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Closer to Nature: The Role of MSCs in Recreating the Microenvironment of the Hematopoietic Stem Cell Niche in vitro

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Keywords

Hematopoietic stem cells · Hypoxia · Mesenchymal stromal cells · Microbioreactor · Microenvironment · Stem cell niche

Abstract

Background: The stem cell niche in human bone marrow provides scaffolds, cellular frameworks and essential soluble cues to support the stemness of hematopoietic stem and progenitor cells (HSPCs). To decipher this complex structure and the corresponding cellular interactions, a number of in vitro model systems have been developed. The cellular microenvironment is of key importance, and mesenchymal stromal cells (MSCs) represent one of the major cellular determinants of the niche. Regulation of the self-renewal and differentiation of HSPCs requires not only direct cellular contact and adhesion molecules, but also various cytokines and chemokines. The C-X-C chemokine receptor type 4/stromal cell-derived factor 1 axis plays a pivotal role in stem cell mobilization and homing. As we have learned in recent years, to realistically simulate the physiological in vivo situation, advanced model systems should be based on niche cells arranged in a three-dimensional (3D) structure. By providing a dynamic rather than static setup, microbioreactor systems offer a number of advantages. In addition, the role of low oxygen tension in the niche microenvironment and its impact on hematopoietic stem cells need to be taken into account and are discussed in this review. ***Summary:*** This review focuses on the role of MSCs as a part of the bone marrow niche, the interplay between MSCs and HSPCs and the most important regulatory factors that need to be considered when engineering artificial hematopoietic stem cell niche systems. ***Conclusion:*** Advanced 3D model systems using MSCs as niche cells and applying microbioreactor-based technology are capable of simulating the natural properties of the bone marrow niche more closely than ever before.

Introduction

The stem cell niche in human bone marrow provides essential factors that regulate the proliferation and differentiation of human hematopoietic stem and progenitor cells (HSPCs). Direct cellular contact mediated by adhesion molecules, scaffolds, extracellular matrix, paracrine factors, spatial distribution, mechanical and shear forces and oxygen tension plays a role in governing stem cell fate [1–6]. As a result, modeling the highly dynamic framework of the hematopoietic stem cell niche in a three-dimensional (3D) environment is warranted. In recent years, microbioreactor-based systems have been able to mimic a number of crucial factors [7–10], but finding an optimal set of read-out assays to measure the effects on stem cells and their functional properties is challenging.

In this review, we analyze the most important regulatory factors and consider some of the most promising examples of 3D coculture and microbioreactor systems. Mesenchymal stromal cells (MSCs) represent an important part of the bone marrow niche and support normal hematopoiesis. However, there is mounting evidence

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that the niche microenvironment can also contribute to the pathogenesis of myeloid malignancies. Finally, the role of oxygen tension in the bone marrow niche is discussed.

Regulatory Factors in the Stem Cell Niche and Their Effects on Stem Cell Engineering

A number of regulatory factors in the stem cell niche have been identified in recent years, and their relevance for stem cell engineering has been addressed in various studies. Some of these important factors could be more precisely controlled in microfluid-based cell culture arrays or microbioreactors [9]. Table 1 gives an overview of the most important publications on the key issues (but is by no means exhaustive).

Scaffold and Cellular Determinants of the Niche

The scaffold has a substantial impact on the niche as well as on stem cells. It constitutes the macro- and microstructure of the niche and may also facilitate paracrine and autocrine activities of the matrix. For instance, higher cell proliferation rates could be observed under higher mechanical stresses. Additionally, control of cell shape via substrate size directs human MSC differentiation [11–13]. MSCs represent one of the cellular determinants of the human stem cell niche in the bone marrow, along with numerous other cells, such as sympathetic neurons, macrophages, osteoblasts and endothelial cells, which all significantly influence the self-renewal and differentiation behavior of HSPCs [14–18]. However, it must be taken into account that the origins of MCSs – bone marrow, adipose tissue, cord blood – have a profound impact on the resulting cell population [19–24]. Similarly, the type of culture and expansion medium has an impact on the resulting cell population. The application of different MSC isolation and culture protocols has significantly hampered the comparability of experimental and clinical data from different laboratories and has posed a major obstacle for multicenter clinical trials [25, 26]. Manufacturing of cell products for clinical application in the European Community must be conducted in compliance with Good Manufacturing Practice (GMP). For GMP-compliant MSC expansion for clinical utilization, the use of xeno-free culture medium is warranted [27]. The strategy for quality control testing depends on the product's cell composition, the manufacturing process and the indication and target patient population. Important quality criteria in this sense are, among others, the immunophenotype of the cells, composition of the culture medium and the risk for malignant transformation, as well as aging and the immunosuppressive potential of the manufactured MSCs [28].

While the niche microenvironment supports not only normal hematopoiesis, it can also contribute to the pathogenesis of myeloid malignancies such as myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) [29], which led to the establishment of an experimental concept of "niche-induced" oncogenesis [30, 31]. MSCs from AML patients exhibited significant growth deficiency and impaired osteogenic differentiation capacity, which was molecularly reflected by a specific methylation signature affecting pathways involved in cell differentiation, proliferation and skeletal development [32]. Geyh et al. [33] demonstrated that MSCs from patients with several MDS subtypes showed reduced growth and proliferation behavior accompanied by premature replicative senescence. This was associated with specific methylation patterns that clearly separated MDS-MSCs from healthy controls. Furthermore, in MDS-MSCs, altered expression of key molecules involved in the interaction with bone marrow-derived (BM) HSPCs was demonstrated, particularly osteopontin, Jagged1, kit-ligand and angiopoietin, as well as several chemokines. Functionally, this translated into a significantly diminished ability to support BM HSPCs in long-term culture-initiating cell assays associated with reduced cell cycle activity [33].

Medyouf et al. [34] showed in a xenograft model that patient-derived MSCs (MDS MSCs) displayed a disturbed differentiation program and were essential for the propagation of MDS-initiating $Lin^{-}CD34^{+}CD38^{-}$ stem cells in orthotopic xenografts. Overproduction of niche factors such as N-cadherin, IGFBP2, VEGFA and LIF was associated with the ability of MDS MSCs to enhance MDS expansion. On the other hand, healthy MSCs adopted MDS MSC-like molecular features when exposed to hematopoietic MDS cells, indicative of instructive remodeling of the microenvironment. There is functional and molecular evidence for the view that MDS involves both the hematopoietic and stromal compartments and that a specific pattern of MSC hematopoietic cell interaction exists within the diseased BM that most likely contributes to the progressive BM clonality and fibrosis frequently observed in MDS patients [34]. This is in line with the findings of Chen et al. [35], who conducted massive parallel transcriptome sequencing of prospectively isolated mesenchymal elements from human low-risk MDS, revealing a common molecular signature, distinct from both normal and ex vivo expanded cells, characterized by cellular stress and upregulation of genes encoding inflammation-associated secreted factors with established inhibitory effects on hematopoiesis [35].

Figure 1 shows a summary of artificial hematopoietic niche approaches based on coculture with MSCs. However, to recreate a physiological niche microenvironment, it is necessary not only to obtain adequate niche cells (e.g., MSCs), but also to monitor the influence of the physical properties of the artificial compartment on the niche cells themselves. MSCs are responsive to not only biochemical,

Table 1. Overview of key technical features for niche formation and interaction [7, 8, 15, 16, 18, 50, 64, 68, 70–74, 78, 99–114, 116–120]

Author, year	Technical features	Species
Scaffold and advanced 2D and 3D culture techniques		
Zhao et al. [7], 2007	Effects of shear stress on 3D human mesenchymal stem cell construct development in a perfusion bioreactor system	Human
Mendez-Ferrer et al. [16], 2010	Cell depletion experiments and spheroid formation, LTC-IC assay	Mouse
Kim and Ma [70], 2012	Perfusion bioreactor flow experiments using 3D scaffolds	Human
Cimetta et al. [8], 2013	Microfluidic bioreactor for dynamic regulation in human pluripotent stem cells	Human
Ding and Morrison [101], 2013	Mouse stem cells on methylcellulose culture structures	Mouse
Zhang et al. [102], 2013	Coculture techniques and transplants using hMSC and hCML in NSG mice	Human Mouse
Choi et al. [103], 2015	hHSC on scaffold-based 3D biomaterial; 2D biomaterial platforms for stem cell niche engineering	Human
Bai et al. [73], 2019	3D culture of human CB-HSPCs in a degradable zwitterionic hydrogel	Human
Kafi et al. [104], 2019	3D collagen scaffold; cell proliferation assay; gene expression analysis	Human
Wilkinson et al. [72], 2019	Defined, albumin-free culture system that supports the long-term ex vivo expansion of functional mouse HSCs	Mouse
Hafner et al. [105], 2020	Micromechanics ECM using HUVEC and MSC on porous scaffolds	Human
Coculture of HSPCs and niche cells		
Walenda et al. [71], 2010	HSPC/MSC coculture, RT-PCR, immunophenotypic and immunoblot analysis	Human
Ehninger and Trumpp [106], 2011	Nestin+ MSC and G-CSF stimulation in HSC niche	Human
Walenda et al. [107], 2011	Coculture hHSPC/MSC; CFU and murine transplant model	Human Mouse
Ludwig et al. [68], 2014	Flow cytometry analysis of HPC, confocal laser scanning on stained MSC	Human
Wuchter et al. [74], 2016	HSPC/MSC coculture, RT-PCR and Western blot, colony-forming cell assay	Human
Ramalingam et al. [108], 2017	Coculture human BM-EC and human HSPC and hEC, fluorescent cell labeling and imaging	Human Mouse
Gottwald et al. [100], 2019	Chip manufacturing and bioreactor setup, coculture of hHSPC/MSC	Human
Animal model systems		
Calvi et al. [15], 2003	Transgenic analysis and antibody staining on bone tissue sections	Mouse
Sieburg et al. [109], 2006	Cell clone dilution techniques and kinetics	Mouse
Anthony and Link [110], 2014	Gene regulation and cytokine expression to establish a murine model of HSC niche	Mouse
Zhou et al. [111], 2014	Immunofluorescent cell/DNA staining and expression analysis	Mouse
Busch et al. [112], 2015	Knock-in mice/flow cytometry/PCR genotyping/single-cell transplantation	Mouse
Kusumbe et al. [113], 2016	MSC differentiation assay and ELISA, BM transplantation experiments	Mouse
Asada et al. [114], 2017	RNA-Seq-analysis and competitive cell transplantation	Mouse
Pinho et al. [115], 2018	Immunocell-labeling and FACS, cell depletion and transgenic mouse models	Mouse
Hypoxia		
Rehn et al. [116], 2011	FACS analysis on hypoxic niche	Mouse
Nombela-Arrieta et al. [117], 2013	Hypoxic status induction and multiphoton microscopy, LSC analysis	Mouse
Cheloni et al. [99], 2017	CML cell lines cultured at hypoxic conditions; kinetics of BCR/Abl suppression	Human
Kwon et al. [78], 2017	Hypoxia enhances cell properties of hMSCs	Human
Functional studies		
Schaniel et al. [118], 2011	Hematopoietic progenitor cell assay, cell cycle analysis	Mouse
Lee-Thedieck et al. [119], 2012	Hydrogel matrix elasticity analysis and atomic force microscopy	Human
Wuchter et al. [64], 2014	HPC transwell migration assay, BM-MSC immunofluorescent staining and ELISA	Human
Velten et al. [120], 2017	RNA sequencing and functional studies	Human Mouse
Monzel et al. [50], 2018	Cell adhesion experiments and live-cell imaging	Human

but also physical cues, such as substrate topography and stiffness [36, 37]. Frank et al. [38] aimed to simulate the dynamic structures of extracellular environments of the marrow in vivo and designed a "dynamic in vitro niche." They used a surrogate substrate for marrow-derived MSCs based on physically cross-linked hydrogels whose elasticity could be adapted dynamically by chemical stimuli. Under frequent mechanical stress, MSCs grown on hydrogel substrates maintained the expression of STRO-1 (a protein marker of MSCs) over 20 days, irrespective of substrate elasticity. Upon exposure to the corresponding induction media, these cultured MSCs could undergo adipogenesis and osteogenesis without requiring cell transfer onto other substrates. The surrogate substrate

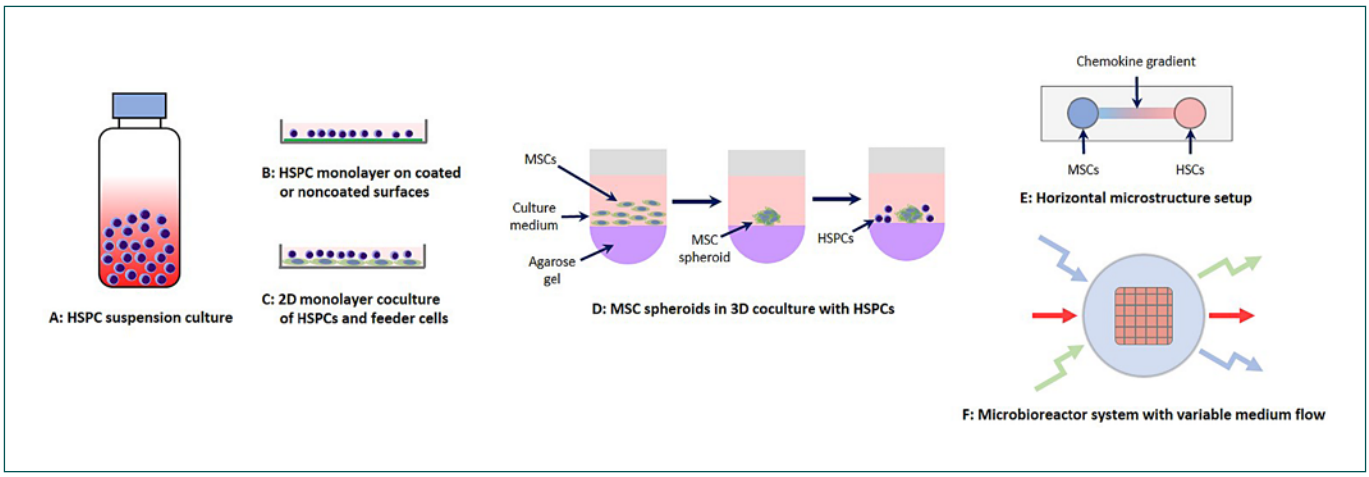

Fig. 1. Scheme for stepwise development of artificial HSPC niche systems. Step A: suspension culture is the simplest form of HSPC culture; however, even with added cytokines, it is not capable of maintaining stemness adequately for more than a few days (compare Walenda et al. [71]). B: HSPCs can be grown as monolayers on coated and noncoated surfaces, such as supporting membranes (compare Monzel et al. [50]). C: 2D coculture systems using a monolayer of feeder cells (e.g., MSCs) result in better HSPC survival and maintenance of stemness compared to suspension culture of HSPCs (compare Ludwig et al. [68]). D: MSC spheroids generated on agarose gel in 3D coculture with HSPCs. E: horizontal microstructure setup for the analysis of chemotactic HSPC migration toward MSCs. MSCs are pre-incubated in one of the reservoirs, and HSPCs are added to the other reservoir after medium exchange (compare Wuchter et al. [64]). F: The use of microbioreactor systems allows further control of medium flow, shear force and oxygen tension (compare Gottwald et al. [100], Kim et al. [70] and Wuchter et al. [74]).

suppressed the proliferation of MSCs by up to 90% without any loss of multiple lineage potential when changing the substrate elasticity every 2nd day. Such an approach can be used to obtain a better understanding of the effect of dynamic mechanical stresses on the fate and functionality of MSCs.

Extracellular Matrix

The complex microenvironment of the bone marrow comprises many different cell types (e.g., macrophages, adipocytes, fibroblasts), which together secrete a specialized extracellular matrix (ECM) [39]. ECM molecules represent an essential part of the stem cell niche and can actively modulate cell functions. Muth et al. [40] demonstrated that human umbilical cord blood (UCB) HSPC adhesion depends on the type of ligand, i.e., the type of ECM molecule, and the lateral, nanometer-scaled distance between the ligands (and the ligand type influences the dependency on the latter). For small fibronectin-derived peptide ligands, the critical adhesive interligand distance for UCB-HSPCs was below 45 nm. Fibronectin-derived and osteopontin-derived protein domains also supported cell adhesion at greater distances. In addition, the expression of the ECM protein thrombospondin-2 in UCB-HSPCs depends on the presence of the ligand and its nanostructured presentation. Functionally, thrombospondin-2 was proven to mediate the adhesion of UCB-HSPCs. In conclusion, UCB-HSPCs are sensitive to the nanostructure of their microenvironment, and they are able to actively modulate their environment by secreting ECM factors.

Kräter et al. [41] used BM-mimetic decellularized ECM scaffolds derived from MSCs to study the interaction between ECM and granulocyte colony-stimulating factor-mobilized peripheral blood HSPCs. Seeding freshly isolated mobilized peripheral blood HSPCs, adherent and nonadherent cells were found. Enhanced expansion and active migration of adherent cells mediated by ECM-incorporated stromal-derived factor could be detected. Probing cell mechanics, adherent cells displayed naïve cell deformation compared to nonadherent cells, indicating physical recognition of ECM material properties by focal adhesion. Integrin αIIb (CD41), αV (CD51) and β3 (CD61) were found to be induced. Integrin β3 induction was identified to facilitate cell adhesion and migration and mediate ECM physical cues to modulate mobilized peripheral blood HSPC function. Scaffold physical parameters might be transduced through outside-in signaling via integrin αVβ3 and lead to mechanical and functional adaptation, resulting in increased stromal cell-derived factor 1 (SDF-1) recognition and chemotactic migration [41].

Adhesion Molecules, Cytokines and Chemokines

Adhesion molecules such as N-cadherin, CD44 or the cytokine SDF-1α and its ligand CXCR4 are essential for the control of vital HSPC functions, including mobilization and homing [42–51]. Burk et al. [52] used planar lipid membranes with precisely defined concentrations of specific ligands to determine the binding strength between human UCB-HSPCs and the BM niche. The relative significance of UCB-HSPC adhesion to the surrogate

niche models via CXCR4/SDF-1α or N-cadherin was quantified by the fraction of adherent cells, the area of tight adhesion and the critical pressure for cell detachment. The authors demonstrated that the binding of UCB-HSPCs to the niche model is a cooperative process, and the adhesion mediated by the CXCR4/SDF-1α axis is stronger than that mediated by hemophilic N-cadherin binding. Statistical image analysis of stochastic morphological dynamics revealed that UCB-HSPCs dissipated energy by undergoing oscillatory deformation.

The CXCR4 antagonist plerixafor has been proven to be highly effective in clinical use for the mobilization of HSPCs [53–63], but the complexity of its interaction with HSPCs and the niche is still not fully understood. In a previous study by our group, the impact of plerixafor on the interaction between human BM-derived MSCs and human UCB-HSPCs was quantitatively assessed [64]. Measurement of SDF-1α levels in the supernatant of MSC cultures revealed that exposure to plerixafor led to a transient increase in SDF-1α levels but had no long-term effect. In a series of Transwell experiments, the addition of SDF-1α significantly stimulated UCB-HSPC migration; this stimulation was almost completely abolished by the addition of plerixafor, confirming the direct impact of the CXCR4/SDF-1α interaction on the migration capacity of UCB-HSPCs. In a microstructural niche model, the chemotactic sensitivity of UCB-HSPCs was determined. UCB-HSPCs migrated actively along an SDF-1α gradient within the microchannels and showed a surprisingly high sensitivity of UCB-HSPCs: a linear SDF-1α concentration gradient of 20 pg ml^{-1} mm^{-1} was calculated [64]. This result indicated that UCB-HSPCs are able to follow a subtle concentration gradient toward the direction of the source of attractant. We further hypothesized that two synergistic effects of plerixafor occur simultaneously, consequently inducing the mobilization of HSPCs from the bone marrow into the peripheral blood: (1) HSPCs lose much of their ability to "sense" the natural SDF-1α gradient in the bone marrow, and (2) the SDF-1α gradient in the BM temporarily collapses due to the release of high amounts of SDF-1α by MSCs after plerixafor treatment. For confirmation, we analyzed the direct effect of plerixafor on the migration of human UCB-HSPCs in a Transwell setup. We confirmed, in agreement with previous findings, that SDF-1α is a potent trigger of HSPC migration, thereby acting as a chemokine [47, 48, 65–68]. The addition of plerixafor led to an almost complete inhibition of this effect, underlining the impact of the SDF-1α/CXCR4 interaction on UCB-HSPC migration [64].

However, the involvement of additional factors or chemokines in this process in vivo is very likely. For instance, Steinl et al. [69] demonstrated the occurrence of highly elevated serum concentrations of matrix metalloproteinase-8 in patients during granulocyte colony-stimulating factor-induced UCB-HSPC mobilization. SDF-1α can be proteolytically processed by treatment with metalloproteinase-8, and this degradation has a strong inhibitory effect on UCB-HSPC migration. This might also explain the high mobilization efficiency of the combination of granulocyte colony-stimulating factor and plerixafor in the clinical setting.

Taken together, these findings illustrate the high sensitivity of HSPCs to SDF-1α and emphasize the extremely fine-tuned nature of the interaction between HSPCs and their niche via the SDF-1α/CXCR4 axis, thereby making it a relevant subject to analyze in niche model systems.

Shear Stress and Medium Flow

Shear stress is an important parameter in regulating MSC growth and development. Zhao et al. [7] analyzed the biomechanical characteristics of MSCs within a 3D perfusion bioreactor system for two flow rates. A higher proliferation rate, higher CFU-F formation, and more fibronectin and HSP-47 secretion were observed at a lower flow rate. The higher flow rate upregulated the osteogenic differentiation potential, as measured by the expression of alkaline phosphatase activity and calcium deposition in the matrix, after 14 days of osteogenic induction.

Medium flow in bioreactor systems is generally controlled in either a parallel or transverse manner, creating different cellular and biomechanical microenvironments in the 3D constructs. Kim et al. [70] used a custom-designed modular perfusion bioreactor system that was operated under either parallel or transverse flow. The influence of the flow patterns on the characteristics of the MSC cellular microenvironment and subsequent construct development was investigated. The parallel flow configuration retained ECM proteins and mitogenic growth factors within the scaffold, effectively preserving MSC progenicity and proliferation potential, whereas transverse flow induced MSC osteogenic differentiation, with higher ALP activity and calcium deposition and upregulation of osteogenic bone markers.

There is strong evidence that macroscopic flow plays a regulatory role in the 3D cellular microenvironment of MSCs. However, configuring the medium flow to specifically direct MSC fate and 3D niche formation in perfusion bioreactor systems remains challenging.

Cellular Composition of (Co)culture Systems

In numerous studies, MSCs maintained the stemness of human HSPCs more efficiently through direct cell-cell contact in two-dimensional coculture systems than in suspension culture [1, 2, 14, 68, 71]. However, very recently, Wilkinson et al. [72] described the development of a defined, albumin-free culture system that supports the long-term ex vivo expansion of functional mouse hema-

topoietic stem cells (HSCs). If these findings could be confirmed with human primary cells, it might be an important step toward enabling in vitro expansion of human HSPCs for transplantation. This represents a particularly relevant aim for clinicians who use UCB-HSCs for allogeneic transplantation because in this setting, the number of available HSPCs is naturally limited.

The next step for a more physiological in vitro environment was the development of 3D (co)culture systems. Bai et al. [73] have recently used a 3D culture system of human HSPCs in a degradable zwitterionic hydrogel and achieved substantial expansion of phenotypically primitive CD34+ cord blood and BM-derived HSPCs. This culture system led to a 73-fold increase in long-term HSC frequency, as demonstrated by limiting dilution assays, and the expanded HSPCs were capable of hematopoietic reconstitution in immunocompromised mice. Both the zwitterionic characteristics of the hydrogel and the 3D format were important for HSPC self-renewal. The authors concluded that the impact of 3D zwitterionic hydrogel culture on mitigating HSPC differentiation and promoting self-renewal might result from an inhibition of excessive reactive oxygen species production via suppression of O_2-related metabolism [73].

In our hands, a 3D coculture system based on a custom-made chip could be established as an in vitro model system of the human HSC niche [74]. An array of up to 625 microcavities, with a size of 300 μm in each orientation, was inserted into a microfluidic bioreactor. The microcavities of the microarray chip were inoculated with human BM MSCs together with umbilical cord blood HSPCs. The cells were mixed and inoculated into the microcavity chip, which was mounted into the microbioreactor, allowing active medium and gas supply. MSCs used microcavities as a scaffold to build a complex 3D mesh. UCB-HSPCs were distributed three-dimensionally inside this MSC network and formed β-catenin- and N-cadherin-based intercellular junctions with the surrounding MSCs. A proportion of UCB-HSPCs maintained the expression of CD34 throughout a culture period of 14 days. Based on colony-forming unit assays, UCB-HSPC potency remained similar after bioreactor coculture. These results support the notion that the 3D microenvironment created within microcavity arrays and bioreactors seems to preserve the stem cell functions of HSCs more efficiently than conventional coculture systems.

However, it remains challenging to evaluate stemness, as most classical functional assays, such as colony-forming cell assays or even long-term culture-initiating cell assays, actually assess the activity of progenitor cells rather than stem cells. In addition, HSC heterogeneity must be considered, as well as the presence of lineage-biased HSCs and lineage-restricted progenitors within the HSC compartment [75]. The most important aspect that needs to be addressed in future studies is therefore to establish potency assays that indicate (preferably on a single-cell level) the impact of the specific microenvironment on HSPCs and their differentiation potential.

Microbioreactor Systems as an Advanced Means to Mimic Nature

Schmid et al. [10] developed a perfusion microbioreactor system that allowed the cultivation of 3D cell cultures in an oxygen-controlled environment in up to 4 independent operating bioreactors. A uniform flow distribution in the microbioreactor could be demonstrated using a computational fluid dynamics model. For oxygen measurements, microsensors were integrated into the bioreactors to measure the oxygen concentration in the geometric center of the 3D cell cultures. Furthermore, an automated cell seeding protocol was implemented. The human mesenchymal stem cell line SCP-1 was seeded on a bovine cancellous bone matrix and cultivated in the developed microbioreactor system at different oxygen levels. Oxygen control was capable of maintaining preset oxygen levels over a cultivation period of several days. In combination with the automated cell seeding procedure, this setup may allow more reproducible experiments, with the goal of generating tissue-engineered grafts in an oxygen-controlled environment.

Cimetta et al. [8] developed a microbioreactor providing time sequences of space-resolved gradients of multiple molecular factors in 3D cell culture settings, along with high-throughput operation and imaging compatibility. These microbioreactors provided multiple gradients of molecular factors in 3D cell culture settings, with complex sequences of time- and space-resolved gradients and the application of rapid dynamic changes in environmental signals. Due to the rapid establishment of steady-state conditions, the microbioreactors ensured the maintenance of precise and time-invariant compositions of the soluble microenvironment to which individual 3D stem cell aggregates were exposed. Embryoid bodies obtained from human embryonic and induced pluripotent stem cells were exposed to concentration gradients of Wnt3a, activin A, BMP4 and their inhibitors to analyze early-stage fate specification and mesodermal lineage commitment. The initiation of mesodermal induction was evaluated by measuring and correlating the gene expression profiles to the concentration gradients of mesoderm-inducing morphogens. The study demonstrated the modulation of pathway activation by the local microenvironment, resulting in nonlinear cell responses to linear concentration gradients. This example shows that microbioreactor systems combining spatial and temporal gradients of molecular and physical factors with human embryonic stem cell and induced pluripotent stem cell cultures may form a basis for predictable in vitro models of development and disease.

The Role of Oxygen Tension in the Bone Marrow Niche

In the bone marrow niche, a low partial oxygen pressure has been reported in the range of 1–7% [76–79]. However, there have been varying data published regarding the functional role of oxygen tension in the BM and its impact on HSPCs [80–87]. Hypoxic conditions apparently lead to increased self-renewal of human embryonic, hematopoietic, mesenchymal and neural stem/progenitor cells and improve the efficiency of genetic reprogramming to induce pluripotency [88]. When investigating enriched stem cell fractions, there is evidence that their metabolism depends on hypoxic conditions and glycolysis [86, 89].

Hypoxic conditions enhance osteogenic differentiation and allow stem cells to remain in an undifferentiated state and thus maintain their potential for multilineage differentiation [90–94]. Hypoxia-inducible factor-1α is one of the most important regulators of cell metabolism. It is stabilized under low oxygen tension. With numerous downstream target genes and crosstalk with other signaling pathways, hypoxia-inducible factor-1α regulates various metabolic pathways, such as the oxidative stress response, glycolysis and mitochondrial respiration [76, 95, 96]. Furthermore, hypoxia-inducible factor-1α is involved in the process of homing and mobilization of HSPCs [77, 91, 97].

Drolle et al. [98] reported that while no difference in hypoxia level could be detected between BM infiltrated by acute myeloid leukemia (AML) and healthy BM, physiological hypoxia of 1% O_2 led to cell cycle arrest of AML blasts in the G_0/G_1 phase, with upregulation of p27 and a consecutive decrease in cells in the S phase. In addition, the susceptibility of AML blasts to cytarabine, an S phase-dependent drug, was found to be significantly decreased, supporting the notion that low oxygen tension in the bone marrow niche might contribute to the chemoresistance of AML blasts. Cheloni et al. [99] reported that driver oncogenic proteins of several leukemias were suppressed following cell incubation at oxygen concentrations compatible with stem cell niche physiology. This suppression might represent a key positive regulator of leukemic stem cell survival and maintenance within the stem cell niche. It appeared that incubation at very low oxygen tension determined the suppression of driver oncogenic proteins and signals in a number of leukemia cell populations, leading to the hypothesis that this suppression is a more common phenomenon occurring in different types of cancers. In all of the tested types of leukemia in this study, oncogene suppression occurred only after incubation in very low oxygen, indicating that induction of the suppression mechanism was more likely related to the onset of severe energy restriction than simply "adaptation to hypoxia." Thus, leukemic stem cells hosted in stem cell niches seemed suited to sustain the long-term maintenance of therapy-resistant minimal residual disease.

Conclusions

The human hematopoietic stem cell niche in the BM is three-dimensional, and any model system that aims to simulate its physiology should also be three-dimensional. In recent years, substantial progress has been made in constructing microbioreactor-based 3D systems to overcome the limitations of static cultivation techniques. Some of them take a number of the above-mentioned factors into account, which contribute to vital niche functions. We are just starting to understand the full potential of such systems. However, an open challenge is the development of precise read-out assays to determine the impact of niche properties on HSPC function. There is a need for new approaches that enable verification of the functional properties of the cells and might serve as potency assays in the context of novel cellular therapies.

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Conflict of Interest Statement

The authors declare no conflicts of interest, except the following: P.W. – Member of Advisory Boards for Sanofi-Aventis.

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Author Contributions

P.W. substantially contributed to the conception of the work, the drafting and editing of the paper. A.D. and H.K. substantially edited and revised the paper critically for important intellectual content.

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