Chapter 8
Microfluidic Organs-on-Chips to Reconstitute Cellular Microenvironments

Yu-suke Torisawa

Abstract Recent advances in microsystems technology and tissue engineering have led to the development of biomimetic microdevices to model key functional units of human organs, known as organs-on-chips. By mimicking natural tissue architecture and microenvironmental chemical and physical cues within microfluidic devices, this technology realizes organ-level function *in vitro* that cannot be recapitulated with conventional culture methods. Since the physiological microenvironments in living systems are mostly microfluidic in nature, microfluidic systems facilitate engineering of cellular microenvironments. Microfluidic systems allow for control of local chemical gradients and dynamic mechanical forces, which play important roles in organ development and function. This organ-on-a-chip technology has great potential to facilitate drug discovery and development, to model human physiology and disease, and to replace animal models for efficacy and toxicity testing. This chapter shows an overview of the organ-on-a-chip technology to recapitulate cellular microenvironments and especially focuses on bone marrow-on-a-chip that enables culture of living bone marrow with a functional hematopoietic niche as a novel type of approach to develop organs-on-chips.

Keywords Organs-on-chips · Microfluidics · Tissue engineering · Cellular microenvironment

8.1 Introduction

8.1.1 Cellular Microenvironment

Cellular functions are precisely controlled by their specific microenvironment where they normally reside. The microenvironment contains a complex set of structural,

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chemical, and mechanical signals which are necessary to maintain cellular viability and function [1–3]. Since stem cells cannot maintain their stemness without specific stem cell niches [3–5], cellular microenvironments are crucial for maintaining cellular function, whereas current conventional culture methods do not contain these microenvironmental cues correctly. Because of this microenvironmental gap, cellular functions and responses *in vitro* are very different from those *in vivo*, and thus conventional 2D cultures cannot accurately predict cellular functions and responses inside the body [6–8]. It is necessary to reconstitute cellular microenvironments for developing reliable *in vitro* methods. Development of biomimetic microdevices that can recapitulate tissue structure and microenvironmental cues could be a useful platform to facilitate drug discovery and development and to develop predictive models of human physiology and diseases.

8.1.2 *Cellular Microenvironments Within Microfluidic Systems*

The physiological microenvironments inside the body contain largely microfluidic structures such as vascular networks and sinusoids. Thus, the use of microfluidic systems facilitates engineering of cellular microenvironments. Cell culture conditions in the microfluidic systems are very different from those in conventional culture dishes (Fig. 8.1); the volume of culture medium in the dish culture is much greater compared to cellular volume, whereas a majority of the volume in a tissue is taken up with cells inside the body [9]. This difference in fluid volume to cell volume ratio causes differences in the chemical microenvironment. The volume of culture medium in the microfluidic systems is nanoliter-scale which is similar to *in vivo*. Because of physiological ratios of cells to liquid volumes, microfluidic systems enable to maintain cellular interactions based on autocrine and paracrine signals, whereas dish cultures do not maintain these signals [9, 10]. This feature also facilitates the formation of local chemical gradients that may otherwise not be possible to be studied. Furthermore, microfluidic devices can apply fluidic shear stress, enabling reconstitution of the physiological mechanical microenvironment inside the body [11].

8.1.3 *Control of Chemical Microenvironments Using Microfluidic Systems*

Microfluidic cell culture systems are typically made from poly(dimethylsiloxane) (PDMS) using the soft lithography technique [12]. Microfluidic systems have been used to engineer chemical microenvironments such as concentration gradients of biochemicals. Gradients of biochemical signals (e.g. growth factors, hormones,

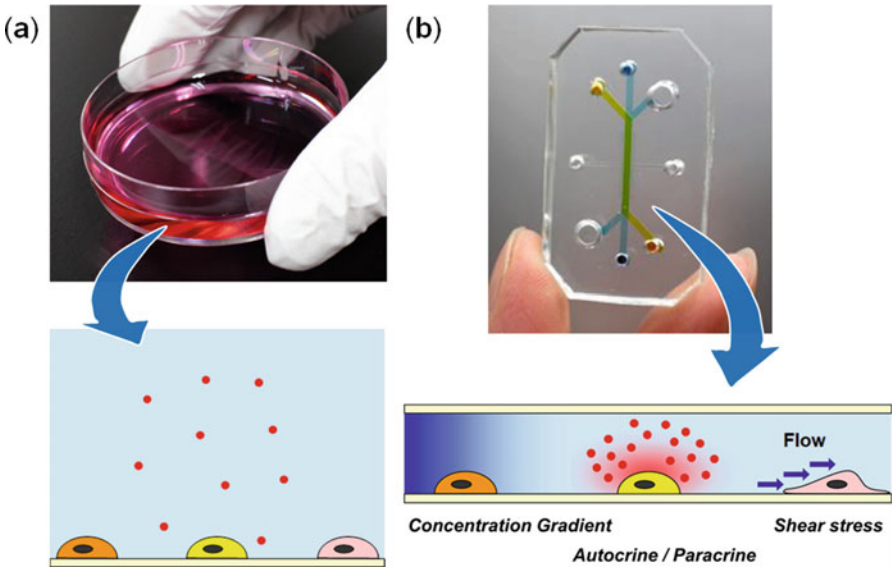

Fig. 8.1 Cellular microenvironment in a microfluidic culture and a conventional dish culture. (**a**) Cells cultured in a dish are maintained in a large volume of culture medium under static condition. (**b**) Cells cultured in a microfluidic device are maintained in a small volume of culture medium under static or dynamic conditions. Concentration gradients can be generated by flowing chemicals or cellular secretion and consumption and also fluid flow can generate shear stress within a microfluidic device

morphogens, and chemokines) play an important role in a wide range of biological processes including development, immune response, wound healing, and cancer metastasis [13, 14]. Although these biochemical gradients are very difficult to produce and maintain in conventional culture systems, microfluidic systems can generate arbitrary shapes of biochemical gradients [15, 16]. Thus, these microfluidic systems to generate concentration gradients have been used to study of immune responses, cancer metastasis, and stem cell biology [16–19]. Recently, development-on-chip has been developed by mimicking spatial and temporal chemical environments found *in vivo* during neural tube development using a microfluidic device [20]. This system was able to maintain simultaneous opposing and/or orthogonal gradients of developmental morphogens, resulting in neural tube patterning analogous to that observed *in vivo*.

Chemical gradients can be generated using cellular secreted factors by patterning different types of cells within microfluidic devices (Fig. 8.2). Cancer metastasis was modeled in a compartmentalized microfluidic device in which cancer cells were hydrodynamically patterned in a microchannel at spatially defined positions relative to source cells that secrete chemokines and sink cells that scavenge the chemokines (Fig. 8.2a) [21]. This system enabled to recreate a physiological cancer microenvironment, resulting in efficient chemotaxis under much shallower chemoattractant gradients than previously possible in other *in vitro* systems. These concentration

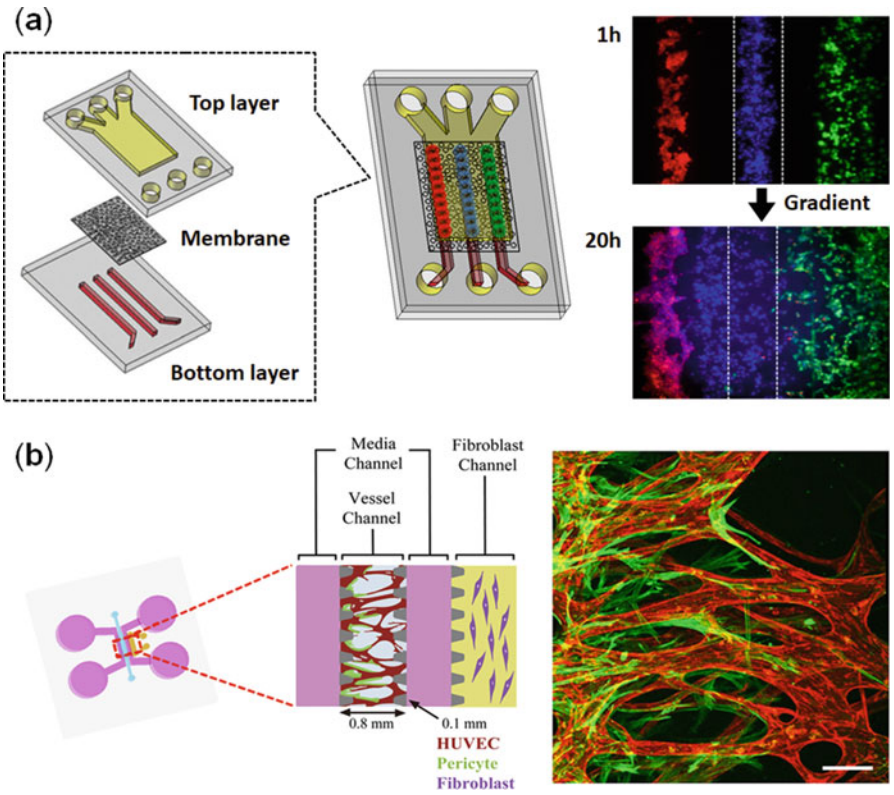

Fig. 8.2 Microfluidic co-culture systems to recreate chemical microenvironment. (**a**) A cancer metastasis model was engineered in a microfluidic device consisting of two PDMS microchannels separated by a porous membrane. Cancer cells (blue) were hydrodynamically patterned in the top channel at spatially defined positions relative to source (red) and sink cells (green), which generated chemoattractant gradients that induced cancer cell migration. (Reproduced from Ref. [21] with permission from the Royal Society of Chemistry). (**b**) A microfluidic system to form a 3D perfusable vascular network. The device consists of a central vessel channel, two adjoining media channels, and the outermost fibroblast channel. The vascular network (red) covered by pericytes (green) was formed in the central channel with assistance from the lateral fibroblasts. (Reproduced from Ref. [26]. Copyright 2015, Public Library of Science)

gradients of cellular secreted factors are very difficult to be maintained in conventional dish cultures. Using 3D cell culture techniques with microfluidic devices, 3D functional microvascular networks can be engineered [22–27]. Jeon's group has developed a microfluidic device which enables spatially patterned 3D co-culture of endothelial cells with stromal fibroblast cells [23] known to induce angiogenesis and support vascular formation (Fig. 8.2b) [25]. This system was able to model natural cellular programs found during development and angiogenesis to form perfusable networks of intact 3D microvessels. This system enabled the formation of 3D blood vessels with perfusable lumina that are similar in 3D architecture, intact barrier function, long term stability and salient biochemical markers to their *in vivo*

counterparts. This model can be used to study interactions between pericytes and endothelial cells [26] and the role of interstitial flow during the formation of neovessels [27]. Since vascular networks are crucial to the maintenance of cellular viability and function in tissues and organs [28], engineering perfusable 3D vascular networks that can deliver nutrients and oxygen as well as cells could be a powerful platform to develop *in vitro* systems. This method has recently been applied to novel methods to vascularize 3D cell spheroids where a perfusable vascular network connected to microchannels is formed [29, 30]. These microfluidic devices may provide a novel approach to culture 3D tissues as well as organoids [31].

8.2 Organs-on-Chips to Mimic Cellular Microenvironmental Signals

Organs-on-chips are microfluidic cell culture devices made from elastomer, typically PDMS. By recapitulating tissue architectures and chemical and mechanical microenvironments, these microfluidic devices reconstitute organ-level cellular functionality not possible with conventional culture methods [32–35]. Especially, these devices can mimic biomechanical signals which cells normally experience inside the body.

8.2.1 Lung-on-a-Chip

A representative example of organs-on-chips is the lung-on-a-chip which reconstitutes alveolar function in the human lung by mimicking tissue-tissue interface and breathing mechanical environment (Fig. 8.3a) [36]. This system consists of a compartmentalized microfluidic device in which human alveolar epithelial cells are cultured in apposition with human pulmonary microvascular endothelial cells on a thin porous ECM-coated PDMS membrane that resembles the *in vivo* alveolar-capillary interface. This system is integrated with a mechanical actuation system to mimic breathing motions by applying cyclic suction to distort the PDMS microdevice which cyclically stretches the thin PDMS membrane. This biomimetic microdevice enables the reconstitution of organ-level cell responses not normally observed in conventional culture systems, such as recruitment of immune cells in responses to bacteria. Moreover, this system has revealed unexplained adverse effects of breathing-induced mechanical forces; the cyclic breathing motions increased cellular uptake of silica nanoparticles and translocation of nanoparticles from the alveolar airspace to the vascular compartment. This effect was confirmed in a mouse *ex vivo* model. This system also enables to model pulmonary edema induced by toxicity of the cancer drug interleukin-2 (IL-2) [37]. Clinical relevant concentration of IL-2 administration into the vascular channel caused continuous

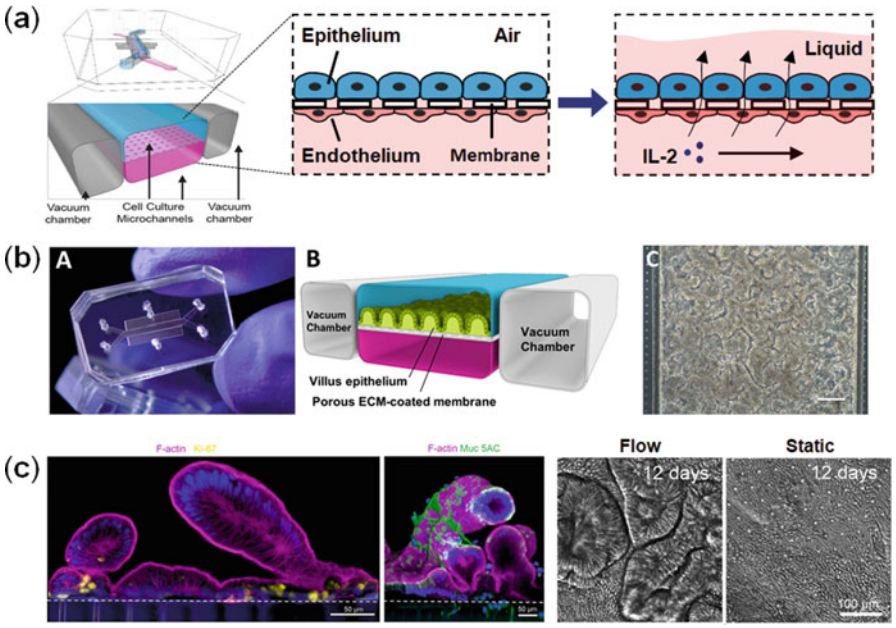

Fig. 8.3 Microfluidic organs-on-chips to recapitulate tissue architecture and mechanical microenvironment. (**a**) Lung-on-a-chip recapitulates the alveolar-capillary interface by culturing human alveolar epithelial cells on top of a thin porous PDMS membrane and human capillary cells on bottom. Breathing motions are recapitulated by applying cyclic suction to the side chambers, which deform the PDMS membrane to which the cell layers are attached. Administration of interleukin-2 (IL-2) into the vascular channel resulted in fluid leakage into the alveolar channel. (Reproduced from Ref. [44]. Copyright 2018, Nature Publishing Group). (**b**) Gut-on-a-chip. Photograph (A) and a schematic illustration (B) of the device in which human Caco-2 intestinal epithelial cells are cultured on a thin PDMS membrane to form 3D villi-like structures by applying peristalsis-like cyclic mechanical strain as well as fluid flow. (C) Micrograph of Caco-2 cells cultured for 6 days in the Gut-on-a-chip. Scale bar, 100 μm. (Reproduced from Ref. [43]. Copyright 2015, Public Library of Science). (**c**) Primary human small intestine-on-a-chip. A confocal image showing a cross-section of primary intestinal epithelium immunostrained (magenta, F-actin; yellow, Ki67; green, Muc5AC). Optical images of primary intestinal epithelium cultured on-chip for 12 days under continuous flow compared with a static culture condition. Formation of intestinal villi-like structures occurred only in the presence of flow. (Reproduced from Ref. [44]. Copyright 2018, Nature Publishing Group)

leakage of vascular fluid into the alveolar channel and complete flooding of the airspace (Fig. 8.3a). Importantly, this system revealed that the mechanical forces produced by breathing motions contribute to the development of increased vascular leakage and pulmonary edema. Thus, this system enabled the identification of novel therapeutics that inhibited mechanotransduction pathways. The lung-on-a-chip microdevice faithfully recapitulates organ-level cell function and predict physiological and pathological responses *in vivo*.

This system has been applied to a human lung small airway-on-a-chip which contains a differentiated, mucociliary bronchiolar epithelium exposed to air and an

underlying microvascular endothelium that experiences fluid flow [38]. This system enabled active synchronized cilia beating and mucociliary transport whose velocity was nearly identical to that in healthy human lung airway. Exposure of the epithelium to IL-13 in this device reconstituted the goblet cell hyperplasia, cytokine hypersecretion, and decreased ciliary function of asthmatics. The small airway-on-a-chip generated with epithelial cells from individuals with chronic obstructive pulmonary disease reconstituted features of the disease such as selective cytokine hypersecretion, increased neutrophil recruitment, and clinical exacerbation by exposure to viral and bacterial infections. Thus, this device allows for analysis of organ-level lung pathophysiology *in vitro*. Furthermore, this device enabled modeling of smoke-induced lung injury by connecting to a smoking instrument that inhales and exhales whole smoke from burning cigarettes in and out of the epithelium-lined microchannel of the device [39]. This system was used to compare the effects of inhaled smoke on chips containing bronchiolar epithelium isolated from normal lungs or from lungs of chronic obstructive pulmonary disease (COPD) patients. This model led to identification of ciliary micropathologies, COPD-specific molecular signatures, and epithelial responses to smoke generated by electric cigarettes. This smoking airway-on-a-chip provides a tool to study normal and disease-specific responses of human lung to inhaled smoke across molecular, cellular, and tissue-level responses.

8.2.2 Gut-on-a-Chip

A similar compartmentalized microfluidic system was used to create a human gut-on-a-chip system that mimics physiological peristaltic-like motion and fluid flow of the intestine (Fig. 8.3b) [40–43]. The device consists of two layer of cell culture channels separated by a thin porous PDMS membrane in which human intestinal epithelial Caco-2 cells (colorectal carcinoma line) are cultured. Caco-2 cells cultured with fluid flow and cyclic mechanical distortion in the microfluidic device spontaneously formed 3D structures that resemble the architecture of the intestinal villus. This system enabled to reproduce the intestinal barrier function, high levels of mucus production, and intestinal absorptive characteristics, which will be useful for drug testing as well as development of intestinal disease models. This system also enabled long-term co-culture of various gut microbes with the epithelial cells by recapitulating the dynamic mechanical microenvironment, whereas conventional culture methods cannot maintain co-culture of microbes. Lack of mechanical signals was shown to trigger bacterial overgrowth similar to that observed in patients with ileus and inflammatory bowel disease. Thus, the human gut-on-a-chip can be used to analyze contributions of microbiome to intestinal pathophysiology and disease mechanisms in a controlled manner.

Caco-2 cells are widely used to estimate intestinal barrier function; however, these cells are cancer-derived cells and have some issues such as enzymatic activity. Recently, primary human small intestine-on-a-chip has been developed using

intestinal biopsies [44]. Primary intestinal epithelial cells harvested from 3D organoids are cultured in a microfluidic device with human intestinal microvascular endothelium in a parallel microchannel under fluid flow. The intestinal epithelium formed villi-like structures lined by polarized epithelial cells that undergo multi-lineage differentiation (Fig. 8.3c). Transcriptomic analysis demonstrated that the intestine chip more closely mimicked whole human duodenum than duodenal organoids. This study demonstrated that recapitulation of the dynamic mechanical environment and tissue-tissue interface enabled the formation of 3D villi-like structures as well as the maintenance of intestinal functions. Since this system can maintain normal human intestinal function, it will be a powerful platform to study drug pharmacokinetics and to model human intestinal diseases.

8.2.3 Kidney-on-a-Chip

In vitro systems to model kidney functions are crucially needed to predict drug-induced kidney injury which is often observed in pharmacotherapy; however current available models do not recapitulate the biological functions of the kidney and poor predictions of drug-induced kidney injury [45]. A kidney proximal tubule-on-a-chip microdevice was developed that is lined by human proximal tubule epithelial cells exposed to physiologically relevant fluidic flow [46]. Mechanical forces caused by fluid flow have been recognized as a key determinant of cellular functions in the kidney. This microfluidic device consists of a luminal flow channel and an interstitial compartment separated by a thin porous membrane on which the kidney cells are cultured. This device demonstrated that low levels of fluid shear stress (0.2 dyn cm^{-2}) similar to that observed in the collecting ducts and proximal tubules of the kidney enhanced differentiation, increased molecular and drug transport functions, and produced more *in vivo*-like toxicity responses. Thus, this kidney proximal tubular model can mimic the structural, mechanical, transport, absorptive, and physiological properties of the human kidney. A 3D flow-directed kidney proximal tubule system has also been developed using a microfluidic device [47]. Human proximal tubular epithelial cells are seeded onto collagen extracellular matrix within a microchannel so that the cells self-assemble to form a 3D tubular structure. This system recapitulates the perfusion delivery and transport pathway of a solute which is perfused into a surrogate vascular channel, diffuses through the pseudo-interstitial space, and undergoes uptake and efflux across the epithelial barrier into the flowing perfusate in the tubular luminal channel. This model replicates the polarity of the proximal tubule, retains polarized expression and function of protein essential for reabsorptive and secretory transport, responds to physiological stimuli, and performs critical biochemical synthetic activities. This system provides a platform for modeling of renal drug clearance and drug-induced nephrotoxicity.

A kidney glomerulus model has recently been developed using a compartmentalized microfluidic device with human iPS (induced pluripotent stem) cell-derived podocytes which can produce glomerular basement-membrane collagen [48]. This

device enabled to recapitulate the natural tissue-tissue interface of the glomerulus and the differential clearance of albumin and inulin by co-culturing podocytes and human glomerular endothelial cells under physiological fluid flow and cyclic mechanical strain. This device demonstrated that application of physiological relevant mechanical cues further augmented podocyte differentiation and maturation. Since existing immortalized podocyte cells and cultures poorly mimic glomerular function, this device could provide a better *in vitro* system for predicting nephrotoxicity and therapeutic development.

8.3 Bone Marrow-on-a-Chip to Reconstitute Hematopoietic Niche Physiology

Bone marrow is the only permanent hematopoietic organ in humans. Given the importance of bone marrow as the source of all blood cells, an *in vitro* culture system that can reconstitute function of living bone marrow will be a useful platform. The bone marrow microenvironment contains a complex set of cellular, chemical, structural, and physical cues necessary to maintain the viability and function of the hematopoietic system [4, 49]. This bone marrow microenvironment regulates the function of hematopoietic stem cells (HSCs), facilitating a balance between self-renewal and differentiation into progenitors that generate all mature blood cells. Current *in vitro* culture methods do not accurately model bone marrow physiology; hence, studies relevant to the hematologic system are usually conducted in animals [50–52]. Engineering an artificial bone marrow capable of reproducing the bone marrow microenvironment could be potentially used to study blood development and physiology, model diseases, and serve as a platform for drug development and toxicity studies.

8.3.1 Engineering of Bone Marrow In Vivo

Because of the complexity of the bone marrow microenvironment necessary to support HSC function as well as blood production, it has not been possible to recapitulate the complex bone marrow microenvironment as well as blood forming functions *in vitro* [52–55]. To overcome this challenge, we used a tissue engineering approach to first induce formation of new bone containing marrow *in vivo*, and then we surgically removed it whole and maintained it in a microfluidic device *in vitro* (Fig. 8.4a) [56]. New bone formation was induced in the subcutaneous tissue of a mouse by implanting a PDMS device containing cylindrical hole filled with a collagen gel and bone-inducing materials (demineralized bone powder and BMPs). This method produced a cylindrical shaped bone containing a bony cortex and an internal trabecular bone network that was filled with a normal appearing marrow

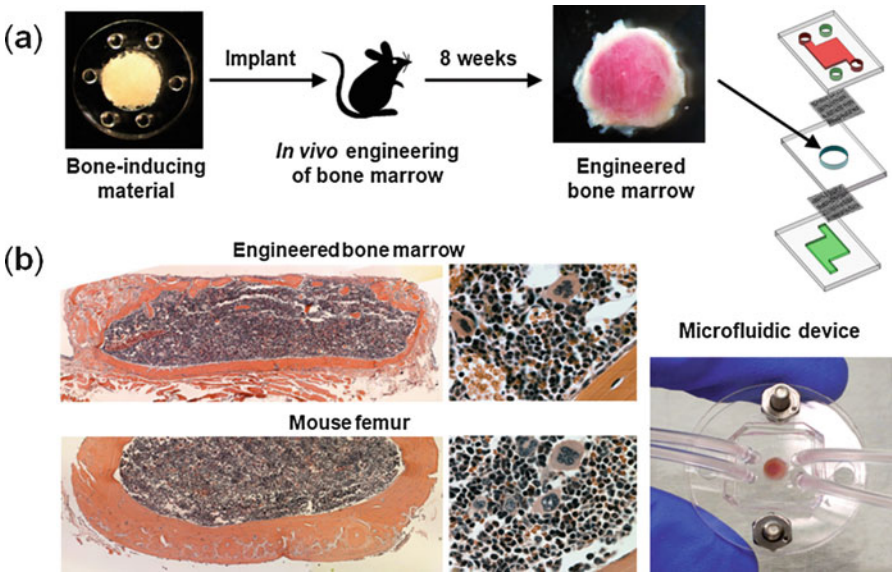

Fig. 8.4 Bone marrow-on-a-chip. (**a**) PDMS devices containing a cylindrical chamber filled with bone-inducing materials were implanted subcutaneously on the back of a mouse for 8 weeks and then surgically removed. The engineered bone marrow that formed within the PDMS device was placed into a similar shaped central chamber in a microfluidic system and then maintained in culture *in vitro*. (**b**) Low- (left) and high-magnification views (right) of histological hematoxylin-and-eosin-stained sections of the engineered bone marrow formed in the PDMS device at 8 weeks following implantation (top) compared with a cross section of bone marrow in the normal mouse femur (bottom). (Reproduced from Ref. [56] with permission from the Nature Publishing Group)

with a blood cell composition virtually identical to that of natural bone marrow (Fig. 8.4b). Previous reports showed that tissue engineering approaches with the bone-inducing materials resulted in the formation of marrow largely inhabited by adipocytes [57–59] which are known to inhibit hematopoiesis [60]. To reduce adipocyte content in the marrow, we used a PDMS device to restrict access of cells or soluble factors from the overlying adipocyte-rich hypodermis to the bone-inducing materials while maintaining accessibility to the underlying muscle tissue through the lower opening. Thus, the use of PDMS devices enabled to engineer bone marrow that resembles to natural bone marrow (Fig. 8.4b). We demonstrated that our method produced bone containing marrow with a hematopoietic cell composition nearly identical to that of natural bone marrow. Immunohistochemical analysis also confirmed that endothelial and perivascular cells as well as hematopoietic stem cells were located in their normal spatial positions in the engineered bone marrow. The presence of key cellular and molecular components of the hematopoietic niche indicated that the cellular content of the engineered bone marrow closely resembles the natural bone marrow microenvironment.

Several approaches have been reported that bone marrow can be engineered ectopically in mice. Human mesenchymal stem cells (MSCs) were seeded into

collagen-based scaffolds and cultured *in vitro* to form a hypertrophic cartilage tissue [61]. When the engineered cartilage tissues were implanted into mice for 12 weeks, these tissues formed fully-fledged bone containing marrow with hematopoietic stem and progenitor cells which can reconstitute multilineage long-term hematopoiesis in lethally irradiated mice. Matrigel has also been used to engineer bone marrow ectopically [62]. When human MSCs were suspended in Matrigel and subcutaneously injected into immunodeficient (NSG) mice, MSCs spontaneously formed a bone marrow cavity through a vascularized cartilage that was progressively replaced by hematopoietic tissue and bone. This humanized MSC-derived microenvironment permitted homing and maintenance of long-term mouse hematopoietic stem cells (HSCs) as well as human HSCs after cord blood transplantation. This xenotransplantation model also enables robust engraftment of primary acute myeloid leukemia samples at levels much greater than those in unmanipulated mice [63]. Direct intraossicle transplantation accelerated engraftment and resulted in the detection of higher leukemia-initiating cell frequencies. These humanized ossicle xenotransplantation systems allow to model wide variety of human hematologic diseases. Therefore, human MSCs can induce the formation of bone marrow ectopically *in vivo* when they are implanted with bone-inducing materials. The *in vivo* engineering of bone marrow offers a new approach for analysis and study of hematopoiesis and hematologic disease.

8.3.2 In Vitro *Culture of Bone Marrow with a Functional Hematopoietic Niche*

Various *in vitro* culture systems have been developed to maintain and expand HSCs and hematopoietic progenitors because the expansion of HSCs would greatly improve bone marrow transplantation and facilitate the development of advanced cell therapies for many blood disorders and malignant diseases [64–68]. Bone marrow stromal cells are mostly used to mimic the bone marrow microenvironment *in vitro* [68]. Recently, 3D culture systems have been develop using scaffolds or hydrogels in which bone marrow stromal cells are cultured with hematopoietic cells [53, 69–72]. Bone-derived materials have also been used as a scaffold to recreate the bone marrow microenvironment [73]. Frozen human bones were decellularized, demineralized, and cut into pieces to make bone-derived scaffolds so that cells can be cultured with those scaffolds in conventional culture plates. Bone marrow stromal cells such as mesenchymal stromal cells (MSCs) and osteoblasts were cultured within the bone-derived scaffolds with hematopoietic stem and progenitor cells (HSPCs), resulting in enhancing expansion of HSPCs compared with conventional 2D culture systems. Microfluidic culture systems with scaffolds also have been developed to maintain HSPCs for a long time. A 3D co-culture model based on a hydroxyapatite coated zirconium oxide scaffold consisting of human MSCs and HSPCs enabled the culture of HSPCs for 4 weeks within a microfluidic device

[74]. Although HSCs rapidly expand after transplantation *in vivo*, *in vitro* studies indicate that control of HSPC self-renewal and differentiation in culture remains difficult. There is currently no method to recreate the entire bone marrow microenvironment and to expand hematopoietic stem cells *in vitro* because of the complexity of the bone marrow microenvironment.

As a proof of concept, we tested whether the engineered bone marrow could maintain a functional hematopoietic system *in vitro* [56]. The engineered bone marrow formed 8 weeks after implantation was surgically removed from the mouse, perforated with a surgical needle to permit fluid access, placed in a similar shaped chamber in a microfluidic device, and perfused continuously with culture medium for *in vitro* culture (Fig. 8.4). Flow cytometric analysis of cellular components of the cultured 'bone marrow-on-a-chip' revealed that the presence of hematopoietic stem and progenitor cells in similar proportions to those of freshly harvested bone marrow for up to 7 days on-chip. In contrast, bone marrow cultured on a stromal feeder cell layer in a culture dish, which is the current benchmark, exhibited a significant decrease of long-term HSCs and a concomitant increase in hematopoietic progenitor cells relative to freshly isolated from natural mouse bone marrow. Thus, the long-term HSCs, which are the only cells capable of self-renewal and multilineage potential, appeared to be differentiating into more specialized progenitor cells in the static stroma-supported culture system as previously reports noted [68–72]. Thus, the bone marrow-on-a-chip enabled maintenance of a significantly higher proportion of long-term HSCs while more effectively maintaining distribution of hematopoietic progenitors as well as mature blood cells. Moreover, because the engineered bone marrow autonomously produces factors necessary to maintain hematopoiesis, this system was able to maintain HSCs and hematopoietic progenitors in normal proportions in the cultured chip without the addition of exogenous cytokines. Importantly, the full functionality and robustness of this engineered bone marrow tissue was confirmed by demonstrating that bone marrow cultured on-chip could be used to fully reconstitute the entire blood system when transplanted into lethally irradiated mice. Cells harvested from the engineered bone marrow after *in vitro* culture successfully engrafted the mice at a similar rate to that of freshly isolated bone marrow and repopulated all differentiated blood cell lineages. Thus, the bone marrow-on-a-chip retains fully functional, self-renewing, multipotent HSCs as well as a functional hematopoietic niche *in vitro*. Furthermore, because of microfluidic perfusion culture, this bone marrow-on-a-chip can produce blood cells continuously and release them into the microfluidic circulation, while maintaining HSCs and hematopoietic progenitors in normal *in vivo*-like proportions inside the microfluidic device for at least 2 weeks in culture [75]. This system was able to induce red blood cell production by adding erythropoietin (EPO) which is known to stimulate erythrocyte formation. When EPO was administrated in the bone marrow chip, we detected a continuous increase in the number of erythrocytes released in the outflow. Thus, this bone marrow-on-a-chip can be used to study and test continuous blood cell production *in vitro*. This method enabled to maintain an intact 3D bone marrow microenvironment with functional hematopoietic cells *in vitro*.

8.3.3 Testing of Drugs Using Bone Marrow-on-a-Chip

To evaluate whether the maintenance of an intact bone marrow microenvironment could be effective for efficacy and toxicity testing, we tested whether the bone marrow-on-a-chip can be used to test radiation toxicity and pharmacological countermeasures that might help radiation injured bone marrow repair itself [56, 75]. Despite an active search for medical countermeasures to radiation toxicity, no effective drug has been approved by FDA that could help protect or reconstitute the hematopoietic system [76]. Current drug development studies rely on animal studies; however, these animal models rarely reflect human response [77]. Thus, there is a critical need for predictive *in vitro* models to test effects of radiation countermeasures. When the bone marrow-on-a-chip was exposed to varying doses of γ-radiation, it showed a significant radiation dose-dependent decrease in the proportion of HSCs and hematopoietic progenitors which closely mimics what is observed in the bone marrow of live irradiated mice. In contrast, the stroma-supported static culture exhibited suppressed responses and was significantly more resistant to the effects of radiation toxicity. We then evaluated radiation-protecting effects of two potential therapeutic proteins, granulocyte-colony stimulating factor (G-CSF) [78] and bactericidal/permeability-increasing protein (BPI) [79], which have been reported to accelerate recovery of hematopoiesis after radiation-induced bone marrow failure *in vivo*. When G-CSF was administrated to the bone marrow chip 1 day after exposure to γ-radiation, we detected a significant increase in the number of HSCs and progenitors within the bone marrow chip compared to untreated irradiated bone marrow chips, and furthermore we detected significant increases in the number of HSCs, progenitors, and myeloid cells in the outflow of the chips. When BPI was administrated to the bone marrow chip 1 day after exposure to γ-radiation, it also significantly increased the number of HSCs and myeloid cells in the outflow of the chips. Thus, these radiation countermeasure drugs were able to accelerate recovery of blood cell production after radiation injury in the bone marrow-on-a-chip *in vitro* as they have previously been reported *in vivo*. In contrast, when a conventional static culture was exposed to γ-radiation, it was not able to detect any effect of BPI. Therefore, the bone marrow-on-a-chip effectively mimics the *in vivo* response of living bone marrow to radiation countermeasure drugs, whereas the conventional static bone marrow cultures do not. These results suggest that the presence of a hematopoietic microenvironment is crucial for modeling radiation toxicity and testing effects of potential countermeasure drugs *in vitro*. Thus, it is because conventional culture methods do not accurately recapitulate the bone marrow microenvironment that drug development studies currently rely on animal models for efficacy and safety testing. Since the bone marrow-on-a-chip system contains a functional bone marrow microenvironment, this system can mimic physiological responses to radiation countermeasure drugs normally only observed *in vivo* and thus this system could offer a novel approach to replace animal testing. Therefore, recapitulation of cellular microenvironment is crucial for efficacy and

toxicity testing and thus the organ-on-a-chip technology has great potential to generate reliable predictions of drug efficacy and toxicity in humans.

8.4 Culture of iPS Cells in Organs-on-Chips

The organ-on-a-chip technology has been used to culture human pluripotent stem cells including induced pluripotent stem cells (iPSCs) [80]. A microfluidic method has been reported to induce functional differentiation of human pluripotent stem cells on-chip [81]. This method demonstrated that extrinsic signal modulation though optimal frequency of medium delivery can be used as a parameter for improved germ layer specification and cell differentiation. This method enabled accurate spatiotemporal control of the soluble microenvironment around cells through regulation of periodic perfusion frequencies and achieved effective induction of hepatocytes on-chip with higher activity of hepatocyte specific functions under optimized perfusion conditions.

A disease model has been reported to reproduce disease pathophysiology *in vitro* using the organ-on-a-chip technology. A heart-on-a-chip was used to replicate contractile pathophysiology of Barth syndrome (BTHS) using engineered myocardial tissue assembled from BTHS or control iPSC-derived cardiomyocytes (iPSC-CMs) [82]. When iPSC-CMs were cultured onto thin elastomers micropatterned with fibronectin lines for 5 days, the iPSC-CMs self-organized into laminar anisotropic myocardium tissues. During electrical field stimulation, control iPSC-CM tissues contracted rhythmically, whereas BTHS iPSC-CM tissues developed significantly lower twitch and peak systolic stress compared to controls, demonstrating that BTHS engineered myocardial tissues replicate the BTHS myopathic phenotype. Importantly, this method enabled to model disease correction; treatment of BHTS iPSC-CMs with TAZ modRNA for 5 days restored contractile function of the cardiomyocyte tissues to levels comparable to those of controls. Patient-derived iPSCs have considerable potential to facilitate study of human disease and enable therapeutic screening [83]; however, currently there is the lack of *in vitro* models that reproduce disease pathophysiology. Thus, the use of organ-on-a-chip systems with the iPS cell technology could be a powerful platform to develop *in vitro* disease models [84].

Microfluidic systems have recently been utilized to engineer blood cells from pluripotent stem cells. To produce platelets, microfluidic bioreactors have been developed by mimicking the bone marrow and blood vessel microenvironments [85, 86] because platelets are generated from megakaryocytes in the bone marrow vasculature *in vivo* [87]. These bioreactors recapitulate extracellular matrix components, stiffness, endothelial cell contacts, and vascular shear forces. These systems demonstrated that physiological shear stress triggers proplatelet production and platelet release. By recapitulating physiological microenvironments, these bioreactors enabled the production of functional human platelets from megakaryocytes derived from adult hematopoietic progenitor cells as well as human iPSCs. The

iPSC-based technology has recently enabled to produce stable immortalized megakaryocyte progenitor cell lines [88]. These iPSC-derived megakaryocyte progenitor cells can be expanded, cryopreserved, and differentiated into functional platelets. Producing platelets from iPSCs *in vitro* could offer donor-free blood products as well as genetically modified products and provide a way of autologous transfusion [89].

Microfluidic systems that can recapitulate biomechanical forces have also been used to engineer HSCs. It has been demonstrated that biomechanical forces, such as blood flow-induced shear stress, play an important role in hematopoietic development and the emergence of HSCs [90, 91]. The use of microfluidic systems enabled to apply physiological fluid shear stress (5 dyn cm^{-2}), demonstrating that fluid shear stress endows long-term multilineage engraftment potential on early hematopoietic tissues not previously shown to harbor HSCs [92]. This study revealed that effects on hematopoiesis are mediated in part by a cascade downstream of shear stress that involves stimulation of prostaglandin E_2 (PGE_2)-cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling axis. Using mouse embryonic stem cells differentiated *in vitro*, it has been demonstrated that fluid shear stress increases hematopoietic colony-forming potential and expression of hematopoietic markers [93]. Thus, the use of the organ-on-a-chip technology that can recapitulate cellular microenvironment and biomechanical signals could facilitate engineering of HSCs [94]. Recently, production of HSCs from iPSCs has been achieved using transcription factors as well as *in vivo* transplantation [95]. Human iPSCs were differentiated into hemogenic endothelial cells *in vitro* and then treated with cocktails of transcription factors to endow the potential to engraft multi-lineage hematopoiesis *in vivo*. This method requires *in vivo* microenvironment to mature HSCs, suggesting signals from the microenvironment is necessary for HSC maturation. Therefore, the organ-on-a-chip technology may provide a way to produce HSCs *in vitro* by mimicking the microenvironment inside the body.

8.5 Future Prospects

Organ-on-a-chip microdevices produce organ-level function *in vitro* by recapitulating natural tissue arrangements and microenvironmental signals. Since these microdevices are created using human cells including primary human cells and cell lines as well as pluripotent stem cell-derived cells, these devices can predict human responses to medications, which provides reliable prediction of drug efficacy and safety in humans. These systems have also potential to serve as a platform to differentiate pluripotent stem cells into specific cell types. The use of iPS cells with organ-on-a-chip microdevices is especially attractive because iPS cells provide sources of patient-specific cells and enable genome editing. These systems enable to model human physiology and disease and further will provide opportunities to model organs that have not yet been possible to be studied *in vitro*.

As a novel approach to develop organs-on-chips, we have developed the bone marrow-on-a-chip in which an organ, bone marrow, is engineered inside a device *in vivo* and then maintained in culture. This system can sustain an intact bone marrow with a functional hematopoietic microenvironment *in vitro*, enabling to mimic complex tissue-level responses to radiation toxicity and to therapeutic agents. This system provides an interesting alternative to animal models because this offers the ability to manipulate individual hematopoietic cell populations or to insert other cell types such as cancer cells *in vitro* as well as *in vivo* before analyzing the response of the intact bone marrow to relevant clinical challenges. It is also possible to generate human bone marrow models by engineering bone marrow in immunocompromised mice and inserting human hematopoietic cells or human leukemia cells [63]. Therefore, this method offers a new approach for analysis of drug responses and toxicities and for study of hematopoiesis and hematologic diseases.

These organ-on-a-chip microdevices can be integrated via microfluidic linkage to model physiological interplay between different organs. Body-on-a-chip systems have been developed by integrating several organ models (typically 2~4 types) into a microfluidic device [96–100]. These systems realize to estimate the adsorption, distribution, metabolism, and elimination (ADME) of drugs *in vitro*. For example, when a drug was introduced into a microfluidic device, the drug converted in a liver compartment into its reactive metabolites and then circulated to other organ compartments such as a lung compartment or a cancer compartment to detect drug toxicity or effect. It might be possible to develop 'human body-on-a-chip' consisting of fluidically linked chambers representing different organs that could reliably predict drug toxicity and efficacy inside the human body in the future. Although the organs-on-chips have great potential, this technology still requires further validation and improvement. For integration of different organ chips, it requires a novel common culture medium, such as blood substitute, that can be perfused through the entire system to maintain viability and functions of all the cells on a chip. Furthermore, although PDMS devices are widely used, the use of PDMS could raise a problem for drug discovery applications because it absorbs small hydrophobic molecules. By improving materials, cell culture techniques, and tissue engineering techniques, organs-on-chips could provide novel approaches for drug discovery and development, which could reduce or replace animal testing in the future.

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