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Engineered tissue models to replicate dynamic interactions within the hematopoietic stem cell niche

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Abstract

Hematopoietic stem cells are the progenitors of the blood and immune system and represent the most widely used regenerative therapy. However, their rarity and limited donor base necessitates the design of *ex vivo* systems that support HSC expansion without the loss of long-term stem cell activity. This review describes recent advances in biomaterials systems to replicate features of the hematopoietic niche. Inspired by the native bone marrow, these instructive biomaterials provide stimuli and cues from co-cultured niche-associated cells to support HSC encapsulation and expansion. Engineered systems increasingly enable study of the dynamic nature of the matrix and biomolecular environment as well as the role of cell-cell signaling (e.g., autocrine feedback vs. paracrine signaling between dissimilar cells). The inherent coupling of material properties, biotransport of cell-secreted factors, and cell-mediated remodeling motivate dynamic biomaterial systems as well as characterization and modeling tools capable of evaluating a temporally evolving tissue microenvironment. Recent advances in HSC identification and tracking, model-based experimental design, and single-cell culture platforms facilitate the study of the effect of constellations of matrix, cell, and soluble factor signals on HSC fate. While inspired by the HSC niche, these tools are amenable to the broader stem cell engineering community.

Graphical Abstract

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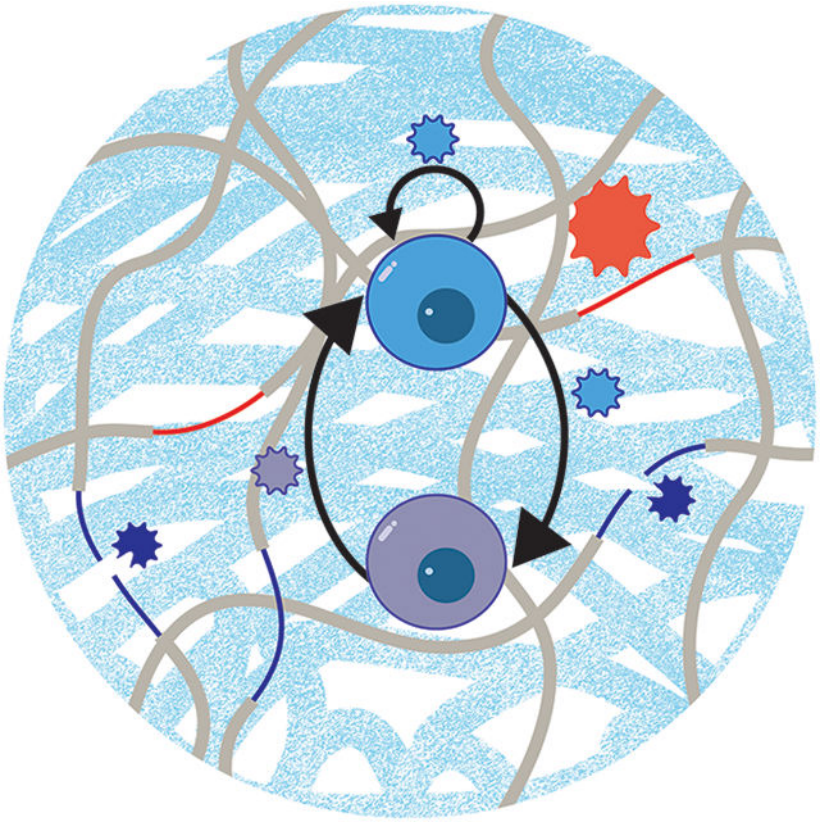

Hematopoietic stem cells (HSCs) are regulated by the complex milieu of the surrounding microenvironment. Biomaterial approaches offer an advantageous route to stimulate HSC expansion through presentation of biophysical, biomolecular, and cellular cues. Synergy among factors (e.g., matrix regulation of cell-secreted factors) has led to the development of dynamic biomaterials and model-based approaches that identify essential features of an artificial niche.

Keywords

hematopoietic stem cell; hydrogel; artificial niche; remodeling; signaling

1. Hematopoietic stem cells in homeostasis and disease

The body's entire complement of blood and immune cells is produced by a small population of cells, termed hematopoietic stem cells (HSCs), that reside in specialized compartments of the bone marrow. HSCs display characteristic traits including the capability to produce multiple progeny, the ability to self-renew, and the ability to remain quiescent (dormant) for long periods of time. Although they make up just a small fraction of the bone marrow (~0.007% murine; ~0.05% human) HSCs produce approximately half a trillion cells daily.[1] The inherent potential of HSCs is limited and defined by intrinsic factors, e.g. DNA, but the continuous process of producing and maintaining a stable blood and immune system is also modulated by instructive cues that arise from the surrounding microenvironment.[2] And while HSCs can be found throughout the body, they reside primarily in the

adult bone marrow, in distinct compartments termed *niches*. Within each niche, the local microenvironment (on the scale of 10^2 μm) is comprised of cellular, biophysical, and soluble factors that provide extrinsic cues to maintain or activate hematopoietic activity.[3] Depending upon external pressures, HSCs may self-renew or differentiate through a series of intermediate cell phenotypes to produce terminal cells within the blood (myeloid) or immune (lymphatic) systems (Figure 1).[1c] Alternatively, HSCs may enter into a quiescent state, in which they maintain a low metabolic rate and are protected from internal and external stresses, such as proliferation or DNA injury, providing an "untapped" source of hematopoietic progeny across the lifespan.[4]

1.1. Identification and isolation of HSCs

Studies of HSCs and their interaction with a defined niche environment date back almost a half century,[5] making them particularly useful for evaluating external regulatory pressures in cell fate decisions. A large area of research has been dedicated to the identification and isolation of HSCs harvested from either bone marrow or peripheral blood using a series of membrane-bound antigens, i.e. surface markers (Figure 1).[6] The increasing accessibility of Fluorescence-Activated Cell Sorting (FACS; flow cytometry) and single cell sequencing has enabled easy identification and isolation of putative HSCs. Advances in the field have identified minimal combinations of surface markers that identify populations of cells that contain the repopulation functionality of HSCs, with a high purity (~50%).[7] A hematopoietic stem and progenitor cell (HSPC) population is typically identified via the presence of stem cell antigen 1 (Sca-1) and tyrosine-protein kinase Kit (c-kit), with the absence of specific lineage markers (lineage negative): Lin- Sca1+ cKit+ (LSK).[6d] Adding additional markers, such as the SLAM family of surface markers (CD150, CD48), further improves the purity of long-term repopulating HSCs.[7-8]. However, surface markers are only an expression of phenotype and require additional verification of cell identify by assessment of functional activity.[9] The gold standard of hematopoietic phenotype analysis is a functional repopulation assay, in which a putative HSC is placed into a mouse which has undergone ablative irradiation therapy, eliminating their autologous ability to undergo hematopoiesis. Any hematopoiesis is therefore dependent upon the transplanted HSC producing a blood and immune system. Additionally, the period of time that hematopoiesis is sustained in the ablated mouse distinguishes long-term repopulating HSCs (LT-HSC) and short-term repopulating HSCs (ST-HSCs), with LT-HSCs sustaining hematopoiesis for 4 – 6 months and ST-HSCs 1 month.[9] The most stringent definition of LT-HSC requires confirmation of self-renewal capacity and uses serial transplantation of putative HSCs from reconstituted mice to demonstrated repeated and sustained repopulation.[10] Alternative, and less time consuming functional assays, such as Colony Forming Unit (CFU) assay require culture of a putative HSC in agar/methylcellulose and, following expansion and differentiation, the cultures are examined for multiple cell types indicative of stem cell potential.[9, 10b]

1.2. Clinical significance: bottleneck in expansion

In addition to their utility as a model stem cell system, HSCs are a valuable therapeutic tool. HSC transplants (HSCTs) are used to treat a range of disorders of the blood (anemias) and immune (leukemias) systems, and are arguably the most common clinical regenerative

therapy due to their capacity to completely regrow a functional hematopoietic system.[11] Instances of HSCT have been increasing steadily since 2000, and in 2019 there were 24,000 HSCTs (United states; 60% autologous).[12] However, despite the increasing use of HSCTs, there is an associated degree of risk, with mortality 1-year post-transplant reaching 50% in some demographics.[13] A nontrivial percentage of these HSCT-related deaths is due to the inability of donor HSCs to successfully home and engraft into the recipient's bone marrow niche,[13] along with enhanced graft versus host disease (GVHD), due to the destroyed bone microenvironment.[14] Autologous transplants, in which HSCs are sourced from the recipient patient, have higher rates of success than allogeneic (non-self) as this reduces the concern of GVHD; however, autologous transplants have an increased rate of disease relapse compared to allogenic transplants, accounting for 80% (autologous) of deaths compared to 27% (allogenic), due in part to contaminating cancer cells and reestablishment of a malignant environment.[13b] Methods to reduce GVHD include removal of T-cells and conditioning regiments, which have led to positive patient outcomes for acute leukemia, however this method is extremely strenuous and is an additional burden on the patient.[15] Additionally, lymphoid deficiency following allogenic HSCT leads to an increased risk of infection and mortality.[16] Typically, allogenic transplants are restricted to younger patients who can better cope with the condition regiment required for successful grafting, while autologous transplants are preferred for solid tumors, lymphoma, and myeloma.[17] It is clear that there are several challenge that must be overcome in order to improve clinical outcomes. However, meta-analyses of patient data have identified a positive correlation between the number of transplanted HSCs and the likelihood of survival post-transplant transplant, with low numbers of HSCs increasing the risk of engraftment failure and delayed lymphoid reconstitution.[18] A rational route towards increasing survival is the use of increased number of HSCs per transplant. However, this is stymied by the rarity of the stem cell population and is further exasperated by the difficulty in finding appropriate human leukocyte antigen (HLA)-matched donors (8 and 19% for patients of African and Asian ancestry, respectively, as of 2010).[19] While the gap in HLA-matched donors has been somewhat lessened by the use of umbilical cord blood (UCB), which poses reduced risk of rejection and immunoresponse from mismatched HLA, UCB transplants still require large cell doses and have delayed onset of hematopoiesis.[20]

These challenges have motivated efforts to develop *ex vivo* culture methods to expand donor HSCs prior to transplantation. There have been instances of astonishing expansion of hematopoietic progenitors via the use of soluble factors. Stimulation of peripheral blood (PB) hematopoietic progenitor cells (CD34+) by pre-transplant exposure to granulocyte-colony stimulating factor (G-CSF) or combinations of interlueken-6 (IL-6), stem cell factor (SCF), thrombopoietin (TPO), and FMS-like tyrosine kinase 3 (FLT-3) have led to increased mobilization and expansion of HSPCs.[21] Mobilized PB contains a high proportion of T-cells and increased risk of GVHD, however it is the most common source of stem cells for HSCT.[20, 22] The use of soluble factors to mobilize and expand HSCs in bone marrow, UCB, and PB, has led to only modest increases in successful homing and engraftment of HSPCs to the niche and overall survival rates, highlighting the need to provide the correct sequences of external cues to provoke sustainable hematopoiesis, all while meeting good manufacturing practices (GMPs).[23] Simple expansion of hematopoietic progenitors and

progeny is not sufficient to maintain hematopoiesis. A rationale for the lack of notable increase in engraftment and survival rate with increased HSPC numbers may depend upon two features of HSCs. One feature is the HSC quiescent state, in which cells have exited the cell cycle and are in a dormant G0-state. However stimulation by exogenous factors can force entry into the cell cycle (G1 – S – G2 – M) whereby the cell proliferates or differentiates.[4b] Ultimately this can lead to exhaustion of the stem cell and failure to maintain hematopoiesis, which requires a balanced pool of quiescent and activated HSCs.[24] The other feature is the heterogeneity in HSC potential. Extensive work by Müller-Sieburg and others, have demonstrated that HSCs can have disparate differentiation capacity, with biases towards production of myeloid-lineage, lymphoid-lineage, or self-renewal.[25] To adequately provide sustainable hematopoiesis, a diverse population of HSCs with diverse potential must be expanded. The combination of these two features, exhaustion and potential, has created an unmet challenge within the field, with no existing protocol established to reliably expand and maintain a stem cell population without exhaustion.[26]

The inability to expand HSCs *ex vivo* is, in part, due to a bottleneck in replicating the complex sequences of signals present in the *in vivo* niche that lead to HSC fate decisions. For example, megakaryocytes regulate HSCs through local secretion of matrix remodeling enzymes and release of release growth factors for quiescence (transforming growth factor beta 1) and for activation (Fibroblast Growth Factor 1) during myeloablative stress.[27] Biomaterials provides the means to replicate megakaryocyte signaling (e.g., matrix remodeling and growth factor release) by presenting a dynamic environment with *in situ* modification of stiffness[28] and sequential presentation of growth factors.[29] The complexity and difficulty of *in vivo* analysis has resulted in an on-going understanding of the spatial and temporal organization of the native niche, with new discoveries leading to an evolving model of the niche. Ultimately, the local microenvironment is responsible for directing hematopoietic response, and design of a culture system for HSC expansion and maintenance will be, by necessity, informed by *in vivo* cues.[30]

2. Defining the HSC niche microenvironment

Our current understanding of the hematopoietic niche suggests a complex, multicellular, microenvironment marked by dynamic changes in biophysical properties, cell-cell contact, and biomolecular factors. While we briefly describe the current understanding of the mechanical, cellular, and soluble factor cues that direct HSC fate, we also wish to point readers to a series of excellent recent reviews that describe the *in vivo* composition of the niche.[2b, 2c, 2e, 31]

2.1. Structure, vasculature, and hypoxia of the bone marrow

The resident home of adult HSCs is the femur and tibia of mice and the axial skeleton (cranium, sternum, ribs, vertebrae, and ilium) in humans. Bone marrow is a complex organ with an endosteal (bone) region and an internal red marrow (hematopoietic region) or yellow marrow (fatty region with little to no hematopoietic activity). The marrow contains a central artery and vessels which branch off and run towards the bone surface (endosteum) to form a dense network of capillaries before returning via sinusoids into the central vein (Figure

2).[2b, 2c, 31g] The network of blood vessels in the bone leads to heterogeneous pressure potentials and velocities, which allow for lodging and settling of mobilized HSCs into areas of low velocity and subsequent immobilization in the bone marrow.[32] Blood flow also provides biomechanical stimuli that regulates hematopoietic activity through hydrostatic and shear forces. Hydrostatic pressure has been shown *in vitro* to preferentially support a hematopoietic progenitor population,[33] and shear stress *in vivo* enhances hematopoietic progenitor potential.[34] Depending upon the species and age, blood flow in the bone marrow is 10 – 20 mL min^{-1} (per 100g tissue),[35] with a hydrostatic pressure ranging from 1 – 15 kPa.[36] The bone marrow is hypoxic (< 5% O_2) and a notable feature of the niche is the gradient in oxygen concentration, with an increased partial pressure of oxygen near the endosteum due to the highly branched and dense vasculature.[37] The hypoxic nature of the hematopoietic niche has been implicated in maintenance, self-renewal, and expansion of HSCs, and hypoxia-inducible factor (HIF) pathways are a putative mechanism by which hypoxia impacts stem cell fate decisions.[38] Immunofluorescent staining of whole bone marrow shows that BrDU retaining (quiescent) HSCs reside in the hypoxic sinusoidal niche.[39] Hypoxia pretreatment enhances homing and engraftment by stabilization of C-X-C Motif Chemokine Receptor 4 (CXCR4) through HIF-1α,[37a, 40] while loss of HIF-1α leads to expansion of the HSC population but a loss of a quiescent population.[41] Marrow hypoxia also acts indirectly in maintaining the niche, with increased levels of HIF-1α in niche-associated cells, including mesenchymal stromal progenitor cells (MSPCs) and megakaryocytes, leading to decreased differentiation of supportive niche cells, enabling a homeostatic and quiescent HSC population.[37a, 38]

2.2. Mechanical properties

There are transitions in mechanical properties within the bone marrow, leading from highly stiff endosteal (bone) to a softer interior (red marrow). Mechanical transitions can extend across several orders of magnitude, such as shifts in the porcine marrow from 0.25 to 25 kPa.[42] Near vasculature, the matrix is soft (0.3 – 2 kPa), while near the endosteum the matrix is quite stiff (>35 kPa).[43] Taken as a whole, the bone marrow is a soft, viscoelastic material with an elastic modulus of <1 kPa, that changes during development, maturation, and age.[44] The viscosity of bone marrow is composition sensitive with a range of 35 – 400 mPa s, depending upon the cellular makeup.[36b, 44] And measurements of the apparent diffusion coefficient of water via MRI show a range of diffusivity (~200 - 600 $\mu m^2/s$), again depending upon the spatial organization of cells and matrix.[45] The bone may also be considered a poroelastic material, in which deformation of the porous bone induces fluid pressure upon cells within the marrow, such as during physiological loading, with an induced shear stress of ~1 kPa.[46] The mechanical properties of the niche are determined predominantly by the distribution of cells and the extracellular matrix (ECM) of the bone marrow which is largely comprised of collagen types I, II, III, IV, X, fibronectin, and laminin, among others.[47] However, while the role of ECM in the HSC niche is not entirely understood, studies have shown the importance of integrin binding, fibronectin, collagen type IV, and tenascin-c.[48] Increasing evidence has highlighted the role of mechanical cues on stem cell fate; while this work is difficult to translate to the weakly adherent HSCs, it is clear that the mechanics of the niche play an important role in hematopoiesis.[33]

2.3. Cellular and secreted factors

The cellular composition of the bone marrow is rich and diverse. The rarity of HSCs suggests these HSCs have the potential to interact with a wide range of niche-associated cells from hematopoietic, vascular, and nervous origins, though recent emphasis in the literature has focused on mesenchymal stromal cells.[31b, 31g, 31h, 49] Cells of the niche influence HSC fate decisions by direct cell-cell contact, secretion of soluble factors, and alteration of the local marrow matrix. The exact origin of secreted factors is difficult to ascertain, however megakaryocytes (hematopoietic origin) and non-myelinating Schwann cells are producers of transforming growth factor beta 1 (TGFβ-1), which has a direct role in promoting quiescence [2e, 4a, 50]. Further, C-X-C motif chemokine ligand 12 (CXCL12) and SCF are active players in maintaining hematopoiesis and are secreted by endothelial cells, mesenchymal stromal cells, and adipolineage cells (Figure 2).[31g, 51] Hypoxic conditions can also stimulate niche cells to secrete soluble factors, with low oxygen inducing expression of vascular associated factors including vascular endothelial growth factor A (VEGFA), which aids in maintaining HSC engraftment within the niche.[37a]

Mesenchymal stromal cells (MSCs) have been strongly linked to HSC activation and dormancy, improved success of HSC transplant engraftment to the reciepient niche, and are co-localized with HSCs in the perivascular regions and central marrow.[31b, 52] While the term MSC broadly defines a heterogenous compartment of cells, there are efforts to identify subpopulations that regulate the HSC population.[53] Mesenchymal progenitor populations that are Nestin+, CXCL12-abundant reticular (CAR), or Leptin receptor-expressing (LEPR+) have all been shown to maintain and recruit hematopoietic activity.[31i] Recent investigations have demonstrated a subpopulation of stromal cells ($CD45^{-}$ $Ter119^{-}$ $CD31^{-}$) that express PDGFRα+ CD51+ and secrete soluble factors that expand and recruit circulating HSPCs.[54] Additionally, osteoprogenitor MSCs have been shown to provide a niche for the retention, activation, and expansion of HSCs *in vivo*.[55] MSCs are believed to mediate HSC activation and quiescence via cell-cell contact and secretion of biomolecules [2a, 31b, 49b, 51, 56] through production of a variety of factors, including CXCL12,[57] IL-6, [50, 58] and TPO.[59] MSCs are also notable due to their interactions with the surrounding ECM, remodeling the matrix via protein deposition and enzymatic degradation,[60] leading to a feedback loop termed *dynamic reciprocity*.[60d, 61] However, *in vivo* studies of the dynamics of HSC-MSC interactions are limited, and are motivating a new generation of biomaterials platforms to study signaling and remodeling mechanisms linked to HSC expansion and quiescence.

3. Biomaterials approach to HSC culture

Use of biomaterials for generation of HSC niche extends to the early 1980s, with the *in vivo* insertion of cellulose ester membranes to provide a physical structure for the formation of a stromal layer that supported hematopoietic function.[62] Ultimately each feature of the *in vivo* niche is reliant upon the other.[63] Secreted factors recruit resident niche cells; cells alter the surrounding matrix through deposition of ECM and degrative enzymes; the matrix modulates biotransport of secreted factors. Consequently, it is likely that the expansion of HSCs *ex vivo* will require combinations of matrix, soluble factor, and cell

types.[41] Biomaterial culture provides an advantageous route for uncovering combinations of external stimuli that can expand and maintain a diverse population of HSCs. For example, HSCs cultured on a highly elastic tropoelastin substrate or separately cultured in the presence of IL-6, IL-3, and SCF led to an expansion of the LSK population; however the combined conditions of HSC culture on tropoelastin in the presence of the cytokines led an additive effect and a significantly greater expansion.[64] Biomaterials provide a biophysical structure that modulates hematopoietic activity in a similar manner to the *in vivo* bone marrow niche. They also enable incorporation of ECM ligands, mechanostimulation, and biotransport of biomolecules for presentation of combinations of external cues to encapsulated cells.[65] While many early studies used 2D and microwell material formats,[66] biomaterials (hydrogels, scaffolds) provide additional means to alter the architecture (nano/macrostructure), chemistry, and biophysical properties of a culture environment.[67] Material properties of the biomaterial, e.g. elasticity and stress relaxation, impact stem cell fate,[68] and soluble factor and matrix interactions direct biotransport of cell secreted factors.[69]

3.1. ECM ligand presentation

Biomaterial systems presenting marrow ECM-inspired ligands, notably fibronectin, have been a focus for *in vitro* HSC culture (Table 1).[70] 2D cultures suggested improved maintenance of immature hematopoietic cells (HSPCs) on fibronectin-coated surfaces at marrow mimetic stiffness with the adhesive motif, RGD, identified as the putative factor.[71] Translating from 2D to 3D fibronectin-coated polycaprolactone (PCL) cultures of human cord blood cultured showed a significant expansion of CD34+ cells (3D: 38-fold; 2D: 3-fold).[72] Separate studies developed ECM scaffolds from MSC secretions, showing integrin-binding improved maintenance of a CD34+ population as well as the proportion of hematopoietic cells in a more quiescent (G0/G1 phase) state, while non-adherent CD34+ cells showed increased expansion (3-fold) and reduced G0/G1 phase cells.[73] Adding on additional layers of complexity to a fibronectin-based culture system, Kurth et al. 2009 demonstrated confinement of HSCs to small microcavities coated in fibronectin led to increased quiescent maintenance.[74] Interestingly, HSCs could be forced to reenter the cell-cycle depending upon the combination of fibronectin microcavities and cytokines (Figure 3).[74] HSPC activity has also been shown to be responsive to collagens.[75] One such study used collagen-coated carboxymethylcellulose microscaffolds to assemble a stable niche, with similar expansion of HSPCs to a 2D control, but with reduced differentiation to lineage-committed phenotypes.[75a] While a large body of work has utilized features of fibronectin and collagen, there are many additional ECM components that can be implemented in a biomaterial system, including a wide range of marrow-mimetic peptides,[76] to stimulate and maintain hematopoietic activity.[48, 77] Glycosaminoglycans (GAGs) are anionic polysaccharides present throughout the marrow and have been used in *in vitro* culture platforms to expand CD34+ populations. Heparin sulfate, a key regulator of cytokine reservoirs *in vivo*,[78] has been demonstrated to support CD34+ expansion up to 6-weeks in *in vitro* 2D culture, demonstrating the capacity for both enrichment of CD34+ (proportion of CD34+) and an increase in absolute cell numbers.[79] In a follow-up study in 3D, a porous chitosan scaffold that was modified to present N-desulfated heparin was capable of maintaining a CD34+ population with progenitor potential over a 7-day period in a perfusion chamber. Notably, there was an additive effect of oxygen and material, with hypoxia

(<5% O_2) leading to a 30% higher proportion of CD34+ cells compared to normoxia (19% O_2).[80] Using a synthetic biomimetic polymerized high internal phase emulsions (polyHIPEs) system with presentation of Jagged-1 peptide, a porous scaffold was shown to promote egress of encapsulated CD34.[81] While this study did not quantify an expansion of progenitor cells, it does provide a route to study the mechanisms leading to mobilization and retention of CD34+ cells in an artificial niche. In another study using 3D printed porous structures of alginate and 10% gelatin, there was a 33-fold increase in CD34+ CD38− over a 10-day period. However, this expansion effect required the co-encapsulation MSCs as a feeder cell. The study found that both conventional 2D and 3D cultures without MSCs had similar detrimental effects on CD34+ CD38− expansion, demonstrating the synergy between co-culture and material selection for HSC expansion.[82]

3.2. Co-culture with niche-associated cells

Co-cultures with putative niche cells provide direct cell-cell contact as well as diffusive transport of soluble factors (paracrine signals). Co-cultures of HSCs with cells of hematopoietic origin or mesenchymal stromal cells have been linked to increased expansion and maintenance.[83] Co-culture with one of the most abundant bone marrow cell types, BM-adipocytes (bone marrow adipocytes), showed BM-adipocytes supported long-term (5-weeks) survival of co-cultured HSCs. Secretomic and genomic analysis suggested high levels of hematopoietic-associated cytokines were secreted by the BM-adipocytes, notably CXCL12 and TGFβ-1.[84] Other stromal cell types such as endothelial cells have been used as feeder cells, with HSPCs either cultured directly on top of epithelial monolayers or grown in media conditioned by vascular-associated epithelial cells.[85]

Recent efforts have begun to engineer multicellular-multidimensional niches, with work from our lab and the broader stem cell engineering community offering increasingly complex culture platforms, especially to examine MSC-HSC crosstalk. Bone marrow-derived MSCs and their differentiated progeny, osteoblasts, were seeded on decellularized bone with HSPCs, in a tri-culture. This led to an expansion of both long (CD34+ CD38−) and short-term (CD34+ CD38+) repopulating HSCs after a 2-week culture, with maintenance of hematopoietic potential as evidenced by functional assays (e.g. CFU).[86] In another example, MSCs were used to create an ECM-coated hydroxyapatite scaffold which led to an expansion of an HSPC population and sustain proliferation of differentiated hematopoietic cells.[87] Collagen hydrogels cultured with HSCs and bone marrow-derived MSCs supported the formation of an endosteal niche for expansion of hematopoietic progenitors, compared to liquid suspension which resulted in HSC differentiation.[75c]

3.3. Matrix and biotransport of cell-secreted factors

Cell-secreted factors are an essential feature of the *in vivo* and *in vitro* niche. Co-cultures of HSCs and various other cell types (notably MSCs) have established the importance of heterotypic cell signaling in an artificial niche. Soluble factors can play both an inhibitory and promotional role in HSC expansion, and may individually lead to expansion, but in combination lead to maintenance, with effects further mediated by ECM proteins and cell-matrix interactions.[56b, 74] While the matrix environment can directly influence HSC activity, transport of cell-secreted factors is modulated by the dense interconnected network

of cells and the poro- and viscoelastic properties of the tissue environment.[69b, 75b, 88] In a biomaterial-based culture system, biotransport is modulated by the chemical and physical properties. Intrinsic factors such as electrostatic and steric hindrance and ECM-binding motifs (e.g. proteoglycans, amino acid side groups) can lead to sequestration of factors, and extrinsic mechanisms such as perfusion and flow can increase or bias presentation of factors.[89] The synergy between matrix and soluble factors can be exploited by designing matrix properties such that discrete domains of cell signaling (autocrine vs. paracrine signaling) can be engineered into the system. In a matrix with a small mesh size and low diffusivity, there is a limited range in which secreted factors may propagate, leading to a predominantly autocrine feedback rich environment. Conversely, in an environment with a large mesh size and unhindered diffusion, cell-secreted factors can travel farther and have a larger radius of influence, allowing for paracrine signaling. The potential for synergistic coupling of cell signaling and matrix properties has previously been demonstrated for HSCs in collagen and gelatin-based hydrogels. In recent work from our laboratory, we first showed HSPC maintenance was improved in co-cultures with hematopoietic lineage cells in a collagen hydrogel designed with low diffusivity to promote autocrine feedback (Figure 3).[75b] Separately, HSC maintenance in a co-culture with MSCs was dependent upon the transport properties of a gelatin-based culture platform, which allowed for increased communication between neighboring HSCs and MSCs.[88a] The difference in HSC maintenance in high (for MSC co-culture) and low (for Lin+ hematopoietic cell co-culture) diffusivity hydrogels was a function cell-cell crosstalk of the different niche cell populations. These findings highlight the opportunity to alter the local diffusive properties of a hydrogel environment in multicellular culture to selectively improve HSC expansion and quiescence.

The complex interaction between biomolecular signaling and matrix properties offers an opportunity to regulate production and biotransport of cell-secreted signals in a heterotypic culture of HSCs and niche-associated cells. In designing a hydrogel culture for HSC encapsulation, the material properties must be considered to allow for control of autocrine or paracrine signaling. Models that predict mesh size and biotransport[90] are especially important within this space, as they allow for the prediction of cell-signaling domains. The utility of such models is demonstrated by recent work by Axpe et al., which established a Multiscale Diffusion Model (MSDM) which models the diffusion of a solute within a chemically or physically crosslinked hydrogel.[91] Additionally, recent work by Richbourg et al. has extended predictive equations of biotransport and material properties to encompass a broader range of hydrogel chemistries that expand upon idealized tetrafunctional polymer systems.[92] Importantly, translating solute diffusion models of commonly used biomaterials (PEG, PAA, PVA), requires the use of a mesh *radius* over a mesh *size*, which provides a more descriptive view of solute-hydrogel interactions in 3D geometries.[93] Such models are an important step to identifying hydrogel formulations that can optimize hematopoietic expansion and maintenance through cell-signaling domains.

Excitingly, there is increasing evidence that mesenchymal exosomes, packets of protein, RNA, DNA, and/or lipids released into the surrounding environment provide an important avenue for modulating HSCs. While the role of exosomes in modulating hematopoietic activity is relatively new, there exosomes have recently been shown to promote expansion of

primitive hematopoietic cells in both *in vivo* and *in vitro* settings.[94] These findings suggest opportunities to design engineered marrow environments to modulate HSC activity.

3.4. Dynamic environment: new opportunities

The role of matrix in modulating cell-cell interactions via electrostatic and steric hindrance of secreted factors is further complicated by the dynamic nature of the native bone marrow niche. On the organism-wide level, significant remodeling of the marrow microenvironment is seen across the lifetime, with significant shifts in hematopoietic cell number and activity.[95] However, niche remodeling is not confined only to organism-level process, but also dynamic, local remodeling events that shape unique HSC fate decisions. *In vivo* imaging of HSCs in live animal models reveals that the majority of HSCs are mobile and do not remain in a static niche but progress in a non-linear fashion at a non-trivial rate of ~0.15 μm/s.[96] Additionally, HSC motility and mobilization from niches is partially regulated by circadian rhythm, with 1% of HSCs egressing the bone marrow each day.[31f, 97] This movement is in part mediated by proteolytic activity from various metalloproteases which degrades soluble and bound ECM proteins, mainly gelatin, collagen, and fibronectin, and surface proteins.[98] The action of metalloproteases, namely matrix metalloproteases (MMPs) and a disintegrin and metalloproteinase / with thrombospondin motif (ADAM/ADAMT) are essential for maintenance of normal hematopoiesis. By cleaving the ECM, metalloproteases release sequestered ligands and soluble factors that direct hematopoietic activity and even disrupt signaling pathways, e.g., CXCL12 binding to membrane bound CXCR4 is disrupted by MMP1, 2, 3, 9, and 13.[27a] While a host of cells secrete metalloproteases, MSCs play an important role in the remodeling of the *in vivo* and *in vitro* niche by acting as a source of proteolytic enzymes and deposition of ECM proteins.[60] Ultimately the interplay between cell-secreted ECM and matrix-inspired cues leads to a non-static material, and a feedback loop termed *dynamic reciprocity*.[60d, 61]. As a result of the dynamic nature of the *in vivo* niche and *in vitro* cultures, it is essential to investigate initial set-points and temporal changes in biophysical features (modulus, diffusion, chemistry) of the marrow microenvironment. While largely intractable *in vivo*, well-characterized *in vitro* systems provide an opportunity to manipulate dynamic remodeling events and monitor their influence on HSC activity.

Material systems with degradable motifs have been utilized to replicate the dynamic nature of the *in vivo* niche. A zwitterionic hydrogel with MMP-cleavable links enabled cell-mediated degradation for stem and progenitor cell proliferation (Figure 3).[99] Natural biomaterials with inherent degradation properties, e.g. gelatin, have been used in conjunction with MSCs for cell-mediated remodeling to maintain an immature and quiescent hematopoietic population.[88a] However, the reciprocal relationship between cell and matrix is challenging to mimic and control. Altered matrix properties leads to changes in biotransport properties and altered cell-cell interaction pathways, creating a non-static environment in which autocrine or paracrine signaling are spatially and temporally dynamic.[69b, 69c, 100] The nature of cell-matrix interactions via biosynthesis of ECM proteins, secretion of proteolytic enzymes and their inhibitors, and initial material properties, is highly complex;[101] however, incorporation of the complexity of cell-responsive biomaterials will be essential in efforts to engineer HSC fate.[102] Advances in materials development

have pioneered novel crosslinking schema and has led to the ability to alter material properties, either through cell-mediated processes (enzyme degradation) or user defined inputs (light stimulus, strain, chemistry).[103] In an example of enzymatic intervention, SortaseA, a bacterial enzyme that selectively cleaves the LPXTG amino acid sequence, can be used to reversibly stiffen[104] PEG-peptide hydrogels or effectively recover cell-secrete biomolecules[105] that present SortaseA-reactive motifs. We recently showed that Sortase exposure does not cleave surface antigens on primary murine HSPCs,[106] bolstering the potential to use orthogonal crosslinking and degradation pathways to investigate dynsmic processes within engineered niches. Additionally, a variety of light-sensitive Ruthenium polypyridyl complexes are readily available and permit softening of crosslinked hydrogels through visible- and near infrared light stimulation.[107] As dynamic material chemistry becomes more widespread, future design of an artificial HSC niche will be informed by novel materials that provide the opportunity to capture the essential role of dynamic properties in hematopoiesis.

3.5. Hypoxia and reactive oxygen species

The bone marrow niche is an inherently hypoxic environment (< 5% O_2), and the presence of reactive oxygen species (ROS) can lead to abnormal hematopoiesis and artificial aging of the HSC population.[108] Recently, Broxmeyer et al.,[109] showed the negative impact of ambient air (21% O_2) on the functional ability of HSCs to engraft and repopulate the blood and immune systems of deplete murine models. The loss of transplant functionality can be recovered by reducing oxidative stress on HSCs by harvesting under physioxia conditions (3% O_2) or in the presence of oxygen scavengers (antioxidants). This is another area in which advances in materials development can be deployed, using novel chemistry to actively mediate ROS production and oxygen presence. An increasing accessibility of click chemistry reactions for hydrogel crosslinking and insight into supramolecular biomaterial design has helped to reduce the dependence upon radical-initiated crosslinking of hydrogels that can lead to ROS production.[110] Additionally, the biomaterial matrix can act as an oxygen sink, reducing the availability of oxygen and providing a hypoxic environment.[111] This helps to eliminate any confounding variables linked to ROS and supraphysiological oxygen exposure in HSC *in vitro* culture. The alleviation of ROS and oxygen and its impact on HSC expansion specifically, was strikingly demonstrated in the previously described zwitterionic hydrogel system: by mitigating ROS production, pathways linked to senescence and differentiation were downregulated and maintenance of HSPCs was increased, ultimately leading to a 73-fold increase in the HSC population.[99] In a model of hypoxic insult to brain, gelatin–ferulic acid (GelFA) was crosslinked via Laccase, an oxidative enzyme, that resulted in a transiently hypoxic environment as the crosslinking reaction consumed oxygen. In the same system, oxygen generation was stimulated by introduction of hydrogen peroxide with horseradish peroxidase, demonstrating control over local oxygen and ROS activity.[111b] Such systems can be translated to an artificial stem cell niche to manipulate hematopoietic activity via oxygen and ROS exposure.

4. Hierarchy and heterogeneity in hematopoiesis

HSCs are typically subcategorized based upon their ability to maintain hematopoiesis over specific timescales. Long-term repopulating HSCs (LT-HSCs) make up ~0.007% of the murine bone marrow and have infinite self-renewal capability, providing a continual source of stem cells over the course of the organism's lifespan. The slightly more populous short-term repopulating HSCs (ST-HSCs), which make up ~0.04% of the murine bone marrow, possess a limited capacity for self-renewal and are only able to maintain the full complement of blood and immune cells for a short duration.[1a] Multipotent progenitors (MPPs) represent the final stage of committed stem cells, with no self-renewal capacity and are an intermediate cell phenotype prior to differentiation towards myeloid or lymphoid lineage. This classic model assumes a linear progression from the immature HSC to differentiated terminal cell, with transient intermediate cell-states. This has become increasingly challenged, as evidence emerges of cells by-passing previously assumed differentiation pathways.[112] There are competing models of hematopoietic differentiation cascade, with the classic model suggesting a step-wise hierarchy with a cell fate decision occurring at every branch point. Newer models show either more direct routes to termination, with cell fate decision occurring early while the cell has multipotent potential, or they show a continuum in which all intermediate states between multipotent stem cells and terminal cells are highly transient and do not represent defined cell types.[113] The ability for hematopoietic progenitors to 'jump' across states has recently been confirmed via computational models of feedback in hematopoietic cell culture; here, a subfraction of progenitor cells able to jump compartments is required to fit the observed population dynamics of murine hematopoietic cells maintained in liquid culture.[114]

Recent changes to our understanding of hematopoietic differentiation have been influenced by improved understanding of the heterogeneity of HSC populations and *in vivo* niches.[115] Cell populations that were canonically considered homogenous are now known to possess multiple subsets with differing differentiation capabilities, and despite sharing the same HSC phenotype, may be biased towards specific lineages (Figure 4A).[25, 116] This has important implications in the analysis of *in vitro* cultures containing HSCs. While the *in vivo* niche hosts a diverse population of HSCs with heterogeneous potentials,[31h, 112c] *in vitro* platforms likely contain cues that select for specific subsets and may lead to a homogenization of the stem cell population (Figure 4B).[25a, 25c] Clonal expansion, the proliferation of a single subset of HSCs, is an important feature of the niche, however reduction in the number of distinct and diverse clonal populations has been linked to stress and aging of the hematopoietic compartment [117] and does not capture the balance of lineage-biased stem cells required for healthy tissue in hemostasis.[112c]

4.1. Label-free imaging of HSCs

Functional characterization of HSCs via repopulation assays in vivo are time intensive. And phenotypic assays such as surface antigen presentation do not necessarily capture the potential of a heterogeneous population of HSCs. There is a significant opportunity to develop techniques able to characterize the heterogenous nature of the hematopoietic system that complement existing functional and phenotypic assays. Raman-based imaging

combined with machine learning techniques provide such an approach to characterize hematopoietic cell subsets.[118] The obtained spectra also have the additional benefit of providing a host of biological information, including lipid, protein, and cholesterol content that can potentially be linked to functional activity.[119] Other imaging modalities can similarly provide insightful information about the structure and state of the cell such as recent efforts adapting photonic crystal based biosensors able to support label-free imaging of hematopoietic cells and to trace shift in functional activities (proliferation, apoptosis).[120] Light-scattering properties can be used to ascertain physical characteristics (nucleus size, mitochondrial distribution), and metabolic state can be probed via fluorescence lifetime imaging microscopy (FLIM) to measure auto-fluorescence of bound or unbound metabolites.[121] The HSC community has often led efforts to leverage these new label-free imaging techniques, that go beyond surface antigen quantification, to provide descriptive properties of the cell and engineered HSC niche environments, such the efforts of *Schroeder et al.* that developed long-term, single-cell tracing methods to assemble time-lapse data of HSC fate decisions in the context of engineered marrow environments.[122] The incorporation of imaging and single-cell analysis enables individual cell response to be probed, helping to identify subsets of cells that respond to factors that may be obscured by the population average.[123] By pairing novel identification techniques with functional assays there is the potential to identify new subpopulations of HSCs with distinct repopulation capacity.

5. Model-based approaches in HSC culture

The complexity of cell interactions and the large phase-space of materials, soluble factors, and cells necessitates modeling methods in order to parse out features of a culture system that are linked to a desired stem cell response. Statistical approaches to experimental design have the power to identify initial HSC culture conditions that lead to a desired stem cell phenotype. Response surface mapping (RSM) is a subset of design of experiment methodology that has historically been used in the chemical engineering and materials science field to identify initial inputs that lead to a desired output (e.g. dopant concentrations leading to increased toughness, biodiesel production).[124] Such a system can be applied to design of hematopoietic culture, in which a subset of a large number of combinations, e.g. modulus, cytokine concentration, is used to infer optimal combinations that lead to hematopoietic response. By implementing systems biology methodology and modeling designs, a reductionist approach can be applied to identify biophysical, chemical, and cellular cues that lead to desired stem cell fate. There are several instances of modeling procedures performed on HSC cultures to identify soluble factors, secreted by HSCs or co-cultured MSCs, and correlate them to hematopoietic phenotype. Increasingly, a Partial Least Squares Regression (PLSR) approach is being utilized to identify secreted factors, either autocrine or paracrine, that are linked to hematopoietic recovery. In a model of MSC secretions, proteins were identified that were correlated to increased murine survival post irradiation, and *in vitro* mechanical stimulation was used to prime cultured MSCs to secrete elevated levels of recovery-associated proteins prior to *in vivo* transplantation.[125] The idea of model-based culture conditions is further exemplified in work by Muller et al., in which a comprehensive PLSR model was developed that incorporated culture conditions, including

soluble factors, cell-cell contacts, and material properties, into one model that predicted hematopoietic cell numbers (Figure 3).[56b] In a biomaterials and PLSR approach, Gilchrist et al., orthogonally changed material properties of a gelatin-based matrix and seeding density of co-cultured HSCs and MSCs to identify altered secretome profiles that resulted in increased hematopoietic maintenance. Identified factors were then validated *in vitro* to recover hematopoietic activity without the presence of MSCs. [125b] Model design can grow more complex as additional experiments provide insight. A range of response metrics can be adapted to produce a more accurate picture of hematopoietic activity, including phenotypic data (FACs), label-free imaging (Ramen, scattering), and functional activity (CFUs, repopulation assays). Additionally, time-dependent changes in material properties (dynamic modulus, mesh size) and cell-cell interaction (paracrine, autocrine) due to dynamic remodeling can be incorporated to capture essential characteristics of an artificial HSC niche.

Model-based decisions for hematopoietic culture can help to reduce the complexity of the system. However, to build an adequate model requires identification of parameters that impact hematopoietic activity and validation of the model (Figure 4C). The increased use of single-cell analysis techniques such as single-cell gene expression and time-lapse imaging has shown the importance of combination of cues to identify early stages of hematopoietic stem cell and self-renewal capacity.[126] The increase in availability of genomic and proteomic sequencing also lends itself to model development, as these large datasets can be incorporated as either a response (dependent variable) or a predictor (independent variable) of hematopoietic activity. This has been aided by the scaling down of culture systems and development of high-throughput cultures,[127] as demonstrated by the use of microwells by Muller et al., in the development of their PLSR model. In another work, single-cell gene expressions in conjunction with single-cell culture in individual microwells was used to identify unique genomic signatures linked to a quiescent HSC state.[126] Droplet-based cultures have also been employed to rapidly produce a large number of discrete microenvironments to probe stem cell fate.[127b] Stem cell culture in droplet-based environments have the advantage of controlled number of cells per droplet and reduced length scale for biotransport, enabling controlled cell-cell interactions and ease of nutrient diffusion. This was demonstrated in a microfluidic device with agarose droplets and encapsulation of two hematopoietic cell lines. By varying the ratio of each cell type per droplet, paracrine signaling was modulated and the spatial organization of encapsulated cells was altered.[127a] Development of scaled-down, high-throughput culture systems enables rapid and large-scale examination of external factors; paired with model-based approaches, this allows building and validation of models that identify essential features of an artificial HSC niche. Incorporation of time-dependent cues due to matrix remodeling (e.g., dynamic material properties, biotransport of soluble factors, cell-cell communication) is becoming increasingly necessary, and requires model-based approaches to identify time points where intervention/stimulation is the most impactful in expanding an HSC population.

6. Conclusion: expanding into the future

HSCs in the bone marrow navigate diverse and dynamic matrix, metabolic, and cellular selection pressures. Replicating the cascade of signals responsible for controlling stem

cell behavior remains a critical challenge for biology and medicine. The hematopoietic system offers unique advantages for developing multi-dimensional biomaterial tools to accomplish this goal. Sophisticated tissue engineering models of bone marrow combined with imaging, modeling, and bioinformatics methods offer the opportunity to investigate the coordinated effect of multicellular crosstalk, the matrix environment and cell-mediated ECM remodeling, as well as biomolecular signals and hypoxic stress on HSC activity. Our understanding of the *in vivo* niche and the differentiation potential of resident HSCs is evolving, offering us new insights into design paradigms for an artificial stem cell niche. The transient nature of the niche has motivated the development of dynamic material systems that allow for on-demand tuning of material properties (modulus, diffusion) as well as single cell tracking and analysis methods. Advances in theoretical models of biotransport allow for *apriori* design of biomaterials that select for specific domains of cell signaling (autocrine, paracrine). Additionally, biomaterials can be used to create specific oxygen environments, protecting HSCs from oxidative stress and premature senescence. Each of these design parameters will be essential for expanding HSCs without exhaustion and clonal selection. The expansion of a diverse population of HSCs, mimicking the hematopoiesis potential that of the *in vivo* population will be informed by model-based approaches that help to reduce the large phase space of material, cellular, and biomolecule combinations that exist, and select for optimized conditions that engineer stem cell fate. Ultimately, we are at the beginning of an exciting age of dynamic, instructive biomaterials with the potential to shape the expansion of hematopoietic subsets for both basic science (e.g., engineered tissue models) and clinical (e.g., improve HSCT efficacy) applications.

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Biography

Aidan Gilchrist received his PhD in Materials Science and Engineering from the University of Illinois at Urbana-Champaign. Subsequently, he joined the lab of Sarah Heilshorn at Stanford University as a post-doctoral fellow. His research implements biomaterial strategies to study the role of the surrounding (3D) environment in stem cell response via dynamic materials design and shifts in genomic and proteomic expression.

Brendan Harley is the Robert W. Schaefer Professor in the Dept. of Chemical and Biomolecular Engineering at the University of Illinois at Urbana-Champaign. His research group develops biomaterial to dynamically regulate cell behavior for applications in musculoskeletal regeneration, hematopoietic stem cell biomanufacturing, as well as to investigate endometrial pathologies and invasive brain cancer. He is a fellow of AIMBE, BMES, and AAAS. He is exceptionally proud of his lab's collective efforts to build and model an inclusive & supportive research environment that prioritizes justice, collaboration, & accessibility.

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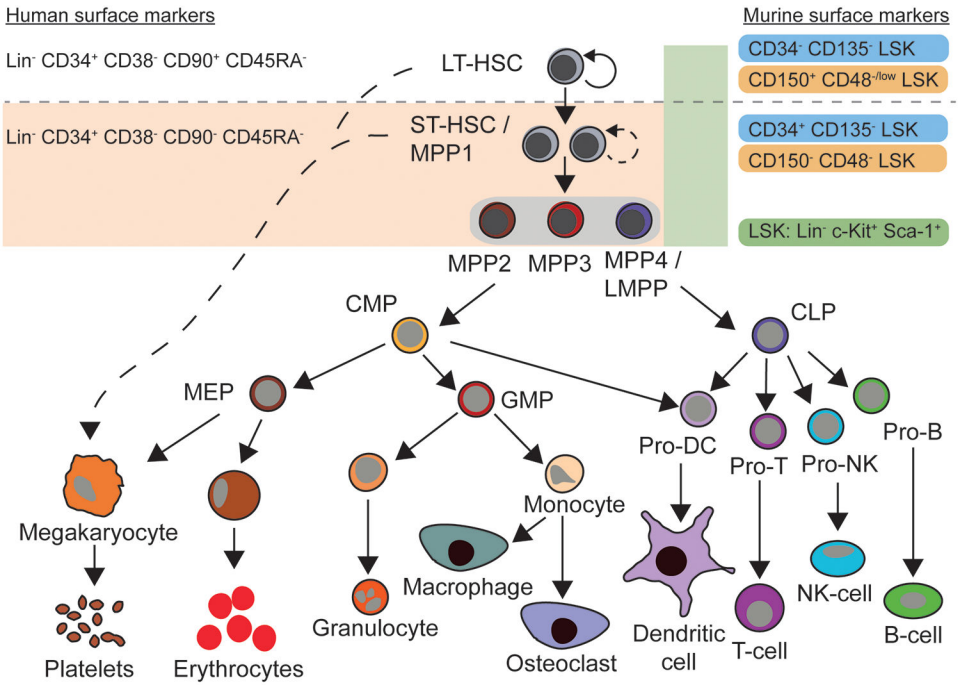

Figure 1: Hematopoietic differentiation hierarchy.

Primitive LT-HSCs, with infinite self-renewal capacity are at the apex of the hematopoiesis process, followed by ST-HSCs with limited self-renewal capacity. ST-HSCs, also known as MPP1, are distinct from the more committed MPPs (2-4) which display no self-renewal. MMPs have potential for either lymphoid or myeloid lineages; however, MMP2-3 are both myeloid-biased, MMP2 is platelet-biased, while the MMP4 (lymphoid-primed multipotent progenitors; LMPP) is lymphoid-biased.[128] A pathway exists for direct differentiation of megakaryocytes from progenitor cells, however the exact path is not yet known.[129]. A number of surface markers exist to isolate and characterize human and murine hematopoietic cells. Lineage markers, CD34, CD38, CD90, and CD45RA separate LT-HSCs from ST-HSC/MMP1-4 in a human system; and a series of different surface marker schemes exist for identification of murine MMP1-4.[1c] For illustration purposes, two such schemes are shown to separate murine LT- and ST-HSCs: the upper (blue) panels denote a CD135 (Flk2/Flt3) approach, and the lower (orange) denote a CD150 (SlamF) approach, the green LSK panel identifies the HSPC population (LT-, ST-HSC, MPP).

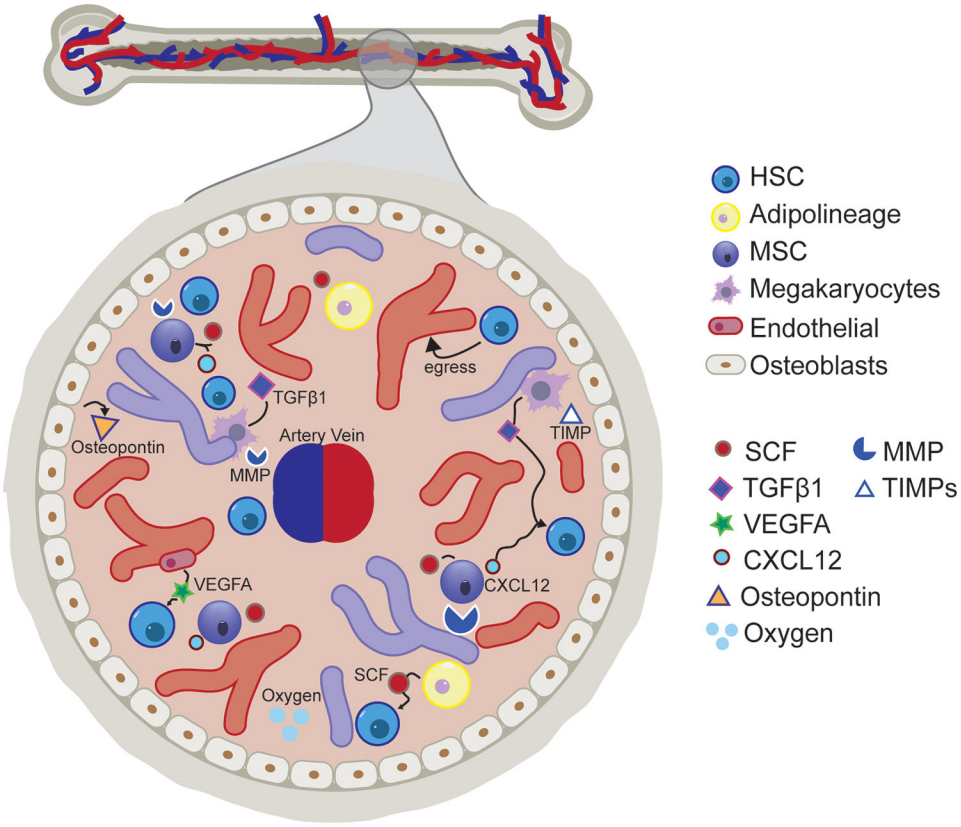

Figure 2: Bone marrow niche.

The bone marrow is a heterogeneous compartment, with a surrounding endosteal (bone) region and radially distributed vascular structures emanating from a central vein, resulting in a gradient of oxygen. Niche-resident cells provide a source of soluble factors and direct cell-cell contact.

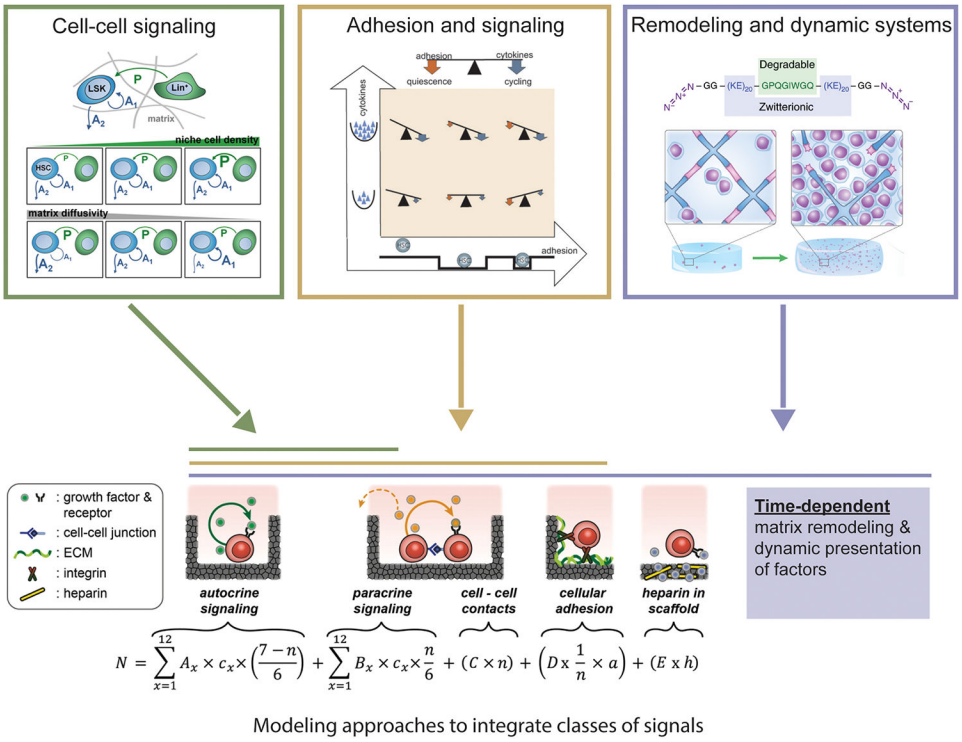

Figure 3: Synergy and integration of distinct extrinsic cues into a cohesive niche.

Individual approaches have identified classes of signals that lead to expansion and modulation of hematopoietic activity. Material properties promote domains of cell-cell signaling dominated by autocrine or paracrine signaling;[75b] Cytokine levels are balanced by adhesion and confinement and adhesion balance to produce regimes of cell cycle-specific stages (quiescence and entry into the cell cycle);[74] Dynamic materials allow for cell-mediated remodeling and time-dependent material properties.[99] Biomaterial approaches have leveraged PLSR modeling to integrate multiple classes of signals (autocrine/paracrine signaling, cell-cell contacts, adhesion) to identify interactions of signals correlated to a desired hematopoietic response.[56b] As models grow in complexity, there are opportunities to incorporate dynamic material systems that provide time-dependent properties and resulting shifts in how cells interaction with their surrounding environment. Panel (Cell-cell signaling) is reprinted from Biomaterials, Mahadik B., et al., Regulating dynamic signaling between hematopoietic stem cells and niche cells via a hydrogel matrix, 2017, 124:54. Panel (Adhesion and signaling) is reprinted from Integrative Biology, Kurth I., et al., Hematopoietic stem and progenitor cells in adhesive microcavities, 2009, 1:427, with permission from Oxford University Press. Panel (Remodeling and dynamic systems) is reprinted from Nature Medicine, Bai T., et al., Expansion of primitive human hematopoietic stem cells by culture in a zwitterionic hydrogel, 2019, 25;1566, with permission from Springer Nature. Panel (Modeling approaches to integrate classes of signals) is reprinted from Scientific Reports, Müller, E., et al., Distinguishing autocrine and paracrine signals in hematopoietic stem cell culture using a biofunctional microcavity platform, 2016, 6:31951, licensed under a Creative Commons Attribution (CC BY) license.

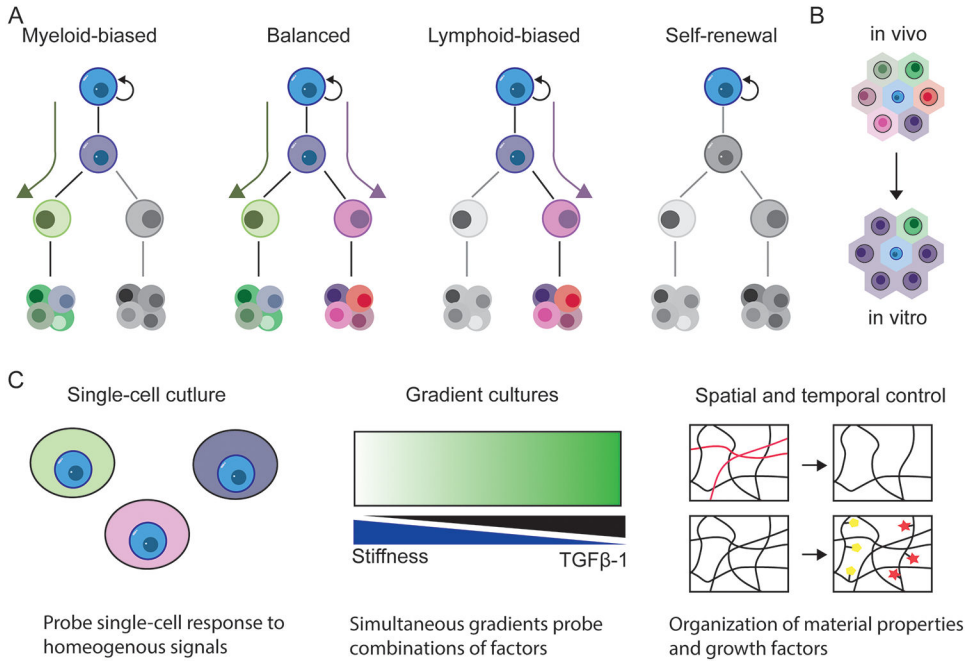

Figure 4: Developing models of hierarchy.

A) HSCs are a heterogeneous population with differing lineage potentials. Individual HSCs may be biased towards specific lineages or self-renewal. **B)** Current in vitro culture approaches lead to a non-uniform clonal expansion, producing an enlarged subset of HSCs that does not represent the *in vivo* HSC pool. **C)** Biomaterial cultures are well suited to probing features of an *in vitro* niche that bias HSC potential: single-cell cultures in microdroplet environments probe the heterogeneous response of HSCs to specific factors,[127a] gradients of factors and material properties identifies combinations and synergies among extrinsic cues,[130] and spatial and temporal presentation of multiple factors at specific time points.[131]

Table 1:

Summary of biomaterial approaches to expand and study hematopoiesis.

Material	Soluble and cellular cues	Ligand presentation	Material properties	HSC response
2-dimensional				
[71]Functionalized polyacrylamide		Fibronectin Collagen Laminin	3.7 – 44 kPa	Endosteal niche mimetic surface: maintenance of myeloid-biased progenitors on 44kPa fibronectin surface
[64]Tropoelastin	IL-3, IL-6, SCF		Highly elastic (extensional)	Mechanisms of LSK expansion from cytokines vs elastic surface were distinct as the combination of cytokines and tropoelastin led to an additive increase in LSK expansion.
3-dimensional				
[72]Polycaprolactone		Fibronectin		38-fold expansion of CD34+ cells. 8-fold increase of CXCR-4 expression in 3D culture compared to 2D control.
[73]Decellularized ECM	MSC-derived ECM. Includes MSC-secreted CXCL12	MSC-derived ECM	Sequestration of MSC-secreted factors	Adherence promoted a morphological and biomechanical state similar to freshly isolated CD34+ cells and maintained a quiescent state.
[74]Fibronectin-coated PDMS	TPO, SCF, FL3	Fibronectin	Microwells Microwell size: 15 – 80 μm 1 to 30 cells per microwell	Quiescence is promoted by confinement and high fibronectin engagement: small well size (15 μm). Effect is abrogated by high levels of cytokines.
[75a]Collagen-coated Carboxymethyl-cellulose	OP9-MSCs HSPC:OP9 1:100, 10:100	Collagen		OP9 cells secrete cytokines and provided a substrate for HSPCs adhesion. Cells self-organized in the scaffold, with OP9 cells lining the pores and HSPCs nestling in the lining.
[80]Chitosan	Hypoxia	Heparin	Perfusion (1.5 mL/min), heparin sequestration, anisotropic pores ~ 4 – 70 μm diameter (bottom – top of scaffold)	Fluid flow, hypoxia, and 3D environment combine to promote enhanced rates of progenitor retention compared to each factor individually
[81]Polymerized high internal phase emulsions (polyHIPEs)			Pore size 10 – 130 μm diameter~13 kPa (unfunctionalized)~44 kPa (functionalized)	Highly porous scaffold hosted a primitive CD34+ population with continuous egress of lineage-committed cells into the surrounding liquid suspension over a 28-day period
[82]Alginate and gelatin	Umbilical cord MSCs		Millimeter-scale pores for nutrient flow. Micrometer-scale pores for cell deposition.	Increased CXCR4 expression in 3D groups. Presence of MSCs was required for expansion of HSPC population over 10-days
[75b]Collagen	Lineage-committed hematopoietic cells		Auto/paracrine signaling regulated by matrix hindrance to biotransport. Decrease of 40% in mesh size (high to low density gels).	Autocrine feedback which promoted expansion of early progenitors.
[87]Hydroxyapatite, ECM	MSC	MSC-derived ECM		Expansion of progenitor and lineage-committed hematopoietic cells in an artificial osteoblastic niche, that promoted increased Nestin+ MSC expression.
[99]zwitterionic poly-carboxybetaine		MMP-cleavable GPQGIWGQ peptide	Initial stiffness: 0.7 kPa Time-dependent material properties over 24-day culture	Significant expansion of a long-term HSC population. Zwitterionic hydrogel inhibited ROS production.