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Impedimetric quantification of cells encapsulated in hydrogel cultured in a paper-based microchamber

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ABSTRACT

Recently, 3D cell culture technique was proposed to provide a more physiologically-meaningful environment for cell-based assays. With the development of microfluidics technology, cellular response can be quantified by impedance measurement technique in a real-time and non-invasive manner. However, handling of these microfluidic systems requires a trained engineering personnel and the operation is not compatible to traditional biological research laboratories. In this work, we incorporated the impedance measurement technique to paper-based 3D cell culture model and demonstrated non-invasive quantification of cells encapsulated in hydrogel during the culture course. A cellulose filter paper was patterned with an array of circular microchambers. Cells were encapsulated in hydrogel and loaded to the microchambers for culturing cells in 3D environment. At the preset schedule during the culture course, the paper was placed on a glass substrate with measurement electrodes for the impedance measurement. Cells in each microchamber was represented by impedance magnitude and cell proliferation could be studied over time. Also, conventional bio-assay was performed to further confirm the feasibility of the impedimetric quantification of cells encapsulated in hydrogel cultured in the paper-based microchamber. This technique provides a convenient, fast, and non-invasive approach to monitor cells cultured in 3D environment. It has potential to be developed for routine 3D cell culture protocol in biological research laboratories.

1. Introduction

Cell culture is a basic and essential technique for most of the studies including investigation of physiology and biochemistry of cells [1–3] and exploration of cellular responses to the tested substances [4–6]. In conventional cell culture practices, cells are attached and cultured on a flat surface, e.g., Petri dish or multi-well microplate, in a monolayer format. It is referred to 2 dimensional (2D) cell culture technique. Most recently, biologists are increasingly turning to 3 dimensional (3D) cell culture because they discovered patterns of gene expression and other biological activities more closely mirror to living organisms [7–9]. In mammalian tissues, cells are supported by extracellular matrix (ECM) containing various proteins. These proteins can help to organize communication between cells embedded within the matrix. As a result, 3D cell culture model provides a more physiologically-meaningful culture condition for cell-based assays compared to 2D cell culture model. One of the strong evidences was shown by differences in phenotype when cells are cultured in 2D and 3D environments. Cells with inhibitory β1-integrin antibody completely changed the behavior of cancerous breast cells grown in 3D culture condition [10]. They became non-cancerous and losing their abnormal shapes and growth patterns. That had never been observed in 2D culture condition. Moreover, chondrocytes lost their phenotypic natures to synthesize surrounding ECM when they were cultured in 2D environment, whereas the phenotype can be restored when transferred to 3D environment [11]. Hence, it is generally believed that 3D cell cultures provide a more physiologically-meaningful culture technique for cell-based assays and bridge the gap between *in vitro* cell culture models to living organisms [12].

In vitro 3D cell culture can be realized by culturing cells encapsulated in polymeric scaffold materials, e.g., agarose hydrogel, in standard multi-well microplate. Because cells are suspended in a volumetric material, chemical gradient in the culture construct may be induced for the test of cell chemosensitivity. Such that, miniaturization of the 3D cell culture construct was proposed to

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tackle this problem [13]. It has been proved that chemical gradient could be greatly eliminated in the miniaturized 3D culture construct. With recent mature development of microfluidics technology, extensive demonstrations have been reported to realize various biomedical applications [14–18]. Microfluidic systems have been also demonstrated on the drug screening applications in the 3D cell culture model [19–21]. Moreover, impedimetric monitoring of proliferation and chemosensitivity of cells encapsulated in 3D hydrogel was realized to provide real-time information during the culture course [22,23]. These microfluidic systems provide well-defined and homogenous 3D culture environment for precise study of the relationship between cellular responses and the extracellular stimulations. However, handling of these microfluidic systems requires a trained engineering personnel and the operation is not compatible to traditional biological research laboratories. Most recently, the concept of paper-based microfluidics was proposed for a new class of analytical devices for remote environment [24,25]. The paper-based microfluidic devices were realized by patterning paper with hydrophobic barriers [26–29]. Aqueous solution could be retained in paper and bounded by hydrophobic barriers. Various kinds of bio-assays were demonstrated to show the advantages of lightweight, ease-of-use, and low cost [30–33]. In addition, because cellulose filter papers are cotton linters with reticulated structure, they are treated as a scaffold to maintain a 3D space with well-defined dimension. Cells encapsulated in hydrogel can be applied to such a 3D space for 3D culture. Therefore, cells/hydrogel construct is contained in a well-defined 3D environment and performed further assays. Paper-supported 3D cell culture was reported to study cellular responses to molecular gradients and to mimic tissue- and organ-level functions [34,35]. Cells were encapsulated in hydrogel and cultured in papers. Oxygen and nutrient gradients in 3D environment could be controlled by stacking layers of paper. Also, molecular and genetic responses of cells in each layer could be analyzed by destacking. Based on this work, various investigations of cellular responses and cell-based assays were reported [36–39]. Paper-based 3D cell culture model inherits the advantages of lightweight, ease-of-use, and low cost. Also, it is compatible to the facilities and skillsets in traditional biological laboratories. In these excellent demonstrations of paper-based microfluidics, quantification of cells was still based on cell staining or bio-assays that require to sacrifice the cultured cells. These quantification methods are end-point assays and thus hamper the subsequent observation of cell activities.

In this work, impedimetric quantification of cells encapsulated in hydrogel was developed for paper-based 3D cell culture model. Impedance measurement technique was widely used for the cell monitoring and is generally believed that it can provide a real-time, non-invasive, and label-free approach [22,23,40–44]. We incorporated the impedance measurement technique to paper-based 3D cell culture and demonstrated non-invasive quantification of cells encapsulated in hydrogel during the culture course. The paper-based 3D cell culture platform was a cellulose filter paper with an array of circular microchambers for culturing cells in 3D environment. Cancer cells were encapsulated in hydrogel and cells/hydrogel suspension was then pipetted to the paper-based microchamber. After gelling, the entire paper was soaked in culture medium and placed in an incubator. At the preset schedule during the culture course, the paper was taken out from the incubator and placed on a glass substrate with coplanar measurement electrodes for the impedimetric measurement. Because the filter paper was thin (~200 μm), the coplanar measurement electrodes were able to generate effective electric field at the optimized frequency to penetrate most of the volume of the 3D culture construct for the measurement. Cells in each microchamber was represented by the impedance magnitude at the measurement frequency of 100 Hz. Correlation between the impedance measurement and the conventional bio-assay was studied to show the appropriate measurement range. Moreover, impedimetric quantification of cell proliferation was performed during the culture course of 3 days. Results revealed that cell proliferation could be quantified by impedance magnitude directly. Also, conventional bio-assay was conducted to further confirm the feasibility of the impedimetric quantification of cells encapsulated in hydrogel cultured in the paper-based microchamber. As a whole, this technique provides a convenient, fast, and non-invasive approach to monitor cells cultured in 3D environment. It has potential to be developed for routine 3D cell culture protocol in biological research laboratories.

2. Materials and methods

2.1. Cell culture

Cancer cells (Cell line: TW06; nasopharyngeal carcinoma (NPC)-derived cell line) kindly provided by Dr. Jenny Liu at Chang Gung University were used throughout this study. Culture medium was Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco-RBL Life Technologies, USA) and antibiotic/antimycotic (100 U/mL of penicillin G sodium, 100 mg/mL of streptomycin, and 0.25 mg/mL of amphotericin B; Gibco-BRL Life Technologies, USA). Cells were cultured on standard polystyrene culture dish and amplified to confluence. Then, cells were trypsinized using 0.05% trypsin for 3 min, centrifuged at 1000 rpm for 5 min, and resuspended in the medium for further experiments.

2.2. Fabrication of the paper-based microchambers

Cellulose filter paper (Thickness: ~200 μm; Model: Grade 4; Whatman, USA) was used and patterned with 10 microchambers for culturing cells in 3D environment. Circular patterns in the diameter of 6 mm were printed on the filter paper by a solid wax printer (Model: ColorQube 8570N; Xerox, Japan). Wax was printed on the surface of the filter paper and subsequently melted by placing the paper on a hot plate at 100 °C for 10 min. Then, melted wax was permeated through the paper. After cooling, the wax was solidified in the paper and became a hydrophobic “wall”. Therefore, cylindrical microchambers with the diameter of 6 mm and the height of 200 μm were fabricated in the paper. Aqueous solution could be trapped in the microchambers. The filter paper with microchambers can be used immediately or stored at room temperature for later use.

2.3. Impedimetric quantification of cell proliferation in the paper-based microchamber

The methodology of the impedimetric quantification of cell proliferation in the paper-based microchamber was illustrated in Fig. 1. Cellulose filter paper is composed of cotton linters with reticulated structures and is suitable for the use of a scaffold to maintain a 3D space. Before cell culture, the paper-based substrate with microchambers was sterilized by ultraviolet light overnight. Then, cells were encapsulated in 1.0% (w/v) agarose hydrogel (Sigma, USA) and 20 μL cells/hydrogel suspension was then pipetted to the microchambers of the paper-based substrate. The suspension was permeated through the volume of the microchambers. After gelling, cells/hydrogel construct was formed and confined by the microchamber with well-defined volume. Subsequently, the entire paper was soaked in culture medium and placed in an incubator (Model: 370; Thermoscientific, USA) for