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Electrical Impedance Determination of Cancer Cell Viability in a 3-Dimensional Cell Culture Microfluidic Chip

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A technique of quantitative determination of cell viability in three-dimensional (3D) cell culture construct is proposed based on on-site electrical impedance measurement in a microfluidic chip. *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 work, a microfluidic chip was developed and is capable of on-site determining cell viability in 3D construct without affecting the cellular behaviors. The chip mainly consisted of a culture chamber, in which a pair of vertical electrodes at its opposite sidewalls was embedded for measurement, and a fluidic channel for perfusion purpose. Cancer cells/agarose suspension was loaded into the culture chamber for 3D cell culture with perfusing anti-cancer drug in different concentrations (6, 12, 18, and 24 μg ml^{-1}) 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 cell viability. The proposed technique has a high potential to develop a fast and easy measurement compared with the conventional cellular analysis techniques.

Keywords: Microfluidics; Electrical impedance; Cell culture; Cell viability; Drug testing.

1. INTRODUCTION

In physiological condition, animal cells inhabit very three-dimensional (3D) environment in tissue. In order to faithfully explore the *in vivo* cellular behaviors *in vitro*, encapsulating cells in a 3D polymeric scaffold material has been recently proposed for the cell culture [1-3]. The 3D cell culture technique is more representative of the cellular behavior in living tissues and thus, provides a more physiologically-meaningful culture condition for cellular assays. Because of the mature development

of microfluidic technology, miniaturized perfusion 3D cell culture technique was reported to further mimic the native cellular microenvironments [4-6]. Miniaturization of the 3D cell culture can eliminate the chemical gradients in the 3D culture constructs, compared to the existing bulky bioreactor systems for 3D cell culture. Also, perfusion-based culture refers to the operation of culturing cells in a closed volume with continuous nutrient supply and waste removal. That provides a more stable culture environment compared to the conventional cell culture that requires the replacement of medium periodically. More importantly, the closed cell culture device is capable of keeping the culture system sterile during the entire culture period. Consequently, it is generally believed that the miniaturized perfusion 3D cell culture can provide a precise, stable, and homogenous culture environment for cellular assays. Cell culture-based assays that study the cellular response to the tested substance such as drug [7] and toxin [8] have been demonstrated.

In general cell culture-based assays, the investigations of cell number and cell viability during culture period are commonly performed to study the cellular responses under various culture conditions explored. Quantification of cell number can provide the information of cell proliferation. Cell viability study can understand the percentage of cell death under a specific tested substance. For the detection of cell viability, it is commonly achieved directly by fluorescent dye staining and accompanied fluorescent microscopic observations [9]. Based on this, live and dead cells in a cell culture can be quantified through cell counting. Alternatively, the cell viability can also be evaluated through the indirect measurement of some indicative metabolites (e.g. lactate) that are released by the dead cells. As a whole, these methods may not be practical in a 3D cell culture model because cells encapsulated in a 3D scaffold are difficult to observe and quantify microscopically. Moreover, these operations are normally labor-intensive and time-consuming that could hamper the high throughput cell culture-based assay works like drug screening or toxin testing. To tackle the technical hurdles, this study proposed to utilize electrical impedance measurement to both effectively and efficiently detect the cell viability in a 3D cell culture system.

In the past decade, electrical impedance measurement has been proposed as an analytical method for the quantification of biological substances [10-12]. The rationale behind is to use a pair of electrodes as an electrical transducer to measure the impedance change caused by the existence 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. Reports in literature have demonstrated the use of the similar principle for the detection of various biological substances such as enzymes [13], antibodies and antigens [14-16], DNA [17], cells [18-20], and bacteria [21-25]. For example, quantification of human $CD4^+$ cells in a minimal volume of blood was demonstrated by impedance measurement using microelectrodes on a glass surface [18]. In such study, the proportional increase of impedance was measured when the number of $CD4^+$ cells captured on the microelectrodes was increased. Also, on-line and real-time monitoring of cell concentration, growth, and physiological state during a cell culture has been demonstrated through the impedance change across the interdigitated electrodes [19, 20]. The measurements can be performed for several days without detectable electrical influence on the cells. Based on this, the influence of serum components and the toxicity of heavy metal ions on the cultured cells can be quantified by the changes of the impedance signals. In addition, impedance measurement

was also reported feasible to monitor the viability of cells in a cell culture through interdigitated electrodes [26]. In that study, the viabilities of tumor cells treated with a combination an 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 polymer cell chip with interdigitated array electrodes integrated was developed for on-chip monitoring of human rhabdomyosarcoma (HR) cell viability under the exposure to cadmium (Cd) with different concentrations [27]. Furthermore, the cell analyzer based on a commercial product called xCELLigence system was reported for real-time detection of neuronal cell death [28]. 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, above promising demonstrations have proved the feasibility of utilizing impedance measurement for various cellular assays. However, most of these studies concerned the quantification of cellular responses in a conventional two-dimensional (2D) cell culture format, which is referred to the culture of cells as a monolayer on a surface of a cell culture vessel. To the best of our knowledge, the utilization of electrical impedance measurement for quantifying the viability of cells in 3D cell culture construct has not yet being explored.

In this work, a 3D cell culture-based microfluidic chip is developed for the determination of cancer cell viability under different anti-cancer drug concentrations through on-chip impedance measurement. The chip mainly consists of a culture chamber, in which a pair of vertical electrodes at its opposite sidewalls was embedded, and a fluidic channel. In this study, the 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, while the detection of cell number was demonstrated in our previous works [29, 30]. This work is found valuable for 3D cell culture-based assays, e.g., drug or toxin testing. Moreover, the presented mechanism is also promising to be integrated in a microfluidic system for the on-chip high throughput cellular analysis.

2. EXPERIMENTAL METHODS

2.1 Design of the perfusion microfluidic chip

The chip is designed for providing a steady and homogenous perfusion 3D cell culture environment and onsite monitoring of cell viability in a 3D cell construct without sacrificing the cultured cells. The microfluidic chip is composed of three layers: an electrode layer, a culture chamber layer, and a fluidic layer. The illustration of the chip is shown in Figure 1. The electrode layer consists of a pair of vertical electrodes ($1\times2\times1$ mm^3) and their electrical contacts to the external measurement equipment. The culture chamber layer has a rectangular opening ($7\times2\times1$ mm^3) for accommodating the

cells/agarose hydrogel construct. The fluidic layer provides fluidic channels for the medium perfusion purpose.

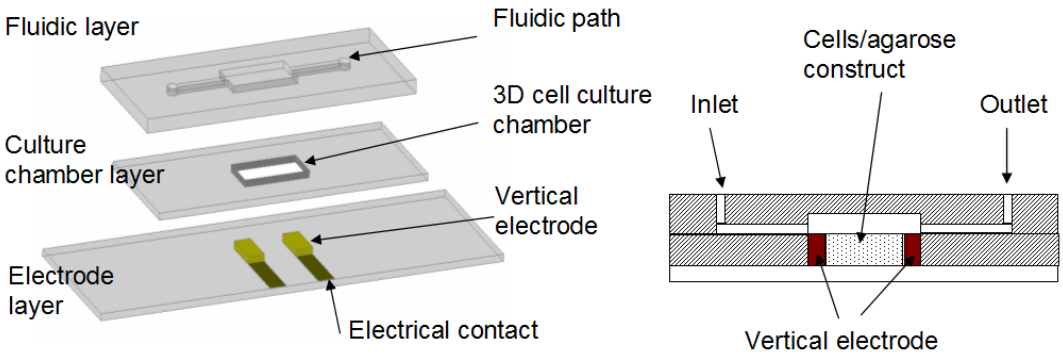

Figure 1. (Left) Illustration of the perfusion 3D cell culture microfluidic chip embedded with vertical electrodes. (Right) Cross-sectional structure of the chip.

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 cells/agarose hydrogel construct can be quantitatively loaded into the culture chamber. Culture medium containing anti-cancer drug is injected though the inlet and the cells in the 3D cell construct can be cultured under different drug concentrations. In this study, the cell viability is monitored by the measurement of electrical impedance through the vertical electrodes on-site. Parallel electric field generated by two opposite vertical electrodes penetrates through the 3D cells/agarose construct and cell viability can be correlated to the impedance changes.

2.2 Fabrication of the perfusion microfluidic chip

The microfluidic chip consists of an electrode layer, a culture chamber layer, and a fluidic layer. The substrate of the electrode layer is glass slide. The culture chamber and fluidic layers are made of polydimethylsiloxane (PDMS) material (Sylgard® 184, Dow Corning, USA) and fabricated by soft lithography. The general process of soft lithography is briefly described. PDMS mixture is first prepared by thoroughly mixing of the PDMS pre-polymer and curing agent in a weight ratio of 10:1 according to the manufacturer's instruction. Then, the mixture is degassed under a vacuum chamber, and followed by pouring onto the polymethylmethacrylate (PMMA) mold with designed structure fabricated by micro-machining technique. After curing at 100 °C for 1 h, the PDMS layer with the desirable structures is obtained through a careful de-molding process. The overall fabrication process of the microfluidic chip is illustrated in Figure 2. A pair of electrodes was first printed on a glass substrate by using conductive paste. The purpose of the electrodes was for the seed layer of

electroplating process and the electrical contacts to the external measurement equipment. Then, a 1mm thick PDMS layer with a rectangular culture chamber was bonded to the glass substrate with appropriate alignment.

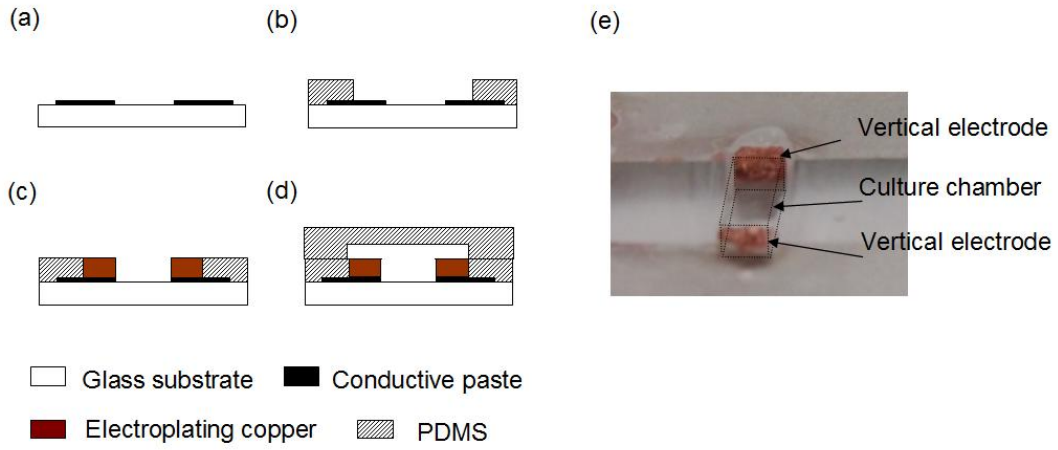

Figure 2. Fabrication process of the microfluidic chip embedded with vertical electrodes in the culture chamber. (a) Definition of the seed conductive layer on a glass substrate. (b) Bonding of the PDMS culture chamber layer with rectangular opening. (c) Copper electroplating to fabricate a pair of vertical electrodes. (d) Bonding of the PDMS fluidic layer. (e) Photograph of the on-site vertical electrodes located at opposite sidewalls of the culture chamber. The dash lines show the location of the culture chamber.

Copper electroplating with the current density of 0.2 A/cm^2 was performed for 16 h. Copper was grown from the seed layer to the upper surface of the culture chamber. 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 was bonded to the culture chamber layer. Anti-cancer drug can be perfused by an external syringe pump to the culture chamber for perfusion cell culture purpose. Cells encapsulated in a 3D agarose scaffold can be cultured in the chamber with continuous drug perfusion and their viability can be monitored by the on-site impedance measurement. A photograph of the vertical electrodes located at opposite sidewalls of the culture chamber is shown in Figure 2(e).

2.3 Perfusion 3D cancer cell culture for anti-cancer drug testing using the proposed microfluidic chip

A human oral cancer cell line (OEC-M1) cell culture was performed to demonstrate the feasibility of using the proposed system for on-line drug testing. Before loading with cancer cell/agarose suspension, the surfaces of fluidic channels, and 3D cell culture chamber were sterilized using 70% (w/v) of ethanol for 30 min. In this study, the cells were encapsulated in 2.0 % (w/v) of agarose gel (low-gelling temperature agarose; Unless otherwise stated all chemicals were purchased from Sigma, Taiwan) at a cell density of 1×10^5 cells ml^{-1}. The prepared cells/agarose suspension was

loaded into the culture chamber by manual pipetting and followed by spreading it horizontally using a glass slide to remove the redundant suspension, as illustrated in Figure 3 (a)-(b). The volume of the cells/agarose suspension was therefore well-defined for qualitative analysis. After gelling, the fluidic layer was assembled with proper alignment to build the chip (Figure 3 (c)).

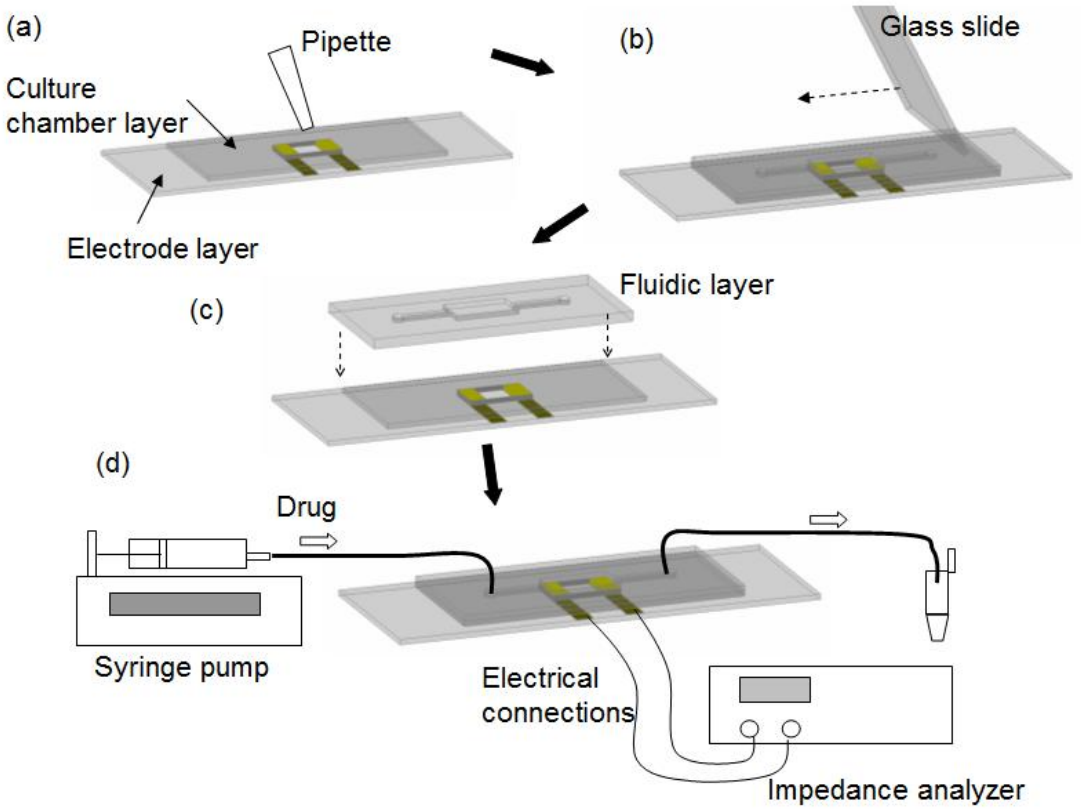

Figure 3. (a) Loading of cells/agarose suspension in the culture chamber by manual pipetting. (b) Spreading horizontally using a glass slide to remove the redundant suspension. (c) Assembly of the fluidic layer. (d) Illustration of the experimental setup for performing perfusion 3D cell culture and impedimetric cell viability monitoring.

Then, the tubing was connected to the inlet and outlet of the chip. A commercial syringe pump (KDS 220, KD Scientific Ltd., USA) was utilized for the drug perfusion (Figure 3 (d)). In the subsequent cell culture process, the 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^{-1}), was perfused to the cell culture chamber at a set flow rate of 15 μl h^{-1} for up to 2 days. After cell culture, the electrical impedance of the treated 3D cell culture constructs was experimentally measured. On the other hand, the cytotoxic effects of Cisplatin toward the OEC-M1 cells were evaluated using a lactate dehydrogenase kit (*In vitro* toxicology assay kit, lactic dehydrogenase–based). Briefly, the lactate dehydrogenase retained inside the cells and released into the culture medium due to the loss of membrane integrity was assayed respectively based

on the manufacturer's instructions. The cell viability percentage (%) is defined herein as the level of lactate dehydrogenase retained inside the cells divided by the total level of lactate dehydrogenase.

2.4 Impedance measurement of 3D cell culture construct

The proposed method aims to detect the viability of cells in a 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 500 Hz 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. Parallel electric field penetrated through the 3D cells/agarose construct and the viability of the cells in the construct 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 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.

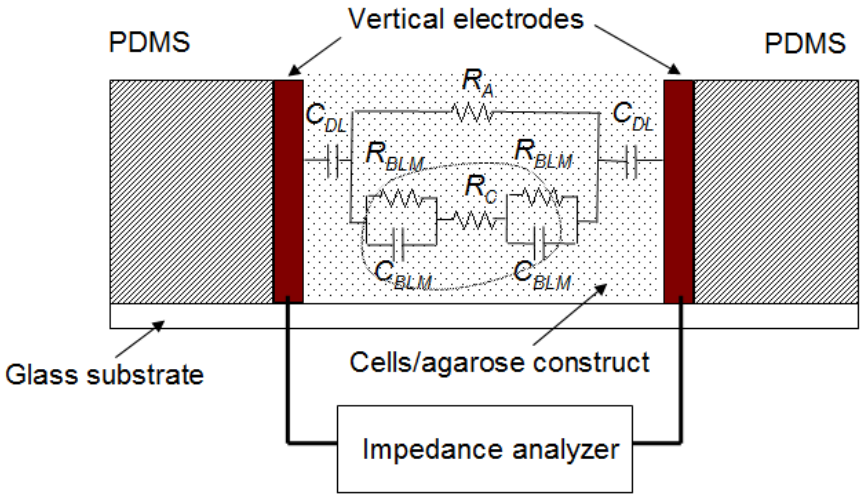

Figure 4. Equivalent circuit of the cells/agarose construct in the culture chamber. C_{DL}: double layer capacitance; R_A: total resistance of the agarose scaffold soaking in drug; C_{BLM}: capacitance of the cell membrane; R_{BLM}: resistance of the cell membrane; R_C: resistance of the cell. The electrical parameters of cells, i.e., C_{BLM}, R_{BLM}, and R_C, are assumed to be an aggregated number.

In this study, the equivalent circuit diagram of the 3D cells/agarose construct across the vertical electrodes is shown in Figure 4. The electrical cell model can be found in the literature [31, 32]. In the

figure, C_{DL} is the double layer capacitance between the electrode and the electrolyte, R_A is the resistance of agarose scaffold soaking in anti-cancer drug, C_{BLM} is the capacitance of the cell membrane, R_{BLM} is the resistance of the cell membrane, and R_C is the resistance of the cell. The electrical parameters of cells, i.e., C_{BLM}, R_{BLM}, and R_C, are assumed to be an aggregated number. In this circuit diagram, the total impedance of the cells/agarose scaffold is suggested to be interpreted as having two parallel branches. One branch represents the impedance of the agarose scaffold and another branch represents the impedance of the cells. Therefore, the cell viability can be estimated by the total impedance change of the construct.

3. RESULTS AND DISCUSSION

3.1. 3D cell culture microfluidic chip for chemosensitivity assay

Cell cultures have been widely used in life science or medicine research for various purposes. Nevertheless, the conventional static 2D monolayer cell cultures have their limitations on providing stable, well-defined, and biologically-relevant cell culture environments for more precise and physiologically-meaningful cellular assays. As a result, for example, drug testing based on conventional cell culture models might provide misleading data regarding *in vivo* response [33]. To address this issue, in this study we propose a 3D cell culture microfluidic chip, which aims (i) to provide a stable and better-defined culture environment due to the perfusion and miniaturized cell culture format [4], and (ii) to create a more biomimetic condition due to the 3D cell culture method. All these characteristic features make it a useful cell culture tool for exploring the *in vivo* cellular responses *in vitro*. In a cell culture-based assay, the observations or detections of cellular responses to the tested conditions are crucial.

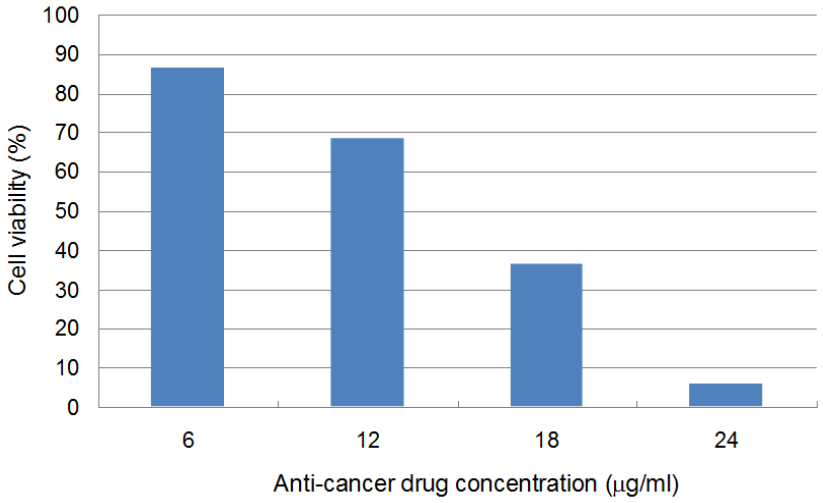

Figure 5. Evaluation of the OEC-M1 cell viability at various concentrations of anti-cancer drug (i.e., Cisplatin). The cell viability was evaluated based on the conventional lactate dehydrogenase assay.

Among various cellular responses, the viability of the cultured cells under a specific culture condition can provide us with information about toxicity, or biocompatibility. Conventional methods for evaluating cell viability are normally labor-intensive and time-consuming. More importantly, the cultured cells commonly have to be sacrificed for assays. These could hinder the use of cell culture-based assays for high throughput research works (e.g. new drug screening). The proposed 3D cell culture microfluidic chip timely addresses the issue by integrating electrical impedance measurement mechanism in a microfluidic cell culture system for on-line and non-invasive cell viability evaluation. In this study, the chemosensitivity assays of cancer cells to anti-cancer drug was demonstrated. Firstly, the viability of OEC-M1 cells treated with different concentrations of Cisplatin was evaluated using a conventional cell viability assay. Results (Figure 5) reveals that the cell viability of OEC-M1 cells decreased with an increase in the concentration of the Cisplatin, in which the viabilities of the OEC-M1 cells were evaluated to be 86.4%, 68.4%, 36.4%, and 5.8% for the drug concentration levels of 6, 12, 18, and 24 μg ml^{-1}, respectively. The obtained data were then correlated with the data from the following electrical impedance measurements to see the feasibility of using proposed method for cell viability detection.

3.2 Electrical impedance measurements for the evaluation of cell viability

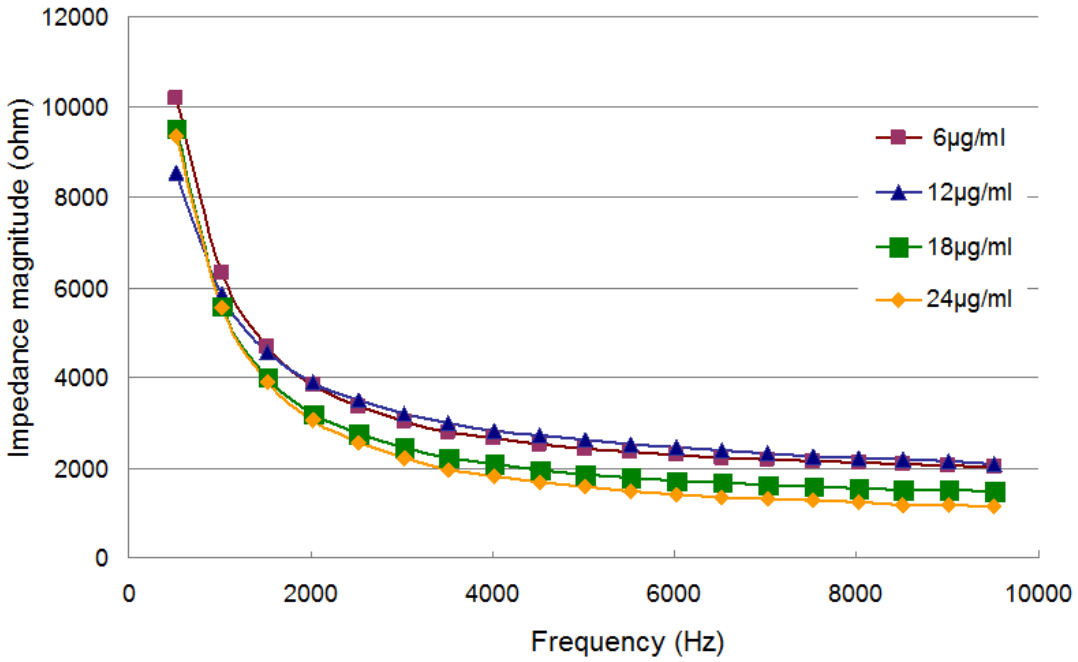

Figure 6. Impedance magnitude of the cells/agarose construct after perfusion of anti-cancer drug for 2 days. The drug in the various concentrations of 6, 12, 18, and 24 μg ml^{-1} was respectively perfused to the culture chambers up to 2 days.

The use of impedance measurement to detect cell number in the 3D cell construct has been demonstrated in our previous works [29, 30]. In the measurements, the use of low potential was not

observed to affect the cellular behaviors. The impedance magnitude showed increased with the increase of the cell number in the 3D cell culture construct. In this study, the correlation of cell viability and the total impedance of the 3D cell culture construct was investigated. OEC-M1 cells with a cell density of 1×10^5 cells ml^{-1} were cultured in the 3D agarose scaffold and perfused with culture medium containing Cisplatin in different concentrations (6, 12, 18, and 24 μg ml^{-1}) for up to 2 days. In the process, the cytotoxic effect of Cisplatin can cause cell death. In this study, the electrical impedance magnitudes of 3D cell culture constructs treated with varied concentration of Cisplatin were measured, as shown in Figure 6.

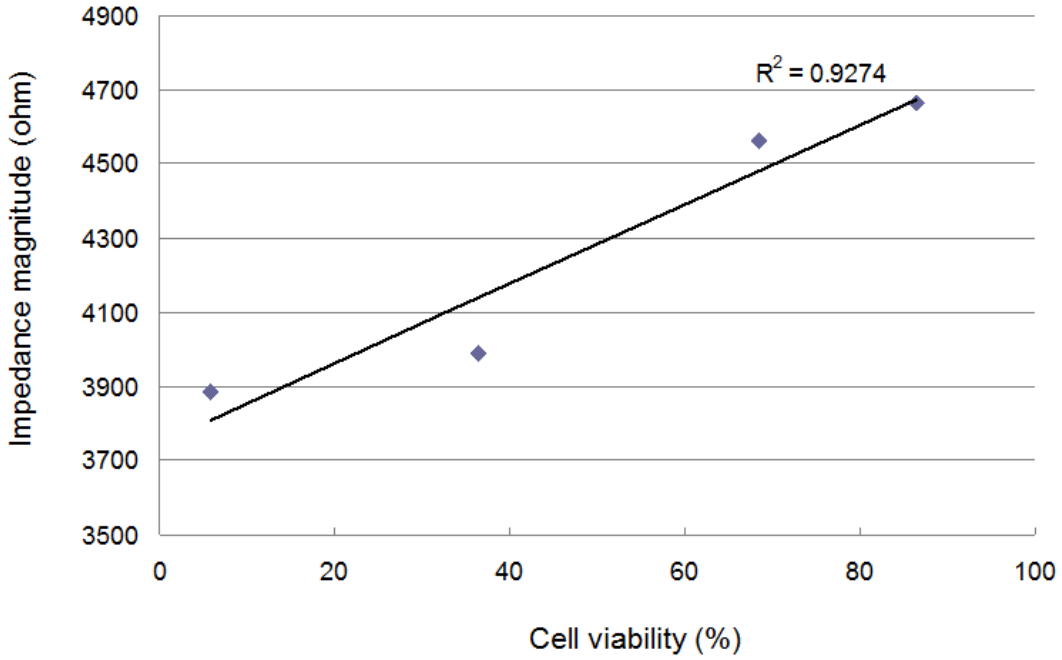

Figure 7. The correlation between cell viability and impedance magnitude of the cells/agarose construct after perfusion of anti-cancer drug for 2 days. The impedance magnitude was measured at 1500 Hz.

The impedance spectrum (Figure 6) shows a typical capacitive property. Capacitances, i.e., C_{DL} and C_{BLM}, dominated at the low frequency and resistances, i.e., R_A, R_{BLM}, and R_C, became significant because the capacitances were minimized at high frequency. Such electrical property was similar to the existing 2D measurement [18-25]. In the previous studies, 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 concentration should lead to more cell damage or death. Generally, impedance magnitude decreased with the increase of the applied drug concentration. However, two sets of our

preliminary results (i.e., drug concentrations of 6 and 12 μg ml^{-1}) did not perfectly follow the tendency. It was suspected that low cell concentration was utilized in the cells/agarose construct loaded in the culture chamber initially. After 2 days culture with perfusing drug in the concentrations of 6 and 12 μg ml^{-1} respectively, cell viability was still higher than 65% (i.e., 86.4% and 68.4%, respectively). The differentiation of such high cell viability in low cell concentration construct is still need to be further investigated. Moreover, correlation between cell viability and impedance magnitude of the cells/agarose construct after perfusion of anti-cancer drug for 2 days is shown in Figure 7. The impedance magnitude was measured at 1500 Hz. It showed that the impedance magnitude was reasonably for representing cell viability. The R-squared value was 0.9274. Further testing is needed to find out the detection resolution and sensitivity. This testing was just to demonstrate the feasibility of using the impedance measurement to determine the cell viability in the 3D cell culture construct.

4. CONCLUSIONS

A perfusion 3D cell culture microfluidic chip has been developed for the impedance determination of cell viability without affecting the cellular behaviors. The chip mainly consisted of a culture chamber, in which a pair of vertical electrodes at its opposite sidewalls was embedded, and a fluidic channel for perfusion purpose. Cells/agarose suspension was loaded into the culture chamber for 3D cell culture with perfusing anti-cancer drug in different concentrations. The total impedance of the cells/agarose construct measured via the vertical electrodes could determine the cell viability in the construct. In this study, cancer cells were cultured under the perfusion of anti-cancer drug in various concentrations (6, 12, 18, and 24 μg ml^{-1}) for 2 days. 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. 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.

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