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AUTHOR'S VIEWS

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.

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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.[1] Decades of fundamental research with murine models have illustrated the crucial role of the different bone marrow (BM) microenvironments or niches to fine-tune HSC functions according to organism demands in physiological conditions,[2,3] but also their contributions to pathological conditions.[4] Despite this accumulative evidence, our knowledge of human BM stem cell 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 murine 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.[5] Recently, in addition to novel patient-derived xenograft (PDX) humanized models, several bioengineering approaches have been proposed to generate humanized BM niches *in vivo*.[6] Nevertheless, even if human cells are able to efficiently engraft and generate a humanized environment, these *in vivo* 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 system 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,[7] 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).[8] 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.[9] 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.[8]

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.[8]

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

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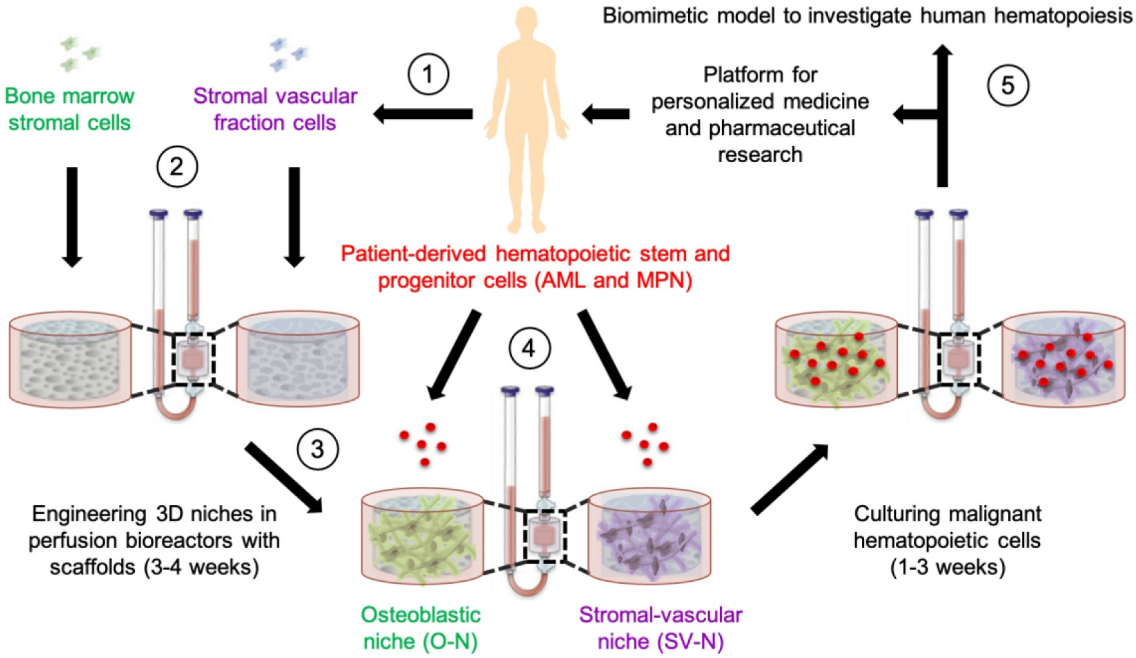

Figure 1. Bioengineering approach to generate patient-derived, biomimetic and customizable 3D niches for malignant hematopoietic cells. These microenvironments are engineered by seeding human primary cells (bone marrow stromal cells or adipose-derived stromal vascular fraction cells) (1) onto 3D scaffolds inside perfusion bioreactors (2). Then, cells are cultured for 3–4 weeks under a constant perfusion flow to generate mature 3D niches (osteoblastic, O-N; or stromal-vascular, SV-N) (3). Finally, patient-derived hematopoietic stem and progenitor cells (AML and MPN) are seeded and cultured in these engineered niches for up to 3 weeks (4). This system can be exploited as biomimetic model to investigate human hematopoiesis in fully humanized niches and/or as platform to test therapeutic candidates for pharmaceutical research, including personalized medicine settings (5) (modified from García-García et al. 2021[8]).

chemotherapy.[10] 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 microenvironment. 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.[8]

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-personalized 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

No potential conflict of interest was reported by the author(s).

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