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Invited Mini Review

Challenges and innovations in hematopoietic stem cell transplantation: exploring bone marrow niches and new model systems

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Hematopoietic stem cell transplantation (HSCT) remains an indispensable therapeutic strategy for various hematological diseases. This review discusses the pivotal role of bone marrow (BM) niches in influencing the efficacy of HSCT and evaluates the current animal models, emphasizing their limitations and the need for alternative models. Traditional animal models, mainly murine xenograft, have provided significant insights, but due to species-specific differences, are often constrained from accurately mimicking human physiological responses. These limitations highlight the importance of developing alternative models that can more realistically replicate human hematopoiesis. Emerging models that include BM organoids and BM-on-a-chip microfluidic systems promise enhanced understanding of HSCT dynamics. These models aim to provide more accurate simulations of the human BM microenvironment, potentially leading to improved preclinical assessments and therapeutic outcomes. This review highlights the complexities of the BM niche, discusses the limitations of current models, and suggests directions for future research using advanced model systems. [BMB Reports 2024; 57(8): 352-362]

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) has been considered a beacon of hope for patients suffering from a variety of hematological disorders, offering a potential pathway to healing through reprogramming of the hematopoietic system. The advances of gene editing technologies, which include CRISPR/Cas9 and next-generation base and PRIME editors, have significantly improved therapeutic feasibility by enabling precise correction of autologous HSCs. In light of this, numerous clinical trials are currently being conducted worldwide, and therapies such as Casgevy (exa-cel) and Lyfgenia (lovotibeglogene autotemcel) have just received conditional clinical approval (1, 2). Despite these advances, the successful engraftment and subsequent long-term persistency of gene-edited HSCs within the recipient's bone marrow (BM) remain pivotal challenges, highlighted by the resilience of HSCs against genotoxic stress and hematopoietic dynamics. While these gene editing treatments are expected to deliver therapeutic benefits, they still carry significant disadvantages. Notably, in treatments such as nula-cel, unexplained cases of pancytopenia in certain cohorts have resulted in clinical trials being halted (3).

To ensure the successful outcome of *ex vivo* HSC gene therapy, the long-term tracking of cell fate is imperative. Preclinical animal models have long served as standard tools, providing insights into the engraftment process and the dynamics of stem cell-niche interactions (4). However, the conventional animal models exhibit significant limitations. Notably, controversial outcomes are often incurred by differences in species-specific niche components (5, 6). Given these constraints, there is a growing interest in developing alternative transplantation models. This review investigates the current landscape of animal models in HSC transplantation, delves into the specific limitations, and discusses the development of alternative transplantation models.

MAIN TEXT

HSC homing and engraftment to BM niches following transplantation

Most hematopoiesis and immune cell production takes place within the BM niches with intricate structure (7), facilitating fine-tuned control over homeostatic and stress responses. Thus, HSC homing and engraftment are crucial steps in the process of HSCT for long-term persistency and the subsequent multiplication of blood lineage cells within the marrow to restore the hematopoietic system (8, 9).

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Intravenously infused HSCs home and engraft via a distinct series of molecular processes. Circulating HSCs are first attracted to home in the BM microvasculature by a gradient of small molecules released by the BM microenvironment. Among prominent small molecules, stromal-derived factor-1 (SDF-1, also known as CXCL-12) plays a crucial role (10, 11). Upon binding of SDF-1 to the corresponding receptor, CXCR4 on the cell surface of HSCs, other binding molecules, such as integrin (VLA-4), are activated, leading to firm adhesion to the target endothelial cells (12). In addition to VLA-4, other molecules that include P-selectin glycoprotein ligand-1 (PSGL-1), type 1 receptor for S1P (S1PR1), and FLT3, on the surface of HSCs slow down and halt the cells by tethering to P-selectin and E-selectin, as well as vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, which partially mirrors the rolling of leukocytes (13, 14). Following adhesion, HSCs transmigrate across the endothelial barrier into the extravascular cord and BM space, the so-called transendothelial migration. While firm adhesion by VLA-4/VCAM-1 and SDF/CXCR4 improve the migration, the regulation of vascular endothelial (VE)-cadherin, a critical molecule forming endothelium integrity, improves the efficiency of transvascular migration (15). HSCs are then redistributed and lodged within the niches. Once in the BM niche, interaction takes place within various kinds of BM constituting cell types, which include both hematopoietic and non-hematopoietic cells, such as mesenchymal and stromal cells (MSCs), nerves, osteoblasts, adipocytes, endothelial cells, and extracellular matrix (ECM) components as well (16). Of note, transplanted HSCs have a propensity to migrate toward the endosteal region for life-long proliferation, while the cells committed to an immediate differentiation toward specific lineage tend to move to near the BM sinusoids (17-19). The perivascular niche close to the sinusoidal blood vessels within the BM central marrow cavity predominantly supports the active formation of myeloid and lymphoid cells, while the endosteal niche located near the trabecular bone preserves dormant long-term HSCs that are capable of replenishment (Fig. 1) (18, 20). The dynamic interaction of HSCs within the niche microenvironment and the balance between these niches are essential factors for the successful engraftment of transplanted HSCs and their long-term persistence.

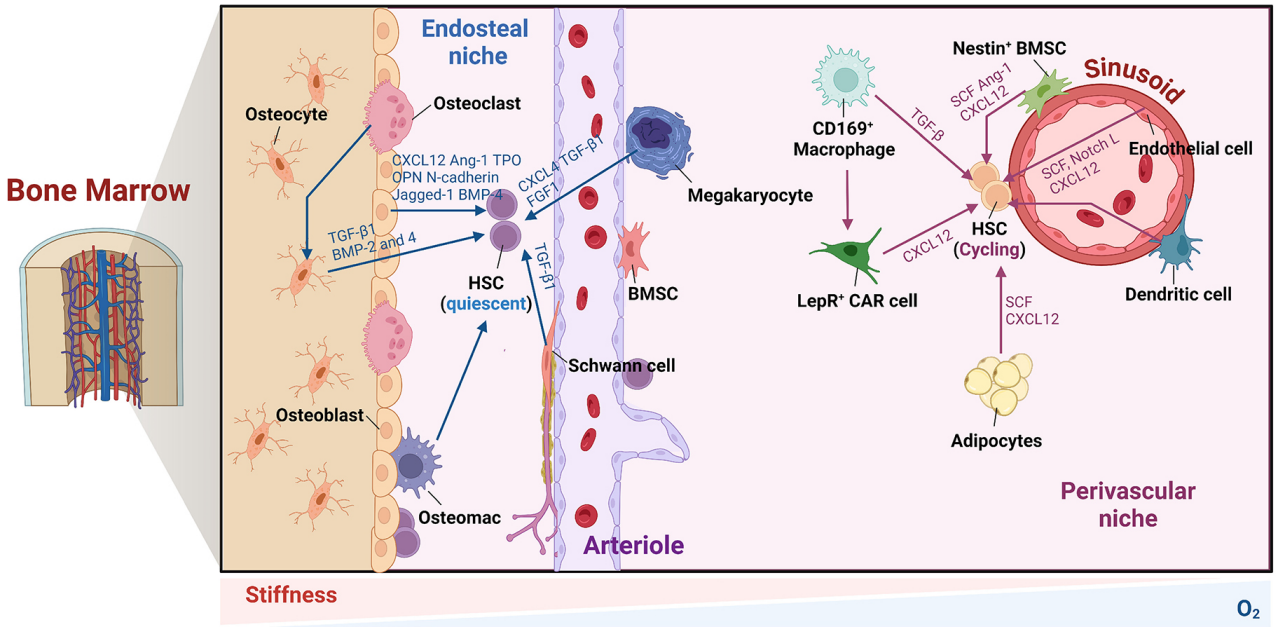

Fig. 1. Hematopoietic niches in bone marrow. Organization and cellular interactions within the hematopoietic stem cell (HSC) niches of adult bone marrow (BM). HSCs are predominantly situated in proximity to the marrow vasculature, in perivascular niches. The niche includes various cell types, such as endothelial cells, CD169$^+$ macrophages, and LepR$^+$ CXCL12-abundant reticular (CAR) cells, dendritic cells (DCs), Nestin$^+$ bone marrow stromal cells (BMSCs), and adipocytes. The niche contributes to HSC self-renewal, proliferation, and differentiation via the expression of CXCL12 and stem cell factor (SCF). Conversely, the endosteal niche houses quiescent HSCs, and is characterized by osteoblast, osteocyte, osteoclast, osteomac, and schwann cells, and megakaryocytes, which provide a specialized cellular architecture for hematopoiesis regulation and quiescence. The factors related to bone metabolisms and transforming growth factor beta-1 (TGF-β1), play a crucial role in the long-term persistence of quiescent HSCs. Various factors in the microenvironment, such as stiffness, calcium levels, oxygen gradients, and reactive oxygen species, help maintain the dormancy and quiescence of HSCs.

The role of perivascular BM niche in HSC proliferation and differentiation

The perivascular niche is rich in endothelial cells and perivascular MSCs that produce factors that are crucial for supporting immediate HSC proliferation and differentiation following transplantation (21, 22). The perivascular niche-specific cells secrete various growth factors and chemokines, ensuring the retention, self-renewal, and differentiation of HSCs (23). Endothelial cells, major cellular components in the niche, secrete SDF-1 and stem cell factor (SCF). As circulating HSCs are encountered most frequently, the cells form a critical cellular population to secrete or express the aforementioned molecules, such as E-selectin, P-selectin, and VCAM-1, as well as SDF-1, facilitating the adhesion and transmigration of HSCs across the blood vessel wall into the BM (22). Among the niche cells, leptin receptor (LepR)-expressing CXCL12 abundant reticular cells (CARs) secrete 100 times more CXCL12 than do endothelial cells, facilitating the active cycling of HSCs (24). Furthermore, these cytokines regulate the balance between the quiescence and active cycling of retained HSCs to promptly proliferate and promote mature multiple cell populations (22, 25, 26). Nestin$^+$ bone marrow mesenchymal stromal cells (BMSCs) and pericytes are also another source for a range of SCF and CXCL12 (27). Of the secretory molecules, angiopoietin-1 and Notch ligand take on the roles of HSC maintenance, lineage commitment, and regeneration (28). Recent studies have elucidated the considerable prevalence of adipocytes and variability in the red marrow, as one of the crucial compartment cells in the perivascular niche, and due to its proximity, closely associated with HSC, as well as sinusoidal vasculature (29). Thus, adipocytes significantly contribute to the hematopoiesis, in alignment with the intrinsic properties of the BM, by secreting factors, such as CXCL12 and SCF (30). Tissue-resident immune cells could also support the maintenance and hematopoiesis of the seeded HSCs. In the perivascular niche, tissue-residing CD169$^+$ macrophages support the survival and retention of HSCs (31). Dendritic cells (DC) can affect the HSC niche through the secretion of cytokines and other factors, leading to the survival and differentiation of B cells (32).

The role of endosteal BM niche in HSC quiescence and long-term maintenance

The trabecular bone surrounding the marrow contributes to forming the porosity, providing the cavities where HSCs settle and reside. The endosteal niche primarily induces HSC dormancy and maintains quiescence to protect HSCs from exhaustion, and maintains their long-term repopulation capacity (20, 21). The endosteal niche provides signals that help retain HSCs in a quiescent state, including osteopontin (OPN), angiopoietin-1 (Ang-1), and thrombopoietin (TPO). Osteoblasts (OBs), crucial for forming the endosteal niche, also secrete CXCL12, but in much greater amounts compared to other cells in the perivascular niche, so contribute to the quiescence of HSCs (33). In addition to CXCL12, Ang-1 and TPO, which bind to the corresponding receptors Tie2 and MPL, respectively, improve HSC quiescence and maintain long-term repopulation regional HSCs (28, 34). OPN plays an essential role, both in determining where HSCs are located, and in serving as a physiological suppressor of the proliferation, eventually leading to HSC quiescence (35). Bone morphogenetic protein (BMP) signaling, particularly subtype 4 within the endosteal niche, contributes to the long-term engraftment of HSC (36). The extent of membrane-bound ligands, such as Jagged-1 and N-cadherin, has been considered to also play a crucial role in the regulation of HSC self-renewal and endosteal-HSC interactions (37, 38). While primarily involved in bone resorption, osteoclasts (OCs) play a role in modulating the BM microenvironment and can influence HSC quiescence via increasing TGF-β1, and BMP2 and 4, derived from the destructing osteocytes (39). The recently discovered cell, osteomacs (OMs), located proximate to osteoblasts, would support HSCs through the formation of endosteal structure, thereby maintaining the integrity of the niche, or augmenting the function of other cellular compartments (40, 41). Megakaryocyte (MK) plays a pleiotropic role in HSC proliferation, but in this niche, it secretes CXCL4, FGF-1, and TGF-β1, subsequently regulating HSC cycling (42). Schwann cells, in conjunction with sympathetic nerves, induce a quiescent state in HSCs secreting TGF-β1, and maintain direct contact with them (43). The dynamic changes in the microenvironment collectively produced by multiple cell types and their structure, such as stiffness, calcium ion level, oxygen gradient, and related ROS and hypoxia-inducible factors (HIFs), also exert the quiescence of HSCs (44). Importantly, under hematologic malignancies, such as acute myeloid leukemia (AML), BM experiences pathophysiological changes, such as imbalance in bone formation, subsequently impairing the engrafting of transplanted HSPCs. Thus, leveraging the appropriate preclinical models that mimic the structural features and cellular makeup of the BM niches can increase the credibility of research.

Current preclinical animal models for human hematopoiesis and HSCT

Limitations and advancements in humanized xenograft mouse models for HSCT research: Even though a range of animal models, such as zebrafish, are utilized, the xenograft mouse is the most employed preclinical HSCT 'standard' model. For human HSCT and subsequent hematopoietic recapitulation, the immunodeficient mice are imperative. Over the past decades, various immunodeficient mouse models, such as NOD/SCID, BALB/c-nude, and Rag2^{null} strain, have been developed, with each strain presenting different extent of immunodeficiency (45-47). Among the immunodeficient mice, the humanized NSG (NOD/SCID/IL2Rγ^{null}) mouse, which lacks mature T-cells, B-cells, and functional NK-cells, and has a mutation in the IL2 receptor gamma chain, is a versatile model that is extensively used in biomedical research (48, 49). Once engrafted, human HSCs in NSG mice partially reconstitute a human immune system introducing T-cells, B-cells, and myeloid cells

into the bloodstream (50).

Although the mouse BM environment, including the endosteal and perivascular niches, somehow supports the survival and persistent hematopoiesis of engrafted human HSCs, they are considered less than ideal, due to species-specific variations between human and murine physiology (46, 47). The features may not fully mirror those found in human BM, such as cytokine profiles, adhesion molecules, and other niche components necessary for the engraftment, survival, and self-renewal of both normal and malignant hematopoietic cells. One example is that BMSCs respond to leukemia cells by modifying their secretion profiles, leading to the development of a competitive environment that supports leukemia stem cells (LSC) (51). To investigate BM failure syndromes, the various lymphoid populations, and non-hematopoietic elements, such as a sympathetic innervation, as seen in the BM of aplastic anemia or myelodysplastic syndrome (MDS) should be elucidated (52). Even AML patient-derived xenograft (PDX) mouse models cannot fully recapitulate AML hematopoiesis, due to insufficient reproducibility caused by irradiation-mediated stroma damage, delayed engraftment period, and subsequently diminished engraftment rate. Thus, these inborn limitations of the model result in the poor engraftment of many human leukemias and other blood disorders in these standard models (53).

To overcome these limitations, a variety of preclinical models beyond humanized NSG mice are being developed. One simple approach is to genetically modify immunodeficient mice to replicate the patient- or disease-specific environment, representing human cytokines or growth factors like SCF, GM-CSF, IL-3, and TPO (54-56), or to transplant together with patient-derived BMSCs (57). Humanized BLT (BM, liver, and thymus) mice are implanted with human BM, liver, and thymus tissues, leading to a more comprehensive reconstitution of the human immune system (58). Enhanced T-cell generation enables the replication of more efficient models of infection or infection-induced tumor formation, such as HIV infection and Kaposi's sarcoma and immune-related diseases, including cytokine-releasing syndrome (59-61). While these systems provide essential insights into human health and disease, each model still has limitations in fully replicating human physiology.

Benefits and challenges of autologous nonhuman primate (NHP) model: Of note, NHPs share significant genetic and anatomical similarities with humans, making them a more comparable model reflecting human physiology. In particular, the large capacity of the hematopoietic cell reservoir complies with the requirement for autologous transplantable cell dose, thus creating an autologous transplantation preclinical model (62, 63). In addition, the similarity in immune system facilitates a more accurate assessment of how the human immune system recovers post-transplant. Utilizing these characteristics, it is possible to conduct research that elucidates new functions of relatively novel blood lineage cells and hematopoietic dynamic under normal or perturbed situations (64). With average lifespan of more than 30 years, the autologous NHP animal model

Table 1. Alternative HSC transplantation model candidates

Model type	Key features	Cell constituents	Key molecules	Applications	Limitations	References
Ossicle-based BMO	Use human BMSCs to form bone-like structures for engraftment	BMSCs (human), leukemia-initiating cells (LIC), HSCs	CXCL12, TGF-β1, VCAM1, ANGPT1, KITLG	Disease modeling, studying niche-HSC interactions	Limited replication of cellular dynamics, challenges in long-term HSC viability, species differences in implantation models	(56), (72), (73), (74), (87), (88), (89), (90)
Self-organizing BMO	3D cultures that self-organize into bone marrow-like structures	BMSCs, fibroblasts, endothelial cells, HSCs	VEGF, BMP4, SCF, IL-3, Flt-3L, TPO, FGF-2	In-depth hematopoiesis study, therapeutic target validation	Incomplete niche components, maintaining long-term hematopoiesis, may not support all cell types involved in BM	(70), (71), (75), (91)
Blood vessel organoid (BVO)	Form vascular-like structures	Endothelial cells, mural cells	VEGF-A, FGF-2, BMP4	Study of vascular biology and HSC interactions	Cannot replicate endosteal BM niches, limited to initial HSC transplantation and vascular interactions	(93), (94)
Thymic organoid (TO)	Mimic the thymic environment for T cell development	Thymic epithelial cells (TECs), HSCs	Activin A, BMP4 inhibition	Study of T cell development, immune responses	Differences from human biology limit clinical translation; focus on immune modeling, not direct hematopoiesis	(96), (97)
BM-on-a-chip	Replicate BM microenvironment in a microfluidic device	MSCs, endothelial cells, osteoblasts, HSCs	Various chemokines, adhesion molecules, nutrients, oxygen	High-throughput drug testing, HSC niche interaction studies	Cannot fully replicate *in vivo* BM complexity; challenges in scaling and standardization for broader applications	(99), (100), (101), (102), (103)

is capable of predicting the clinical outcomes of HSC transplantation, such as *ex vivo* gene therapy from the long-term aspect, assessing engraftment efficiency, persistence, and potential adverse effects (62, 63).

Even though the model system offers numerous benefits, there are several hurdles, the greatest of which is the increasingly stringent ethical standards and regulatory controls due to the cognitive abilities and social needs of NHPs. It is also challenging that NHPs require specialized facilities, which along with their care, results in higher costs, compared to other models. Therefore, there is a requirement for alternative preclinical models that more closely mimic the human BM microenvironment for the simulation of hematopoiesis and immune responses, and that empower long-term tracking, which could further improve the predictive value of preclinical studies for human clinical outcomes (Table 1).

Alternative HSCT models

Key requirements and characteristics of organoids for replicating BM function and structure: Organoids are self-organized 3D cellular structures that mimic the microarchitecture and function of the corresponding organs. Induced pluripotent stem cells (iPSCs), or adult stem and progenitor cells, as well as differentiated cells isolated from the body, are differentiated under defined chemicophysical conditions to replicate the functional and structural features of the original organ (65, 66). Generally, the organoid should meet several key requirements: (a) It should have a three-dimensional structure with cells that either preserve or establish the identity of the organ it aims to model; (b) It should contain various cell types, mirroring the diversity and interactions found in the particular organ; (c) The organoid should be capable of performing some or all of the key functions of the organ it models; (d) It should undergo self-organization according to the same intrinsic developmental processes (67, 68).

Advances in 3D bone marrow organoid (BMO) for hematopoiesis and cellular interactions: Due to the challenges in investigating a hematopoiesis and related cellular interactions *ex vivo*, especially homing and the early engraft phase immediately after HSPC transplantation, there have been efforts for over 30 years to create a 3D model structure for BM mimicry (69-75). BM is highly heterogeneous tissue, necessitating the replication of endosteal and perivascular niches to incorporate hematopoiesis, as well as bone homeostasis, and stromal and vascular support (22, 76, 77). The diversity within the BM results from its mixture of various cells that include hematopoietic, bone, stromal, and endothelial cells, plus ECM molecules (78-80). These cells need to be more than just a cluster; they should be intricately arranged, forming diverse niches that are supported by stromal cells (20, 22, 76, 78). This can be achieved by constituting the space with ECM components, including collagen fibers and glycoproteins, as well as cellular spatiotemporal organization, such as adipocytes and BMSCs. Furthermore, restoring the appropriate stiffness is also required (81). By forming niche structures, it becomes possible to observe the fundamental process of hematopoiesis, as well as the restoration of additional functions, such as the secretion of cell-specific growth factors and cytokines. Finally, these recapitulation processes must occur spontaneously, ensuring sustainability to support long-term hematopoiesis over time. With these key features, BMOs hold promise as tools for transplantation research, because they can be cultured for a long time, and are suitable for transplantation, providing stability and reproducibility. Additionally, BMOs can overcome the limitations of previous models by accurately replicating individual genetic defects in the BM (82, 83). In early research, BMOs were developed by combining biological elements, such as bone fragments, and non-biological components, like synthetic scaffolds. Somehow, the outcomes of these research are more akin to 3D scaffolds supporting *in vitro* hematopoiesis (84-86). Rather than simply imitating 3D structures using biocompatible materials, this review paper will cover research that incorporates the more complex features of the organoids mentioned previously.

Challenges in ossicle-based BMO for humanized hematopoietic and leukemic research: Early research on BMOs involved creating ossicles, small bone fragments, and subsequently transplanting them into mouse models or grafting HSCs. A series of studies have attempted to reproduce a humanized BM environment through the injection of cells of mesodermal origin (87). The Majeti group devised a method for the humanized BM niche microenvironment. They ectopically transplanted the ossicles differentiated from BMSCs into humanized mice (56, 88). Furthermore, they analyzed the engraftment of HSCs from AML, acute promyelocytic leukemia (APL), and myelofibrosis (MF) patients by using immunodeficient mice producing human cytokines, in particular, assessing the increased settlement of leukemia-initiating cells (LIC) (56). Pievani *et al*. further conducted the research by using BMSCs derived from patients to replicate the BM structure unique to AML patients (89). They implemented blood-borne fibroblasts (BF), a unique cell population, into the mouse model and observed the formation of a cortical bone outer layer that surrounded cavities populated with hematopoietic tissues, including the erythroid, myeloid, and megakaryocytic lineages (90). Moreover, this structure contains CD146-expressing stromal cells derived from human cells, thereby complying with the structural requirements to function as a hematopoietic niche. Markedly, this structure embodied comparable populations of $Lin^-/Sca\text{-}1^+/c\text{-}Kit^+$ (LSK) progenitors, LT-HSCs, ST-HSCs, and multipotent progenitors (MPP). In functional aspect, it also expressed hematopoietic and HSC niche marker genes, such as CXCL12, VCAM1, ANGPT1, KITLG, SPP1, and JAG1, that are found in intrinsic BMSCs (73). Given that the hybrid gel enriched primitive HSC and their progeny, including MPPs and multi-lymphoid progenitors (MLPs) in *in vitro* HSPC culture assays (74), applying materials reflecting active moieties of BM tissue, such as hybrid TG-PEG/HA hydrogels, might im-

prove the *in vivo* retention of hematopoietic cells.

The transplantation within immunodeficient mice to form a complete structure of organoids is noteworthy. While the bone metabolisms by OCs and OBs originate from the donor to replicate the cortical bone and medullary cavity, the vascular structures required for the complete differentiation of hematopoietic cells, including endothelial and perivascular cells, originate from either mouse or externally-injected human cells. To overcome these hurdles, Gieger and colleagues have generated structures on a microwell plate by mixing the MSC and vascular endothelial cells (ECs) to create a model that reproduces stromal and vascular structures (72). Within these structures, the proliferation of $ENG^{+}CD146^{+}$ MSCs, adipocytes, and pre-osteoblasts occurs alongside self-organized vasculature. However, there remains controversy over whether to classify these models as organoids or simple spheroids. Moreover, questions remain about the fidelity of the recreated intricate BM structure, leading to the challenge of accurately replicating porosity and density, and the effectiveness in truly replicating hematopoiesis.

Innovations and limitations of self-organizing BMOs for replicating hematopoiesis and BM niche dynamics: The current trend is shifting from cell-to-cell communication model to the natural development of BM niche microenvironments that mirror actual tissue environment. From this perspective, Selami and colleagues have successfully modeled the definitive hematopoiesis, producing HSPCs that are capable of creating RBCs containing adult β-globin and γ-globin, and T-lymphocytes with an extensive T-cell receptor (TCR) repertoire (91). In fact, they generated the structures to replicate the yolk sac, where initial hematopoiesis occurs, since their goal is to produce blood lineages that are applicable for transfusions, allowing the absence of a BM-like structure.

Khan and colleagues have recently developed a promising 3D BM organoid that recapitulates the central myelopoietic BM cavity, including mesenchymal elements, myeloid cells, and sinusoidal-like vasculature (70, 71). This organoid is spontaneously generated through a stepwise differentiation process over 18 days, using specific growth factors, and encompasses four phases: mesodermal aggregates (phase I), vascular and hematopoietic lineages (phase II), vascular sprouting (phase III), and organoid formation (phase IV). Indeed, this process allows for the formation of a 3D structure that is composed solely of BMSCs, fibroblasts, endothelial, and hematopoietic cells, without the need for xenotransplantation, or the mixing with cells from other sources. The transcriptomic profile of each part and the molecules involved in the interaction between niche-blood lineage cells have been shown to be similar to those observed in BM *in vivo*. Concurrently, Frenz-Wiessner and colleagues also reported the successful creation of BMOs using iPSCs (75). Their approach began with the formation of embryoid bodies, followed by hemogenic and endothelial induction, a sprouting phase, and finally, organoid maturation. Unlike the predominantly erythromyeloid cell types in the hematopoietic compartment, as shown by Khan *et al*., this organoid specifically induces spontaneous granulopoiesis. This model advanced to the stage of transplantation experiments, in particular enabling the simulation of a disrupted hematopoietic environment and TGF-β-induced BM fibrosis. Furthermore, gene-edited iPSC-derived BMOs effectively modeled characteristics of an inherited BM failure syndrome by replicating myelofibrosis pathophysiology. This approach modeled human VPS45 deficiency, BM failure with neutropenia, and myelofibrosis, a notable advance over previous challenges faced by preclinical animal models or ossicle-based BMOs (75).

However, it is generally acknowledged that there are limitations to replicating their complex structures. In particular, it is challenging to precisely reproduce stromal cells and vascular structures (70, 92). Though their model can replicate two key aspects of marrow structure, it lacks lymphoid, adipocyte, and osteochondral cells, and does not support bone deposition. Moreover, there might be constraints to support long-term cell maintenance; it also remains questionable whether the HSCs in the BMOs can truly be classified as LT-HSCs or not. Therefore, it is crucial to replicate the endosteal niche to restore the quiescence of HSCs, and to analyze the long-term clonal dynamics of gene-edited cells. In addition, the diminishing proportion of endothelial cells over time suggests that implementing a microfluidic system could address this issue; it would more directly reflect the patterns of HSCs homing via the vascular system.

Developing blood vessel organoids (BVOs) to model HSCT and early hematopoietic processes: BVOs are developed by differentiating stem cells, including pluripotent stem cells, in a 3D culture environment that promotes the formation of endothelial cells and pericytes, and subsequently, vessel-like structures that resemble capillaries or larger blood vessels (93, 94). The presence of specific growth factors, including VEGF-A, FGF-2, and BMP4, together with the 3D culture conditions, facilitate these cells to self-organize (93). BVOs can develop lumen-containing vessel structures that are able to support blood flow, and range from simple capillary networks to more complex structures that include multiple vascular cell types, exhibiting functional similarity to that attributed to blood vessels in the body, including barrier functions and response to shear stress (94).

HSC transplantation on a BVO could offer a novel approach to investigate the complicated process of hematopoiesis, especially focusing on the commencement of HSC homing and engraftment. The BVO could allow the aforementioned elaborate molecular interactions between HSC and perivascular BM niche cells at the homing stage to be examined, and the following HSC self-renewal and differentiation. Pathological conditions, such as myeloproliferative neoplasms or BM failure syndromes, perturb the vasculature and hematopoietic systems (95); hence, the model system can be adapted to simulate the compromised HSC transplantation. Ensuring that transplanted HSCs properly integrate with the organoid vasculature and achieve functional maturation still poses technical difficulties.

What is more, BVOs cannot precisely reproduce the endosteal BM niche structures, thus they may only be useful for observing the initial transplantation process.

Thymic organoids (TOs) to model T cell development and immune responses for research and drug discovery: TOs are engineered constructs that are designed to mimic the microenvironment and functional properties of the thymus, a primary lymphoid organ that is responsible for the maturation of T-cells. Research using TO allows in-depth study of thymic function, T-cell development, and immune responses. TOs are developed by culturing thymic epithelial cells (TECs) and pluripotent stem cells in a scaffold that promotes their self-organization and then recapitulates the thymic microenvironment, including the presentation of self-antigens for negative selection, critical for developing a self-tolerant T-cell repertoire (96, 97).

The concept of HSC transplantation to TOs emerged from the need to study thymic functions and T-cell development, rather than BM hematopoiesis (98). Once HSCs are introduced into these organoids, HSCs are co-cultured with TECs within the organoids, facilitating direct cellular interactions that are essential for T-cell maturation, mimicking the natural thymic selection processes. However, translating findings from organoid models to human treatments involves significant challenges, due to differences between the model systems and human biology. Thus, this model could be used in a limited capacity for preliminary drug discovery, allowing for the screening of immunomodulatory agents and the study of immune-related diseases.

BM-on-a-chip: innovating hematopoietic BM niche replication in a microfluidic device: BM-on-a-chip is an innovative technology that aims to replicate the complex microenvironment of the BM, especially in a microfluidic device, mimicking the bloodstream around the hematopoietic niches (99). Through the convergence of multiple technologies that include tissue engineering, microfabrication, and biomaterials science, the dynamic BM models leverage the micro-engineered environment to mimic the key features of the hematopoietic BM niches under controlled conditions. BM-on-a-chip devices are designed to replicate the physical, chemical, and biological cues of the BM microenvironment, including the spatial organization of cells, ECM composition, and fluid shear stress (99-101).

The chip could be used to elucidate the mechanisms by which HSCs home and engraft. Various types of cells, such as MSCs, endothelial cells, and osteoblasts, within microfluidic chambers embedded in biomaterials that mimic the ECM, provide a 3D structure that supports cell-cell and cell-matrix interactions that are related to HSC maintenance (100). In addition, a dynamic flow system facilitates the simulation of homing and subsequent engraftment of transplanted HSC through the blood stream, and the cellular behavior can be monitored real-time using imaging and other analytical techniques. This flow can be adjusted to study the effects of the transport of nutrients, oxygen, and signaling molecules, such as chemokines and adhesion molecules, and mechanical forces (102). The response to external factors, such as drugs, irradiation, or chemotherapy, in terms of HSC engraftment efficiency, niche occupancy, and post-transplantation recovery, can be assessed in a controlled high-throughput manner (103). Similar to BMOs, BM-on-a-chip models can model diseases that affect HSCs and their niches, such as leukemias or MDS (101). However, despite their advanced capabilities, BM-on-a-chip models still cannot fully replicate the complexity and dynamics of the *in vivo* BM environment, including bone microstructure and long-term changes within the system. Challenges remain in scaling up the technology for larger studies, and in standardizing chip designs and operating procedures to ensure reproducibility.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

This review has explored the complexities of BM niches and the practicality of preclinical animal models for HSCT. While these models offer invaluable insights, they also bear several limitations, which include discrepancies in mimicking human physiological responses, and their burdensome nature in use. To overcome these constraints, alternative preclinical models, such as BMO and BM-on-a-chip, could be employed, suggesting the potential for a more accurate simulation of the human BM microenvironment. Given the enhanced understanding of the BM microenvironment and relevant hematological disorders, the technology behind these innovative models is continually advancing. The authentic replication of human BM allows for more precise modeling of diseases and personalized treatment strategies. These robust platforms serve as essential tools for screening in personalized medicine, providing a tailored approach to treatment that accounts for individual differences.

To serve as a superior model, these models should be refined to ensure their scalability and reproducibility. In addition, it is essential to expand investigations to encompass the repercussions of genetically engineered cells transplanted into the host, as well as their subsequent effects on the marrow milieu (104, 105). Current studies have primarily focused on the impact received by genetically corrected cells and their sustainability within animal models. One promising tactic for the accurate replication of intricate biological structures is the utilization of the assembloid, a type of advanced organoid technology (106). Created by combining different types of organoids, this will allow the modelling of interactions, more specifically between different cell types and tissues, in a more integrated and physiologically-relevant context. It is anticipated that utilizing and fusing BVOs or bone organoids could improve the current limitations of BMOs in replicating vascular development and bone structure. Even though the distinctive properties of HSCs make them ideal candidates for *ex vivo* gene therapy, the end goal is to deliver genetic material directly into the organism, the so-called *in vivo* gene therapy

(107). The alternative transplantation model system offers promising avenues for *in vivo* gene therapy techniques, potentially revolutionizing treatment protocols for genetic and acquired BM disorders.

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CONFLICTS OF INTEREST

The author has no conflicting interests.

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