HHS Public Access

Author manuscript

Exp Hematol. Author manuscript; available in PMC 2022 February 01.

Published in final edited form as:

Exp Hematol. 2021 February ; 94: 20–25. doi:10.1016/j.exphem.2020.11.008.

Engineering hematopoietic environments with ossicle and bioreactor technologies

Pia Sommerkamp[1,2,7], **François E. Mercier**[3,7,*], **Adam C. Wilkinson**[4,7], **Dominique Bonnet**[5,8], **Paul Bourgine**[6,8]

[1]Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ) and DKFZ-ZMBH Alliance, Heidelberg, Germany

[2]Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), Heidelberg, Germany

[3]Lady Davis Institute for Medical Research, Department of Medicine, McGill University, Montreal, Quebec, Canada.

[4]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, USA.

[5]The Francis Crick Institute, London, United Kingdom

[6]Laboratory for Cell, Tissue, and Organ Engineering, Department of Clinical Sciences, Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden.

[7]Co-first authors

[8]Co-senior authors

Abstract

The bone marrow microenvironment contains cellular niches that maintain the pool of hematopoietic stem and progenitor cells and support hematopoietic maturation. Malignant hematopoietic cells also co-opt normal cellular interactions in order to promote their own growth and evade therapy. *In vivo* systems to study human hematopoiesis have mostly been achieved through transplantation into immunodeficient mouse models. However, incomplete cross-compatibility between the murine stroma and transplanted human hematopoietic cells limit the rate of engraftment and the study of relevant interactions. To supplement *in vivo* xenotransplantation models, complementary strategies have recently been developed, including the use of three-dimensional human bone marrow organoids *in vivo*, generated from bone marrow stromal cells seeded onto osteo-inductive scaffolds, as well as the use of *ex vivo* bioreactor models. These topics were the focus of the Spring 2020 International Society for Experimental Hematology New Investigator webinar. We review here the latest advances in generating humanized hematopoietic organoids and how they allow for the study of novel microenvironmental interactions.

*Corresponding author: francois.mercier@mcgill.ca.

Keywords

Ossicle; bioreactor; hematopoiesis; hematopoietic stem cell; niche

Introduction

Hematopoietic stem and progenitor cells (HSPCs), primarily residing within the bone marrow (BM), sustain life-long hematopoiesis[1–4]. The BM microenvironment is composed of a range of cell types – from mesenchymal stromal cells (MSCs) and endothelium to various mature hematopoietic cell types – and provide various supportive extrinsic cues[5, 6]. The accumulation of somatic mutations within HSPCs is well described as driver of a range of hematological disorders such as myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), and leukemias[7–12]. However, there is increasing recognition for the interplay between mutant HSPCs and altered BM stroma in the development and progression of hematological diseases[13].

While hematopoietic niches have long been studied in model organisms, particularly mouse and zebrafish, understanding the microenvironmental interactions specific to human hematopoiesis and leukemogenesis are essential to the development of new therapeutic strategies. Replicating the human BM microenvironment *in vitro* is challenging and, correspondingly, supporting primary HSPCs in culture has also remained difficult[14]. Additionally, how purified populations of HSPCs grow *ex vivo* may differ to their activities within the context of their native cellular niche. The development of mutant and genetically engineered mouse strains expressing human hematopoietic factors has greatly improved the engraftment of human HSPCs[15], but uncertainties remain regarding the ability of these models to replicate all relevant microenvironmental interactions.

Recent advances in humanized BM organoids provide an approach to grow human hematopoietic cells in a conspecific microenvironment for better engraftment and experimental interrogation of cellular interactions[3, 16, 17]. Engineered hematopoietic environments were the focus of the Spring 2020 International Society for Experimental Hematology New Investigator Committee Webinar. This webinar (also available here: https://www.youtube.com/watch?v=R3XSTALtmjs&feature=youtu.be) included presentations from Drs. Dominique Bonnet and Paul Bourgine, who discussed humanized ossicle and bioreactor technologies, respectively, and was moderated by Dr. François Mercier. In this *Perspective*, we introduce traditional xenotransplantation assays and then discuss recent progress in the humanization of the hematopoietic niche using ectopic ossicle and bioreactor technologies.

Traditional xenotransplantation assays

The transplantation of human hematopoietic cells into immunodeficient mice has been pivotal in defining the nature of human hematopoiesis and leukemia including phenotyping human HSPCs and hematopoietic hierarchies[18–20], providing evidence for leukemic stem cells and leukemic evolution[12, 15, 21, 22], and evaluating new therapeutic strategies[15, 23, 24]. However, there remain limitations to xenotransplantation. In particular, individual cases of

acute myeloid leukemia (AML) and MDS display variable engraftment[25], as the ability of hematological malignancies to engraft is usually characteristic of cases with an aggressive clinical course. It has also been observed that clonally heterogeneous mixtures of leukemic cells do not always engraft representatively[26].

Since the development of the first NOD/Scid immunodeficient mouse model[18, 21], various derivatives of this mouse strain have been developed to further improve human hematopoiesis in mice[15, 23]. This includes the NOD/Scid/IL2Rg-KO (NSG) mice, *Kit*-mutant NSG mice (NSG-W41 and NBSGW), and human cytokine-expressing NSG mice (e.g., the NSG-S and MISTRG) strains[27–32]. Expression of human cytokines has improved the development of human innate immune cells in mice compared to earlier models (e.g. NOD/Scid), thereby considerably improving the engraftment of different patient samples in these xenotransplantation settings[15]. The generation of murine recipient strains that are even more permissive for human hematopoiesis, native or diseased, is pursued through the suppression of innate immune responses or humanization of additional ligands (and is reviewed in detail elsewhere[33, 34]). However, all these models rely on the engraftment of human HSPCs within mouse hematopoietic organs, particularly the mouse bone marrow and spleen. Although these microenvironments can support the long-term engraftment of human cells, species differences mean these microenvironments are not fully analogous to human. For example, numerous ligand-receptor pairs are yet to be humanized in these mouse models. For the study of these specific interactions in human hematopoiesis, generating human hematopoietic niches de novo offers flexible, complementary strategies.

In vivo ossicle models

Over the last decade, several groups including Dr. Bonnet's have developed methods to model the human BM microenvironment in mice (Figure 1A) using subcutaneous humanized ossicles[35–41]. These protocols often involve the seeding of human MSCs onto a 3D scaffold composed of extracellular matrix, then subcutaneously implanting the scaffold into NSG mice. However, other methods such as those characterized by the Majeti laboratory[36, 37] subcutaneously inject MSCs mixed in an extracellular matrix gel to generate the ossicle (Figure 1A). Within the mouse, these humanized ossicles become colonized with mouse endothelium and mouse hematopoietic cells[38]. The microenvironment within the ossicle allows engraftment of injected human HSPCs and supports human HSPC expansion and differentiation. Highlighting that much remains to be learned about the human HSPC-niche interactions, unknown differences between MSC donors cause major variations in the composition and engraftment of human hematopoietic lineages in these models[38].

Various improvements to this basic ossicle formation protocol have been made. For example, to further improve endochondral ossification within the scaffold, the Bonnet lab incorporated bone morphogenic protein 2 (BMP-2), an osteo-inductive signal, into the collagen scaffold[38]. Similarly, Bourgine and colleagues primed MSCs for cartilage differentiation via transgenic expression of stromal-derived factor 1 alpha (SDF1α, also known as CXCL12), with the aim of mimicking the endochondral ossification pathway seen during embryonic bone development[41]. These methods also highlight the potential to genetically modify the MSCs to study the role of specific signaling pathways in these humanized hematopoietic

microenvironments. Additionally, the Bonnet lab showed that seeding the scaffold with human endothelial cells can lead to formation of human vasculature within the ossicle, further humanizing this BM niche[38].

One goal of ossicle technology is to overcome the variable engraftment of hematological malignancies in mice[25]. A comparison between the engraftment of samples derived from patients with AML, using traditional xenotransplantation assays and ossicle transplantation assays, found that ossicles were superior[38,36]. In particular, humanized ossicles allowed the engraftment of AML samples that engrafted poorly otherwise[38]. This highlights the utility of the ossicle system and its advantages over traditional xenotransplantation assays. Ossicle technology is now being expanded to other diseases, such as MDS, in which patient samples poorly engraft in traditional murine xenotransplantation and are therefore difficult to study *in vivo*[42]. To date, MSCs have been collected from healthy donors, but there is also great interest to generate ossicles from patient derived MSCs in the future. Using ossicle models, it should be possible to dissect the cellular and molecular interactions within the human BM niche in health and disease, and ultimately help to identify new therapeutic targets for disease intervention.

Ex vivo bioreactors models

While the development of humanized ossicles represents a unique opportunity to study hematologic diseases *in vivo*[43], some limitations remain (Table 1). For example, established *in vivo* models remain chimeric, since vasculature as well as nerve fibers are of murine origin. This also holds true for some circulating cytokines, which are not always conserved between mouse and human, such as GM-CSF[44]. Thus, additional techniques are needed to facilitate the study of human HSPCs and hematopoietic malignancies, including engineered *in vitro* BM systems[45]. These methods can be used for HSPC expansion, drug screening or disease modeling. Several models have been established, from 2D cultures to static 3D scaffold systems up to dynamic 3D setups, which implement the component of perfusion.

Bourgine and colleagues recently engineered a dynamic human 3D *in vitro* BM niche[43] (Figure 1B). This bioreactor consists of four components: (I) a porous ceramic material, which mimics the bone structure, (II) human MSCs, (III) human HSPCs, and (IV) perfusion of serum-free medium. Briefly, MSCs were allowed to colonize the ceramic scaffold for one week, and osteogenesis primed for three weeks. After this initial period of engineered niche (eN) formation, $CD34^+$ HSPCs and recombinant growth factors (SCF, TPO, Flt3-L) were added. Time-course analysis identified gradual seeding of hematopoietic cell populations within the eN after addition of HSPCs. In comparison to the control condition, where only the ceramic scaffold was used, the eN setting promoted the expansion of phenotypic HSPCs. Functional analysis revealed *in vitro* maintenance of stem cells. However, stem cell performance was still reduced compared with freshly isolated human HSPCs. Within the eN, formation of extracellular matrix (ECM) and osteocalcin at the scaffold surface was observed, characterizing the eN as osteoblastic. Interestingly, a functional compartmentalization could be observed, which mimicked the biological cellular distribution of normal BM. The human HSCs were preferentially located in the ECM close to MSCs, while more committed progenitors were also detected in the supernatant. The

presented model exhibits characteristic features of a human osteoblastic BM niche and can be used to study the effects of different extrinsic factors on HSCs or for disease modeling.

In a proof-of-concept experiment, Dr. Bourgine showed that overexpression of SDF1α in human MSCs led to increased maintenance and quiescence of cultured human HSCs[43]. In addition, niche injury was modeled *in vitro* by application of the DNA-damaging compound bleomycin. Bleomycin led to a decrease in HSPC numbers in the ECM compartment of the bioreactor and an increase in the number of cycling cells. These proof-of-principle approaches demonstrated that such bioreactor systems can be used to analyze the influences of different biological and chemical factors on the MSC and HSPC compartments. Current applications of this bioreactor technology include the *ex vivo* maintenance and study of HSPCs derived from patients suffering from MPNs. This approach will potentially allow for mechanistic analyses and drug screenings to be performed using patient-derived MPN HSPCs *ex vivo*, investigations that have been challenging to date.

Together, these studies demonstrate that complex biological environments such as the human BM can be engineered *in vitro*, allowing for improved functional maintenance of HSPCs. The platform can be used to investigate the role of different factors and disease development. In the future, it will be of interest to study the long-term culture of HSPCs using bioreactor systems. Recently, long-term culture of mouse HSPCs has been improved considerably[46, 47]. Refined compositions of culture medium in combination with engineered BM niches could facilitate culture and expansion of HSPCs. In addition to the analysis of HSPCs derived from patients suffering from hematological diseases, it will be also important to have a closer look at matched patient-derived MSCs. Such studies could reveal additional mechanistic interactions within the diseased niche and enable the identification of potential niche-specific therapeutic targets.

Conclusions

The development of new models to study human hematopoiesis and leukemogenesis and the bone marrow microenvironment, including *in vivo* ossicle models and *ex vivo* bioreactors, are providing new biological and biomedical insights. In the recent ISEH webinar, Drs. Bonnet and Bourgine presented how humanized BM organoids can be used to improve the engraftment of hard-to-transplant patient samples, characterize the effect of microenvironmental perturbations on HSPCs, and expand human hematopoietic progenitor cells *ex vivo*. We expect that these applications are only the start for these powerful technologies. Further optimization and characterization of these engineered microenvironments will undoubtedly yield new fundamental and practical insights in the regulation of normal and aberrant hematopoiesis.

Acknowledgments:

We thank ISEH staff and colleagues on the ISEH New Investigator Committee for their support.

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Highlights:

- A review of the use of ossicle models to model human hematopoiesis
- Discusses the use of bioreactors to grow human HSPCs *ex vivo*

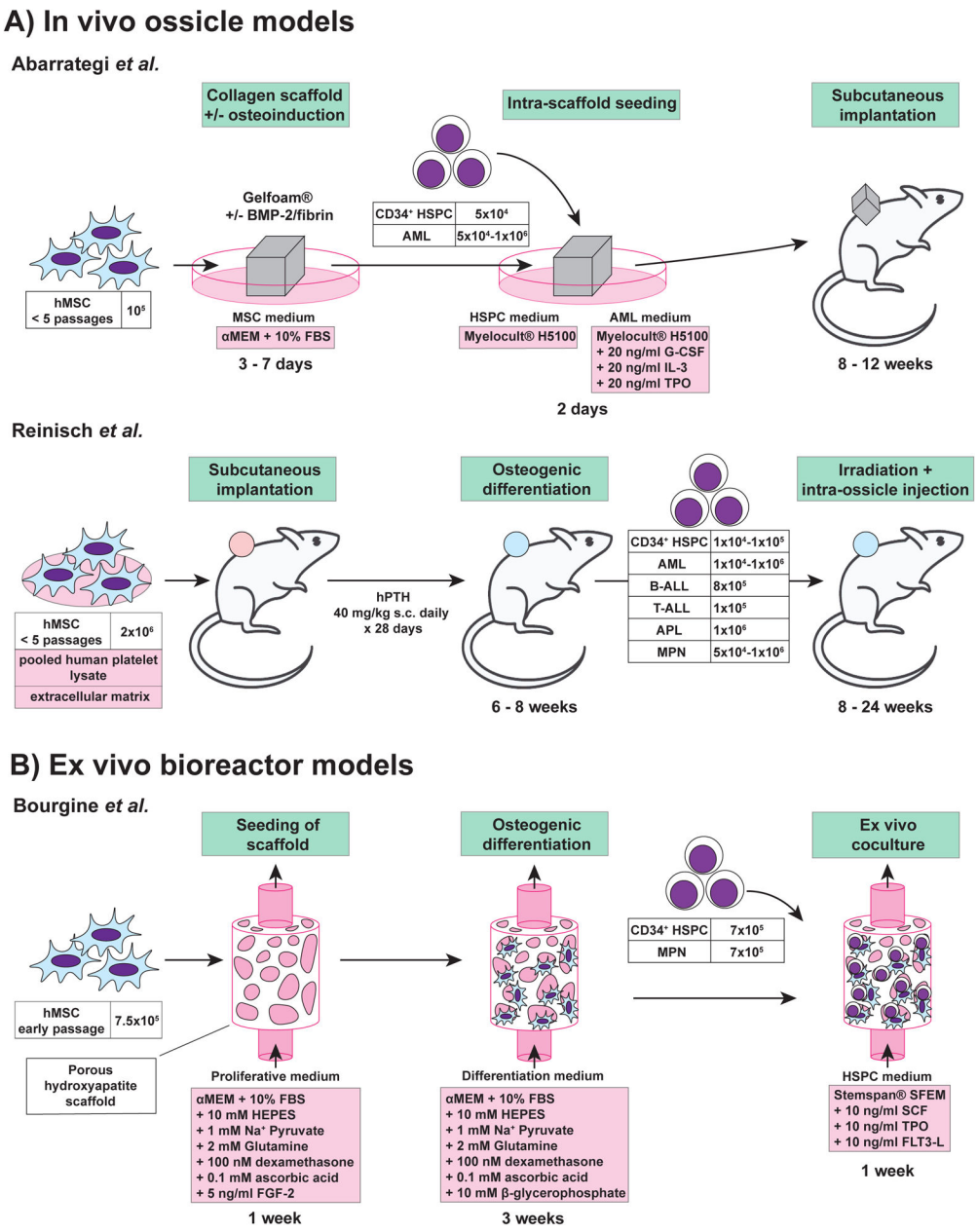

Figure 1: Schematic summary of ossicle and bioreactor technologies

Approaches for engineering tri-dimensional hematopoietic environments. **A.** In vivo ossicle models. **B.** Ex vivo bioreactor model. ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; BMP-2: bone morphogenetic protein; FBS: fetal bovine serum; FGF-2: fibroblast growth factor-2; FLT3-L: Fms-related tyrosine kinase 3 ligand; G-CSF: granulocyte colony stimulating factor; HSPC: hematopoietic stem and progenitor cells; hMSC: human mesenchymal stromal cells; hPTH: human parathyroid hormone; IL-3: interleukin-3; MPN: myeloproliferative neoplasm; SCF: stem cell factor; TPO: thrombopoietin.

Table 1:

Comparison of ossicle and bioreactor technologies

Model	Major Pros	Major Cons
In vivo ossicle models	Support functional human HSCs long-term as well as various myeloid malignancies; offers an in vivo model of the human bone marrow niche	Requires use of immunodeficient animals; ossicles can become chimeric with infiltration of mouse cells; batch-to-batch variability between donor MSCs
Ex vivo bioreactor technology	Fully defined 3D microenvironment that can be easily modulated real-time; amenable to time course analysis, perturbations, etc.	Current bioreactor technology cannot currently stably support HSCs long-term; requires constant perfusion