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Body on a Chip

Re-creation of a living system in vitro.

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THE RECENT ADVANCEMENT OF LAB-ON-a-chip (LOC) technology based on nano/microfabrication for applications in biology has led to the establishment of in vitro tissue systems that achieve a deeper understanding of tissue functions. Nowadays, this type of device is called "organ on a chip." As an advancement from this technology, a new research trend called body on a chip is emerging. This new technology enables the re-creation of physiological and pathological conditions of the human body in an in vitro system and, therefore, could be a powerful tool not only for studying fundamental biology but also for drug discovery.

However, body on a chip is still in the proof-of-concept stage, and more research and technological advancements are clearly necessary to achieve its practical application. We provide an overview of the ongoing trends in developing individual building blocks and some trials on developing body on a chip, as well as its future prospects.

The body consists of many organs, including the brain, heart, liver, kidneys, and blood, as well as the central nervous system, that are well organized with one another. Body on a chip refers to a device that is integrated with multiple microtissues to mimic the whole body of an animal or a human (Figure 1). Such a device would be extremely useful for studying human physiology and pathologies.

In addition to fundamental biological studies, pharmaceutical companies and government research institutions have shown an interest in the development of body-on-a-chip technology because of its capacity to act as an in vitro system for mimicking the human body as an alternative to preclinical tests. For drug development and screening, pharmaceutical companies need to screen a large number of drug candidates before

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developing a new medicine, which is a time-consuming process.

At present, the development of a drug costs at least US$1 billion and requires about ten years to carry out since it involves screening, animal testing, and clinical trials before the drug can be marketed. In addition, most drugs fail during clinical trials because of their toxicity. This is because of the lack of sufficient preclinical trials (i.e., cell-based assays and animal tests) to understand the efficacy and toxicity of drug candidates. In general, cell-based assays are used with established cell lines, but these cannot be considered representative of primary cells harvested from the human body. Furthermore, animal systems do not accurately represent human systems. Therefore, there is a need to establish new methods for preclinical trials as an alternative to these conventional methods.

In 2012, the National Institutes of Health and the Defense Advanced Research Projects Agency in the United States started to support the development of human body on a chip to create a system that would mimic human physiological conditions in the laboratory as well as to establish an array of human microtissues. The European Union has also shown similar interest in obtaining a deep understanding of the relationships between drugs/metabolites and human tissues. Many countries have started paying a lot of attention to the development of body-on-a-chip technology and its potential impact. In this article, we introduce the concept of body on a chip and highlight its enabling technologies.

ORGAN ON A CHIP

Recently, there has been an ongoing effort to establish organ on a chip. In the field of microfluidics-based methodologies, we have been continuously working on developing LOC technologies, and the development of organ on a chip falls along this line in terms of the application of LOC in cell and tissue biology. This is because microfluidic technology has numerous advantages over conventional biological methodologies, including the controllability of small fluids, manipulability of three-dimensional (3-D) cellular geometry, the capacity to be high throughput, and automated settings. In addition, the development of organ on a chip provides insight into the mechanisms of cell organization in an organ as well as organ-specific diseases. In combination with micro- and nanoelectromechanical systems (MEMS/NEMS), which we will describe later, this technology confers the ability to manipulate and quantify cellular behavior. Therefore, various organ-on-a-chip technologies have been developed, and we would like to introduce the current status of these technologies.

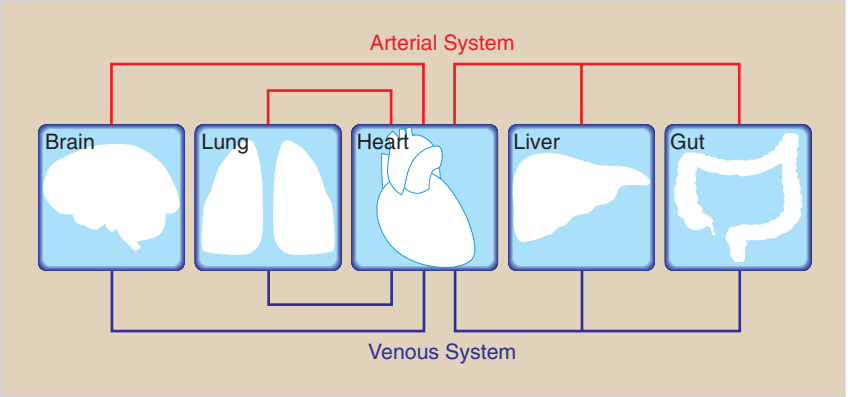


FIGURE 1 A conceptual illustration of the fabrication of a body on a chip by integrating multiple microtissues within a single device. Circulating systems are mimicked using a flow channel.

INDIVIDUAL ORGAN ON A CHIP

Currently, numerous organ on a chips for the brain [1]–[4], the brain–blood barrier (BBB) [5], the liver [6], [7], the lungs [8]–[11], the heart [12]–[16], and the kidneys [17], [18] have already been developed.

For example, the BBB plays an important role in brain homeostasis and also prevents toxic materials from accessing the brain. To develop new drugs to treat neurodegenerative diseases, it is required that they pass through the BBB. Griep et al. [5] mimicked the BBB on a microfluidic chip. They introduced hCMEC/D3 human brain endothelial cells on to a microfluidic device and evaluated the functionality of an established endothelial cell layer as a model BBB by measuring the trans-endothelial electrical resistance (TEER). They confirmed that the endothelial cells in the microfluidic device formed tight junctions. In comparison with conventional trans-well techniques, the BBB chip showed higher TEER activity, which was suitable for evaluating a new drug.

The liver performs various kinds of physiological and pharmacological roles, such as detoxification, protein synthesis, and the production of bioactive molecules. Most drugs pass through the liver, and therefore, we need to investigate the influence of drugs on liver function and metabolism. To investigate this in detail, an in vitro assay is beneficial to reduce its complexity. However, until now, it has been a challenge to establish a mature and functional microliver tissue structure in either a culture dish or a microfluidic device. Therefore, it is necessary to establish a 3-D microliver in vitro.

A miniaturized gut had also been developed in a microfluidic device by using human Caco-2 intestinal epithelial cells to mimic the complicated structure of the living intestine [19]. Caco-2 cells were introduced on one side of a porous membrane that was coated with extracellular matrix proteins, and the porous membrane was used to establish a transwell in a microfluidic chamber. In this microfluidic device, two vacuum chambers were placed next to a cell culture chamber to apply mechanical strain on the intestinal cells placed on a porous membrane. The authors found that mechanical strain cycles induced the formation of 3-D intestinal structures such as crypts and increased the TEER. They also successfully used the same techniques to develop a lung in a microfluidic device [10].

Grosberg et al. [12] developed a heart on a chip by using a microthin film to

measure the contractive force of cardiomyocytes in combination with an action potential sensitive dye and fluorescent microscopy to perform physiological and pharmacological tests. In previous studies on cardiovascular pharmacology, it was a challenge to use cardiomyocytes for in vitro drug testing because of the inability to replicate in vivo conditions in in vitro settings as well as to measure the contractility and electrophysiology. In their device, Grosberg et al. used a platform that they had previously developed, muscular thin films (MTFs), [20], [21] that allow the monitoring of tissue contraction by observing the 3-D deformation of MTF. Cardiomyocytes were placed on a micropatterned surface of elastic polydimethylsiloxane (PDMS) to form an anisotropic monolayer, which mimicked in vivo heart tissue. They then measured the diameter of the length of the films that were deformed as a result of cardiomyocyte contraction. They were able to assess drug effects on cardiomyocytes by monitoring the change in the contraction force, propagation of the action potential, the beating frequency, and the cellular architecture as determined by immunocytochemistry.

MULTIPLE ORGANS ON A CHIP

As described earlier, tremendous efforts are under way to create an organ on a chip by introducing tissue-specific cells into a microfluidic device. As a basis for these advancements toward creating a real body on a chip, some attempts to combine two or more organs in a single device have been reported [22]–[24].

Toward developing human-on-a-chip technology, Zhang et al. [25] constructed a microfluidic device that is capable of culturing multiple types of human cells. In this microfluidic device, adipose, kidney, lung, and liver cells were connected and perfused. During cell culture, the cells released cytokines, nutrients, and waste.

If one were to construct a circulation system in a device, factors that are released from one type of cell in the device would affect the secreting cells and other type of cells, similar to autocrine and paracrine effects. Therefore, we need to consider the effects of factors that are released from cells. Zhang et al. found that a common medium supplemented with growth factors enabled them to maintain their collection of cells; however, transforming growth factor (TGF)-β1 facilitated the function of A549 lung cancer cells but inhibited that of C3A liver cells. Therefore, gelatin-based microspheres containing TGF-β1 were placed in the cell culture chamber with A549 cells. TGF-β1 that was released from the microspheres facilitated A549 function but did not influence the C3A cells. Thus, to construct a body on a chip, we need to consider the cellular microenvironment for each type of cell and tissue.

Microvessels play a role in delivering nutrients to tissues and cells, and they define cellular microenvironments in the body. To interconnect the microtissues within a body on a chip, it would be very important to properly form microvessel networks [26], [27]. Zheng et al. [26] fabricated a microfluidic device with a chamber that was filled with collagen gel to study angiogenesis and thrombosis in vitro. Prevascular cells that were placed in the collagen gel were able to form a 3-D tubular structure. Sakaguchi et al. [27] also fabricated in vitro microvessels under layered cardiomyocyte sheets to supply nutrients and gases to deep tissues. They found that although they were able to establish thin-layered heart tissues without a vascular network, the formation of healthy thicker tissues required a vascular network.

DISEASE MODELING THROUGH ORGAN-ON-A-CHIP TECHNOLOGY

Body-on-a-chip technologies could be used to understand diseases and establish new treatments [28]–[31]. For example, Huh et al. [28] fabricated a microfluidic device that was cultured with two types of human lung cells: alveolar epithelial cells and vascular endothelial cells. As shown in Figure 2, epithelial and endothelial cells were separately cultured on porous membranes and were exposed to cyclic mechanical distortion to mimic breathing. By introducing interleukin-2 (IL-2), a cytokine used in cancer therapy that can be toxic and causes pulmonary edema in cancer patients, into the sides of alveolar epithelial cells, this microfluidic device was able to serve as a human

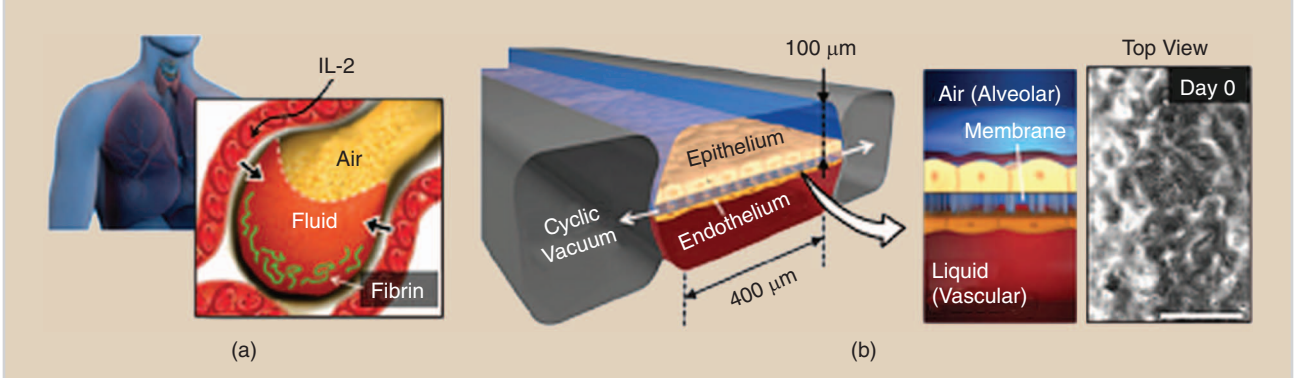


FIGURE 2 A lung on a chip that mimics human pulmonary edema is shown [29]. (a) Human pulmonary edema caused by IL-2 cancer treatment. (b) A microfluidic device for mimicking human pulmonary edema by introducing alveolar epithelial cells and vascular endothelial cells on a porous membrane in combination with a cyclic mechanical stress. (Reproduced with permission from the American Association for the Advancement of Science.)

pulmonary edema model. By using this device, the authors identified new potential therapeutics, angiopoietin-1 and transient receptor potential vaniloid 4 inhibitor (GSK2193874), that could abrogate the IL-2 induced toxicity.

MEMS/NEMS TOWARD ORGAN ON A CHIP

MEMS/NEMS technologies have allowed the integration of mechanical and electrical functionalities into a microfluidic device. This is now emerging as a platform for more accurate and cost-effective cell culturing and drug development/testing because of its small length scale (at the micrometer to millimeter level), reduced sample volumes (below the microliter level), and well-developed micro/nanofabrication techniques [32]–[37]. These microfluidic devices enable a real-time examination of cellular responses to mechanical or chemical stimuli with a more precise control of the cellular environment than conventional macroscale methods. This technology, therefore, holds the potential to contribute to the development of body on a chip.

MATERIALS AND FABRICATION

There are three main factors to consider when choosing materials and fabrication methods for creating a microfluidic system: the required function, the degree of integration, and the application [38]. During the first few years, the microfluidic devices were fabricated from inorganic materials such as silicon and glass; as the field advanced, other materials such as polymers and paper were employed. Indeed, polymer-based materials, such as poly(methyl methacrylate), PDMS: poly(dimethylsiloxane), and epoxy-based photoresist: SU-8 [39] or TMMR/F[40], have replaced silicon and glass as preferred materials to fabricate microfluidic devices, as biomedical applications demand disposable devices to eliminate the risk of sample contamination and permit ease of fabrication [41]. In our previous study, we showed that these materials, when used for MEMS/NEMS fabrication, caused alternations in the gene expression patterns of stem cells [42]. Therefore, we need to consider how these materials affect cellular functions in body on a chip.

There are several published reviews that list all potential materials and the fabrication techniques used in the development of microfluidic devices [43], [44]. For example, Waldbaur et al. [44] focused on the chemical compatibility of commonly used polymer materials for microfluidic devices and the unique fabrication approaches for creating true 3-D structures. In a tissue, cells and cellular microenvironments are highly regulated and organized in a 3-D fashion, and these 3-D fabrication approaches are advantageous for establishing in vitro 3-D microtissues.

> Body on a chip refers to a device that is integrated with multiple microtissues to mimic the whole body of an animal or a human.

PDMS-BASED MICROFLUIDIC DEVICES

Most conceptual microfluidic devices are still fabricated in PDMS by using soft lithography [45]. In soft lithography, device molds are formed via conventional machining or photolithography methods, and PDMS microstructures are cast and cured on these molds. Because of its low elastic modulus, relative ease of fabrication, excellent optical properties for both light transmission and fluorescence microscopy, and its biocompatibility [46], PDMS is suitable for biological applications. However, using PDMS also has some drawbacks: it is permeable to small hydrophobic molecules, shows limited solvent resistance, and requires complicated fabrication processes that contain assembly steps such as slow thermal curing or manual handling of thin elastomeric films for large-scale manufacturing. Therefore, several groups have attempted to improve the material properties of new elastomeric formulations to facilitate the use of elastomeric polymers in the creation of microfluidic devices [47]–[50].

PDMS also allows one to seamlessly combine diverse functional elements into a microfluidic system such as microvalves, micropumping systems, and cell-culture chambers [51]–[55]. For example, Nishijima et al. [55] developed an ingenious mechanism for cell culturing and manipulation by using pneumatically actuated microwells. Each microwell is composed of a thin PDMS membrane, and the shape of each microwell is controlled by applying pressure to the membrane. By using such active microwells, selective manipulation and parallel analysis can be performed.

MEMS PLATFORMS FOR ELECTROPHYSIOLOGY

Electrical recording of cell activity is a fundamental method for studying the behavior of ion channels in cell membranes and investigating cellular functions [56]. The activity of electrically excitable cells, such as neurons, cardiomyocytes, muscle cells, and pancreatic β cells, can be observed by measuring changes in the action potential across the cell membrane or the extracellular ion concentration [57]. The action potential across the cell membrane is detected by patch-clamp techniques, which measure ion currents flowing through the cell membrane ion channels. Conventional patch-clamp techniques that involve using glass micropipettes are cumbersome and have a low throughput that can only be applied to single or small numbers of cells. MEMS technologies permit the development of chip-based patch-clamp microsystems to circumvent the low-throughput bottleneck through robust automated operation and parallelization of electrophysiological measurements [58]–[60]. Tang et al. [59] integrated microfabricated planar patch-clamp substrates and PDMS microfluidic components in a 1,536-well format. Substrates with cell-patch-site aperture diameters ranging from 300 nm to 12 μm were fabricated

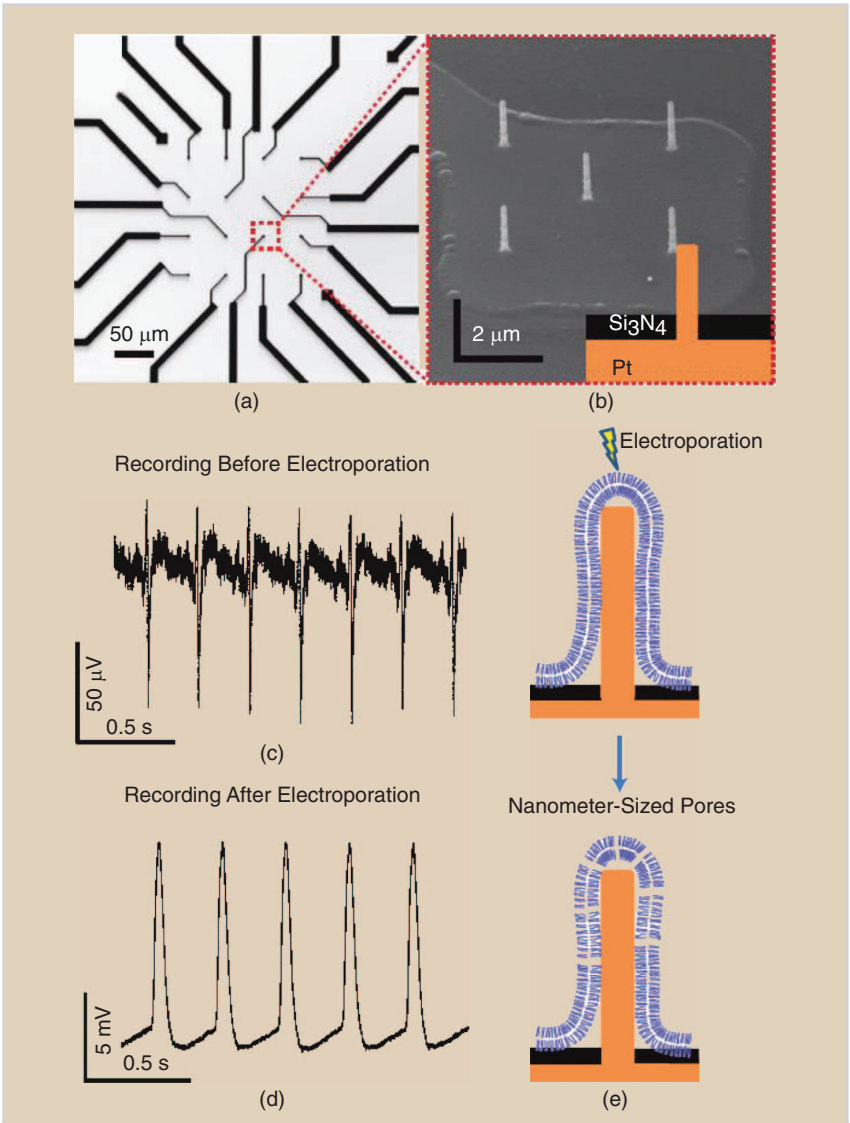


FIGURE 3 A nanopillar array device for the intracellular recording of action potentials is shown [66]. (a) An optical micrograph of a nanopillar array. (b) A scanning electron micrograph of nanopillars for electroporation and intracellular recording. An intracellular recording of the action potential of HL-1 cardiomyocytes (c) without electroporation and (d) with electroporation. (e) An illustration of electroporation to generate nanometer-sized pores in a cell membrane to obtain better signals for intracellular recordings. (Reproduced with permission from Nature Publishing Group.)

using standard MEMS fabrication techniques. In addition, a tight cell/chip aperture seal is an important requirement for on-chip electrophysiological measurements of living cells.

On the other hand, substrate-integrated microelectrode arrays (MEAs) are commonly used to measure extracellular electrophysiological activity [61]. Complementary metal–oxide semiconductor (CMOS) MEAs enable the measurement of ion concentrations in their vicinity based on the extracellular voltage or current, which changes according to the activity of the cell. For example, monolithic bidirectional CMOS MEAs that record extracellular activity at high spatial and temporal resolution and have stimulation and recording capabilities have been extensively developed by the ETH Zurich group [62]–[64]. They have fabricated high-density CMOS MEAs in combination with additional post-CMOS processing steps [64]. The device permits recordings from 126 electrodes that are selected out of 11,000 electrodes.

To further develop MEA technologies for electrophysiological analysis, Xie et al. [65] developed a nanopillar array in combination with an electroporation technique to perform intracellular recordings of cell membrane action potentials (Figure 3). They fabricated a nanopillar-electrode array in which nanopillars can be introduced onto a cell membrane and electroporated to generate nanometer-size pores in the membrane. This array allows the recording of action potentials with high precision.

MEMS PLATFORMS IN MECHANOBIOLOGY

MEMS platforms have had an impact on cell mechanobiology and are used to apply localized forces to single cells or to measure localized forces that are exerted by a cell in a quantitative and accurate fashion [66], [67]. Although mechanical forces are important parameters in cellular physiological behavior, this area remains largely underexplored. The progress in MEMS was facilitated by new capabilities in applying and measuring forces and displacements with pico-Newton and nanometer resolutions. For example, cell traction plays important roles in cell adhesion and migration. Microfabricated PDMS pillars have been developed to measure forces that are exerted by single adhesion sites of cells [68]–[71]. Cell traction forces can be calculated through a visual measurement of the micropillars' deflections, which have a well-defined relationship with local forces, following Young's modulus of PDMS that is used for pillars. However, these micropillars suffer from a critical drawback: the regulation of pillars' stiffness is limited to lateral dimensional control. Thus, Sochol et al. [72] recently used direct-write laser lithography to fabricate arrays of microsprings. By adjusting the geometric characteristics of the individual springs, the lateral and vertical stiffness of the cellular substrate can be customized on a micrometer scale.

NANO/MICROENGINEERED CELLULAR MICROENVIRONMENTS

In the body, cellular microenvironments, which are also called niches, play a central role in regulating the cellular

processes that culminate in the establishment of well-organized tissue structures (Figure 4). If cellular microenvironments could be engineered and manipulated as we desire, it could become feasible to establish controllable tissues. However, it is still challenging for the conventional macroscopic experimental settings of cell biology and tissue engineering to create in vitro models in a high-precision manner due to limited controllability in terms of size and time. Therefore, nano/microfabrication technologies could be useful for mimicking the in vivo cellular microenvironments and recreating them as in vitro systems.

Nanoengineered cellular substrates can be used to stimulate stem cells to facilitate their functions. For example, in a conventional cell culture dish, cells have limited numbers of focal adhesion complexes to promote their growth due to the flatness of the culture dish. Recent studies indicate that nanoengineered substrates strongly influence stem cell self-renewal and differentiation [73]–[75]. For example, nanofibers are often used as cellular scaffolds for tissue engineering because of their unique characteristics, such as the ability to form 3-D structures and the easy availability of large amounts of nanofibers. Subramony et al. [76] demonstrated that aligned nanofibers, in combination with mechanical stimulation, induced lineage-specific differentiation of mesenchymal stem cells into ligament fibroblast-like cells, while unaligned nanofibers modulated only cellular morphology without any induction of differentiation (Figure 5).

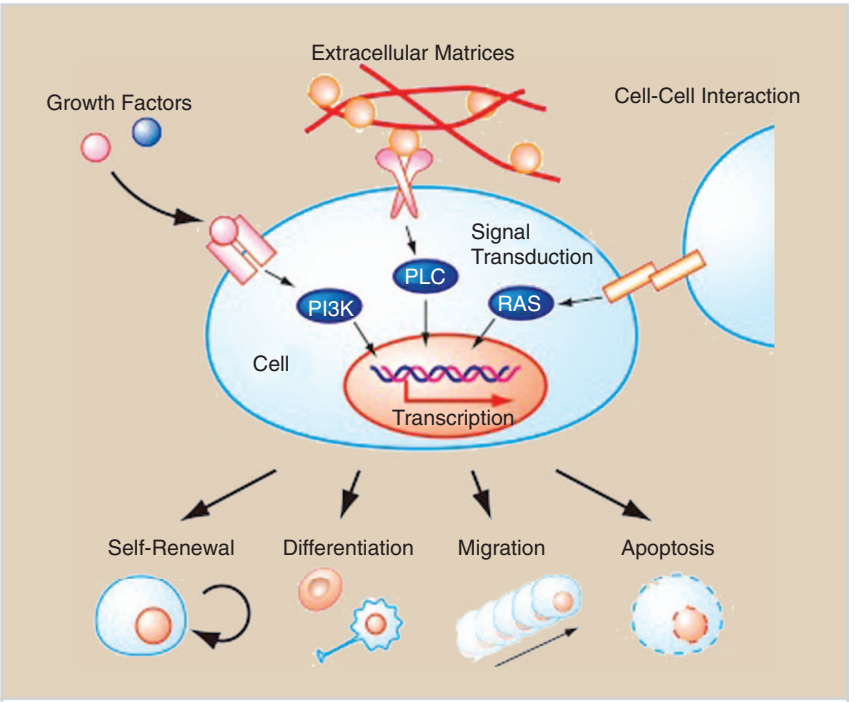
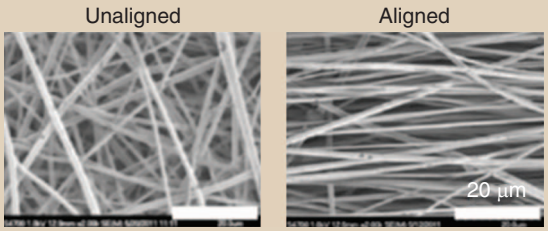


FIGURE 4 Cellular microenvironments, which are composed of growth factors, extracellular matrices, as well as cell–cell interaction, regulate cell function as well as tissue organization.

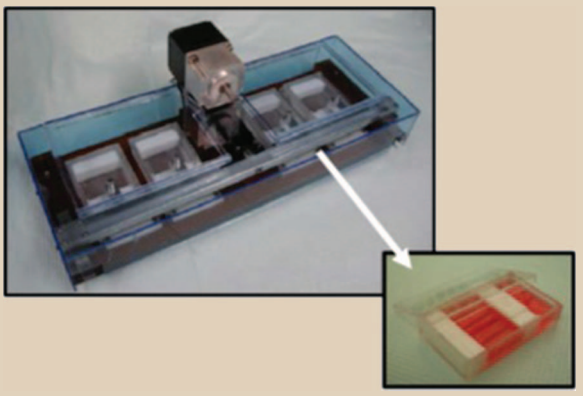
HUMAN PLURIPOTENT STEM CELLS FOR BODY ON A CHIP

Human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) [77] and human induced pluripotent stem cells (hiPSCs) [78]–[80], are beneficial for developing body on a chip because of their unique characteristics, such as unlimited self-renewal without abnormal chromosomes, as well as the ability to differentiate into any kind of cell in the body (Figure 6). Therefore, we could generate multiple



	Unaligned	Aligned
Fiber Diameter (nm)	667 ± 128	615 ± 152
Pore Size (μm)	4.91 ± 0.78	4.23 ± 1.06
Porosity (%)	81.76 ± 3.93	80.75 ± 2.97

(a)



(b)

FIGURE 5 The unaligned and aligned nanofibers in combination with cycled mechanical stress for inducing the differentiation of mesenchymal stem cells (MSCs) into ligament fibroblast-like cells. (a) Unaligned and aligned nanofibers and their parameters. (b) Custom bioreactor for culturing and mechanical loading of MSCs.

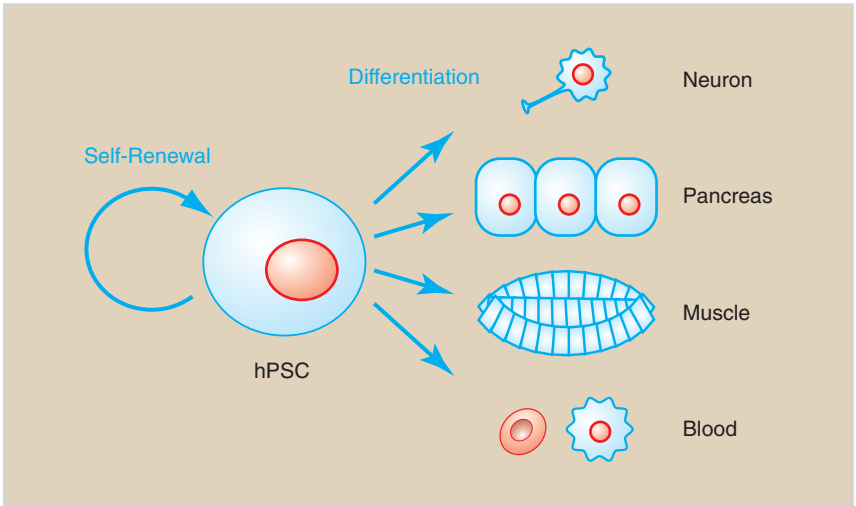


FIGURE 6 An illustration of the hPSCs, which have unique characteristics of unlimited self-renewal and differentiation into almost any kind of cells in the body.

types of tissue-specific cells from a single cellular origin and integrate them within a single device. Previously, we needed to collect several types of tissue-specific cells that were harvested from different people, but these cell collections did not represent any one person (Table 1). These established tissue-specific cells are often immortalized through their expansion in vitro or originate from tumors, and thus, they are very different from normal tissue cells. However, primary cells that are harvested from healthy people are difficult to expand in culture.

In addition to the unique advantages of using hPSCs for body-on-a-chip technology, patient- and disease-specific hiPSCs are also beneficial for recreating disease models in vitro as well as in the understanding of disease mechanisms [81], [82]. In particular, it has been very challenging to cure patients with rare diseases until now. hiPSCs derived from these patients will provide new insight into their diseases and help in the development of new therapies. Furthermore, recent advancements in genome editing (i.e., zinc finger nuclease (ZFN) [83], transcription activator-like effector nuclease (TALEN) [84], and clustered regularly interspaced short palindromic repeats (CRISPR) [85], [86]) now allow the insertion of genes of interest into hPSCs to study their functions. Therefore, we are able to create disease-specific hPSCs by introducing disease-related genes and generate disease-specific body-on-a-chip systems.

TABLE 1 Characteristics of available cell types.

	CELL LINE	PRIMARY CELL	PLURIPOTENT STEM CELL
Cell growth	Many passages	A few passages	Unlimited passages
Source	Cell bank	Volunteers or patients	Cell bank or handmade
Culture method	Established	Challenges	Established, but room for improvement
Differentiation ability	Some, but limited	Some, but limited	Almost any kinds of cells
Chromosomes	Most of case, abnormal	Basically normal	Basically normal, but editable
Gene modification	Easy	Difficult	Possible, but difficult
Disease models	Easy	Difficult	Possible
Tissue-specific cells	Possible	Possible, but difficult	Possible, but time consuming

Drug discovery/screening and toxicological tests are best suited for the application of hPSCs. Various types of human cells with the same genomic information can be derived from cells that have been obtained from a single source. Because hPSCs have unlimited self-renewal and differentiation capabilities, we can obtain sufficient numbers of human tissue cells for screening. Therefore, many pharmaceutical companies are attempting to use hPSCs in drug development and screening.

Previously, several groups, including ours, have successfully developed microfluidic devices for culturing, differentiating, and analyzing hPSCs [3], [87]–[90]. For example, Park et al. [3] successfully developed neural progenitor cells from hESCs by applying a cytokine-concentration gradient within a microfluidic cell culture chamber. To create functional retinal tissue from hPSCs, an array of microchannels with microfabricated rod structures that mimicked in vivo retinal tissue was fabricated [90]. hESCs that were cultured on the rod structures cocultured with retinal pigment epithelial cells were able to differentiate into photoreceptor cells. These results indicate that hPSCs could be used to create microtissues within a microfluidic device, thereby resulting in a body on a chip.

CONCLUSION

Body on a chip could contribute to human health and be an extremely useful application in nano/microengineering. However, in reality, body on a chip is still at the proof-of-concept stage, such as in terms of the placement of multiple tissues within a single device, and much effort is required for its development into a practical and useful technology.

To achieve this goal, it is necessary to advance individual technologies such as micro/nanoengineering, tissue

engineering, and stem cell manipulations and the integration of these technologies. This means that the development of body on a chip requires interdisciplinary research and, if achieved, it would not only represent a vast advancement in drug discovery but would also increase our understanding of specific disease mechanisms. In particular, in conjunction with hiPSCs, which are specified for rare diseases, body on a chip will serve as a new platform to study unsolved and unstudied mechanisms of rare diseases and provide cures for such diseases.

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