Building bones for blood and beyond: the growing field of bone marrow niche model development

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Highlights

- Bone marrow (BM) models are becoming increasingly biomimetic.
- These models help investigate the impact of BM elements on haematopoiesis.
- Future models could enhance therapies for BM-associated disorders and diseases.

Building bones for blood and beyond: the growing field of bone marrow niche model development

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Abstract

The bone marrow (BM) niche is a complex microenvironment that provides the signals required for regulation of hematopoietic stem cells (HSCs) and the process of haematopoiesis they are responsible for. Bioengineered models of the BM niche incorporate various elements of the *in vivo* BM microenvironment, including cellular components, soluble factors, a 3D environment, mechanical stimulation of included cells, and perfusion. Recent advances in the bioengineering field have resulted in a spate of new models that shed light on BM function and are approaching precise imitation of the BM niche. These models promise to improve our understanding of the *in vivo* microenvironment in health and disease. They also aim to serve as platforms for HSC manipulation, or as preclinical models for screening novel therapies for BM-associated disorders and diseases.

Highlights

- Incorporating various elements of the BM niche has resulted in increasingly biomimetic BM models.
- The influence of several properties of the *in vivo* BM niche have been clarified by these models.

- Future BM models have the potential to expand HSCs *in vitro* for curative transplants to treat BM-associated malignancies, and aid development of novel treatments for BM-associated disorders and diseases.

1. Introduction

Models of the bone marrow (BM) niche promise to revolutionize our understanding of both physiological and pathological haematology and immunology, while also facilitating therapeutic advancements targeting BM-associated disorders and diseases. One of the primary goals of these niches is to manipulate haematopoietic stem cells (HSCs), a rare population of cells (~0.01-0.04% of total BM mononuclear cells (Zon, 2008)) which reside within the niche and are responsible for the process of haematopoiesis, the continuous production of blood and immune cells throughout an organism's life (Seita and Weissman, 2010). HSCs exist in a hierarchy at the top of which sits naïve, long-term HSCs (LT-HSCs), a self-renewing, multipotent subpopulation of HSCs that lasts for an individual's entire lifespan (Wilson *et al.*, 2008; Fares, Calvanese and Mikkola, 2022) and is responsible for the production of more proliferative progenitors (Cheshier *et al.*, 1999; Yamamoto *et al.*, 2013). LT-HSCs maintain long-term engraftment capacity (Morrison and Weissman, 1994; Dykstra *et al.*, 2007; Till and McCulloch, 2011), and are critically important for HSC transplant (HSCT), a curative treatment for BM malignancies and disorders (Schmitz *et al.*, 2002; Locatelli, 2005; Hulbert and Shenoy, 2018; Willasch *et al.*, 2020). Occasionally LT-HSCs will produce short term HSCs (ST-HSCs) via asymmetrical cell division (Busch *et al.*, 2015). ST-HSCs are more metabolically and mitotically active than LT-HSCs, and possess reduced repopulation capacity (Wilson *et al.*, 2008). ST-HSCs differentiate into multipotent progenitors (MPPs), which in turn produce lineage restricted progeny via an oligopotent cell step (Zon, 2008; Pietras *et al.*, 2016).

The BM niche itself is a highly complex organ. It maintains the HSC pool and haematopoietic homeostasis using a variety of stimuli. The niche is found within the lumen of long and axial bones (Morrison and Scadden, 2014), and is permeated by a network of tiny blood vessels (Nilsson *et al.*, 1998; Kunisaki *et al.*, 2013; Acar *et al.*, 2015). Advances in *in vivo* imaging techniques have demonstrated that around this vasculature there exists two BM "sub-niches", named the sinusoidal and arteriolar niches after the blood vessels they are located

around. Sinusoids carry deoxygenated blood and are distributed throughout the softer central BM cavity (Nilsson et al., 1998) while arterioles carry oxygenated blood and are primarily found close to the stiff endosteum's surface (Pinho and Frenette, 2019). Despite conflicting evidence regarding the exact position of HSCs within the BM (Kunisaki *et al.*, 2013; Acar *et al.*, 2015), these sub-niches are strongly believed to influence the HSCs that reside within them, with the arteriolar niche enriched for LT-HSCs, even though a larger total HSC population is found in the sinusoidal niche (Pinho and Frenette, 2019). Various non-haematopoietic cell types also reside within the BM niche and have been demonstrated to influence HSCs' function and maintenance (see **Table 1**). Another important component of the BM niche is the extracellular matrix (ECM) (Lee-Thedieck, Schertl and Klein, 2022) which interacts with cells via non-uniform distribution of ECM proteins such as collagen, fibronectin and laminin (Coutu *et al.*, 2017), as well as variable mechanical properties within a characteristically low stiffness range (Jansen *et al.*, 2015; Chaudhuri *et al.*, 2020; Chen *et al.*, 2020). In addition, oxygen saturation plays a key role in the niche's interaction with HSCs (Spencer *et al.*, 2014; Ross *et al.*, 2019), as does blood flow (North *et al.*, 2009; Winkler *et al.*, 2010; Bixel *et al.*, 2017). Non-cellular elements of the BM niche, their role within the niche, and how they have been utilised in model BM niches are summarised in **Table 2**.

Other extramedullary sites of haematopoiesis exist over the course of development (Mikkola and Orkin, 2006; Crane, Jeffery and Morrison, 2017) and have been observed in response to acute haematopoietic stress (Inra *et al.*, 2015; Crane, Jeffery and Morrison, 2017). Although these niches are spatially distinct from the BM niche, many similarities are observed; they are highly vascularised and populated by stromal and endothelial cells with similar protein expression profiles to those observed in the BM niche (Gekas *et al.*, 2005; Inra *et al.*, 2015; Khan *et al.*, 2016). While this review will focus on BM niche models, research on these alternative haematopoietic sites could further elucidate the precise mechanisms of haematopoiesis and provide new avenues for its manipulation (Michaels *et al.*, 2023).

BM niche models emulate the *in vitro* BM niche. The current gold standard for BM models are animal models (Haas et al., 2018; P. Zhang et al., 2022). However, a lack of comparability

with human biology, complexity obscuring specific mechanisms of the niche, and a lack of reproducibility and availability (Ingber, 2020; Lee-Thedieck et al., 2022) hamper the success of animal models. Bioengineered *in vitro* BM models that replicate aspects of the BM niche are now emerging as a viable alternative. Early *in vitro* BM niche models aimed to expand HSCs for HSCT (reviewed: Chatterjee *et al.*, 2021; Fares, Calvanese and Mikkola, 2022). However, this ambition was met with mixed results, primarily due to expanded HSCs' heterogeneity, with the cells produced mostly being ST-HSCs, MPPs, and lineage-restricted progenitors, leading to reduced long-term engraftment capacity (Wagner *et al.*, 2016). A few recent systems successfully demonstrated the plausibility of HSC expansion without the associated loss of engraftment capacity, yet these systems lack integration of the physical and functional properties of the bone marrow niche, instead relying on small molecules or hydrogels (Meaker and Wilkinson, 2024). Over the past decade, *in vitro* niche models have shifted their objective away from HSC expansion and towards accurately replicating aspects of the BM niche to shed light on the haematopoietic process, harnessing it for clinical applications, and understanding how it goes wrong in haematological diseases and disorders (Méndez-Ferrer *et al.*, 2020). Foundational research in simple *in vitro* and animal models, combined with recent advances in the tissue engineering and haematology fields, has galvanised *in vitro* BM niche models' development. This has resulted in a wealth of new models, the current state of which will be discussed (see **Figure 1a**), as well as potential applications and the future of the field (see **Figure 1b**).

Table 1. Bone marrow niche cellular components and their role within the BM niche.

BM niche cell type	**Role of cell type**	**References**
HSCs	HSCs are responsible for haematopoiesis. They produce haematopoietic cells that regulate that form the blood and immune systems.	(Morrison and Weissman, 1994; Cheshier *et al.*, 1999; Dykstra *et al.*, 2007; Wilson *et al.*, 2008; Zon, 2008; Seita and Weissman, 2010; Till and McCulloch, 2011; Yamamoto *et al.*, 2013; Morrison and Scadden, 2014; Fares, Calvanese and Mikkola, 2022)
MSCs	MSCs reside near HSCs in the BM niche. Nestin+ MSCs are particularly	(Nichols *et al.*, 2009; Méndez-Ferrer *et al.*, 2010; De Barros *et al.*, 2010; Tiwari *et al.*, 2012; Lai *et al.*, 2013; Lima *et al.*, 2013;

	important, as they produce many of the paracrine signals associated with HSC maintenance.	Rödling *et al.*, 2017; Bray *et al.*, 2017; Futrega *et al.*, 2017; Bourgine *et al.*, 2018; Aleman *et al.*, 2019; Bianco *et al.*, 2019; Ma *et al.*, 2020; David B Chou *et al.*, 2020; Goranov *et al.*, 2020; Nelson *et al.*, 2021a; Donnelly *et al.*, 2022; Glaser *et al.*, 2022)
Osteoblasts	Osteoblasts have been hypothesised to regulate haematopoiesis through paracrine and juxtracrine signalling. However, recent studies demonstrated that HSCs do not spatially associate with osteoblasts, and that osteolineage cells don't significantly contribute to the signalling milieu that influences HSC maintenance.	(Calvi *et al.*, 2003; Nichols *et al.*, 2009; De Barros *et al.*, 2010; Bromberg *et al.*, 2012; Greenbaum *et al.*, 2013; Lai *et al.*, 2013; Bowers *et al.*, 2015; Ma *et al.*, 2020; Nelson *et al.*, 2021a)
Osteoclasts	Osteoclasts influence MSCs, allowing them to attract HSCs for colonisation of the nascent BM niche during development. They also remodel the perinatal niche and aid with vascularisation.	(Mansour *et al.*, 2012; Zeytin *et al.*, 2022)
Macrophages	Macrophages produce several soluble factors	(Hur *et al.*, 2016; Li *et al.*, 2018)

	and present surface antigens that influence haematopoiesis in a paracrine and juxtracrine manner, respectively.	
Endothelial cells	HSCs colocalise with vasculature in the *in vivo* BM niche. There is a possibility that these cells influence HSCs.	(Kiel *et al.*, 2005; De Barros *et al.*, 2010; Ding *et al.*, 2012; Perlin, Sporrij and Zon, 2017; Bray *et al.*, 2017; Aleman *et al.*, 2019; Braham *et al.*, 2019; David B Chou *et al.*, 2020; Goranov *et al.*, 2020; Ma *et al.*, 2020; Nelson *et al.*, 2021b; Heil *et al.*, 2021)
Megakaryocytes	Megakaryocytes are derived from HSC progenitors, produce platelets, regulate HSC fate and interact with many cells within the BM niche, contributing to its regulation.	(Stone, Nascimento and Barrachina, 2022)
Fibroblasts	Fibroblasts provide mechanical/physical support to HSCs during proliferation and differentiation by secreting and modulating the ECM.	(LeBleu and Neilson, 2020; Lee-Thedieck, Schertl and Klein, 2022)
Adipocytes	Adipocytes have potential suppressive effects on	(Naveiras *et al.*, 2009)

	haematopoiesis within the bone-marrow niche.	
Nervous cells	Sympathetic nerves have been implicated in HSC mobilisation, maintenance and recovery following genotoxic insult.	(Rameshwar and Gascon, 1995; Broome and Miyan, 2000; Katayama *et al.*, 2006; Lucas *et al.*, 2013; Maryanovich *et al.*, 2018; Xu *et al.*, 2018)

Table 2. BM niche components and their impact when included in niche models

Model BM niche element	**Impact of the element**	**References**
BM niche cells	Inclusion of BM resident cells simulates the complex cellular composition of the BM niche, with included cells interacting with HSCs via juxtracrine and/or paracrine signalling.	(Pinho and Frenette, 2019; Xiao, McGuinness, *et al.*, 2022)
Soluble factors/cytokines	The use of soluble factors has been shown to improve HSC proliferation, however often the expanded cells are in the ST-HSC or progenitor compartment and lack long-term engraftment capacity	(Boitano *et al.*, 2010; Lai *et al.*, 2013; Bray *et al.*, 2017; Wilkinson *et al.*, 2019; Sánchez-Lanzas, Kalampalika and Ganuza, 2022)
Extracellular matrix (ECM)	ECM components such as fibronectin, collagens,	(Salmerón-Sánchez and Dalby, 2016; Donnelly *et al.*, 2022; Xiao, Donnelly, *et al.*,

components	vitronectin and laminin have been shown to affect MSCs' and HSCs' phenotypes by sequestering and presenting signalling molecules and growth factors, as well as interacting with the cells mechanically.	2022)
Hydrogel	BM is a low-stiffness natural hydrogel. The use of hydrogels allows the 3D nature of the *in vivo* BM niche to be replicated. Gels can also provide physical, mechanical, and chemical stimuli to cells in a BM niche model.	(Leisten *et al.*, 2012; Bray *et al.*, 2017; Rödling *et al.*, 2017; Aleman *et al.*, 2019; Braham *et al.*, 2019; David B Chou *et al.*, 2020; Nelson *et al.*, 2021a; Donnelly *et al.*, 2022)
Non-hydrogel scaffold	Non-hydrogel scaffolds that mimic the structure of cortical bone, such as ceramaics, colloidal crystals and hydroxyapatite, possess many of the same 3D benefits as hydrogels and afford the opportunity to add other properties, such as control over cellular localisation e.g. using magnetised cells and	(Nichols *et al.*, 2009; Bourgine *et al.*, 2018; Goranov *et al.*, 2020)

	a magnetised scaffold.	
Decellularized matrix	Using decellularized animal BM matrix to culture human BM cells has met with success due to the conserved BM architecture and physical properties.	(Tiwari *et al.*, 2012; Lai *et al.*, 2013; Bianco *et al.*, 2019)
Blood flow/perfusion	Perfusion models replicate blood flow, which could impact HSCs that natively reside alongside blood vessels. HSCs may also enter the circulatory system, making this another important element of the BM niche.	(Wright *et al.*, 2001; Lapidot and Petit, 2002; Rödling *et al.*, 2017; Bourgine *et al.*, 2018; David B Chou *et al.*, 2020; Goranov *et al.*, 2020; Ma *et al.*, 2020; Nelson *et al.*, 2021a; Patra, 2021; Glaser *et al.*, 2022)

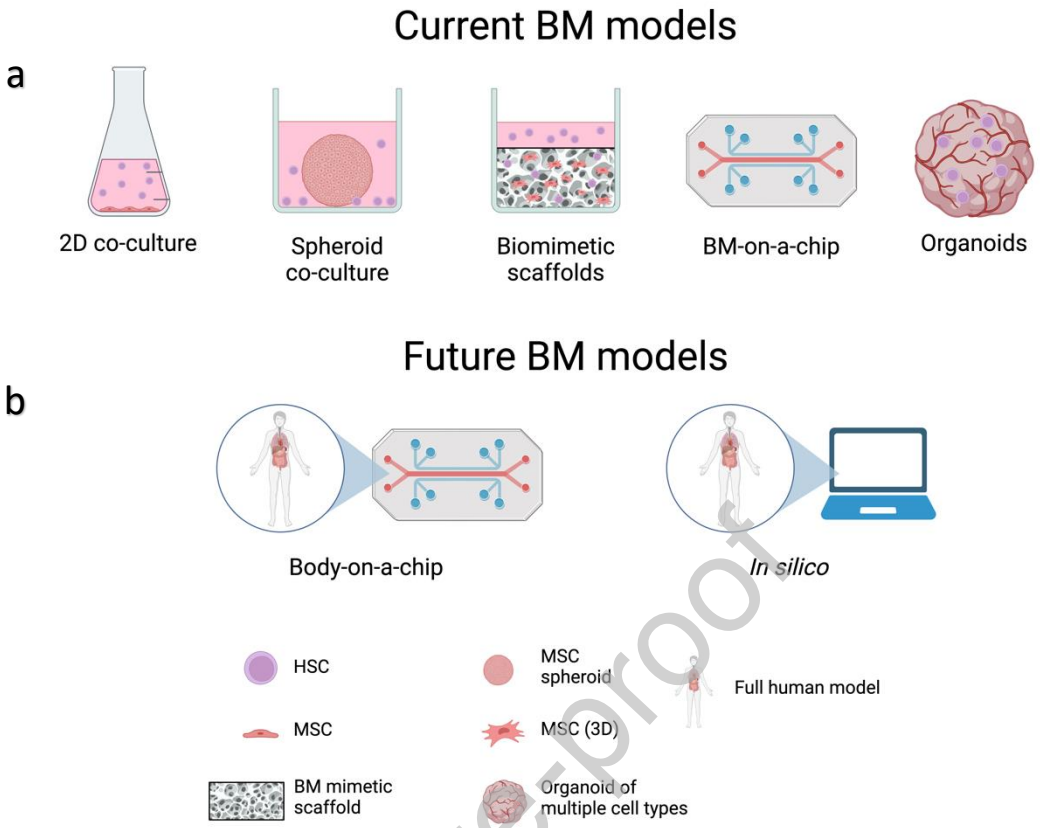

Figure 1. Illustrations of (a) current and (b) potential future bone marrow niche models.

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2. Current state of BM niche models

2.1 MSCs and soluble factors

Developing models of the BM niche for studying and manipulating normal and pathological haematopoiesis presents various challenges, not least of which being that HSCs rapidly differentiate when cultured under standard tissue culture conditions (Jaroscak *et al.*, 2003). To counteract this, soluble factors are often used. Soluble factors found within the niche promote maintenance and expansion within the putative HSC compartment (Delaney *et al.*, 2010; Horwitz *et al.*, 2014; Wagner *et al.*, 2016; Lampreia, Carmelo and Anjos-Afonso, 2017; Xiao, McGuinness, *et al.*, 2022). Stem cell factor (SCF) (Asada *et al.*, 2017), fms-like tyrosine kinase 3 (Flt3) ligand (FL) (Sitnicka *et al.*, 2002) and thrombopoietin (TPO) (Qian *et al.*, 2007) have all been implicated in HSC maintenance and are used routinely for HSC culture *in vitro* (Rödling *et al.*, 2017; Bai *et al.*, 2019; Vannini *et al.*, 2019; Donnelly *et al.*, 2022). Of note, systems that solely utilised soluble factors showed limited success in growing HSCs with long term-engraftment capacity (Delaney *et al.*, 2010; Horwitz *et al.*, 2014; Wagner *et al.*, 2016). The earliest of these systems sought to identify and deploy the source of these soluble factors. This resulted in the discovery of a secondary, adherent cell type present in the BM niche without which the HSCs were unable to survive (Dexter, Allen and Lajtha, 1977). We now know that these cells are mesenchymal stromal cells (MSCs), which interact with HSCs and maintain haematopoietic homeostasis through excreted soluble factors and juxtracrine signalling (Méndez-Ferrer *et al.*, 2010; Pinho *et al.*, 2013). As a result, many modern BM niche models include MSCs.

MSCs included in BM models have several sources. MSCs sourced directly from donors' BM are the gold standard, but they are difficult to acquire due to their relative rarity within the niche, as well as low availability of high quality BM samples from young, healthy patients (Pittenger *et al.*, 1999; Hass *et al.*, 2011; Li *et al.*, 2016; Bhat *et al.*, 2021). Extramedullary sources of MSCs from patients include adipose tissue (Tsuji, 2014), peripheral blood (Li *et al.*, 2015), and birth-associated tissues (Shang, Guan and Zhou, 2021). MSCs can be acquired easily from these sources following liposuction, birth or from a blood sample, and MSCs are more abundant in these locations, making them a popular alternative to BM MSCs (Hass *et al.*, 2011). Induced pluripotent stem cell (iPSC)-derived MSCs also present various

advantages, namely that they can be grown from patients' somatic cell and have the potential to self-organise and reproduce various cellular aspects of the BM niche simultaneously (Khan *et al.*, 2023; Frenz-Wiessner *et al.*, 2024). However, this technology is relatively new and more research is required before iPSCs' pluripotency can be fully understood and manipulated to reliably produce MSCs (Dupuis and Oltra, 2021; Thanaskody *et al.*, 2022). Immortalised MSCs, which are easy to use due to their long-term maintenance of differentiation competency, have been used as a BM MSC alternative as well (James *et al.*, 2015).

2.2.1 2D co-culture

Lima et al., (2013) attempted to expand HSCs from umbilical cord blood by co-culturing cord blood samples with BM-derived MSCs and various cytokines. They demonstrated remarkable expansion of putative HSCs using this method. However, when the expanded cells were transplanted in patients, long-term engraftment was significantly lower compared to transplantation with unmanipulated cord blood. These results suggested that a simple two-dimensional (2D) co-culture system was insufficient to support the maintenance of the LT-HSC population that is required for successful long term engraftment (Seita and Weissman, 2010).

2.2.2 Spheroids

Later research attempted to improve upon simple co-culture models by assembling MSCs into spherical masses, termed spheroids, prior to co-culture (Méndez-Ferrer *et al.*, 2010; Lewis *et al.*, 2017). Spheroid MSCs adopted a more niche-like phenotype than those cultured in a monolayer (Isern *et al.*, 2013; Lewis *et al.*, 2016), however the number of putative HSCs after expansion was shown by one study to be comparable when MSC spheroids or monolayers were used for co-culture (Futrega *et al.*, 2017). Other studies reported a substantial increase in putative HSC count under similar conditions (Isern *et al.*, 2013; Pinho *et al.*, 2013). This was largely attributed to soluble factors produced by the MSCs, rather than juxtracrine signalling. The advent of spheroids in BM niche models demonstrated the advantages of integrating the stromal cell component of the BM niche in 3D.

2.3 Physical properties of the cellular microenvironment

Material properties, such as stiffness, porosity and topography, of HSCs' microenvironment directly affect their maintenance and proliferation (Gvaramia *et al.*, 2017). Various approaches have sought to capitalise on this, for example by using decellularized BM (Tiwari *et al.*, 2012; Lai *et al.*, 2013; Bianco *et al.*, 2019) or 3D BM mimetic scaffolds (Nichols *et al.*, 2009; Raic *et al.*, 2014; Bourgine *et al.*, 2018) to influence HSCs. An early example of this was Nichols et al. (2009), who used inverted colloidal crystals to mimic the porosity and stiffness of the *in vivo* BM niche, and demonstrated that this system was capable of expanding HSCs, with the inclusion of MSCs. Raic et al. (2014) expanded upon this approach using bio-functionalised macroporous hydrogels seeded with MSCs, resulting in a system that was capable of transient maintenance of seeded HSCs' differentiation capacity. Collectively, these models illustrated the need to incorporate BM mimetic materials into future BM niche models, as well as the combinatorial effect of materials coupled with soluble factors. Interestingly, Raic et al. (2014) found a population of HSCs which did not remain within the scaffold and instead settled underneath it. They demonstrated that this cell population had much higher levels of naivety and differentiation capacity than HSCs that remained within the niche, implying the simulation of multiple BM niche compartments which differentially influenced the HSCs within, akin to the sub-niches observed in the native BM niche.

2.4 The multifaceted nature of the BM niche

Bourgine et al. (2018) also encapsulates some of the complexity of the BM niche using a porous hydroxyapatite scaffold functionalised with human MSCs. MSCs were grown within the scaffold, causing them to deposit elements of the native BM ECM. This system was assembled in a perfusion bioreactor which mimicked the blood flow present within the *in vivo* BM niche. HSCs were added to this system, filling the scaffold and overflowing above it to form separate artificial ECM and supernatant environments, similar to the static sub-niches observed by Raic et al. (2014). However, unlike Raic et al. (2014), Bourgine et al. (2018) demonstrated that a higher proportion of the HSCs found within the scaffold displayed surface markers typically associated with LT-HSCs. It's possible that the artificial ECM compartment, which was designed to mimic the structure of bone, assumed the role of the arteriolar niche, with putative, more quiescent HSCs preferentially localising within it, while more actively dividing HSCs localised to the supernatant. Similar models favoured this

assessment (Leisten *et al.*, 2012; Nelson *et al.*, 2021a). These two-compartment models replicate the multifaceted nature of the BM niche and could help to elucidate some of its complexities.

2.5 Combinatorial niche models

In an attempt to encapsulate various other aspects of the BM niche, some researchers have focussed on engineering BM-on-a-chip models. Nelson et al. (2021) created a system using a complex co-culture of MSCs, osteoblasts, human umbilical vein endothelial cells and HSCs. These cells were cultured in separate wells connected via microfluidic channels, allowing nutrient exchange between them. Different combinations of cells and medias with or without the inclusion of a hydrogel were applied to each well during the assembly of the model, resulting in an interconnected system of wells each of which simulated some aspect of the *in vivo* BM niche, including angiogenesis. A similar model developed by Glaser et al. (2022) even incorporated perfusion. These highly complex systems and others like them (Aleman *et al.*, 2019; David B Chou *et al.*, 2020) encompassing more BM niche elements and are more biomimetic than the simpler models discussed,. This makes them excellent candidates for furthering our understanding of the BM niche in both a healthy and diseased state, and for screening treatments for BM-associated diseases and disorders.

2.6 Organoid models

Recently, efforts have been made to produce miniaturised, self-organising, multicellular, 3D models of the BM niche, termed organoids (Rossi, Manfrin and Lutolf, 2018). Two parallel papers outline similar multiphase pipelines to produce complex, vascularised organoids that mimic many aspects of the BM microenvironment (Khan *et al.*, 2023; Frenz-Wiessner *et al.*, 2024). Each phase of organoid production utilised a different cocktail of media components. Initially, induced pluripotent stem cells (iPSCs) were encouraged to form embryoid body (EB) aggregates. Once established, the EB aggregates had a mesodermal phenotype induced, which was appropriate as BM niche cells are derived from the mesoderm over ontogeny (Mikkola and Orkin, 2006; Vodyanik *et al.*, 2010). Next, the EBs were primed for vascular and haematopoietic differentiation, and subsequently embedded in a hydrogel. Within the hydrogel, angiogenesis was observed as the EBs began to sprout (Wimmer *et al.*, 2019). Finally, sprouting EBs were removed from the gel and cultured in ultra-low adhesion plates,

resulting in the formation of vascularised organoids consisting of multiple cell types found within the native BM niche. Organoid models present a unique opportunity to further our understanding of haematological development over ontogeny using a pluripotent *in vitro* system. In addition, they have shown potential for the maintenance of otherwise difficult to culture primary cancer cells (Khan *et al.*, 2023), and as pathological models for diseases such as myelofibrosis and neutropenia (Khan *et al.*, 2023; Frenz-Wiessner *et al.*, 2024). While these models are impressive, future challenges remain in incorporating a complete BM niche cellular cohort, and in self-organisation into the multiple sub niches observed in the *in vivo* BM niche. Also, due to the immaturity of the cells produced, organoid models more closely resemble foetal rather than adult BM. Yet organoids represent a significant advance in the field; the ease with which iPSCs can be produced compared to other BM stem cell sources makes this an attractive model for HSC expansion and disease modelling, and further optimisation of organoid production promises to yield even better *in vitro* BM models.

Similar results were produced using organoid-like constructs termed ossicles, which were assembled *in vitro* then transplanted into murine models (Reinisch *et al.*, 2016, 2017). Although these constructs recapitulated some aspects of the BM niche and were subsequently invaded by murine hematopoietic tissue, they still possess many of the issues inherent to animal models compared to fully humanised models (Ingber, 2020).

3. Future of BM niche models

3.1 Limitations of current BM niche models

While the current crop of BM niche models is promising, the challenge remains to produce a system which can consistently expand a population of HSCs without sacrificing the cells' differentiation or engraftment potential. This is in part due to the highly heterogenous nature of HSCs (Rossi *et al.*, 2011), which makes it difficult to identify LT-HSCs isolated from models (Kiel *et al.*, 2005; Rossi *et al.*, 2011; Futrega *et al.*, 2017; Sonoda, 2021). Also, LT-HSCs rarely multiply (Zhang *et al.*, 2019), meaning it is likely that any expansion observed occurs out with the LT-HSC compartment, resulting in a population of cells with reduced engraftment and differentiation capacity. Furthering our understanding of haematopoietic niches, especially foetal niches from which HSCs are initially derived (Mikkola and Orkin, 2006), could provide an avenue to better understand mechanisms of LT-HSCs expansion, which could in turn allow HSCT treatment to move away from the current one donor one recipient system and towards a one donor multiple recipient system, greatly increasing its availability (Wilkinson, Igarashi and Nakauchi, 2020). Alternative, simpler approaches to HSC expansion have been employed with increasing success in recent years, including the addition of small molecules that prevent loss of the LT-HSC phenotype when expanding HSCs using cytokine cocktails (Peled *et al.*, 2012; Wilkinson *et al.*, 2019; Cohen *et al.*, 2023), and the use of hydrogels for 3D HSC culture (Bai *et al.*, 2019). These systems hold great clinical potential, especially nicotinamide expanded HSCs which recently gained clinical approval for the HSCs produced to be used for HSCT (Meaker and Wilkinson, 2024). The question remains if the HSCs they generate are adequate replacements for the fresh cells typically used for HSCT, especially when produced at scale. Strategies that blend these simple systems with more complex BM model elements warrant investigation.

Another hurdle for BM model development is the reliance on extracellular stimuli such as perfusion or artificially separated cell populations. This is an adequate compromise while tissue engineering technology continues to develop. However, this approach does not reproduce the complex interplay that occurs within a cellularly diverse, mechanically heterogenous organ such as the BM niche, not to mention interactions the BM has with other organs within an individual. Fully cellular organoid models, and other emerging approaches discussed below, may offer a revolutionary solution, despite their development

still being in its infancy. These systems may pave the way for highly accurate models that can facilitate research on the BM niche and expand understanding of the organ and how it influences haematopoiesis.

Finally, recent advances have led to highly complex BM models that recapitulate various aspects of the native BM niche. These models have furthered our understanding of the cellular and niche signals' interplay in this complicated microenvironment. However, this complexity is itself a limitation, due to the inherent cost, time and level of expertise required to assemble these models. These challenges must be overcome for BM models to be accepted and more widely utilised in research and industry.

3.2 Potential applications of BM niche models

A prospective application of BM models is their usefulness for studying and developing treatments for BM-associated disorders and diseases. Novel treatments such as chimeric antigen receptor T-cell (CART) therapy show huge promise (Kim *et al.*, 2018) but are often inaccurately assessed with animal trials due to differences in animal and human biology, preventing potential treatments from reaching the market, or allowing ineffective or unsafe ones to do so (Akhtar, 2015; Ingber, 2020). Pathological *in vitro* BM models that more accurately replicate diseased human biology, reduce animal suffering and can be produced at scale have the potential to revolutionise our approach to drug testing, either as a screening step prior to animal testing or as a complete substitution (Haddrick and Simpson, 2019).

BM models have already been used to further our understanding of the BM niche and haematopoiesis, as previously discussed. They have shown that HSC maintenance is reliant on MSCs and the physical properties of the HSCs' microenvironment, that soluble factors produced by BM cells also play an integral role, and that the BM niche is formed of distinct, interconnected sub-niches. As BM niche models continue to evolve so will our understanding of the *in vivo* BM niche, haematopoiesis, and HSCs (see **Figure 2**).

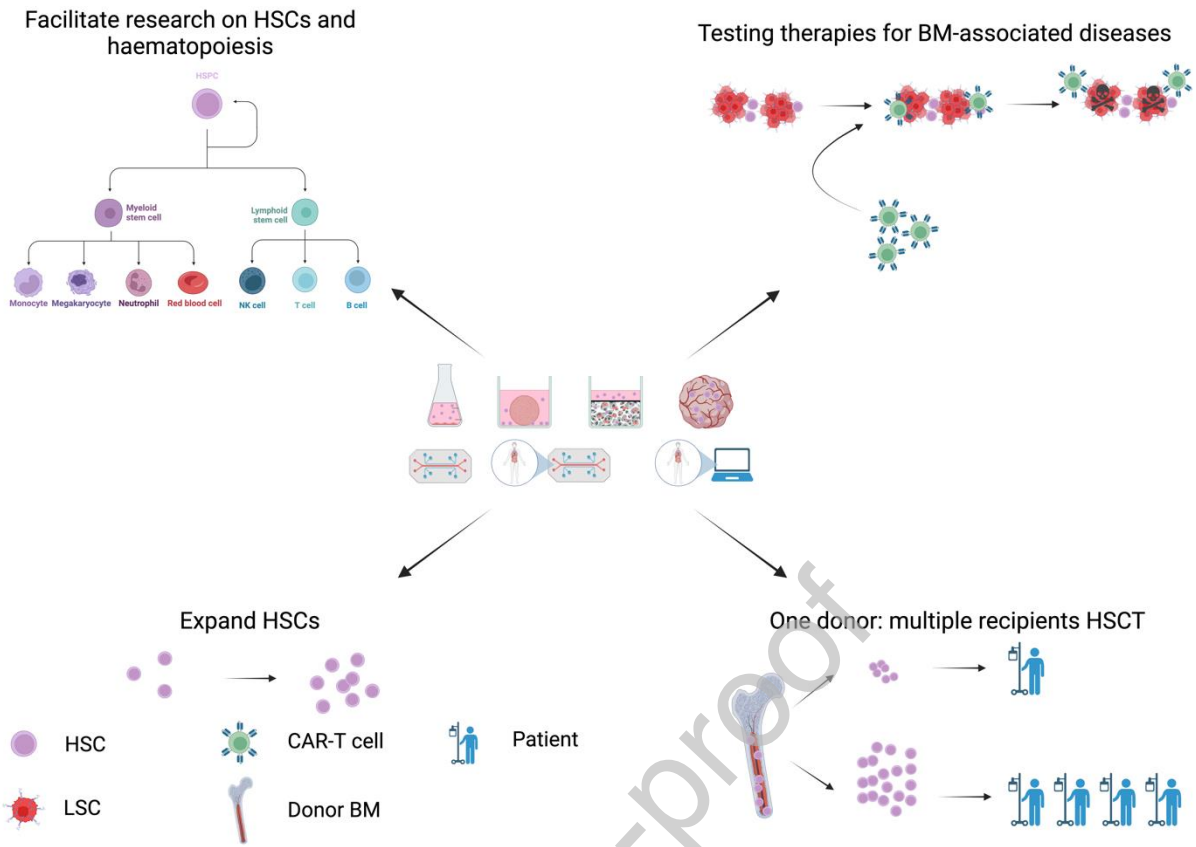

Figure 2. Potential applications of a model BM niche.

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3.3 Theoretical future BM niche models

Future BM niche models could seek to replicate the *in vivo* niche more accurately by incorporating a complete BM cellular cohort or by precisely replicating the physical, mechanical, and chemical composition of the *in vivo* niche. If achieved this would result in BM niche models with high biomimicry, furthering our understanding of the *in vivo* niche's physiology, biochemistry, and biophysics. Other future models could include simplified niches specialised to accomplish a specific goal, such as LT-HSC maintenance (Donnelly *et al.*, 2022) and expansion, or as platforms to test novel therapies in which the HSCs are easily accessible for treatment and evaluation (David B. Chou *et al.*, 2020). As our understanding of the BM niche continues to develop it may even become possible to eliminate the need for inherently complex co-cultures and replicate the effect of BM cellular components in HSC monoculture systems, simplifying artificial BM niches substantially.

Next generation models could also be developed. These may include whole body models which mimic several or all human organs (Novak *et al.*, 2021), providing a platform for a more comprehensive assessment of a treatment's efficacy and safety on an individual, and possibly leading to the development of a body-on-a-chip (Sung *et al.*, 2019).

In silico models of the BM or whole individuals could also emerge. These might take the form of a digital twin, mimicking the function of biological systems based on experimental datasets and/or biological principles (Geris *et al.*, 2018). Simple *in silico* models are already in use, and have demonstrated the ability to assist with optimisation of experimental parameters (Baker *et al.*, 2023), confirm hypotheses (Chang *et al.*, 2010; Stratmann *et al.*, 2014), and shed light on previously poorly understood biological systems (Edelman, Eddy and Price, 2010). Recent advances in artificial intelligence technology could facilitate further development of these revolutionary new models by assisting with data compilation from diverse sources (Homeyer *et al.*, 2022) and through utilisation of techniques such as deep learning and machine learning to collate and utilise large datasets for predicting the impact of stimuli such as novel drugs on biological processes (Sarker, 2022). As such, *in silico* models have the potential to exponentially accelerate research by identifying optimum parameters for experimentation, and, with further developments potentially leading to fully realised digital twins, may offer an alternative to animal and *in vitro* models for clinical trials. One of the key benefits of whole body next generation models, both *in silico* and *in vitro*, is their ability to assist with prediction of pharmacokinetics and off-target toxicity, which are not explorable using single organ models (Bender *et al.*, 2007; Chang *et al.*, 2010).

These types of models currently remain a challenge as they require extensive knowledge of the simulated organs to develop, or large, reliable, relevant datasets and systems which can interpret and utilise them. However, due to the rapidly evolving nature of the field they may be closer than they appear.

4. Conclusion

Within the last half a century BM models have gone from simple liquid suspensions of HSCs to highly complex *in vitro* organ models specialised to achieve specific goals. This has greatly improved our understanding of the *in vivo* BM niche, allowing for the exponential pace of BM model development seen recently. As this field continues to develop it promises to deliver several benefits, namely an improvement over animal models to facilitate more efficacious and cheaper development of treatments for BM-associated disorders, and a potential method for the expansion of LT-HSCs for HSCT.

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