# A PERFUSION 3D CELL CULTURE BIOCHIP WITH ON-CHIP VERTICAL ELECTRODES FOR DETECTING CELL NUMBER BY ELECTRICAL IMPEDANCE MEASUREMENT

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## ABSTRACT

A perfusion three dimensional (3D) cell culture biochip embedded with on-chip vertical electrodes has been developed and is capable of onsite detecting the cell number in the 3D cell culture construct without sacrificing the cultured cells. Conventional monitoring of the cell number in 3D cell culture construct is normally based on the detection of the indicative cellular components (e.g., DNA assay), which requires sacrificing the cultured cells. In this study, the proposed biochip provides a well-defined and homogenous perfusion 3D culture environment and onsite monitoring of the cell number in the 3D culture construct by impedance measurement.

## KEYWORDS

Biochip, vertical electrode, cell culture, cell number, impedance measurement.

## INTRODUCTION

In the past decade, microfluidic systems or micro total analysis systems are rapidly developed and their study becomes a new interdisciplinary field. The main reason is because the micro-fabrication technique becomes mature. For example, soft lithography is capable of producing structures at micrometer scale inexpensively and rapidly [1]. Microfluidic systems can manipulate tiny quantities of liquids and perform biological assays on single chip [2]. Because of their miniaturization and automation, there are a number of advantages such as less sample/reagent consumption, reduced risk of contamination, less cost per analysis, lower power consumption, enhanced sensitivity and specificity, and higher reliability.

In the study of cell biology, cells are conventionally cultured as a monolayer on a surface of a cell culture vessel, which is referred to two dimensional (2D) cell culture. This technique is widely used because of easy operation and observation. However, because cells inhabit environments with very specific 3D features in animal tissue, *in vitro* 3D cell culture has recently been interpreted for faithfully representation of the *in vivo* cellular behavior in living tissues [3]. Cells are encapsulated in a 3D polymeric scaffold material for the culture, providing a more physiologically-meaningful culture condition for cell-based assays [3]. To study the cell proliferation in 3D culture construct, the commonly used analytical technique is based on the measurement of cellular components such as DNA [4]. The cultured cells need to be lysed in order to release the DNA molecules; as a result, this technique hampers the use of the cultured cells for further cellular assays.

In order to determine the cell number without sacrificing the cultured cells, the use of electrical impedance has been proposed in the 2D cell culture [5]. The rationale behind is to utilize a pair of electrodes to detect the impedance change of the biological substance. For example, the detection of *Legionella* bacteria was demonstrated using a pair of indium tin oxide (ITO) interdigitated microelectrodes on glass substrate [6]. Analyte-specific antibody, i.e., anti-*Legionella* IgG, was first immobilized on the electrode surface for capture of bacteria specifically. Therefore, bacterial concentration could be determined by the impedance change across the electrodes. Moreover, a miniaturized impedance imaging system was proposed for the observation of cell growth [7]. Cell migration and stratification can be monitored by the generated 2D images. Although reports in literature have demonstrated the use of impedance measurement of cell number, most of these studies were for the 2D cell culture format. In this study, a biochip is developed and provides a well-defined and homogenous perfusion 3D cell culture environment and onsite determination of cell number in the 3D cell culture construct without sacrificing the cultured cells. The proposed biochip consists of a culture chamber with on-chip vertical electrodes embedded at opposite sidewalls of the chamber. The measurement of cell number is based on the impedance change of the construct. This method suggests a fast and easy measurement and has the potential to be a total solution of 3D cellular assays.

## DESIGN AND FABRICATION OF THE BIOCHIP

The proposed biochip consists of a glass electrode layer, a polydimethylsiloxance (PDMS) chamber layer and a PDMS fluidic layer. The fabrication process of the biochip is illustrated in Figure 1. A pair of electrodes was first printed on a glass substrate by using conductive paste. The electrodes were used for the seed layer of electroplating process and the electrical contacts to the external measurement equipment. Then, a 1mm thick PDMS chamber layer with a rectangular opening worked as a culture chamber was bonded to the glass substrate with appropriate alignment. Copper electroplating with current density of 0.2 A/cm$^2$ was performed for 16 hours. Copper was grown from the seed layer to the upper surface of the culture chamber. Therefore, a pair of vertical electrodes (L×W×H: 1×2×1 mm$^3$) located at the opposite sidewalls of the culture chamber (L×W×H: 5×2×1 mm$^3$) was fabricated. Finally, a PDMS fluidic layer fabricated by soft lithography was bonded to the chamber layer for medium perfusion purpose. Finally, the proposed biochip was fabricated. A photo of the vertical electrodes located at opposite sidewalls of the culture chamber is shown in Figure 1(e).



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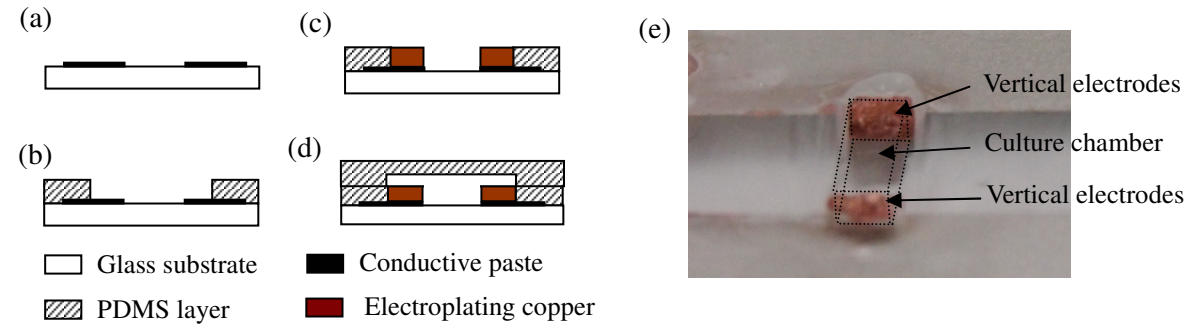


Figure 1. Fabrication of the biochip. (a) Definition of the seed conductive layer on a glass substrate. (b) Bonding of the PDMS layer to form the culture chamber. (c) Fabrication of the vertical electrodes by copper electroplating. (d) Bonding of the fluidic layer. (e) Photograph of the biochip embedded with on-chip vertical electrodes located at two opposite sidewalls of the culture chamber.

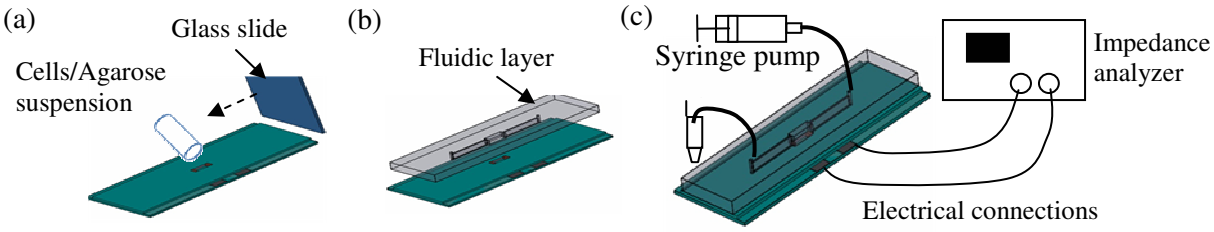


Figure 2. Experimental process of perfusion 3D cell culture and cell monitoring. (a) Loading of cells/agarose suspension in the culture chamber and followed by spreading horizontally using a glass slide to remove the redundant suspension. (b) Bonding of the fluidic layer. (c) Connection of fluidic paths for medium perfusion and electrical connections for impedance measurement.

## EXPERIMENT

The proposed biochip provides a well-defined and homogenous perfusion 3D cell culture environment and onsite determination of cell number in the 3D cell culture construct without sacrificing the cultured cells. In this study, breast cancer cell (cell line: OECM1) was utilized to demonstrate the feasibility of using the proposed biochip. The cells were encapsulated in 2.0% (w/v) agarose gel (low-gelling temperature agarose, Sigma, Taiwan) to perform the 3D cell culture. The experimental process is schematically illustrated in Figure 2. Before the experiment, the biochip was sterilized using 70% (w/v) ethanol for 6 hours. To load the cells/agarose suspension to the culture chamber, a certain amount of the prepared suspension was pipetted to the culture chamber and followed by spreading it horizontally using a glass slide to remove the redundant suspension. Therefore, volume of such sample can be quantitatively defined by this procedure. After gelling, the fluidic layer was assembled with proper alignment to build the biochip. Fluidic tubing was connected to the inlet and outlet of the biochip. A commercial syringe pump (KDS 220, KD Scientific Ltd., USA) was utilized for the medium perfusion. Medium (DMEM with 1000mg/l glucose, 25mM HEPES, without sodium bicarbonate; pH 7.5) supplemented with 10% of fetal bovine serum (FBS) (Invitrogen, Taiwan) and 2% antibiotic/antimycotic solution was perfused to the cell culture chamber at the flow rate of 10 μl/h. Such miniaturized culture chamber can eliminate the chemical gradients existing in the 3D culture construct and the perfusion setting provides continuous nutrient supply and waste removal [8]. After 40 minutes perfusion cell culture, impedance measurement was performed by an impedance analyzer (HP 4192A). Potential of 0.1 V was applied across the vertical electrodes and the impedance magnitude was measured from 5 kHz to 100 kHz. Parallel electrical field penetrated through the cells/agarose construct and the cell number in the construct can be estimated by the impedance change.

## RESULTS

Impedimetric spectroscopy of the cells/agarose construct is shown in Figure 3 and was conducted with the cells in $10^4$ cells/ml. From the figure, the response at frequency above 50 kHz shows resistive phenomenon, i.e., the magnitude shows relatively constant across the measuring frequencies. That indicates the response was dominated by the resistances contributed from agarose scaffold, medium, and cells. The capacitances from double layer between electrode and the electrolyte and cell membrane were minimized. At frequency below 50 kHz, the response showed a combination of resistive and capacitive effects. From the previous reports of 2D measurement, interdigitated electrodes were utilized for the detection of cells or bacteria laid on the electrode surface [9, 10]. Impedance behavior was affected by blocking of the applied electric field. In the current study of 3D measurement, a pair of vertical electrodes was used for the detection of cells suspended in the agarose scaffold. Cells were sitting in the agarose scaffold and treated as dopant from the viewpoint of electric characteristic. It is obvious that the increase of resistances from cells is due to the increase of cell number. Consequently, the total impedance measured at frequencies above 50 kHz is suggested to be suitable for the estimation of cell number in 3D culture construct.

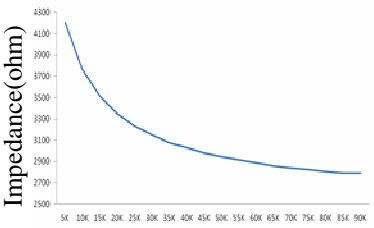


Figure 3. Impedimetric spectroscopy of 3D cell culture construct. The cell number of $10^4$ cells/ml was utilized.

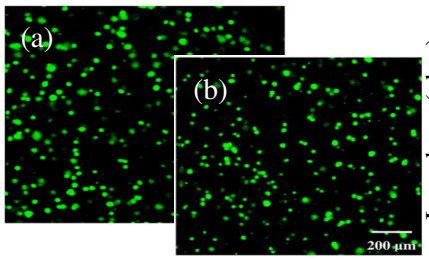


Figure 4. Fluorescent microscopic observation of cell viability (a) before and (b) after measurement. The green and red (not found) dots represent live and dead cells, respectively.

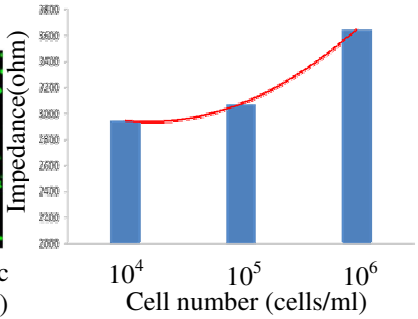


Figure 5. Impedance magnitude of the cell number in the 3D construct.

Impedance measurement is achieved by the application of an electric field across the sample under a certain frequency. In our study, cells/agarose construct was measured under a potential of 0.1 V. The electric field used to conduct the impedance measurement was found no harm to the cells. The cultured cells kept cell viability as high as 94-97%. This can be observed from Figure 4 that most of the cells were live (green dots). Therefore, breast cancer cells in different cell number of $10^4$, $10^5$, and $10^6$ cells/ml were encapsulated in the agarose gel for the impedance measurement. The cells/agarose suspensions were loaded in the culture chamber respectively. Medium was applied to the culture chamber for 40 minutes before the measurement. The impedance magnitudes of the cell number in the construct measured at the frequency of 50 kHz is shown in Figure 5. The magnitude was directly proportional to the cell number in the construct. The result indicates the cell number in the 3D culture construct can be estimated by the impedance magnitude.

## CONCLUSION

A biochip for perfusion 3D cell culture and onsite detection of cell number has been demonstrated. The proposed biochip mainly consists of a culture chamber embedded with a pair of vertical electrodes located at opposite sidewalls of it and a fluidic channel for medium perfusion. A well-defined and homogenous perfusion 3D cell culture environment and onsite impedimetric detection of cell number in the 3D cell culture construct are provided. Generally, the impedance magnitude of the construct was proportional to the cell number in the construct. By analyzing the impedimetric spectroscopy, it is suggested that the measurement is suitable in the frequency above 50 kHz. The proposed biochip provides a physiologically-meaningful culture environment and a fast and easy measurement technique. Therefore, it is potentially to develop a total solution of 3D cell culture based assays with the capability of on-chip cell analysis.

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