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LEADING ARTICLE

Organ-On-A-Chip: Development and Clinical Prospects Toward Toxicity Assessment with an Emphasis on Bone Marrow

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Abstract Conventional approaches for toxicity evaluation of drugs and chemicals, such as animal tests, can be impractical due to the large experimental scale and the immunological differences between species. Organ-on-a-chip models have recently been recognized as a prominent alternative to conventional toxicity tests aiming to simulate the human in vivo physiology. This review focuses on the organ-on-a-chip applications for high-throughput screening of candidate drugs against toxicity, with a particular emphasis on bone-marrow-on-a-chip. Studies in which organ-on-a-chip models have been developed and utilized to maximize the efficiency and predictability in toxicity assessment are introduced. The potential of these devices to replace tests of acute systemic toxicity in animals, and the challenges that are inherent in simulating the human immune system are also discussed. As a promising approach to overcome the limitations, we further focus on an in-depth analysis of the development of bone-marrow-on-a-chip that is capable of simulating human immune responses against external stimuli due to the key roles of marrow in immune systems with hematopoietic activities. Owing to the complex interactions between hematopoietic stem cells and marrow microenvironments, precise control of both biochemical and physical niches that are critical in maintenance of hematopoiesis remains a key challenge. Thus, recently developed bone-marrow-on-a-chip models support immunogenicity and immunotoxicity testing in long-term cultivation with repeated antigen stimulation. In this review, we provide an overview of clinical studies that have been carried out on bone marrow transplants in patients with immune-related diseases and future aspects of clinical and pharmaceutical application of bone-marrow-on-a-chip.

Key Points

Organ-on-a-chip systems use microscale technologies to replicate the physiological environment at the cellular level.

Combined with bone-marrow-on-a-chip as the hub of the mammalian immune system, the organ-on-a-chip system can be used to study the effect of drugs on organ function or toxicity of various chemicals on tissues.

1 Introduction

Three-dimensional (3D) cell and tissue culture models are being developed with the aim of reproducing the physiological microenvironment in vitro for efficiency and toxicity evaluation of drugs, chemicals, and biochemical agents. The combination of microfluidic and microelectromechanical (MEMS) devices with 3D culture systems

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has further facilitated the evolution of 'lab-on-a-chip' into 'organ-on-a-chip' devices to investigate cellular behavior in response to exogenous substances [1–5]. Current approaches to in vitro drug assessment (e.g., drug screening) focus on developing standardized platforms to overcome limitations regarding toxicity testing in laboratory animals due to the lack of suitable test subjects, practicality of testing conditions, and ethical issues [6]. In this context, organ-on-a-chip devices have rapidly advanced to improve early toxicity assessments of candidate drugs as an alternative to conventional animal tests. Therefore, it may be possible to standardize time-consuming pre-clinical screenings and carry them out efficiently and cost effectively while including many more experimental variables than what is feasible with conventional methods.

2 Organ-on-a-Chip: Towards Improved Toxicity Assessment

According to the United Nations (UN), many pharmaceutical products have been banned, withdrawn, severely restricted, or not approved by governments after toxicity tests [7]. Moreover, to assess the potential hazard of new chemicals for regulation, pre-clinical toxicity testing commonly requires the use of a large number of animals, raising the cost of drug development [8]. This cost of toxicity testing challenges the effectiveness of the current evaluation system, thus high-throughput methodologies for drug screening could represent a viable alternative. The effectiveness of conventional substance testing approaches—in vivo animal testing and human cell-culture-based testing—is limited by short substance exposure times (from a few hours to a few days), a lack of human-like properties to replicate a physiologic ratio of blood to cells [9], and inefficient evaluation of drug combinations [10]. Thus, many drugs in development fail the clinical tests due to toxic effects on tissue that were not revealed in previous screenings [11]. The inability to predict the side effects of drugs using animal models may come, for example, from inter-species differences in immune systems [12]. Therefore, organ-on-a-chip systems have emerged as an efficient platform for understanding human physiological responses of engineered organs to various drugs. In this context, many studies have focused on generating organ-on-a-chip models of the liver and kidney due to the importance of theses organs in drug metabolism and clearance [13–15]. Models of other important organs, e.g., lung [16, 17], skin [18–20], and the gastrointestinal (GI) tract [21, 22], have also been developed for toxicity testing, since those organs are closely related to allergic and immunogenic disorders caused by exogenous substances [23, 24].

The concept of organs-on-a-chip for high-throughput screening of candidate drugs against toxicity originates in a combination of microfabrication and tissue engineering techniques [25, 26] aiming to mimic in vivo physiology with accurate spatiotemporal regulation [27, 28]. Commercial cell lines are generally used in the design and optimization stages of organ-on-a-chip development, whereas primary and pathological cells can be implemented once the system has proven to be reliable. Cell lines are useful in initial screening steps due to less complicated culture conditions and fast growth, which benefits high-throughput analysis. However, primary cells are often more sensitive to certain pharmaceuticals than commercial cell lines, therefore providing more predictive toxicity results relevant to the given tissue of interest. Moreover, recent studies have demonstrated that induced pluripotent stem cells (iPSCs) can recapitulate the phenotype of several known diseases, making iPSCs a promising cell source for predictive drug screening [11, 29]. For instance, differentiation of iPSCs (or embryonic stem cells [ESCs]) can be controlled using organ-on-a-chip systems to generate specific responses [30]. Hence, the concept of organ-on-a-chip is a promising platform for the testing of pharmaceuticals [9] and the screening of existing therapies [31] as a cost-efficient and effective alternative to animal models.

3 Fundamental Strategies to Maximize the Efficiency and Predictability of Organs-on-a-Chip in Toxicity Assessment

Natural tissue environments are highly complex, exhibiting a high degree of hierarchical organization [28]. Tissue functions are influenced by this hierarchical organization, starting with a single cell and leading to functional subunits that form the tissue and maintain suitable organ function [32]. Thus, a major goal in designing organs-on-a-chip is developing structure–function relationships in engineered constructs by mimicking the hierarchical tissue architectures such as those of laminar cardiac muscle [33], human intestinal villi [34], and hepatic cord. For instance, Nakao et al. [35] proposed a microfluidic device to replicate a biomimetic structure helping hepatocytes to remain viable and maintain liver-specific functions for longer periods than on a planar two-dimensional (2D) culture environment. Vascularization is another essential element in reconstructing functional subunits in vitro to maintain cell viability and tissue functions [36]. For example, Sudo et al. [37] co-cultured primary rat hepatocytes and rat/human microvascular endothelial cells (rMVECs/hMVECs) on the sidewall of a collagen gel scaffold under controlled flow conditions. This work demonstrated that the 3D scaffold architecture in the organ-on-a-chip platform allows for

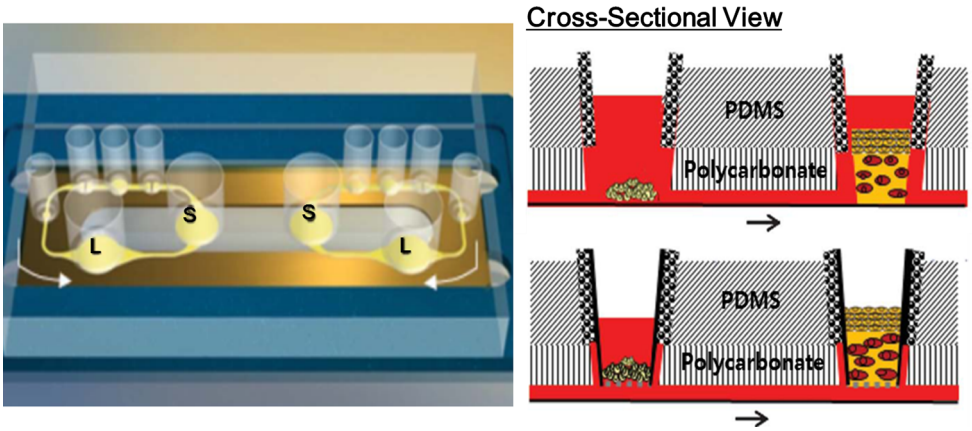

Fig. 1 Multiple-organs-on-a-chip model. A three-dimensional scaffold-on-chip model containing microfluidic paths in a poly(dimethylsiloxane)-glass chip was positioned into a multiple-organ-on-a-chip holder (*blue*). *Arrows* indicate the direction of fluid movement. Device compartments could support both submersed tissue cultures under fluid flow (*upper right image*) and cultures shielded from it (*lower right image*). Reproduced from Wagner et al. [20] with permission from The Royal Society of Chemistry. *L* liver culture compartments, *PDMS* poly(dimethylsiloxane), *S* skin culture compartments

cellular interactions that promote 3D capillary morphogenesis through vascular cells. The presence of shear stress was also shown to be responsible for the formation of endothelial vessel linings [38]. Such mechanical stimuli associated with physiological organ motion provide physical cues enabling specific function beyond static tissue culture [27]. Mechanical forces, e.g., cyclic pressure and shear stress from the blood stream, and tensile and compressive forces among cells can induce various cell responses [39, 40]. A well-known example was developed by Huh et al. [17], whose lung-on-a-chip model, along with a mechanically active cell culture environment, was used to investigate a human disease model. This device lent functional properties to the human pulmonary epithelial and endothelial cells by mimicking breathing movements and allowed for further observation of toxic side effects of anti-cancer drugs.

To overcome the poor predictability of in vitro drug toxicity, pharmacokinetic and pharmacodynamic (PK–PD) models are utilized to predict the time course of pharmacological effects. (Pharmacokinetics refers to a time-dependent concentration of a substance, whereas pharmacodynamics refers to pharmacological effect of a drug [41]). Combined with PK–PD mathematical modeling, multiple studies are geared towards multiple-organ-on-a-chip (MOC) devices to mimic in vivo physiological complexities and test, for example, for drug side effects. Sung and colleagues [42, 43], for example, combined colon cancer cells (HCT-11), hepatoma cells (HepG2/C3A), and myeloblasts (Kasumi-1) into 3D hydrogel cell cultures in separate bioreactor chambers to test colon cancer drugs. In this device, anti-cancer drugs were used to detect and observe complex interactions among multiple tissues because metabolism of drugs (e.g., conversion of a pro-drug) by specific liver enzymes may result in toxic events at a different organ. This MOC model was able to reproduce the metabolism of drugs by HepG2/C3A cells, and the effect of the drug on tumor cells was demonstrated using the PK–PD model-on-a-chip. The MOC device was found to correctly predict the physiological response in multiple tissues to this anti-cancer drug. In another application, Wagner et al. [20] developed a MOC model composed of separate standardized tissue culture spaces connected by microfluidic channels (Fig. 1). This study indicated that an improved dose-dependent sensitivity to a toxic substance can be achieved using an MOC platform for toxicity assays. Although this concept is still in the developmental stage, these devices will contribute to research and development of new medical compounds by enabling faster, more precise, and more sophisticated testing.

4 Development of Bone-Marrow-on-a-Chip through Systemic Optimization

4.1 Micro- and Macro-Environmental Requirements for Implementation of Engineered Bone Marrow

Bone-marrow-on-a-chip, a recent addition to the organ-on-a-chip field, is expected to bring forth a new breakthrough in research by offering a more controlled environment to study the tissue's physiological response [44]. Bone marrow is a soft, flexible, sponge-like tissue contained inside flat bones or in the cancellous (trabecular) bones of long bones. This tissue serves as the center of continuous production for blood and immune cells in the human body. In recent years, several attempts have been made to engineer

in vitro bone marrow, inspired by the natural structure. Since a great number of factors influence the development of artificial bone marrow, it is important to recapitulate its critical roles and their importance. In addition, hematopoietic stem cells (HSCs) in red marrow hardly maintain their multi-potency when physicochemical microenvironments fail to mediate their phenotype, leading to impaired hematopoietic activities. Thus, reciprocal interactions between hematopoietic, stromal, and osseous compartments are critical to maintain hematopoiesis. Thus, all of these factors should be considered as important determinants in recapitulating bone marrow to provide appropriate niches.

4.2 Unveiling the Component Mechanisms 1: Cellular Components and Biological Niches

Red marrow, occupying the majority of parenchyma, is the site where HSCs self-renew and differentiate into mature blood cells such as myeloids (e.g., white blood cells), erythroids (e.g., red blood cells), and other cells (e.g., macrophages, lymphocytes, or thrombocytes) that are distributed via the systemic circulation [45].

Yellow marrow makes up the majority of stroma and is no longer hematopoietically active, but is an indirect hematopoietic regulator that provides physiological microenvironments with physical cues, soluble factors, and feeder cells. Mixed populations of stromal cells are precisely controlled, and their significance is described in Table 1. Nestin$^+$ marrow stromal cells [known as mesenchymal stem cells (MSCs)] are found to be a critical player regulating the fate of HSCs as they tightly control HSC migration (or 'homing') [46] and express several HSC-maintenance transcription factors such as chemokine (C-X-C motif) ligand 12 (CXCL12) [47, 48], vascular cell adhesion molecules (VCAMs)-1 [49, 50], stem cell factor (SCF) [46], or osteopontin (OPN) [51] in notably higher amounts. Those two stem cells, MSCs and HSCs, are simultaneously regulated via neuronal (e.g., sympathetic nervous system) [47] and hormonal stimulations (e.g., parathormone) [52]. Accordingly, co-infusion of MSCs after myeloablative treatments showed positive clinical outcomes with improved hematopoietic recovery [53]. The multi-lineage characteristic of MSCs is responsible for creating hematopoietic supporting tissues or adipocytes inside bone marrow [54, 55]. Other cell types in stroma related to HSC niches have also been identified; perivascular cells, osteoblasts, endothelial cells, immune cells, and sympathetic nerves are described in Table 1 along with their roles in regulating the fate of HSCs.

Many in vitro studies have successfully expanded HSCs co-cultured solely with MSCs; among commercially available MSCs, bone marrow originated cells are favorable in HSC maintenance in comparison with those originating from the umbilical cord [56]. Raic et al. [56] have recently developed an RGD-functionalized

Table 1 Critical elements required in bone marrow niches

Descriptions	Examples
Cell-mediated interactions	
1. Cell–cell interactions between parenchymal and stromal cells	1. Mesenchymal stem cell—HSC mobilization and homeostasis (SCF, CXCL12, and Ang-1 [51, 65])
2. Synthesis of extracellular matrices	2. Perivascular cells—transit HSC and immune cells located near endosteum and vasculature (melanoma-associated cell adhesion molecule, CXCL12 [66])
3. Production of soluble molecules including growth factors, cytokines, hormones, and neurotransmitter	3. Endothelial cells—HSC maintenance and regeneration (E-selectin [67], CXCL12 [66])
4. Presenting membrane-bound ligands	4. Osteoblast—HSC and progenitor cell frequency (G-CSF, IL-1, and IL-6 [68, 69])
	5. Immune cells (macrophage, B- or T- lymphoid cell, dendritic cell, neutrophil, monocyte, antibody-secreting plasma cell [70])
	6. Sympathetic nervous system – HSC migration (norepinephrine [48]), nestin$^+$ MSC proliferation and osteoblastic differentiation (parathormone [51])
Mechanical environments	
1. 3D structure of scaffolds	1. Pore size of 15–25 μm [57] or below 100 μm [56]
2. High porosity for cellular migration and nutrient diffusion	2. CXCR4-SDF1α axis [71] for HSC migration
3. Large surface area for cell attachment	3. Adhesion mediated via integrin beta 1 [61]
4. Variable feasibility of structures for inducing chemical gradients (oxygen, SDF 1α or calcium)	4. Presence of regional hypoxia [72, 73] as a protectant against oxidative stress and to maintain stem cell properties
	5. Chemoattractive properties of calcium ion (Ca^+) [65]

Ang angiopoietin, *G-CSF* granulocyte colony-stimulating factor, *HSC* hematopoietic stem cells, *IL* interleukin, *SCF* stem cell factor, *SDF* stroma cell-derived factor

poly(ethylene glycol) diacrylate (PEGDA) hydrogel with a highly porous structure (40–100 μm pore size) induced by salt leaching. This allowed a mixed population of MSCs and HSCs to spontaneously migrate into the matrix, while the lyophilized scaffold was rehydrated with media [56]. However, in vivo functions of this analog have not been demonstrated. In other studies, the development of actual bone marrow was unsatisfactory when single cell types (HS-5 and hFOB) were used as feeder cells, indicating that a cocktail of supporting cells would be favorable [57].

In fact, forging such delicate and dynamic niches of human bone marrow has so far been difficult, as HSCs interact with multiple niches simultaneously and the microstructure within the marrow are hardly uniform, but rather variant (perivascular space, endosteum, stroma, hematopoietic space, sinusoids, artery, and capillaries). Heretofore, it is clear that no engineered environment is a perfect copy of the physiological one, but further research on standardized culture conditions and simplified essential elements of microenvironments may help to provide reproducible results.

4.3 Unveiling the Component Mechanisms 2: Mechanical Environments

In addition to bio-chemical cues, physical parameters, including temperature or the presence of shear forces, oxygen, or ion concentration gradients (e.g., Ca^{+}), also regulate HSC fate through cell–stroma interactions [45]. These factors are regarded as important aspects in mimicking natural physiology when engineering functional bone marrow (Table 1). In addition, highly porous structures of the cancellous bone enclosing the bone marrow accommodate the active transport of soluble molecules (e.g., growth factors or cytokines) that are vital to long-term maintenance, whereas sponge-like properties are important for stress and strain resistance [58]. The mean pore size of a scaffold needs to be optimized to provide a sufficiently large surface area to facilitate initial cell attachment and also to allow cells to infiltrate the construct [59]. Several studies revealed that 2D scaffolds failed to mediate the cultivation of engineered bone marrow with respect to the diffusion of soluble factors and cell–cell contacts [60, 61]. For instance, human CD34+ cells co-cultured with MSCs on 3D Puramatrix gel showed superior potency in maintaining primitive HSCs compared with 2D systems, with higher expression of Nestin and stroma cell-derived factor α (SDF1α) by MSCs [61]. The SDF1α/C-X-C chemokine receptor type 4 (CXCR4) axis is known to be essential for the retention of HSCs in the marrow [62]. Strikingly, the unique hypoxia gradient present in the in vivo niche was greater in the 3D matrix responsible for HSC quiescence than in the 2D case [61]. Nichols et al. [57] fabricated hexagonally packed spheres (100 μm diameter) with silicate or acrylamide hydrogels via inverted colloidal crystal geometry with interconnected pore size of 15–25 μm coated with nanocomposites for improved biocompatibility. The repeated and connected porous structures allowed continuous mass flow through the pores. A mixed population of primary bone marrow stromal cells including osteoblast lineage were co-cultured with primary CD34+ HSCs from bone marrow. About 40 % of the population remained undifferentiated for 28 days. During lipopolysaccharide (LPS) stimulation, B-lymphocytes were successfully activated, followed by the production of immunoglobulin M (IgM) antibodies against influenza infections in vitro. This observation was confirmed in vivo by implanting matrices in the backs of severe combined immunodeficiency (SCID) mice, resulting in the expression of mature leukocyte markers in circulation [57].

Several studies revealed that engraftment of osteo-conductive matrix with growth factors such as bone morphogenetic proteins (BMPs) within a collagen-based matrix was successful in bone tissue regeneration [44, 63, 64]. Along with these techniques, Torisawa et al. [44] showed a novel approach combined with microfluidic techniques to maintain in vivo bone marrow that was originally created in vitro, as a form of bone-marrow-on-a-chip. Type 1 collagen gel matrix containing demineralized bone powder (DBP) and BMP-2 was subcutaneously implanted in the back of mice, and a poly(dimethylsiloxane) (PDMS) cylindrical mold with open ports at the bottom was used as a carrier device. After 8 weeks, a bone-like tissue with a central marrow region was recovered exhibiting a proportion of hematopoietic cells similar to the native tissue. Interestingly, the in vitro culture of the bone-marrow-on-a-chip device was not significantly affected by the absence of exogenous cytokines, indicating that engineered bone marrow properly serves as a hematopoietic niche. This was further confirmed through the engraftment of green fluorescent protein (GFP)-labeled bone-marrow cultured on chip into γ-irradiated mice: differentiated lineages of immune cells were identified in normal proportions up to 16 weeks after transplantation [44]. This could potentially provide a functional platform to simulate bone marrow microenvironments, leading to the use of these artificial stem cell niches in a clinically relevant fashion.

5 Future Aspects of Clinical/Pharmaceutical Applications

Since the mid-twentieth century, HSC transplants have been performed in patients with hematological diseases, particularly in hematologic and lymphoid cancer patients. Numerous clinical studies have also been carried out on

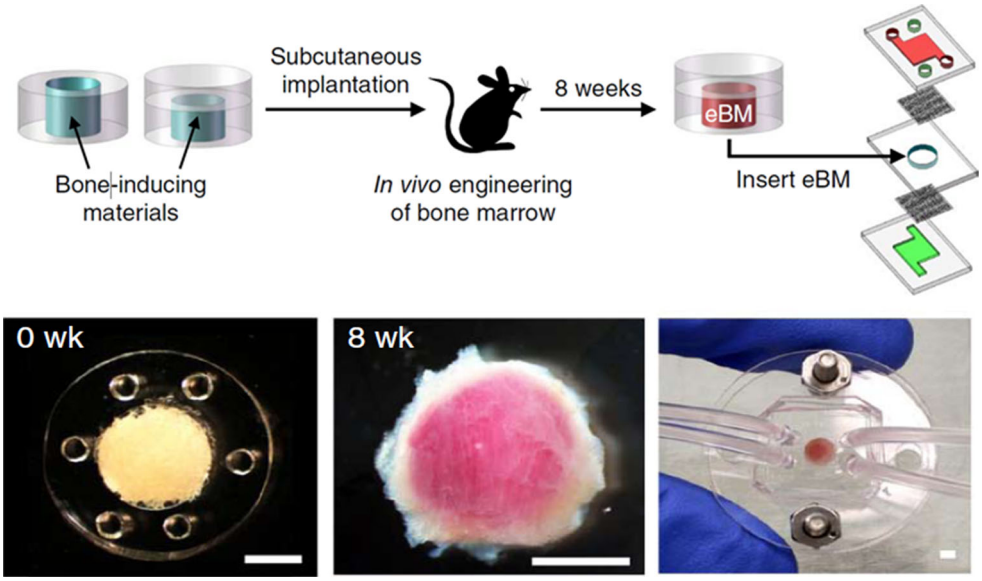

Fig. 2 Bone-marrow-on-a-chip model. The engineered bone marrow was formed in a poly(dimethylsiloxane) device and then cultured in a microfluidic system. Development of white cylindrical bone with *pink* marrow was evident after implantation (8 weeks). *Scale bar* 2 mm. Reproduced from Torisawa et al. [44] with permission from Nature Publishing Group. *eBM* engineered bone marrow

transplants in patients with immune-related diseases [74] such as myeloma [75] and acute lymphoblastic leukemia [76–79]. However, about one-third of patients in need of an allogeneic transplant are currently not matched with proper donors [80], and the cost for allogeneic transplantation is high (exceeding $US 200,000 per patient during first 100 days post-transplantation from year 2007–2009) [74]. Bone marrow donors within human leukocyte antigen (HLA)-identical siblings are even more rare [81]. Meanwhile, it has been shown that a single mouse CD34+ HSC was capable of restoring the irradiated lympho-hematopoietic system [82]. Along with aiding in the understanding of hematopoiesis mechanisms [83, 84], engineered bone marrow has risen as a potential cell source through ex vivo expansion and differentiation of HSCs and MSCs due to their innate self-renewal properties [85, 86].

Even if the patient's HLA matches closely with the allogeneic donor, the recipient will require immunosuppressive medications to mitigate an immune rejection of donor cells [i.e., graft-versus-host disease (GVHD)] [87]. Before an engraftment procedure, patients undergo either chemotherapy, radiation treatment, or both, called a 'conditioning' regimen. The myeloablative effects suppress the host's immune response after the transplant and allow new stem cells to grow. Herein, artificial bone marrow can provide a useful platform to test the potential outcomes. For instance, altered bone marrow cell proportions, such as neutropenia, anemia, or thrombocytopenia, is one of the side effects involved in chemotherapy or radiation therapy, resulting in critical imbalances in the immune system [88]. White blood cells are usually most susceptible, and tend to quickly drop in number due to their short lifespan, which in turn results in a high risk of infection. Moreover, this trend is not consistent with all types of drugs, pathological status, and the patient's treatment history. If the effect of treatments on the population of specific lineages can be predicted in engineered marrow, then the drug selection can theoretically be adjusted for different individuals. In this regard, Torisawa et al. [44] simulated the in vitro bone marrow response to drug toxicity and to the effects of poisoning protective drugs against γ-radiation in a dose-dependent manner on engineered bone marrow (Fig. 2). In this work, engineered bone marrow that underwent γ-radiation resulted in cell compositions comparable with the bone marrow of live irradiated mice. Moreover, the bone-marrow-on-a-chip validated the effects of the radiation countermeasure drug [granulocyte-colony stimulating factor (G-CSF)] showing that G-CSF accelerated recovery from radiation-induced toxicity as previously reported in vivo.

So far, studies on engineered bone marrow have shown improved clinical potential for research on disease development mechanisms due to the relevance of marrow in a wide range of pathologies. Researchers can track specific biomarkers and their changes in different microenvironments in certain diseases such as bone marrow-malignant cells interaction [89], hematopoiesis and hematologic diseases [44, 90], which paves the way for drug discovery as well as dealing with drug resistance. For example, Zhang et al. [89] demonstrated that patients with different stages of multiple myeloma (MM) show variant MM cell populations and drug resistance levels that are related to adhesion-mediated interactions with surrounding osteoblasts. This platform demonstrated that it can manipulate various biochemical cues to simulate complex and minute mechanisms that are not feasible in animal models. While the niches for stem cells and downstream blood cells in bone marrow are major concerns in drug testing,

adaptive immune response is a separate niche that is responsible for antibody secretion and maintenance of T and B cells. In the human immune system, bone marrow adaptive immunity functions such as antibody secretion in plasma cells and maintenance of the B- and T-cell vaccination memory result from re-infection by known pathogens. T and B memory lymphocytes and plasma cells in the red marrow are distributed to non-lymphoid organs (e.g., gut, lung, skin, and liver) through the bloodstream [23]. Thus, the immunity function of non-lymphoid organs should be considered together with bone-marrow immunity when the bone-marrow-on-a-chip device is established for systemic immune mechanisms. Therefore, bone-marrow-on-a-chip combined with chip-based non-lymphoid organ models is a promising alternative to efficiently replicate the human immune system for understanding human immunogenicity covering autoimmune diseases [91], inflammation [92], and the development of targeted therapeutics.

6 Concluding Remarks

In most cases, conventional animal studies show limited throughput capacity for drug testing [93, 94] and are impractical due to the large experimental scale and the distinctive immunological differences between species [12]. In fact, animal models such as murine tissues would not be representative of human physiology, due to their differential genomic responses in inflammatory stress [95] and expression of CD34 markers [96]. Any differences in immune phenotype or immunological process limit the applicability of the results to the human immune system [12]. Organ-on-a-chip models would be a more efficient way when testing multiple combinations of drugs [10] and when personalized therapeutics are to be developed (e.g., patient-specific 3D tissue model for multiple myeloma treatment [89]). However, in vitro cultures using on-chip devices are limited in their ability to simulate human metabolism (e.g., conversion of a pro-drug) that occurs as a result of organ–organ or tissue–tissue interactions. This leads to poor prediction of therapeutic action or toxic side effects of the drug [97]. Unforeseeable immunogenicity against drugs is another challenge for drug screening. In this regard, bone-marrow-on-a-chip would be a breakthrough for toxicity assessment. However, due to the extremely intricate structure of bone marrow, a precise copy of the tissue topography and geometry has been difficult to achieve. As current studies have focused on regional mechanisms, models combining bone marrow with other engineered organs are certain to improve the understanding of the entire mechanism. Moreover, MOCs replicating the human immune system would present an advantageous approach in evaluating effects of secondary metabolites on different tissues and emulating multi-organ interactions (e.g., liver, brain cortex, and bone marrow [98]; liver, tumor, and marrow [42]). However, as these systems are currently unable to replicate the human immune system (e.g., immune cell distribution to non-lymphoid organs), it is therefore an area of research that will require significant effort in coming years.

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