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Review article

Rebuilding the hematopoietic stem cell niche: Recent developments and future prospects☆

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ABSTRACT

Hematopoietic stem cells (HSCs) have proven their clinical relevance in stem cell transplantation to cure patients with hematological disorders. Key to their regenerative potential is their natural microenvironment – their niche – in the bone marrow (BM). Developments in the field of biomaterials enable the recreation of such environments with increasing preciseness in the laboratory. Such artificial niches help to gain a fundamental understanding of the biophysical and biochemical processes underlying the interaction of HSCs with the materials in their environment and the disturbance of this interplay during diseases affecting the BM. Artificial niches also have the potential to multiply HSCs *in vitro*, to enable the targeted differentiation of HSCs into mature blood cells or to serve as drug-testing platforms. In this review, we will introduce the importance of artificial niches followed by the biology and biophysics of the natural archetype. We will outline how 2D biomaterials can be used to dissect the complexity of the natural niche into individual parameters for fundamental research and how 3D systems evolved from them. We will present commonly used biomaterials for HSC research and their applications. Finally, we will highlight two areas in the field of HSC research, which just started to unlock the possibilities provided by novel biomaterials, *in vitro* blood production and studying the pathophysiology of the niche *in vitro*. With these contents, the review aims to give a broad overview of the different biomaterials applied for HSC research and to discuss their potentials, challenges and future directions in the field.

Statement of significance

Hematopoietic stem cells (HSCs) are multipotent cells responsible for maintaining the turnover of all blood cells. They are routinely applied to treat patients with hematological diseases. This high clinical relevance explains the necessity of multiplication or differentiation of HSCs in the laboratory, which is

Abbreviations: BM, Bone marrow; BCC, Breast cancer cell; CaP, Calcium phosphate; CFU, Colony forming unit; GEMM, Granulocyte Erythrocyte Macrophage Megakaryocyte; GM, Granulocyte Macrophage; G, Granulocyte; M, Macrophage; Mk, Megakaryocyte; E, Erythrocyte; DLL, Delta-like ligand; EC, Endothelial cell; ECM, Extracellular matrix; Flt3L, Fms-like tyrosine kinase 3 ligand; FN, Fibronectin; GAG, Glycosaminoglycan; GelMA, Gelatin methacrylate; HA, Hyaluronic acid; HAp, Hydroxyapatite; HSC, Hematopoietic stem cell; HSPC, Hematopoietic stem and progenitor cell; HUVEC, Human umbilical vein endothelial cell; ICC, Inverted colloidal crystal; LN, Laminin; LTC-IC, Long-term culture-initiating cell; MNC, Mononuclear cell; MSC, Mesenchymal stem/stromal cell; NF, Nanofiber; OB, Osteoblast; OC, Osteoclast; PA, Polyacrylamide; PB, Peripheral blood; PCL, Polycaprolactone; PDLLA, Poly (D,L-Lactic acid); PEG-DA, Poly(ethylene glycol) diacrylate; PEMA, Poly(ethylene-*alt*-maleic anhydride); PGA, Poly(glyconic acid); PMMA, Poly(methyl methacrylate); PLGA, Poly(lactic-co-glycolic acid); PLLA, Poly(L-lactic acid); PU, Polyurethane; PVA, Polyvinyl alcohol; RBC, Red blood cell; SCF, Stem cell factor; TCP, Tissue culture plastic; TIPS, Thermally-induced phase separation; TNC, Total nucleated cell; TPO, Thrombopoietin; UCB, Umbilical cord blood; YAP, Yes-associated protein.

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hampered by the missing natural microenvironment – the so called niche. Biomaterials offer the possibility to mimic the niche and thus overcome this hurdle. The review introduces the HSC niche in the bone marrow and discusses the utility of biomaterials in creating artificial niches. It outlines how 2D systems evolved into sophisticated 3D platforms, which opened the gateway to applications such as, expansion of clinically relevant HSCs, *in vitro* blood production, studying niche pathologies and drug testing.

Contents

1. Introduction

Blood cells need to be constantly produced, as their loss due to immune response, injury or an intrinsically limited lifespan demands their dynamic replenishment. In adult humans, this process typically takes place in the bone marrow (BM) and is performed by hematopoietic stem cells (HSCs) [1]. HSCs are defined by their ability to self-renew as well as giving rise to lineage-determined daughter cells, that are able to differentiate into all cell types of the hematopoietic system [2]. Maintenance of these remarkable features of HSCs can only be achieved within specialized microenvironments, called the HSC niches, located in the BM of trabecular bones [1,26]. Hematopoiesis, production of blood cells from HSCs, is a highly regulated and hierarchical process. The multitude of factors involved in the physiology of the HSC niche makes the production of biomaterial-based artificial niches challenging. However, the potential of such platforms has stirred the interest of many research groups, as they could be used to study the mechanisms involved in the maintenance of the healthy niche and the pathophysiology of the diseased BM [5,6]. Most importantly, biomimetic materials can be utilized for efficient expansion of HSCs *ex vivo* without the loss of stemness for which there is a dire need in the current clinical landscape due to the limited pool of available donors [7,8]. In addition, targeted HSC differentiation for *in vitro* blood production can be enhanced by biomaterial-based artificial niches [9–11]. Attempts to recreate the disordered niche offer insight into the resulting malignancies and provide drug-testing platforms to counter them [12–15] (Fig. 1). Usually, such tasks are carried out using animal models, which are not fully comparable to the human system. More importantly, they have to be implemented and accepted under ethical standpoints into the 3Rs principle to Reduce, Refine and Replace animal models [16]. With this review, we aim to highlight recent developments in the field of biomaterials for HSC culture. Starting with the description of the biology and biophysics of the natural archetype, we will discuss 2D biomaterial-based approaches to dissect the niche complexity for fundamental studies. Then we move on to the diverse 3D culture systems used for mimicking the natural niche as well as the importance of

biomaterials for *in vitro* blood production. Finally, we discuss the attempts made in replicating the niche under certain pathological circumstances and the importance of such systems to further understand and intervene in the resulting diseases.

2. Biology of the HSC niche

First put forth by Schofield et al. in 1978 [17], the niche concept has grown from a mere hypothesis into a widespread model of hematopoiesis that comprises distinct biological sites with different purposes [1]. The endosteal niche, located near the endosteum of spongy bone in the metaphysis of long and flat bones, keeps HSCs predominantly in a quiescent state with high self-renewal potency [18]. Besides HSCs, numerous different cell types are part of the niche. It is populated with osteoblasts (OBs) that modulate HSC fate by activation of the Notch-pathway and release of thrombopoietin (TPO) in mice [19,20]. An important subset of OBs in the murine endosteal niche are spindle shaped N-cadherin expressing OBs that partly mediate the interaction with HSCs [21]. In addition, osteocytes are essential for the mobilization of hematopoietic stem and progenitor cells (HSPCs) mediated through granulocyte colony-stimulating factor [22,23]. This specific niche provides HSCs with a quite stiff matrix (>35 kPa) and high ionic calcium and low oxygen concentrations [24,25].

The vascular niche, which hosts HSCs in association with the sinusoidal endothelium, is thought to foster HSCs involved in the regular production of blood cells, given its intimate connection to the vascular system [26,27]. Apart from the endothelial cells (ECs) that form the inner lining of blood vessels, other relevant cell types in this niche include e.g. perivascular mesenchymal stem/stromal cells (MSCs) [28], subtypes of them such as CXCL12-abundant reticular cells [28,29] and macrophages [30]. These and other niche cells present and release important mediators of HSC migration and maintenance, including E-selectin, CXCL12, stem cell factor (SCF), Leptin and Nestin [31,32]. MSCs are one of the major suppliers of cytokines to HSCs [33,34]. Due to the proximity to blood vessels, the vascular niche provides a quite soft matrix to HSCs, (0.3 kPa in the marrow [35], 0.5–2 kPa in endothelium [36] and 5–8 kPa for the vessel walls [37,38]). Sinusoidal and arteriolar niches are described as vascular subniches [39,40]. However, HSC niches and locations seem to be interconnected and are still a matter of ongoing research.

Cell-cell interactions, both direct and indirect, are of vital importance in the niche. Direct interactions are mediated mostly by the receptors of the cadherin and selectin families [18,41,42]. Moreover, direct cell-cell contact between HSCs and MSCs has proven to be important in inducing self-renewal and supporting migratory potential of HSCs [43–45]. Indirect communications are mainly conveyed by soluble factors such as cytokines, growth factors, hormones and small molecules and they play important roles in the HSC niche. SCF is an early acting cytokine, which binds to the c-Kit receptor, a receptor tyrosine kinase type III. Binding of SCF results in an activation of phosphatidylinositol 3-kinase, JAK/STAT and MAPK pathways. It is known that c-Kit activation through SCF is a very important physiologic regulator of HSC activity *in vivo* (reviewed in [46]). TPO is another important cytokine, which promotes self-renewal and expansion of HSCs. TPO binds to its receptor cMpl and can activate several different downstream signaling pathways, which seem to be important for the survival and proliferation of $CD34^+$ HSPCs (reviewed in [47]). Fms-like tyrosine kinase 3 ligand (Flt3L), which binds to Flt3, was observed to support the survival of human HSCs [48]. Due to their HSC supporting activities SCF, TPO and Flt3L are used as soluble additives in many HSPC expansion protocols. Besides these, many other soluble factors, such as interleukin 3 and 6, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, CXCL12, transforming growth factor-beta, osteopontin, angiopoietin-1 and insulin-like growth factor 1, have proven to play a role in the HSC niche ([49,50] and reviewed in [51]).

The extracellular matrix (ECM) of the HSC niche, secreted by the niche cells, is composed largely of structural proteins such as collagen I and IV, glycoproteins like fibronectin (FN) and laminin (LN) and glycosaminoglycans (GAGs, e.g. hyaluronic acid (HA)), which consist of chains of repeating disaccharide units. Most GAGs are found as a part of proteoglycans with a respective protein core [52–54]. Mainly, the integrin family mediates the interaction of cells with their surrounding ECM. Integrins are membrane bound heterodimeric receptors having several α- and β-subunits, which in combination form 24 different types [18,55]. These do not only recognize specific ECM substrates but also initiate respective cell responses [4,56,57]. Most importantly, integrins connect the ECM with the cytoskeleton and present bidirectional force transmission sites, thereby providing the platform for mechanotransduction [4].

A well-organized interplay between HSCs, different niche cells and the ECM is crucial for a proper niche and thereby HSC function. In this context, not only the biological and biochemical components of the niche, but also biophysical conditions like stiffness, topography, or shear stress have an impact on HSC fate [4,25].

3. Biophysics of the HSC niche

The mechanobiology of HSPCs is governed both by the ECM of the niche and their neighboring cells [4,58]. The sensitivity of cells towards the ECM stiffness and associated forces not only maintains normal cellular functions and tissue homeostasis but also determines the differentiation fates of stem cells [7,59]. This is usually achieved by specific receptor-ligand interactions, which are not purely biochemical but can also discern biophysical cues, such as matrix architecture, pliability, rigidity and roughness. Simply put, cells are simultaneously subjected to various physical, chemical and biological stimuli which they can integrate and respond to appropriately [4,60]. The influence of biophysical parameters in the HSC niche is still a topic of ongoing research. HSCs undergo a certain degree of deformation while crossing the endothelial barrier during transmigration and trafficking. Under these circumstances, lamins, which are intermediate filament proteins of the nucleoskeleton, could play a vital role. Shin et al. showed that a certain stoichiometry between both isoforms lamin-A and -B contributes to human BM-derived $CD34^+$ HSPC transmigration, trafficking and differentiation [61]. Mechanical stimuli in cells can not only result in prompt responses like adhesion or migration but also in the long run, influence expansion and differentiation. Particularly important in this context are the transcription factors Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif, which have been identified as mediators of mechanical signals to the nucleus. In a recent study, Lundin et al. used a human dorsal aorta-on-a-chip platform to depict that hemodynamic YAP-mediated mechanotransduction plays a role in *de novo* human HSPC production [62]. In 2010, Holst et al. showed that increased substrate elasticity promoted the expansion of human umbilical cord blood (UCB)-derived mononuclear cells (MNCs) and murine BM-derived $Lineage^-Sca1^+c\text{-}Kit^+$ (LSK) HSPCs, using tropoelastin, a highly elastic biomolecule [63,64]. Several hydrogel studies have also revealed the mechanosensitivtiy of HSPCs and are reviewed in the chapter 4.3.

Systems exploring the basic physics of the ECM (geometry, stiffness, ligand spacing, micro- and nanotopography) formed the grassroot level to investigate the impact of biophysical parameters on HSPCs. With their help, it was shown that HSPCs are sensitive to matrix mechanics and shear forces (discussed in chapters 4 and 6), 3D architecture and gradients occurring in 3D sys-

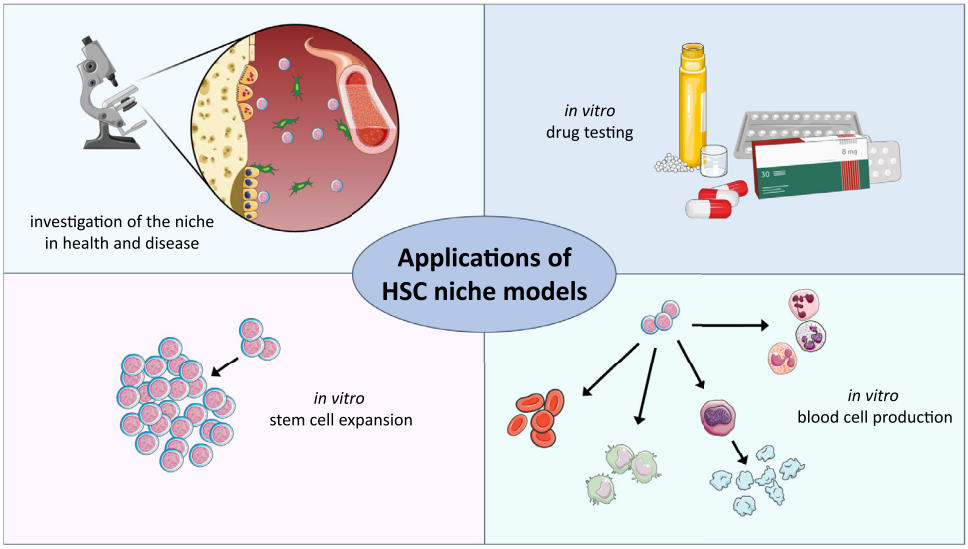

Fig. 1. Applications of HSC niche models. One of the most prominent applications of HSC niche models is the investigation of the healthy or diseased niche under well-defined conditions (upper left). As artificial HSC niches resemble the *in vivo* conditions better than standard 2D culture, they allow *in vitro* drug testing, thereby avoiding animal models (upper right). In addition to *in vitro* HSC expansion (lower left), a diverse range of blood cells, for example, erythrocytes, monocytes, platelets or granulocytes, for cellular therapies can be produced with these systems (lower right). (Figure was created with the help of Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License).

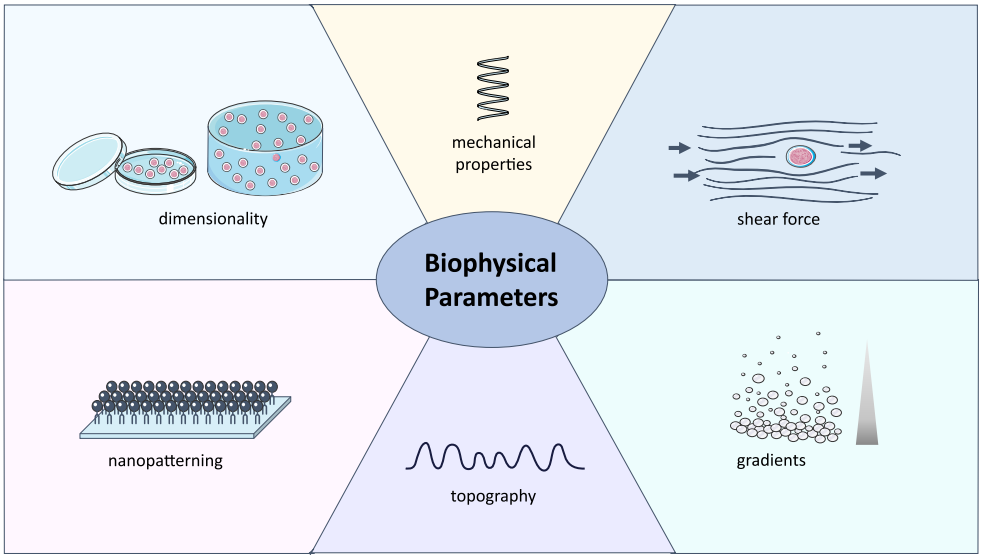

Fig. 2. Biophysical parameters influence HSPCs. These include dimensionality and 3D architecture as well as mechanical properties, nanopatterning and topography of the environment. Furthermore, shear forces occurring by fluid flow and gradients of soluble factors such as growth factors or cytokines play a role in controlling HSPC behavior. (Figure was created with the help of Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License).

tems (discussed in chapter 6.1) as well as nanopatterning and –topography (discussed in chapters 4.2 and 5.1) of the environment (Fig. 2). Studies on time-dependent properties (matrix degradability, viscoelasticity, compressibility, stiffening, surface mobility) of the microenvironment, investigation of cell-cell and cell-matrix interactions, cell polarity, auto- and paracrine signaling as well as addition of soluble factors and other biomolecules enabled deeper insights in the mechanotransduction pathways of other types of stem cells and are yet to be determined for HSPCs. [40,59,65]. Together with the in-depth knowledge on the HSC niche biology, fundamental studies on the role of biophysics in the niche form the basis for engineering the HSC niche *in vitro* using biomaterials, which is a complex and multifaceted task. The incorporation and investigation of more than one niche factor is vital in biomimetic niche models. The following chapters take a walk through the different biomaterial-based approaches, their evolution from conventional 2D cultures to sophisticated 3D systems and give an insight into what the future holds for HSC research using biomaterials. An overview of the different biomaterials discussed in this review is shown in Fig. 3.

4. Dissecting the hematopoietic niche complexity in 2D culture systems

4.1. Incorporation of niche components in conventional cultures

In traditional cell culture [66], HSCs with a size of approximately 10 µm in diameter [67] perceive the solid culture vessel walls as a large, flat 2D surface that is homogeneous in composition. Maintenance of HSPCs *in vitro* is enhanced when biological niche components, like cells, ECM proteins or soluble factors, are incorporated in the culture container. Since the middle of the last century, researchers have utilized feeder-cell layers to culture mouse BM cells *in vitro* [68]. It has been shown

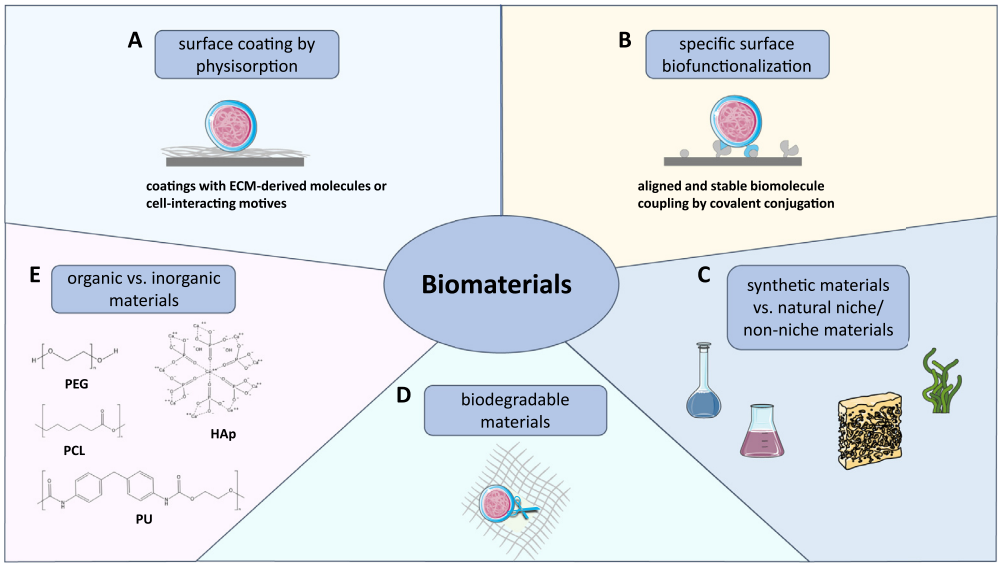

Fig. 3. The variety of biomaterials offers numerous possibilities for HSC research. Surface coatings by physisorption (A) using ECM-derived molecules or cell-interacting motifs such as collagen, FN or LN result in non-specific adsorption, whereas the covalent conjugation of selected biomolecules enables a more defined and specific biofunctionalization (B). In general, materials to mimic HSC niches can consist of ECM components that naturally occur in the niche, natural molecules that are not found in the niche or synthetic polymers (C). Biodegradable materials (D) can be natural ECM substances with cleavage sites for cell secreted metalloproteases, molecules with weak physical bonds or synthetic polymers with incorporated cleavable linkers. Reconstitution of the natural HSC environment, including all aspects of the bone, the soft BM and vasculature, can be best mimicked by combining organic (e.g. PEG, PCL, PU) and inorganic (e.g. HAp) biomaterials (E). (Figure was created with the help of Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License).

that direct contact between human UCB-derived CD34$^+$ HSPCs and BM-derived ECs (used as a feeder layer or separated by a microporous membrane) can regulate proliferation of progenitor cells by elaboration of lineage-specific cytokines [69]. To date, clinical trials have demonstrated that co-culturing human UCB-derived CD34$^+$ HSPCs *ex vivo* with MSCs supported their expansion without loss of stemness and significantly improved engraftment when transplanted into patients [70,71]. Cocktails of cytokines and growth factors such as TPO, SCF, Flt3L, IL-3 and IL-6 supplemented to the medium can expand the total yield of cells carrying the HSPC marker CD34 multifold [72–74]. When such cocktails are combined with small molecules such as the aryl hydrocarbon receptor antagonist StemReginin1, the pyrimidoindole derivative UM171 or valproic acid the differentiation of the expanded cell populations can be halted [73,75–77]. Recently, polyvinyl alcohol (PVA) was identified as a replacement for serum albumin in HSC culture. Murine CD34$^-$ LSK HSCs cultured in the presence of PVA, 100 ng/ml TPO and 10 ng/ml SCF showed increased expansion and maintenance of functional HSC activity *ex vivo*. Similarly, for human UCB-derived CD34$^+$ HSPCs PVA was shown to be able to replace serum albumin. However, in contrast to murine HSCs, human CD34$^+$CD38$^-$CD90$^+$CD49f$^+$ HSCs were not sensitive to the hydrolysis states of PVA [78].

The modification of the culture surface with cellular ligands imitating the surface of a cell layer, ECM molecules or immobilized soluble factors offers the possibility to dissect the *in vivo* niche into molecular pieces. Hence, 2D platforms are well suited to evaluate molecular-driven effects on HSPCs given the simplicity in production and handling, easy access to cells for harvest, monitoring and subsequent analyses, and the potential to scale up for high throughput.

4.2. Biofunctionalized rigid surfaces

Coatings of culture surfaces with biomolecules is the most frequently used biofunctionalization technique in 2D culture approaches. Either the full-length molecule or, if specific cell-interacting motifs are known, shorter versions are tethered, whereby engineered fragments can shrink in size to only harbor the minimal cell recognition motif and a few flanking groups. Surface coatings apply a deposition of target biomolecules or its engineered constructs from solution to the surface by either physisorption, electrostatic interaction (Fig. 3A) or covalent binding (Fig. 3B). Many ligands suspected to regulate HSPCs have been investigated via surface immobilization and will be discussed below in more details.

4.2.1. Non-covalent immobilization

Ligands of the Notch pathway participate in direct cell-cell contact. Delaney et al. immobilized the engineered Notch ligand DLL-1 ext-IgG together with the FN fragment CH-296 on tissue culture plastic (TCP) in varying concentrations. The growth and differentiation of UCB-derived CD34$^+$ CD38$^-$ HSPCs at moderately low coating concentration and the resulting respective surface density increased the absolute number of CD34$^+$ cells, whereas relatively high surface densities induced apoptosis leading to a decreased CD34$^+$ cell yield [79]. In phase I trials, these Notch-mediated *ex vivo* expanded CD34$^+$ progenitors were transplanted and could successfully engraft in patients [80,81].

Investigation of cell-matrix interactions can be realized with coated ECM proteins to create adhesive surfaces for cell attachment. Celebi et al. used culture surfaces coated with either rat collagen I, human collagen IV, FN or LN with increasing concentrations of ECM protein solutions and additionally tested two ECM protein-mix complexes for UCB-derived CD34$^+$ cell culture. While the total nucleated cell (TNC) count did not differ significantly between the different individual protein coatings, LN-coatings at the lowest concentration showed the most beneficial impact on myeloid and megakaryocyte (Mk) progenitor expansions at day 6, shown by means of colony forming unit (CFU) assay. A mixed coating of all four ECM proteins increased CD34$^+$ cell expansion and promoted greater myeloid progenitor expansion than the coating mix without collagen I as revealed again by CFU assay [82]. ECM proteins of the hematopoietic microenvironment are often in

close contact to GAGs of the proteoglycans. Madihally et al. immobilized GAGs onto culture surfaces by forming insoluble ionic complexes with the amino-polysaccharide chitosan. In their study, only chondroitin sulfate B-chitosan and heparin-chitosan complex membranes significantly enhanced the expansion of the human $CD34^+$ population[2] with a maximum growth within the first 2 and 4 weeks of culture, respectively. They also colorimetrically evaluated the partial dissociation of the complexes during culture and concluded that soluble GAGs contributed concentration-dependently to the observed proliferative effect [83].

4.2.2. Covalent immobilization

Coatings based on biomolecule physical adsorption onto TCP surfaces can desorb with time under cell culture conditions. Non-specific adsorption is further subject to random orientation of the adsorbed biomolecule. More defined biofunctionalized surfaces present aligned and stable biomolecule coupling to the underlying biomaterial surface that is achieved by covalent conjugation. Franke et al. tested a set of different ECM coatings. They covalently immobilized FN, HA, tropocollagen I, heparin, heparan sulphate, and in addition co-fibrils of collagen I and heparin or HA onto thin films of poly(ethylene-*alt*-maleic anhydride) (PEMA). The adherence of human peripheral blood (PB)-derived $CD133^+$ HSPCs to these biofunctionalized surfaces was evaluated and no significant adhesion to tropocollagen but a selectin-mediated adhesion to heparin and an integrin-mediated adhesion to FN using reflection interference contrast microscopy was observed [84]. Moreover, FN-derived peptides that were covalently immobilized on polymer surfaces enabled a higher expansion of human UCB-derived $CD34^+$ HSPCs[3] [85,86].

As cell to material distances are on a nanoscale level (5 to 30 nm) [87], studies using well-defined bioadhesive substrates revealed the importance of the spatial distribution of ligands. Integrin-dependent cell adhesion of human UCB-derived $CD34^+$ HSPCs was shown to be dependent on the lateral distance of ECM-derived ligands [3,88] accompanied by induced lipid raft clustering [3] as well as the size of the nanopatterned ligand [88]. While ECM-derived ligands require a nanotopographical presentation to efficiently mediate responses in HSPCs, cellular ligands such as DLL-1 are dependent on their density to enable receptor clustering for promoting the expansion of human UCB-derived $CD34^+$ HSPCs as observed by flow cytometry and cell enumeration [89]. These studies underscore the importance of paying attention to the nanoscale features in functionalized materials when designing substrates for HSPC culture. The use of fibers is another approach to achieve nanotopographical structures. These can imitate the fibrous ECM proteins and will be discussed in chapter 5.1.

4.3. Biofunctionalized elastic substrates

Rigid surfaces typically used for cell culture, e.g. polystyrene flasks, are often hydrophobic, whereas typical biocompatible softer surfaces are of hydrophilic nature. Hydrogels are hydrophilic polymer networks that retain a significant fraction of water [90]. Therefore, they are often used to investigate 2D soft surfaces. HSPCs are sensitive to the mechanical properties of the underlying culture substrate. Conventionally used cell culture substrates are highly-rigid surfaces (elastic modulus of glass ~70 GPa [91]) that is why covalently crosslinked hydrogels are most commonly employed to study substrate stiffness effects. This stems from the advantage that hydrogel formulations can be adjusted to cover the range of physiologically relevant values of substrate elasticity found in BM. Elasticity sensing and responsiveness of HSPCs to substrates was explored by the culture of murine LSK HSPCs on top of 2D collagen-coated polyacrylamide (PA) substrates. Analysis of 2D microscopy images (computation of spread area and cell shape index of the cells), showed that the stiffer the substrate and the higher the collagen ligand density, the more spread the cells are [92]. Stiffness-dependent cell morphological changes could further be confirmed with different ligand types, namely collagen I, FN or LN. Murine LSK HSPCs cultured on soft gels remained rounded, whereas on stiffer substrates, they appeared more polarized and spread [93]. For human HSPCs, cell adhesion and motility are influenced by the stiffness of FN-functionalized hydrogels. Manual tracking of HSPCs in time-lapse movies showed that they adhered better and migrated further on stiffer hydrogels [94]. Apart from that, stiff FN-coated substrates maintained most primitive myeloid progenitors in short-term (24 h) culture, whereas stiff LN-coated substrates promoted differentiation towards erythroid lineages[4] in comparison to their respective softer substrates [93]. The same study substantiated that the effect of FN on the maintenance of primitive HSPCs versus induction of differentiation is controlled by intracellular tension and a selective engagement of integrin receptors [93]. Conclusively, only enabling cell adhesion is not sufficient for HSPC maintenance *in vitro*, but it ensures close proximity to the substrate and its inherent properties. In this line, a poly(ethylene glycol) diacrylate (PEG-DA) hydrogel surface proved the interplay of immobilized soluble factors, SCF and IFNγ, in combination with the cell adhesion ligands RGDS and connecting segment 1. Both, the combination of RGDS and SCF or RGDS and IFNγ promoted proliferation and maintenance of murine HSPCs [95].

5. Heading for the next dimension: from 2D to 3D

5.1. Nanofibers

Nanofibers (NFs) can be termed a stepping stone from 2D to 3D culture and have been widely used in the past two decades due to their ability to mimic the ECM structure of many human tissues, including BM [96]. Several strategies have been used to produce NFs, e.g. phase separation, self-assembly and electrospinning [97]. Among them, electrospinning has been of major interest owing to its ability to produce NFs similar to the fibrous structure of ECM, the ease of controlling their diameter, its applicability for a wide range of materials and cost-effectiveness. Natural polymers, e.g. collagen, silk, gelatin and elastin or synthetic polymers such as polycaprolactone (PCL), poly(glycolic acid) (PGA), poly(L-lactic acid) (PLLA), poly(lactic-co-glycolic acid) (PLGA) and polyurethanes (PU) have been used in NF fabrication [98] but they all have advantages and disadvantages. While natural polymers, in general, have a good biocompatibility and hydrophilicity, they suffer from weak mechanical properties and low processability. Synthetic polymers, in contrast, have good mechanical properties and offer greater flexibility to tune their properties during synthesis or post polymerization modification, but they are mostly hydrophobic and have low cell affinity due to the lack of cell-binding sites [99]. To overcome these challenges, many approaches have been tested. Chemical surface treatment has been done to introduce functional groups onto the surface of synthetic polymer NFs. Human $CD34^+$ HSPCs cultured on aminated polyethersulfone NF meshes showed stronger adherence as well as greater expansion and maintenance of multilineage progenitors[5] compared to HSPCs cultured on unmodified, hydroxylated, or carboxylated NF meshes or aminated films [100]. This study demonstrates that even simple surface chemistry can

[2] Cell phenotype analyzed by flow cytometry.
[3] Cell phenotype analyzed by flow cytometry and differentiation potential assessed by CFU assay.
[4] Differentiation potential assessed using CFU assay.
[5] Phenotypic analysis was performed using flow cytometry (CD34, CD45) and their differentiation potential was shown using CFU assay.

affect HSPCs in the microenvironment. Many studies suggest the coating of synthetic polymer NFs with natural polymers, such as FN or collagen, to increase the biocompatibility of the fibers for human UCB-derived $CD34^+$ HSPCs [101]. It was also shown that the large surface area-to-volume ratio provided by PCL-NF and the cell-binding domain of FN or collagen promoted cell-cell and cell-matrix interaction and resulted in higher cell proliferation compared to TCP[4] [102]. Expansion of BM-derived HSCs could also be enhanced by using NFs prepared from a PLGA/collagen I blend. In this study, coating blended NFs with E-selectin raised the efficiency of capturing HSCs [103]. Co-culturing human UCB-derived $CD133^+$ HSPCs with MSCs on these PLLA NFs was able to further increase the expansion of $CD133^+$ HSPCs [104]. Batnyam et al. designed a biohybrid, beehive-like 3D scaffold composed of three overlaid layers [105]. Each layer was covered with PU electrospun fibers, which aligned during the fabrication process to result in two types of scaffold either transversely isotropic (scaffold I) or anisotropic (scaffold II). Both scaffolds were seeded with MSCs as a feeder layer. Scaffold II showed a significant increase of JAG1 expression in MSCs compared to scaffold I. Both scaffolds, however, enhanced the proliferation of human $CD34^+$ and $CD34^+CD38^-$ cells compared to the 2D control culture.

Although using NFs can be considered as a step forward in capturing the fibrous structure of the ECM, it is not sufficient to mimic the hierarchical structure of this niche, including the spatial distribution of different biomaterials and cells surrounding the HSCs.

5.2. Microcavities

Mechanically soft hydrogel layers can exhibit an elastic creasing instability of their 2D surface. This microtopography formed as a result of surface creasing can affect *in vitro* cell behavior [106]. Extending the cell-substrate contact area to precisely trap one cell can in turn be utilized for sorting HSPCs [107]. The underlying correlation between migration behavior, lodgment and stem cell maintenance can better be observed by single cell culture in microcavities. Single human PB-derived $CD133^+$ HSPCs adhering inside FN-functionalized microstructures were found to slow down cell cycling as detected by BrdU incorporation and cytometric analysis [108]. A reduced expansion was found for adherent human $CD133^+$ HSPCs in collagen I- and heparin-functionalized microcavities, with the effect being more pronounced on collagen I[6] [109]. The spatial constraints were assumed to maintain isolated murine Lin^- HSPCs in a quiescent and multipotent state[1] which is vital for repopulation of a depleted hematopoietic system [110]. On the other hand, allowing direct HSPC cell-cell contact and unhindered para- and juxtacrine signaling in multicell-sized microcavities resulted in higher expansion of human $CD34^+$ HSPCs than in single cell-sized cavities, even on a soft background [111]. In these artificial single cell niches, individual HSPCs explore spatial confinements that are defined by substrate stiffness, ECM ligand type, density and spatial distribution, as well as available soluble factor levels – all found in a 3D environment. Within recent years, engineered HSPC culture substrates intensively focused on 3D environments to resemble the natural niche more closely. Many 3D biomaterial-based models convincingly expanded and maintained HSPCs more efficiently compared to a 2D substrate.

6. Recreating the hematopoietic niche using 3D systems

6.1. Hydrogel-based models

Hydrogel-based systems are one of the most promising tools utilized in mimicking the 3D environment in *in vitro* cultures. There are several reasons why researchers favor 3D hydrogels for designing experiments to study the HSC niche. To begin with, they can imitate the biophysics of the ECM (stiffness, architecture), enable the signals which are generated during cell-ECM interactions and finally provide scope for remodeling [112]. Next, they provide a 3D environment in which the cells can form contacts to the matrix and maintain their natural shape, without polarizing in a non-physiological way as in 2D cultures [112]. Furthermore, they support cell-cell communication [113] and mimic the influence of the niche ECM on diffusion and concentration gradients of soluble components, such as signaling and waste molecules, better than 2D systems [114]. Finally, hydrogel properties are quite tunable which makes them advantageous for certain applications or analysis methods. The use of hydrogel-incorporated systems in the field of biomaterial-based *in vitro* studies is vast and diverse. Therefore, we will highlight some studies in the text and give an overview of selected studies in supplementary Tables S1 and S2.

Organic molecules used to fabricate hydrogels for 3D approaches can be natural niche ECM components (e.g. Matrigel®, collagen, fibrin; see suppl. Table Sections 1.1 and 2.1), natural non-niche ECM molecules (e.g. alginate, chitosan, pullulan, cellulose, silk; see suppl. Table sections 1.2 and 2.2) or synthetic polymers (e.g. PEG, PU, PLGA, PCL; see suppl. Table Sections 1.3 and 2.3) (Fig. 3C). However, the line between the categories blurs when blends of synthetic and natural polymers are used (e.g. star-PEG and heparin [115] HA, gelatin and PEG-DA [116] (suppl. Table Sections 1.4 and 2.4)) or when natural polymers are synthetically modified (e.g. gelatin methacrylate (GelMA) [117–119] or cationized bovine serum albumin [120] (suppl. Table Sections 1.1 and 2.1)). It is not only the diverse array of available materials that makes hydrogels an interesting platform but also the multitude of techniques one can apply in their preparation. Each technique results in a different kind of hydrogel, some of which will be discussed in the subsequent chapters.

6.1.1. Approaches to mimic the 3D environment of HSPCs with hydrogels

The ways in which hydrogels are used to provide a 3D environment for HSPCs can be subdivided into two main approaches – encapsulation of cells within the gels or using them as macroporous scaffolds into which cells can be seeded. While the first approach is suited to recreate the interaction of HSPCs with the ECM of the soft BM, macroporous scaffolds mimic the porous architecture of cancellous bone, which hosts the niche-harboring red BM.

During encapsulation cells are mixed with a precursor solution, which is then crosslinked around the cells so that the cells are completely enveloped by the matrix. This ensures that the entire surface of the cell is in contact with the biomaterial. As cells are present during the crosslinking, this technique requires non-cytotoxic methods. Often, soft hydrogels are applied in such approaches, which resemble the soft ECM of the BM [112]. An important factor of encapsulation is the mesh size of the polymer network, which influences the stiffness of a gel as well as the diffusion of small molecules [121] including the disposal of waste substances, the supply by nutrients and oxygen [122] and the signaling between cells. The Harley group developed hydrogels with different mesh sizes, which supported either paracrine (big meshes) or autocrine (small meshes) signaling and studied the effect on the cells. They showed that murine LSK HSPCs proliferated more in a paracrine signaling supporting GelMA matrix when kept alone, but the expansion was much higher when HSPCs were co-cultured with MSCs in an autocrine signaling supporting gel [118]. Sometimes, encapsulating gels can have fibrillary structures [123,124], such as in collagen gels, which closely resemble the microscale architecture of the ECM [125] (more examples discussed in suppl. Table S1).

[6] Characterized by flow cytometric analysis of CD133 and/or CD34 expression.

Macroporous hydrogels have a structure with interconnected pores that are large enough to harbor cells. The cells present in the pores have less contact to the surrounding material and a greater contact to the medium compared to encapsulating systems but can still maintain a natural shape [126]. If designed with appropriate stiffness and architecture, porous scaffolds resemble the trabeculae of the cancellous bone. Therefore, they can be used to create a representation of the endosteal niche. In such systems, the scaffold is first prepared and then the cells are added. Hence, contrary to encapsulation gels, porous structures are usually prepared under harsh conditions. To produce the porous complexes, several techniques are used, such as emulsion templating [127], phase separation [128], salt leaching [126,129–131], cryogelation [120,132,133], electrospinning [134], 3D printing [135] or inverted colloidal crystal (ICC) templating [136,137].

Congruent to the mesh size in encapsulating hydrogels, pore size, porosity and interconnectivity of the pores play an important role in these systems. In general, it has been observed for certain cell types, such as MSCs and OBs, that the pore size and porosity influence proliferation, differentiation, migration and aggregate formation [138]. Taqvi et al. observed that a decreasing pore size increases hematopoietic differentiation of murine embryonic stem cells [139]. Some of the effects arising from high porosity might be due to a higher nutrient and oxygen transport [138]. Exchange of soluble factors in macroporous scaffolds can be enhanced by introducing a medium flow into the system, which was shown to influence HSPC fate in co-cultures with MSCs [130,131,133,140]. Rödling et al. showed that perfusion of macroporous scaffolds led to an enhanced exchange of hematopoietic cytokines released by the inherent cells which fostered differentiation of human $CD34^+$ HSPCs[2] [130]. Some studies have demonstrated that non- or very slowly perfused setups can be used to mimic the endosteal niche and promote HSPC expansion, whereas perfused cultures resemble the vascular niche and support differentiation and blood cell production [130,131,133,140] (more studies in suppl. Table S2).

To capture the chemical complexity of the ECM in *in vitro* cultures is a challenging task. In this context, the use of decellularized matrices is worth mentioning as it is an approach to produce hydrogels from a biological source. These scaffolds are derived from tissues or organs in which the cellular contents have been eliminated, but the ECM composition and 3D structure are preserved. Hence, they are biocompatible and retain certain biological activities [141]. After the decellularization process, the tissues can be used as macroporous scaffolds (see suppl. Table Section 2.5).

The majority of these studies have observed that a more primitive HSPC subpopulation is maintained in 3D hydrogel systems compared to 2D cultures. In some studies, the 3D expanded HSPCs were able to engraft and repopulate in mice [132,142–144]. Some have also succeeded in achieving lineage-specific differentiations [123,124,136,137,145]. The important observations and results obtained from these studies are briefly summarized in suppl. Tables S1 and S2.

6.1.2. Tunable Hydrogel Properties for HSPC culture

In addition to providing a 3D architecture, hydrogels also allow the adjustments in their biochemical functionalization, mechanical properties and degradability to resemble the natural archetype. Similar to 2D, synthetic polymers in 3D hydrogels need to be modified with cell adhesion motifs to accommodate efficient cell-material interactions. This can be accomplished by coating a porous scaffold with ECM molecules [101,134,146] or by incorporating ECM molecules [147] or only small peptide sequences [126,129] into encapsulating or macroporous hydrogels. As discussed in chapter 2, natural ECM molecules not only provide binding sites and facilitate cell adhesion but also trigger signaling pathways. In synthetic systems, researchers can select the desired biomaterial and functionalization and hence control the signals generated by the artificial ECM, tailored to suit the aims of the study. Some examples are the functionalization with Jagged-1 [127], Delta-like ligand-1 (DLL-1) [136], SCF or IFNγ [95,148]. In the natural environment of HSPCs, signals such as chemokines [29,149], calcium [150] and oxygen [151] can guide the motion of HSPCs. They are often present in hotspots [152] or gradients [153]. Mahadik and colleagues could generate cell density gradients and signaling factors in 3D murine LSK HSPC cultures [148,154]. Marturano-Kruik et al. introduced an oxygen gradient in a human bone perivascular niche-on-a-chip using MSCs, ECs and breast cancer cells (BCC) [155].

Similar to 2D systems, matrix stiffness influences the fate of HSPCs in 3D as well. Zhang and co-workers reported a higher expansion of murine LSK HSCs[1] in stiffer collagen gels compared to softer hydrogels with a stiffness lower than 100 Pa (14.64 and 36.96 Pa) [156]. In contrast, Gvaramia et al. observed a reduced human $CD34^+$ HSPC expansion[7] with increasing stiffness of a star-PEG heparin gel in ranges much higher than 100 Pa (0.5–3 kPa) [115].

Biodegradable materials are used to mimic the remodeling of the natural ECM [118] (Fig. 3D). These can be either natural components of the ECM, which have cleavage sites for cell-secreted metalloproteases (e.g. collagen and collagenase) or synthetic polymers with incorporated cleavable linkers [95,115,118,142]. Bai et al. used zwitterionic hydrogels with peptide cleavage sites to expand human UCB- and BM-derived HSPCs for several generations. A 73-fold increase of long-term HSCs could be reached as well as a reconstitution for at least 24 weeks in immunocompromised mice (details of evaluation methods in suppl. Table S1 Section 1.3) [142].

Another feature of some materials in HSPC culture is the ability to regain the original shape after having been compressed. This attribute is used to generate injectable scaffolds [157] (see suppl. Table S2 Section 2.4). Tavakol and colleagues were even able to create an artificial *in vitro* system with different niche cells and factors, which could be completely injected while maintaining *in vitro* generated ECM molecules and cell-cell contacts [132].

6.1.3. From hydrogels to more complex niche mimetics

Mimicking the niche requires the combination of its chemical and biological components while ensuring appropriate biophysical properties. It was shown that some effects on HSPCs only arise if two or more properties of the system are combined [118,126]. It is not possible to achieve this task only by hydrogel systems. For a near complete representation of the natural HSC microenvironment *in vitro*, aspects of the bone, the soft BM and vasculature should be considered by combining inorganic (discussed in chapter 6.2) and organic materials (Fig. 3E). Additionally, one should take into account the biophysics of the niche by including medium flow, appropriate 3D architecture, niche cells as well as chemical niche factors including ECM molecules and cytokines. An attempt towards this goal was made by Bourgine et al. in 2018 by combining an inorganic scaffold modified with ECM by stromal cells in a bioreactor which resulted in an artificial BM system that supported HSPC maintenance [140]. Hydrogels in combination with 3D bioprinting (discussed in chapter 6.3), on-a-chip devices (discussed in chapter 7) and bioreactors [145,158] (more examples in suppl. Table S2) could bring us a step closer to achieving a model which would recapture the niche complexities to a higher extent. Some researchers have already used 3D printed grids as macroporous scaffolds with favorable results [134,135]. In 2018, Braham et al. designed the endosteal and vascular HSC niche using 3D bioprinting and studied the survival and proliferation of primary human

[7] Cell phenotype analyzed by flow cytometry ($CD34^+$, $CD133^+$) and differentiation potential assessed using long-term culture-initiating cell (LTC-IC) assay.

BM-derived CD138$^+$ myeloma cells. They used a bioprintable pasty calcium phosphate (CaP) cement scaffold seeded with osteogenic MSCs to model the endosteal niche and endothelial progenitor cells and MSCs encapsulated in Matrigel® to model the perivascular niche. The resulting system highlighted the importance of the perivascular niche in supporting myeloma cells. This study is an important example of an integrated 3D system, which makes use of both organic and inorganic substances and combines 3D printing technology with encapsulating hydrogel systems to mimic the niche [135].

6.2. Inorganic materials used in BM scaffolds

The bone is constantly and dynamically remodeled, a process mainly controlled by osteoclasts (OCs) resorbing calcified bone material and by OBs rebuilding the bone matrix. As OBs are known to regulate HSC functions [19,20,159], materials that mimic highly calcified bone and enable the *in vivo* processes of bone remodeling are needed to realize such processes *in vitro*. Inorganic materials are best suited to fulfill these criteria. Bone is mainly composed of collagen fibers being mineralized by hydroxyapatite (HAp), which consists of CaP. A biomimetic material hosting OBs and OCs should therefore contain CaP. Till date, there are different materials with diverse CaP phases available in the form of bone pastes, cements, injectable foams, 3D-printed scaffolds, coatings or nanoparticles (reviewed in [160]). Besides CaP, other phosphates such as magnesium phosphate have been established as suitable bone replacement materials [161]. Along with classical phosphate materials, also scaffolds made of calcium silicate or bioactive glass have been developed [162–165]. In addition to calcium and magnesium there are other inorganic ions present in bone, such as zinc [166], strontium [167] or manganese [168], which therefore have been incorporated into bone mimicking materials as well. These diverse substances in all their possible blends lead to a great variety of bone-mimetic materials, which is even expanded as these inorganic materials can also be combined with organic substances. By adding polymers to a CaP cement, the setting time of the paste, washout resistance, injectability, macroporosity, mechanical properties, long-term degradation and drug release properties of the material can be varied [169]. In theory, all inorganic materials which are being successfully used in biomedical applications or bone remodeling studies can be adapted for hematopoietic endosteal niche models to mimic the cancellous bone. In these models, besides HSCs, supporting niche cells such as MSCs or OBs are also seeded into these scaffolds. It was shown that MSCs from BM and adipose tissue cultured on porous HAp scaffolds, produced by foam-gel technique, were able to support murine c-Kit$^+$ HSPC maintenance in mice post transplantation [170,171]. For biphasic CaP scaffolds it was shown that human CD34$^+$ HSPC adhesion and the formation of proliferating colonies[3] is dependent on the stoichiometry of HAp and β-tri CaP; the best results were obtained on a material composed of 50% HAp and 50% β-tri CaP [172]. Composite materials of magnesium-doped HAp and collagen I, which formed a porous and highly fibrous structure, were transplanted into rabbits. They induced the formation of trabecular and cortical bone-like tissue and supported the formation of an osteogenic as well as a hematopoietic niche [173]. Zhang et al. used biphasic calcium-infiltrated HAp foams, in which they co-cultured human Lin$^-$CD34$^+$CD38$^-$ HSPCs and OBs and showed that calcium-infiltration improved HSPC maintenance[2] most likely due to increased local calcium concentrations [174]. Schmelzer et al. embedded an open-porous HAp layer between hollow-fiber membrane layers, which was set-up in a perfusion bioreactor. Therein they cultured human BM-derived MNCs, which turned out to be metabolically more active compared to static culture conditions. The long-term maintenance of human Lin$^-$CD34$^+$CD38$^-$ HSPCs[2] was found to be enhanced by this system [175]. The best mimicry of bone, however, is provided by nature itself which is why the usage of decellularized bone for modeling the niche [155] and HSPC culture [176] is not uncommon. Using this material a maintenance and expansion of human CD34$^+$CD38$^-$ HSPCs could be achieved[2] [176,177].

6.3. Bioprinted scaffolds

3D bioprinting can be defined as precise positioning of biomaterials and living cells using an automated computer-aided layer-by-layer deposition approach to fabricate a 3D functional living tissue or organ [178]. It is a promising technique for a wide spectrum of applications and research fields [179]. This technique allows scientists not only to fabricate scaffolds with specific geometric shapes using one or more types of biomaterials and cells but also gives them the spatial control over the distribution of these materials and cells within the printed scaffold. Muerza-Cascante et al. fabricated a model to mimic the endosteal microenvironment using 3D melt electrospinning writing technology [180]. In this study, the authors fabricated a 10-layered scaffold with 100–200 µm pores using PCL-NF. Therein, primary human OBs were cultured under osteogenic conditions for 35 days followed by introduction of HSCs for 5 days using cytokine-free and cytokine-containing conditions. The addition of cytokines significantly increased the number of CD45$^+$ cells in the 3D co-culture and supported a higher ratio of expansion compared to 2D. Kang et al. prepared a hierarchical scaffold by using 3D printing (rapid prototyping) together with electrospinning, followed by a coating of FN or collagen I for the *ex vivo* expansion of human UCB-derived CD34$^+$ HSPCs. PCL mixed with 10% (w/v) HAp was used for printing the main body of the scaffold and PU was electrospun onto each layer of the scaffold during the process. Later, the surface of the scaffold was treated either with FN or collagen I while the TCP remained untreated. The scaffold coated with FN showed the highest number of TNCs (164-fold), highest ratios of CD45$^+$CD34$^+$ (35%) and CD34$^+$CD38$^-$ (32%) populations[2] after 7 days in culture [181]. In another study, a similar scaffold treated with vitronectin instead of FN was used to study the effects of applying intermittent hydrostatic pressure as a kind of mechanical stimulus on the expansion of human UCB-derived CD34$^+$ HSPCs[8]. The results revealed that the combination of the hierarchical structure and physical stimulation could enhance *ex vivo* expansion of HSPCs while maintaining their phenotypic characteristics [134]. Although the number of studies related to the application of 3D bioprinting for HSCs is relatively low, this technique is quite encouraging for scientists who aim to develop a hierarchical 3D scaffold, which is able to mimic the microenvironment of the HSC niche.

6.4. Approaches to mimic the vascular HSC niche

Probably due to the later discovery of the vascular niche [39,182], most studies mimicking the human HSC niche recapture the endosteal niche. When mimicking the vascular niche, the multitude of different cell types and the biophysical properties characteristic of this microenvironment, e.g. the need for softer substrates compared to endosteal niche models, have to be considered [93,183]. Additionally, the development of a functional microvascular network and sufficient vascularization throughout the material is crucial for an appropriate vascular niche model but difficult to achieve. Despite these challenges, there are a few promising approaches in capturing the complexity of the vascular

[8] Observations were based on flow cytometric analysis of CD34$^+$CD38$^-$ population, CFU and LTC-IC assays.

niche. ECs play an important role in the vascular niche. They have been shown to tightly interact with HSPCs and to enhance their long-term expansion, engraftment, differentiation and regeneration [1,184–188]. Introduction of the adenoviral *E4ORF1* gene into ECs augments their cultivability while maintaining their HSPC supporting properties [189,190]. Many studies use co-cultures of MSCs and ECs as their close interaction is essential when it comes to neovascularization and the formation of a stable vasculature [191,192]. The combination of ECs and MSCs in an appropriate scaffold enables the generation of pre-vascularized bone or tissue with a network of vessel-like structures [193,194]. 3D culture systems using biomaterials as platforms, incorporating HSPCs with stromal cells and relevant ECs, would be an ideal approach for biomimicry of the vascular niche.

The work of Yang et al. [195] underlines the importance of using MSCs and ECs for a vascular niche model as they observed enhanced vascularization, the formation of more capillary-like tubes and positive effects on osteogenesis in the co-cultured samples. They incorporated human vascular ECs and human BM-derived MSCs into an *in situ* forming and injectable double-network hydrogel, which was characterized by a condensed polymer network, enhanced mechanical properties and lower degradation kinetics compared to the respective single-network hydrogels. Another advantage of the used hydrogel is the reversibility of the crosslinks, which enables the proliferation of the cells within the network structure. Even though this hydrogel was not tested with HSPCs so far, it is an encouraging approach meeting some of the above addressed requirements and could serve as a supportive niche-like native ECM model. Another study closely mimicking the BM vascular architecture included controllable shear forces and flow rates using a perfusion bioreactor system aiming for an *ex vivo* platform for human platelet generation from Mks [133]. Tozzi et al. fabricated porous, spongy scaffolds out of silk fibroin and achieved cell seeding and perfusion through channels of 0.5 or 1 mm diameter. The fabricated silk sponge was mounted into the bioreactor and the Mks were seeded into the channels. After 36 h, the bioreactor was connected to a pump and platelets were collected due to applied shear forces. With this multi-channel vascular system, they could imitate the structure and flow dynamics of BM capillaries. The incorporated channels enabled perfusion and the procession of large amounts of cells combined with a highly porous scaffold that provides a large surface area for cell attachment. Further development of this system could also incorporate HSCs or ECs, thus stepping towards a more complex model of the vascular niche. Another interesting approach provided a perfusable 3D microvessel system to mimic the perivascular niche [196]. The 3D microfluidic system consisted of a collagen I gel with uniformly embedded BM-derived fibroblast cell lines or MSCs creating a 100 µm diameter network. By perfusing the channels of this network with human umbilical vein endothelial cells (HUVECs), which adhered to the collagen, functional microvessels formed. After cultivating the fabricated vessels for 3-4 days, healthy or leukemic human $CD34^+$ cells were added to the EC-MSC/BM-derived fibroblast cell co-culture via perfusion through the channels. With this model, Kotha et al. were able to demonstrate the dynamics of the multicellular interactions that they additionally could spatiotemporally control and change.

7. Bone marrow-on-a-chip

In terms of complexity, one is only limited by imagination. A very high complexity is achieved in multicellular and self-organizing organoids, which represent life-like models [197,198]. To create dynamic culture conditions and get closer to reality, such multicellular tissue arrangements were cultured in microfluidic devices to generate so-called organs-on-chips [199]. These models consider 3D architecture, cell-cell and cell-matrix interactions and allow a permanent perfusion with cell culture medium. Torisawa et al. were able to establish a BM-on-a-chip, in which they achieved the cultivation of murine LSK HSPCs for at least a week in *in vivo*-like proportions. To do so, they implanted a collagen I gel comprising demineralized bone powder into mice and received a nearly true-to-nature engineered BM containing HSPCs and their progeny[9]. This BM was afterwards surgically removed and placed in a microfluidic device, where the BM was supplied with nutrients [200]. Later, Sieber et al. developed a human cell-based BM-on-a-chip model. MSCs from BM and HSPCs were seeded on an HAp-coated zirconium oxide ceramic scaffold and transferred into the microfluidic device. With this system a long-term culture of up to 28 days of primitive human $CD34^+CD38^-$ HSPCs was enabled [201]. Aleman et al. also used a human cell-based system but deconstructed the HSC niche into the following four niche constructs: periarterial using arterial ECs, perisinusoidal using sinusoidal ECs, mesenchymal using MSCs and osteoblastic using osteogenically differentiated MSCs. All of the cells were encapsulated in a hydrogel consisting of HA and gelatin and placed into four separate chambers of a microfluidic device. Healthy or malignant HSPCs were infused by a circulating channel system. Human $CD34^+$ HSPC interactions with the four different niche constructs could be monitored in real-time using a cell tracking technology [116]. Different niche models combined in one device were also used by Chou et al. They created a system that could balance proliferation, multilineage differentiation and continued progenitor survival while also improving $CD34^+$ cell maintenance compared to conventional culture methods [202]. They fabricated a poly-di-methyl-siloxane (PDMS) chip consisting of two chambers connected through a porous membrane. The top “hematopoietic” chamber was filled with a 3D co-culture of human $CD34^+$ HSPCs and BM-derived MSCs in a fibrin gel representing the endosteal niche. The bottom “vascular” channel, which was lined by HUVECs, assured perfusion of the system with medium containing supporting cytokines. This model spatially mimicked the endosteal and the vascular niche simultaneously, separating them locally. Furthermore, the perfusion was only obtained through the vascular channel similar to the delivery of nutrients throughout the vasculature *in vivo*.

8. Biomaterial-based approaches in hematopoietic differentiation

The *in vitro* production of blood and immune cells holds great promise in the field of regenerative medicine [203,204], immunotherapy [205] and transfusion medicine [206]. Despite the small proportion of $CD34^+$ cells in BM (1–3%), UCB (~1%) and PB (<0.1%) [207], HSCs remain a good starting point for the directed *in vitro* blood production. However, since HSCs are mainly obtained from blood donations, applications for *in vitro* differentiation are limited by their small number [207] and new strategies have to be developed to make current differentiation protocols more efficient. The *in vitro* production of blood cells from HSCs is a promising strategy to gain new insights into the biology of hematopoietic differentiation. It has the potential to make a major contribution towards the availability of safe blood cells [208,209], improved and eventually personalized treatment options and the concomitant reduction of transfusion induced adverse reactions [210–212]. In the last few years, a lot of improvements in the *ex vivo*

[9] Cell phenotype analyzed by flow cytometry; mature, lineage-restricted blood cells ($Lin^-Sca1^+cKit^+CD34^{+/-}$, $Lin^-Sca1^+cKit^+CD150^{+/-}CD48^{-/+}$), hematopoietic progenitor cells (Lin^-Sca1^+, Lin^-cKit^+, Lin^-CD34^+, Lin^-CD135^+), mature erythrocytes ($Ter119^+$), lymphocytes (T cells-$CD45^+CD3^+$; B cells-$CD45^+CD19^+$) and myeloid cells ($CD45^+Mac1^{+/-}Gr1^{+/-}$).

generation of HSC-derived Mks and platelets in different types of perfusion systems, cellular interpenetrating network hydrogels and BM-mimicking biocompatible scaffolds were made, and have been extensively reviewed elsewhere [9–11]. To circumvent the limited availability of donor-derived red blood cells (RBCs), efforts have been made to establish new designs for large-scale blood production with the introduction of several bioreactor-based culturing designs [213–215]. Nevertheless, these approaches are not optimal as under these culture conditions HSCs are pushed into lineage commitment and rapidly exhaust the initial stem cell pool, which raises the costs of *in vitro* production many times over donor-based transfusion [216].

In general, there are two different methods aiming to overcome these issues: (i) the reduction of added supplement concentrations [128] and (ii) the development of a self-sustaining differentiation system [127,217]. However, the avoidance of cytokine stimulation might be disadvantageous in $CD34^+$ monocultures, as the cells are in need of the necessary stimulation to differentiate towards desired cell lineages. On the other hand, Mortera-Blanco et al. indicated that a well compartmentalized 3D culturing environment, based on PLGA or PU scaffolds, may serve as an important counterbalance to cytokine stimulation during hematopoiesis [128]. On this basis, the next major step towards applicable *in vitro* differentiation procedures would be to design a regenerating cell culture system that can maintain human $CD34^+$ HSPC stemness while promoting efficient hematopoietic differentiation as demonstrated by Severn et al. in 2016[10] and 2019[11] [127,217]. These studies clearly show that the use of scaffolds as BM surrogates such as collagen I-coated PU [128,217] and scaffolds prepared by polymerized high internal phase emulsions [127] work well as sustainable culturing models. They provide a fully defined cellular and chemical composition facilitating the maintenance of an initial $CD34^+$ cell population inside the scaffolds over 28 days, while releasing the progenitor cells that can be further differentiated.

Platelets and erythrocytes need specific micro niches within the BM for their maturation. Tight cell contacts are necessary to transmit intercellular stimulatory signals [218] providing opportunities to enhance current differentiation models. This was recently demonstrated by the formation of erythroid progenitors in 3D cell aggregates [219], which are able to imitate the observed *in vivo* cell proximity within erythroblastic islands and support the terminal enucleation of human UCB-derived $CD34^+$ HSPC-derived RBCs. In 2019, Allenby et al. provided evidence that a combinational approach to address current challenges of *in vitro* differentiation, namely low yield, unphysiologically high cytokine concentrations and exhaustion of stem cell pools while maintaining high cell densities, is possible. Using a 3D hollow fiber reactor, consisting of four permeable ceramic hollow fibers surrounded by a collagen coated PU scaffold, they reported the evolution of dynamic hematopoietic microenvironments and a reconstruction of the vascular system and the HSC niche in BM only with SCF and erythropoietin supplementation [220].

T-cells mature in the thymus under the control of Notch receptor-DLL and HLA-T-cell receptor signaling, provided by cell-cell contacts with stromal thymic cells [221]. Therefore, early approaches on T-cell generation were contingent on DLL expressing feeder cells [222–224]. One of the first steps towards stromal-free *in vitro* differentiation was provided by the successful differentiation of human $CD34^+CD38^{-/low}$ HSPCs on Fc-DLL-1-coated plates using surface immobilized Notch ligands [225]. However, these approaches lack clinical applicability as completely xeno-free culturing conditions are required. Kratzer et al. developed a nanostructured bifunctional PEG-DA hydrogel functionalized with RGD and DLL-1 [226], thereby providing a completely xeno-free culturing system. However, in comparison to thymus, the relative simplicity of the system only allowed for an unnatural T-cell differentiation of human UCB-derived HSPCs. The use of ICC scaffolds with a highly geometrical platform and adaptable features, such as pore interconnectivity, size and density, has already provided promising results in supporting both B- and T-cell differentiation *in vitro* [136,137,227]. Lee et al. used a layer-by-layer molecular assembly technique to immobilize DLL-1 Notch ligands on the ICC surface to differentiate human BM-derived $CD4^+$ depleted $CD34^+$ HSPCs into $CD8^-/CD4^+$ T-cells [136].

Considering the varying composition of specific stromal cells in HSC subniches in the BM and the resulting variance in extracellular signals maturing HSCs are exposed to [228], the reconstitution of a biologically reliable imitation for HSC differentiation studies still remains a challenging task.

9. Biomaterials for studying the pathophysiology of HSC niches

Both infectious and malignant diseases can affect BM. While there are only a few biomaterial-based models for infectious BM pathologies [120], most research in this field focusses on malignant diseases. In the subsequent chapters, we will discuss the current scenario and future prospects of ongoing research in biomaterial-based *in vitro* studies investigating malignant diseases affecting the HSC niche in the BM.

9.1. Acute myeloid leukemia

Acute myeloid/myelogenous leukemia (AML) is a malignancy of the hematopoietic system characterized by the accumulation of abnormal blasts in the BM and impairment of normal hematopoiesis [229]. Even if remission rates for newly diagnosed patients are acceptable [230], high relapse rates [231] constitute a major hurdle in the treatment of this disease, which is associated with a low 5-year survival rate [232]. This reflects the inadequacy of currently available options for clinical management of AML, underscoring the need for novel alternatives. An ideal solution would be to design a platform for drug testing that captures enough complexity of the BM, so it has predictive power while being tractable, and does not rely on animal models. Leukemic cells are known to reconfigure their environment, modifying the healthy HSC niche to one that is amicable to their own survival [233]. This niche is where leukemic stem cells are thought to reside. These cells can remain in a quiescent state which makes them resistant to chemotherapy, contributing to relapse after treatment [234].

An early effort [235] explored several FDA-approved materials to create highly porous scaffolds. PLGA, PU, PMMA, PDLLA, PCL and polystyrene foams were obtained using thermally-induced phase separation (TIPS) with pore sizes ranging from 100 to 250 μm. These polymers showed a varying degree of success in maintaining long-term (2 months) viability of several leukemia cell lines, with PU and PLGA performing best. Coating of the scaffolds with ECM proteins (FN or collagen I) significantly improved cell growth. Nair et al. successfully cultured AML cell lines on 3D structures made of a PU/PLLA composite in a range of proportions of the two components [14]. This material was subjected to TIPS to obtain a culture platform with a micro- and nanostructure reminiscent of a decellularized BM and subsequently coated with FN. Markedly higher resistance of the cells to anti-neoplastic drugs was observed compared to TCP and FN-coated 2D cell culture devices, underscoring the key effect of dimensionality. A recent work by Karimpoor et al. [236] used a microfluidic bubbling technique to produce an

[10] Results were affirmed by flow cytometry (CD34, CD36, CD14, CD235a, CD61 and CD233) and immunofluorescence stainings (IgG1, CD44, CD34, CD235a, CD42b, Calprotectin).

[11] Results were affirmed by flow cytometry (CD34, CD36, CD14, CD235a, CD61) and immunofluorescence stainings (IgG1, CD44, CD34, CD235a).

alginate foam to which HAp nanoparticles were added to study chemotherapy sensitivity of primary AML cells. When cultured in these alginate foams, AML cells showed a significant decrease in their sensitivity to doxorubicin.

While the results of AML cell monoculture in 3D platforms are enlightening, other cell types can influence the behavior of leukemic cells. Aljitawi et al. [237] demonstrated that co-culture of leukemia cells and MSCs in PLGA scaffolds was feasible. They showed a general trend of increased resistance of AML cell lines to doxorubicin when co-cultured with MSCs in a 3D culture device. MSCs are not the only cells involved in sustaining the leukemic niche. ECs are known to play a relevant role in AML cell survival [238] and the maintenance of residual disease [239]. Several biomaterials have been used to explore the interaction between leukemic cells and ECs. A starPEG-heparin hydrogel, functionalized with the integrin-binding RGD peptide containing an MMP degradable crosslinker, was used to establish a tri-culture of AML cells (either primary or cell lines), MSCs and ECs. Drug response studies showed increased AML cell resistance to daunorubicin and cytarabine in tri-culture versus monoculture, both in 2D and 3D. Combination of these drugs resulted in total, or near total, disruption of the cultures [240].

9.2. Multiple myeloma

Multiple myeloma (MM), the second most common hematological cancer, is a neoplasia that derives from the proliferation of malignant plasma cells [229]. As in AML, traditional cell culture obviates the role of the microenvironment, which can lead to contradictory results [241,242].

To better study MM in its niche, Kirshner et al. [243] used Matrigel® for *in vitro* 3D studies of MM samples. This system, which included cells from the stroma, was used to study both MM and stromal cell response *in vitro* to melphalan and bortezomib. Despite the significant results reported in this work, the use of Matrigel® is not considered ideal given its batch-to-batch variability and incompletely defined composition [244]; more defined alternatives are preferred. Calimeri et al. [245] used porous PCL scaffolds as an alternative to the human fetal bone chips needed in the SCID-hu mouse model of MM [229]. Seeding of the scaffolds with unsorted BM-derived MNCs from MM patients and implantation into mice achieved *in vivo* expansion of MM cells on a synthetic platform. A 3D culture platform that does not depend on animals was reported by La Puente et al. [246]. They used a crosslinked plasma fibrinogen scaffold and BM cells (stromal cells, ECs and MM cells), both components derived from MM patients, to establish a 3D culture with drug and oxygen gradients in which MM cells showed increased resistance to antineoplastic drugs. Building up on this platform, they were also able to show a recapitulation of patient-specific response to the treatment [247]. Reagan et al. used porous silk scaffolds to establish 3D cultures of MSCs and an MM cell line. The former were induced to deposit mineral on the material to better mimic the structure of the BM, so the MM cells got physiologically relevant environmental cues. As with the other works cited, they found an increased resistance of MM cells to bortezomib in 3D co-cultures [15].

9.3. Bone metastatic carcinomas

Breast, prostate and renal carcinomas commonly metastasize to the bone and result in very poor prognosis and high fatality rate [248]. In 1889, Dr. Stephan Paget shed some light on this phenomenon with his “seed and soil” theory, suggesting that the incidence of metastasis in certain organs is not by chance [249]. The presence of dense vascular networks, different cells and a rich milieu of soluble factors makes the BM a preferable “soil” for tumor cells or the “seeds”. [250]. Several studies have drawn parallels between the homing of HSCs and tumor cell migration to the BM [251]. Signals from the primary tumor sites recruit supportive stromal components in the BM and lay the groundwork for tumor cell arrival, establishing the so-called “pre-metastatic niches”. Furthermore, it has been observed that these cells compete with the HSCs to occupy their niches, thereby giving rise to the concept of “hijacked niches” [250]. Thus, it makes sense to mimic the potential permissive niches for understanding the mechanisms of tumor progression in bone metastasis. Allocca and colleagues observed that human BCCs preferentially migrated to the OB- and microvasculature-rich regions in a mouse model. A rise in the number of BCCs homing to the bone after HSPC mobilization predicated a niche overlap [252]. Similarly, human prostate cancer cell lines directly competed with HSPCs to occupy murine HSC niches and utilized the CXCL12/CXCR4 pathway to gain access and egress from the HSC niche area [253]. Often it has been seen that tumor cells acquire stem cell characteristics, mainly self-renewal and quiescence, resulting in overt metastasis and chemoresistance, respectively [250]. Shiozawa et al. used a mouse model to show that prostate cancer cells migrating to the BM, specifically to the osteoblastic niche, acquired stem cell characteristics [254]. Cancer cells are also mechanosensitive [255] hence it is very likely that they take over the mechanotransduction pathways of the stem cells. Considering all scenarios, it is quite important to investigate the events occurring in the BM HSC niche post tumor cell arrival. Starting with simple hydrogels to more complex systems involving microfluidics (reviewed in [256]), on-chip devices (reviewed in [257,258]) or bioprinted scaffolds, biomaterials have indeed progressed the study of metastasis to a great extent. The lionś share of *in vitro* biomaterial-based studies focusing on tumor-stromal-endothelial interactions in BM have used MSCs, OBs, ECs or fibroblasts, which have clarified many mechanisms, opened up new therapeutic possibilities and paved the way for further research. For example, Bray et al. designed an advanced biphasic *in vitro* microenvironment that mimicked breast tumor tissue (MCF-7 and MDA-MB-231 in a hydrogel) spatially separated with a mineralized bone construct (human primary OBs in a cryogel). 3D printed nanocomposite matrices have served as a tool to study BCC-MSC [259] and BCC-OB [260] interactions. A 3D microfluidic model developed by Bersini et al. recreated a vascularized bone microenvironment with a tri-culture of MDA-MB-231 cells, osteogenically differentiated human BM-derived MSCs and HUVECs. They observed that MSC-conditioned medium promoted extravasation and bone-secreted CXCL5 and BCC-secreted CXCR2 played important roles in the process [261]. Microfluidic devices have also enabled investigations about circulating tumor cell tropism to distant secondary organs [262], extravasation [263] and mechanoregulation [264]. In 2018, Marturano-Kruik et al. developed a perfused bone perivascular niche-on-a-chip to investigate the progression and drug resistance of BCCs colonizing the bone. It included a functional human tri-culture using MSCs, ECs and MDA-MB-231 cells with stable vascular networks within a 3D native bone matrix cultured on a microfluidic chip. The incorporation of controlled flow velocities, shear stresses and oxygen gradients led to long-lasting, self-assembled vascular networks without the need to supplement with angiogenic factors. The authors observed that the MDA-MB-231 cells, exposed to interstitial flow within the system, persist in a slow-proliferative state associated with increased drug resistance. They concluded that interstitial flow promoted the formation of stable vasculature and mediated cancer cell colonization [155].

Progress in tissue engineering has prompted the integration of bone scaffolds into animal models in numerous studies with very promising results. One of the first experiments was performed in 2007, when Moreau et al. successfully incorporated a cell-laden silk

scaffold in mice and studied the combined effect of BMP-2 and BM-derived MSCs in breast cancer bone metastasis [265]. An interesting study from 2018 used collagen I-coated ICC hydrogel scaffolds, incorporated with stromal cells to design a "pre-metastatic niche". A vascular niche was formed post implantation, which recruited circulating tumor cells, released from a human prostate tumor xenograft [266]. As is evident from the discussed references the majority of experiments conducted to explore the effects of metastasis on HSPCs and vice versa, are *in vivo* studies. Indeed, biomaterial-based xenografts have the potential to mimic the complexity of the niche and can be considered a powerful approach. However, owing to the dissimilarities between murine and human hematopoietic systems, the capability of these models to capture the long-term evolution of the metastatic niche and their relevance to humans remains a good question. *In vitro* studies incorporating primary human HSPCs and metastatic cancer cells along with other niche components utilizing a biomaterial platform are quite underrepresented. The fact that there is an immediate need for biomaterial-based *in vitro* models of various metastatic niches, which delve deeper into the human HSC and metastatic cancer cell interactions, demands the attention of researchers.

10. Future prospects

Looking back at the last 20 years of research on HSC niches, fast progress was made by assessing them and their biology in animal models – mostly mice. In these settings, the influence of individual factors was studied in "top-down" approaches by looking at the entire functional system in the animal and eradicating, influencing or adding particular factors and studying the effect of these alterations on the hematopoietic system. Attempts to recapitulate the HSC niche *in vitro* have mainly been guided by the perspective of producing hematopoietic cells for cellular therapies or testing drugs in biomimetic systems. However, these systems can also be used in fundamental research with "bottom-up" creation of artificial HSC niches. These simplified artificial niches allow to add one factor after another or to modulate parameters independently and thus, to study the influence of each individually added factor on the HSC niche – a "bottom-up" approach of research synergistically supplementing the "top-down" approaches in animal studies. Biomaterials play an important role in the designing of artificial HSC niches and the rapid progress in this field allows engineering more and more complex systems. In this way, artificial niches will become a valuable tool for elucidation of the HSC niche as well as for clinical and pharmaceutical applications. This includes not only the biology and biochemistry of the HSC niche but also its biophysics. The importance of biophysics in the HSC niche is becoming increasingly clear; however, many studies are phenomenological. The molecular pathways underlying the sensitivity of HSCs to biophysical parameters such as matrix stiffness or surface patterning is yet to be discovered. These studies will be enabled by biomaterials that allow tuning individual parameters such as surface patterning, matrix stiffness, shear forces or porosity precisely and independently.

Advances in the fields of biomaterials, biochemistry and cell biology create more and more complex artificial HSC niches that get closer and closer to their natural archetype. As biomaterials are the focus of this review, on-chip-devices and bioprinting need mentioning as examples for such advances. BM-on-chips have already been established. They allow integrating artificial HSC niches with microfluidics. Furthermore, on-chip-devices open the avenue to investigate BM- and HSC niches-on-chips in combination with other organs-on-chips and are promising for drug screening purposes. This also applies when pathophysiological processes affecting multiple organs such as bone metastasis are mimicked. Bioprinting allows combining different types of cells and materials within one printed model to an extent, which is difficult to achieve with classical biomaterial synthesis routes. This is particularly important for BM analogs, in which a multitude of different cell types along with materials mimicking soft marrow and stiff bone tissue need to be added and precisely positioned for a full biomimicry. Combining bioprinting with on-chip-devices will greatly enhance the possible complexity that can be used to rebuild the HSC niche *in vitro*.

Mimicking pathophysiological conditions of the BM with the help of biomaterials is an emerging field of research. Often, materials for 3D culture or mimicking healthy BM are applied and brought to a diseased phenotype by applying disease-derived primary cells or cell lines. However, in many diseases not only the cells but also their environment and its material properties are changed. Biomaterials will allow to mimic also these diseased environments and thus bring disease models to a new level of biomimicry. Fabrication of 3D systems incorporating multiple cell types which facilitate differentiation of HSCs to mature blood cells is another application that will largely benefit from such complex biomimetics.

Overall, biomaterials have brought new aspects into research on HSCs and their possible applications. The potential of combining tailor-made biomaterials with HSC research is not fully unlocked yet. Novel developments in the field of biomaterials will further enhance the chances of this interdisciplinary approach for fundamental research on HSCs and their applications.

11. Summary and conclusions

Biomaterials have paved the way for unraveling the importance of cell-material interactions in attempts to control stem cell behavior *in vitro,* both for fundamental research as well as applications including cellular therapies or drug-testing platforms. HSCs present a specific case in comparison to other stem cells. This is because their niches in the BM, on which they depend, are highly complex and intricate microenvironments, and hence are difficult to mimic *in vitro*.

2D biomaterial systems allow to dissect the niche complexity into particular parameters and factors and to investigate their influence on HSCs independently. These studies led to the identification of crucial ECM proteins and cellular ligands for *in vitro* HSC culture and to the observation that they are not only sensitive to the biochemical but also to the physical properties of their environment such as stiffness or ligand nanopatterning and topography. 3D biomaterial systems allowed a closer mimicry of the natural scenario. Hydrogels and inorganic bone-mimicking scaffolds are generally used and have shown that the 3D architecture, direct and indirect communication of cells, diffusion of soluble molecules and their availability within the 3D structures play important roles. Co-cultures of HSPCs with niche cells proved to be efficient in realizing important niche characteristics *in vitro*, namely allowing for balanced HSC maintenance (to some degree) and differentiation. While maintaining HSCs *in vitro* is the fundamental basis for applications aiming at HSC multiplication for transplantation, targeted differentiation in an artificial environment is the basis for *in vitro* blood production. Therefore, biomaterial-based approaches gain importance in studies regarding *in vitro* production of mature blood cells.

The tight balance of the niche gets disturbed in malignant diseases of the hematopoietic system or bone metastasis. *In vitro* models of such diseases enable fundamental studies on the steps in disease progression that are difficult to assess in human patients or regarding processes that differ between human beings and animal models. Furthermore, biomaterial-based approaches allow the preparation of drug-testing platforms for different diseases and patient-specific models for individualized medicine. While several studies show the principal applicability of HSC niche or BM

models for drug testing, the transfer to industrial or clinical settings is yet to be established.

The emergence of technologies like bioprinting and on-chip devices have empowered the development and study of more and more complex *in vitro* models of the HSC niche. Furthermore, stimuli-responsive and degradable materials are ready to include time-dependent properties such as matrix stiffening, degradation or growth factor release into niche models. The more complex the designed systems, the closer they get to the natural niche. However, increasing complexity can also hinder concise experiments or the transfer of techniques into application. Therefore, the used system has to be carefully chosen according to the scientific question to be addressed, the needs of the respective experiment, or the particular application. For example, for research on the influence of individual biophysical parameters on HSCs, 2D biomaterial substrates that allow to tune these parameters independently from others, might be better suited than a complex 3D co-culture system. For applications aiming to mimic natural processes of the niche, the complexity of the niche has to be reduced to the essentials for this specific process concerning different types of cells, ECM molecules, soluble factors, 3D architecture and arrangement of the constituents. In this way, simplified artificial HSC niches can be created that are as complex as necessary for the particular purpose and at the same time as simple as possible.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actbio.2021.03.061.

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