila*. *Microelectron. Eng.* **2012**, *91*, 174–177.
18. Lei, K.F.; Wu, M.H.; Liao, P.Y.; Chen, Y.M.; Pan, T.M. Development of a micro-scale perfusion 3D cell culture biochip with an incorporated electrical impedance measurement scheme for the quantification of cell number in a 3D cell culture construct. *Microfluid. Nanofluid.* **2012**, *12*, 117–125.
19. Meyvantsson, I.; Beebe, D.J. Cell culture models in microfluidic systems. *Ann. Rev. Anal. Chem.* **2008**, *1*, 423–449.
20. Ni, M.; Tong, W.H.; Choudhury, D.; Rahim, N.A.A.; Iliescu, C.; Yu, H. Cell culture on MEMS platforms: A review. *Int. J. Mol. Sci.* **2009**, *10*, 5411–5441.
21. Hung, P.J.; Lee, P.J.; Sabounchi, P.; Aghdam, N.; Lin, R.; Lee, L.P. A novel high aspect ratio microfluidic design to provide a stable and uniform microenvironment for cell growth in a high throughput mammalian cell culture array. *Lab Chip* **2005**, *5*, 44–48.
22. Gomez-Sjoberg, R.; Leyrat, A.A.; Pirone, D.M.; Chen, C.S.; Quake, S.R. Versatile, fully automated, microfluidic cell culture system. *Anal. Chem.* **2007**, *79*, 8557–8563.
23. Saum, A.G.E.; Cumming, R.H.; Rowell, F.J. Use of substrate coated electrodes and ac impedance spectroscopy for the detection of enzyme activity. *Biosens. Bioelectron.* **1998**, *13*, 511–518.
24. Grant, S.; Davis, F.; Law, K.A.; Barton, A.C.; Collyer, S.D.; Higson, S.P.J.; Gibson, T.D. Label-free and reversible immunosensor based upon an ac impedance interrogation protocol. *Anal. Chem. Acta* **2005**, *537*, 163–168.
25. Chiriaco, M.S.; Primiceri, E.; D’Amone, E.; Ionescu, R.E.; Rinaldi, R.; Maruccio, G. EIS microfluidic chips for flow immunoassay and ultrasensitive cholera toxin detection. *Lab Chip* **2011**, *11*, 658–663.
26. Gupta, S.; Kilpatrick, P.K.; Melvin, E.; Velez, O.D. On-chip latex agglutination immunoassay readout by electrochemical impedance spectroscopy. *Lab. Chip* **2012**, *12*, 4279–4286.
27. Ma, K.S.; Zhou, H.; Zoval, J.; Madou, M. DNA hybridization detection by label free *versus* impedance amplifying label with impedance spectroscopy. *Sens. Actuators B* **2006**, *114*, 58–64.

28. Javanmard, M.; Davis, R.W. A microfluidic platform for electrical detection of DNA hybridization. *Sens. Actuators B* **2011**, *154*, 22–27.
29. Mishra, N.N.; Retterer, S.; Zieziulewicz, T.J.; Isaacson, M.; Szarowski, D.; Mousseau, D.E.; Lawrence, D.A.; Turner, J.N. On-chip micro-biosensor for the detection of human CD4⁺ cells based on AC impedance and optical analysis. *Biosens. Bioelectron.* **2005**, *21*, 696–704.
30. Krommenhoek, E.E.; Gardeniers, J.G.E.; Bomer, J.G.; van den Berg, A.; Li, X.; Ottens, M.; van der Wielen, L.A.M.; van Dedem, G.W.K.; van Leeuwen, M.; van Gulik, W.M.; *et al.* Monitoring of yeast cell concentration using a micromachined impedance sensor. *Sens. Actuators B* **2006**, *115*, 384–389.
31. Yang, L.; Li, Y.; Griffis, C.L.; Johnson, M.G. Interdigitated microelectrode (IME) impedance sensor for the detection of viable *Salmonella typhimurium*. *Biosens. Bioelectron.* **2004**, *19*, 1139–1147.
32. Ehret, R.; Baumann, W.; Brischwein, M.; Schwinde, A.; Stegbauer, K.; Wolf, B. Monitoring of cellular behavior by impedance measurements on interdigitated electrode structures. *Biosens. Bioelectron.* **1997**, *12*, 29–41.
33. Lei, K.F.; Wu, M.H.; Hsu, C.W.; Chen, Y.D. Real-time and non-invasive impedimetric monitoring of cell proliferation and chemosensitivity in a perfusion 3D cell culture microfluidic chip. *Biosens. Bioelectron.* **2014**, *51*, 16–21.
34. Varshney, M.; Li, Y. Interdigitated array microelectrodes based impedance biosensors for detection of bacterial cells. *Biosens. Bioelectron.* **2009**, *24*, 2951–2960.
35. Pethig, R.; Markx, R.H. Applications of dielectrophoresis in biotechnology. *Trends Biotechnol.* **1997**, *15*, 426–432.
36. Giaever, I.; Keese, C.R. Monitoring fibroblast behavior in tissue culture with an applied electric field. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3761–3764.
37. Radke, S.M.; Alocilja, E.C. A high density microelectrode array biosensor for detection of *E. coli* O157:H7. *Biosens. Bioelectron.* **2005**, *20*, 1662–1667.
38. Varshney, M.; Li, Y.; Srinivasan, B.; Tung, S. A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of *Escherichia coli* O157:H7 in food samples. *Sens. Actuators B* **2007**, *128*, 99–107.
39. Yang, L. Electrical impedance spectroscopy for detection of bacterial cells in suspensions using interdigitated microelectrodes. *Talanta* **2008**, *74*, 1621–1629.
40. Thakur, M.; Mergel, K.; Weng, A.; Frech, S.; Gilabert-Oriol, R.; Bachran, D.; Melzig, M.F.; Fuchs, H. Real time monitoring of the cell viability during treatment with tumor-targeted toxins and saponins using impedance measurement. *Biosens. Bioelectron.* **2012**, *35*, 503–506.
41. Yeon, J.H.; Park, J.K. Cytotoxicity test based on electrochemical impedance measurement of hepg2 cultured in microfabricated cell chip. *Anal. Biochem.* **2005**, *341*, 308–315.
42. Liu, Q.; Yu, J.; Xiao, L.; Tang, J.C.O.; Zhang, Y.; Wang, P.; Yang, M. Impedance studies of bio-behavior and chemosensitivity of cancer cells by micro-electrode arrays. *Biosens. Bioelectron.* **2009**, *24*, 1305–1310.
43. Diemert, S.; Dolga, A.M.; Tobaben, S.; Grohm, J.; Pfeifer, S.; Oexler, E.; Culmsee, C. Impedance measurement for real time detection of neuronal cell death. *J. Neurosci. Methods* **2012**, *203*, 69–77.

44. Cheng, W.; Klauke, N.; Sedgwick, H.; Smith, G.L.; Cooper, J.M. Metabolic monitoring of the electrically stimulated single heart cell within a microfluidic platform. *Lab Chip* **2006**, *6*, 1424–1431.
45. Rodrigues, N.P.; Sakai, Y.; Fujii, T. Cell-based microfluidic biochip for the electrochemical real-time monitoring of glucose and oxygen. *Sens. Actuators B* **2008**, *132*, 608–613.
46. Abbot, A. Cell culture: Biology's new dimension. *Nature* **2003**, *424*, 870–872.
47. Cukierman, E.; Pankov, R.; Stevens, D.R.; Yamada, K.M. Taking cell-matrix adhesions to the third dimension. *Science* **2001**, *294*, 1708–1712.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).