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MICROFLUIDIC CELL CULTURE SYSTEMS AND CELLULAR ANALYSIS

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Abstract - **Microfluidic systems have significant implications for the fields of cell biology and cell-based assay as they enable conventional assays to be conducted using an automated and high-throughput approach. Unlike conventional in vitro cell culture methods, microfluidics can provide small and complex structures mimicking the in vivo environment of cells. Recent research has shown that microfluidic cell culture systems convey more reliable results due to their ability to grow cells as biological systems do, and because they outperform those from conventional cell cultures and assay systems. According to a variety of cell types, different microfluidic platforms have been reported. The performance of microfluidic devices highly depends on the inner structure providing cells with the in vivo-like environment to cells. In this paper, the microfluidic cell culture systems developed are reviewed and categorized according to their cell type and assay development. Potential applications of current microfluidic cell culture systems are also addressed.**

Keywords - **Microfluidics, Microfabrication, Cell culture platform, Cell-based assay.**

I. INTRODUCTION

Cell culture is a key step in cell biology, tissue engineering, biomedical engineering, and pharmacokinetics for drug development[1]. In vitro cell culture methods make it possible not only to culture various cells in large amounts, but also to alternate current animal tests for drug screening[2]. Although the in vitro cell culture technique is widely used in conventional laboratory experiments, it is doubted by some that cells grown in vitro are identical to cells grown in vivo as in vitro methods provide a static and macroscale environment that is entirely different from the environments of biological systems. Living organisms have more complex and well organized two- or three-dimensional microscale systems composed of multilayers, membranes, protein channels, and many other elements. Within these structures, cells grow through interactions and communication with other cells.

Microfluidic technology can be used to supply and transfer media, buffers, and even air while the waste products by cellular activities are drained in a way resembling the human circulatory system[3]. In addition, many studies have focused on analytical microsystems that are integrated into a microfluidic platform that carries out sample mixing, buffer exchange, as well as cell seeding, transferring and separation in a microchannel. Therefore, microfluidic systems can provide an in vivo-like environment for a cell culture as well as a reaction environment for a cell-based assay. Previously, a simple twodimensional microstructure for a cell culture was widely used to construct a microfluidic cell culture system. However, as microfluidic devices have become sophisticated in an effort to realize a perfect in vivo environment on a chip, they have been adapted for use with three-dimensional microstructures and polymer scaffolds8, ensuring multiple layers for co-cultures or three-dimensional cell cultivation.

In this paper, a variety of methods for fabricating microfluidic cell culture devices are introduced, and the developed microfluidic systems are described according to their cell type and target analytes. Furthermore, a suggestion concerning in vivo-like microenvironment construction in a microfluidic cell culture system is addressed.

II. MICROTECHNOLOGIES IN BIOLOGICAL APPLICATION

Microfluidics refers to the devices, systems, and methods for the manipulation of fluid flows with characteristic length scales in the micrometer range. Microfluidic devices are especially suitable for biological applications, particularly on the cellular level, because the scale of channels corresponds with that of cells and the scale of the devices allows important factors to accumulate locally, forming a stable microenvironment for cell cultures. Compared with traditional culture tools, microfluidic platforms provide much greater control over the cell microenvironment and a rapid optimization of media composition using relatively small numbers of cells. Given that a group of cells can more easily maintain a local microenvironment within a microchannel than in a macroscale culture flasks, cells in microchannels grow significantly slower than they would in a traditional culture flask[3]. Microtechnologies including microfabrication and microfluidics continuously provide practicable opportunities in cell biology with the development of biocompatible materials and other supplement tools for cell cultures and cellular analysis with high-throughput screening (HTS).

In this paper, the microfluidic cell culture platform is described according to categorized cell culture methods (Figure 1). In addition, major results about cell culture platform are presented.

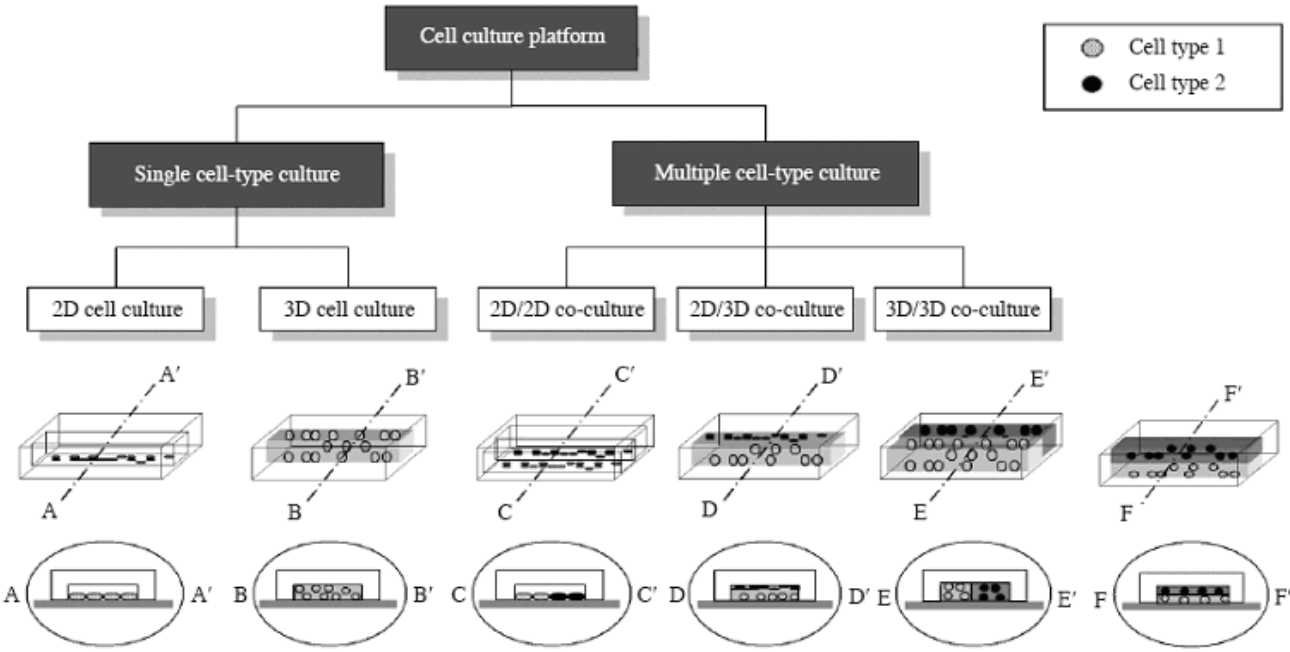

Fig. 1. Classification of a microfluidic cell culture platform.

2-dimensional cell culture. Mammalian cell culture has played a fundamental role in the development of biotechnology, including drug screening procedures and the large scale production of proteins. Most in vitro experiments with adherent human cells are performed using a two-dimensional platform in which cells are plated onto a surface-treated plastic plate to stimulate cell binding. A two-dimensional culture method makes it easy to control a single well-defined cell type, and simplifies the manipulation of large quantities of cells, the direct detection of the cellular behavior using fluorescence detection method, as well as the repeated acquiring of this after cellular analysis[5]. For this reason, most microfluidic cell culture systems adapt a two-dimensional culture method.

There are several examples of two-dimensional culture platform that use the cell patterning, laminar flow, and complex structure of PDMS. Patterning techniques based on photo- and soft-lithography have been widely used to modify surface properties for a variety of applications, such as separating cells, positioning cells in confined region, and detecting cellular responses. Recent reports on the differentiation or separation of muscle cells, the isolation of central nervous system (CNS) axons, the surface geometry effects of cell growth, and the stretch response of actin symbolize the importance of the patterning process in the two-dimensional platform. In particular, primary rat neurons were patterned via a simple plasma-based dry etching method used to pattern cell-adhesive and non-adhesive areas. This was maintained for up to six days inside a microfluidic device.

Laminar flow in a microchannel implies a flow of liquid with low viscosity. Using laminar flows, it is possible to control the deposition of multiple cells, reagents and drugs in a microfluidic channel. A patterning method using a laminar flow does not require a mask or a complex channel structure. Takayama et al. used the laminar flow of liquids in capillary systems to perform a patterned cell deposition. This method offers a means to control all characteristics of the surface in order to selectively attach cells. However, as it is difficult to control the fluid flow while patterning, the shape of the patterned structure can easily be deformed by shear stress of the media flow and shrinkage caused by the solidification of biodegradable materials. Therefore, it is necessary to have a precise control system for the fluid flow and ensured reproducibility for the patterned structure.

3-dimensional cell culture. In animals, cells typically reside in environments with very specific threedimensional features. Of great interest to an in vivolike environment for cell biology, a three-dimensional cell culture system manufactured using a biodegradable polymer or a microstructure that creates a threedimensional environmental and circulatory system, has been developed using cell culture models for a better reproduction of the in vivo functions involved. There are many efforts for constituting threedimensional cellular structures by fabricating an improved bioreactor and patterning a laminar flow using a biocompatible polymer. Among these methods, the fabrication of a bioreactor is useful because it is possible to fabricate the PDMS structure directly using prototype molds without damage to the cells. In addition, microfluidic bioreactors are potentially advantageous for cellular applications as they provide a large surface-area-to-volume ratio as well as many other biomimetic properties. Leclerc et al. reported a microfabricated PDMS bioreactor for continuous perfusion culture of HepG2 cells, which was composed of multiple layers of PDMS with dedicated structures for the oxygen supply and cell culture. However, in the bioreactors, medium fluids and chemical agents are not diffused efficiently into the cellular structure.

By using polymers, it is possible to entrap cells during the gelling process, allowing a more uniform distribution of cells throughout a construct. Recently, three-dimensional cell patterning methods have been attempted for use with a biocompatible polymer. With three-dimensional patterning, unlike two-dimensional patterning, the polymer and cells are printed in a confined region and can be cultured extensively as shear stress is minimized by the mechanical strength of polymer. There are various examples of three-dimensional patterning technologies constructed from three-dimensional cell sheets using two layers of patterned cells in addition to patterned cells entrapped with a polymer in a microfluidic channel. It was also reported that polymers and cells were three-dimensionally immobilized in the channel of a microfluidic device using a laminar flow and were encapsulated without any additional surface treatment. In addition, using various biodegradable polymers with a laminar flow, the creation of a concentration gradient of drugs or reagents[5] was realized. These techniques include bacteria trapping using a laminar flow of hydrogel, and a hepatocyte culture using collagen and terpolymer. Kim et al. reported a novel microfluidic platform for cell-based assays using a peptide hydrogel that encapsulated HepG2 cells. A novel cell-based assay scheme was also constructed using a concentration gradient in a microfluidic channel. However, the concentration gradients inside the polymer are unstable, and controlling their shapes is difficult. Therefore, system reproducibility should be improved by combining polymer microfabrication and microfluidics.

Co-culture. A functional organ normally possesses multiple cell types organized in a unique structure that perform physiological roles. As a co-culture system based on cell-to-cell and cell-to-environment interaction has been a crucial tool for various biological processes with the development of microtechnology, it is possible to create interactions between cells and an in vivo-like environment using a multicellular structure in a microfluidic channel. There have been many efforts toward an understanding of the relationship between tissue structure and organ function and toward the development of new techniques for reconstructing organs consisting of complex tissue using a co-culture system. As shown in Figure 1, a co-culture system can be thought of as a division of 2D and 3D cell culture platforms into 2D/2D, 2D/3D, and 3D/3D co-culture systems.

The 2D/2D co-culture system implies that different cells are positioned on the surface of a structure using various microfabrication and microfluidics tools. Examples of a 2D/2D co-culture platform include a blood-brain barrier utilizing a nanofabricated membrane and a patterning of multiple cells using laminar flows in capillary networks. In particular, micropatterned interactions of hepatocytes and fibroblasts were presented for toxicological study. Recently, it was reported that heterogeneous liver cells were patterned using dielectroporesis (DEP). Unlike a planar microfluidic system, Chiu et al. presented a patterning method for the deposition of cells and proteins onto surfaces using three-dimensional microfluidic systems, and the patterned cells were cultured for 24 h to grow and spread into a confluent layer.

More in vivo-like cellular properties using a microenvironmental culture condition are reflected in a three-dimensional culture than in two-dimensional culture systems. In a 2D/3D co-culture platform, human umbilical vein endothelial cells (HUVEC) were cultured using a three-dimensional platform and fibroblasts were cultured on the HUVEC surfaces using a 3D structure. In a 3D/3D co-culture system, different cells were cultured in a three-dimensional structure simultaneously. For example, a multilayer structure for a biomimetic 3D/3D culture condition was developed using layer-by-layer microfluidics. This multilayer system was created using vascularcell types within heterogeneous types of biopolymers to mimic the structure and composition of blood vessel walls. In an effort to realize the vital liver function, hepatocytes and endothelial cells were cultured using a photosensitive biodegradable polymer in a 3D/3D co-culture platform. In addition, multi phenotype cell arrays using hydrogel microstructures and a three-dimensional platform that utilizes cell docking inside microwells within microfluidic channels have been presented. Furthermore, a three-dimensional collagen gel co-culture system was used in the in vitro maturation of human oocytes and cumulus cells. These three-dimensional culture methods will be developed continuously with microfabrication technologies for cell biology, tissue engineering, and drug development systems.

III. Cell Types and Application

Table 1 shows categorized microfluidic cell culture systems by cell type and by application to cell biological research. The representative research for each cell type is also represented in this table.

TABLE 1

CATEGORIZED MICROFLUDIC CELL CULTURE SYSTEM ACCORDING TO CELL TYPES AND APPLICATION.

Cell type	Cell line	Application
Liver cells	SV-40 transformed murine hepatocytes	Cytochrome P450 assay
	Hepatocyte carcinoma cell line (HepG2)	3D tissue construction
	Primary rat hepatocytes and fibroblast	Toxicology study and liver tissue engineering
	Hepatocyte carcinoma cell cline (HepG2) and human umbilical vein endothelial cells (HUVECs)	Lobule-mimetic cell patterning
Muscle cells	Murine skeletal muscle cell line (CSC12)	Differentiation from myoblast to myotube
	Human dermal microvascular endothelial cells (HDMECs)	Mechanotransduction Muscle cells

Neural cells	Neuronal cell line (Mz-1 cells)	Drug testing
	Primary rat cortical neuron	Basic neuroscience
	Central nervous cell (CNS) axon	Controlled chemical treatment
	Neutrite and glial cells	Cellular response to neurotrophin-3
Stem cells	Human neural stem cells	Stem cell growth and differentiation
	Adult hippocampal progenitor cells	Stem cell proliferation
Other cells	Human umbilical vein endothelial cells (HUVECs) and human lung fibroblast	Multiple cell patterning for tissue engineering
	Endothelial cell and astrocyte	Blood-brain barrier model
	Human ovary arcinoma cell line (HeLa)	Cell-based assay
	Fetal monkey kidney cell (COS7)	Physiological study
	Immature human oocytes and cumulus cell	Oocytes maturation
	Mouse calvarias osteoblastic cells (MC3T3-E1)	Bone tissue engineering

IV. CONCLUSION

Microfluidic cell culture is a basic tool for all cellbased applications including toxicological studies, drug discovery studies, cell and tissue engineering efforts. Recent reports have shown that many novel microfluidic systems, including cell culture on a chip, are worthy of scientific and industrial attention. However, many microfluidic cell culture devices have thus far only been tested with culturing cells without the consideration of individual cell culture conditions for various cell types. In consideration of the microfluidic cell cultivation condition, it is necessary to design the most suitable cell culture platform according to the specific cell type. For example, cells with a two-dimensional culture condition show proper activity when cultured on the surface of a culture dish. For obtaining three-dimensional cell cultivation, the *in vivo*-like properties of 3D patterned multi cellular structure are necessary to form the biomimetic cellular structure. Another limitation of current microfluidic cell culture systems is that most culture systems do not utilize a variety of cellular analyses; the purpose of cell culture is not apparent for cellular applications. Moreover, integration and automation Microfluidic Cell Culture Systems are important issues for further application of HTS and in the manufacture of commercially functional devices. In terms of both advanced microtechnologies and cellular analysis, microfluidic platforms are very useful alternatives for future cell biology studies and cell-based assays.

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