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Microphysiological systems in the evaluation of hematotoxicities during drug development

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Abstract

Microphysiological systems are progressively entering the pharmaceutical industry, and various systems have already proven to be highly valuable at different stages of the drug development process. The field of hematotoxicity research has so far received only minor attention, even though microphysiological systems might provide key benefits over current assays. In this review, we will highlight the need for more complex human *in vitro* assays, and how emerging technologies such as microphysiological systems present novel solutions for the study of adverse hematologic effects.

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Introduction

The hematopoietic tissue is an elaborately orchestrated system responsible for producing all major blood cell types. The high proliferation rate of most of the associated hematopoietic stem and progenitor cells (HSPCs), and the extensive blood perfusion make this system a sensitive target for xenobiotic interference. At the extreme, a compound can be toxic directly to the sensitive cells of the bone marrow leading to anemia, neutropenia, thrombocytopenia, or pancytopenia. These may result in poor oxygen saturation, susceptibility to infection, clotting deficiencies, or respective combinations. The seriousness of these effects makes high demands on the preclinical screening tools and assays for the detection of hematotoxicity. Biotherapeutics were initially thought to have only limited potential for side-effects because of their specificity of binding. However, unexpected hematologic effects of biotherapeutics were reported to be 1.4-fold more frequent than in small-molecule pharmaceuticals [1]. Thrombocytopenia is most commonly observed. Most hematotoxic effects of biotherapeutics are species-specific, immune-mediated and of low incidence [2]. Therefore, the use of a purely human test system to detect these toxicities is especially important. The toxicity caused by a biotherapeutic can be related directly to the activity of the substance or can be indirect, for example, because of autoimmunity, biological cascades, or antidrug antibodies [2]. The types of toxicity that are generally most difficult to study under *in vitro* conditions are those requiring the interaction of various cell types or even different tissues. Exemplarily, a hematologic effect that arises as a consequence of a toxicity to cells that influence hematopoiesis, such as the cells of the hematopoietic niche, is difficult to detect using current *in vitro* technologies. In addition, a secondary toxicity that may be because of the activity of a metabolic product also requires highly complex assays and, therefore, is currently often undetected.

In vitro organ models aiming to mimic their *in vivo* counterparts often make use of three-dimensional culturing techniques and specific extracellular matrix components or scaffolds. The combination of these models with a microfluidic perfusion increases the fidelity of the cultures because of the enhancement of control over culture conditions. The resulting microphysiological systems (MPSs) have been proven previously to be highly useful during the drug development process [3]. These systems are envisioned to model human biology at the smallest possible scale under physiological or pathological conditions. Their ability to host three-dimensional organ models in a controlled microenvironment under constant media perfusion enables them to create and maintain homeostasis [4,5]. Devices targeting specific drug-induced toxicities, such as pulmonary edema induced by interleukin-2 or an anti-EGFR antibody-related inhibition of physiological skin cell turnover, have been published previously [6,7]. Furthermore, immune cells have been integrated in MPS as single cells or even in combination with other organs [8]. So far, only a very few devices have been designed for the study of the hematopoietic system but results are promising [9,10].

Status quo of hematotoxicity testing and current needs

The hematopoietic system comprises a hierarchy of cells. This starts with an extremely small number of stem and progenitor cells which differentiate through three major lineages—erythroid, myeloid, and lymphoid—to produce multiple precursor cells with lower proliferative potential. Finally, mature cells restricted to single blood cell types are formed [11]. Therefore, assays studying the effects of substances on specific hematopoietic progenitor proliferation and differentiation—the so-called colony-forming unit (CFU, or colony-forming cell) assays—were established. Cells of primary origin (mononuclear bone marrow, cord blood, or cell populations enriched for CD34+) are cultured here in semisolid matrices and stimulated with appropriate cytokines to produce *in vitro* colonies of specific cells [12]. A morphological assessment allows the discrimination of distinct progenitor lineages and reveals whether an applied substance shows lineage-specific effects. Not only the number of colonies but also individual cell morphology can be studied to reveal effects even at low substance concentrations. Commonly used CFU assays include the granulocyte macrophage colony-forming units (CFU-GM), the erythroid burst-forming units, and megakaryocyte colony forming units assays [13]. The CFU-GM assay has even been validated by the European Centre for the Validation of Alternative Methods for the prediction of drug-induced hematotoxicity *in vitro* [14,15]. However, there have been some constraints when using these assays. This is due partly to their length, making them relatively low throughput, and due partly to the inherent subjectivity in the microscopic evaluation and enumeration of colonies [12,16]. It has been reported that there is only a little statistical correlation in the colony counts between different laboratories, emphasizing the fact that the colonies produced are so diverse that standardizing colony counting is extremely difficult to achieve [11]. Furthermore, additional analysis for more in-depth characterization of the cells other than counting the colonies and morphologically categorizing them is not possible. A liquid-culture, microplate-based assay has recently been introduced that allows not only for high-throughput screening but also further endpoint analyses, such as flow cytometry [17]. Nevertheless, major drawbacks, such as the inability to detect secondary toxicities and a lack of mechanistic information because of incomplete or restricted lineage maturation, remain [18]. Additionally, effects on the hematopoietic niche supporting the stem and progenitor cells cannot be detected. Furthermore, assays do not allow for a chronic treatment overlooking gradual interferences with the cells of the hematopoietic niche or the stem and progenitor cells themselves.

Status quo of bone marrow-on-a-chip cultures

The bone marrow stem cell niche in which the HSPCs reside is vital for their maintenance. Signals from the niche are critical for the regulation of stem cell self-renewal and quiescence and for the differentiation and maturation of hematopoietic lineages [19]. The structural and biological microenvironment within the niche is highly heterogeneous, and HSPCs are known to interact with multiple niches simultaneously (endosteal and perivascular niche, stroma, and capillaries). The most quiescent HSPCs were reported to reside in the perivascular niche close to blood vessels [20]. Several studies have attempted to recreate these biological niches under *in vitro* conditions. The addition of extracellular matrix components, three-dimensional culture, and co-culture with stromal cells has been most successful regarding the creation of artificial niches and human HSPC expansion [21–25]. However, optimal culture conditions for HSPC maintenance, proliferation, and differentiation into the different lineages vary and partly are yet to be devised. Efforts targeting the *ex vivo* expansion of HSPCs for transplantation, for example, have identified promising substances allowing for up to 50-fold stem cell expansion over 21 days of culture [19]. Furthermore, the perfusion of *in vitro* cultures has allowed a more precise control over culture parameters—like continuous levels of exogenous cytokines, oxygen concentration and pH—allowing to selectively influence HSPC fate [26,27].

Similarly, MPS cultures benefit from a continuous perfusion and, therewith, from a more *in vivo*-like supply of nutrients, oxygen, and physical stimuli. The first MPS-based bone marrow culture targeting the long-term survival of HSPCs allowed the culture of a tissue-engineered bone marrow model *in vitro* while retaining HSPCs in normal proportions [10]. In this study, bone marrow was engineered by subcutaneous implantation of a device filled with bone-inducing material on the backs of C57BL/6 mice. After 8 weeks, a bone-like tissue with a central marrow region was recovered and inserted into a microfluidic circulation. This murine bone marrow-on-a-chip enabled the maintenance of long-term hematopoietic stem cells, while the distribution of mature blood cells produced remained constant. An exposure of the device to gamma radiation resulted in the reduction of leukocyte production which was nearly identical to the proportions measured in whole marrow from live mice that underwent similar irradiation [10]. Treating the system with two potential therapeutics, granulocyte-colony stimulating factor and bactericidal/permeability-increasing protein, induced significant increases in the number of hematopoietic stem cells and myeloid cells in the fluidic outflow [28]. Interestingly, the cultured cells were not significantly affected by the absence of exogenous cytokines. This indicates that the engineered bone marrow MPS was able to self-sustain its hematopoietic niche.

The first human bone marrow-on-a-chip system targeting the long-term survival of HSPCs was presented by Sieber et al. [9]. Here, a three-dimensional co-culture model, on the basis of a hydroxyapatite-coated zirconium oxide scaffold seeded with mesenchymal stromal cells (MSCs) and cord blood-derived HSPCs, was able to maintain the stem cell population for up to 28 days. Specialized bone marrow stromal cells and endothelial cells are the most important cells associated with HSPC preservation [29]. The MSCs are known to have multilineage differentiation potential and, consequently, are able to create the required partner cells in the niche [30]. Signaling molecules and essential extracellular matrix components are also produced by MSCs [31]. The HSPCs in the co-culture model were found to stay in their primitive state and remained capable of granulocyte, erythrocyte, macrophage, and megakaryocyte colony formation. The microenvironment formed by the MSCs also showed molecular and structural similarity to the *in vivo* bone marrow niche [9].

Both systems, from Sieber et al. [8] and Torisawa et al. [10], were able to generate a biological niche sustaining HSPCs for prolonged *in vitro* culture periods. Compounds that are specifically toxic to the stromal cells of the hematopoietic niche and, therefore, may have inadvertent effects on various stem cell functions can be identified here. Under *in vitro* conditions, the differentiation into mature blood cells may take several weeks [32]. Therefore, hematopoiesis has to be monitored for prolonged culture periods to get a comprehensive evaluation of the effects of a drug. Especially substances that specifically target very late-stage maturation of hematopoietic cells require a system able to maintain the viability and ability to differentiate and produce mature blood cells *in vitro* for up to several weeks.

Challenges and opportunities for MPS-based hematotoxicity testing

The high complexity of the bone marrow niche is very difficult to reproduce using purely *in vitro* technologies. The long-term maintenance of HSPCs was shown to be possible; however, a major challenge remains regarding the degree of maturity the differentiated progeny obtains and the plurality of different lineages that are produced simultaneously. When using a defined culture setup, the models still require the addition of cytokines and specialized media formulations. The respective conditions promote the maturation of distinct lineages, while others may not be supported or differentiation halts at a preliminary stage. Previous studies on bone marrow-on-a-chip cultures aimed at retaining the primitive phenotype of HSPC and optimized the culture conditions respectively. Sieber et al. were able to reduce the number of cytokines added to the medium to only two (TPO and FLT3-L) [9]. No data are given for the cells that differentiated out of the bone marrow model. The *in vivo*—engineered bone marrow of Torisawa et al. was cultured without exogenous cytokines for 1 week while maintaining all cell populations in normal proportions [10]. In a later study, they report that mainly myeloid cells and only few erythrocytes and lymphoid cells were produced in the bone marrow-on-a-chip culture. The medium was, similarly, optimized to preferentially support survival of HSPC [28]. Hence, the optimal conditions supporting a maturation of all major cell types is still to be developed.

An increase in model complexity, by the addition of, for example, another cell type, might represent a way of solving this issue. Endothelial cells were reported to be fundamental for priming the perivascular niche [19,29]. Technologies for the integration of a vascular bed in MPS are readily available [33–35]. Combining these existent vascularization techniques with bone marrow-on-a-chip cultures may bring about several opportunities. The addition of a further cell type will increase the number of factors secreted by the supporting stroma, probably rendering the addition of exogenous factors unnecessary. Additionally, a perfused vascular structure will provide a more physiologically relevant route for substance delivery and cell migration in and out of the niche. This might come in handy when the pharmacokinetic profile of a substance is to be analyzed in parallel. The higher physiological relevance enhances the transferability of data from the MPS to the *in vivo* situation.

The platform described by Sieber et al. [9], furthermore, allows for a co-culture of the bone marrow model with secondary organ models, such as the liver. This allows the study of the effects of metabolites on the HSPCs. Multitissue co-cultures using the same device with different organ constructs were able to show organ—organ crosstalk between liver and skin, intestine, tumor, and pancreatic islet tissue models [7,36,37].

With an increase in biological complexity—by co-culturing multiple organ models in a MPS—rises the complexity of monitoring the condition of the different cells. To monitor the stability of the primitive states or likewise, the progression of maturation in the bone marrow-on-a-chip system various assays may be performed. Medium supernatants can be sampled daily in MPS and may be analyzed for secreted factors. Similarly, cells released from the bone marrow model into the culture medium may be sampled at regular intervals, and endpoint studies on the cells remaining in the bone marrow model may be performed.

Furthermore, the source of cells is an important issue when going from single-organ to multiorgan models. Single bone marrow-on-a-chip cultures may be assembled out of commercially available cryopreserved cells or of freshly isolated cells from cord blood or bone marrow.

Multiorgan models including immunologic tissues and other organ models in a common media circulation are required to be compatible to avoid inadvertent immune reactions. At present, the field of MPS research is heading toward human-on-a-chip or body-on-a-chip devices. These systems aim to reproduce whole body physiology and responses to substance applications. High effort is, therefore, put into the development of induced pluripotent stem cell–derived organ models and their co-culture. Induced hematopoietic progenitors were able to mature into erythroid, megakaryocytic, and myeloid cells [38,39]. Erythroid-lineage cells displayed enhanced expression of adult β-globin indicating definitive pathway patterning [38].

These developments are envisioned to culminate in the establishment of patient-on-a-chip assays, which may further improve investigational toxicology [40]. Here, patient-derived induced pluripotent stem cells are used to generate individual body-on-a-chip devices for the study of specific medication options. This leap toward precision medicine applications will be of high value in understanding the roles of patient factors. It is well known that factors such as preexisting disease and genetics can influence the occurrence of hematotoxicity [2].

Finally, having a high-content assay early in the drug development process allowing one to generate data predictive of the clinical situation will be highly useful in identifying toxic compounds early and excluding them before costly clinical trials. Cultivating a bone marrow model in an MPS provides manifold opportunities. First, systems are able to maintain HSPCs in their niche. Therefore, direct or indirect hematotoxicities can be observed. Second, devices primed for the production of immune cells by the bone marrow model allow the study of directed and undirected immunologic effects. Finally, a self-sustained production of erythrocytes increases the physiological relevance of oxygen supply to the various tissue in the MPS. Summarizing, bone marrow-on-a-chip cultures are envisioned to be highly advantageous in various fields of research.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: ED, AW, and UM are employees of TissUse GmbH which commercializes MPS platforms.

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- •• of outstanding interest

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