35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Non-invasive Measurement of Cell Viability in 3-Dimensional Cell Culture Construct*

Kin Fong Lei, Min-Hsien Wu, Che-Wei Hsu, Yi-Dao Chen

***Abstract*—In this work, a non-invasive measurement technique for the quantitative determination of cell viability in a three-dimensional (3D) cell culture construct is proposed. This technique is based on on-site electrical impedance measurement. A microfluidic chip with a 3D culture chamber is fabricated to demonstrate this technique. *In vitro* 3D cell culture has been interpreted for faithfully representation of the *in vivo* cellular responses in living tissues. However, monitoring of the cellular responses in 3D cell culture construct is normally time-consuming and labor-intensive. In this study, the microfluidic chip consists of a culture chamber, in which a pair of vertical electrodes at its opposite sidewalls was embedded, and a fluidic channel for drug perfusion. Cancer cells encapsulated in agarose gel were loaded into the culture chamber to perform 3D cell culture under the perfusion of culture medium and anti-cancer drug in different concentrations (6, 12, 18, and 24 μg/ml) for 2 days. Since higher drug concentration led to more cell damage or death, the total impedance magnitude of the culture construct was shown to be reasonably proportional to the anti-cancer drug concentration. Moreover, cell proliferation can be also monitored using this technique. The proposed measurement method can determine cell viability without affecting the cellular behaviors during culture. It has a high potential to develop a fast and easy measurement compared with the conventional cellular analysis techniques.**

I. Introduction

Cell culture is a widely used technique for various biological investigations. For example, it has been used to study the physiology and biochemistry of cells [1] and explore the cell response to the substance tested such as drug [2], toxin [3], or biomaterials [4]. This technique is an operation of culturing cells outside the biological tissue from which they are harvested. Conventionally, two-dimensional (2D) cell culture, which cells attach on a substrate surface in a monolayer format, is normally adopted due to its simple operation and observation. Gases can be exchanged in this semi-closed system and medium requires to be replaced periodically. Such culture setting cannot be well controlled. 2D cell culture may hinder the precise quantification of the study of cellular responses under specific culture conditions, especially for the mammalian cell culture [1]. Recently, three-dimensional (3D) cell culture has been interpreted for faithfully representation of the *in vivo* cellular behavior in living tissues [5]. Cells are encapsulated in a 3D polymeric scaffold materials and it provides a more physiologically-meaningful culture condition for cell-based assays [5, 6]. However, evaluation of the cellular response, e.g., cell number and viability, becomes difficult for the 3D cell culture setting. Conventional methods including directly counting cells microscopically, detecting the turbidity of a cell suspension optically, or indirectly quantifying cellular components such as DNA are required to sacrifice the cultured cells. It would hamper the subsequent cellular assays in the 3D construct.

Electrical impedance measurement has been proposed as an analytical method for the quantification of biological substances, such as antibodies and antigens [7-9], DNA [10], cells [11], and bacteria [12, 13]. The general working principle is to use a pair of electrodes to measure the impedance change caused by the presence of the biological substances. This technique provides a label-free, non-invasive, and real-time measurement that is found practically useful for the detection of substances in miniaturized analytical devices like microfluidic chips. For example, detection of *Legionella* bacteria was demonstrated using a pair of indium tin oxide (ITO) interdigitated microelectrodes on a glass substrate [13]. Analyte-specific antibody, i.e., anti-*Legionella* IgG, was first immobilized on the electrode surface for the capture of bacteria specifically. Then, bacterial concentration could be determined by the impedance change across the electrodes. In addition, impedance measurement was also reported the feasibility of monitoring the viability of cells in a cell culture through interdigitated electrodes [14]. In that study, the viabilities of tumor cells treated with a combination of epidermal growth factor-based targeted toxin and particular plant glycosides were assayed via impedance detections. Results revealed that the measured impedance magnitude was correlated well with the cell viability of treated tumor cells. Moreover, a commercial available system called xCELLigence system was utilized for real-time detection of neuronal cell death [15]. A good correlation between the impedance measurements and endpoint cell viability assays in hippocampal neurons (HT-22 cells) and neuronal progenitor cells (NPC) was demonstrated for detecting cell death kinetics. Overall, electrical impedance measurement has been widely used for the monitoring of the cellular responses during culture. However, most of these

*Research supported by National Science Council, Taiwan (NSC101-2221-E-182-003-MY3).

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studies were the demonstrations of cellular responses in a conventional 2D cell culture format.

In this work, a microfluidic chip is developed for performing 3D cancer cell culture and determining cell viability after perfusion of anti-cancer drug for 2 days. The chip consists of a culture chamber, in which a pair of vertical electrodes at its opposite sidewalls was embedded, and a fluidic channel for drug perfusion. In this study, cancer cells are encapsulated in agarose hydrogel scaffold and cultured in the culture chamber with continuous drug perfusion for up to 2 days. After exposure to anti-cancer drug, the viability of the cancer cells treated with different concentrations of drug was detected through impedance changes without sacrificing the cultured cells. As a whole, the proposed technique provides an effective and efficient scheme to monitor the cell viability in a 3D cell culture construct. This work is found valuable for 3D cell culture-based assays, e.g., drug or toxin testing.

II. Design and Fabrication of the Microfluidic Chip

The microfluidic chip mainly consists of a culture chamber and a fluidic channel, as shown in Figure 1. It is composed of 3 layers: an electrode layer, a culture chamber layer, and a fluidic layer. A pair of vertical electrodes (1×2×1 mm^3) and their electrical contacts to the external measurement equipment are fabricated on the electrode layer. The culture chamber layer has a rectangular opening (7×2×1 mm^3) for accommodating the cells/agarose hydrogel construct. The fluidic layer provides fluidic channels for the drug perfusion purpose. Three layers are assembled with appropriate alignment to build the chip. Such that, the vertical electrodes are located at the opposite sidewalls of the cell culture chamber. Fluidic path is located at the top layer of the cell culture chamber. Therefore, the volume of the cell culture chamber (5×2×1 mm^3) is quantitatively defined.

The fabrication process of the microfluidic chip is illustrated in Figure 2. A pair of electrodes was first printed on a glass substrate by conductive silver paste. It is used for the seed layer of subsequent electroplating process and electrical contacts to the external measurement equipment. Then, a 1 mm thick polydimethylsiloxane (PDMS) layer (Sylgard® 184, Dow Corning, USA) with a rectangular opening was bonded to the glass substrate after oxygen plasma treatment. Hence, copper electroplating with the current density of 0.2 A/cm^2 was conducted to grow copper from seed layer. Therefore, a pair of vertical electrodes located at the opposite sidewalls of the culture chamber was fabricated for impedance measurement purpose. Finally, a PDMS fluidic layer fabricated by soft lithography was bonded to the culture chamber layer.

III. Impedance Measurement of the 3D Cell Culture Construct

Human oral cancer cell (cell line: OEC-M1) was utilized to demonstrate the determination of the cell viability in 3D cell culture construct after drug perfusion. The experimental procedure is illustrated in Figure 3. Cells were encapsulated in 2.0 % (w/v) of agarose gel (low-gelling temperature agarose,

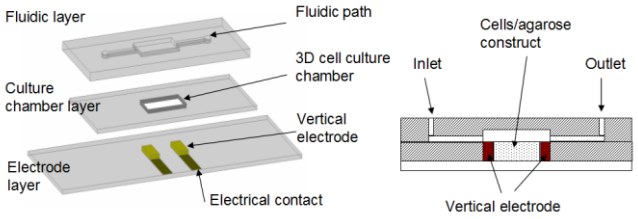

Figure 1. Design of the microfluidic chip.

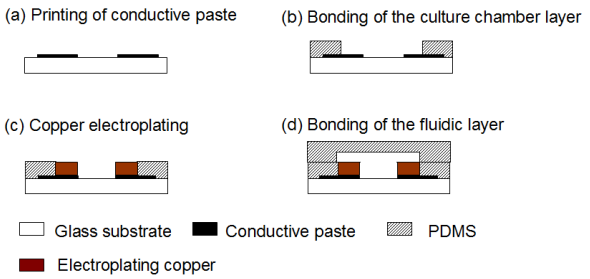

Figure 2. Fabrication process of the microfluidic chip. (a) Printing of conductive paste on glass substrate. (b) Bonding of the culture chamber layer with a rectangular opening, i.e., culture chamber. (c) Copper electroplating for building a pair of vertical electrodes at the opposite sidewalls of the culture chamber. (d) Bonding of the fluidic layer for providing drug perfusion.

Sigma, Taiwan) at a cell density of 1×10^5 cells/ml. Cells/agarose suspension was loaded into the culture chamber and spread horizontally using a glass slide for removing the redundant suspension. After gelling, the fluidic layer was assembled and the tubing of inlet and outlet was connected. A commercial syringe pump (KDS 220, KD Scientific Ltd., USA) was utilized for the drug perfusion. Culture medium (DMEM containing 1000 mg l^{-1} glucose, 25 mM HEPES, but without sodium bicarbonate; pH 7.4), supplemented with Cisplatin (i.e., anti-cancer drug) at various concentrations (6, 12, 18, and 24 μg/ml), was perfused to the cell culture chamber at flow rate of 15 μl/h for up to 2 days. Subsequently, electrical impedance of the treated 3D cell culture constructs was experimentally measured.

The proposed method aims to detect the cell viability in the 3D cell culture construct without affecting the cell culture process. In this study, the electrical impedance of the treated 3D cell culture construct was directly measured by an impedance analyzer (HP4284A) via the on-chip vertical electrodes. Briefly, potential of 0.1 V was applied and the impedance magnitude was measured from 1 kHz to 10 kHz. In order to apply a uniform electric field across the 3D construct, vertical electrodes were fabricated at the opposite sidewalls of the culture chamber in our design. Parallel electric field penetrated through the 3D cells/agarose construct and the cell viability could be estimated by the impedance change. In this study, cells were encapsulated in the agarose scaffold and assumed to be uniformly distributed. The impedance magnitude of the construct represented the total impedance

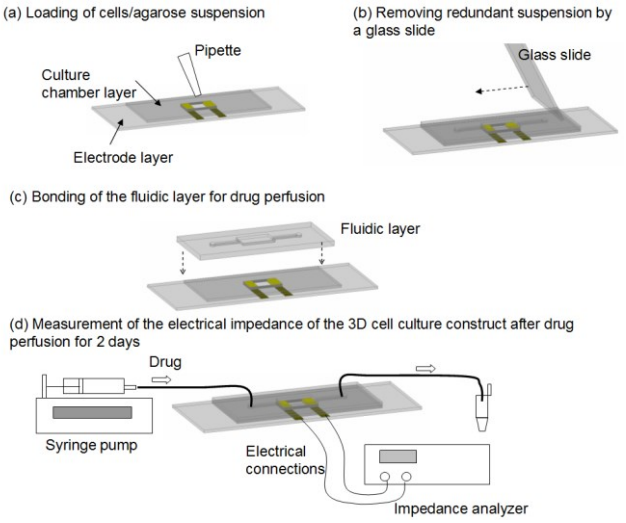

Figure 3. Experimental procedure from loading of cells/agarose suspension to the microfluidic chip to measurement of the electrical impedance of the 3D cell culture construct. The cancer cells were culture and perfused with anti-cancer drug at various concentrations for up to 2 days. Cell viability after 2 days was estimated by the electrical impedance of the 3D cell culture construct.

combining with agarose scaffold and cells, including live and dead cells. Here, the impedance of the agarose scaffold was not changed during the measurement. Therefore, the impedance change of the 3D construct was mainly dominated by the cell viability. The electric property of live and dead cells might be inherently different. This is mainly because that the integrity of the cell membrane of a dead cell might loose, which could in turn influence its electrical property.

IV. Experimental Results

Cancer cells with a cell density of 1×10^5 cells/ml were cultured in the 3D agarose scaffold and perfused with culture medium containing Cisplatin in different concentrations (6, 12, 18, and 24 μg/ml) for up to 2 days. In the process, the cytotoxic effect of Cisplatin can cause cell death. In this study, the correlation of cell viability and the total impedance of the 3D cell culture construct was investigated. Electrical impedance magnitudes of 3D cell culture constructs after treating with varied concentration of Cisplatin were respectively measured, as shown in Figure 4. The impedance spectrum shows a typical capacitive property, i.e., high impedance at low frequency and low impedance at high frequency. Such electrical property is similar to the existing 2D measurement [11-13]. Interdigitated electrodes were utilized for the 2D impedance measurement. Cells or bacteria laid on the electrode surface and blocked the current flow. Impedance magnitude was changed by the cell number on the electrode surface. In our study, cells were sitting in the agarose scaffold and treated as dopant from the viewpoint of electric characteristic. It is obvious that cells blocked the current flow and led to the change of the impedance magnitude of the construct. The impedance magnitude was proportional to the cell number in the construct. Higher drug

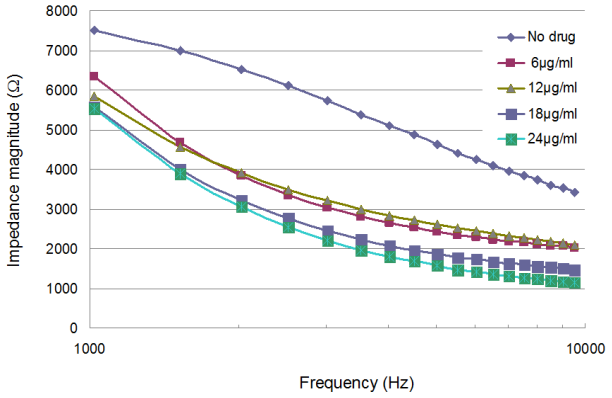

Figure 4. Impedance spectrum of 3D cell culture construct after treating with culture medium (i.e., No drug) and varied concentration of anti-cancer drug (i.e., 6, 12, 18, and 24 μg/ml) for 2 days.

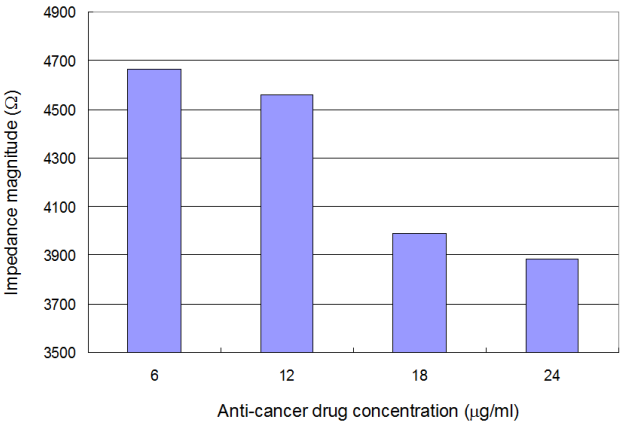

Figure 5. The correlation of anti-cancer drug concentration and the total impedance of the 3D cell culture construct at 1.5 kHz.

concentration should lead to more cell damage or death. Generally, impedance magnitude decreased with the increase of the applied drug concentration. Result shown in Figure 4 reveals this general tendency. The correlation of the anti-cancer drug concentration and the total impedance of the 3D cell culture construct at 1.5 kHz was plotted in Figure 5.

Moreover, another interesting observation is when cells were cultured without drug for 2 days. That is, cells were cultured under the perfusion of culture medium. The total impedance magnitude was measured and shown in Figure 4. Impedance magnitude was observed much higher than the others, i.e., cells were cultured with drug. It was suspended that cells grew in the 3D cell culture construct during the 2-days culture process. The total cell number was increased, leading to the increase of the impedance magnitude. That implies this impedance measurement method can monitor the cell proliferation in 3D cell culture construct. This testing was just to demonstrate the feasibility of using the impedance measurement to determine the cell viability in the 3D cell culture construct. However, it provides solid information of this proposed technique.

V. Conclusion

A non-invasive measurement technique for the quantitative determination of cell viability in 3D cell culture construct has been demonstrated. A microfluidic chip was developed and a pair of vertical electrodes at the opposite sidewalls of the culture chamber was fabricated for the impedance measurement. Cancer cells encapsulated in agarose gel were loaded into the culture chamber for 3D cell culture under the perfusion of culture medium and anti-cancer drug in different concentrations (6, 12, 18, and 24 μg/ml) for 2 days. The total impedance of the cells/agarose construct measured via the vertical electrodes could determine the cell viability in the construct. Because higher drug concentration led to more cell damage or death, impedance magnitude was shown to be reasonably proportional to the cell viability in the construct. Moreover, cell proliferation was observed based on the increase of the impedance magnitude. These preliminary results demonstrated the feasibility of using the impedance measurement to determine the cell viability in the 3D cell culture construct. The proposed technique has a high potential to develop a fast and easy measurement compared with the conventional cellular analysis techniques.

Acknowledgment

We would like to thank Prof. Tung-Ming Pan at the Department of Electronics Engineering, Chang Gung University, Taiwan for the instrumentation support.

References

[1] M.H. Wu, J.P.G. Urban, Z.F. Cui, Z. Cui, X. Xu, "Effect of extracellular pH on matrix synthesis by chondrocytes in 3D agarose gel", *Biotechnology Progress*, vol. 23, 2007, pp.430-434.

[2] M.H. Wu, Y.H. Chang, Y.T. Liu, Y.M. Chen, S.S. Wang, H.Y. Wang, C.S. Lai, T.M. Pan, "Development of high throughput microfluidic cell culture chip for perfusion 3-dimensional cell culture-based chemosensitivity assay", *Sens. Actuators B*, vol. 155, 2011, pp.397-407.

[3] Z.F. Cui, X. Xu, N. Trainor, J.T. Triffitt, J.P. Urban, U.K. Tirlapur, "Application of multiple parallel perfused microbioreactors and three-dimensional stem cell culture for toxicity testing", *Toxicol In Vitro*, vol. 21, 2007, pp.1318-1324.

[4] J.M. Anderson, "Biological responses to materials", *Annu. Rev. Mater. Res.*, vol. 31, 2001, pp.81–110.

[5] A. Abbot, "Cell culture: biology's new dimension", *Nature*, vol. 424, 2003, pp.870-872.

[6] E. Cukierman, R. Pankov, D.R. Stevens, K.M. Yamada, "Taking cell-matrix adhesions to the third dimension", *Science*, vol. 294, 2001, pp.1708-1712.

[7] S. Grant, F. Davis, K.A. Law, A.C. Barton, S.D. Collyer, S.P.J. Higson, T.D. Gibson, "Label-free and reversible immunosensor based upon an ac impedance interrogation protocol", *Anal. Chem. Acta.*, vol. 537, 2005, pp.163-168.

[8] K.F. Lei, "Electrical detection of sandwich immunoassay on indium tin oxide interdigitated electrodes", *Micro & Nano Letters*, vol. 6(3), 2011, pp.157-160.

[9] K.F. Lei, "Quantitative electrical detection of immobilized protein using gold nanoparticles and gold enhancement on a biochip", *Meas. Sci. Tech.*, vol. 22, 2011, 105802 (7pp).

[10] K.S. Ma, H. Zhou, J. Zoval, M. Madou, "DNA hybridization detection by label free versus impedance amplifying label with impedance spectroscopy", *Sens. Actuators B*, vol. 114, 2006, pp.58-64.

[11] N.N. Mishra, S. Retterer, T.J. Zieziulewicz, M. Isaacson, D. Szarowski, D.E. Mousseau, D.A. Lawrence, J.N. Turner, "On-chip micro-biosensor for the detection of human CD4+ cells based on AC impedance and optical analysis", *Biosens. Bioelectron.*, vol. 21, 2005, pp.696-704.

[12] E.E. Krommenhoek, J.G.E. Gardeniers, J.G. Bomer, A. Van den Berg, X. Li, M. Ottens, L.A.M. van der Wielen, G.W.K. van Dedem, M. Van Leeuwen, W.M. wan Gulik, J.J. Heijnen, "Monitoring of yeast cell concentration using a micromachined impedance sensor", *Sens. Actuators B*, vol. 115, 2006, pp.384-389.

[13] K.F. Lei, P.H.M. Leung, "Microelectrode array biosensor for the detection of *Legionella pneumophila*", *Microelectron. Eng.*, vol. 91, 2012, pp.174-177.

[14] M. Thakur, K. Mergel, A. Weng, S. Frech, R. Gilabert-Oriol, D. Bachran, M.F. Melzig, H. Fuchs, "Real time monitoring of the cell viability during treatment with tumor-targeted toxins and saponins using impedance measurement", *Biosens. Bioelectron.*, vol. 35, 2012, pp.503-506.

[15] S. Diemert, A.M. Dolga, S. Tobaben, J. Grohm, S. Pfeifer, E. Oexler, C. Culmsee, "Impedance measurement for real time detection of neuronal cell death", *J. Neuroscience Methods*, vol. 203, 2012, pp.69-77.