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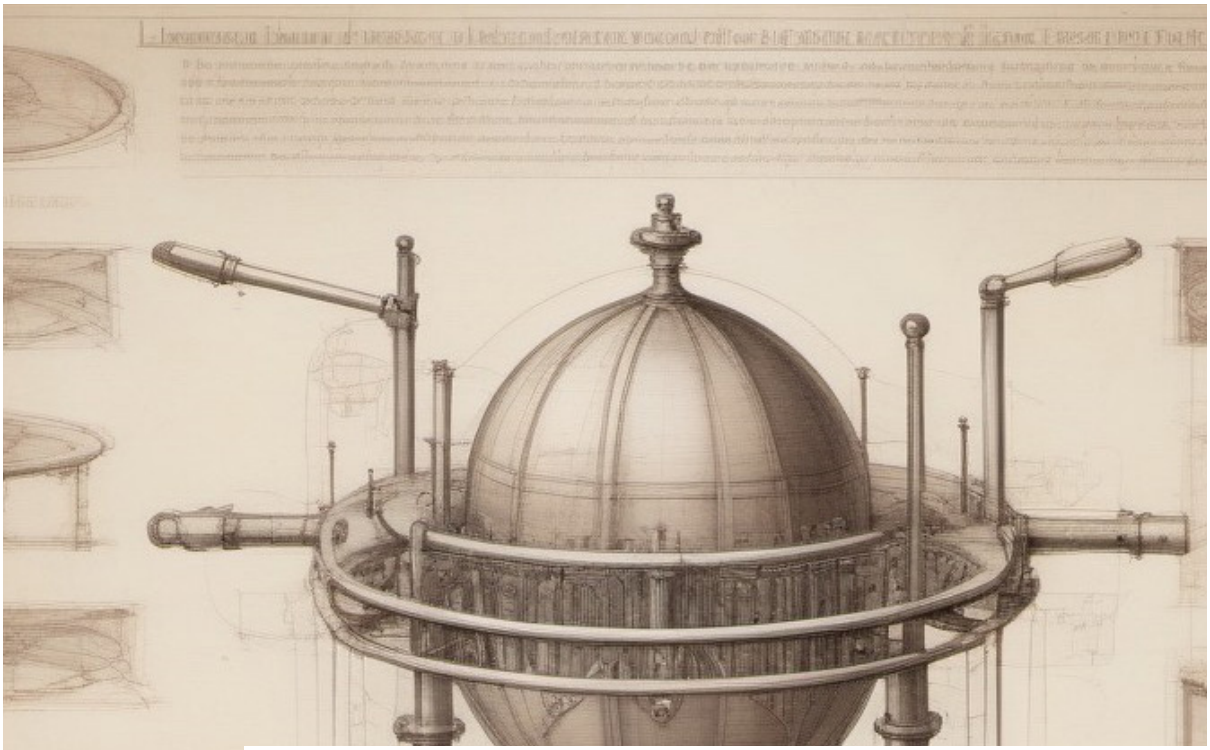

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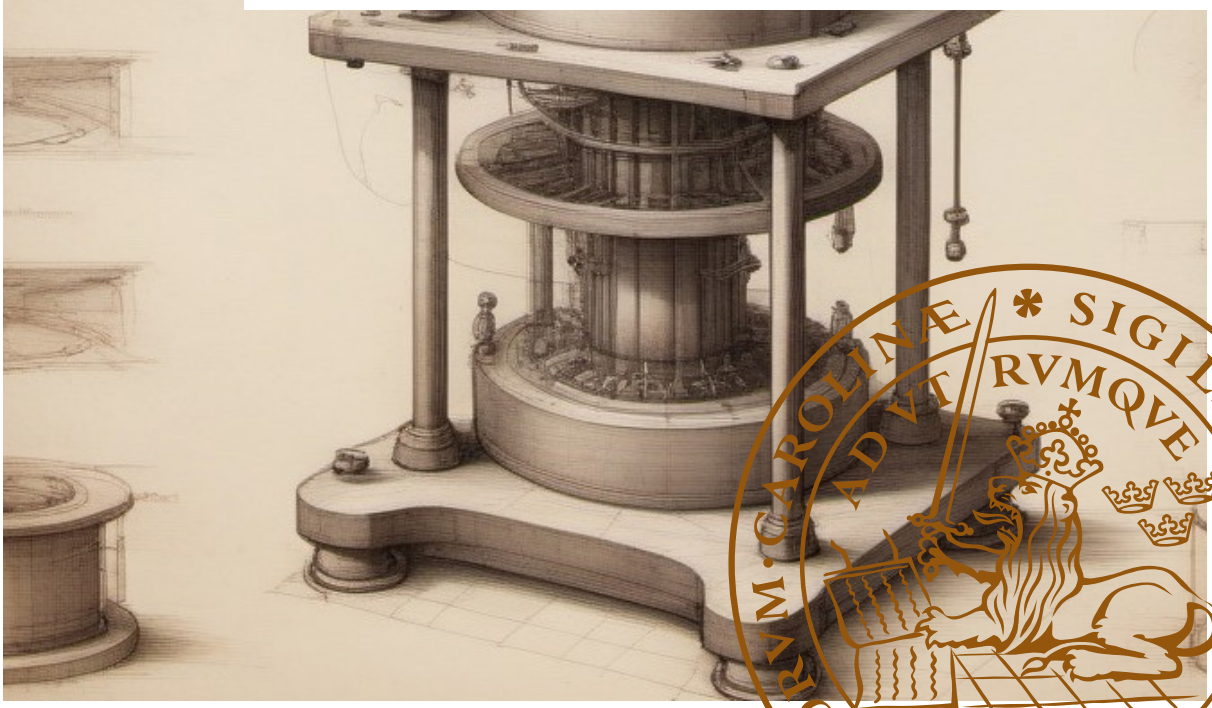

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Steven J. Dupard

LUND
UNIVERSITY

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Abstract:

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This thesis opens on the possibility to harness MSCs' ability to form humanized ossicles (hOss), a human BM organ hosting hematopoiesis in mice. While hOss provides an advanced in vivo model for human niche investigation, we report here that the lack of (1) a standardized protocol, (2) a stable MSCs source, and (3) functional characterization limits its exploitation. We thus aim at addressing these shortcomings. We subsequently report the reproducible generation of hOss using the human MSCs line MSOD-B (Mesenchymal Sword of Damocles Bone morphogenetic protein type-2). Using this standardized tool, we demonstrate the robust hOss formation, offering superior engraftment of human healthy and leukemic blood cells in hOss compared to mouse bones. This was correlated to the MSOD-B capacity to reconstitute the mesenchymal elements of the human BM niche. These results prompted us to explore if our MSCs line could form hematopoietic niche in vitro.

The in vitro engineering of human BM niches is performed by combining scaffolding material and 3D perfusion bioreactor systems. Using such set-up, we describe the generation of osteoblastic niches through the functionalization of a collagen scaffold by directed human MSCs differentiation. HSPCs are then infused, leading to niche interactions reconstitution. Standardization is then achieved by combining MSOD and our open-source 3D printed perfusion bioreactor. We established the rapid design of human hematopoietic niche of custom sizes and further biologically validated our system for up to two weeks. We observed MSCs-HSPCs interactions throughout the niche leading to the phenotypic expansion of the blood stem cell populations.

The last part of the thesis focuses on the study of mitochondrial transfer between MSCs and HSPCs, a key aspect of niche communication. This recently proposed niche interaction route remains cryptic, and the exploitation of bioengineering systems would largely help uncovering this mechanism. To this end, a MSCs line bearing inducible mCherry mitochondrial tag was first generated, the iMSOD-mito. By exploiting iMSOD-mito in 2D and 3D in vitro culture systems, we evidenced significant mitochondrial transfer from mesenchymal to leukemic and healthy HSPCs. Most importantly a preferential transfer towards phenotypic CD34+/CD38-/CD45RA-/CD90+/EPCR+ stem cells was identified. We further associated this transfer with retained quiescence in single-cell divisional assays.

In summary, this thesis presents the development and exploitation of advanced standardized models of the human BM niche. We envision that this work will facilitate the understanding of the mesenchymal regulation of hematopoiesis in both healthy and malignant contexts.

Key words: Bone marrow microenvironment, Hematopoietic niche modeling, mitochondrial transfer.

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À mes parents.

À Mme Charles, M. L'Huissier et M. Lagneau pour avoir cultivé ma passion pour l'écriture, la science and le dépassement de soi.

"Ti ash i koup gro bwa"
Proverbe réunionnais

Table of Contents

Abstract

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In summary, this thesis presents the development and exploitation of advanced standardized models of the human BM niche. We envision that this work will facilitate the understanding of the mesenchymal regulation of hematopoiesis in both healthy and malignant contexts.

Résumé

La niche de la moelle osseuse est un microenvironnement cellulaire, moléculaire et physique complexe capable d'héberger et de soutenir l'hématopoïèse. Elle se caractérise par sa capacité à maintenir et à stimuler le renouvellement et la différenciation des cellules souches et progénitrices hématopoïétiques (CSPH), donnant lieu à la génération de tous les types de cellules sanguines. Cependant, les interactions entre les éléments de la niche dans un contexte humain restent largement incertaines en raison des difficultés à concevoir et à exploiter des modèles humains pertinents. Pour surmonter ces limitations, cette thèse explore la génération de nouveaux outils et systèmes cellulaires pour la bio-ingénierie in vivo et in vitro des niches de la moelle osseuse humaine. Les travaux se concentrent principalement sur l'exploitation des cellules stromales mésenchymateuses (CSM) humaines, qui constituent les principaux éléments régulateurs de la niche de la moelle osseuse.

Cette thèse s'ouvre sur la possibilité d'exploiter la capacité des CSM à former des ossicules humanisés (hOss), un organe de la moelle osseuse humaine hébergeant l'hématopoïèse après transplantation chez les souris. Bien que les hOss offrent un modèle in vivo avancé pour l'investigation de la niche humaine, nous rapportons ici que l'absence (1) d'un protocole standardisé, (2) d'une source stable de CSM et (3) d'une caractérisation fonctionnelle de la niche formée limite son exploitation. Nous nous donc choisi de remédier à ces lacunes.

Nous confirmons ici la génération de hOss de manière reproductible à l'aide de la lignée de CSM humaines MSOD-B (Mesenchymal Sword of Damocles Bone morphogenetic protein type-2). En utilisant cet outil standardisé, nous démontrons la formation robuste de hOss, offrant une greffe supérieure de cellules sanguines humaines saines et leucémiques dans les hOss en comparaison aux os de souris. Cette performance est corrélée à la capacité de MSOD-B à reconstituer les éléments mésenchymateux de la niche de la moelle osseuse humaine. Ces résultats nous ont incités à explorer si notre lignée de CSM pouvait également former une niche hématopoïétique in vitro.

L'ingénierie in vitro des niches de la moelle osseuse humaine est réalisée en combinant un matériau de support biologique et des systèmes 3D de bioréacteurs à perfusion. En utilisant un tel dispositif, nous décrivons ici la génération de niches ostéoblastiques réalisées par la fonctionnalisation d'une matrice de collagène par différenciation dirigée des CSM humaines. Les CSPH sont ensuite infusés, ce qui conduit à la reconstitution des interactions de la niche. La standardisation est ensuite obtenue en combinant MSOD et notre bioréacteur à perfusion imprimé en 3D et disponible en open-source. Nous avons établi la conception rapide de niches hématopoïétiques humaines de tailles personnalisées et avons validé

biologiquement notre système jusqu'à deux semaines de culture. Nous avons observé des interactions CSM-CSPH tout au long de la niche, conduisant à l'expansion phénotypique des populations de cellules souches sanguines.

La dernière partie de la thèse se concentre sur l'étude du transfert mitochondrial entre les CSM et les CSPH, un aspect clé de la communication de la niche. Cette voie d'interaction de la niche récemment découverte reste cependant cryptique, et l'exploitation de systèmes de bio-ingénierie contribuerait largement à élucider ce mécanisme. Dans ce but, une lignée de CSM portant une étiquette mitochondriale mCherry inductible a été générée, l'iMSOD-mito. En exploitant iMSOD-mito dans des systèmes de culture in vitro 2D et 3D, nous avons mis en évidence un transfert mitochondrial significatif des cellules mésenchymateuses vers les CSPH leucémiques et saines. Plus important encore, un transfert préférentiel vers les cellules souches CD34+/CD38-/CD45RA-/CD90+/EPCR+ a été identifié. Nous avons également associé ce transfert à une quiescence conservée dans des essais divisionnels.

En résumé, cette thèse présente le développement et l'exploitation de modèles avancés et standardisés de la niche de la moelle osseuse humaine. Nous pensons que ce travail facilitera la compréhension de la régulation mésenchymateuse de l'hématopoïèse dans les contextes sains mais aussi pathologiques.

Populärvetenskaplig sammanfattning

Benmärgsnischen (BM) består av en komplex cellulär och molekylär mikromiljö. Den kan fungera som ett hem och ett stöd för hematopoesen och kännetecknas av förmågan att underhålla och driva differentieringen och självförnyelsen av hematopoetiska stam- och progenitorceller (HSPC), som i slutändan genererar alla blodcellstyper. Hur nischelementen interagerar i ett mänskligt sammanhang är dock oklart på grund av svårigheterna att konstruera och nyttja relevanta mänskliga specifika modeller. För att ta itu med dessa begränsningar utforskar denna avhandling genereringen av nya cellulära verktyg och system för in vivo och in vitro bioteknik av mänskliga BM-nischer. Arbetet är till stor del centrerat på exploatering av mänskliga mesenkymala stromaceller (MSC), de viktigaste reglerande delarna av BM-nischen.

Denna avhandling öppnar för möjligheten att utnyttja MSCs förmåga att bilda humaniserade ossiklar (hOss), ett mänskligt BM-organ som fungerar som värd för hematopoes hos möss. Medan hOss tillhandahåller en avancerad in vivo-modell för mänsklig nischundersökning, rapporterar vi här att avsaknaden av (1) ett standardiserat protokoll, (2) en stabil MSC-källa och (3) funktionell karakterisering begränsar dess utnyttjande. Vi strävar därigenom efter att åtgärda dessa brister.

Vi rapporterar därefter om den reproducerbara genereringen av hOss med hjälp av den mänskliga MSCs-linjen MSOD-B (Mesenchymal Sword of Damocles Bone morfogenetic protein type-2). Med detta standardiserade verktyg demonstrerar vi den robusta hOss-bildningen, som erbjuder en överlägsen intransplantation av humana friska och leukemiska blodkroppar i hOss jämfört med musben. Detta korreleras till kapaciteten av MSOD-B att rekonstituera de mesenkymala elementen i den mänskliga BM-nischen. Dessa resultat får oss vidare att undersöka om vår MSC-linje kan bilda hematopoetisk nisch in vitro.

In vitro-konstruktion av mänskliga BM-nischer kan uppnås genom att kombinera ställningsmaterial och 3D-perfusionsbioreaktorsystem. Med hjälp av att använda oss av en sådat system beskriver vi genereringen av osteoblastiska nischer, genom funktionaliseringen av en kollagenställning genom riktad mänsklig MSC-differentiering. HSPCs infunderas sedan, vilket leder till rekonstitution av nischinteraktioner. Standardisering uppnås sedan genom att kombinera MSOD och vår öppen källkod 3D-printad perfusionsbioreaktor. Vi etablerar den snabba designen av human hematopoetisk nisch av anpassade storlekar och ytterligare biologiskt validerade vårt system i upp till två veckor. Vi observerar MSCs-HSPCs interaktioner i hela nischen som leder till den fenotypiska expansionen av blodstamcellspopulationerna.

Den sista delen av avhandlingen fokuserar på studiet av mitokondriell överföring mellan MSCs och HSPCs, en nyckelaspekt av nischkommunikation. Denna nyligen föreslagna nischinteraktionsväg förblir kryptisk, och utnyttjandet av biotekniksystem skulle till stor del hjälpa till att avslöja denna mekanism. För detta ändamål genereras först en MSCs-linje som bär inducerbar mCherry-mitokondrietagg, iMSOD-mito. Genom att utnyttja iMSOD-mito i 2D och 3D in vitro odlingssystem, visar vi betydande mitokondriell överföring från mesenkymal till leukemi och friska HSPCs. Viktigast av allt identifieras en preferensöverföring mot fenotypiska CD34+/CD38-/CD45RA-/CD90+/EPCR+ stamceller. Vi förknippar vidare denna överföring med bibehållen vila i encellsdelningsanalyser.

Sammanfattningsvis presenterar denna avhandling utvecklingen och utnyttjandet av avancerade standardiserade modeller av den mänskliga BM-nischen. Vi tror att detta arbete kommer att underlätta förståelsen av den mesenkymala regleringen av hematopoesen i både friska och maligna sammanhang.

Abbreviations

AKT-PI3K	Protein kinase B - Phosphatidylinositol 3-kinase pathway
AML	Acute myeloid leukemia
ANGPT1	Angiopoietin 1
ATP	Adenosine triphosphate
BM	Bone marrow
BMP2	Bone morphogenic protein 2
CAR cells	CXCL12-abundant reticular cells
CDs	Clusters of differentiation
Cho	Chondrogenic
CLPs	Common lymphoid progenitors
CMPs	Common myeloid progenitors
Col1	Collagen 1
CrL-Col1	Crosslinked Col1
CXCL12	C-X-C motif chemokine ligand 12
CXCR4	C-X-C motif chemokine receptor 4
DAPI	4′,6-diamidino-2-phenylindole
DKK1	Dickkopf-1
DLL4	Delta like canonical Notch ligand 4
DOX	Doxycycline
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EPCR	Endothelial protein C receptor
EPO	Erythropoietin
FLT3LG	FMS-like tyrosine kinase 3 ligand
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMPs	Granulocyte/macrophage progenitors
GFP	Green fluorescent protein

HLA-DR	Human leukocyte antigen - DR isotype
HSCs	Hematopoietic stem cells
HSPCs	Hematopoietic stem and progenitor cells
IgG	Immunoglobulin G
IL2RG	Interleukin 2 receptor subunit gamma
ILs	Interleukins
ISCT	International Society for Cell and Gene Therapy
JAG1	Jagged 1
LTC-IC	Long-term culture initiating cells
LepR	Leptin Receptor
M-CSF	Monocyte colony-stimulating factor
MEPs	Megakaryocyte/erythrocyte progenitors
MPPs	Multipotent progenitor
MSCs	Mesenchymal stem/stromal cells
MSOD	Mesenchymal Sword of Damocles
MSOD-B	MSOD-BMP2
mtDNA	Mitochondrial DNA
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NK cells	Natural killer cells
Ost	Osteogenic
OXPHOS	Oxidative phosphorylation
PBS	Phosphate-buffered saline
PDMS	Polydimethylsiloxane
PEG	Polyethylene glycol
PLA	Polylactic acid
PVA	Polyvinyl alcohol
ROS	Reactive oxygen species
RUNX2	Runt DNA-binding domain 2
SCF	Stem cell factor

scRNA-seq	Single cell RNA sequencing
Stro-1	Stromal cell antigen-1
ST-HSCs	Short-term hematopoietic stem cells
TCA	Tricarboxylic acid
THPO	Thrombopoietin
TGFß1	Transforming growth factor beta-1
UM171	Small molecule compound
VCAM1	Vascular cell adhesion protein 1
VEGFC	Vascular endothelial growth factor C
WTN5	Wnt family member 5A

Introduction

Key cellular and structural component of the bone marrow hematopoietic niche

Etymologically, "marrow" finds its roots from the old English word "mearg," meaning "substance in the interior of a bone." (Ringe and Taylor, 2014). Beyond its literal meaning, 'marrow' assumes a figurative significance, often symbolizing the vital essence of a larger entity – which parallels our current understanding of the bone marrow (BM) function. It is a soft, spongy tissue found within certain bones, such as the hip, spine, and breastbone, responsible for the production of all circulating cells supplying the entire organism. This production is a regulated cellular maturation process called hematopoiesis.

The BM hematopoietic niche is a specialized microenvironment homing and supporting hematopoiesis (**Figure 1**). It is characterized by its ability to both maintain hematopoietic stem cells (HSCs) in a quiescent state (dormant and undivided) and to drive HSCs self-renewal and differentiation into hematopoietic stem and progenitor cells (HSPCs) which ultimately generate all blood cell types (Morrison and Scadden, 2014a). During fetal development, the hematopoietic centers are located outside the forming BM, the generation of blood cells from these centers are referred to as *primitive* hematopoiesis. After birth, blood cell production resides essentially in the BM and is then referred to as *definitive* hematopoiesis (Morrison and Scadden, 2014a).

The hematopoietic niche is not a defined structural unit. When grasping about the concept of the hematopoietic niche, one should rather refer to the multiplicity of hematopoietic niches. The BM itself varies in cellular composition, stiffness, and extracellular matrix composition, thus there are as many hematopoietic niches as there are variations of these tissular elements (Bianco, 2011). Moreover, the same hematopoietic niche is not a fixed unit throughout adulthood. Cellular composition and stiffness dramatically change during aging and the development of malignancies and diseases, impacting the output of definitive hematopoiesis (Ho and Méndez-Ferrer, 2020).

As previously defined, multiple criteria are expected from hematopoietic niche cell candidates (Flores-Figueroa *et al.*, 2012). Firstly, as HSCs represents 0.01% of the

BM nucleated cells and because the niche is saturable (Flores-Figueroa *et al.*, 2012), niche cells are likely to also be rare populations. Secondly, to exert paracrine or cell-to-cell contact regulation, these cells need to be in close physical proximity. Thirdly, to be part of the hematopoietic niche, they need to express HSCs maintenance gene or provoke the expression of such genes in other niche cells. Lastly, niche cells are expected to interpret and get activated by environmental signals to impact the hematopoietic output of the hematopoietic niche.

Hereafter we describe the known components of the hematopoietic niche.

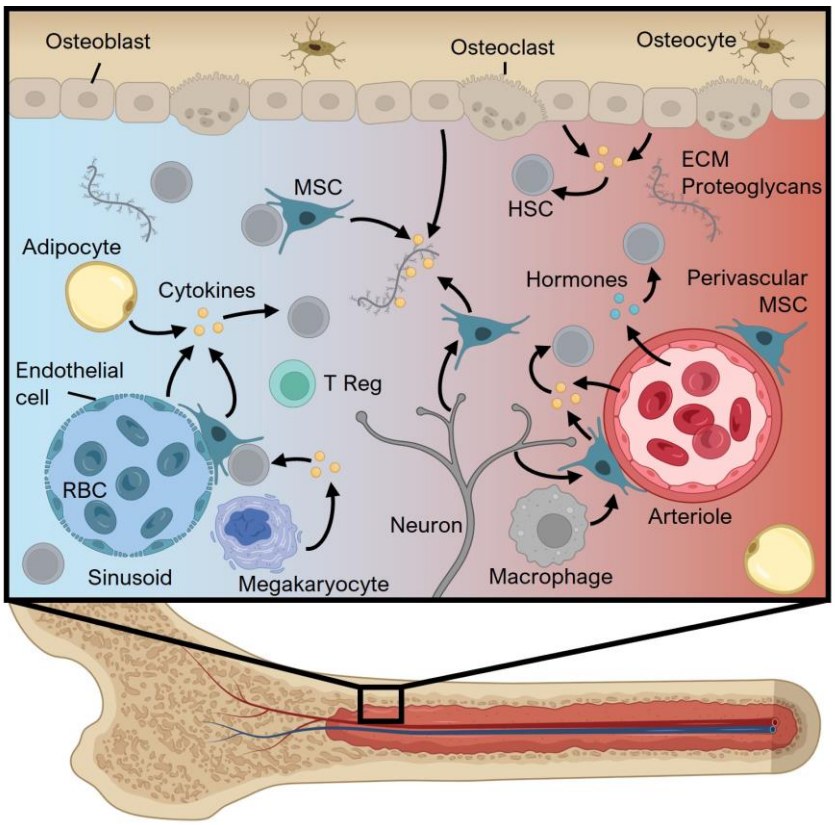


Figure 1. Interactions within the bone marrow hematopoietic niches.
Hematopoietic stem cells (HSCs) predominantly reside adjacent to sinusoids throughout the bone marrow, where they are supported by endothelial cells and mesenchymal stromal cells (MSCs). These cells contribute to HSC maintenance by producing essential hematopoietic factors. In addition to sinusoids, similar supportive cell populations may exist around other types of blood vessels, such as arterioles, further promoting HSC preservation. The HSC niche also comprises various other cell types that contribute to its regulation. For instance, osteoblasts located near bone surfaces in trabecular-rich areas play a significant role in maintaining the HSC niche. Sympathetic neurons, macrophages, osteoclasts, and osteocytes are additional cell types that participate in the regulation of HSC niches. Regulatory T cells (T Reg), for example, play a crucial role in maintaining an immune-privileged niche environment that safeguards hematopoietic stem and progenitor cells (HSPCs) from potential damage

by effector T cells. Moreover, the extracellular matrix (ECM) plays a crucial role in HSC regulation. By binding hematopoietic factors to proteoglycans within the ECM, the microenvironment provides a supportive milieu for HSC maintenance and function. This intricate network of cellular and non-cellular components collectively contributes to the complex regulation of HSC niches within the bone marrow. Furthermore, systemic signals, such as hormones, influence the hematopoietic niche, contributing to its overall regulation. These long-range signaling molecules reach the niche and interact with the local microenvironment, modulating HSC behavior and function. This interplay between systemic signals and the local niche highlights the intricate and dynamic nature of HSC regulation within the bone marrow. *Illustrated with BioRender. Abbreviations: CLP, common lymphoid progenitor; CMP, common myeloid progenitor; ECM, Extracellular matrix; GMP, granulocyte/macrophage progenitor; HSC, hematopoietic stem cell; MSC, Mesenchymal stromal cells; RBC, Reb blood cells; T Reg, Regulatory T cells.*

Hematopoietic stem and progenitor cells (HSPCs)

HSPCs is an umbrella term that groups immature blood cells with varying degree of self-renewal and multipotency, involved in hematopoiesis (Wilkinson, Igarashi and Nakauchi, 2020). The cellular diversity arising from hematopoiesis is typically classified into two main blood lineages (**Figure 2**). The myeloid lineage gives rise to red blood cells, megakaryocytes, platelets, and granulocytes which compose the innate immune system. On the other hand, the lymphoid lineage, with the generation of T-cells, B-cells and natural killer (NK) cells constitute the adaptative immune system (Doulatov *et al.*, 2012).

Hematopoietic homeostasis consists of regulating mechanisms by which the blood cells output is maintained or adapted to the environment (Boettcher and Manz, 2017; Pinho and Frenette, 2019). These mechanisms act upon a hierarchy of HSPCs from which the stemness and multipotency of cells decrease, and the commitment towards a lineage increase, along a cascade of asymmetric cellular divisions initiated by the long-term HSCs, which ultimately ends in fully mature blood cells (Eaves, 2015; Wilkinson, Igarashi and Nakauchi, 2020) (**Figure 2**).

While still debated, the hematopoietic cellular hierarchy (from stem to committed) runs broadly as follows: the long-term HSCs gives rises to short-term HSCs and to the multipotent progenitor (MPPs), from here the MPPs branch into either the myeloid or lymphoid commitment with the first cells engaged in these lineage being respectively the common myeloid progenitors (CMPs) and the common lymphoid progenitors (CLPs); CMPs then further ramify into megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/macrophage progenitors (GMPs) (Doulatov *et al.*, 2012; Wilkinson, Igarashi and Nakauchi, 2020) (**Figure 2**). Of note, lineage commitment also occurs in a non-stepwise manner, directly from HSCs to late progenitors, which could constitute a response to stress (Naik *et al.*, 2013; Yamamoto *et al.*, 2013). The HSPCs mentioned above constitute broad populations which can be further divided into subtypes, for sake of clarity we will not explore these sub-populations in this thesis.

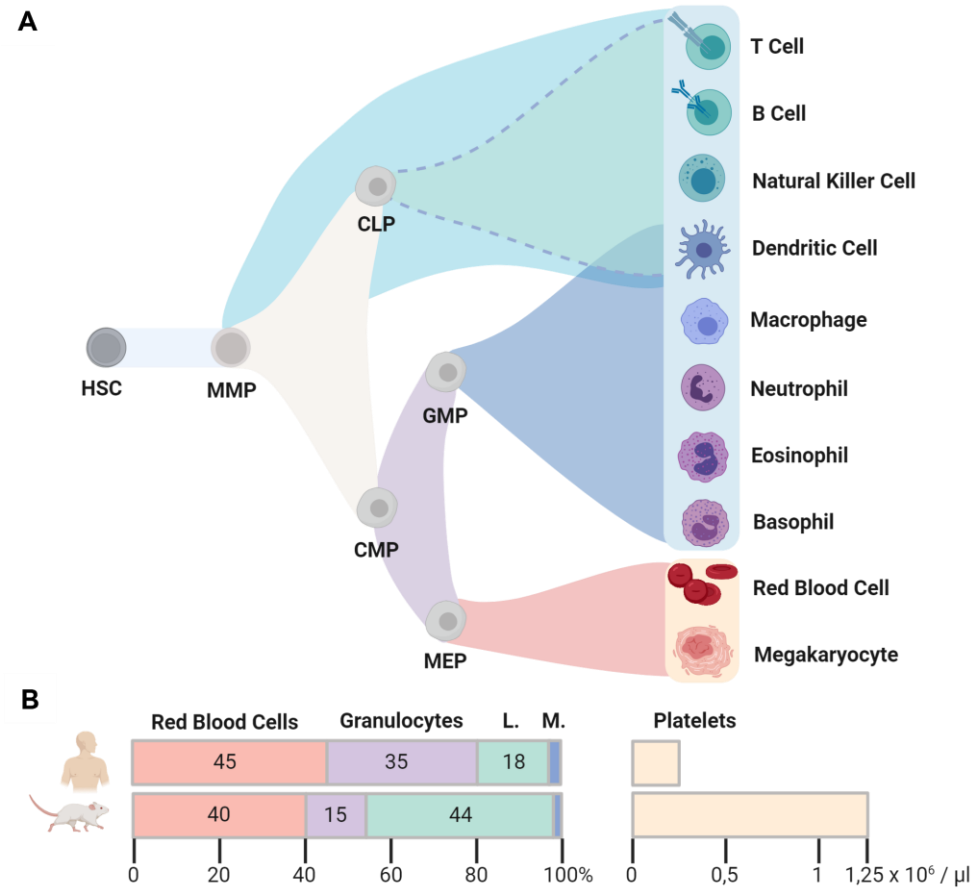


Figure 2. Hematopoiesis and interspecies difference in hematopoietic output.
(A) Multipotent hematopoietic stem cells (HSCs) give rise to all mature blood cell types through differentiation via a spectrum of hematopoietic stem and progenitor cells (HSPCs). During this process, self-renewal capacity and multipotency are progressively lost. Multipotent progenitors (MPPs) emerge from HSCs, subsequently losing multipotency upon differentiation into lymphoid-primed MPPs or common myeloid progenitors (CMPs). Further lineage specification follows lymphoid-primed MPPs differentiate into common lymphoid progenitors (CLPs), which ultimately give rise to B cells or T cells. In parallel, CMPs differentiate into either granulocyte-macrophage progenitors (GMPs), leading to the generation of neutrophils or monocytes, or megakaryocyte-erythrocyte progenitors (MEPs), which eventually differentiate into erythrocytes or platelet-producing megakaryocytes. Although simplified models of hematopoiesis suggest that HSC differentiation occurs through discrete, stepwise transitions, evidence indicates that hematopoietic differentiation is, in fact, a continuous process. The intricate balance between self-renewal and differentiation ensures the proper generation and maintenance of various blood cell types throughout an organism's lifespan. **(B)** Cellular blood composition comparison of the median blood count of adult humans and adult wild-type mice. *Adapted from* (Dupard *et al.*, 2020). *Abbreviations: CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte/macrophage progenitor; HSC, hematopoietic stem cell; L., lymphocytes; M., monocytes; MEP, megakaryocyte/erythrocyte progenitor; MMP, multipotent progenitor.*

Despite the wide variation in cellular architecture and shape observed in the mature blood population, morphological difference is minimal in HSPCs and cannot be solely employed to discriminate them. Though, of note, with sensitive equipment, a slight increase in cellular size can be observed with reduced clonogenic potential (Lengefeld *et al.*, 2021). Instead, analyzing the composition and the level of lineage of commitment of HSPCs is done via the use of antibodies specifically targeted towards an array of clusters of differentiation (CDs) (Doulatov *et al.*, 2012). CDs are proteins present at the surface of the cell membrane. By their absence (-) or their presence (+), detected by tagged antibodies, we can determine the level of commitment of human HSPCs (Pellin *et al.*, 2019). Long-term HSCs for instance, typically harbor CD45+CD34+CD45ra-CD90+EPCR+CD49f+, more committed lymphoid progenitor will display the expression of CD10 and myeloid cells CD33 (Velten *et al.*, 2017; Pellin *et al.*, 2019; Anjos-Afonso *et al.*, 2022).

While helpful, it is important to note that this method of distinction only provides crude HSPC populations. This arise from the imperfect nature of this selection but also from the biology of the cells themselves: a cell harboring a particular phenotype on its surface may have engaged the transcriptional machinery to transition to the next phase of commitment, which will corelate with its surface phenotype only at a later timepoint (Liu, Beyer and Aebersold, 2016). For instance, long-term HSCs identified via CDs are then referred to as "phenotypical long-term HSCs" because while the population of long-term HSCs was enriched, not all the cells isolated from these CDs will display all the characteristics of quiescence, self-renewal and multipotency of long-term HSCs (Haas, Trumpp and Milsom, 2018).

As any adult multipotent cells, HSPCs are subject to tight regulation to guide them towards the right maturation cascades and to prevent malignancy to arise from such a wide cellular output: $9x10^{15}$ blood cells produced over a lifetime, or about 4 million per seconds in adults (Sender and Milo, 2021). This guidance is two-fold: from intrinsic regulatory signals arising from the HSPCs themselves (i.e., epigenetics, repressed or activated transcription factors, autocrine factors); and extrinsic regulatory factors, which arise from external sources (i.e., niche cells, secreted factors, environment) (Rieger and Schroeder, 2012; Wei and Frenette, 2018). Hence, when aiming to understand HSPCs fate decision, one needs to consider a holistic perspective of the cellular and structural elements of the niche.

Mesenchymal stem/stromal cells (MSCs)

Defined by the International Society for Cell and Gene Therapy (ISCT), BM mesenchymal stem/stromal cells (MSCs) are rare multipotent cells characterized by their CDs phenotype (CD105+CD73+CD90+CD45-CD34-CD14-HLADR-CD19-), their ability to adhere to plastic and to differentiate into chondrocytes, adipocytes, and osteoblasts *in vitro* (Mohamed and Franceschi, 2017; Viswanathan *et al.*, 2019)

(**Figure 3**). The large array of CDs necessary to define the MSCs fraction in BM samples reflects the fact that (1) no markers can solely define MSCs and (2) these markers are also expressed in a multitude of other BM cells (Boxall and Jones, 2012). This makes the isolation of a pure and uniform MSC population directly from BM challenging (Boxall and Jones, 2012). While this ISCT definition helped the establishment of a standard for MSCs' terminology, observed discrepancies in stemness and hematopoietic niche support led to the identification of additional CD markers. CD271, CD146 or Stro-1 partially addressed these shortcomings by providing more uniform populations of clonogenic BM MSCs (Quirici *et al.*, 2002; Gronthos *et al.*, 2003; Tormin *et al.*, 2011; Fitter *et al.*, 2017). These new markers also correlate to MSCs' physical proximity to HSPCs *in vivo*. Indeed, across human BM sections, approximately 86% of CD34+ hematopoietic cells costained with CD271+ MSCs (Flores-Figueroa *et al.*, 2012). However, for CD146 and Stro-1 especially, *in vitro* culture interfere early-on with their expression (Gronthos *et al.*, 2003; Blocki *et al.*, 2013; Fitter *et al.*, 2017), which reduce their relevance for selection of expanded cells, and denotes the difficulty to maintain *in vivo* MSCs phenotype *in vitro*.

In vivo, defining MSCs remains a challenge. MSCs can be segregated according to their origin, most notably by their proximity to HSCs or to the BM vasculature (Morrison and Scadden, 2014a). However, despite this topographical distinction, their MSCs status relies on *in vitro* criteria discussed above, where their singularity seems to be lost when used *ex vivo*. Indeed, MSCs isolated from the avascular, HSCs-distal, articular cartilage performed similarly to HSC-proximal and vascular MSCs in *in vitro* assay (Barbero *et al.*, 2003). Moreover MSCs derived from adipocytes can be differentiated to mineralizing osteoblast as efficiently as BM MSCs *in vitro*, but are not capable of forming bone *in vivo* without preconditioning with cytokines (Yoon *et al.*, 2007; Stockmann *et al.*, 2012). Altogether, these discrepancies between *in vivo* localization and function and *in vitro* characteristics reflect the rapid loss of phenotype of MSCs during *in vitro* isolation. To reduce this artefact, researchers relied on in situ manipulation of MSCs to obtain a more faithful picture of their role in the hematopoietic niche. Naturally, such investigation was only possible in animal models. Genetic manipulation of MSCs in situ, either by alteration of their number or prospective gene allowed to measure their relevance to the niche within their tissue of origin.

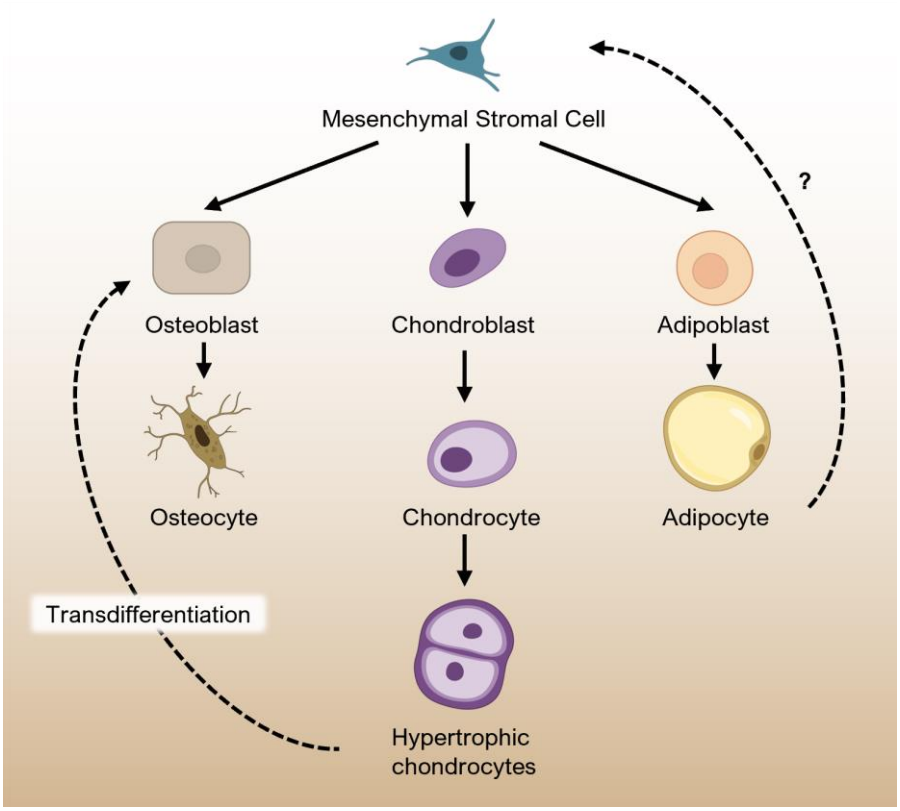


Figure 3. Mesenchymal progeny.
The bone marrow contains mesenchymal stromal cells (MSCs) that act as progenitors for skeletal tissue-forming cells. These MSCs have the capacity for self-renewal, producing identical cells that can then differentiate into multiple lineages responsible for the formation of the skeleton and bone marrow stroma. MSCs give rise to mineralizing osteoblasts, which become integrated into the bone matrix and further develop into osteocytes. Moreover, precursor cells for chondroblasts and adipoblasts can differentiate into hypertrophic chondrocytes, which contribute to cartilage formation, and adipocytes, which store fat. Intriguingly, recent research indicates that adipocytes might be capable of dedifferentiating into MSCs during myeloablation, although this process has yet to be observed in unmanipulated bone marrow (Hirakawa *et al.*, 2023). The transdifferentiation of hypertrophic cartilage into osteoblasts is another example of the complex interplay between various cell types within the bone marrow microenvironment (Zhou *et al.*, 2014).

Several MSCs population have been identified in relation to their expression of niche factors – secreted proteins contributing to hematopoietic niche regulation (Méndez-Ferrer *et al.*, 2010; Pinho and Frenette, 2019). This characterization, initiated in mice, allowed to uncover phenotypically distinct MSCs population supportive of HSPCs. In mice genetically engineered to express the green fluorescent protein (GFP) within the Cxcl12 locus, researchers were able to pinpoint

a distinct group of perivascular reticular cells, termed CAR cells (CXCL12-abundant reticular cells). CAR cells were found to be in close proximity to HSCs, hinting at the possibility that the CAR cell population harbored specialized niche cells (Sugiyama *et al.*, 2006). When depleted, this population led to HSCs loss in the BM (Sugiyama *et al.*, 2006). The authors also revealed that this MSC population is responsible for the production of most of the CXCL12 and stem cell factor (SCF) in the BM, essential niche factors for HSCs maintenance and function (Sugiyama *et al.*, 2006). It is, however, more difficult to identify their human equivalent, and more importantly their role in human hematopoiesis is still the subject of ongoing research effort (Bianco, 2014). However, through the use of coculture and clonogenic assay, expression of CXCL12 by MSCs allow direct contact with hematopoietic cells through the CXCR4 receptor (Jing *et al.*, 2010; Walenda *et al.*, 2010). Disruption of this interaction limits the clonogenic capacity of hematopoietic cells (Jing *et al.*, 2010), suggesting a crucial role of MSCs to HSPCs cell-cell contact through CXCL12-CXCR4 in the regulation of hematopoiesis. However, this relationship is yet to be observed *in vivo* or in situ.

Nestin expression in mouse perivascular MSCs cells also met the criteria defining a niche cell, they are proximal to most HSCs and HSC are rapidly lost from the BM upon genetic depletion of Nestin+ cells (Méndez-Ferrer *et al.*, 2010). Although mouse MSCs have not been reported to express CD146, further studies are needed to determine whether Nestin+ cells are the mouse equivalent of human CD45−CD146+ perivascular cells that were also shown to reconstitute hematopoietic niche activity in heterotopic transplantations and to highly express HSC maintenance genes (Sacchetti *et al.*, 2007).

Similarly to Nestin+ MSCs, Leptin Receptor (LepR)-expressing mouse MSCs, another perivascular stromal population identified to contribute to hematopoiesis, is also considered a potential equivalent to CD146+ perivascular human MSCs. Ablation of SCF expression from Leptin receptor (Lepr)-expressing perivascular MSCs resulted in depletion of HSCs number in the BM (Ding *et al.*, 2012). Surprisingly, the authors did not observe a contribution from perivascular Nestin+ cells when ablating their SCF expression. This suggests that LepR+ and Nestin+ cells are distinct. However, some overlap between MSCs population contributing to hematopoiesis was observed. Indeed, Nestin+ cells express high levels of both Scf and Lepr (Méndez-Ferrer *et al.*, 2010), and LepR+ cells express high levels of CXCL12 (Ding *et al.*, 2012); which limits the distinction of Nestin+, LepR+ and CAR MSCs.

While the identification and functional study of MSCs is difficult in a human context, the identification of their committed progeny in the hematopoietic niche is easier as they present unique phenotype and distinct functions.

Osteoblasts and osteoclasts

Osteoblasts are bone-resident cells of mesenchymal origin (**Figure 3**). These are the cells responsible for the deposition and remodeling of the bone extracellular matrix, the osteoid, which in turn will mature into bone after calcification (Long, 2012). The osteoblasts are lining the external surface of the BM niche (**Figure 1**), they thus constitute the more distal cellular layer of the BM niche, at the interface with the calcified bone (Long, 2012; Morrison and Scadden, 2014b).

The first human cell type identified to provide support to HSPCs via secreted factors where osteoblasts isolated from the trabecular BM (Taichman and Emerson, 1994). In coculture with HSPCs, these osteoblasts maintained HSPCs' clonogenic potential for up to three weeks, with the identification of G-CSF as niche factors produced by osteoblasts (Taichman and Emerson, 1994). This pioneering work motivated further investigation on the biology of osteoblasts and their role in the hematopoietic niche.

Osteoblast not only provides maintenance of the structural support for the hematopoietic niche, they also directly regulate HSPCs, via direct contact in addition to secreted factors (Taichman, 2005). While the exact role of human osteoblast towards hematopoiesis is not fully resolved, in mouse, ablation of osteoblast leads to depletion of early lymphoid progenitors (Visnjic *et al.*, 2004; Zhu *et al.*, 2007). This results in pancytopenia in the following weeks, a deficit in all blood population which suggests an impairment of the maintenance and repopulating capacity of HSCs. Further ablative and osteoblast studies led to the identification of the delta like canonical Notch ligand 4 (DLL4) as a potent regulator of lymphopoiesis. Furthermore, the HSCs regulator genes thrombopoietin (THPO), angiopoietin 1 (ANGPT1) and Wnt family member 5A (WTN5) were all expressed by osteoblasts (Ding *et al.*, 2012). Altogether these results, thought obtained in mice, strongly support the role of osteoblasts in maintaining more primitive hematopoietic populations. In contrast, osteoclasts, the bone resorbing cells, appear to display a distinct activity towards hematopoiesis.

Osteoclasts are responsible for bone resorption and coordinate with osteoblasts for bone remodeling. This delicate balance between bone formation and resorption is essential for maintaining skeletal tissue. Osteoclasts are not derived from the skeletal lineage but from the monocytic lineage (Taichman, 2005). They are thus mature hematopoietic cells, and they impact their own cells of origin within the niche. It is believed that osteoclast impacts the hematopoietic cells through the breaking down of the calcified bone matrix (Adams *et al.*, 2006). The release of calcium ions thought this decalcification provokes the mobilization of HSCs within the niche, which senses the increasing calcic concentration, resulting in reduced engraftment capacity of the HSPCs (Olszak *et al.*, 2000).

Adipocytes

Adipocytes are stromal cells responsible for the accumulation and release of energy primarily via lipolysis of their characteristic cytoplasmic lipid droplets (Li, Wu and Kang, 2018; Cuminetti and Arranz, 2019). Representing 10% of the body mass of healthy human and filling 90% of the BM cavity (Cuminetti and Arranz, 2019), the BM adipose tissue tends to increase with aging and during pathological conditions such as osteoporosis (Devlin and Rosen, 2015). In BM, adipocytes are present as the yellow adipocyte (**Figure 1**). This yellow terminology is not to be assimilated to the other yellow adipocytes found in other tissues, as recent evidence suggests that BM adipocytes are a separate subtype (Suchacki *et al.*, 2020). Aside from their energetic role, adipocytes provide an extensive signaling function notably via the secretion adipokines (Devlin and Rosen, 2015).

While extensive, the relation between adipocytes and hematopoiesis was mainly drawn from mouse models and *in vitro* human MSCs-derived adipocytes, which warns caution from interspecies differences (Lempesis *et al.*, 2022) and *in vitro*/*in vivo* inconsistencies (Soukas *et al.*, 2001). Nonetheless, it appears that adipocytes contribute to the regulation of hematopoiesis via their secretory function. Leptin, the satiety signal protein, and adiponectin are the principal secreted cytokines from adipocytes. These two adipokines both impact hematopoiesis in different ways (DiMascio *et al.*, 2007; Claycombe, King and Fraker, 2008). In humans, leptin levels correlate with leukocyte counts (Wilson *et al.*, 1997). Though the direct relationship between leptin and HSPCs' proliferation remains unclear, in mice devoid of leptin expression, monocyte and lymphocytes number decrease (Claycombe, King and Fraker, 2008). Adiponectin, on the other hand, promotes HSPCs proliferation during emergency hematopoiesis (i.e., during bacterial infection) by suppressing inflammatory signaling molecule such as the tumor necrosis factor (Masamoto *et al.*, 2016). Adiponectin ablation has however no impact on hematopoiesis of healthy animals (Masamoto *et al.*, 2016).

Endothelial cells and Blood Vessels in the bone marrow Niche

The BM vasculature is composed of a vast interconnected capillary network of arterioles and sinusoids – fenestrated capillaries (**Figure 1**). Sinusoids are larger than arterioles and converge towards a central sinus within the BM (Kopp *et al.*, 2005). Sinusoids are composed of a single layer of endothelial cells that do not possess a supportive connective tissue which provide high permeability for surrounding cells (Kopp *et al.*, 2005). The BM niche is often subdivided into the "arteriolar BM niche" and the "sinusoidal BM niche" to reflect the variations in feature of the vasculature of these niches (Calvi and Link, 2015).

Both arterioles and sinusoids are constituted of endothelial cells lining the lumen of the vessel (Kopp *et al.*, 2005), at the interface between the niche and the systemic circulation (Pinho and Frenette, 2019) (**Figure 1**). It is important to note that endothelial cells present in both vessels and in similar vasculature throughout the body are very diverse and tissue specific (Nolan *et al.*, 2013). Though surface markers are similar among endothelial cells, their function and pattern of expression diverge widely, and impact the hematopoietic niche in divergent fashion (Kopp *et al.*, 2009). Endothelial cells secrete a range of hematopoietic factors such as SCF, which is produced in much higher quantity by the arteriolar endothelial cells than their sinusoidal counterpart providing a more quiescent signal to HSPCs (Pinho and Frenette, 2019).

Sinusoidal endothelial cells are essential for megakaryocyte maturation. Indeed, megakaryocyte breaches through the endothelial layer and shed part of their cytoplasmic membrane into the sinusoidal lumen which leads to the formation of mature platelets (Kopp *et al.*, 2005). Thus, endothelial cells, by their nature and organization in the niche are required for hematopoiesis.

Other important niche cells

Mature hematopoietic cells also contribute to the regulation of the niche. Innate immune cells such as macrophages not only play a crucial role in bone maturation and repair, but they are actively involved in the regulation of hematopoietic cells within the niche (Kaur *et al.*, 2017). Macrophages act indirectly on HSCs quiescence thought a interaction with MSCs (**Figure 1**), leading to their expression of hematopoietic retention genes, such as CXCL12 (Chow *et al.*, 2011). Macrophages in close proximity to hematopoietic primitive cells also act directly by preventing HSPCs exhaustion via the production of Prostaglandin E2 and clearance of neutrophils during inflammation of the niche (Ludin *et al.*, 2012; Kawano *et al.*, 2017). Adaptative immune cells also return to the hematopoietic niche after maturation in other organs to protect HSPCs. Regulatory T cells for instance helps promote an immune-privileged niche environment to protect HSPCs integrity from damage of effector T cells (Fujisaki *et al.*, 2011). Lastly, megakaryocytes help maintain HSPCs homeostasis via the secretion of a plethora of hematopoietic factors, most notably thrombopoietin (THPO) and CXCL4 (Bruns *et al.*, 2014; Nakamura-Ishizu *et al.*, 2014).

Neural cells are also a member of the hematopoietic niche (**Figure 1**). Innervation of the BM niche is composed of both sympathetic and sensory nerves as well as parasympathetic nerves, the latter being only present in the bone at the periphery of the niche (Pinho and Frenette, 2019). Sympathetic nerve act upon the niche via the release of the noradrenaline neurotransmitter, influencing the expression of CXCL12 by niche cells, which gradient impacts HSPCs quiescence and

mobilization (Katayama *et al.*, 2006). Most notably, this signal is responsible for the circadian oscillation of the CXCL12 niche concentration at the origin of the diurnal HSPCs egress from the niche to the systemic circulation (Méndez-Ferrer *et al.*, 2008).

Extracellular matrix and signalling molecules in the niche

In addition to the cellular contribution to the hematopoietic niche, a variety of extracellular matrix (ECM) proteins and molecules involved in its regulation have been identified. For sake of simplicity, we will hereafter focus on the best characterized effectors.

The BM niche ECM is primarily composed of proteoglycans and fibrous proteins, the most abundant being fibronectin and collagen proteins (Lee-Thedieck, Schertl and Klein, 2022). As structural proteins, they regulate changes of stiffness and ductility of the BM niche which varies upon the niche location in the bone cavity (Lee-Thedieck, Schertl and Klein, 2022). These changes are sensed by hematopoietic cells via cell-ECM interactions mediated mainly via integrins – cell-membrane proteins which binds ECM components (Zanetti and Krause, 2020). This sensing and probing of their environment via integrins lead to migration, survival or differentiation of HSPCs (Zanetti and Krause, 2020). For instance, ß1 and ß2 integrins are necessary for homing and colonization of HSPCs to the BM niche, with deficiency in ß2 leading to high level of leukocytes in the systemic circulation (Bouvard *et al.*, 2001). The interactions between fibronectin and HSPCs' α4 integrins are also central in the development of myeloid and lymphoid cells, as migration within the niche is required in their maturation (Bouvard *et al.*, 2001).

Another important role of ECM is the presentation and storage of proteins and molecules produced by niche cells influencing hematopoiesis (**Figure 1**). The transforming growth factor beta-1 (TGFß1) for instance, found in abundance in the BM ECM, is released when the ECM integrity is altered leading to an inflammatory response and recruitment of MSCs (Crane and Cao, 2014). One of the most abundant proteoglycans in the BM ECM is the heparan sulfate Perlecan. Perlecan is able to immobilize a wide array of hematopoietic factors such as, among other, interleukins (ILs), stem cell factor (SCF), FMS-like tyrosine kinase 3 ligand (FLT3LG) and CXCL12 (Klein *et al.*, 1995; Papy-Garcia and Albanese, 2017). *In vitro* assays provided evidence that the potency of these hematopoietic factors towards HSPCs was increased by this immobilization in the ECM compared with unbound factors (Zandstra, Lauffenburger and Eaves, 2000; Doran *et al.*, 2009).

Hematopoietic factors are proteins or molecules that regulate the quiescence, differentiation and production of all the blood cells from progenitors within the BM. Hematopoietic factors can be distinguished in two classes, the hormones and the

cytokines (Kaushansky, 2006). This distinction comes from the production sites of these factors, hormones are mainly produced by specialized glands and act distally while cytokines are produced locally by cells within the BM niche. Hematopoietic factors bind to their respective receptor on hematopoietic cells to induce the signaling cascade leading the hematopoietic differentiation or quiescence of HSPCs (Pinho and Frenette, 2019). These factors often enhance or antagonize each other in coactivation/inhibition of their respective receptors. This phenomenon occurs via heterotypic receptor interactions and cross-phosphorylation of downstream effectors (Link and Prentice, 1993; Duarte and Frank, 2002; Lee *et al.*, 2002). It is then important to stress that a pool of hematopoietic factors coordinate together the cellular response, and not solely on their own in a binary manner. This results in the observed pleiotropy of hematopoietic factors towards HSPCs.

Hematopoietic hormones include erythropoietin (EPO) and thrombopoietin (THPO), the former being produced in the kidney's renal cortex and outer medulla, and the latter in the liver's hepatocytes and proximal tubular cells in the kidneys (Kaushansky, 2006). EPO stimulated the production of red blood cells in the BM niche upon sensing a lower oxygen level in the bloodstream (Kaushansky, 2006). THPO regulates the production and maturation of platelets from megakaryocytes, its expression is a response to low platelets count in the systemic circulation (Kaushansky, 2006).

The best characterized hematopoietic cytokines include SCF, FLT3LG, interleukin IL7, TGFß1, CXCL12, and the granulocyte-macrophage, monocyte, and granulocyte colony-stimulating factors, respectively GM-CSF, M-CSF, and G-CSF. The colony-stimulating factors are produced by immune, mesenchymal, and endothelial cells. They induce the production of granulocytes and monocytes and act as late-stage maturation signals for HSPCs within the niche. SCF, expressed by MSCs and endothelial cells, is involved in the maintenance of HSPCs in the BM, as well as granulocyte cells maturation (Lennartsson and Rönnstrand, 2012). IL7 is required for the development of human T-cells and is expressed by MSCs in the BM (Puel *et al.*, 1998; Chen *et al.*, 2021). FLT3LG, which is produced by MSCs and HSPCs, provoke the proliferation of HSPCs only when other cytokines have activated their target, in a synergic induction of the FLT3LG signaling cascade (Jacobsen *et al.*, 1995; Tsapogas *et al.*, 2017). Finally, as previously stated CXCL12 and TGFß1 are important cytokines produced by niche cells which support quiescence and retention of HSPCs for the former and inflammation for the later (Pinho and Frenette, 2019).

Hematopoietic factors are the messengers between local niches cells as well as distant organs with the hematopoietic system. The interplay of the different factors constitutes a complex input of information for HSPCs which act and interpret them in as many outputs of proliferation, quiescence, and maturation.

Mitochondria transfer in the BM niche

Physical contact between niche cells and HSPCs, putatively guided by membrane-bound cytokines, provides another array of behavior which leads to exchange of cellular material between cells in response to external stimuli. We will hereafter focus on mitochondria transfer in the BM niche.

The exchange of mitochondria between cells, known as cell-to-cell mitochondrial transfer or horizontal mitochondrial transfer, was initially demonstrated in laboratory settings through the restoration of mitochondrial function in A549 p° cancer cells lacking mitochondria (Spees *et al.*, 2006). In live organisms, evidence of this transfer was first observed through variations in mitochondrial DNA (mtDNA) in transmissible canine venereal tumors (Rebbeck, Leroi and Burt, 2011). Since these initial findings, researchers have documented such transfers across various tissues as a mechanism to alleviate mitochondrial dysfunction in pathological conditions (Islam *et al.*, 2012; Vallabhaneni, Haller and Dumler, 2012; Hayakawa *et al.*, 2016), benefiting the recipient cells. Notably, experiments with engineered inter-mitochondrial mouse chimeras – where tissues are composed of cells with distinct mtDNA haplotypes – have demonstrated that mitochondrial transfer is a ubiquitous physiological process in normal tissue development and maintenance in adults (Marti Gutierrez *et al.*, 2022).

Mitochondria play pivotal roles in various essential cellular processes, encompassing oxidative phosphorylation (OXPHOS), apoptosis, regulation of reactive oxygen species (ROS), the tricarboxylic acid (TCA) cycle, calcium signaling, and heme synthesis. Specifically, they serve as the primary site for adenosine triphosphate (ATP) production through OXPHOS, making them the metabolic hub of the cell. During OXPHOS, ROS are generated as by-products of mitochondrial respiration (Filippi and Ghaffari, 2019). HSCs possess relatively abundant mitochondria, which, interestingly, remain in an inactive state, correlating to their low levels of ROS (Filippi and Ghaffari, 2019). Recent research indicates that mitochondria stability preserves HSC quiescence and their capacity to swiftly transition from a dormant to an active metabolic state (Filippi and Ghaffari, 2019; Chakrabarty and Chandel, 2021).

BM MSCs have the ability to transfer mitochondria via tunneling nanotubules (TNTs), microvesicules and gap junctions, to a wide array of hematopoietic cells from the niche and other cells outside the BM (Liu *et al.*, 2021; Singh and Cancelas, 2021). Generally, this transfer of mitochondria is beneficial for the recipient cells and is one of the protection mechanisms that malignant blood cells exploit to survive therapeutic treatment (Liu *et al.*, 2021). Transfer to MSCs from HSPCs also occurs when HSPCs experience oxidative stress or inflammation to eliminate dysfunctional/damage mitochondria (Singh and Cancelas, 2021). While our comprehension of how BM MSCs transfer mitochondria remains incomplete,

several key insights stand out. Notably, the involvement of gap junctions in facilitating this transfer alongside the activation of AKT-PI3K pathways and the expression of CD38 on recipient cells was shown to correlate with mitochondrial transfer (Islam *et al.*, 2012; Marlein *et al.*, 2019; Mistry *et al.*, 2019). Nonetheless, numerous limitations hinder the practical application of these findings. Specifically, these observations are confined to specific tissue types or disease models and may not accurately represent the widespread occurrence of mitochondrial transfer in tissue maintenance (Marti Gutierrez *et al.*, 2022). Moreover, inhibiting the identified molecular players only partially disrupts mitochondrial transfer. This suggests the existence of undiscovered redundancy in the pathways governing this process. Most notably, there is a scarcity of reports on human-exclusive mitochondrial transfer, impeding the translational potential of this mechanism. As the importance of human mitochondrial transfer becomes more evident, the development of a standardized protocol exclusively for human studies is imperative to validate crucial molecular and cellular components for potential exploitation.

In vivo human hematopoietic niche modelling

Historical context of human hematopoietic niche research

The second half of the 1800s was a transformative era for biology. Spearheaded by the theories of Charles Darwin (1809-1882) and Alfred Russel Wallace (1823-1913), the relationship between living beings and their habitat was rationalize and evidenced by the mutual and reciprocal relationships species undergo with respect to their environment. The *Theory of Evolution*, and its new discipline *Evolutionary Biology*, radically shaped modern biology by guiding a more holistic reflection on biological systems: the environment is an intrinsic element required for their understanding.

In 1913 the term niche – from the French for nest – was first coined by American field biologists Grinnell and Swarth in the description of two species of wood rats: "[the two species] occupy almost identically the same ecologic **niche**, with resulting similarity in habits" ('Grinnell and Swarth on the Birds and Mammals of the San Jacinto Area of Southern California', 1914). While still elusive, the term was further clarified and define by English zoologist and animal ecologist Charles Elton in 1927 as: "what [an animal] is doing in its community,… its place in the biotic environment, its relations to food and enemies" (Mann and Elton, 1966).

Taking this rational from the ecological to the cellular level, the British hematologist Raymond Schofield at the Paterson Laboratories in Manchester, first proposed the theory of the stem cell niche in his seminal 1978 paper "The relationship between

the spleen colony-forming cell and the haemopoietic stem cell" (Schofield, 1978). Schofield built his niche theory upon the work of Canadian researchers J.E. Till and E.A. McCulloch, who evidenced in 1961 the direct correlation between the number of BM cells (spleen colony-forming cell) injected intravenously and the number of colonies in the spleen in irradiated mice (Till and McCulloch, 1961). More importantly, they showed that a single cell can produce an abundant differentiated progeny. Schofield recognized that stem cells recovered from the spleen colonies have reduced hematopoietic reconstitution capabilities than cells from the BM, and thus proposed that the environment (the niche) of these cells influence their behavior.

Schofield's colleague, the hematologist Michael Dexter sought out to identify the element of this HSCs niche. Dexter showed that stromal cells could maintain HSCs *ex vivo*, referred to as "*epithelial*" and "*giant fat*" cells, likely mesenchymal stromal cells and adipocytes (Dexter, Allen and Lajtha, 1977). These first results, and later work, confirmed that cells of stromal origin can maintain HSCs *ex vivo*, which opened the possibility for *in vitro* niche modeling.

Niche biology at that stage identified the BM as the center of regulation of hematopoiesis, and BM cells as providing the signals and support needed for regulating its functions. Like any interconnected system, the interplay of all cells present in the BM is responsible for the overall regulation of hematopoiesis. How this interplay and lack thereof influence hematopoiesis remained still elusive. Fundamental and enlightening, early work on niche support to hematopoiesis was performed in the murine context, human-centered investigation was then needed for potential therapeutic application of findings.

Early hematopoietic niche studies were conducted in mice because the identification and measurement of HSCs can only be achieved through functional repopulation assays. Consequently, this poses a clear obstacle for studying the human HSCs niche biology, for normal hematopoiesis but also for modeling pathological conditions. To partially circumvent this hurdle, aspects of the human hematopoietic niche in mouse could be recreated using xenotransplantation in immunodeficient mice, whereby human hematopoietic cells engraft in the depleted BM of transgenic mice. This method was first implemented by the laboratory of John Dick in 1988 by intravenous injection of human BM cells in irradiated *bg/nu/xid* mice. Due to their mutations, these mice have a reduced cytotoxicity for foreign cells. After 5 weeks post-*in vivo* xenotransplantation, human hematopoietic cells were capable of myeloid colony formation (Kamel-Reid and Dick, 1988). This seminal work opened the development of further engineered mouse strains to recapitulate a more human niche microenvironment and notably via the constitutive expression of human hematopoietic cytokines.

Nonetheless, despite humanization efforts, the BM niche in these animals remained mainly murine, hindering the extrapolation of insight gathered from xenograft models (Stripecke *et al.*, 2020). Further evidence highlighted major divergence in the regulation of hematopoiesis between the two species, notably the higher demands and long-term survival of human hematopoietic cells (Parekh and Crooks, 2013). Moreover, the inactivity of mouse hematopoietic cytokines on human cells (IL-3, EPO, M-CSF, GM-CSF), despite engineered mouse strain, revealed to be a major roadblock to guide human hematopoietic diversity and maturation *in vivo* (Manz, 2007). Thus, more advanced methods of *in vivo* and *in vitro* assay were developed to bridge this mouse-human gap. We will hereafter give an overview on the main modeling methods that enabled the investigation of interactions between human bone niche cells and human hematopoietic cells.

Species-specific differences in the hematopoietic niche

Advancements in xenotransplantation models, *in vitro* culture systems, and immunophenotyping offered new insights into interspecies variations. Although many aspects of hematopoiesis remain similar between mice and humans, it is essential to consider several differences before extrapolating findings from mouse studies to humans, especially when aiming to create more accurate models (Doulatov *et al.*, 2012; Sykes and Scadden, 2013; Engert *et al.*, 2016). Below, we emphasize the key interspecies differences.

In humans, definitive hematopoiesis takes place exclusively within the BM. Interestingly, in mice, it occurs not only in bones but also in the spleen, a fetal trait retained postnatally in mice but lost in humans. This "extramedullary" hematopoiesis, accounting for up to 30% of hematopoiesis and producing all hematopoietic lineages, holds significance when interpreting hematologic phenotypes in xenotransplantation context as the hematopoietic niche in the spleen does not relate to the BM niche (O'Connell *et al.*, 2015).

Notably, mice and humans display considerable differences in overall blood composition, reflecting distinct hematopoietic and immunologic requirements (**Figure 2**). In the lymphoid lineage, mice blood predominantly comprise lymphocytes (70–80%), while humans exhibit a higher proportion of neutrophils (60%) among white blood cells. Additionally, rodents possess a significantly higher number of platelets in circulation (900,000–1,600,000 per microliter of blood) compared to humans (150,000 to 400,000 per microliter) (Dean, 2005; Ryan, 2016). These characteristics imply potential biases towards lineage commitment in both species, possibly influenced by differences in receptors, molecular signals, and expression patterns which needs to be considered in xenograft models.

The BM in rodents is characterized by lower adiposity compared to humans. Although this difference has not been established as interspecies variation, it is widely recognized that adipocytes predominantly impede hematopoietic potential within the hematopoietic stem cell (HSC) niche (Naveiras *et al.*, 2009). Additionally, species-specific distinctions are observed in the expression patterns of cellular markers (Seita and Weissman, 2010). For instance, in mice, long-term HSC activity in transplantation assays is identified by Lin− Sca+ Kit^{hi} CD150+ CD48− cells (Kiel *et al.*, 2005; Sintes *et al.*, 2008). However, CD150, CD48, and Sca-1 are not prevalent in human HSCs (Kiel *et al.*, 2005; Sintes *et al.*, 2008). Human hematopoietic stem and progenitor cells (HSPCs) do not express CD150 but express CD48 (Doulatov *et al.*, 2012), and long-term human HSCs can be obtained from the Lin− CD34+ CD38− fraction of BM cells expressing the Fms-like tyrosine kinase 3 (Flt-3) receptor. On the other hand, long-term murine HSCs do not express CD34 or Flt-3 but do express CD38 (Sitnicka *et al.*, 2003; Doulatov *et al.*, 2012).At the molecular level, both long-range and short-range extrinsic signals, such as SCF, THPO, FLT3LG, CSFs, and ILs, play a crucial role in HSC fate decisions, modulating differentiation, self-renewal, and quiescence in both species (Pinho and Frenette, 2019). Nevertheless, there are several core differences: for instance, SCF was identified as a more potent survival factor than Flt-3 for murine HSCs, whereas the opposite seems to be true for human cells (Adolfsson *et al.*, 2001; Sitnicka *et al.*, 2003). In lymphoid lineage commitment, IL-7 signaling through the common γ-chain receptor (IL2RG) is necessary for murine B lymphopoiesis but is not essential for human B lymphopoiesis (Doulatov *et al.*, 2012).

These variations between human and mouse models not only complicate analysis of the findings on hematopoietic regulation but also hinder their capacity as predictive preclinical model. Hence for many decades, 2D *in vitro* systems for human-only hematopoietic niche modeling were developed to bypass murine models' shortcomings. Furthermore, existing *in vivo* models were refined to provide better predictive potential in both normal and pathological hematopoiesis.

Overview of *in vivo* methods for studying the human hematopoietic niche

Over the past few decades, numerous xenotransplantation mouse models have been meticulously designed to facilitate the engraftment and investigation of both normal and abnormal human hematopoiesis (Ito *et al.*, 2012). Through continuous refinement of the strains used, researchers have progressively improved the development of human hematopoiesis in mouse bones. In this pursuit, genetically modified mouse strains such as NSGS, MISTRG, and the latest SRG-15 have been engineered to express human hematopoietic cytokines by replacing their corresponding mouse homologs (Beyer and Muench, 2017). However, it is

important to note that these host mice may manifest a higher incidence of hematologic disorders (such as anemia) and premature mortality, which limits their exploitation for gathering human hematopoietic niche insights (Nicolini *et al.*, 2004; Herndler-Brandstetter *et al.*, 2017). Naturally, niches cells and the niche architecture are in majority of murine origin.

In vivo methods for experimenting with the human BM niche aimed at providing not only human cell interactions in mice but also the human niche architecture. This aim implies to create a human BM tissue and insert it into a mouse for nutrient/waste removal and oxygen support. The graft of human tissue in mice for hematopoietic purpose was spearheaded by Irvin Weissman's lab in 1988 with the graft of human fetal liver and thymus into immunodeficient mice for the generation of mature human T and B cells and subsequent production of immunoglobulin G (IgG) (McCune *et al.*, 1988). The graft was maintained for 13 weeks and was proof of the possibility to sustain human tissue in mice for extended time.

More than 20 years later, the development of the now-standard *in vivo* human BM niche engineering was first established. A polyacrylamide hydrogel seeded with human BM niche cells was implanted under the skin (ectopically) of immunodeficient mice and further infused with normal or cancerous human hematopoietic cells. After 4 weeks post-implantation, the construct displayed an arborescent vasculature with marrow tissue hosting both normal and cancerous human HSPCs (Lee *et al.*, 2012). Further iteration of this method takes advantage of the process of endochondral ossification and endochondral myogenesis, respectively forming bone and BM during skeletal development and repair (Reinisch *et al.*, 2017; Bourgine *et al.*, 2019). This endochondral pathway consists of the progressive calcification of a matrix by MSCs and remodeling into mature bone and BM by osteoblasts, osteoclasts, and the forming vasculature. Its exploitation to form human chimeric BM microenvironment result in a tissue called humanized ossicle (hOss) from a cartilage template laden with human BM cells (Dupard *et al.*, 2020) (**Figure 4**). Stemming from this work, multiple iterations of the ectopic implantation of human bone-like tissue has created a versatile and more faithful interrogation of human hematopoietic interactions with its humanized niche environment (Dupard *et al.*, 2020). However, this system also suffers from a lack of standardization due to the BM cells heterogeneity as well as the presence of murine cells and factors that complicate the human extrapolation of this system's insights. These two points will be discussed later in this thesis (Dupard *et al.*, 2020).

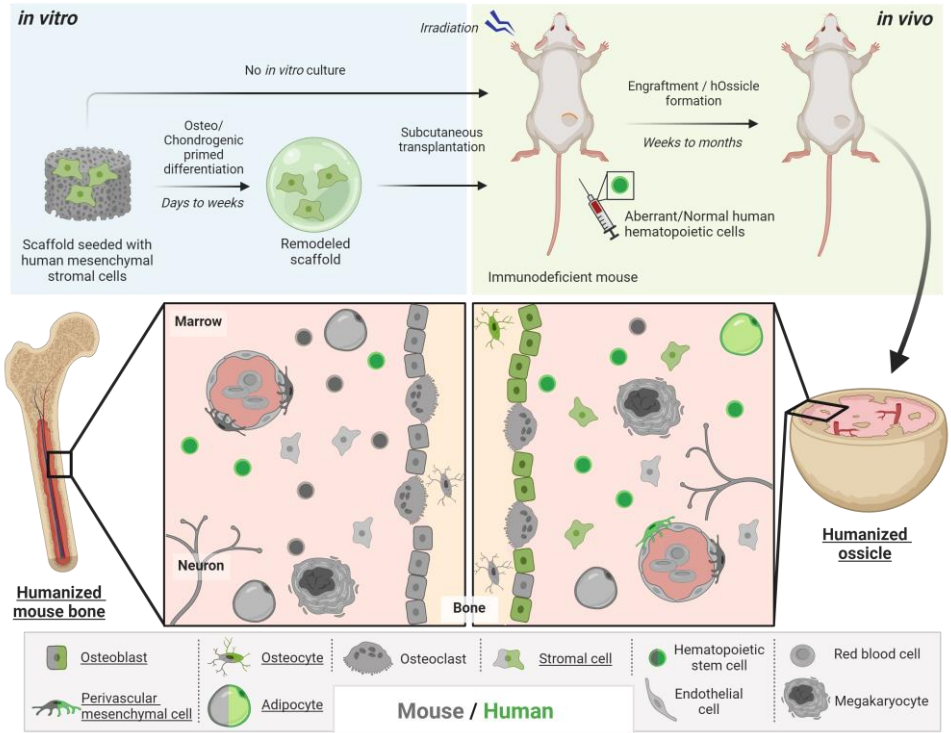


Figure 4. Formation of Humanized Ossicles (hOss) in mice.
Human mesenchymal cells are seeded onto a scaffold for preculture (with chondrogenic or osteogenic priming) or are directly implanted subcutaneously into the back of immunodeficient mice. Following a first in vivo remodeling period (>4 weeks), the animal is transplanted with human hematopoietic stem cells (HSCs) delivered intravenously or directly within the forming hOss (intraossicle injection). As an alternative, HSCs can be seeded onto the scaffold before in vivo implantation (not illustrated). By contrast to humanized mouse bones, the resulting hOss is composed of both niche and hematopoietic cells of human origin. Illustration from (Dupard *et al.*, 2020).

The lack of standardization stemming from the mesenchymal sources was recently addressed in our latest work exploiting the capacity of our MSCs line to create a cartilage template that remodels in bone and marrow tissue after mouse implantation ectopically (Grigoryan *et al.*, 2022). The hOss produced as a result comprise fully developed bone and BM structures that create a mesenchymal niche for human HSCs preserving their stem cell characteristics. In comparison to mouse bones, these hOss models demonstrate superior engraftment of human cord blood hematopoietic cells and primary acute myeloid leukemia samples, difficult to engraft in humanized mouse strain (Grigoryan *et al.*, 2022). However, this system still presents a murine vasculature that supplies murine cells and other hematopoietic factors.

Although these approaches allow hematopoiesis to be studied for long-term maintenance of HPSCs in a biologically complex environment, the lack of a controllable, fully human niche prompted the development of in vitro alternatives.

2D *in vitro* model of the human hematopoietic niche

Due to the cellular heterogeneity of BM and the difficulties to provide support for all niche components, 2D *in vitro* model can often accommodate only a few cell types as proxies for recapitulating human HSPCs-niche interactions. These systems thus focus on the support provided by stromal/endothelial cells towards HSPCs maintenance, differentiation, and quiescence. Hereafter we focus on human-only 2D systems.

Cell types and 2D methods commonly used in co-culture systems

Coculture systems commonly consist of a stromal cells layer on which hematopoietic cells are seeded and their output analyzed. Sources of HSPCs and niche cells can vary. For HSPCs, the most common sources of primary samples are the CD34+ fraction of cord blood or BM mononuclear cells. Hematopoietic cell lines are usually cancerous cells harvested from patients. For the stromal layer, MSCs are the principal cell types used. Sourced from BM (biopsy, bone fragments) or cord blood, MSCs can also be differentiated *in vitro* into osteoblasts and adipocytes. These differentiated cells can also be harvested directly from biopsies or bone fragments. Endothelial cells can similarly be harvested from primary hematopoietic tissue. A wide variety of stromal/endothelial cell lines are routinely used for 2D culture, which vary in their capacity to faithfully mimic their primary counterpart. HS-5 and HS-27a are two human MSC lines used in hematopoietic coculture but suffer from lack of differentiation potential (Adamo *et al.*, 2020). While its murine origin limits its modelling capacity, the OP-9 mouse line is a popular MSCs for coculture experimentation for support and differenciation of human HSPCs (De Smedt, Hoebeke and Plum, 2004; Vodyanik *et al.*, 2005).

Early attempts to engineer such 2D cultures follow the Dexter method previously mentioned, to identify the phenotype and cytokines of human stromal cells capable of supporting the clonogenicity of HSPCs (Dexter, Allen and Lajtha, 1977). The Dexter methods describe the harvesting and culture of mice femoral BM cells into a glass flask for 3 weeks to keep a layer of adherent cells (later identified as stromal cells) and remove non-adherent ones. This stromal layer is then “refed” with freshly harvested BM cells, sustaining hematopoietic cells with some clonogenic activity for several months (Dexter, Allen and Lajtha, 1977). Thus, early iterations of the

Dexter methods in a human-only context sought to identify cells and hematopoietic factors that contribute to the support and clonogenicity of cultured HSPCs.

A decade later, in order to narrow the stromal cells able to provide such support in a human-only context, MSCs isolated from BM based on their capacity to adhere to plastic flask and differentiate into osteoblasts, chondrocytes and adipocytes, were cultured to confluence, irradiated and used for hematopoietic co-culture as stromal layer (Majumdar *et al.*, 1998). CD34+ HSPCs, which comprise a diverse population of HSPCs with varying clonogenic potential, were laid on top of the MSCs stromal layer for 2 weeks. The number of hematopoietic colonies produced was used as a determinant for the capacity of MSCs to support CD34+ HSPCs, an assay termed as long-term culture initiating cells (LTC-IC). The authors not only provide evidence that MSCs can be used to support HSPCs but also provided the description of expressed hematopoietic factors by these human cells during support such as FLT3LG and various ILs (Majumdar *et al.*, 1998). Thus, these early 2D coculture systems provide a simplified framework by which the fitness of hematopoietic cells can be quantified when in presence of different niches cells.

Using this method, human osteoblasts and endothelial BM cells were evidenced to both support HSPCs via cell-cell contact and via the production of human hematopoietic cytokines (Berneman *et al.*, 1989; Rafii *et al.*, 1995; Taichman, Reilly and Emerson, 1996). While BM adipocytes show some support for HSPCs, it was minimal compared to other niche cells (Touw and Lowenberg, 1983). Furthermore, this method refined the supplementation of media with small molecules (i.e., PVA, UM171) and hematopoietic factors (i.e., SCF, THPO, FLT3LG, IL7) to either direct the differentiation of HSPCs or to expand their number for clinical applications (Wilkinson, Igarashi and Nakauchi, 2020).

Due to the limited success of expanding HSCs in culture without the use of feeder cells, there has been growing interest in incorporating niche-inspired structural or mechanical cues as a possible strategy to manipulate HSC fate choices. Further modeling modifications were implemented to mimic aspects of the BM ECM in 2D and investigate their impact on HSPCs. A seminal study uncovered that HSPCs ability to attach to the ECM is increase with commitment (Coulombel *et al.*, 1988). Coating and functionalization of the 2D surface with ECM proteins such as collagen, fibronectin and glycosaminoglycan revealed different behavior from HSPCs (Franke *et al.*, 2007). Collagen and fibronectin strongly promoted cell adhesion to the 2D surface while glycosaminoglycan did not favor adhesion. Modification of the stiffness and granularity of the culture surface showed to play a major role in the migration and differentiation of niche cells and HSPCs (Chua *et al.*, 2007; Hsiong *et al.*, 2008).

2D culture methods provided invaluable insights in the interplay between niche cells and their relation to their environment. These insights soon reveal the limit of 2D

systems to faithfully provide a BM proxy with relevant physical, structural and cellular environement.

Limitations and challenges of 2D Models

Cell culture in a 2D arrangement offers a straightforward approach and valuable material for investigating uniform cell populations. Nevertheless, it overlooks significant aspects of cell physiology, including cell-to-cell and cell-to-microenvironment communication, as well as interactions with adjacent molecules present on a more complex ECM structure (Duval *et al.*, 2017; Jensen and Teng, 2020). Stemming from its simplicity, one of the main limitations of this methodology is its inability to fully mimic animal physiology, as a single layer of cells fails to replicate the intricate cellular microenvironment found in the original tissue. Cells can sense the shape of their surrounding environment, a planar environment is almost never present in the biological niches, thus providing a more intricate and complex environment will more faithfully allow the cells to self-organize in a niche-like manner. The hematopoietic niche is ductile and niche cells remodel its architecture, constantly providing varying gradient of stiffness and granularity to the environment (Choi, Mahadik and Harley, 2015).

Moreover, when attempting to reproduce the hematopoietic environment in 2D, one quickly renders the surface of culture confluent with the stromal layer, which triggers morphological and expression patterns which differ throughout the culture period. This over-confluence is often circumvented by irradiation of the stromal cells layer but at the cost of disrupting the biology of the affected cells.

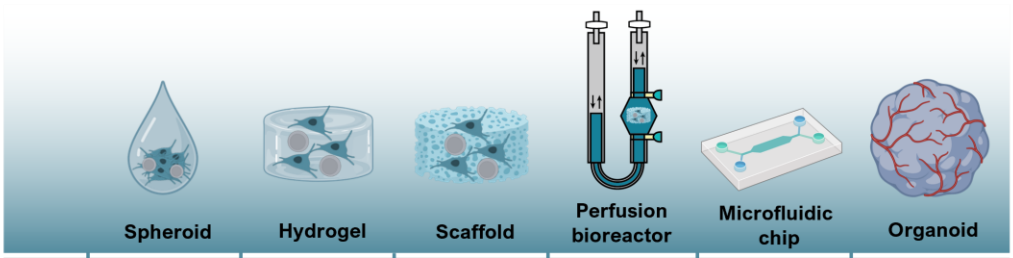
Lastly, 2D culture is often performed in a static environment. Hematopoietic factors produced by stromal cells also extend their paracrine reach through with the interstitial fluid flow present in the niche, a flow which by itself influences the niche homeostasis. This flat environment also impairs the polarization of the cells in culture: with one side facing a rigid and abiotic surface and the other open to the media devoid of extensive cell interaction (Chatterjee *et al.*, 2021).

Further systems such as 3D niche modelling is the natural next step for addressing these modelling shortcomings.

3D Modelling of the Hematopoietic Niche

3D cells culture methods have distinct impact towards differentiation, proliferation, migration and interaction of HSPCs and niche cells compared to 2D models (Duval *et al.*, 2017). Establishing a benchmark for the superiority of one system to another

ultimately relies on the scientific question being asked with the system used (**Figure 5**). To tackle such question, 3D systems provide higher cellular and structural complexicity while retaining the ability to fully control the modelled microenvironment. We hereafter describe the advantages of static and dynamic 3D models while highlighting the limits inherent to the method used.



	Spheroid	Hydrogel	Scaffold	Perfusion bioreactor	Microfluidic chip	Organoid
Benefits	– Simplicity – Cost – Uniformity – Parallelization	– Customizable – Scalable – Cost	– Customizable – Tissue-like architecture	– Dynamic – Cell seeding – Tissue size – Customizable	– Inter-connectivity – Tissue-like architecture	– Self organization – Parallelization – Complexity
Limits	– Size/Shape – Limited complexity	– Tissue size – Limited complexity – Variable	– Tissue size – Cell seeding – Variable	– Handling – Cost – Parallelization	– Handling – Low throughput	– Variable – Embryo-like – Size

Figure 5. Comparison of in vitro 3D methods for bone marrow modelling.
The spheroid approach involves culturing cells under low-attachment conditions, promoting cell aggregation and cell-cell interactions. This is achieved through non-adherent culture surfaces, hanging drop methods, or specialized plates. In the hydrogel and scaffold-based approaches, biocompatible materials are employed to create a 3D structure that emulates the bone marrow microenvironment. Cells are seeded onto these scaffolds, fostering cell-cell and cell-matrix interactions. Perfusion bioreactors consist of a chamber or scaffold containing cells or tissue, connected to a network of channels or tubing for continuous circulation of culture medium. The microfluidic device or organ-on-a-chip approach utilizes interconnected channels to model the dynamic nature of the bone marrow microenvironment. This includes fluid flow, gradients of soluble factors, and compartmentalization of vessel and marrow environments. Lastly, the organoid method involves cells self-assembling into three-dimensional structures that recapitulate key aspects of bone marrow architecture and function relevant in modeling cell behavior and tissue development.

Overview of common approach of 3D co-culture systems in hematopoietic niche modelling

One of the simpler approaches to promote the formation of a static 3D structures is by providing MSCs with a low adherent surface to self-organize as spheroid (Isern *et al.*, 2013). Within spheroids, MSCs keep an undifferentiated state and have enhanced self-renewal. Using this method MSCs could also be propagated over 2 months. Interestingly, they displayed higher support towards HSPCs via increased secretion of hematopoietic factor (Isern *et al.*, 2013).

Another widely used method for static 3D culture consists in the seeding of niche cells within hydrogels. Hydrogels are a type of 3D crosslinked polymer network of hydrophilic molecules, either synthetic (polyethylene glycol PEG) or natural (Collagen). These molecules are capable of absorbing and retaining large amounts of aqueous solutions without dissolving (Caliari and Burdick, 2016). They can be designed to have different degrees of flexibility, porosity, and swelling capacity, which allows tailoring their properties to suit specific niches architecture modelling purposes (i.e.: functionalization with matrix proteins) (Leisten *et al.*, 2012; Caliari and Burdick, 2016). The degree of stiffness of the hydrogel also directs the differentiation of MSCs, typically favoring osteoblastic differentiation with high stiffness and adipocytic differentiation with low stiffness (Dupont *et al.*, 2011; Mao, Shin and Mooney, 2016).

Niches cells are typically encapsulated by hydrogel before polymerization. Collagen was first used to mimic the endosteal hematopoietic niche, believed to sustain more primitive HSPCs *in vivo*. HSPCs migration for instance, was observed in collagen-based hydrogel homing coculture between BM MSCs and umbilical cord blood HSPCs (Leisten *et al.*, 2012). HSPCs segregated between the hydrogel and the media directly above the hydrogel – in suspension. MSCs within the hydrogel significantly enhanced the number of primitive CD34+CD38- after 14 days. In contrast, the suspension of HSPCs led to their differentiation (Leisten *et al.*, 2012). Furthermore, the porosity of the hydrogel provided extensive culture surface, bypassing the culture limitation of conventional 2D systems. The MSCs cultured within the hydrogel also laid matrix proteins typical of the BM niche ECM, such as collagens, laminin, fibronectin and osteopontin (Leisten *et al.*, 2012).

In a similar fashion, static 3D culture within a scaffold has been widely adopted by the research community. Scaffolds are open-cell foam structures aimed at mimicking the architecture of the trabecular bone. Various fabrication techniques (i.e., lyophilization, solvent casting, crosslinking) have been employed to make scaffolding material of tuned porosity from natural (i.e., collagen, fibrin) or synthetic (i.e., polycaprolactone, poly[lactic-co-glycolic acid], poly[L-lactide], polyurethane) sources (Bruschi *et al.*, 2022). Similar hematopoietic niche modeling output were established with such scaffolds, with B-cell differentiation acquired with specific applications (Nichols *et al.*, 2009). As opposed to hydrogels, polymerization is not required for cells to be embedded within the scaffold.

As stated previously, the human hematopoietic niche is not only a 3D environment, but it is also dynamic. Thus, new approaches aiming at providing fluid flow for enhance mass transport, nutrient supply, waste removal and enhanced cell-cell interaction were developed (Cabrita *et al.*, 2003; Martin, Wendt and Heberer, 2004). One of the first approaches was the fixed bed perfusion bioreactor wherein MSCs were immobilized on porous glass carrier beads continuously perfused with media

containing HSPCs (Meissner *et al.*, 1999). Over several weeks of culture, this device yielded expansion of primitive and committed HSPCs capable of forming colonies of multiple hematopoietic lineages (Meissner *et al.*, 1999). Another method, the perfusion chamber bioreactors, was developed to increase the number hematopoietic cells for clinical application. Early devices consisted of a chamber filled with collagen microsphere for adherence of niche cells, held by ultrafiltration membranes (Wang and Wu, 1992). A continuous flow of media was perfused through this chamber over 14 days. This method yielded a 30-fold expansion of colony forming human HSPCs (Wang and Wu, 1992). Since this seminal research, perfusion bioreactors have been modified to further refine human hematopoietic niche modeling.

The combination of porous scaffolding materials and perfusion bioreactor allowed modeling of both the architecture and the dynamic environment of the hematopoietic niche (Di Maggio *et al.*, 2011). To uniformly seed niche cells in such bioreactor, cell-containing media was perfused into the scaffold in an oscillating manner. (Wendt *et al.*, 2003). The resulting scaffold seeded with BM mononuclear cells – containing human HSPCs and MSCs, and perfused for 19 days, provided more reproducible and uniform bone tissue when subsequently implanted ectopically in mice (Braccini *et al.*, 2005). Furthermore, the perfusion enables the functionalization of the scaffold by human MSCs, benefiting the fitness of human HSPCs and enabling the uniform differentiation of MSCs for the formation of adipocyte rich, osteoblastic or stem-rich niche environment (Braccini *et al.*, 2005; Bourgine *et al.*, 2018). Lastly this 3D culture method also provides a compartmentalization of the HSPCs in a gradient of stemness. Indeed committed progenitors were enriched in the perfused media and primitive population were enriched in the scaffold (Bourgine *et al.*, 2018). With the advent of cell engineering, human MSCs could be engineered to enhance or disrupt the expression of hematopoietic factors in this dynamic context to interrogate their pleiotropy towards human HSPCs (Bourgine *et al.*, 2018).

Microfluidic devices also emerged in the last decade for modeling other aspects of the hematopoietic niche, most notably the interface between the BM niche and the endothelial vasculature (Chou *et al.*, 2020). BM on-a-chip devices commonly consist of two compartments separated by a porous Polydimethylsiloxane (PDMS) membrane allowing nutrient/waste exchange. One side of the PDMS membrane is colonized by endothelial cells and continuously fed with fresh media, while the other contains BM cells embedded within a hydrogel which can also be feed with its own separated media (Chou *et al.*, 2020). A BM chip was developed using a combination of human CD34+ HSPCs and MSCs from BM with adjacent vasculature composed of immortalized human endothelial cells. This chip homed the differentiation and maturation of various blood cell lineages over 28 days in culture (Chou *et al.*, 2020). As the chip's primary goal was to be an alternative to

animal use in late-stage preclinical drug development pipeline, the authors set out to test pharmacological response of cells to commonly used myeloablation (recurring pretreatment for leukemia). The device accurately replicated toxicities caused by exposure to 5-fluorouracil and γ-radiation, mimicking patient dose-responses to such treatments as opposed to static culture (Chou *et al.*, 2020). Furthermore, this BM on-a-chip was seeded with HSPCs from Shwachman-Diamond Syndrome (SDS) patients and successfully replicated aspects of this hematological disorder (Chou *et al.*, 2020).

Lastly, innovative new avenues for BM modelling *in vitro* recently opened with the organoid technology (Chou *et al.*, 2020; Kim, Koo and Knoblich, 2020). This technology is based on a 12 days multiphasic differentiation of induced pluripotent stem cells spheroids. These pluripotent spheroids are directed towards mesodermal, vascular and hematopoietic cells differentiation using a sequence of growth and hematopoietic factors supplementation (Khan *et al.*, 2023). The resulting multi-lineage cell aggregates are then embedded in hydrogels and placed on low-adherence plate for a week. Harvested organoids presented a sinusoid network with lumen, BM stroma architecture and myeloid cells such as megakaryocytes, with extensive intercellular interactions both physical and paracrine. These organoid niches also engrafted primary cells from human blood malignancies (Khan *et al.*, 2023). Most notably, organoids give rise to a varied cell population reflecting the cellular complexity of the BM niche in a manner hardly obtainable with other 3D culture methods. Whether this complexity can be harvested to answer previously challenging questions for human BM niche understanding remains to be uncover as this technology is at it first faltering steps.

Limitations and challenges of 3D Models

Similarly to 2D approaches, the passage to the third dimension opened new scientific opportunities but also exposed new limitations in BM modelling (**Figure 5**).

MSCs spheroids, for instance, appear to negatively impact the quiescence of HSCs when in direct contact. This feature is not observed when the HSCs and the MSCs spheroids are separated by a transwell. (Isern *et al.*, 2013). Moreover, only a small fraction of primary human MSCs from the BM can form spheroid in culture, raising concerns on which aspect of MSCs-HSPCs this system can model (Isern *et al.*, 2013).

Hydrogels and static scaffold approaches also suffer from shortcomings. Their reliance on passive diffusion of nutrients and oxygen can lead to necrotic cores, limiting the dimension of such systems (Cabrita *et al.*, 2003; Martin, Wendt and

Heberer, 2004). In addition, some hydrogel formulations necessitate photopolymerization which leads to phototoxicity of embedded cells.

Perfusion bioreactor addressed many of the 3D static approaches' pitfalls but falls short in terms of cellular complexity. Indeed, these systems were developed to host a limited variety of niche cell types. While the MSCs to HSPCs interactions were faithfully recapitulated, the lack of an endothelial-vascular and even neuronal contribution limits their modeling capability.

Microfluidic systems on the other hand provide an endothelial compartment and marrow compartment to interrogate interactions of niches cells with a vascular proxy. However, the miniaturization leads to low experimental output which complicates multiplex analysis of the BM niche modeling outcomes. The PDMS membrane of these devices also poses a challenge for hydrophobic drugs testing as PDMS retains such drugs and warrant the use of microfluidic device for their pharmacokinetic investigation (Ingber, 2022).

Organoid protocols also present challenges. As for 3D static methods, the size is limited by the lack of flow and the formation of a necrotic core in the growing tissue. Batch-to-batch variability is also a concern with current iteration of organoid formation (Hofer and Lutolf, 2021). The main benefit of organoid technology is high-throughput production of miniaturized organs for parallelization of drug-testing. However current methods fall short of providing essential BM niches cells such as osteoblasts, adipocytes and lymphoid cells (Khan *et al.*, 2023). Via the use of iPSCs, one concern is that the tissue generated might more closely model an embryonic-like BM niche rather than an adult microenvironment.

Organoid and BM on-a-chip technologies represent recent advancements in the field. As these technologies are still emerging, variations in chip designs and experimental configurations are expected. Achieving standardization and validation of these models across diverse research groups and laboratories will further refine their modeling potential.

Overall, all 3D methods are steppingstones in the creation of the next model of the human BM niche. One can envision a system exploiting all the advantages and bypassing the limitations of mentioned techniques. For instance, a system presenting the functionalization of a microporous scaffold with organoid-generated tissue in a perfused and compartmented bioreactor would provide a dynamic bone like architecture with cellular complexity and high throughput. However, more steps will be needed to reach this degree of modeling. The cost and the lack of standardization across methods and laboratory will have to be overcome to define the future contour of human BM modelling.

Most models previously described rely on the use of primary human BM samples. Primary sources rapidly reached senescence in culture and donor genome, age, sex,

and other environmental factors leads to high variability, even from the same donor. This renders studies comparison across laboratory and methods challenging and calls for a more standardized approach to BM modeling. One solution is the use immortalized human cells lines to provide reproducible outcomes. Cell lines of different niche cell populations already exist and have been used in some iteration of 2D and 3D methods. Even though cell lines could be seen as oversimplifying the modeling capability, one should consider their potential to answer specific research questions that does not require the full modelization of the BM. For instance, understanding the cause and outcome of mitochondrial transfer between the BM MSCs and HSPCs in healthy and diseased context could be empowered using MSC cell lines to allow mitochondria tracing and genetic modification. However, and as opposed to analog murine cell, available immortalized lines suffer from a lack of extensive characterization which warrant their implementation in study designs. Thus, more research is needed to define these tools for more actionable and reproducible human BM modeling outcomes.

Aims of the thesis

I. To provide a comprehensive analysis of the unique features, potential, and limitations of human ossicle models as a tool for exploring cellular and molecular mechanisms underlying the establishment and functions of human skeletal and hematopoietic compartments.

II. To examine the ability of a standardized hOss model to recreate a mesenchymal niche conducive to both healthy and pathological human hematopoiesis, as well as supportive of solid tumor metastasis.

III. To describe the application of a 3D perfusion bioreactor for the in vitro generation of human hematopoietic niches.

IV. To develop a customizable 3D perfusion bioreactor system using fused deposition modeling of polylactic acid filament in 3D printing, enabling the rapid generation and modification of perfusion chambers harboring engineered human bone marrow hematopoietic niches.

V. To generate and utilize an immortalized human bone marrow mesenchymal stromal cell line for identifying discriminants and consequences of mitochondrial transfer to normal and malignant human blood cells.

Summary of key results

Paper I: Development of Humanized Ossicles: Bridging the Hematopoietic Gap

Human bone ossicle (hOss) models have emerged as valuable tools for exploring the cellular and molecular processes that establish and maintain human skeletal and hematopoietic compartments. However, variations in current hOss protocols hinder robust comparisons and standardization, which consequently restricts the potential of these models to offer valuable insights into human hematopoiesis and skeletal development. In our **first paper**, we highlighted these limitations and suggested directions to tackle these challenges.

One major challenge associated with hOss models is the employment of diverse scaffolds, BM MSC numbers, and differentiation pathways, resulting in a wide range of intrinsic variabilities. These variations could potentially affect human blood engraftment and functions, making it difficult to identify and compare the most effective hOss strategies. Additionally, inconsistencies in in vitro engineering methods, transplantation routes, the number and purity of transplanted cells, and the readout window further complicate the comparison and identification of hOss strategies that offer higher or more reproducible engraftment.

Another significant challenge lies in the inadequate characterization of the humanized BM environment within hOss models. Most studies have neglected to quantify human-derived BM MSCs in the niche or examine their fate. This lack of comprehensive understanding impedes the evaluation of the degree of niche humanization achieved and the assessment of whether different protocols might generate distinct types of niches. It is plausible that some hOss strategies result in differential niche specification, such as osteoblastic, stromal, or vasculogenic, which can differentially impact human hematopoietic functions. Although a first quantitative approach to human cell populations was provided for one model, a significant decrease (90%) in the number of human MSC-derived cells was observed upon hOss remodeling, indicating limited MSC self-renewal in hOss. This raises questions about the possibility of further optimizing the protocols to enhance the long-term survival of human MSCs.

The hOss microenvironment remains largely chimeric and immune defective, with both the nervous system and vasculature being of mouse origin, despite the detection of human mesenchymal perivascular cells in one study. These considerations challenge the applicability of hOss models for investigating the function of putative niche cellular or molecular factors, as the influence of their mouse counterparts must be carefully considered when studying specific cytokines.

Another limitation of hOss models is the dependence on primary MSCs isolated from healthy donors, which exhibit substantial heterogeneity and variability in terms of proliferation and differentiation. Only select batches efficiently form hOss, and post-formation, hOss display significant differences in terms of human BM niche reconstitution and human blood engraftment. The lack of standardization poses challenges for the full exploitation of the models and necessitates screening for potent MSC donors and experimental iterations. The time-consuming in vitro engineering and in vivo development of hOss further complicate the exploitation of the technology. Additionally, primary MSCs have a limited lifespan ex vivo, associated with a progressive loss of differentiation potential, and their genetic manipulation for gain/loss-of-function studies remains laborious.

To address these challenges and fully harness the potential of hOss models, future developments should prioritize standardizing protocols, enhancing the characterization of the humanized BM environment, and developing dedicated mesenchymal lines to reduce variability and enable genetic modifications. This will facilitate a more comprehensive understanding of human skeletal and hematopoietic compartments and pave the way for the use of hOss models in various applications, such as studying human mesenchymal cell fate and function during bone development, organ-scale quantification of hOss populations, and as a cellular/molecular screening platform.

Moreover, hOss models could serve as an alternative to mouse engineering approaches that aim to incorporate human cytokines into the murine genome, which has limitations such as time-consuming single-gene targeting and non-tissue-specific side effects. By engineering BM MSCs to overexpress a combination of cytokines, the modifications will be confined within the hOss, not affecting mouse organs while displaying superior reconstitution of hematopoietic lineages. Furthermore, the generation of patient-derived hOss from mesenchymal and transplanted blood cells from the same patient in an autologous setting could enable the study of patient mesenchymal niche interactions with their cancer HSCs, ultimately holding great promise for the development, testing, and personalized selection of drugs. However, overcoming the challenges associated with patient-derived MSCs, such as impaired proliferation/differentiation and limited availability, is crucial for the successful development of autologous hOss. These are the challenges we aimed to tackle in **Paper II**.

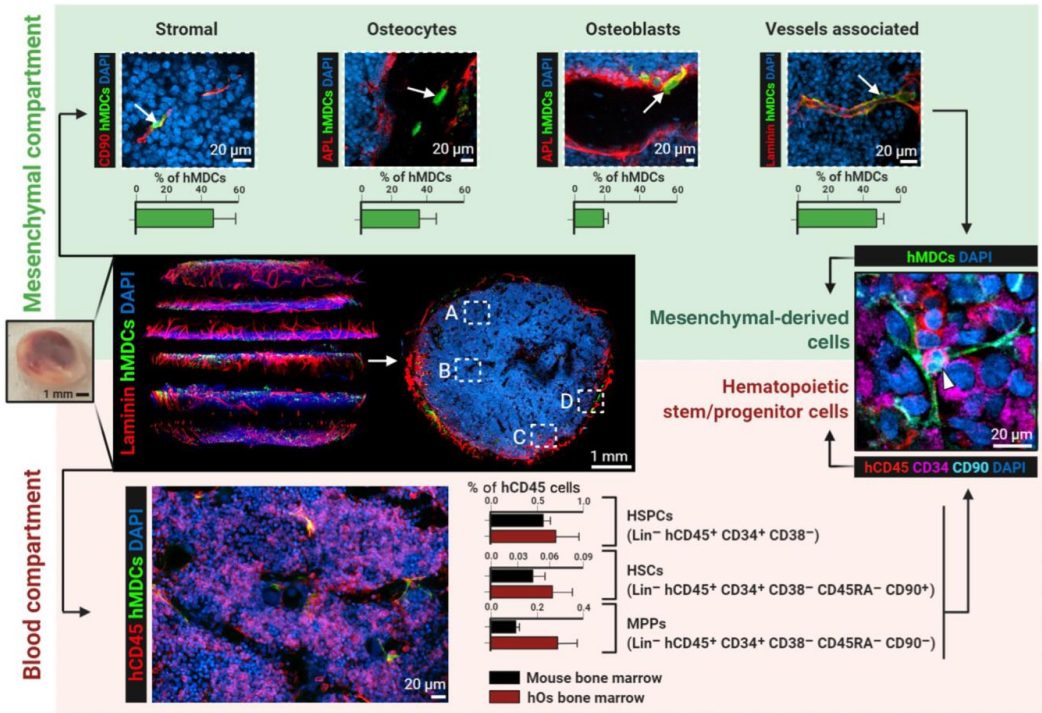


Figure 6. Cellular Composition and Interactions of the Mesenchymal and Blood Compartments in Humanized Ossicles (hOss).
Retrieved hOss can be analyzed as an organ system. Multidimensional confocal fluorescence allows the identification and organization of both human mesenchymal-derived cells (green) and human blood compartments (red). The respective populations can be quantified by designing isosurface strategies complemented by flow cytometry readouts and also providing spatial information. The interactions and distances between humanized BM niche–blood cells–structure (bone, vessel) can be precisely identified and quantified. The overall strategy offers a comprehensive understanding of distribution and functions of human components of hOss. *Figure adapted from (Bourgine et al., 2019). Abbreviations: ALP, alkaline phosphatase; CD, cluster of differentiation; DAPI, 4',6-diamidino-2-phenylindole; hMDCs, human mesenchymal-derived cells; HSCs, hematopoietic stem cells; HSPCs, hematopoietic stem and progenitor cells; Lin, lineage; MPPs, multipotent progenitors.*

Paper II: Engineering human mini-bones for the standardized modelling of healthy hematopoiesis, leukemia, and solid tumor metastasis

In **Paper II**, we initially examined the potential of mesenchymal sword of Damocles BMP2 (MSOD-B) cells to form ectopic bone organs by seeding them on collagen scaffolding material and subjecting them to either chondrogenic (Cho) or osteogenic (Ost) differentiation cues. After 3 weeks of in vitro differentiation, Cho tissues were composed of a collagen 2 and collagen 10-rich ECM, characteristic of mature hypertrophic cartilage, while Ost tissues consisted of a mineralized collagen structure resembling the osteoid matrix preceding bone formation. These tissues were implanted in NSG mice for further development. In vivo observations revealed cartilage and its progressive digestion in Cho tissues, while cartilage was consistently absent in Ost tissues. However, Ost tissues accumulated osteocalcin. Four weeks after in vivo implantation, both Cho and Ost grafts led to the successful formation of human ossicles (hOss) with a mature cortical structure and a hematopoietic cavity.

Within Cho hOss, MSOD-B cells contributed to the formation of a multilineage mesenchymal niche by differentiating into osteoblasts, adipocytes, and osteocytes, reconstituting a humanized microenvironment marked by the abundant presence of human-specific ECM proteins. MSOD-B cells and their progeny persisted in hOss even 24 weeks after implantation. Retrieved MSOD-B cells were able to form secondary hOss, suggesting the presence of MSOD-B cells with mesenchymal stem cell–like properties in engineered hOss.

To investigate the potential of MSOD-B hOss to support human hematopoiesis, NSG mice were implanted with Cho and Ost tissues and irradiated 4 weeks later for intravenous injection of human cord blood CD34+ HSPCs. 96.25% of Cho and 96.7% of Ost tissues successfully developed into mature bone organs. However, structural differences were observed between hOss types. Cho tissues developed into ~40 mm3 organs with a progressive decrease in bone volume/total volume, linked to a reduction in trabecular and cortical bone thickness and a concomitant expansion of the BM cavity of high cellularity. In contrast, Ost hOss appeared to be smaller organs (~20 mm3), predominantly composed of bone tissue with reduced BM space of lower cellularity. Quantitative analysis showed an overall very high hematopoietic engraftment in Cho and Ost hOss, as well as in mouse BM, with balanced lymphoid and myeloid lineage differentiation 20 weeks after transplantation. Notably, Cho hOss showed higher engraftment compared to both Ost hOss and mouse BM.

Based on their higher BM cellularity and hematopoietic engraftment, Cho hOss were selected for subsequent experiments. To further analyze the human

mesenchymal microenvironment, MSOD-B–derived cell populations were isolated from Cho hOss 4 weeks after implantation for single-cell RNA-seq (scRNA-seq). This time point corresponds to when further humanization, such as CB-CD34+ HSPC transplantation, is performed in hOss. scRNA-seq analysis revealed a relatively homogeneous transcriptional profile, although four clusters were identified. Cluster 1 displayed an overall enrichment in genes involved in angiogenesis. Cluster 2 was enriched in genes associated with multipotent MSCs and concomitant expression of chondrogenic (Aggrecan) and osteogenic genes (Alkaline phosphatase and RUNX2, Runt DNA-binding domain 2) suggests a skeletal progenitor population. Cluster 3 exhibited the strongest expression of osteogenic genes, likely identifying it as osteoblasts and osteocytes. Lastly, cluster 4 displayed greater segregation from other clusters and was enriched in genes associated with antiproliferative functions and adipogenic genes.

MSOD-B cells were shown to express important regulators of hematopoietic regeneration (DKK1, Dickkopf-1; VEGFC, Vascular endothelial growth factor C and JAG1, jagged 1) as well as critical factors regulating HSC retention and maintenance in the BM (VCAM1, Vascular cell adhesion protein 1; CXCL12; SCF, and ANGPT1). These factors were predominantly expressed by clusters 1 and 2. As these populations form a human mesenchymal BM niche, expressing key ECM genes and critical factors reported to regulate and support human hematopoiesis development, we hypothesized that this human microenvironment may even be further permissive to the engraftment of leukemia.

To validate the use of MSOD-B Cho hOss for standardized acute myeloid leukemia (AML) modelling, 10 patient BM aspirates from different clinical risk categories were investigated. In NGS mice without hOss, only one sample engrafted, while 80% of samples engrafted in hOss with substantial human CD45+ detection. A detailed phenotypic analysis of engrafted AML patient cells in hOss revealed similar patterns to initial patient samples, with poor lymphoid and high myeloid reconstitution, including CD34+ containing stem cell-like fractions. Overall, we demonstrated that MSOD-B hOss provide a superior microenvironment for AML patient cell engraftment compared to mouse BM, demonstrating their potential for improved AML modelling.

Lastly, we explored the potential of MSOD-B hOss as a tumor model for bone colonization by assessing its capacity to act as a bone metastatic site in a breast cancer context. Breast cancer cell lines bearing luciferase were intravenously injected into NSG mice carrying Cho hOss. Despite the short in vivo period, up to 55% of hOss exhibited bioluminescence on day 35 after transplantation. The breast cancer cells migrating to bone sites were predominantly identified in either mouse femurs or hOss, indicating that hOss can act as bone metastatic sites for human breast cancer cells. We further explored this metastatic site capacity with neuroblastoma cells which primarily develops in the adrenal gland and rapidly metastasizes to other organs, with bone and marrow being the preferential metastatic

sites. Intra-ossicle and intra-femoral injections of luciferase-labelled neuroblastoma cells were performed to evaluate engraftment and growth. Bioluminescence signals confirmed the positive engraftment of neuroblastoma cells after injections in mouse femurs and hOss, with faster growth and higher tumor burden observed in hOss compared to their corresponding mouse femurs. Through 3D scanning of large tissue sections, a spectacular tumor development was evidenced in injected hOss, with the presence of neuroblastoma cells both within the BM cavity and in the form of an outgrown mass engulfing the whole cortical structure. An increase in osteoclastic activity was also identified in neuroblastoma-engrafted hOss compared to controls, suggesting the recapitulation of osteolytic disease-associated patterning leading to skeletal alterations in neuroblastoma patients.

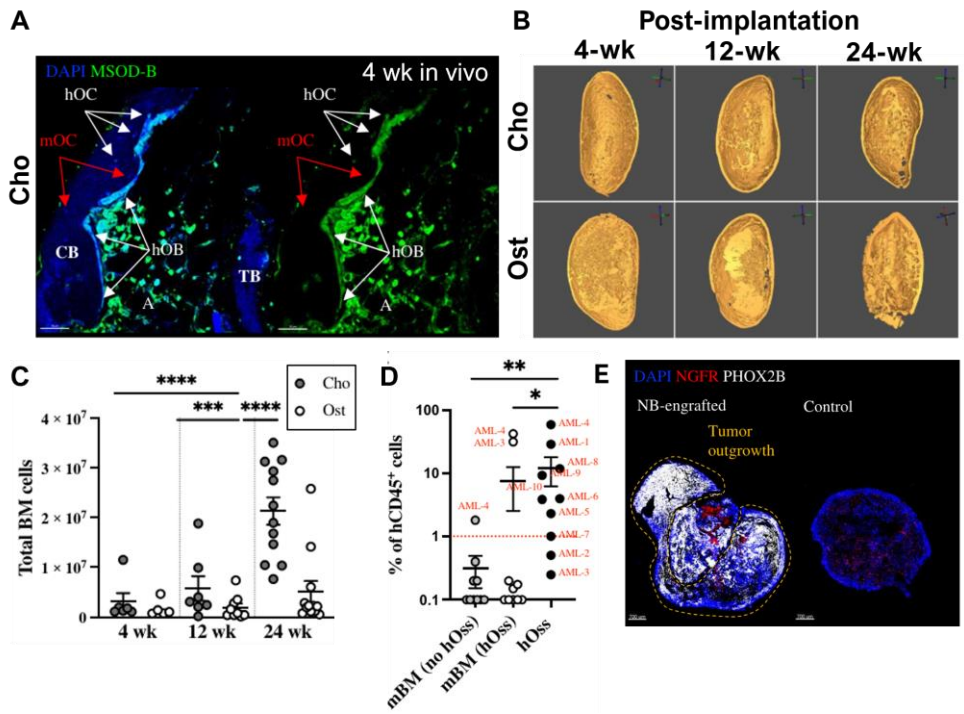


Figure 7. Characterization of MSOD-B humanized ossicles for bone and bone marrow modelling. **(A)** Immunostaining of MSOD-B cells (GFP+) in 4-week implanted Cho hOss. n=3. Respective cells and structures are shown with arrows. Scale bar, 50 µm. **(B)** µCT images of bones from Cho and Ost hOss explanted 4, 12, and 24 weeks after implantation. **(C)** Number of total BM cells isolated from Cho and Ost hOss 4, 12, and 24 weeks after implantation. n=5 to 12. Statistical values were determined by one-way ANOVA followed by Tukey's post hoc test. ** = p-value <.01 and *** = p-value <.001. **(D)** Percentage of hCD45+ leukemic cells in mBM of NSG mice not bearing hOss (mBM no hOss, intravenous injection) and in mBM (hOss) and hOss 24 weeks after intra-ossicle transplantation. n = 11 NSG mice for intravenous transplantation; n = 2 to 4 per AML patient sample for intra-ossicle transplantation, 10 AML patient samples in total. Statistical values were determined by Friedman's test followed by Dunn's post hoc test. * = p-value <.05 and ** = p-value <.01 . **(E)** Representative staining of NGFR+PHOX2B− MSOD-B (Red) and PHOX2B+ neuroblastoma cells (White) in engrafted and control hOss. Nuclei are stained

with DAPI. Yellow dashed line shows areas of neuroblastoma tumor outgrowth. Scale bars, 700 µm. *Abbreviations: CB, cortical bone; TB, trabecular bone; hOC and mOC, human and mouse osteocytes; OB, osteoblasts; A, adipocytes; wk, week; hOss, humanized ossicle; Cho, chondrogenic; Ost, osteogenic; BM and mBM, bone marrow and mouse bone marrow; AML, acute myeloid leukemia; NB, neuroblastoma; MSOD-B, mesenchymal sword of Damocles BMP2; DAPI, 4',6-diamidino-2-phenylindole; NGFR, Nerve growth factor receptor; PHOX2B, Paired-like homeobox 2b.*

Paper III: 3D Engineering of Human Hematopoietic Niches in Perfusion Bioreactor

In **Paper III**, we described the method for the creation of 3D hematopoietic niches using primary human BM MSCs and cord blood CD34+ HSPCs in a perfusion bioreactor system. Elements of this method, briefly summarized hereafter, are subsequently used in **Paper IV** and **V**.

Isolated primary human BM MSCs from BM aspirate are collected from the buffy coat obtained after cell density gradient centrifugation, cells are then enriched with plastic adherent over 2 weeks of culture. Collection of CD34+ HSPCs is performed similarly with the addition of CD34 microbead for magnetic separation.

To establish an osteoblastic niche in the 3D perfusion bioreactor, MSCs are seeded in the bioreactor and cultured with proliferation media for one week to ensure scaffold colonization. The MSCs are then exposed to osteoblastic media for 3 weeks to prime their osteoblastic differentiation and extracellular matrix deposition. HSPCs are resuspended in coculture media and seeded in the bioreactor. The perfusion program is run overnight at a high speed for dynamic seeding and homogeneous cell distribution, and then reduced to a lower speed for culture. The culture media is changed twice a week for one week, with the HSPCs in the pulled media being retrieved and reinjected in the system during each change.

The final step consists in harvesting of samples for flow cytometry and histology. For flow cytometry, floating and loosely attached cells are harvested by collecting the culture media and performing a perfused wash with buffer. Cells present on the scaffolding material are harvested by perfusion with collagenase and trypsin solutions. The cells are then centrifuged and resuspended in ice-cold buffer for immediate use. For histology, the scaffold is removed from the bioreactor, fixed in 4% formalin, and decalcified with 15% Ethylenediaminetetraacetic acid (EDTA) for at least one week before being processed for histological analysis.

Paper IV: Customizable 3D printed perfusion bioreactor for the engineering of stem cell microenvironments

Paper IV describes the design and validation of a 3D printed oscillating perfusion bioreactor for the culture of human mesenchymal and hematopoietic cells. The bioreactor chamber is composed of printed parts, designed in Fusion360™ and printed using a cost-effective Prusa™ MK3 printer with biodegradable bioplastic polylactic acid (PLA). The design accommodates the printing process, supports fluidic and airtightness, and includes a thread fitting 50 mL tubes for sample collection. The bioreactor was tested for airtightness, and disinfection methods were investigated, with isopropanol immersion proving effective.

This study then focused on the culture of a pre-established human mesenchymal stromal cell line MSOD on collagen type I scaffolds (Col1) and an alternative hexamethylene diisocyanate-crosslinked collagen scaffold (CrL-Col1) within the bioreactor. The CrL-Col1 scaffold showed better cell colonization potential in long-term culture due to less degradability and was selected for the rest of the study. The 3D printing approach was demonstrated to be versatile for engineering stromal environments of different dimensions (6 to 12 mm diameter), with homogenous MSOD cell distribution observed within the scaffolding materials under dynamic perfusion culture. The study also demonstrated the reusability of the 3D printed system, with similar MSOD cell growth observed across multiple experimental rounds using the same bioreactors.

The suitability of the 3D printed bioreactor for engineering a BM microenvironment supporting HSC survival was assessed. Physical interactions between mesenchymal and hematopoietic cells were observed, and a 3D stack of the established BM microenvironment highlighted complex networks of intertwined mesenchymal cytoplasmic protrusions.

After a week of coculture in the 3D printed bioreactor, hematopoietic cells were harvested for quantitative phenotypic analysis by flow cytometry. The number of CD34+ HSPCs retrieved from the engineered tissue remained stable over the 7 days of coculture, while more committed myeloid-primed and lymphoid-primed progenitors decreased in both the scaffold and niche settings. The stem CD34+ CD45ra- population also decreased over time in both conditions, though to a lower extent in the presence of MSOD cells. Importantly, a stark difference in stem subpopulation outputs was observed, with a significant increase in HSCs and MPPs CD90-EPCR+ in the presence of MSOD cells, respectively 13.30- and 97.87-fold increase.

In summary, the study validates the generation of a human BM niche using a 3D printed perfusion bioreactor, with superior maintenance of phenotypic HSCs and MPPs CD90-EPCR+ on engineered microenvironments compared to 2D culture and non-stromal conditions.

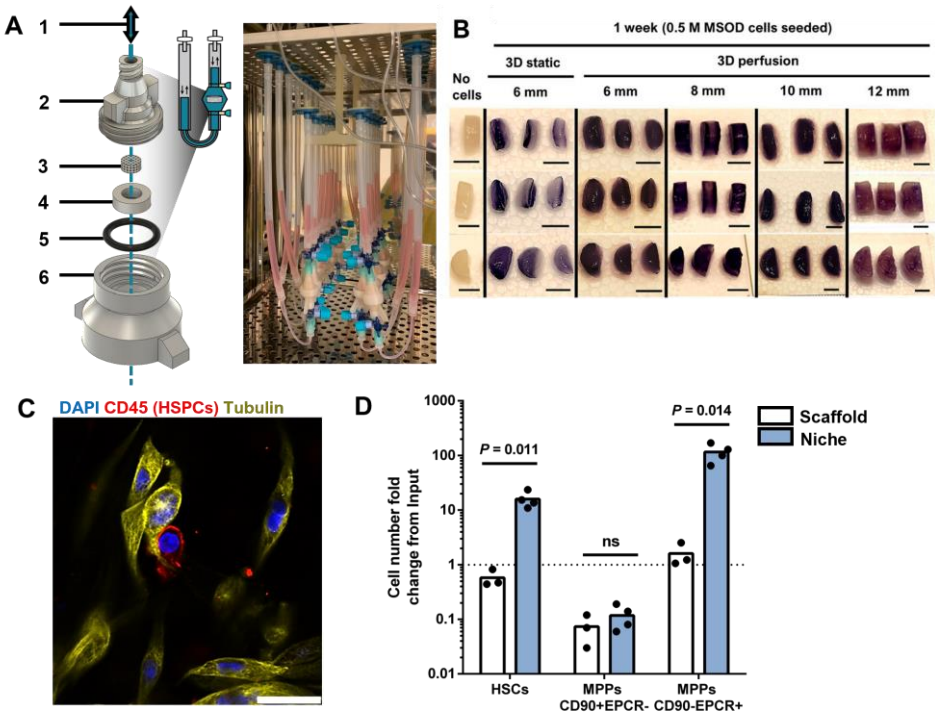


Figure 8. Our customizable 3D printed perfusion bioreactor enables the establishment of a human hematopoietic niche benefiting HSCs self-renewal.
(A) Diagram of the chamber components of the 3D printed perfusion bioreactor and photograph of an array of 14 3D printed bioreactor in use within a cell culture incubator. 1: Representation of the alternating perfusion of culture media (blue) through the bioreactor; 2: Upper stage of the bioreactor chamber; 3: Collagen I (Col1) scaffold; 4: Scaffold holder; 5: Silicon O-ring; 6: Lower stage of the bioreactor chamber. Components 2; 4 and 6 are 3D printed with polylactic acid (PLA), components 3 and 5 are commercially available. **(B)** MTT assay for assessment of the cellular metabolic activity within the 6 to 12 mm diameter CrL-Col1 scaffolds following 1 week of perfusion bioreactor culture with MSOD. The scaffold holder size was adjusted (bottom) to the size of the scaffold. The scaffold was cut through the median plane for better visualization of the scaffold core (n=3). Scale bar= 2 mm. **(C)** Confocal microscopy picture of the engineered Niche 2 days after addition of UCB-CD34+ cells. Physical interactions between the mesenchymal (MSOD) and blood compartments (CD45) could be identified (arrow). Tubulin (Cy3; Yellow) delineates both MSOD and HSPCs while CD45 (CF633; Red) identify blood cells only. DAPI stains nuclei (blue). Scale = 20 µm. **(D)** Stem populations fold change from the UCB-CD34+ input (dotted line) at the end of the culture. The “Scaffold” condition refers to maintenance in culture without stromal cells, as opposed to the “Niche” condition. Unpaired t-test (n ≥ 3). ns = p-value > 0.1. *Abbreviations: MSOD, mesenchymal sword of Damocles; DAPI, 4',6-diamidino-2-phenylindole; HSC, hematopoietic stem cell; MMP, multipotent progenitor; EPCR, Endothelial protein C receptor; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide.*

Paper V: Human mitochondrial transfer modelling reveals biased delivery from mesenchymal-to-hematopoietic stem cells

Paper V reported the development of a human BM-derived mesenchymal stem cell line (iMSOD-mito) capable of transferring functional mitochondria to human leukemic and normal hematopoietic cells. The transfer was observed to occur through non-stochastic cell-to-cell interactions. For healthy hematopoietic cells, the transfer was unequal across different phenotypes, with phenotypic HSCs (CD34+CD45ra-CD90+EPCR+) being the primary receivers of mitochondria.

Co-culture of MOLM13, an AML cell line, with iMSOD-mito for 3 days, resulted in a significant fraction of the MOLM13 cells receiving mCherry mitochondria (15.15% ± 2.57). Within the mCherry+ MOLM13 population, the mean fluorescence intensity of EGFP (cytoplasmic expression in MSOD) was unchanged compared to mCherry- MOLM13, suggesting that no-to-limited cytoplasmic elements were transferred alongside the mitochondria. By correlating MOLM13 cell divisional history and their mitochondrial mass, the study observed a slight decrease of mCherry mitochondria in cells that underwent two divisions, suggesting a continuous rather than discrete mitochondria transfer. mCherry+ MOLM13 displayed a greater mitochondrial membrane potential and reactive oxygen species level than mCherry- MOLM13, suggesting a higher metabolic activity in recipient cells and the transfer of functional mitochondria.

We further found that the mitochondrial transfer from iMSOD-mito to MOLM13 exclusively relies on non-stochastic cell-to-cell interactions. The transfer efficacy was not influenced by the mesenchymal density, suggesting that the transfer is not guided by stochastic interactions between MOLM13 and iMSOD-mito.

iMSOD-mito was able to transfer mitochondria to primary, healthy hematopoietic populations, with over 8.5% (± 4.41) of CD34+ cells receiving mitochondria. The transfer frequency was unequal across hematopoietic phenotypes, with phenotypic HSCs (CD34+CD45ra-CD90+EPCR+) being significantly more targeted than the parent populations (25.51% ± 11.19).

We next aimed to reproduce this finding by comparing the mitochondrial transfer capacity of iMSOD-mito with primary BM MSCs from healthy donors. When primary BM MSCs from three healthy donors were transduced with the mCherry-mitochondria labelling system, all donors exhibited mitochondrial transfer capacity to MOLM13, but at a reduced extent compared to iMSOD-mito (3.38% ± 0.92 against 15.3% ± 2.25). The transfer in various healthy hematopoietic populations revealed a similar biased clear increased frequency in HSCs, with HSCs constantly receiving mitochondria the most among hematopoietic populations in the 2D setting.

The establishment of mesenchymal niches in 3D perfusion bioreactors was found to impact the mitochondrial transfer, resulting in a greater percentage of mitochondria populations receiver and a higher mitochondrial mass transferred to CD34+ cells across all primary donors. In 3D, there was a two-fold increase for HSCs (11.93 to 19.84% in 2D versus 3D respectively) and for CD34+ cells (6.57 to 12.3% in 2D versus 3D respectively) in the percentage of mitochondria populations receiver. There was also a consistently greater mitochondrial mass transferred to CD34+ cells across all primary donors in 3D (1.35-fold increase ± 0.02).

Finally, we found that CD34+ cells receiving mitochondria were more quiescent over a 7-day culture period compared to non-recipient CD34+ cells.

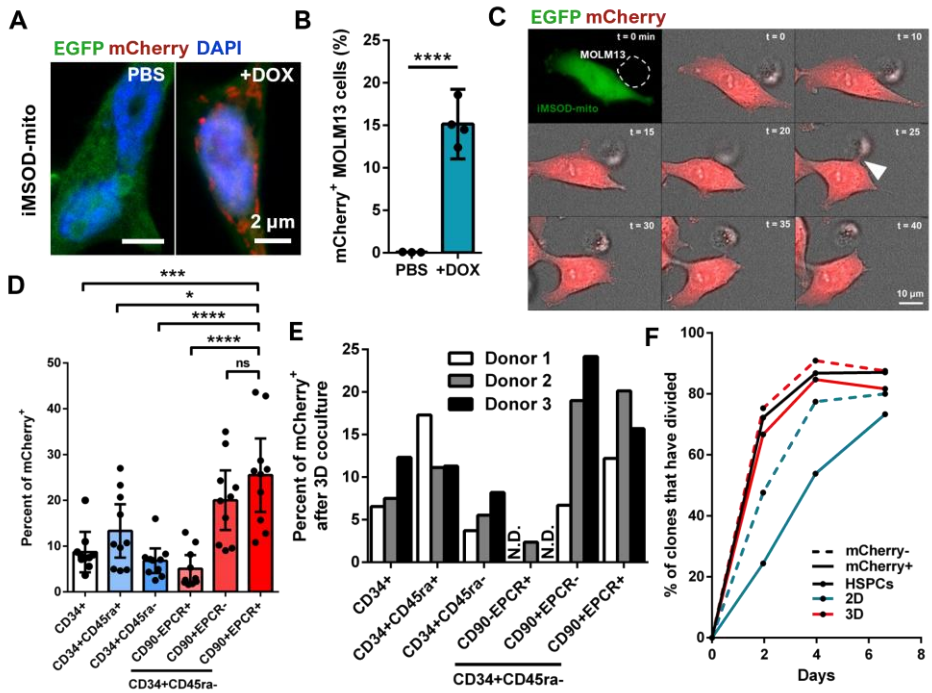


Figure 9. Mitochondrial transfer towards MOLM13 and healthy HSPCs.
(A) Confocal microscopy of iMSOD-mito after 24h exposure to phosphate-buffered saline (PBS) or 150 ng/mL of doxycycline (DOX). **(B)** Percentage of mCherry+ MOLM13 cells after coculture with (+DOX) or without (PBS) induction of iMSOD-mito. Unpaired t-test with logit transformation (n = 4). **(C)** Live imaging snapshots of the mitochondrial transfer from induced iMSOD-mito (EGFP and mCherry) to MOLM13 (dashed contour). After cell-cell contact is established, mCherry mitochondria transfer is initiated at t = 25 min (arrowhead). **(D)** Percentage of mCherry+ in hematopoietic subpopulations after coculture with induced iMSOD-mito. Unpaired t-test with logit transformation (n = 10). **(E)** Percentage of mCherry+ in hematopoietic subpopulations after coculture with induced primary MSCs-mito in 3D. **(F)** Evolution of the percentage of CD34+ clones which have undergone division overtime. **** = p-value <.0001; *** = p-value <.001; * = p-value <.05; ns = p-value >.05. *Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; HSPCs, hematopoietic stem and progenitor cells; MMP, multipotent progenitor; EPCR, Endothelial protein C receptor; PBS, phosphate-buffered saline; DOX, doxycycline; N.D., Not Detected.*

Discussion and future perspectives

Paper I

The variability in hOss protocols exposed in **Paper I** poses challenges in comparing the complexity of the reconstituted human BM niche and potential for subsequent human blood engraftment. The use of different scaffolds, BM-MSC numbers, and differentiation pathways generates intrinsic variabilities that may influence human blood engraftment and functions. In addition, differences in transplantation routes, transplanted cell numbers and purity, and readout windows make it difficult to identify hOss strategies that offer higher or more reproducible engraftment. In addition to the poor characterization of the resulting BM niche, this hinders a comprehensive understanding of the degree of niche humanization and evaluation of whether different protocols generate different types of niches.

Another limitation is the largely chimeric and immune-deficient hOss microenvironment, with both the nervous system and vasculature being of mouse origin (Bourgine *et al.*, 2019). This challenges the applicability of hOss models for probing the function of putative niche cellular or molecular factors and requires careful consideration of the influence of their mouse counterparts.

All reported hOss approaches are based on the use of primary MSCs isolated from healthy donors, which are subject to substantial heterogeneity and variability in terms of proliferation and differentiation (Bianco, Robey and Simmons, 2008; Sacchetti *et al.*, 2016). Although MSC heterogeneity does not appear to be essential for hOss formation, the lack of standardization challenges the full exploitation of the models and requires screening for potent MSC donors (Sacchetti *et al.*, 2007; Bianco *et al.*, 2013). Finally, primary MSCs display a limited lifespan ex vivo, associated with progressive loss of differentiation potential, and their genetic manipulation for gain/loss-of-function studies remains laborious.

As evidenced later in **Paper II**, addressing MSC variability through the generation of dedicated mesenchymal lines can improve the reproducibility of hOss formation and human hematopoietic reconstitution. This would also facilitate genetic modifications of MSCs towards the generation of customized hOss.

hOss can be used to screen for human cellular/molecular factors that regulate HSC functions. The model could refine the function of elusive candidate molecules, particularly those with poor homology to their mouse counterparts. Furthermore,

genetic modification of BM-MSCs can control their differentiation into particular lineages upon hOss formation. This could address debates concerning the function of vascular versus osteoblastic niches (Acar *et al.*, 2015; Crane, Jeffery and Morrison, 2017) and provide a human model of BM aging through induced adipogenicity (Ambrosi *et al.*, 2017). In a similar fashion, hOss can also serve as a substitute for mouse engineering approaches, confining modifications within the hOss and not affecting mouse organs.

Paper II

In **Paper II**, we presented a novel hOss model that served as a versatile tool for studying human hematopoiesis, AML engraftment, and solid tumor metastasis. Our innovation involves the use of a mesenchymal cell line, MSOD-B, which efficiently generates bone organs with consistent results (over 1000 hOss generated). Our approach includes a 3-week in vitro priming followed by in vivo implantation, resulting in the formation of mature bone organs within 4 weeks. These hOss closely mimic native bones, hosting a functional human mesenchymal niche with expression of factors crucial for supporting both healthy and malignant bone-residing cells. Compared to traditional methods using primary MSCs, our approach significantly reduces the time required for differentiation and development (Scotti *et al.*, 2013).

Furthermore, we demonstrate the feasibility of engineering and analyzing a human BM niche at the single-cell level, allowing for the exploration of BM transcriptional changes upon cancer engraftment. Despite these advancements, we faced challenges that remain to be solved for isolating specific mesenchymal populations due to their scarcity and susceptibility to digestion procedures.

The MSOD-B line not only streamlines the creation of hOss but also enables the manipulation of specific genes, offering opportunities for studying molecular pathways involved in hematopoiesis and cancer (Carretta *et al.*, 2017; Bourgine *et al.*, 2019). Additionally, the model shows promise for personalized drug testing, particularly for AML patient samples with limited quantities. Our study suggests the potential of the MSOD-B hOss model in various pathological contexts beyond AML, including other leukemia subtypes and solid tumors (Meads, Hazlehurst and Dalton, 2008; Paczulla *et al.*, 2017; Boutin *et al.*, 2020). By recapitulating disease-specific patterns, such as osteolytic lesions in bone metastasis, this model holds promise for preclinical studies aiming to understand cancer progression and resistance to treatment and test therapeutic interventions (Meads, Hazlehurst and Dalton, 2008; Boutin *et al.*, 2020).

However, limitations such as the chimeric nature of the model and its reliance on mouse vasculature underscore the need for further optimization and humanization.

Nevertheless, our findings support the MSOD-B hOss as a valuable in vivo platform for both fundamental research and preclinical drug testing, paving the way for standardized human-specific studies and accelerated development of personalized therapies.

Paper IV

In **Paper IV**, we present a 3D printed perfusion bioreactor that can be reused and is suitable for the culture of both adherent and non-adherent human stem cells. We showcased the rapid prototyping of bioreactors of various sizes, enabling the engineering of tissues with customized dimensions. The effectiveness of our system was confirmed by generating a human bone marrow proxy, where the 3D environment and interactions between hMSC-HSPCs led to improved maintenance and expansion of phenotypic HSCs.

While oscillating perfusion bioreactors offer dynamic 3D cell culture, they often have rigid designs and require significant resources. To address this, we utilized PLA, a biocompatible material, in conjunction with an affordable 3D printer, resulting in a cost-effective, open-source bioreactor (Schmelzer *et al.*, 2016).

Insights gained from our printing process to allow for airtight and culture compatible design could be implemented by other researchers to develop, prototype, and manufacture their own cell culture systems. Unlike the rigid devices of commercially available alternatives, we provide a design that can be quickly adapted to meet specific experimental requirements, allowing for miniaturization or scaled-up tissue generation. This flexibility is particularly useful when conducting multiple experiments in micro-bioreactor systems or for engineering larger tissues, providing superior cellular throughput for various readouts.

Existing 3D printed bioreactor devices are limited by the toxicity of the processed used (resin-based stereolithography) or the lack of adaptability, reusability, and comprehensive biological validation (Costa *et al.*, 2015; Zhu *et al.*, 2015; MacDonald *et al.*, 2016; Schmid *et al.*, 2018; Janvier, Canty-Laird and Henstock, 2020). Moreover, most of these devices are integrated within a continuous media flow, which makes their use with non-adherent cells challenging. In contrast, our reusable system has been validated for long-term culture, is compatible with various scaffolding materials in oscillating dynamic perfusion with primary human cells, including human HSCs.

While this study does not aim to prove the superiority of 3D dynamic cultures, our data suggests that MSOD cells' supportive capacity is enhanced in a 3D dynamic setting. This understanding could also inform the mechanisms leading to increased maintenance and expansion, ultimately benefiting transplantation and gene editing

therapies (Wilkinson, Igarashi and Nakauchi, 2020). Our 3D printed bioreactor and MSOD combination could also be applied to other tissue modelling application requiring a more democratized 3D culture system.

Paper V

In **Paper V**, iMSOD-mito was generated as the first human MSCs line with inducible mitochondria labelling, capable of mitochondrial transfer to both malignant and healthy human hematopoietic cells. Furthermore, we exposed a transfer biased towards phenotypic HSCs which was enhanced with culture in dynamic 3D hematopoietic niche. Receiving hematopoietic CD34+ cells displayed increased quiescence compared to non-receiving CD34+ HSPCs.

As opposed to Mitotracker and equivalent chemical dyes, mCherry labelling is not cytotoxic and does not depend on the mitochondrial membrane potential (Neikirk *et al.*, 2023). Furthermore, mCherry is not targeted by xenobiotic efflux pump which expel mitochondrial dyes and complicates their interpretation for mitochondrial transfer (de Almeida *et al.*, 2017; Mansell *et al.*, 2021). This is especially relevant for HSPCs, which are known to contain high levels of ABC xenobiotic efflux pumps (de Almeida *et al.*, 2017) potentially leading to biased interpretation of transfer rates in stem populations.

Our standardized cell line was instrumental to uncover the preferential transfer towards HSCs and can be used to further discriminate the transfer mechanism leading to this preference. Genetic disruption and screening for new drugs could be used with iMSOD-mito to elucidate the yet not fully resolved mechanisms of transfer which has been implicated in drug resistance mechanism in multiple leukemia models (Moschoi *et al.*, 2016; Wang *et al.*, 2018; Saito *et al.*, 2021).

Primary BM MSCs labelled with the same system exhibited lower and more variable mitochondrial transfer, a characteristic of primary BM MSCs observed elsewhere (Polak *et al.*, 2015). As discussed in this thesis, primary BM MSCs are not a uniform population as opposed iMSOD-mito. The immortalization of iMSOD-mito might lead to a cancer-associated fibroblast (CAF) phenotype which was shown to increase mitochondrial transfer to leukemic cells (Burt *et al.*, 2019). Strikingly, despite these differences, the same pattern of preferential transfer towards HSCs was observed across all donors, advocating for the iMSOD mito capacity to mirror the transfer mechanism of primary cells.

The process of mitochondrial transfer is often associated with stress conditions, during which the metabolic function of the cells receiving the mitochondria is restored (Islam *et al.*, 2012; Mistry *et al.*, 2019). Among HSPCs, HSCs possess the greatest mitochondrial mass per unit volume compared to other types of stem cells

(de Almeida *et al.*, 2017). Interestingly, this fact contradicts the common understanding that HSCs residing in the bone marrow are primarily inactive (Passegué *et al.*, 2005; Takihara *et al.*, 2019), implying low metabolic requirements. The observed buildup of mitochondria within stem cell populations aligns with the findings of our in vitro research. While our study aligns with these observations, we here reveal that the cells receiving the mitochondria exhibit a higher degree of quiescence compared to the HSPCs that do not receive any. Therefore, it is plausible that our experimental conditions mirror the in vivo absorption of mitochondria by HSCs.

This mitochondrial transfer within the niche could be a contributing mechanism to sustain such a high demand of functional mitochondria within HSCs. While correlative, multiple insights contributed to our understanding of its mechanism. Notably, the role of gap junctions facilitating this transfer in conjunction to AKT-PI3K pathways activation, and the CD38 expression on recipient cells (Islam *et al.*, 2012; Marlein *et al.*, 2019; Mistry *et al.*, 2019). However, these findings are bound to a particular tissue or disease model and does not reflect the ubiquitous nature of mitochondrial transfer in tissue maintenance (Marti Gutierrez *et al.*, 2022). In addition, disruption of identified pathways does not completely abrogate mitochondrial transfer, pointing at a level of redundancy leading to mitochondrial transfer not yet resolved. Importantly, the numbers of human-only mitochondrial transfer reports are scarce which limits translational applications of this mechanism. As the significance of human mitochondrial transfer unravels, a human-only standardized protocol is needed to validate key molecular and cellular players for exploitation, which our current design can provides.

Summary of key methods

We here present the key methods which have been instrumental for the successful performance of the experimentation presented in this thesis. Further details for each method can be found in the material and method's section of corresponding papers.

Umbilical Cord Blood CD34+ HSPCs isolation and storage

Central to **Paper II, III, IV and V**, cord blood samples collected from Lund University, Malmo and Helsingborg Hospitals constituted the source of healthy hematopoietic cells in each experiment. Human umbilical cord blood CD34+ HSPCs are a readily available source of human primary material for hematopoietic studies as alternative to the more invasive bone marrow or peripheral blood cell collection methods. Indeed CD34+ HSPCs from cord blood and from bone marrow share hematopoietic potential that make them a surrogate for human bone marrow hematopoietic cell experimentation (Rubinstein *et al.,* 1998). However, CD34+ HSPCs from cord blood are more clonogenic and better engraft immunodeficient mice than bone marrow CD34+ HSPCs. Thus, the sourcing advantages that cord blood provide is to be tampered with the variation in functionality compared to cells of BM origin (Dong Ku *et al.,* 1999; Ueda *et al.,* 2001).

To avoid bias from individual cord blood variability, we pooled a minimum of three isolated cord blood CD34+ samples together for each experiment. Only samples presenting 90% of CD34 positivity and 95% of viability after isolation were used in this thesis. Hereafter we describe the method used to first isolate and then store CD34+ HSPCs from human umbilical cord blood.

To maximize the recovery of primitive cells from cord blood sample, we used samples collected at most 24h prior to isolation (Barini *et al.,* 2011). Mononuclear cells were collected by Ficoll separation and CD34+ cells were isolated using the CD34 MicroBead kit (Miltenyi Biotec #130-046-702) according to the manufacturer's instructions. Post isolation, cells were resuspended in 1 mL of freezing media per million cells (25% Iscove's modified Dulbecco's media [IMDM]; 15% dimethyl sulfoxide [DMSO]; 60% fetal bovine serum [FBS]) and

placed in cryotube at -80°C for 48h. Cryotube were then transferred to liquid nitrogen.

Primary mesenchymal stromal cells isolation

Used in **Paper III and V**, primary MSCs were obtained from healthy donors. Similarly to HSPCs, mononuclear cells containing primary MSCs, were collected via Ficoll separation. 15 million cells are then plated on plastic flask in media supplemented with Fibroblast Growth Factor 2 (FGF-2) to prevent in-culture MSCs differentiation. When MSCs reach confluence, cells are harvested and stored for further use.

MSCs-HSPCs 2D coculture

This method was used for **Paper IV and V**. We initiated 2D culture in Nucleon™ Delta Surface 12-well plate (ThermoFisher #140675) by first establishing a confluent layer of MSCs with subsequent introduction of HSPCs.

In **Paper IV,** 10.000 MSOD cells were seeded and cultured to confluency over 7 days. At confluence, we stopped proliferation by irradiation with 4 Gy, performed with the CellRad X-ray source by Flaxitron. Cells were then left to recover for another 24 h in fresh culture media. 35.000 HSPCs were then seeded on the MSOD confluent layer with coculture media. The coculture media is composed of low calcium media and a serum substitute supplemented with SCF, TPO and FLT3LG all at 10 ng/mL. The coculture was then carried out for 7 days prior to analysis.

In **Paper V**, 144.000 iMSOD-mito or MSCs-mito were culture for 48h in the presence of 150 ng/mL of DOX to reach confluency and to labelling mitochondria with mCherry. 45.000 HSPCs were introduce and coculture for 72h before mitochondrial transfer analysis or sorting was performed.

MSCs-HSPCs 3D coculture

The method described hereafter present the particularities of each paper, however the method to perform assembly, media change and collection of the cells from culture were the object of **Paper III**.

In **Paper IV**, 5 million MSOD cells were suspended in culture media and infused 3D perfusion onto crosslinked collagen 1 scaffold of 6 mm diameter. A first

overnight infuse/withdraw perfusion cycle speed of 2.8 mL/min (seeding speed) with displacement goal at 2 mL allowed dynamic cell seeding on the scaffold; for the rest of the 3D culture the infuse/withdraw perfusion cycle speed was lower at .28 mL/min (culture speed). After 7 days, 35.000 HSPCs were then infuse in coculture media with the seeding and culture cycles and culture for 1 week. During media change, the medium from each bioreactor is harvested and spun down. The pelleted cells are then resuspended in fresh CoCM and injected back in the corresponding bioreactor.

In **Paper V**, we reduced the seeding of iMSOD-mito and MSCs-mito to 0.5 million cells per bioreactor. In analogy to 2D coculture, cells were exposed to DOX 48h prior introduction of 45.000 HSPCs, mitochondria transfer analysis or sorting was then performed after 72h.

Generation of MSOD-B hOss

The following method of generation of ossicle from MSOD-B cells was fundamental to **Paper II** and the utilization of the resulting humanized ossicle as a standard model for human hematopoietic niche. More than a thousand ossicles were generated from it. We will hereafter describe this method in the generation of chondrogenic and osteogenic ossicles.

After trypsinization from a confluent layer, 3.5 million MSOD-B cells were seeded onto collagen I scaffolds (8 mm x 3 mm [d x l]; Ultrafoam, Davol), to achieve a density of 3.5×10^3 cells/cm^3. For chondrogenic differentiation, the seeded scaffolds were then cultured for 3 weeks in Cho or Ost medium containing the following:

Chondrogenic Medium	**Osteogenic Medium**
Dulbecco's modified Eagle's medium	Minimum Essential Medium Eagle Alpha Modification
0.12% Bovine serum albumin	10% FBS
0.1 mM Ascorbic acid	0.1 mM Ascorbic acid
10^{-7} M Dexamethasone	10^{-4} M Dexamethasone
Transforming growth factor–β3 (10 ng/ml)	0.01 M α-glycerophosphate
1% Insulin, transferrin, and selenium	
Linoleic acid (0.47 mg/ml)	

After 3 weeks of in vitro culture, MSOD-B–derived in vitro engineered tissues were subcutaneously implanted in NSG mice for the formation of hOss.

Cell Transplantation

As for the generation of hOss, this method is exclusively used for **Paper II**.

150.000 Human cord blood CD34+ cells were intravenously injected four weeks post implantation into NSG female mice, 24 h after sub-lethal irradiation (200 cGy). Peripheral blood chimerism was surveyed by flow cytometry every 4 weeks up to 20 weeks post-transplantation. The transplantation experiment was performed 2 times with a cohort of five recipient mice (each mouse bearing 4-6 hOss) per transplant.

For malignant human hematopoietic cells, AML cells were first sorted for CD45+/CD3-/CD19-/CD34+ or CD45+/CD3-/CD19- populations were sorted and resuspended with Myelocult H5100 in a volume of 15 µl for intra-ossicle and 250 µl for intravenous injections. As for HSPCs, AML cells were intravenously injected four weeks post implantation into NSG mice, 24 h after sub-lethal irradiation. For the CD45+ /CD3- /CD19- phenotype, 250.000 cells were injected per ossicle and 500.000 intravenously per mouse. For CD45+ /CD3- /CD19- /CD34+ cells, 100.000 cells were injected per hOss and 200.000 cells intravenously per mouse.

For other cancer cells transplantation, 1 million breast cancer or neuroblastoma cells were injected intravenously after four weeks post-implantation and subsequently monitored for tumor engraftment in vivo via bioluminescence imaging.

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References

Acar, M. *et al.* (2015) 'Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal', *Nature*, 526(7571), pp. 126–130. Available at: https://doi.org/10.1038/nature15250.

Adamo, A. *et al.* (2020) 'HS-5 and HS-27A Stromal Cell Lines to Study Bone Marrow Mesenchymal Stromal Cell-Mediated Support to Cancer Development', *Frontiers in Cell and Developmental Biology*, 8, p. 1227. Available at: https://doi.org/10.3389/fcell.2020.584232.

Adams, G.B. *et al.* (2006) 'Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor', *Nature*, 439(7076), pp. 599–603. Available at: https://doi.org/10.1038/nature04247.

Adolfsson, J. *et al.* (2001) 'Upregulation of Flt3 expression within the bone marrow Lin-Sca1+c-kit+ stem cell compartment is accompanied by loss of self-renewal capacity', *Immunity*, 15(4), pp. 659–669. Available at: https://doi.org/10.1016/S1074-7613(01)00220-5.

de Almeida, M.J. *et al.* (2017) 'Dye-Independent Methods Reveal Elevated Mitochondrial Mass in Hematopoietic Stem Cells', *Cell Stem Cell*, 21(6), pp. 725-729.e4. Available at: https://doi.org/10.1016/j.stem.2017.11.002.

Ambrosi, T.H. *et al.* (2017) 'Adipocyte Accumulation in the Bone Marrow during Obesity and Aging Impairs Stem Cell-Based Hematopoietic and Bone Regeneration', *Cell Stem Cell*, 20(6), pp. 771-784.e6. Available at: https://doi.org/10.1016/j.stem.2017.02.009.

Anjos-Afonso, F. *et al.* (2022) 'Single cell analyses identify a highly regenerative and homogenous human CD34+ hematopoietic stem cell population', *Nature Communications*, 13(1), pp. 1–13. Available at: https://doi.org/10.1038/s41467-022-29675-w.

Barbero, A. *et al.* (2003) 'Plasticity of clonal populations of dedifferentiated adult human articular chondrocytes', *Arthritis and Rheumatism*, 48(5), pp. 1315–1325. Available at: https://doi.org/10.1002/art.10950.

Barini, R. *et al.* (2011) 'Does the time between collecting and processing umbilical cord blood samples affect the quality of the sample?', *Einstein (São Paulo)*, 9(2), pp. 207–211. Available at: https://doi.org/10.1590/s1679-45082011gs1809.

Berneman, Z.N. *et al.* (1989) 'A quantitative and dynamic study of endothelial cells and megakaryocytes in human long-term bone marrow cultures', *Leukemia*, 3(1), pp. 61–67. Available at: https://europepmc.org/article/med/2462657 (Accessed: 6 August 2023).

Beyer, A.I. and Muench, M.O. (2017) ‘Comparison of human hematopoietic reconstitution in different strains of immunodeficient mice’, *Stem Cells and Development*, 26(2), pp. 102–112. Available at: https://doi.org/10.1089/scd.2016.0083.

Bianco, P. (2011) ‘Bone and the hematopoietic niche : a tale of two stem cells’, *Blood*, 117(20), pp. 5281–5289. Available at: https://doi.org/10.1182/blood-2011-01-315069.

Bianco, P. *et al.* (2013) ‘The meaning, the sense and the significance: Translating the science of mesenchymal stem cells into medicine’, *Nature Medicine*, 19(1), pp. 35–42. Available at: https://doi.org/10.1038/nm.3028.

Bianco, P. (2014) ‘“Mesenchymal” stem cells’, *Annual review of cell and developmental biology*. Annual Reviews, pp. 677–704. Available at: https://doi.org/10.1146/annurev-cellbio-100913-013132.

Bianco, P., Robey, P.G. and Simmons, P.J. (2008) ‘Mesenchymal Stem Cells: Revisiting History, Concepts, and Assays’, *Cell Stem Cell* [Preprint]. Available at: https://doi.org/10.1016/j.stem.2008.03.002.

Blocki, A. *et al.* (2013) ‘Not all MSCs can act as pericytes: Functional in vitro assays to distinguish pericytes from other mesenchymal stem cells in angiogenesis’, *Stem Cells and Development*, 22(17), pp. 2347–2355. Available at: https://doi.org/10.1089/scd.2012.0415.

Boettcher, S. and Manz, M.G. (2017) ‘Regulation of Inflammation- and Infection-Driven Hematopoiesis’, *Trends in Immunology*. Elsevier, pp. 345–357. Available at: https://doi.org/10.1016/j.it.2017.01.004.

Bourgine, P.E. *et al.* (2018) ‘In vitro biomimetic engineering of a human hematopoietic niche with functional properties’, *Proceedings of the National Academy of Sciences of the United States of America*, 115(25), pp. E5688–E5695. Available at: https://doi.org/10.1073/pnas.1805440115.

Bourgine, P.E. *et al.* (2019) ‘Fate Distribution and Regulatory Role of Human Mesenchymal Stromal Cells in Engineered Hematopoietic Bone Organs’, *iScience*, 19, pp. 504–513. Available at: https://doi.org/10.1016/j.isci.2019.08.006.

Boutin, L. *et al.* (2020) ‘Mesenchymal stromal cells confer chemoresistance to myeloid leukemia blasts through Side Population functionality and ABC transporter activation’, *Haematologica*, 105(4), pp. 987–998. Available at: https://doi.org/10.3324/haematol.2018.214379.

Bouvard, D. *et al.* (2001) ‘Functional consequences of integrin gene mutations in mice’, *Circulation Research*. Lippincott Williams & Wilkins, pp. 211–223. Available at: https://doi.org/10.1161/hh1501.094874.

Boxall, S.A. and Jones, E. (2012) ‘Markers for characterization of bone marrow multipotential stromal cells’, *Stem Cells International*, 2012. Available at: https://doi.org/10.1155/2012/975871.

Braccini, A. *et al.* (2005) ‘Three-Dimensional Perfusion Culture of Human Bone Marrow Cells and Generation of Osteoinductive Grafts’, *STEM CELLS*, 23(8), pp. 1066–1072. Available at: https://doi.org/10.1634/stemcells.2005-0002.

Bruns, I. *et al.* (2014) ‘Megakaryocytes regulate hematopoietic stem cell quiescence through CXCL4 secretion’, *Nature Medicine*, 20(11), pp. 1315–1320. Available at: https://doi.org/10.1038/nm.3707.

Bruschi, M. *et al.* (2022) ‘Functionalized 3D scaffolds for engineering the hematopoietic niche’, *Frontiers in Bioengineering and Biotechnology*. Frontiers Media S.A., p. 968086. Available at: https://doi.org/10.3389/fbioe.2022.968086.

Burt, R. *et al.* (2019) ‘Activated stromal cells transfer mitochondria to rescue acute lymphoblastic leukemia cells from oxidative stress’, *Blood*, 134(17), pp. 1415–1429. Available at: https://doi.org/10.1182/blood.2019001398.

Cabrita, G.J.M. *et al.* (2003) ‘Hematopoietic stem cells: from the bone to the bioreactor’, *Trends in Biotechnology*, 21(5), pp. 233–240. Available at: https://doi.org/10.1016/S0167-7799(03)00076-3.

Caliari, S.R. and Burdick, J.A. (2016) ‘A practical guide to hydrogels for cell culture’, *Nature Methods*. Nature Publishing Group, pp. 405–414. Available at: https://doi.org/10.1038/nmeth.3839.

Calvi, L.M. and Link, D.C. (2015) ‘The hematopoietic stem cell niche in homeostasis and disease’, *Blood*. American Society of Hematology, pp. 2443–2451. Available at: https://doi.org/10.1182/blood-2015-07-533588.

Carretta, M. *et al.* (2017) ‘Genetically engineered mesenchymal stromal cells produce IL-3 and TPO to further improve human scaffold-based xenograft models’, *Experimental Hematology*, 51, pp. 36–46. Available at: https://doi.org/10.1016/j.exphem.2017.04.008.

Chakrabarty, R.P. and Chandel, N.S. (2021) ‘Mitochondria as Signaling Organelles Control Mammalian Stem Cell Fate’, *Cell Stem Cell*. Cell Press, pp. 394–408. Available at: https://doi.org/10.1016/j.stem.2021.02.011.

Chatterjee, C. *et al.* (2021) ‘Rebuilding the hematopoietic stem cell niche: Recent developments and future prospects’, *Acta Biomaterialia*. Elsevier, pp. 129–148. Available at: https://doi.org/10.1016/j.actbio.2021.03.061.

Chen, D. *et al.* (2021) ‘Interleukin-7 Biology and Its Effects on Immune Cells: Mediator of Generation, Differentiation, Survival, and Homeostasis’, *Frontiers in Immunology*. Frontiers Media S.A., p. 747324. Available at: https://doi.org/10.3389/fimmu.2021.747324.

Choi, J.S., Mahadik, B.P. and Harley, B.A.C. (2015) ‘Engineering the hematopoietic stem cell niche: Frontiers in biomaterial science’, *Biotechnology Journal*. John Wiley & Sons, Ltd, pp. 1529–1545. Available at: https://doi.org/10.1002/biot.201400758.

Chou, D.B. *et al.* (2020) ‘On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology’, *Nature Biomedical Engineering*, 4(4), pp. 394–406. Available at: https://doi.org/10.1038/s41551-019-0495-z.

Chow, A. *et al.* (2011) ‘Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche’, *Journal of Experimental Medicine*, 208(2), pp. 761–771. Available at: https://doi.org/10.1084/jem.20101688.

Chua, K.N. *et al.* (2007) 'Functional nanofiber scaffolds with different spacers modulate adhesion and expansion of cryopreserved umbilical cord blood hematopoietic stem/progenitor cells', *Experimental Hematology*, 35(5), pp. 771–781. Available at: https://doi.org/10.1016/j.exphem.2007.02.002.

Claycombe, K., King, L.E. and Fraker, P.J. (2008) 'A role for leptin in sustaining lymphopoiesis and myelopoiesis', *Proceedings of the National Academy of Sciences of the United States of America*, 105(6), pp. 2017–2021. Available at: https://doi.org/10.1073/pnas.0712053105.

Costa, P.F. *et al.* (2015) 'Additively manufactured device for dynamic culture of large arrays of 3D tissue engineered constructs', *Advanced Healthcare Materials*, 4(6), pp. 864–873. Available at: https://doi.org/10.1002/adhm.201400591.

Coulombel, L. *et al.* (1988) 'Lineage- and Stage-Specific Adhesion of Human Hematopoietic Progenitor Cells to Extracellular Matrices From Marrow Fibroblasts', *Blood*, 71(2), pp. 329–334. Available at: https://doi.org/10.1182/BLOOD.V71.2.329.329.

Crane, G.M., Jeffery, E. and Morrison, S.J. (2017) 'Adult haematopoietic stem cell niches', *Nature Reviews Immunology* [Preprint]. Available at: https://doi.org/10.1038/nri.2017.53.

Crane, J.L. and Cao, X. (2014) 'Bone marrow mesenchymal stem cells and TGF-β signaling in bone remodeling', *Journal of Clinical Investigation*. American Society for Clinical Investigation, pp. 466–472. Available at: https://doi.org/10.1172/JCI70050.

Cuminetti, V. and Arranz, L. (2019) 'Bone marrow adipocytes: The enigmatic components of the hematopoietic stem cell niche', *Journal of Clinical Medicine*. Multidisciplinary Digital Publishing Institute (MDPI). Available at: https://doi.org/10.3390/jcm8050707.

Dean, L. (2005) 'Chapter 1: Blood and the cells it contains. [Internet]', in *Blood Groups and Red Cell Antigens*. Bethesda (MD): National Center for Biotechnology Information (US). Available at: https://www.ncbi.nlm.nih.gov/books/NBK2263/ (Accessed: 8 November 2019).

Devlin, M.J. and Rosen, C.J. (2015) 'The bone-fat interface: Basic and clinical implications of marrow adiposity', *The Lancet Diabetes and Endocrinology*. Elsevier, pp. 141–147. Available at: https://doi.org/10.1016/S2213-8587(14)70007-5.

Dexter, T.M., Allen, T.D. and Lajtha, L.G. (1977) 'Conditions controlling the proliferation of haemopoietic stem cells in vitro', *Journal of Cellular Physiology*, 91(3), pp. 335–344. Available at: https://doi.org/10.1002/jcp.1040910303.

DiMascio, L. *et al.* (2007) 'Identification of Adiponectin as a Novel Hemopoietic Stem Cell Growth Factor', *The Journal of Immunology*, 178(6), pp. 3511–3520. Available at: https://doi.org/10.4049/jimmunol.178.6.3511.

Ding, L. *et al.* (2012) 'Endothelial and perivascular cells maintain haematopoietic stem cells', *Nature*, 481(7382), pp. 457–462. Available at: https://doi.org/10.1038/nature10783.

Dong Ku, K. *et al.* (1999) 'Comparison of hematopoietic activities of human bone marrow and umbilical cord blood CD34 positive and negative cells', *Stem Cells*, 17(5), pp. 286–294. Available at: https://doi.org/10.1002/stem.170286.

Doran, M.R. *et al.* (2009) 'Surface-bound stem cell factor and the promotion of hematopoietic cell expansion', *Biomaterials*, 30(25), pp. 4047–4052. Available at: https://doi.org/10.1016/j.biomaterials.2009.04.043.

Doulatov, S. *et al.* (2012) 'Hematopoiesis: A human perspective', *Cell Stem Cell*, 10(2), pp. 120–136. Available at: https://doi.org/10.1016/j.stem.2012.01.006.

Duarte, R.F. and Frank, D.A. (2002) 'The synergy between stem cell factor (SCF) and granulocyte colony-stimulating factor (G-CSF): Molecular basis and clinical relevance', *Leukemia and Lymphoma*. Taylor & Francis, pp. 1179–1187. Available at: https://doi.org/10.1080/10428190290026231.

Dupard, S.J. *et al.* (2020) 'Development of Humanized Ossicles: Bridging the Hematopoietic Gap', *Trends in Molecular Medicine*. Elsevier Ltd, pp. 552–569. Available at: https://doi.org/10.1016/j.molmed.2020.01.016.

Dupont, S. *et al.* (2011) 'Role of YAP/TAZ in mechanotransduction', *Nature*, 474(7350), pp. 179–184. Available at: https://doi.org/10.1038/nature10137.

Duval, K. *et al.* (2017) 'Modeling physiological events in 2D vs. 3D cell culture', *Physiology*. American Physiological Society, pp. 266–277. Available at: https://doi.org/10.1152/physiol.00036.2016.

Eaves, C.J. (2015) 'Hematopoietic stem cells: Concepts, definitions, and the new reality', *Blood*, 125(17), pp. 2605–2613. Available at: https://doi.org/10.1182/blood-2014-12-570200.

Engert, A. *et al.* (2016) 'The european hematology association roadmap for european hematology research: A consensus document', *Haematologica*, 101(2), pp. 115–208. Available at: https://doi.org/10.3324/haematol.2015.136739.

Filippi, M.-D. and Ghaffari, S. (2019) 'Mitochondria in the maintenance of hematopoietic stem cells: new perspectives and opportunities', *Blood*, 133(18), pp. 1943–1952. Available at: https://doi.org/10.1182/blood-2018-10-808873.

Fitter, S. *et al.* (2017) 'The Mesenchymal Precursor Cell Marker Antibody STRO-1 Binds to Cell Surface Heat Shock Cognate 70', *Stem Cells*, 35(4), pp. 940–951. Available at: https://doi.org/10.1002/stem.2560.

Flores-Figueroa, E. *et al.* (2012) 'Distinctive contact between CD34+ hematopoietic progenitors and CXCL12+ CD271+ mesenchymal stromal cells in benign and myelodysplastic bone marrow.', *Laboratory investigation; a journal of technical methods and pathology*, 92(9), pp. 1330–41. Available at: https://doi.org/10.1038/labinvest.2012.93.

Franke, K. *et al.* (2007) 'Engineered matrix coatings to modulate the adhesion of CD133+ human hematopoietic progenitor cells', *Biomaterials*, 28(5), pp. 836–843. Available at: https://doi.org/10.1016/J.BIOMATERIALS.2006.09.031.

Fujisaki, J. *et al.* (2011) 'In vivo imaging of T reg cells providing immune privilege to the haematopoietic stem-cell niche', *Nature*, 474(7350), pp. 216–220. Available at: https://doi.org/10.1038/nature10160.

Grigoryan, A. *et al.* (2022) 'Engineering human mini-bones for the standardized modeling of healthy hematopoiesis, leukemia, and solid tumor metastasis', *Science Translational Medicine*, 14(666), p. 6391. Available at: https://doi.org/10.1126/scitranslmed.abm6391.

'Grinnell and Swarth on the Birds and Mammals of the San Jacinto Area of Southern California' (1914) *The Auk*, 31(1), pp. 110–112. Available at: https://doi.org/10.2307/4071861.

Gronthos, S. *et al.* (2003) 'Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow', *Journal of Cell Science*, 116(9), pp. 1827–1835. Available at: https://doi.org/10.1242/jcs.00369.

Haas, S., Trumpp, A. and Milsom, M.D. (2018) 'Causes and Consequences of Hematopoietic Stem Cell Heterogeneity', *Cell Stem Cell*, 22(5), pp. 627–638. Available at: https://doi.org/10.1016/j.stem.2018.04.003.

Hayakawa, K. *et al.* (2016) 'Transfer of mitochondria from astrocytes to neurons after stroke', *Nature*, 535(7613), pp. 551–555. Available at: https://doi.org/10.1038/nature18928.

Herndler-Brandstetter, D. *et al.* (2017) 'Humanized mouse model supports development, function, and tissue residency of human natural killer cells', *Proceedings of the National Academy of Sciences of the United States of America*, 114(45), pp. E9626–E9634. Available at: https://doi.org/10.1073/pnas.1705301114.

Hirakawa, H. *et al.* (2023) 'Cellular plasticity of the bone marrow niche promotes hematopoietic stem cell regeneration', *Nature Genetics* [Preprint]. Available at: https://doi.org/10.1038/s41588-023-01528-2.

Ho, Y.H. and Méndez-Ferrer, S. (2020) 'Microenvironmental contributions to hematopoietic stem cell aging', *Haematologica*. Ferrata Storti Foundation, pp. 38–46. Available at: https://doi.org/10.3324/haematol.2018.211334.

Hofer, M. and Lutolf, M.P. (2021) 'Engineering organoids', *Nature Reviews Materials*, pp. 402–420. Available at: https://doi.org/10.1038/s41578-021-00279-y.

Hsiong, S.X. *et al.* (2008) 'Differentiation stage alters matrix control of stem cells', *Journal of Biomedical Materials Research - Part A*, 85(1), pp. 145–156. Available at: https://doi.org/10.1002/jbm.a.31521.

Ingber, D.E. (2022) 'Human organs-on-chips for disease modelling, drug development and personalized medicine', *Nature Reviews Genetics 2022 23:8*, 23(8), pp. 467–491. Available at: https://doi.org/10.1038/s41576-022-00466-9.

Isern, J. *et al.* (2013) 'Self-Renewing Human Bone Marrow Mesenspheres Promote Hematopoietic Stem Cell Expansion', *Cell Reports*, 3(5), pp. 1714–1724. Available at: https://doi.org/10.1016/j.celrep.2013.03.041.

Islam, M.N. *et al.* (2012) 'Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury', *Nature Medicine*, 18(5), pp. 759–765. Available at: https://doi.org/10.1038/nm.2736.

Ito, R. *et al.* (2012) 'Current advances in humanized mouse models', *Cellular and Molecular Immunology*. Nature Publishing Group, pp. 208–214. Available at: https://doi.org/10.1038/cmi.2012.2.

Jacobsen, S.E.W. *et al.* (1995) 'The FLT3 ligand potently and directly stimulates the growth and expansion of primitive murine bone marrow progenitor cells in vitro: Synergistic interactions with interleukin (IL) 11, IL-12, and other hematopoietic growth factors', *Journal of Experimental Medicine*, 181(4), pp. 1357–1363. Available at: https://doi.org/10.1084/jem.181.4.1357.

Janvier, A.J., Canty-Laird, E. and Henstock, J.R. (2020) 'A universal multi-platform 3D printed bioreactor chamber for tendon tissue engineering', *Journal of Tissue Engineering*, 11. Available at: https://doi.org/10.1177/2041731420942462.

Jensen, C. and Teng, Y. (2020) 'Is It Time to Start Transitioning From 2D to 3D Cell Culture?', *Frontiers in Molecular Biosciences*. Frontiers Media S.A., p. 33. Available at: https://doi.org/10.3389/fmolb.2020.00033.

Jing, D. *et al.* (2010) 'Hematopoietic stem cells in co-culture with mesenchymal stromal cells -modeling the niche compartments in vitro', *Haematologica*, 95(4), pp. 542–550. Available at: https://doi.org/10.3324/haematol.2009.010736.

Kamel-Reid, S. and Dick, J.E. (1988) 'Engraftment of immune-deficient mice with human hematopoietic stem cells', *Science*, 242(4886), pp. 1706–1709. Available at: https://doi.org/10.1126/science.2904703.

Katayama, Y. *et al.* (2006) 'Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow', *Cell*, 124(2), pp. 407–421. Available at: https://doi.org/10.1016/j.cell.2005.10.041.

Kaur, S. *et al.* (2017) 'Role of bone marrow macrophages in controlling homeostasis and repair in bone and bone marrow niches', *Seminars in Cell and Developmental Biology*. Academic Press, pp. 12–21. Available at: https://doi.org/10.1016/j.semcdb.2016.08.009.

Kaushansky, K. (2006) 'Lineage-Specific Hematopoietic Growth Factors', *New England Journal of Medicine*, 354(19), pp. 2034–2045. Available at: https://doi.org/10.1056/nejmra052706.

Kawano, Y. *et al.* (2017) 'G-CSF-induced sympathetic tone provokes fever and primes antimobilizing functions of neutrophils via PGE2', *Blood*, 129(5), pp. 587–597. Available at: https://doi.org/10.1182/blood-2016-07-725754.

Khan, A.O. *et al.* (2023) 'Human Bone Marrow Organoids for Disease Modeling, Discovery, and Validation of Therapeutic Targets in Hematologic Malignancies', *Cancer Discovery*, 13(2), pp. 364–385. Available at: https://doi.org/10.1158/2159-8290.CD-22-0199.

Kiel, M.J. *et al.* (2005) 'SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells', *Cell*, 121(7), pp. 1109–1121. Available at: https://doi.org/10.1016/j.cell.2005.05.026.

Kim, J., Koo, B.K. and Knoblich, J.A. (2020) 'Human organoids: model systems for human biology and medicine', *Nature Reviews Molecular Cell Biology 2020 21:10*, 21(10), pp. 571–584. Available at: https://doi.org/10.1038/s41580-020-0259-3.

Klein, G. *et al.* (1995) 'Perlecan in human bone marrow: A growth-factor-presenting, but anti-adhesive, extracellular matrix component for hematopoietic cells', *Matrix Biology*, 14(6), pp. 457–465. Available at: https://doi.org/10.1016/0945-053X(95)90003-9.

Kopp, H.G. *et al.* (2005) ‘The bone marrow vascular niche: Home of HSC differentiation and mobilization’, *Physiology*. American Physiological Society, pp. 349–356. Available at: https://doi.org/10.1152/physiol.00025.2005.

Kopp, H.G. *et al.* (2009) ‘Functional heterogeneity of the bone marrow vascular niche’, in *Annals of the New York Academy of Sciences*. John Wiley & Sons, Ltd, pp. 47–54. Available at: https://doi.org/10.1111/j.1749-6632.2009.04964.x.

Lee-Thedieck, C., Schertl, P. and Klein, G. (2022) ‘The extracellular matrix of hematopoietic stem cell niches’, *Advanced Drug Delivery Reviews*. Elsevier, p. 114069. Available at: https://doi.org/10.1016/j.addr.2021.114069.

Lee, J. *et al.* (2012) ‘Implantable microenvironments to attract hematopoietic stem/cancer cells’, *Proceedings of the National Academy of Sciences of the United States of America*, 109(48), pp. 19638–19643. Available at: https://doi.org/10.1073/pnas.1208384109.

Lee, Y. *et al.* (2002) ‘Enhancement of intracellular signaling associated with hematopoietic progenitor cell survival in response to SDF-1/CXCL12 in synergy with other cytokines’, *Blood*, 99(12), pp. 4307–4317. Available at: https://doi.org/10.1182/blood.V99.12.4307.

Leisten, I. *et al.* (2012) ‘3D co-culture of hematopoietic stem and progenitor cells and mesenchymal stem cells in collagen scaffolds as a model of the hematopoietic niche’, *Biomaterials*, 33(6), pp. 1736–1747. Available at: https://doi.org/10.1016/j.biomaterials.2011.11.034.

Lempesis, I.G. *et al.* (2022) ‘Of mice and men: Considerations on adipose tissue physiology in animal models of obesity and human studies’, *Metabolism Open*, 15, p. 100208. Available at: https://doi.org/10.1016/j.metop.2022.100208.

Lengefeld, J. *et al.* (2021) ‘Cell size is a determinant of stem cell potential during aging’, *Science Advances*, 7(46), p. 271. Available at: https://doi.org/10.1126/sciadv.abk0271.

Lennartsson, J. and Rönnstrand, L. (2012) ‘Stem cell factor receptor/c-Kit: From basic Science to clinical implications’, *Physiological Reviews*, 92(4), pp. 1619–1649. Available at: https://doi.org/10.1152/physrev.00046.2011.

Li, Q., Wu, Y. and Kang, N. (2018) ‘Marrow adipose tissue: Its origin, function, and regulation in bone remodeling and regeneration’, *Stem Cells International*. Hindawi Limited. Available at: https://doi.org/10.1155/2018/7098456.

Link, H. and Prentice, G. (1993) ‘Haematopoietic growth factors’, in *Bone Marrow Transplantation*. John Wiley & Sons, Ltd, pp. 1–13. Available at: https://doi.org/10.1002/9780470015902.A0001287.PUB3.

Liu, D. *et al.* (2021) ‘Intercellular mitochondrial transfer as a means of tissue revitalization’, *Signal Transduction and Targeted Therapy*. Nature Publishing Group, pp. 1–18. Available at: https://doi.org/10.1038/s41392-020-00440-z.

Liu, Y., Beyer, A. and Aebersold, R. (2016) ‘On the Dependency of Cellular Protein Levels on mRNA Abundance’, *Cell*. Cell Press, pp. 535–550. Available at: https://doi.org/10.1016/j.cell.2016.03.014.

Long, F. (2012) ‘Building strong bones: Molecular regulation of the osteoblast lineage’, *Nature Reviews Molecular Cell Biology*. Nature Publishing Group, pp. 27–38. Available at: https://doi.org/10.1038/nrm3254.

Ludin, A. *et al.* (2012) ‘Monocytes-macrophages that express α-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow’, *Nature Immunology*, 13(11), pp. 1072–1082. Available at: https://doi.org/10.1038/ni.2408.

MacDonald, N.P. *et al.* (2016) ‘Assessment of biocompatibility of 3D printed photopolymers using zebrafish embryo toxicity assays’, *Lab on a Chip*, 16(2), pp. 291–297. Available at: https://doi.org/10.1039/c5lc01374g.

Di Maggio, N. *et al.* (2011) ‘Toward modeling the bone marrow niche using scaffold-based 3D culture systems’, *Biomaterials*, 32(2), pp. 321–329. Available at: https://doi.org/10.1016/J.BIOMATERIALS.2010.09.041.

Majumdar, M.K. *et al.* (1998) ‘Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells’, *Journal of Cellular Physiology*, 176(1), pp. 57–66. Available at: https://doi.org/10.1002/(SICI)1097-4652(199807)176:1<57::AID-JCP7>3.0.CO;2-7.

Mann, K.H. and Elton, C. (1966) ‘Animal Ecology’, *The Journal of Animal Ecology*, 35(3), p. 567. Available at: https://doi.org/10.2307/2493.

Mansell, E. *et al.* (2021) ‘Mitochondrial Potentiation Ameliorates Age-Related Heterogeneity in Hematopoietic Stem Cell Function’, *Cell Stem Cell*, 28(2), pp. 241-256.e6. Available at: https://doi.org/10.1016/j.stem.2020.09.018.

Manz, M.G. (2007) ‘Human-Hemato-Lymphoid-System Mice: Opportunities and Challenges’, *Immunity*. Cell Press, pp. 537–541. Available at: https://doi.org/10.1016/j.immuni.2007.05.001.

Mao, A.S., Shin, J.W. and Mooney, D.J. (2016) ‘Effects of substrate stiffness and cell-cell contact on mesenchymal stem cell differentiation’, *Biomaterials*, 98, pp. 184–191. Available at: https://doi.org/10.1016/j.biomaterials.2016.05.004.

Marlein, C.R. *et al.* (2019) ‘CD38-driven mitochondrial trafficking promotes bioenergetic plasticity in multiple myeloma’, *Cancer Research*, 79(9), pp. 2285–2297. Available at: https://doi.org/10.1158/0008-5472.CAN-18-0773.

Marti Gutierrez, N. *et al.* (2022) ‘Horizontal mtDNA transfer between cells is common during mouse development’, *iScience*, 25(3), p. 103901. Available at: https://doi.org/10.1016/j.isci.2022.103901.

Martin, I., Wendt, D. and Heberer, M. (2004) ‘The role of bioreactors in tissue engineering’, *Trends in Biotechnology*, 22(2), pp. 80–86. Available at: https://doi.org/10.1016/J.TIBTECH.2003.12.001.

Masamoto, Y. *et al.* (2016) ‘Adiponectin Enhances Antibacterial Activity of Hematopoietic Cells by Suppressing Bone Marrow Inflammation’, *Immunity*, 44(6), pp. 1422–1433. Available at: https://doi.org/10.1016/j.immuni.2016.05.010.

McCune, J.M. *et al.* (1988) ‘The SCID-hu mouse: Murine model for the analysis of human hematolymphoid differentiation and function’, *Science*, 241(4873), pp. 1632–1639. Available at: https://doi.org/10.1126/science.2971269.

Meads, M.B., Hazlehurst, L.A. and Dalton, W.S. (2008) 'The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance', *Clinical Cancer Research*, 14(9), pp. 2519–2526. Available at: https://doi.org/10.1158/1078-0432.CCR-07-2223.

Meissner, P. *et al.* (1999) 'Development of a fixed bed bioreactor for the expansion of human hematopoietic progenitor cells', *Cytotechnology*, 30(1–3), pp. 227–234. Available at: https://doi.org/10.1023/a:1008085932764.

Méndez-Ferrer, S. *et al.* (2008) 'Haematopoietic stem cell release is regulated by circadian oscillations', *Nature*, 452(7186), pp. 442–447. Available at: https://doi.org/10.1038/nature06685.

Méndez-Ferrer, S. *et al.* (2010) 'Mesenchymal and haematopoietic stem cells form a unique bone marrow niche', *Nature*, 466(7308), pp. 829–834. Available at: https://doi.org/10.1038/nature09262.

Mistry, J.J. *et al.* (2019) 'ROS-mediated PI3K activation drives mitochondrial transfer from stromal cells to hematopoietic stem cells in response to infection', *Proceedings of the National Academy of Sciences of the United States of America*, 116(49), pp. 24610–24619. Available at: https://doi.org/10.1073/pnas.1913278116.

Mohamed, F.F. and Franceschi, R.T. (2017) 'Skeletal Stem Cells: Origins, Functions and Uncertainties.', *Current molecular biology reports*, 3(4), pp. 236–246. Available at: https://doi.org/10.1007/s40610-017-0075-5.

Morrison, S.J. and Scadden, D.T. (2014a) 'The bone marrow niche for haematopoietic stem cells', *Nature*. NIH Public Access, pp. 327–334. Available at: https://doi.org/10.1038/nature12984.

Morrison, S.J. and Scadden, D.T. (2014b) 'The bone marrow niche for haematopoietic stem cells', *Nature*. Nature Publishing Group, pp. 327–334. Available at: https://doi.org/10.1038/nature12984.

Moschoi, R. *et al.* (2016) 'Protective mitochondrial transfer from bone marrow stromal cells to acute myeloid leukemic cells during chemotherapy', *Blood*, 128(2), pp. 253–264. Available at: https://doi.org/10.1182/blood-2015-07-655860.

Naik, S.H. *et al.* (2013) 'Diverse and heritable lineage imprinting of early haematopoietic progenitors', *Nature*, 496(7444), pp. 229–232. Available at: https://doi.org/10.1038/nature12013.

Nakamura-Ishizu, A. *et al.* (2014) 'Megakaryocytes are essential for HSC quiescence through the production of thrombopoietin', *Biochemical and Biophysical Research Communications*, 454(2), pp. 353–357. Available at: https://doi.org/10.1016/j.bbrc.2014.10.095.

Naveiras, O. *et al.* (2009) 'Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment', *Nature*, 460(7252), pp. 259–263. Available at: https://doi.org/10.1038/nature08099.

Neikirk, K. *et al.* (2023) 'MitoTracker: A useful tool in need of better alternatives', *European Journal of Cell Biology*, 102(4), p. 151371. Available at: https://doi.org/10.1016/j.ejcb.2023.151371.

Nichols, J.E. *et al.* (2009) ‘In vitro analog of human bone marrow from 3D scaffolds with biomimetic inverted colloidal crystal geometry’, *Biomaterials*, 30(6), pp. 1071–1079. Available at: https://doi.org/10.1016/j.biomaterials.2008.10.041.

Nicolini, F.E. *et al.* (2004) ‘NOD/SCID mice engineered to express human IL-3, GM-CSF and steel factor constitutively mobilize engrafted human progenitors and compromise human stem cell regeneration’, *Leukemia*, 18(2), pp. 341–347. Available at: https://doi.org/10.1038/sj.leu.2403222.

Nolan, D.J. *et al.* (2013) ‘Molecular Signatures of Tissue-Specific Microvascular Endothelial Cell Heterogeneity in Organ Maintenance and Regeneration’, *Developmental Cell*, 26(2), pp. 204–219. Available at: https://doi.org/10.1016/j.devcel.2013.06.017.

O’Connell, K.E. *et al.* (2015) ‘Practical murine hematopathology: A comparative review and implications for research’, *Comparative Medicine*, 65(2), pp. 96–113.

Olszak, I.T. *et al.* (2000) ‘Extracellular calcium elicits a chemokinetic response from monocytes in vitro and in vivo’, *Journal of Clinical Investigation*, 105(9), pp. 1299–1305. Available at: https://doi.org/10.1172/JCI9799.

Paczulla, A.M. *et al.* (2017) ‘Long-term observation reveals high-frequency engraftment of human acute myeloid leukemia in immunodeficient mice’, *Haematologica*, 102(5), pp. 854–864. Available at: https://doi.org/10.3324/haematol.2016.153528.

Papy-Garcia, D. and Albanese, P. (2017) ‘Heparan sulfate proteoglycans as key regulators of the mesenchymal niche of hematopoietic stem cells’, *Glycoconjugate Journal*, 34(3), pp. 377–391. Available at: https://doi.org/10.1007/s10719-017-9773-8.

Parekh, C. and Crooks, G.M. (2013) ‘Critical differences in hematopoiesis and lymphoid development between humans and mice’, *Journal of Clinical Immunology*, pp. 711–715. Available at: https://doi.org/10.1007/s10875-012-9844-3.

Passegué, E. *et al.* (2005) ‘Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates’, *Journal of Experimental Medicine*, 202(11), pp. 1599–1611. Available at: https://doi.org/10.1084/jem.20050967.

Pellin, D. *et al.* (2019) ‘A comprehensive single cell transcriptional landscape of human hematopoietic progenitors’, *Nature Communications*, 10(1), pp. 1–15. Available at: https://doi.org/10.1038/s41467-019-10291-0.

Pinho, S. and Frenette, P.S. (2019) ‘Haematopoietic stem cell activity and interactions with the niche’, *Nature Reviews Molecular Cell Biology*, 20(5), pp. 303–320. Available at: https://doi.org/10.1038/s41580-019-0103-9.

Polak, R. *et al.* (2015) ‘B-cell precursor acute lymphoblastic leukemia cells use tunneling nanotubes to orchestrate their microenvironment’, *Blood*, 126(21), pp. 2404–2414. Available at: https://doi.org/10.1182/blood-2015-03-634238.

Puel, A. *et al.* (1998) ‘Defective IL7R expression in T-B+NK+ severe combined immunodeficiency’, *Nature Genetics*, 20(4), pp. 394–397. Available at: https://doi.org/10.1038/3877.

Quirici, N. *et al.* (2002) ‘Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies’, *Experimental Hematology*, 30(7), pp. 783–791. Available at: https://doi.org/10.1016/S0301-472X(02)00812-3.

Rafii, S. *et al.* (1995) ‘Human bone marrow microvascular endothelial cells support long-term proliferation and differentiation of myeloid and megakaryocytic progenitors’, *Blood*, 86(9), pp. 3353–3363. Available at: https://doi.org/10.1182/blood.v86.9.3353.bloodjournal8693353.

Rebbeck, C.A., Leroi, A.M. and Burt, A. (2011) ‘Mitochondrial capture by a transmissible cancer’, *Science*. American Association for the Advancement of Science, p. 303. Available at: https://doi.org/10.1126/science.1197696.

Reinisch, A. *et al.* (2017) ‘Generation and use of a humanized bone-marrow-ossicle niche for hematopoietic xenotransplantation into mice’, *Nature Protocols*, 12(10), pp. 2169–2178. Available at: https://doi.org/10.1038/nprot.2017.088.

Rieger, M.A. and Schroeder, T. (2012) ‘Hematopoiesis’, *Cold Spring Harbor Perspectives in Biology*, 4(12), p. a008250. Available at: https://doi.org/10.1101/cshperspect.a008250.

Ringe, D. and Taylor, A. (2014) *The Development Of Old English*. Oxford University Press.

Rubinstein, P. *et al.* (1998) ‘Outcomes among 562 recipients of placental-blood transplants from unrelated donors’, *New England Journal of Medicine*, 339(22), p. 1565.

Ryan, D.H. (2016) ‘Examination of Blood Cells’, in M.H. Professional (ed.) *Williams Hematology*. 9th edn, p. 15.

Sacchetti, B. *et al.* (2007) ‘Self-Renewing Osteoprogenitors in Bone Marrow Sinusoids Can Organize a Hematopoietic Microenvironment’, *Cell*, 131(2), pp. 324–336. Available at: https://doi.org/10.1016/j.cell.2007.08.025.

Sacchetti, B. *et al.* (2016) ‘No identical “mesenchymal stem cells” at different times and sites: Human committed progenitors of distinct origin and differentiation potential are incorporated as adventitial cells in microvessels’, *Stem Cell Reports*, 6(6), pp. 897–913. Available at: https://doi.org/10.1016/j.stemcr.2016.05.011.

Saito, K. *et al.* (2021) ‘Exogenous mitochondrial transfer and endogenous mitochondrial fission facilitate AML resistance to OxPhos inhibition’, *Blood Advances*, 5(20), pp. 4233–4255. Available at: https://doi.org/10.1182/bloodadvances.2020003661.

Schmelzer, E. *et al.* (2016) ‘Response of Primary Human Bone Marrow Mesenchymal Stromal Cells and Dermal Keratinocytes to Thermal Printer Materials In Vitro’, *Journal of Medical and Biological Engineering*, 36(2), pp. 153–167. Available at: https://doi.org/10.1007/s40846-016-0118-z.

Schmid, J. *et al.* (2018) ‘A perfusion bioreactor system for cell seeding and oxygen-controlled cultivation of three-dimensional cell cultures’, *Tissue Engineering - Part C: Methods*, 24(10), pp. 585–595. Available at: https://doi.org/10.1089/ten.tec.2018.0204.

Schofield, R. (1978) ‘The relationship between the spleen colony-forming cell and the haemopoietic stem cell. A hypothesis’, *Blood Cells*, 4(1–2), pp. 7–25. Available at: https://www.scinapse.io/papers/1861988602 (Accessed: 6 August 2023).

Scotti, C. *et al.* (2013) ‘Engineering of a functional bone organ through endochondral ossification’, *Proceedings of the National Academy of Sciences of the United States of America*, 110(10), pp. 3997–4002. Available at: https://doi.org/10.1073/pnas.1220108110.

Seita, J. and Weissman, I.L. (2010) ‘Hematopoietic stem cell: Self-renewal versus differentiation’, *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 2(6), pp. 640–653. Available at: https://doi.org/10.1002/wsbm.86.

Sender, R. and Milo, R. (2021) ‘The distribution of cellular turnover in the human body’, *Nature Medicine*, 27(1), pp. 45–48. Available at: https://doi.org/10.1038/s41591-020-01182-9.

Singh, A.K. and Cancelas, J.A. (2021) ‘Mitochondria Transfer in Bone Marrow Hematopoietic Activity’, *Current Stem Cell Reports*. Springer Science and Business Media Deutschland GmbH, pp. 1–12. Available at: https://doi.org/10.1007/s40778-020-00185-z.

Sintes, J. *et al.* (2008) ‘Differential expression of CD150 (SLAM) family receptors by human hematopoietic stem and progenitor cells’, *Experimental Hematology*, 36(9), pp. 1199–1204. Available at: https://doi.org/10.1016/j.exphem.2008.03.015.

Sitnicka, E. *et al.* (2003) ‘Human CD34+ hematopoietic stem cells capable of multilineage engrafting NOD/SCID mice express flt3: Distinct flt3 and c-kit expression and response patterns on mouse and candidate human hematopoietic stem cells’, *Blood*, 102(3), pp. 881–886. Available at: https://doi.org/10.1182/blood-2002-06-1694.

De Smedt, M., Hoebeke, I. and Plum, J. (2004) ‘Human bone marrow CD34 + progenitor cells mature to T cells on OP9-DL1 stromal cell line without thymus microenvironment’, *Blood Cells, Molecules, and Diseases*, 33(3), pp. 227–232. Available at: https://doi.org/10.1016/j.bcmd.2004.08.007.

Soukas, A. *et al.* (2001) ‘Distinct Transcriptional Profiles of Adipogenesis in Vivo and in Vitro’, *Journal of Biological Chemistry*, 276(36), pp. 34167–34174. Available at: https://doi.org/10.1074/jbc.M104421200.

Spees, J.L. *et al.* (2006) ‘Mitochondrial transfer between cells can rescue aerobic respiration’, *Proceedings of the National Academy of Sciences of the United States of America*, 103(5), pp. 1283–1288. Available at: https://doi.org/10.1073/pnas.0510511103.

Stockmann, P. *et al.* (2012) ‘Guided bone regeneration in pig calvarial bone defects using autologous mesenchymal stem/progenitor cells - A comparison of different tissue sources’, *Journal of Cranio-Maxillofacial Surgery*, 40(4), pp. 310–320. Available at: https://doi.org/10.1016/j.jcms.2011.05.004.

Stripecke, R. *et al.* (2020) ‘Innovations, challenges, and minimal information for standardization of humanized mice’, *EMBO Molecular Medicine*, 12(7). Available at: https://doi.org/10.15252/emmm.201708662.

Suchacki, K.J. *et al.* (2020) ‘Bone marrow adipose tissue is a unique adipose subtype with distinct roles in glucose homeostasis’, *Nature Communications*, 11(1), pp. 1–18. Available at: https://doi.org/10.1038/s41467-020-16878-2.

Sugiyama, T. *et al.* (2006) ‘Maintenance of the Hematopoietic Stem Cell Pool by CXCL12-CXCR4 Chemokine Signaling in Bone Marrow Stromal Cell Niches’, *Immunity*, 25(6), pp. 977–988. Available at: https://doi.org/10.1016/j.immuni.2006.10.016.

Sykes, S.M. and Scadden, D.T. (2013) ‘Modeling human hematopoietic stem cell biology in the mouse’, *Seminars in Hematology*, 50(2), pp. 92–100. Available at: https://doi.org/10.1053/j.seminhematol.2013.03.029.

Taichman, R.S. (2005) ‘Blood and bone: Two tissues whose fates are intertwined to create the hematopoietic stem-cell niche’, *Blood*, 105(7), pp. 2631–2639. Available at: https://doi.org/10.1182/blood-2004-06-2480.

Taichman, R.S. and Emerson, S.G. (1994) ‘Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor’, *Journal of Experimental Medicine*, 179(5), pp. 1677–1682. Available at: https://doi.org/10.1084/jem.179.5.1677.

Taichman, R.S., Reilly, M.J. and Emerson, S.G. (1996) ‘Human osteoblasts support human hematopoietic progenitor cells in in vitro bone marrow cultures’, *Blood*, 87(2), pp. 518–524. Available at: https://doi.org/10.1182/blood.v87.2.518.bloodjournal872518.

Takihara, Y.J. *et al.* (2019) ‘High mitochondrial mass is associated with reconstitution capacity and quiescence of hematopoietic stem cells’, *Blood Advances*, 3(15), pp. 2323–2327. Available at: https://doi.org/10.1182/bloodadvances.2019032169.

Till, J.E. and McCulloch, E.A. (1961) ‘A direct measurement of the radiation sensitivity of normal mouse bone marrow cells’, *Radiation research*, 14, pp. 213–222. Available at: https://doi.org/10.2307/3570892.

Tormin, A. *et al.* (2011) ‘CD146 expression on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization’, *Blood*, 117(19), pp. 5067–5077. Available at: https://doi.org/10.1182/blood-2010-08-304287.

Touw, I. and Lowenberg, B. (1983) ‘No stimulative effect of adipocytes on hematopoiesis in long-term human bone marrow cultures’, *Blood*, 61(4), pp. 770–774. Available at: https://doi.org/10.1182/blood.v61.4.770.770.

Tsapogas, P. *et al.* (2017) ‘The Cytokine Flt3-Ligand in Normal and Malignant Hematopoiesis’, *International Journal of Molecular Sciences 2017, Vol. 18, Page 1115*, 18(6), p. 1115. Available at: https://doi.org/10.3390/IJMS18061115.

Ueda, T. *et al.* (2001) ‘Hematopoietic Capability of CD34 + Cord Blood Cells : A Comparison With CD34 + Adult Bone Marrow Cells’, *International Journal of Hematology*, 73, pp. 457–462.

Vallabhaneni, K.C., Haller, H. and Dumler, I. (2012) ‘Vascular smooth muscle cells initiate proliferation of mesenchymal stem cells by mitochondrial transfer via tunneling nanotubes’, *Stem Cells and Development*, 21(17), pp. 3104–3113. Available at: https://doi.org/10.1089/scd.2011.0691.

Velten, L. *et al.* (2017) ‘Human haematopoietic stem cell lineage commitment is a continuous process’, *Nature Cell Biology*, 19(4), pp. 271–281. Available at: https://doi.org/10.1038/ncb3493.

Visnjic, D. *et al.* (2004) ‘Hematopoiesis is severely altered in mice with an induced osteoblast deficiency’, *Blood*, 103(9), pp. 3258–3264. Available at: https://doi.org/10.1182/BLOOD-2003-11-4011.

Viswanathan, S. *et al.* (2019) ‘Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature’, *Cytotherapy*, 21(10), pp. 1019–1024. Available at: https://doi.org/10.1016/j.jcyt.2019.08.002.

Vodyanik, M.A. *et al.* (2005) ‘Human embryonic stem cell-derived CD34+ cells: Efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential’, *Blood*, 105(2), pp. 617–626. Available at: https://doi.org/10.1182/blood-2004-04-1649.

Walenda, T. *et al.* (2010) ‘Co-culture with mesenchymal stromal cells increases proliferation and maintenance of haematopoietic progenitor cells’, *Journal of Cellular and Molecular Medicine*, 14(1–2), pp. 337–350. Available at: https://doi.org/10.1111/j.1582-4934.2009.00776.x.

Wang, J. *et al.* (2018) ‘Cell adhesion-mediated mitochondria transfer contributes to mesenchymal stem cell-induced chemoresistance on T cell acute lymphoblastic leukemia cells’, *Journal of Hematology and Oncology*, 11(1), pp. 1–13. Available at: https://doi.org/10.1186/s13045-018-0554-z.

Wang, T. -Y and Wu, J.H.D. (1992) ‘A Continuous Perfusion Bioreactor for Long-Term Bone Marrow Culture’, *Annals of the New York Academy of Sciences*, 665(1), pp. 274–284. Available at: https://doi.org/10.1111/j.1749-6632.1992.tb42591.x.

Wei, Q. and Frenette, P.S. (2018) ‘Niches for Hematopoietic Stem Cells and Their Progeny’, *Immunity*. Cell Press, pp. 632–648. Available at: https://doi.org/10.1016/j.immuni.2018.03.024.

Wendt, D. *et al.* (2003) ‘Oscillating perfusion of cell suspensions through three-dimensional scaffolds enhances cell seeding efficiency and uniformity’, *Biotechnology and Bioengineering*, 84(2), pp. 205–214. Available at: https://doi.org/10.1002/BIT.10759.

Wilkinson, A.C., Igarashi, K.J. and Nakauchi, H. (2020) ‘Haematopoietic stem cell self-renewal in vivo and ex vivo’, *Nature Reviews Genetics* [Preprint]. Available at: https://doi.org/10.1038/s41576-020-0241-0.

Wilson, C.A. *et al.* (1997) ‘Relationship of the white blood cell count to body fat: Role of leptin’, *British Journal of Haematology*, 99(2), pp. 447–451. Available at: https://doi.org/10.1046/j.1365-2141.1997.3873201.x.

Yamamoto, R. *et al.* (2013) ‘Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells’, *Cell*, 154(5), pp. 1112–1126. Available at: https://doi.org/10.1016/j.cell.2013.08.007.

Yoon, E. *et al.* (2007) ‘In vivo osteogenic potential of human adipose-derived stem cells/poly lactide-co-glycolic acid constructs for bone regeneration in a rat critical-sized calvarial defect model’, *Tissue Engineering*, 13(3), pp. 619–627. Available at: https://doi.org/10.1089/ten.2006.0102.

Zandstra, P.W., Lauffenburger, D.A. and Eaves, C.J. (2000) ‘A ligand-receptor signaling threshold model of stem cell differentiation control: A biologically conserved mechanism applicable to hematopoiesis’, *Blood*. W.B. Saunders, pp. 1215–1222. Available at: https://doi.org/10.1182/blood.v96.4.1215.h8001215_1215_1222.

Zanetti, C. and Krause, D.S. (2020) '"Caught in the net": the extracellular matrix of the bone marrow in normal hematopoiesis and leukemia', *Experimental Hematology*. Elsevier, pp. 13–25. Available at: https://doi.org/10.1016/j.exphem.2020.07.010.

Zhou, X. *et al.* (2014) 'Chondrocytes Transdifferentiate into Osteoblasts in Endochondral Bone during Development, Postnatal Growth and Fracture Healing in Mice', *PLoS Genetics*. Edited by M.L. Warman, 10(12), p. e1004820. Available at: https://doi.org/10.1371/journal.pgen.1004820.

Zhu, F. *et al.* (2015) '3D printed polymers toxicity profiling: a caution for biodevice applications', in *Micro+Nano Materials, Devices, and Systems*. SPIE, p. 96680Z. Available at: https://doi.org/10.1117/12.2202392.

Zhu, J. *et al.* (2007) 'Osteoblasts support B-lymphocyte commitment and differentiation from hematopoietic stem cells', *Blood*, 109(9), pp. 3706–3712. Available at: https://doi.org/10.1182/blood-2006-08-041384.

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STEVEN J. DUPARD was born in 1992 in L'Aigle, Normandie, France. From a young age, his fascination for biology led him to obtain his bachelor's degree specialized in applied health biotechnological systems at the Institut de Biologie Fondamentale et Appliquée at l'Université de Caen Basse-Normandie. Steven then obtained a trinational double master's degrees in Biotechnology Engineering and High Throughput Analysis from l' École Supérieure de Biotechnologie de Strasbourg (France), Albert-Ludwigs-Universität Freiburg (Germany) and Universität Basel (Switzerland). After performing his master thesis at the Institute of Molecular Biotechnology in Vienna (Austria), and New England Biolabs in Massachusetts (USA), Steven decided to pursue a career in bioengineering. He started his PhD studies at Lund University in Sweden in 2019. Here he worked within the Lund University Stem Cell Center in developing new models for the study of human bone marrow hematopoietic niches.

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