Biomimetic human bone marrow tissues: models to study hematopoiesis and platforms for drug testing

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ABSTRACT

We propose an in vitro 3D culture system combining perfusion bioreactors, scaffolds and human primary cells to engineer fully-humanized, biomimetic and customizable bone marrow tissues. This system could serve as a model to investigate human hematopoietic stem cell niches, but also as a drug testing platform for pharmaceutical research and patient-personalized medicine.

Hematopoietic stem cells (HSCs) emerge among other adult stem cells as the most studied and characterized tissue-specific stem cell in the organism, and HSC transplant is still the gold-standard therapy to treat hematopoietic malignancies. In decades of fundamental research with murine models we have learned the crucial role of the different bone marrow (BM) microenvironments or niches to fine-tune HSC functions according to organ demands in physiological conditions, but also their contributions to pathological conditions. Despite this accumulative evidence, our knowledge of human BM stem niches and their impact on human pathophysiological hematopoiesis remains very limited due to reduced accessibility to intact BM biopsies (for technical and ethical issues). In general, it is assumed that mechanisms governing human BM stem cell niches also apply in the human system and only studies with humanized mouse models have shed light in the regulation of human HSC niches. Recently, in addition to novel patient-derived xenograft (PDX) humanized models, several bioengineering approaches have been proposed to generate humanized BM niches in vitro. Nevertheless, even if human cells are able to efficiently engrave and generate a humanized environment, these in vitro models always remain chimeric and it is very challenging to exclude the influence of the surrounding murine cells. Of special relevance is the contribution of the murine vasculature, which facilitates the exposure of human cells to mouse-derived signals.

In order to bypass these limitations, our lab has developed an in vitro 3D culture system based on the use of perfusion bioreactors, scaffolding materials and human primary BM mesenchymal stromal cells (MSCs) to engineer fully humanized (xeno-free) biomimetic tissues that recapitulate many physiological features of native BM osteoblastic niches (O-N). This system has been validated for the maintenance and expansion of healthy human cord blood HSCs, and more recently for the long-term culture of malignant HSCs isolated from patients diagnosed with hematological malignancies, such as acute myeloid leukemia (AML) or myeloproliferative neoplasms (MPN). In particular, we showed that engineered O-N not only enable malignant cell expansion in the bioreactor system, but they also promote HSC maintenance within the niche tissue through the expression of key chemotactic signals like C-X-C Motif Chemokine Ligand 12 (CXCL12) and Vascular Cell Adhesion Molecule 1 (VCAM1). In contrast, mature hematopoietic cells are continuously released to the fluidic phase of the bioreactor, recapitulating the in vivo traffic of hematopoietic cells between the BM and the bloodstream.

Since not only osteoblastic niches but also perivascular niches regulate healthy and malignant hematopoiesis, we explored the customization potential of our 3D culture system by engineering different models of perivascular BM niches. On one hand, we took advantage of the angiogenic potential of cells derived from the stromal vascular fraction (SVF) of human adipose tissue to vascularize the previously validated osteoblastic niche. This vascularized O-N, recapitulating features of native endosteal perivascular microenvironments, sustains the maintenance of primitive HSCs better than avascular osteoblastic niches. On the other hand, SVF cells were also used to generate a non-osteogenic stromal-vascular niche (SV-N), which mimics features from perivascular niches in central BM.

The long-term culture of the same leukemic cells in engineered niches recapitulating osteoblastic vs perivascular features in central BM (O-N vs. SV-N) allowed us to investigate and compare human leukemia development when cells are exposed to different niche signals. Our results confirmed that these environmental signals differentially influence the functions of leukemic cells and thus validated our system as a relevant model to study leukemogenesis mechanisms in different fully humanized niches.

The BM microenvironments not only play an important role in the pathogenesis of hematopoietic malignancies, but they also contribute to the resistance of leukemic cells to...
chemotherapy. In fact, the failure of many anti-leukemic therapies might be anticipated if those drug candidates could be tested on leukemic cells surrounded by their native micro-environment. In this regard, we provided a proof-of-principle with gold-standard chemotherapy (Cytarabine; Ara-C) for the application of these 3D biomimetic niches as a drug testing platform. Our recent results revealed that, in comparison to classic 2D culture systems, engineered 3D niches provide a large protection to leukemic cells against the chemotherapeutic treatment, confirming the need of considering the niche when testing the efficacy of new drug candidates.

In summary, we propose an in vitro 3D culture system based on perfusion bioreactors to engineer biomimetic and customizable BM niches that can be applied as animal-free model to study fundamental aspects of human hematopoiesis and as drug testing platform (Figure 1). This platform might have potential for clinical research in the context of patient-perso- nalized medicine, if both the hematopoietic and stromal fractions used to generate the niches are derived from a single patient. From a different but complementary angle, these engineered niches might be optimized and standardized to create a 3 R principles-conform platform for the pre-clinical drug screening and validation assays performed in pharmaceutical research. Future research will have to exploit the potential implications of this biotechnological tool for gaining fundamental knowledge on human hematopoiesis, reducing animal experimentation and improving medical therapies.

**Author contributions**

A.G-G and I-M prepared the figure and wrote the manuscript.

**Disclosure statement**

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**References**

1. Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. Am J Pathol. 2006;169(2):338–3. doi:10.2353/ajpath.2006.060312
2. Morrison SJ, Scadden DT. The bone marrow niche for hematopoietic stem cells. Nature. 2014;505(7483):327–334. doi:10.1038/nature12984.
3. Pinho S, Frenette PS. Hematopoietic stem cell activity and interactions with the niche. Nat Rev Mol Cell Biol. 2019;20(5):303–320. doi:10.1038/s41580-019-0103-9.
4. Mendez-Ferrer S, Bonnet D, Steensma DP, Hasserjian RP, Ghobrial IM, Gribben JG, Andreeff M, Krause DS. Bone marrow niches in haematological malignancies. Nat Rev Cancer. 2020;20(5):285–298. doi:10.1038/s41568-020-0245-2.

5. Doulatov S, Notta F, Laurenti E, Dick JE. Hematopoiesis: a human perspective. Cell Stem Cell. 2012;10(2):120–136. doi:10.1016/j.stem.2012.01.006.

6. Abarrategi A, Mian SA, Passaro D, Rouault-Pierre K, Grey W, Bonnet D. Modeling the human bone marrow niche in mice: from host bone marrow engraftment to bioengineering approaches. JExpMed. 2018;215(3):729–743. doi:10.1084/jem.20172139.

7. Bourgine PE, Klein T, Paczulla AM, Shimizu T, Kunz L, Kokkaliaris KD, Coutu DL, Lengerke C, Skoda R, Schroeder T, et al. In vitro biomimetic engineering of a human hematopoietic niche with functional properties. ProcNatlAcadSciUSA. 2018;115(25):E5688–E5695. doi:10.1073/pnas.1805440115.

8. Garcia-Garcia A, Klein T, Born G, Hilpert M, Scherberich A, Lengerke C, Skoda RC, Bourgine PE, Martin I. Culturing patient-derived malignant hematopoietic stem cells in engineered and fully humanized 3D niches. Proc Natl Acad Sci U S A. 2021;118(40):e2114227118. doi:10.1073/pnas.2114227118.

9. Born G, Nikolova M, Scherberich A, Treutlein B, Garcia-Garcia A, Martin I. Engineering of fully humanized and vascularized 3D bone marrow niches sustaining undifferentiated human cord blood hematopoietic stem and progenitor cells. J Tissue Eng. 2021;12:20417314211044855. doi:10.1177/20417314211044855.

10. Zhou HS, Carter BZ, Andreeff M, Zhou H-S, Z. Carter B, Andreeff M. Bone marrow niche-mediated survival of leukemia stem cells in acute myeloid leukemia: yin and Yang. Cancer Biol Med. 2016;13(2):248–259. doi:10.20892/j.2095-3941.2016.0023.