

Microfluidic Cell Culture Systems for 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

Introduction

Cell culture is a key step in cell biology, tissue engineering, biomedical engineering, and pharmacokinetics for drug development¹⁻⁴. *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^{5,6}. 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^{7,8}. 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. However, in an *in vitro* cell culture environment, there are no cellular structures or polymer scaffolds to be adhered to in the culture dish. As there are no other cell types with which to interact or communicate, *in vitro* cultured cells do not play their roles in the way components of organs do, with the exception of proliferation. Consequently, *in vitro* cultured cells frequently alter their cellular properties related to their growth rate, morphology, and intracellular metabolic activities^{3,5,7,8}. On the basis of the inconsistencies between *in vitro* and *in vivo* environments, novel approaches are needed to establish an *in vivo*-like microenvironment.

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^{3,5,7-12}. 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^{1,8}. 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 two-dimensional microstructure for a cell culture was widely used to construct a microfluidic cell culture system¹³⁻¹⁵. 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.

Microtechnologies for Cell Biology

Microfabrication is a collective term for the technologies used to fabricate a micrometer-sized device or system. Microfabrication technologies originate from the microelectronics industry, and microfabricated devices are typically created on silicon wafers or even on glass, plastic, and many other types of substrate materials. Microfabrication processes are performed by several processes, including photolithography, etching, thin-film deposition, thermal oxidation, and wafer cleaning. In the past few years, microfabricated tools have been used increasingly in biomedical and biological applications that cannot easily be achieved using standard biochemical and cellular research tools^{10,11}. In addition, the unprecedented ability of these tools can be exploited to control the cellular microenvironment in a culture and to miniaturize assays for high-throughput applications^{11,16-19}. However, conventional photolithography requires expensive clean room facilities and

photolithographic equipment, and most chemicals used with this method are toxic to cells and are not biocompatible^{11,20}. Recently, soft lithography, a set of microfabrication techniques that uses elastomeric stamps fabricated from patterned silicon wafers to print or mold materials, has come into wider use in the fabrication of microchannels in microfluidics applications as these techniques are convenient, rapid, biocompatible, and inexpensive^{5,11,21-23}. Soft lithographic techniques, including replica molding^{9,13,16-19,24-29}, microcontact printing³⁰⁻³², microtransfer molding and micromolding³³, typically use poly (dimethylsiloxane) (PDMS) as it is biocompatible, optically transparent, permeable to gases, elastomeric, and durable²⁰. With soft lithography based on PDMS, the development of microfluidic systems can be attributed to the adoption of microfabrication technologies enabling the creation of complex miniaturized systems. Figure 1 shows a schematic diagram of the microfabrication procedures for a microfluidic device.

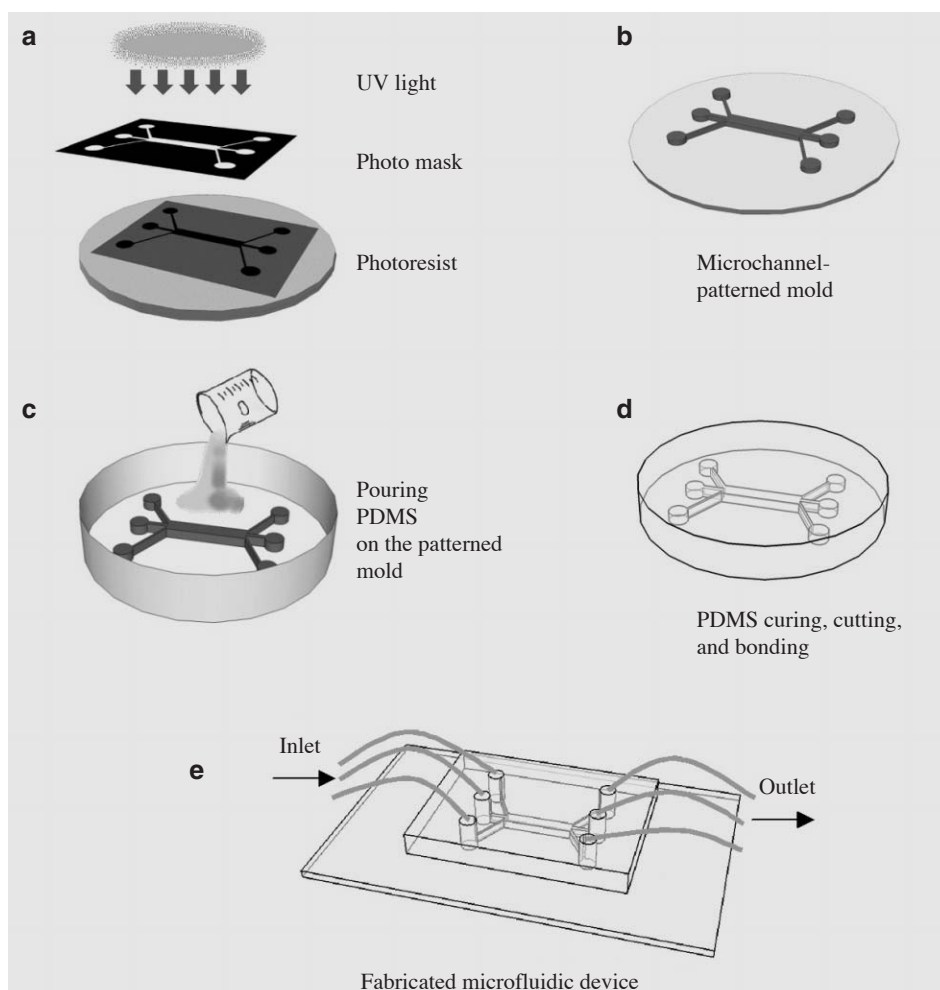


Figure 1. Schematic diagram of microfabrication procedures of a microfluidic device.

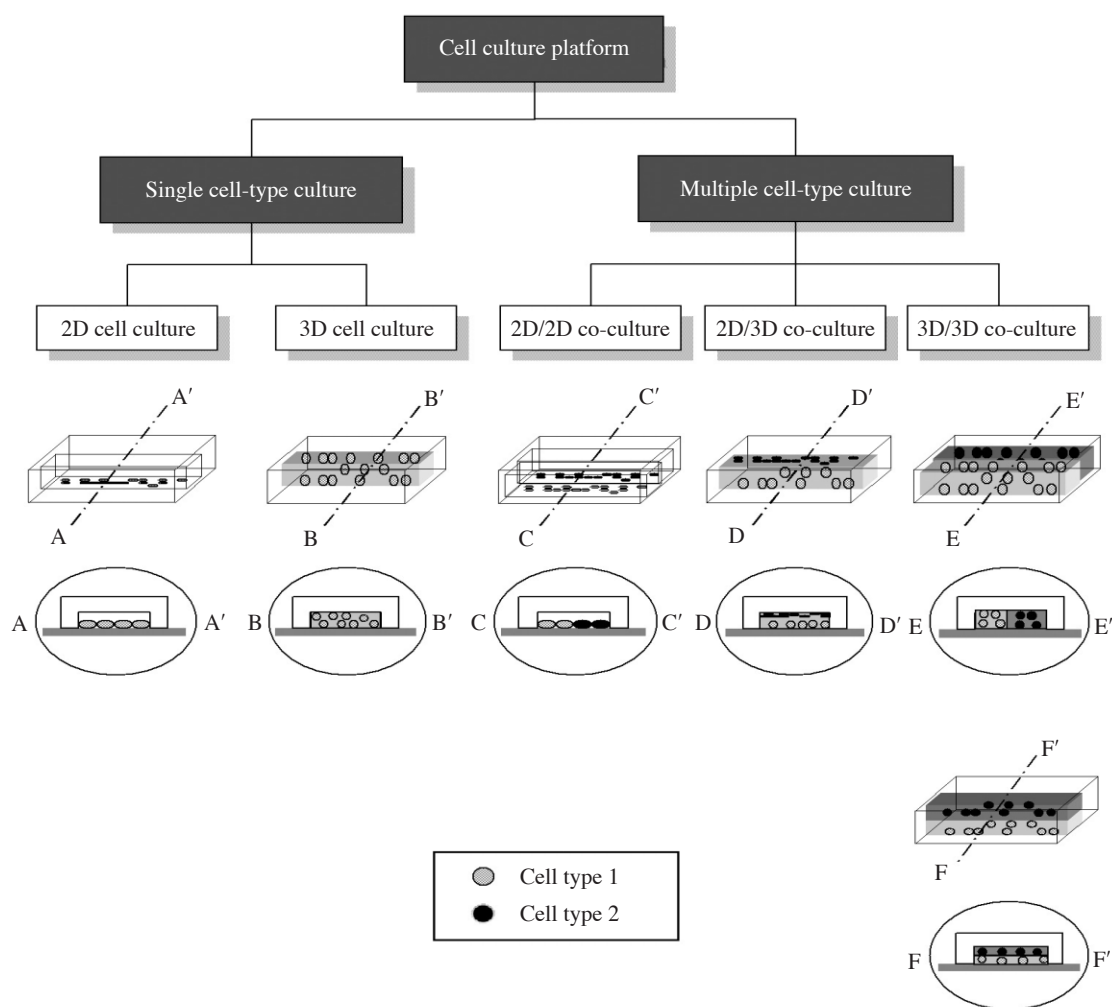


Figure 2. Classification of a microfluidic cell culture platform.

Microfluidics refers to the devices, systems, and methods for the manipulation of fluid flows with characteristic length scales in the micrometer range³⁴. 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^{3,11}. 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^{3,10}. Microtechnologies including microfabrica-

tion 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 2). In addition, major results about cell culture platform are presented.

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^{3,9,10,35,36}. Most *in vitro* experiments with adherent human cells are performed using a two-dimen-

sional platform in which cells are plated onto a surface-treated plastic plate to stimulate cell binding¹⁰. 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^{5,37}. 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^{31,38-42}. Recent reports on the differentiation or separation of muscle cells^{19,43}, the isolation of central nervous system (CNS) axons¹⁵, the surface geometry effects of cell growth^{6,30}, and the stretch response of actin⁴⁴ 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 device³¹.

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^{38,45-49}. 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 deposition⁴⁷. 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.

Thus far, complex PDMS platforms for cell culture^{13,17,28,36,50}, HTS^{9,28}, single cell analysis^{25, 27,51}, and various electromechanical stimulus^{13,52-57} have been developed for use with soft lithography. Lee *et al.* presented a nanoliter scale microbioreactor array which was designed for multiplexed quantitative cell biology including the HeLa cell growth response to varying nutrient concentrations²⁸. However, except

for two-dimensional morphological cells, such as epithelial cells¹⁴, endothelial cells, muscle cells and neural cells, there are a number of limitations in the culturing of certain cell types constituted with three-dimensional construction, such as hepatocytes for the liver, renal cells for the kidney⁵⁸, and osteoblasts for bone organization⁵⁹. Thus, it is important to select a proper cell culture platform according to the cell type for various cellular analyses.

3-dimensional cell culture. In animals, cells typically reside in environments with very specific three-dimensional features¹⁰. Of great interest to an *in vivo*-like environment for cell biology, a three-dimensional cell culture system manufactured using a biodegradable polymer or a microstructure that creates a three-dimensional environmental and circulatory system, has been developed using cell culture models for a better reproduction of the *in vivo* functions involved⁵⁹⁻⁶¹. There are many efforts for constituting three-dimensional cellular structures by fabricating an improved bioreactor and patterning a laminar flow using a biocompatible polymer²¹. 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^{22,26,62-64}. 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 properties¹¹. 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^{26,62}. 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^{21,22,29,63}. 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 polymer¹¹. There are various examples of three-dimensional patterning technologies constructed from three-dimensional cell sheets using two layers of patterned cells²⁹ in addition to patterned cells entrapped with a polymer in a microfluidic channel^{40,65-67}. 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^{45,46,48,68}. In addition, using various biodegradable polymers with a laminar flow, the creation of a concentration gradient of drugs or reagents^{5,11,46,47,69-71} was realized. These techniques include bacteria trapping using a laminar flow of hydrogel⁴⁵, and a hepatocyte culture using collagen and terpolymer⁴⁸. Kim *et al.* reported a novel microfluidic platform for cell-based assays using a peptide hydrogel that encapsulated HepG2 cells⁴⁶. A novel cell-based assay scheme was also constructed using a concentration gradient in a microfluidic channel⁶⁸. 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 channel⁷². 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 system³⁹. As shown in Figure 2, 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 membrane⁷³ and a patterning of multiple cells using laminar flows in capillary networks^{18,47}. In particular, micropatterned interactions of hepatocytes and fibroblasts were presented for toxicological study^{24,74,75}. Recently, it was reported that heterogeneous liver cells were patterned using dielectrophoresis (DEP)^{39,41}. 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 layer³².

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, hu-

man umbilical vein endothelial cells (HUVEC) were cultured using a three-dimensional platform and fibroblasts were cultured on the HUVEC surfaces using a 3D structure⁶⁰. 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^{76,77}. This multilayer system was created using vascular cell 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^{67,78,79}. In addition, multi phenotype cell arrays using hydrogel microstructures⁸⁰ and a three-dimensional platform that utilizes cell docking inside microwells within microfluidic channels¹⁶ have been presented. Furthermore, a three-dimensional collagen gel co-culture system was used in the *in vitro* maturation of human oocytes and cumulus cells⁸¹. These three-dimensional culture methods will be developed continuously with microfabrication technologies for cell biology, tissue engineering, and drug development systems.

Cell Types and Applications

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. Details are described in the following section according to cell type.

Liver cells. Primary liver cells, hepatocytes, are involved in the metabolism of carbohydrates and lipids, and in protein synthesis and detoxification²². To maintain the hepatocyte or hepatocarcinoma cell line *in vitro*, it is necessary to study the pharmacological, toxicological, and metabolic effects, as hepatocytes rapidly lose their liver-specific functions under conventional *in vitro* cell culture conditions^{22,82-84}. There have been many efforts to maintain the hepatocyte *in vivo* function by constituting a microenvironment condition. Recently, various biocompatible polymers were used to construct a three-dimensional microenvironment in a microfluidic channel^{48,68}. It was reported that a novel microfluidic platform used peptide hydrogel encapsulation of HepG2 cells; patterning approaches for hepatocytes and microsomes using a collagen matrix have also been described^{46,65-67,85}.

In addition, a microfabricated bioreactor with a perfusion system was designed to form a tissue-like

Table 1. Categorized microfluidic cell culture system according to cell types and application.

Cell type	Cell line	Application	Year	Reference
Liver cells	SV-40 transformed murine hepatocytes	Cytochrome P450 assay	2005	[65]
	Hepatocyte carcinoma cell line (HepG2)	3D tissue construction	2006	[29]
	Primary rat hepatocytes and fibroblast	Toxicology study and liver tissue engineering	1997/2006	[24, 75]
	Hepatocyte carcinoma cell cline (HepG2) and human umbilical vein endothelial cells (HUVECs)	Lobule-mimetic cell patterning	2006	[41]
Muscle cells	Murine skeletal muscle cell line (CSC12)	Differentiation from myoblast to myotube	2005	[43]
	Human dermal microvascular endothelial cells (HDMECs)	Mechanotransduction of blood vessel cell	2005	[13]
	Human lung fibroblasts, human umbilical vein smooth muscle cells (SMC), and human umbilical vein endothelial cells (HUVECs)	<i>In vivo</i> -like structure creation with cell-cell and cell-matrix interaction	2005	[76, 77]
Neural cells	Neuronal cell line (Mz-1 cells)	Drug testing	2002	[30]
	Primary rat cortical neuron	Basic neuroscience	2005	[31]
	Central nervous cell (CNS) axon	Controlled chemical treatment	2005	[15]
	Neutrite and glial cells	Cellular response to neurotrophin-3	2005	[69]
Stem cells	Human neural stem cells	Stem cell growth and differentiation	2005	[92]
	Adult hippocampal progenitor cells	Stem cell proliferation	2004	[96]
Other cells	Human umbilical vein endothelial cells (HUVECs) and human lung fibroblast	Multiple cell patterning for tissue engineering	2003	[60]
	Endothelial cell and astrocyte	Blood-brain barrier model	2005	[73]
	Human ovary carcinoma cell line (HeLa)	Cell-based assay	2005	[9, 13, 28]
	Fetal monkey kidney cell (COS7)	Physiological study	2005	[58]
	Immature human oocytes and cumulus cell	Oocytes maturation	2005	[81]
	Mouse calvarias osteoblastic cells (MC3T3-E1)	Bone tissue engineering	2006	[63]

structure in which the morphological changes of a hepatocellular spheroid were observed⁸³. A membrane-based PDMS microbio reactor for primary rat hepatocyte was used as a scaffold for cell attachment. It was shown that albumin secretion and ammonium detoxification was increased by triggering a liver-like specific function⁶⁴. Although membrane-based bioreactors are very efficient in the support of bidirectional media flow with a relative large surface area, the properties of attachment to the membrane are not viable due to cell aggregation. In this case, surface treatments are certainly required. It was also reported that microfluidic PDMS bioreactors using a continuous nutrition and oxygen supply show the possibility of useful large-scale hepatocyte culture if an increase in the *in vivo*-like three-dimensional condition could be produced²⁶. A dynamic profiling of the hepatocyte stress response in a microfluidic cell array was reported using green fluorescent protein (GFP)⁸⁴. The activity of the cytochrome P450 metabolism, which is a routine assay of potential drug candidates, was also increased using a microfluidic hepatocyte culture^{65,67}. From this point of view, dynamic organ cultures and kinetic studies of the cellular organizations

will be possible if the liver function can be maintained over an extended period of time.

Muscle cells. The contraction of cardiac myocytes (heart muscle cells) is a crucial factor for maintaining the heart function^{27,86}. If the cardiac myocytes do not work well, cardiovascular diseases and sudden cardiac death⁵⁵ can result. Li *et al.* developed a microfluidic method to study the contraction of a single cardiac myocyte²⁷. This microfluidic device makes it possible to integrate all necessary operations, including microfluidic cell selection and retention, chemical stimulation, and a quantitative analysis of intracellular calcium concentrations. Another group described a partitioning of the extracellular space around single cardiac myocytes under electrical activation⁸⁶. Using this method, two ends of a single cell confined in a microfluidic device can be independently superfused using dual superfusion pipettes. It was also reported that a three-dimensional polymeric substrate can be used to adhere cardiac myocytes. In the creation of an *in vivo*-like environment using a three-dimensional pillar array and cell sheet, the contraction forces of the cells were monitored *in situ*^{87,88}.

Viable/nonviable cardiac myocytes⁸⁹ and myocytes/non-myocytes¹⁹ were separated using dielectrophoresis (DEP) and a size-based microfluidic structure, respectively. Muscle cells have been used for a long-term culture to span the whole process of differentiation from myoblasts to myotubes⁴³, to design localized drug delivery of cellular networks¹⁷, and to examine the stretch response of myocyte hypertrophy⁴⁴. By integrating a microfluidic device with electrochemical and optical sensors, the extracellular potential was measured from a single cardiac myocyte on planar microelectrodes⁵³. A novel microfluidic impedance assay was developed using DEP to monitor endothelin-induced cardiomyocyte hypertrophy⁵⁵. From an electrochemical point of view, this can provide a convenient method to measure cell concentration and increased sensitivity.

Neural cells. Neural cells have great potential as a key element of cell-based biosensors due to their intrinsic electrophysiological characteristics and their specific binding with drugs and toxins. With the binding of these substances, they generate electric signals in a substance-specific and concentration dependent manner, thus the response profiles can be monitored by microelectrodes fabricated on a chip^{5,6,20,90}. It has been demonstrated that a single neuron can be patterned onto an indium tin oxide (ITO) electrode⁵⁴ and planar microelectrode arrays^{52,91} for recording cellular responses according to the extracellular potential. Multiple cells constituting a nervous system were patterned and cultured inside a microfluidic device using microcontact printing and micromolding³¹ after the patterning of neuronal cells by microcontact printing with microfluids by another group³⁰. Recently, Taylor *et al.* isolated CNS axons without somatas or dendrites and localized related physical and chemical treatments¹⁵. It was also reported that a microfluidic chamber was designed to evaluate *in vitro* control of neural cell growth, and a reusable cell culture plate was integrated with a microfluidic media delivery network⁶⁹. However, thus far the electrical measurements of neural activity have not been thoroughly applied in neuroscience and neuroengineering as many of the relevant microfabrication and microfluidic technologies have been developed only in the past few years. Therefore, it is essential to undertake fundamental studies on neural cells using a variety of microfluidic devices combined with electrochemical methods.

Stem cells. Currently, stem cells are regarded as a useful candidate for cell-based therapies as with them it is possible to genetically modify and differentiate a cell into a specific cell type for cellular replacement

or tissue engineering^{92,93}. Conventional static cell cultures are not amenable to a precise control of stem cells. It is very difficult to specify the creation of different cell types and to manipulate a small amount of cells. As a macroscale cell culture requires a large volume of media including growth factor and multiple reagents, microfluidic technology using a perfusion system has been applied to various stem cell cultures⁹²⁻⁹⁷. Chung *et al.* used a concentration gradient of growth factor using continuous laminar flows to human neuronal stem cells in an effort to develop cerebral cortex cells⁹². They succeeded in observing differentiation as concentration-dependent cellular responses according to the growth factor. A microfluidic platform for analyzing the proliferation of neuronal stem cells was developed using a microfabrication technique that created an array of microwells on a glass cover slip, which was compatible with conventional, high-magnification light and fluorescent microscopy⁹⁶. A multiple array using a simple pumping scheme was also used to evaluate the effect of colchicin on myoblasts⁹⁴. Recently, mouse embryos were cultured on endometrium tissue in a membrane-embedded microfluidic device⁹³. It was also shown that the culture of mouse embryonic stem cells over a logarithmic range of flow rates can be realized using a continuous media exchange⁹⁸.

Conclusions

Microfluidic cell culture is a basic tool for all cell-based applications including toxicological studies, drug discovery studies, cell and tissue engineering efforts^{12,50,99}. 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 multicellular 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

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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