Editorial

Engineering bone marrow-on-a-chip

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Bone marrow is the permanent hematopoietic organ; the main function is to produce blood cells for the body through life. The microenvironment of bone marrow contains a complex set of cellular, chemical, and physical signals to maintain the hematopoietic system (1-3). The hematopoietic niche regulates hematopoietic stem cells (HSCs) to facilitate a balance between self-renewal and differentiation into progenitor cells that produce all types of blood cells. Given the complexity of the bone marrow microenvironment and the hematopoietic niche necessary to support HSC function and blood production, it has been difficult to recapitulate the hematopoietic niche as well as blood-forming function *in vitro*. Current *in vitro* systems do not accurately model bone marrow physiology; hence studies on blood cells and hematopoiesis are usually conducted in animals. Thus, engineering a bone marrow mimicry that reconstitutes the bone marrow microenvironment and function will be a powerful platform to study hematopoiesis and bone marrow toxicities as well as to test new therapeutics. Recent advances in microsystems technology and tissue engineering have led to the development organs-on-chips microsystems to model key functional units of human organs by recapitulating natural tissue architecture and microenvironmental chemical and physical cues (4). The organs-on-chips microsystems could provide novel approaches to recapitulate bone marrow structures and functions and to study hematopoiesis and hematologic diseases *in vitro*.

In vitro culture systems were initially developed to maintain and expand HSCs using bone marrow-derived stromal cells (5). Since HSC transplantation can restore a functional hematopoietic system and blood production in recipients, the expansion of HSCs would greatly improve transplantation therapy and facilitate the development of advanced cell therapies as well as gene therapies for blood disorders and malignant diseases. Various types of culture methods have been developed to expand HSCs *in vitro* (5-8); 3D culture systems have recently been developed using scaffolds or hydrogels in which bone marrow stromal cells are cultured to mimic the bone marrow microenvironment (8-10). These 3D culture methods can maintain hematopoietic stem and progenitor cells more efficiently compared with conventional 2D cultures; however, it remains difficult to control HSC self-renewal and differentiation as well as to expand HSCs while maintaining their stemness *in vitro*. To overcome this challenge, we used tissue engineering approach to form entire bone marrow inside a device *in vivo* and then we removed it whole and maintained it *in vitro* (11). New bone formation was induced in the subcutaneous tissue of a mouse by implanting a small device made of poly(dimethylsiloxane) (PDMS) containing a cylindrical cavity filled with bone-inducing materials. This method was able to produce bone containing marrow with a blood cell composition virtually identical to that of natural mouse bone marrow. The use of PDMS devices enabled to engineer bone marrow that closely resembles the natural bone marrow by restricting access of cells and soluble factors. The engineered bone marrow formed 8 weeks after implantation was surgically removed from the mouse and placed in a similarly shaped chamber within a microfluidic device for *in vitro* culture. This bone marrow-on-a-chip microsystem enabled maintenance of hematopoietic stem and progenitor cells as well as mature blood cells, in proportions that were nearly identical to those observed in natural bone marrow. Importantly, this system was able to retain fully functional HSCs and a functional hematopoietic niche *in vitro*. In addition, this bone marrow-on-a-chip system was able to mimic tissue-level marrow responses to radiation toxicity normally only

observed *in vivo*, and to test the therapeutic responses of countermeasure agents that have been shown to accelerate recovery of hematopoiesis after radiation-induced toxicity in animals (11,12). In contrast, these responses could not be replicated by conventional 2D culture methods. This is mainly because conventional culture methods do not recapitulate the bone marrow microenvironment, indicating that the reconstitution of a functional bone marrow microenvironment is crucial for modeling radiation toxicity and testing drugs. Thus, this bone marrow-on-a-chip system offers a new approach for analysis of drug responses and toxicities. Furthermore, since mature blood cells are continuously generated in the outflow from the bone marrow chip (12), this microfluidic system can be used to study blood cell production and may be a new source of generating blood cells for perfusing other organs-on-chips systems or as cellular therapeutics.

This method still uses mice; however, it should be possible to generate human bone marrow models by engineering bone marrow in immunodeficient mice and replacing their marrow cells with human hematopoietic cells. Using human bone marrow-derived mesenchymal stem cells (MSCs), a human hematopoietic niche can be engineered (13). When human MSCs suspended in extracellular matrix were subcutaneously implanted into immunodeficient NSG mice, MSCs spontaneously formed a humanized ossicle with an accessible bone marrow microenvironment that recapitulated bone marrow niche morphology and functions. This humanized ossicle permitted homing and maintenance of human HSCs as well as human leukemia cells. Thus, this xenotransplantation approach provides a way for modeling human hematopoiesis and hematologic diseases, suggesting a possibility of the formation of human bone marrow models.

The use of microfluidic systems also facilitates engineering of cellular microenvironment. Formation of a functional 3D vascular network has been achieved using a microfluidic system (14). Co-culture of vascular endothelial cells with stromal fibroblast cells within a fibrin gel embedded in a microfluidic device enabled the formation of a perfusable network of intact 3D microvessels. This method can be used to engineer vascularized 3D cell constructs in which a vascular network connected to microchannels is formed through 3D cell aggregates (15). Solution containing reagents and cells can be perfused inside 3D cell aggregates through blood vessels by injecting it into a microchannel connected to the blood vessels. This system can model cellular interactions through a vascular network; migration of cancer cells through blood vessels was significantly higher toward bone-like microenvironment formed by osteo-differentiated MSCs compared with the microenvironment formed by undifferentiated MSCs. Since current 3D culture systems lack a vascular system to transport nutrients and reagents as well as cells, engineering vascularized 3D cell constructs could be a useful platform to culture cell spheroids and organoids (16). Furthermore, it might be possible to vascularize the engineered bone marrow tissue formed *in vivo* using this technique. Because a perivascular niche has been demonstrated to play an important role in the maintenance of HSCs, engineering a vascularized bone marrow mimicry could be a powerful tool for the study of hematopoietic function and niches and for modeling bone marrow pathophysiology.

Very recently, a novel human bone marrow-on-a-chip microfluidic system has been developed for modeling bone marrow functions and disease states (17). This system consisted of two microchannels separated by a porous membrane. The top 'hematopoietic' channel was filled with a fibrin gel in which $CD34^+$ hematopoietic progenitor cells were co-cultured with bone marrow-derived stromal cells, while the bottom 'vascular' channel was lined by human umbilical vein endothelial cells. This bone marrow chip could maintain human $CD34^+$ hematopoietic cells and support differentiation and maturation of multiple blood cells over 1 month *in vitro* by perfusing culture medium through the vascular channel. Furthermore, this bone marrow chip could recapitulate human myeloerythroid injury responses to drugs and radiation exposure as well as hematopoietic abnormalities observed in patients with the genetic disorder, Shwachman-Diamond Syndrome. Thus, this human bone marrow-on-a-chip system provides a novel *in vitro* model of human hematopoiesis for modeling clinically relevant features of bone marrow pathophysiology. These human organs-on-chips microsystems have great potential to generate reliable predictions of drug efficacy and toxicity in humans as well as models of human disease.

Another important application of the organs-on-chips is to engineer blood cells including HSCs. A recent study has demonstrated that functional HSCs could be generate from induced pluripotent stem cells (iPSCs) (18). Immature HSCs were generated from iPSC-derived hemogenic endothelial cells using seven transcription factors *in vitro* and were then transplanted into bone marrow of mice to generate functional HSCs. This study suggests that the bone marrow microenvironment plays an important role in the generation of HSCs. Although this method uses mice, it might be possible to engineer HSCs from induced pluripotent stem

(iPS) cells by using bone marrow-on-a-chip that recapitulate the bone marrow microenvironment *in vitro*. Microfluidic systems have also been used to promote blood development by mimicking biomechanical forces observed *in vivo* (19,20). Fluidic shear stress has been demonstrated to promote HSC emergence through endothelial-to-hematopoietic transition (20). Thus, organs-on-chips microsystems provide new avenues to engineer blood cells and HSCs from iPS cells. It might be possible to engineer a bone marrow mimicry using human iPS cells or patient-derived iPS cells in the future. Therefore, human bone marrow-on-a-chip will be a powerful method to study hematopoietic niches and function as well as hematologic diseases.

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