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Bone marrow microenvironment and organ chips: Advances in tumor dormancy research

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Abstract

Bone metastasis presents a major challenge in oncology, often involving prolonged tumor dormancy within the complex bone marrow microenvironment (BMME). This dormancy, characterized by halted proliferation but sustained viability, poses risks for late recurrence and therapy resistance. Recent advancements in bone marrow-on-a-chip (BMOC) technology provide highly controllable, physiologically relevant biomimetic platforms to model the intricate cellular and molecular interactions governing BMME-regulated dormancy. This review focuses on BMOC-based approaches, examining their principles, distinct advantages, applications, and key findings in elucidating mechanisms of tumor dormancy regulation. Critically, it addresses current technical and biological limitations of BMOCs (e.g., replicating full immune component complexity) and propose concrete future directions for enhancing BMOC development and integration with complementary technologies. Enhanced understanding through refined BMOC technology could fundamentally uncover dormancy mechanisms and advance novel therapeutic strategies for metastatic control.

Keywords: Bone marrow microenvironment; Organ-on-a-chip; Microfluidics; Tumor dormancy; Organoid

1. Introduction

Cancer remains a leading cause of global mortality, with metastases driving advanced disease progression.[1,2] Bone, a frequent metastatic site, poses significant clinical challenges due to debilitating skeletal complications and the elusive nature of tumor dormancy.[3] During this clinically silent phase, disseminated tumor cells (DTCs) enter a state of mitotic arrest and metabolic quiescence, evading detection and exhibiting resistance to conventional therapies.[4] Crucially, the bone marrow microenvironment (BMME) governs this dormancy–reactivation balance through complex interactions involving stromal, immune, and cytokine networks.[5,6] Traditional murine models face substantial limitations in capturing the dynamic interplay and multicellular complexity of the human BMME.[7] Key challenges include difficulties in real-time monitoring, a lack of precise microenvironmental manipulation, and ethical/practical constraints highlighted by recent regulatory shifts, such as the FDA Modernization Act 2.0.[8] These shortcomings underscore the need for alternative, human-relevant models.

Bone marrow-on-a-chip (BMOC) technology emerges as a transformative solution. By integrating patient-

derived cells within microengineered platforms that recapitulate physiological cues (e.g., shear stress and cytokine gradients), BMOCs enable unprecedented real-time observation and systematic dissection of dormancy mechanisms.[9] Their modular design allows precise reconstitution of key BMME components—such as angiogenic niches with controlled hypoxia or neuronal interfaces—facilitating mechanistic studies of dormancy induction and escape that are difficult or impossible in animal models.[10] This human-centric approach inherently enhances translational relevance. Coupled with high-throughput screening capabilities, BMOCs represent a powerful, emerging toolset.[11,12]

While BMOC applications in tumor dormancy research are nascent, their potential to decipher the spatiotemporal logic of dormancy and accelerate therapeutic discovery is substantial. This review introduces research progress on cellular crosstalk within the BMME regulating tumor dormancy. It also discusses the conceptual framework and design principles of BMOC platforms for modeling biological mechanisms of the BMME, ultimately aiming to provide novel perspectives and methodologies for tumor dormancy research and therapeutic interventions against bone-metastatic tumors (Figure 1).

2. The BMME: A master regulator of tumor dormancy

While DTCs colonizing distant organs face the challenge of survival within foreign microenvironments, the bone marrow emerges as a critical sanctuary for metastatic cells. The BMME exerts deterministic control over DTC fate through a functionally paradoxical duality: it enables efficient hematogenous dissemination yet actively enforces tumor dormancy through conserved regulatory programs (predominantly characterized in animal models, necessitating verification in human contexts). This dual role positions the BMME as a central rheostat balancing dormancy maintenance and reactivation. Three interconnected mechanisms govern this regulation: (i) cellular crosstalk between DTCs and resident BMME populations (e.g., stromal cells and immune cells); (ii) molecular signaling cascades enforcing cell cycle arrest and metabolic adaptation; and (iii) niche remodeling events triggering dormancy escape. Collectively, hypoxic gradients, cytokine networks, and mechanochemical cues within the BMME establish a dynamic equilibrium where dormant DTCs persist in quiescence while retaining reactivation potential. Decrypting this regulatory logic requires systematic dissection of how BMME components spatiotemporally constrain DTC proliferation—a complexity challenging to recapitulate in conventional models.

2.1. Cellular orchestrators of dormancy within the BMME

The BMME coordinates tumor dormancy through dynamic niche reprogramming, where specialized micro-regional endosteal, perivascular, and immune niches switch between dormancy enforcement and reactivation initiation through cell crosstalk[13,14] (Figure 2). In the endosteal niche, osteoblast-derived growth/differentiation factor 10 and transforming growth factor (TGF)-β2 bind to TGF-β receptor III on DTCs, leading to the activation of p38 mitogen-activated protein kinase (MAPK). This signaling cascade induces phosphorylation of the retinoblastoma protein at N-terminal sites (S249/T252), thereby enforcing G0/G1 cell-cycle arrest and establishing dormancy in DTCs.[15] The perivascular niche utilizes bone marrow mesenchymal stem cells (BMSCs) to guide DTC homing through the C–X–C motif chemokine 12 (CXCL12) gradient[16,17] while inducing dormancy through growth arrest-specific protein 6 signaling[18-20] and exosomal microRNAs-mediated cell cycle inhibition.[21-23] Tumor-derived TGF-β1 transforms BMSCs into cancer-associated fibroblasts,[24] which secrete interleukin-6 (IL-6)/matrix metalloproteinase 9 (MMP-9) to degrade the extracellular matrix (ECM) and activate the integrin–focal adhesion kinase (FAK) reactivation pathway. When (neuron–glial antigen 2-positive) NG2□ BMSCs co-migrate with DTCs via N-cadherin adhesion, it promotes osteogenic colonization.[25]

The immune niche further complicates this balance. Natural killer (NK) cells maintain dormancy through interferon gamma (IFN-γ) surveillance,[26] while myeloid-derived suppressor cells and M2 macrophages disrupt immunity through programmed cell death 1 ligand 1/IL-10, thereby fostering conditions for vascular escape.[27-29]

The limitations of traditional models expose gaps in the study of multicellular dynamics. For example, the Transwell co-culture system employed by Barcellos-de-Souza *et al.*,[30] a system that only transmits signals through conditioned media, cannot simulate the bidirectional regulation of TGF-β1 triggered by direct cell contact *in vivo*. Although Zhang *et al.*'s[25] mouse fracture model recapitulates the bone remodeling process, it is limited by the resolution of *in vivo* imaging and cannot capture the real-time migratory interactions between NG2□ BMSCs and DTCs. In addition, the different production ratios and degradation rates of cytokines among species pose significant difficulties for cross-species studies. In summary, the cellular orchestrators in the BMME provide a foundational framework for tumor dormancy, but reliance on non-human models limits translational insights. Future BMOC-based studies could address these gaps by incorporating human-derived cells and standardized vascular components to validate dormancy mechanisms.

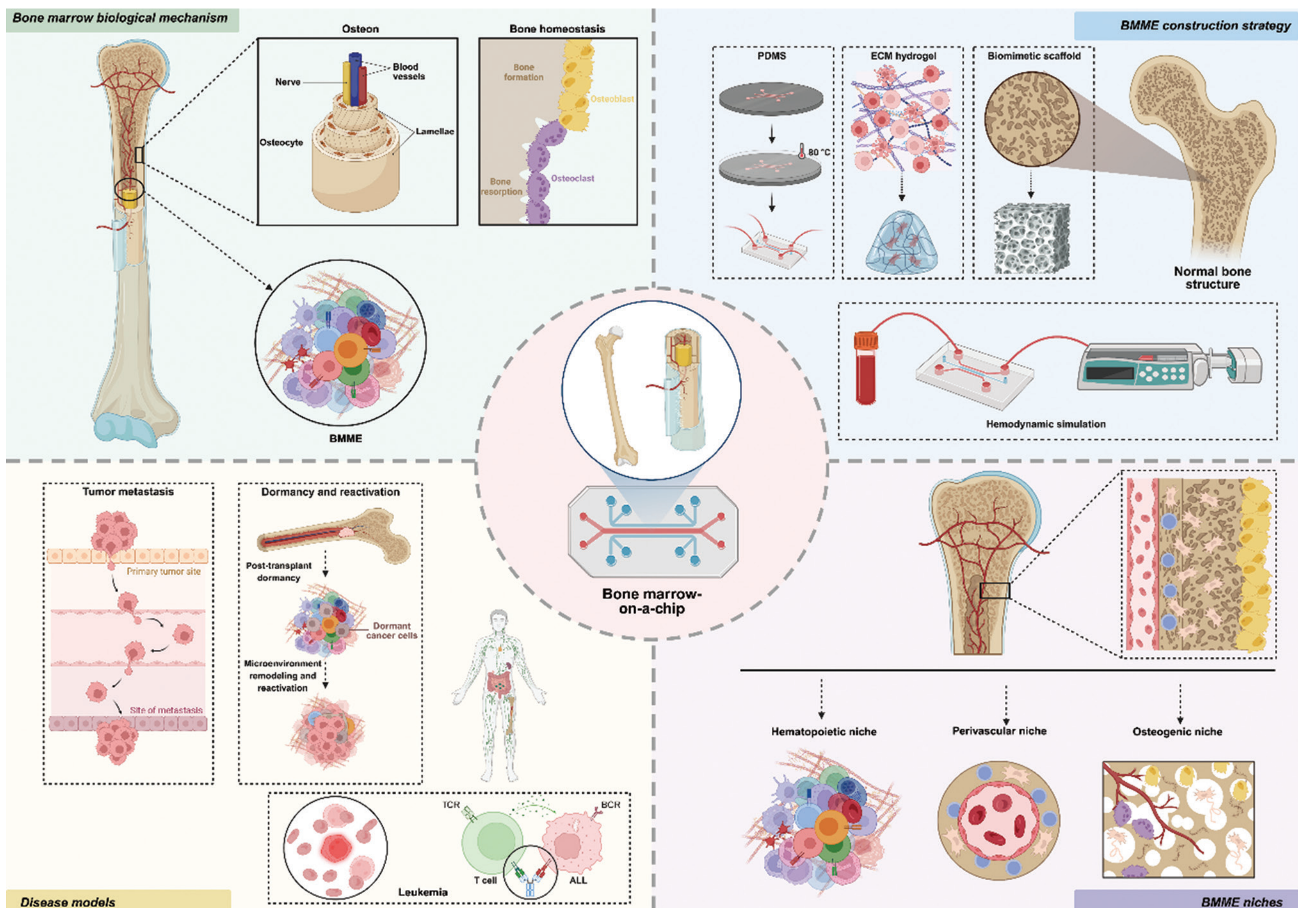

Figure 1. Schematic description of the bone marrow-on-a-chip technique. Created in BioRender. Zhou (2025) https://BioRender.com/ho3468u. Abbreviations: ALL: Acute lymphoblastic leukemia; BMME: Bone marrow microenvironment; ECM: Extracellular matrix; PDMS: Polydimethylsiloxane.

2.2. Molecular pathways governing dormancy induction and maintenance

This section elaborates on the core molecular pathways through which the BMME regulates tumor dormancy, including hypoxic metabolic reprogramming, the CXCL12/C–X–C chemokine receptor type 4 (CXCR4) chemokine signaling, and the TGF-β/bone morphogenetic protein (BMP) growth arrest pathway. Understanding these mechanisms is crucial for identifying vulnerabilities in dormancy and developing targeted interventions.

2.2.1. Hypoxia and metabolic reprogramming

The hypoxic nature of the BMME induces a dormant phenotype in tumor cells, primarily through the hypoxia-inducible factor 1 alpha (HIF-1α) pathway.[31-33] Under hypoxic conditions, HIF-1α accumulates and forms a heterodimer with aryl hydrocarbon receptor nuclear translocator,[34-36] activating downstream target genes (such as *VEGF*, *GLUT1*, and *CDKN1A*) to regulate angiogenesis, metabolic reprogramming, and cell-cycle arrest.[37-40] This pathway collaborates with transcription factors such as forkhead box protein O and myeloid ecotropic viral integration site 1 to enhance the resistance of hematopoietic stem cells (HSCs) and cancer cells to oxidative stress.[38] Notably, HIF-1α effectively mimics the hypoxic environment of the human bone marrow in animal models (e.g., mice). However, the expression of HIF-1α in human cancers often varies due to tumor heterogeneity and microenvironmental complexity. Moreover, the regulatory strength of specific target genes (e.g., *GLUT1*) in animal models has not been fully validated in clinical cohorts, suggesting translational challenges.

2.2.2. CXCL12/CXCR4 axis

The CXCL12/CXCR4 axis regulates bone metastasis and dormancy by mediating the interaction between tumor cells and the BMME. The secretion of CXCL12 creates a chemical gradient that recruits tumor cells with high CXCR4 expression,[41] triggering integrin αvβ3-mediated adhesion[42,43] and an imbalance between MMP-9 and tissue inhibitors of metalloproteinase 2 (TIMP-2),[44] ultimately promoting invasion and immune escape. This axis intersects

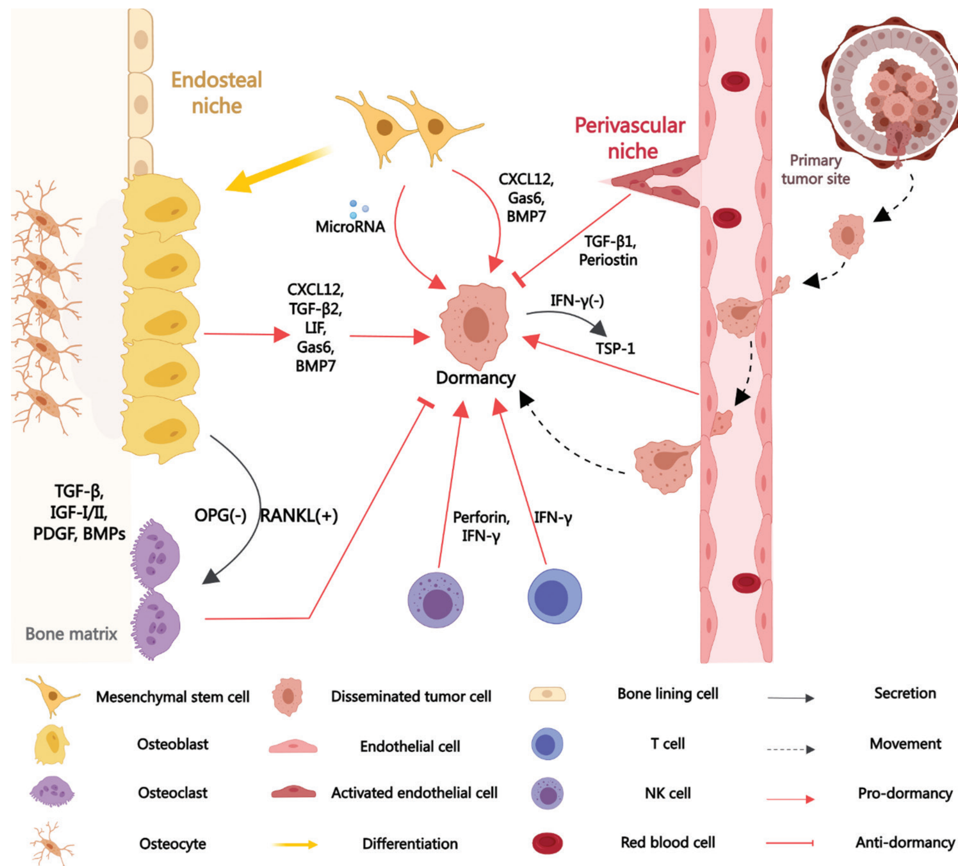

Figure 2. A diagrammatic representation of the bone marrow microenvironment regulating tumor dormancy. Endosteal niche (osteoblasts, bone marrow mesenchymal stem cells, and their secreted factors) and perivascular niche (endothelial cells and their secreted factors) regulate tumor dormancy. T cells and NK cells release IFN-γ and perforin, respectively, contributing to the promotion of the dormancy of tumor cells. Created with MedPeer (medpeer.cn). Abbreviations: BMP: Bone morphogenetic protein; CXCL12: C–X–C motif chemokine 12; Gas6: Growth arrest-specific protein 6; IFN-γ: Interferon gamma; IGF: Insulin-like growth factor; LIF: Leukemia inhibitory factor; NK cell: Natural killer cell; OPG: Osteoprotegerin; PDGF: Platelet-derived growth factor; RANKL: Receptor activator of nuclear factor kappa-B ligand; TGF-β: Transforming growth factor beta; TSP-1: Thrombospondin-1.

with the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT), extracellular signal-regulated kinase (ERK), and human epidermal growth factor receptor 2 (HER2) signaling pathways to maintain a quiescent phenotype.[45] In animal models, CXCR4 inhibition can reverse dormancy.[32] However, human evidence shows that CXCR4 expression is positively correlated with invasiveness, and its synergy with HER2 is more prominent in breast cancer bone metastases.[46] Nevertheless, the limited efficacy of CXCR4 antagonists (e.g., plerixafor) in clinical trials indicates that animal models may overestimate the targetability of a single pathway.

2.2.3. TGF-β/BMP signaling

The TGF-β family (including TGF-βs and BMPs) dynamically regulates tumor dormancy through the mothers against decapentaplegic homolog (SMAD)-dependent pathway: during the inhibitory phase, TGF-β activates p21/p27 to induce G1-phase arrest;[47] during the promotional phase, mutations in *TGFBR2* or deletion of *SMAD4* drive non-canonical signaling (such as MAPK/ERK) to mediate escape from dormancy.[48] This pathway also involves epigenetic reprogramming (e.g., histone deacetylase activation) and microenvironmental remodeling. Clinical evidence (such as *TGFBR2* mutations in colorectal cancer shortening the dormancy period) supports its role in humans.[48] However, the phenotype of *SMAD4* deletion is more consistent in animal models (e.g., transgenic mice), while the TGF-β pathway variations in human cancers are more complex, often accompanied by interference in the immune microenvironment, limiting the direct translation of findings from animal models.

Hypoxic metabolic reprogramming, the CXCL12/CXCR4 chemokine signaling, and the TGF-β/BMP growth arrest pathway jointly coordinate tumor dormancy in the BMME. These mechanisms have been extensively characterized in animal models, but the heterogeneity in human data (such as variations in HIF-1α expression, differences in CXCR4 targeting efficacy, and *TGFBR2* mutation frequencies) highlights unresolved translational

issues, necessitating further research to optimize treatment strategies.

2.3. Triggers of dormancy escape and reactivation

Dormant tumor cells in the BMME can be reactivated through specific perturbations, primarily driven by angiogenic switching, inflammatory stimuli, and ECM remodeling. These mechanisms collectively disrupt BMME homeostasis to reignite tumor proliferation.

2.3.1. Angiogenic switch

This involves a shift from anti-angiogenic to pro-angiogenic signaling. In dormancy, hypoxic stress stabilizes HIFs, inducing metabolic adaptation (e.g., glycolytic dependence) while quiescent endothelial cells secrete thrombospondin-1 (TSP-1) to inhibit angiogenesis.[49] Reactivation occurs when vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) signaling dominates: hypoxia-triggered VEGF surges bind VEGFR on endothelial cells, synergizing with fibroblast growth factor/platelet-derived growth factor (PDGF) to drive neovascularization. This switches endothelial cells from TSP-1 secretion (>80% reduction) to TGF-β1/osteopontin expression, increasing oxygen/nutrient supply and creating a pro-metastatic niche. Critically, tumor cells actively perturb this balance: breast cancer-derived IFN-γ suppresses endothelial cell tryptophan metabolism to reduce TSP-1,[50] while prostate cancer dormancy escape upregulates MMP-9-dependent factors (e.g., VEGF and IL-8) and downregulates TSP-1.[51] Thus, angiogenic switching represents a BMME-specific perturbation where bidirectional tumor–endothelial cell crosstalk overcomes dormancy constraints.

2.3.2. Inflammatory stimuli

This process involves immune cell infiltration and osteoclast-mediated osteolysis. Inflammation reactivates dormant cells by altering BMME signaling landscapes. Key cytokines—tumor necrosis factor alpha, IL-1β, and IL-6—activate nuclear factor kappa B (NF-κB)/signal transducer and activator of transcription 3 pathways in tumor cells, promoting proliferation and survival.[52] Concomitant immune infiltration (macrophages, neutrophils, and T cells) amplifies cytokine release (e.g., IL-8 and C–C motif chemokine 5). It degrades ECM through MMP upregulation.[53] Critically, in the BMME, tumor cells initiate a vicious cycle[54,55] (Figure 3): secretion of parathyroid hormone-related protein binds parathyroid hormone/parathyroid hormone-related peptide receptor on osteoblasts, triggering receptor activator of nuclear factor kappa-B ligand (RANKL) overexpression. This activates the RANKL–RANK axis to promote osteoclastogenesis. Osteoclasts then release various pro-tumor factors, including PDGF, TGF-β, VEGF, BMPs, and Ca^{2+}, further stimulating tumor growth and disrupting dormancy. This self-reinforcing loop of osteolysis–immune crosstalk licenses escape from dormancy in the BMME.

2.3.3. ECM remodeling

This involves altered biomechanical and biochemical cues. The remodeling of the ECM is another key factor for the reactivation of tumor cells from a dormant state.[56] MMP/TIMP imbalance—triggered by inflammation or angiogenesis—degrades bone-specific components (e.g., collagen I and laminin), releasing sequestered growth factors (e.g., VEGF and TGF-β) that activate proliferative pathways.[57] Crucially, collagen and fibronectin in the ECM can activate the FAK and PI3K/AKT signaling pathways through interactions with integrin receptors, promoting cell proliferation and migration.

In summary, angiogenic switch, inflammatory stimulation, and ECM remodeling are the main triggering factors for the reactivation of tumor cells from a dormant state. These mechanisms provide the necessary conditions for the reactivation of tumor cells by changing the oxygen supply, cytokines, and composition of the ECM in the microenvironment.

3. BMOC technology: Engineering the BMME for tumor dormancy research

The engineering of the BMOC platform requires strict coordination of biophysical fidelity and reproducibility. This section summarizes key BMOC studies published in recent years and categorizes them from two main perspectives: device characteristics (device applications, research objectives, materials used, manufacturing methods, fluid mechanisms, and microenvironment materials) and device performance (improved research methods provided compared to other devices) (Table 1). It comprehensively analyzes the ECM and fluid characteristics required for the BMME. It conducts a comprehensive comparison with traditional *in vivo* and *in vitro* systems, highlighting the advantages of the BMOC platform in simulating the human bone marrow niche.

3.1. Foundational design principles of the BMOC technique

3.1.1. Biomaterials mimicking the bone marrow niche

The BMOC platforms leverage advanced biomaterials and fabrication techniques to recapitulate the BMME, offering a dynamic alternative to traditional *in vitro* and *in vivo* models. Soft lithography enables the fabrication of polydimethylsiloxane (PDMS)-based microfluidic devices, providing gas-permeable substrates for real-time imaging and dynamic cell culture.[68] To emulate the ECM, natural polymers like type I collagen and sodium alginate are integrated into PDMS frameworks;[58]

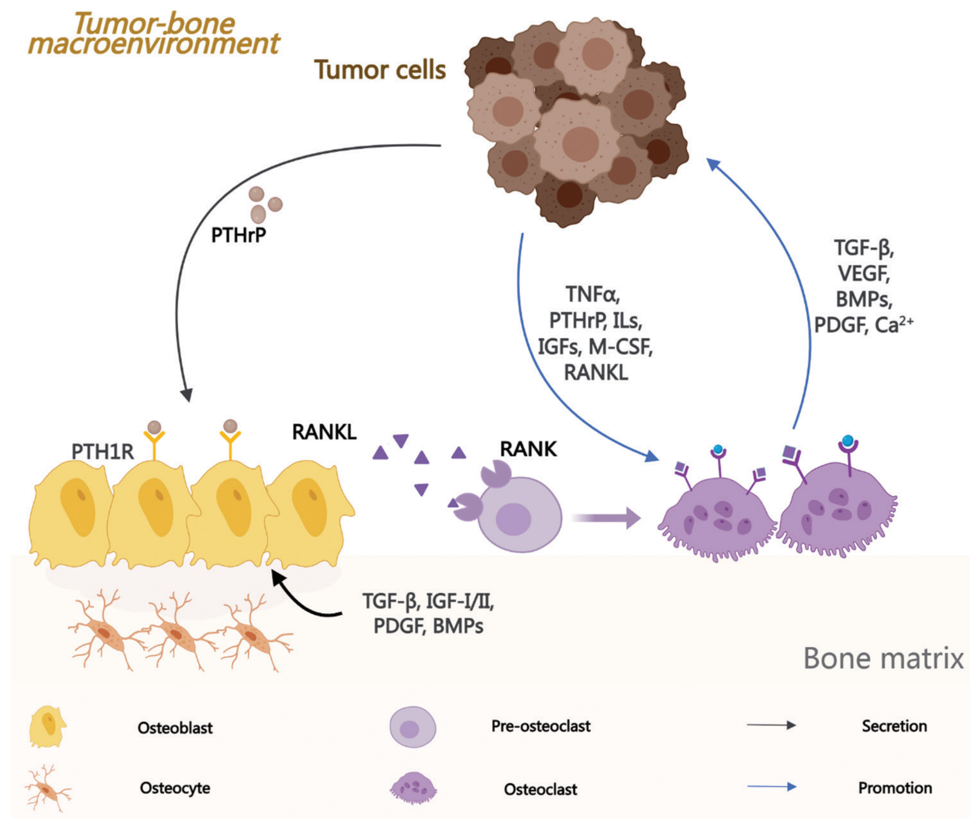

Figure 3. Vicious cycle between bone metastasis and tumor-induced bone destruction. When tumor cells metastasize to bone tissue, they release bioactive substances that stimulate the activation and maturation of osteoclasts, leading to bone destruction and osteoporosis. Subsequently, osteoclasts release tumor-promoting factors that stimulate cell proliferation, forming a vicious cycle promoting tumor growth and spread. Created with MedPeer (medpeer.cn).

Abbreviations: BMP: Bone morphogenetic protein; IGF: Insulin-like growth factor; M-CSF: Macrophage colony-stimulating factor; PDGF: Platelet-derived growth factor; PTH1R: Parathyroid hormone/parathyroid hormone-related peptide receptor; PTHrP: Parathyroid hormone-related protein; RANKL: Receptor activator of nuclear factor kappa-B ligand; TGF-β: Transforming growth factor beta; TNFα: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor.

collagen supports stromal cell adhesion and cytokine sequestration, while alginate hydrogels tune stiffness to mimic marrow viscoelasticity.[66] A key advancement is the use of hydroxyapatite (HAP)-based 3D scaffolds, fabricated through 3D printing or electrospinning, which structurally simulate cancellous bone and enhance oxygen gradients for tumor dormancy niches.[72,73] For example, Ji *et al.*[71] employed 3D-printed HAP scaffolds with cross-linked gelatin methacrylate to model dormancy, encapsulating mesenchymal stem cells (MSCs) and tumor cells under controlled conditions (Figure 4A and B). Fluid dynamics within BMOCs are precisely controlled through peristaltic or syringe pumps, generating physiological shear stresses that regulate endothelial cell alignment and cytokine distribution.[74] While these systems precisely replicate physicochemical properties, current limitations include vascular standardization and immune niche integration, highlighting opportunities for artificial intelligence-enhanced multi-organ platforms to model systemic metastasis.

3.1.2. Integrated vascularization and neural network strategies

In the bone marrow, there is a significant interaction between the neural network and the vascular network.[75,76] Neurons influence angiogenesis and vascular function, while the vascular network regulates neuronal activity and survival. First, the bone marrow contains a special microenvironment called the perivascular niche, which surrounds blood vessels and works together with the bone marrow endothelial niche to support HSCs. This environment is crucial for bone marrow function and affects disease progression, including tumor metastasis and dormancy. Researchers have used the BMOC to replicate the *in vivo* bone marrow vascularization and hydrodynamic mechanisms *in vitro* by mimicking the structure and function of blood vessels. Interestingly, the *in vitro* constructed microfluidic devices can adopt different pulsatile flow patterns to simulate the hemodynamic shear stress and morphological adaptation of vascular endothelial cells under physiological conditions.[77-79] This is of great

Table 1. Key biomimetic parameters and technological advancements provided by microfluidic bone marrow mimetics

References	Device application/research objectives	Device material/ fabrication means	Hydrodynamic mechanism	Main cell type/ microenvironment material	Key benefits and enhancements offered by the device
Sieber *et al.*[58]	3D bone marrow niche-on-chip modeling Achieving long-term HSPC culture through a microfluidic platform that mimics the 3D molecular and structural microenvironment of human bone marrow	Hydroxyapatite-coated zirconium oxide scaffold/none	Peristaltic pump	h-HSPC; h-BMSC/ fibronectin	Co-culture-perfused 3D hydrogel matrix Provides a long-term, molecularly and structurally biomimetic microenvironment compared to conventional models, improving the mechanistic understanding of HSPC homeostasis and multilineage differentiation with functional validation
Kotha *et al.*[59]	Engineered multicellular vascular niche platform Modeling hematopoietic cell trafficking through a tunable multicellular platform that engineers human vascular marrow niches with real-time interaction mapping	PDMS/ soft-lithography	Gravity-induced flow	h-UVEC; human marrow stromal cell (HS-5); human BMNC; human acute myelogenous leukemic cell; h-HSPC/type I collagen	Perfused 3D hydrogel matrix Provides a multicellular and perfused vascular microenvironment compared to conventional static or simplified models, improving the understanding of hematopoietic cell trafficking mechanisms with high spatial and temporal resolution imaging
Marturano-Kruik *et al.*[60]	Perfused bone perivascular niche-on-a-chip modeling Investigating breast cancer metastasis and drug resistance through a triculture niche platform that replicates interstitial flow dynamics and oxygen gradients in human bone perivascular microenvironments	PDMS/ soft-lithography	Syringe pump	h-BMSC; h-UVEC; human breast cancer cell line (MDA-MB-231)/ decellularized 3D bone matrix	Perfused decellularized 3D bone matrix Provides a physiologically accurate perivascular niche with controlled interstitial flow and oxygen gradients compared to conventional static models, improving the understanding of metastatic colonization and drug resistance mechanisms through real-time monitoring of slow-proliferative cancer states
Hao *et al.*[61]	Spontaneous 3D-mineralized bone-on-a-chip modeling Investigating breast cancer bone colonization through a self-mineralizing chip platform that replicates physiologically relevant 3D bone matrices for in vivo-like metastasis hallmark capture	PDMS/ soft-lithography	Reservoir	Mouse pre-osteoblast cell line (MC3T3-E1); MDA-MB-231/ collagen matrix	Co-culture in a perfused 3D hydrogel matrix Provides a spontaneously mineralized and physiologically relevant bone microenvironment compared to conventional induced-differentiation models, improving the capture of breast cancer colonization hallmarks with enhanced spatiotemporal accessibility

(Cont'd...)

Table 1. *(Continued)*

References	Device application/research objectives	Device material/fabrication means	Hydrodynamic mechanism	Main cell type/microenvironment material	Key benefits and enhancements offered by the device
Aleman *et al.*[62]	Deconstructed multi-niche microfluidic platform modeling	PDMS/soft-lithography	Peristaltic pump	h-BMNC; h-BMSC/dithiolated photocrosslinked hydrogel	Co-culture in a perfused 3D hydrogel matrix
	Dissecting normal and malignant HSPC-niche interactions through a deconstructed multi-niche platform that integrates cell tracking and recirculating perfusion within a closed microfluidic system				Provides a structurally deconvoluted and dynamically perfused microenvironment compared to conventional animal models, improving the quantification of normal and malignant HSPC-niche interactions with real-time multi-niche spatial mapping
Ma *et al.*[63]	Organotypic leukemia-on-a-chip modeling	PDMS/soft-lithography	Reservoir	h-BMSC; h-UVEC; human cord blood CD34+cell; h-BMNC; human B-ALL cells/gelatin-fibrin composite hydrogel	Co-culture in a perfused 3D hydrogel matrix
	Deciphering B-ALL chemoresistance through an organotypic chip that replicates patient-derived, multi-niche leukemic microenvironments for targeted therapy screening				Provides a patient-specific and multi-niche leukemic microenvironment compared to conventional homogeneous models, improving the dissection of heterogeneous chemoresistance mechanisms across B-ALL subtypes with spatial-genetic dual-resolution analysis
Chou *et al.*[64]	Vascularized bone marrow-on-a-chip with dual-channel architecture	PDMS/soft-lithography	Peristaltic pump	h-BMSC; h-HSPC; human-derived bone marrow mononuclear cell/fibrin gel	Co-culture in a perfused 3D hydrogel matrix
	Recapitulating clinical bone marrow toxicities through a dual-channel chip platform that models patient-specific pathophysiology for rare disease mechanism discovery				Provides a vascularized and patient-specific pathophysiological microenvironment compared to conventional non-accessible bone marrow models, improving the recapitulation of clinical myelotoxicity and recovery dynamics with week-long temporal resolution
Souquet *et al.*[65]	Compartmentalized bone marrow-on-a-chip fabrication modeling	PDMS/maskless photolithography	Data not available	h-UVEC; h-FOB; h-HSPC/collagen-I and fibrin matrix	Perfused 3D hydrogel matrix
	Deciphering niche-specific HSPC regulation through an optically accessible platform that mimics vascular and endosteal niche segregation with maskless photolithography-engineered microarchitectures				Provides a spatially decoupled and geometrically tunable BMME compared to conventional opaque bone models, improving the understanding of niche-specific HSPC regulation with cellular behavior tracking capabilities

(Cont'd...)

Table 1. *(Continued)*

References	Device application/research objectives	Device material/ fabrication means	Hydrodynamic mechanism	Main cell type/ microenvironment material	Key benefits and enhancements offered by the device
Nelson *et al.*[66]	96-well microfluidic human bone marrow-on-a-chip platform modeling	PDMS/ soft-lithography	Pipette-induced flow	h-BMSC; h-UVEC; h-HSPC/collagen-I and fibrin matrix	Co-culture in a perfused 3D hydrogel matrix
	Elucidating endosteal niche physiology in human bone marrow through a high-throughput platform that integrates osteogenic, vascular, and perivascular microenvironments for disease modeling				Provides a multi-niche microvascularized microenvironment compared to conventional single-niche models, improving the understanding of endosteal niche roles in HSCs maintenance and radiation response with high-throughput reproducibility
Glaser *et al.*[67]	3D human bone marrow vascularized niche modeling	PDMS/ woft-lithography	Peristaltic pump	h-EC; h-HSPC; hFOB 1.19; h-BMSC/fibrin hydrogels	Perfusable 3D hydrogel matrix
	Unraveling mechanisms of human bone marrow function and pathological processes				Provides a more realistic microenvironment compared to conventional models, improving the understanding of human bone marrow function and drug response with high spatial and temporal resolution
Sharipol *et al.*[68]	Modular bone marrow microenvironment-on-chip modeling	Data not available	Peristaltic pump	m-BMSC; m-EC; m-HSPC/type I collagen and fibronectin	Perfused 3D hydrogel matrix
	Advancing bone marrow pathology therapy through a modular chip platform that mimics the vascularized multicellular microenvironment of functional hematopoietic niches				Provides a more realistic and modular BMME compared to conventional murine and 2D/3D models, improving the understanding of bone marrow pathologies, such as marrow failure syndromes and leukemias, with an accessible design for accelerated analysis
Isosaari *et al.*[69]	3D neurovascular network-on-a-chip modeling	Data not available	Gravity-induced flow	Human-induced pluripotent stem cell (hiPSC); h-UVEC; human adipose stem/stromal cell (ASC); h-BMSC/ collagen-I and fibrin matrix	Perfusable 3D hydrogel matrix
	Unraveling dynamic neurovascular interrelationships				Provides a long-term, mural cell-integrated neurovascular microenvironment compared to conventional short-term models, improving the mechanistic understanding of dynamic intercellular crosstalk in health and disease with high-resolution characterization

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Table 1. *(Continued)*

References	Device application/research objectives	Device material/ fabrication means	Hydrodynamic mechanism	Main cell type/ microenvironment material	Key benefits and enhancements offered by the device
Ma *et al.*[70]	Immunocompetent leukemia-on-a-chip platform modeling	PDMS/ soft-lithography	Reservoir	h-BMSC; h-UVEC; hFOB1.19; h-BMNC; human B-ALL cells; anti-CD19 CAR-T cells derived from healthy donors (HD)/patients (PD)/ fibrin hydrogel	Co-culture in a perfused 3D hydrogel matrix
	Modeling CAR-T therapy outcomes through an immunocompetent chip platform that recapitulates human leukemia pathophysiological niches for personalized response prediction				Provides an immunologically active and pathologically accurate leukemia microenvironment compared to conventional non-immunocompetent models, improving the prediction of CAR-T therapy outcomes across remission/resistance/ relapse scenarios with cellular-level spatiotemporal tracking
Ji *et al.*[71]	Tri-niche metastatic bone-on-a-chip platform modeling	PDMS/ soft-lithography	Pipette-induced flow	A549; h-UVEC; THP-1; HS-5; m-BMSC; RAW 264.7/gelatin methacrylate hydrogel	Co-culture in a perfused 3D hydrogel matrix
	Investigating bone metastasis dynamics through a 3D-printed chip platform that mimics premetastatic niche formation for dormancy-reactivation mechanism discovery				Provides a primary cancer-influenced tri-niche metastatic microenvironment compared to conventional single-niche models, improving the elucidation of tumor dormancy-reactivation mechanisms through real-time invadopodia dynamics capture

Abbreviations: B-ALL: B-cell acute lymphoblastic leukemia; BMNC: Bone marrow mononuclear cell; BMME: Bone marrow microenvironment; BMSC: Bone marrow mesenchymal stem cell; CAR-T: Chimeric antigen receptor T-cell; CD19: Cluster of differentiation 19; FOB: Fetal osteoblast; EC: Endothelial cell; HSPC: Hematopoietic stem and progenitor cell; HSC: Hematopoietic stem cell; PDMS: Polydimethylsiloxane; UVEC: Umbilical vein endothelial cell.

significance for constructing a dynamic model to study the spatio-temporal interaction between dormant tumor cells and the perivascular niche. For example, Glaser *et al.*[67] used microfluidics and stem cell technology to simulate a dynamic, perfusable vascular network *in vitro*, replicating *in vivo* bone marrow functions and achieving dynamic interactions between the perivascular and endosteal niches (Figure 4C). This provides a model basis for revealing the mechanisms behind the BMME and tumor dormancy, and addresses the serious limitation of the lack of a model capable of dissecting dynamic events at the niche level.

Second, nerves and blood vessels are interdependent during tissue formation and development. Understanding this interaction is crucial for constructing an *in vitro* BMME model. For example, Isosaari *et al.*[69] created a novel 3D neurovascular chip network model using human cells (including human induced pluripotent stem cell-derived neurons, human umbilical vein endothelial cells, BMSCs, or adipose-derived stem cells) to replicate the physiological process of neurovascular interaction *in vitro* (Figure 4D). This lays an initial platform for the development of vascularized and innervated organ-on-a-chip models and further mechanistic research, and provides the possibility for realistically simulating the BMME and tumor dormancy and related mechanism research *in vitro*.

3.2. The construction of the BMME with the BMOC technique

The human BMME comprises intricate cellular networks, ECM, fluid dynamics, and signaling cues, all of which collectively regulate processes such as tumor dormancy. Traditional models (e.g., 2D cultures and animal systems) fail to fully recapitulate this complexity due to limited spatial organization, static conditions, and species-specific

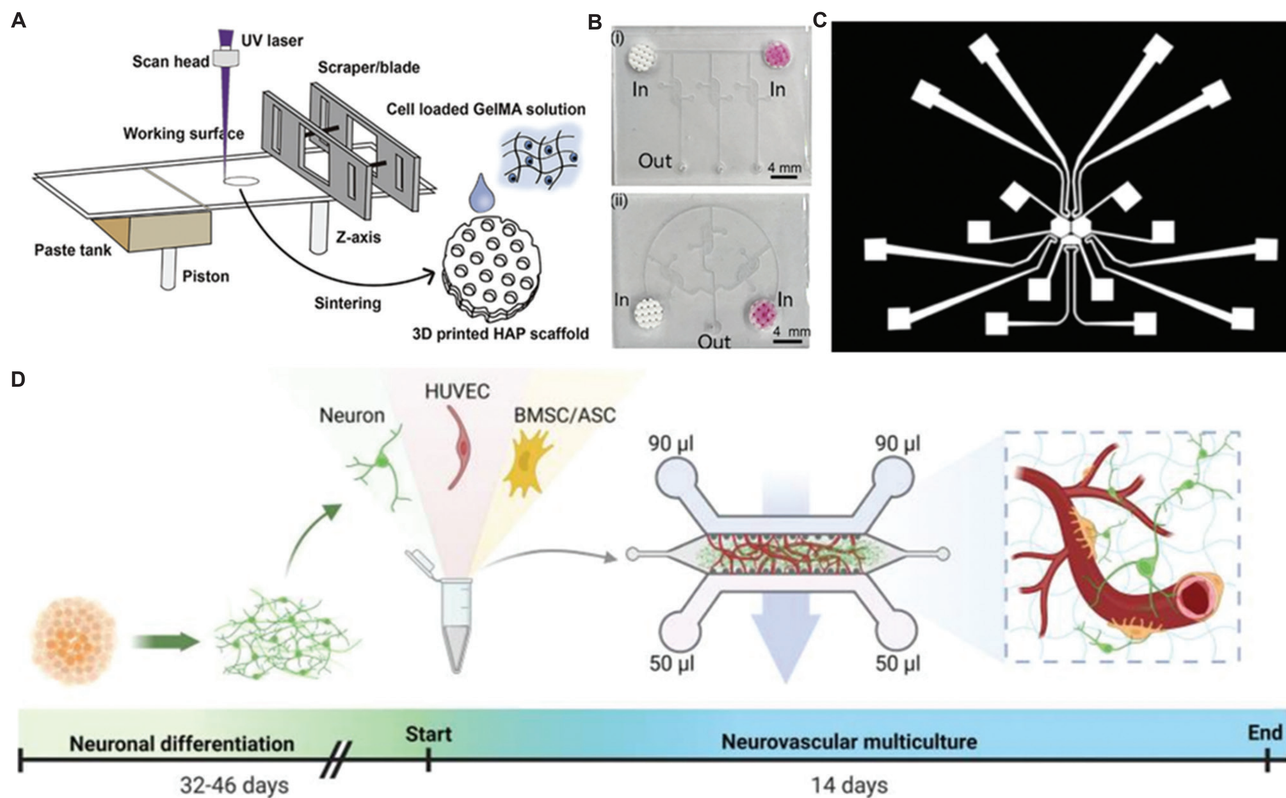

Figure 4. Schematic illustrations of key components and models in bone marrow-on-a-chip techniques. (A) Schematic illustrating the manufacturing process of 3D-printed bone tumor constructs. A549 tumor cells are encapsulated within GelMA hydrogel integrated with MSC-seeded HAP scaffolds, establishing a tumor dormancy niche. (B) Macroscopic appearance of fabricated HAP structures with/without GelMA hydrogel (red) across dual chip platform configurations. (C) A computer-aided design illustration outlines the comprehensive layout of the device, including the interlinked fluidic channels. (D) Chronological progression of a 3D neurovascular network development in a chip platform. Images reprinted with permission from: (A and B) Ji *et al.*[71] Copyright 2023 Wiley-VCH; (C) Glaser *et al.*[67] Copyright 2022 Elsevier; (D) Isosaari *et al.*,[69] licensed under CC-BY 4.0.
Abbreviations: ASC: Adipose-derived stem cell; BMSC: Bone marrow mesenchymal stem cell; GelMA: Gelatin methacrylate; HAP: Hydroxyapatite; HUVEC: Human umbilical vein epithelial cell; MSC: Mesenchymal stem cell.

biases. BMOCs overcome these constraints through engineered designs—integrating multicellular co-cultures, 3D architectures, and dynamic perfusion—to construct physiologically relevant human BMME models. This capability is pivotal for dissecting dormancy-specific microenvironments, such as niche-mediated survival cues. This section systematically compares BMOCs with conventional approaches for BMME construction. It highlights advances in modeling key niches (hematopoietic, endosteal/osteoblastic, and immune niches), emphasizing their translational potential in dormancy research.

3.2.1. Comparative advantages of the BMOC technique in BMME modeling

The BMOC technique provides transformative advantages in constructing human BMME by enabling precision engineering of spatial, biochemical, and biophysical niche parameters—capabilities unattainable in traditional models. While 2D systems (e.g., transwells) lack 3D architecture and dynamic flow, and 3D organoids struggle with standardized niche integration and perfusion control, BMOCs uniquely integrate multicellular co-cultures, programmable fluid dynamics, and modular niche designs (Table 2). This allows faithful recapitulation of dormancy-regulating signals (e.g., CXCL12 gradients and hypoxia) and longitudinal tracking of tumor cell–niche interactions. Animal models, although systemically relevant, exhibit critical species-specific disparities in niche composition (e.g., osteolineage cell ratios) and dormancy regulation mechanisms, which limit their translational value. BMOCs thus offer an ethically superior, human-relevant platform for evaluating dormancy-targeting therapeutics and patient-specific responses (e.g., drug toxicity assays). Nevertheless, scalability bottlenecks and vascular standardization challenges require further innovation to unlock high-throughput applications.

3.2.2. Recent advances in constructing core BMME niches with the BMOC technique

Bone marrow niches play pivotal roles in marrow function, with BMOCs offering distinct advantages for *in vitro* reconstruction of functional bone marrow compartments

Table 2. Comparative analysis of models for constructing the human bone marrow microenvironment

Model feature	Traditional 2D (e.g., Transwell)	3D models (e.g., organoids)	Animal models (*in vivo*)	BMOCs
Spatial complexity	Low (planar)	Medium (3D structure)	High (native architecture)	High (multi-compartment)
Cell–cell/ECM interactions	Limited	Good	Native	Controllable & tunable
Dynamic microenvironment	Minimal (static)	Limited (often static)	Native	High (precise perfusion control)
Multi-niche integration	Difficult	Challenging	Native	High (multi-niche designed)
Human relevance	Medium (human cells)	Medium (human cells)	Low (species difference)	High (human cells, tunable)
Throughput and scalability	High	Medium	Low	Medium–high (depends on design)
Real-time monitoring/imaging	Easy	Challenging	Difficult	Relatively easy

Abbreviations: BMOC: Bone marrow on a chip; ECM: Extracellular matrix.

through multicellular integration, 3D culture systems, modular architectures, and dynamic microenvironment simulation. Current BMOC platforms successfully model key niches—including hematopoietic, perivascular, and endosteal niches—enabling physiologically relevant recapitulation of *in vivo* conditions. These advances provide visualizable platforms for investigating bone marrow physiology and pathological mechanisms.

Hematopoietic stem and progenitor cells (HSPCs), as the source of all blood cell lineages, require specialized niches for long-term maintenance in microfluidic environments.[80] Sieber *et al.*[58] developed a HAP-coated zirconia scaffold-based 3D co-culture model integrating MSCs and umbilical cord blood-derived HSPCs (Figure 5A). This microfluidic multi-organ chip system sustained stable HSPC culture for 28 days while preserving intrinsic biological properties and multilineage differentiation potential, demonstrating significant promise for regenerative medicine applications. Modular chip advancements further accelerated progress. Sharipol *et al.*[68] created a modularly assembled murine BMOC using commercial microfluidic platforms, integrating vascular channels, semi-porous membranes, and marrow compartments with key cellular components to maintain long-term functional HSCs (Figure 5B). Complementarily, Aleman *et al.*[62] incorporated 3D architecture, cell–cell/matrix interactions, and perfusion dynamics to investigate niche interactions with normal and malignant hematopoietic cells.

The BMME constitutes a sophisticated assembly of functionally specialized regions. Perfusion-based 3D co-culture techniques enable comprehensive reconstruction of niche characteristics, particularly for perivascular microenvironments and endosteal compartments. Kotha *et al.*[59] engineered a human perivascular niche platform with tunable multicellular composition to analyze 3D cellular interactions guiding hematopoietic cell trafficking. Evaluations encompassing confocal imaging of marrow fibroblasts/endothelial cells, gene expression profiling, and adhesion/migration assays demonstrated this system's capacity to visualize hematopoietic cell dynamics across fibroblast microenvironments, revealing complex intercellular interactions. Concurrently, Marturano-Kruik *et al.*[60] developed a perfused vascularized niche model to study breast cancer colonization and drug resistance in bone. This platform assessed stable vascular network establishment (through flow rate, shear stress, and oxygen gradient modulation), MSC-supported vasculogenesis, and cancer cell proliferation/drug resistance phenotypes, providing a functional tool to investigate neoplastic dynamics in perivascular niches.

Furthermore, BMOCs effectively model the endosteal niche—a critical microenvironment adjacent to trabecular bone composed of mineralized tissue and stromal cells that regulates HSCs' survival, proliferation, and differentiation while balancing hematopoiesis and bone metabolism. Souquet *et al.*[65] engineered a compartmentalized biomimetic marrow chip with discrete vascular and endosteal niches. Utilizing maskless photolithography for geometric optimization, this platform evaluated HSPC interactions with niche-specific cells, demonstrating that the osteoblast-organized endosteal niche precisely regulates HSPC quiescence and differentiation decisions—overcoming traditional limitations in visualizing opaque bone matrices.

Remarkably, innovations in organ-on-chip technology now enable multi-niche integration within unified BMOC platforms. To delineate relationships among parallel human BMME niches (endothelial, perivascular, and central marrows), Nelson *et al.*[66] created a 96-well high-throughput microfluidic system integrating endosteal, central marrow, and perivascular niches (Figure 5C and D). Through osteogenic differentiation of MSCs forming a bone-like endosteal layer, and endothelial cell/MSC seeding in fibrin-collagen hydrogels generating central marrow and 3D microvascular networks, this model demonstrated significant expression of niche-specific cytokines (e.g., stem

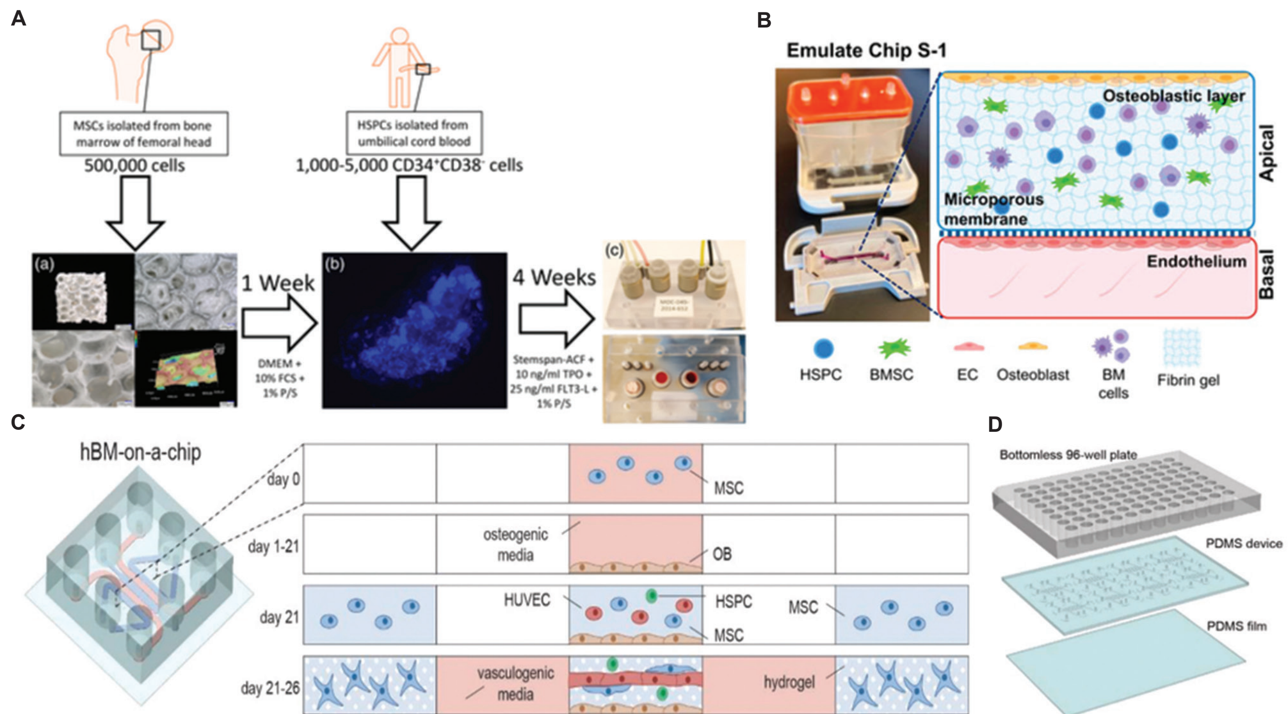

Figure 5. Core bone marrow microenvironment niche models constructed by the bone marrow-on-a-chip technique. (A) 3D bone marrow construct engineering strategy (on-chip). (B) Microfluidic bone marrow microenvironment-on-chip containing osteoblastic, endothelial layers, and entire bone marrow cells. (C) Diagrammatic representation of a five-channel polydimethylsiloxane microfluidic chip (on the left) and an enlarged depiction of the fluidic channels (on the right). (D) The layout of the human bone marrow-on-a-chip within a 96-well plate is depicted in a 3D assembly. Images reprinted with permission from: (A) Sieber *et al.*[58] Copyright 2017 John Wiley and Sons, Ltd; (B) Sharipol *et al.*,[68] licensed under CC BY 4.0; and (C and D) Nelson *et al.*[66] Copyright 2021, Elsevier.
Abbreviations: BMSC: Bone marrow mesenchymal stem cell; EC: Endothelial cell; HSPC: Hematopoietic stem and progenitor cell; HUVEC: Human umbilical vein endothelial cell; MSC: Mesenchymal stem cell; OB: Osteoblast.

cell factor, CXCL12, and jagged-1) and ECM components (fibronectin). Functionally, it mediated enhanced HSC maintenance with suppressed proliferation and reduced radiation-induced apoptosis, faithfully replicating human marrow microphysiology for high-throughput investigation of hematopoietic homeostasis, cancer metastasis, and clinical interventions.

Multi-niche BMOCs represent a significant leap in recapitulating the structural and functional complexity of the human BMME. This complexity is essential for studying tumor dormancy, as dormant cells potentially reside in specific, protective niches.

4. BMOC platforms: Engineering human-specific disease models for dormancy insights

The BMOCs have emerged as transformative tools for recapitulating the dynamic interplay between hematopoietic/stromal components and disease pathologies within the human BMME. This section critically examines how BMOC systems advance disease modeling beyond traditional approaches, specifically focusing on two domains: (i) hematopoietic disorders (e.g., leukemias) where BMOCs enable real-time observation of clonal evolution in a vascularized niche, and (ii) metastatic cancers that colonize bone, where integrated fluid flow and 3D architecture reveal previously inaccessible dormancy-inducing mechanisms. By bridging engineering precision with pathophysiological complexity, these models not only address limitations of animal translation—such as species-specific cytokine signaling—but also establish a unique experimental foundation to systematically dissect tumor dormancy regulation, positioning BMOCs as indispensable platforms for probing therapeutic resistance.

4.1. Human-specific hematopathology modeling

The bone marrow niche orchestrates HSC maintenance and differentiation under physiological conditions, yet in hematological malignancies such as acute leukemia, malignant cells exploit this niche to promote survival and chemoresistance. While traditional 2D or 3D co-culture systems provide accessible platforms for pathological studies, they fundamentally lack the ability to recapitulate critical *in vivo* niche structures—including the central sinus, medullary cavity, and hematopoietic

compartments—limiting mechanistic insights into disease progression.[81,82] To overcome these constraints, BMOCs offer a transformative approach for reconstructing the leukemic BMME, enabling precise dissection of niche interactions and chemoresistance pathways.[83-86] For example, Ma *et al*.[63] developed an innovative "leukemia-on-a-chip" model to emulate the BMME and dissect spatial-genetic heterogeneity in regulating chemotherapy resistance across diverse B-cell acute lymphoblastic leukemia (B-ALL) subtypes (Figure 6A). Unlike traditional *in vitro* co-culture systems, which fail to replicate key niche structures such as the central sinus and endosteal regions, this BMOC platform integrates microfluidic channels and hydrogels to accurately mimic vascularized leukemia niches, enabling real-time observation of dynamic cell–ECM interactions. Comparative analysis with *in vivo* murine models revealed that the chip recapitulates leukemic spatial organization and identifies subtype-specific mechanisms: for example, perivascular and hematopoietic niche-derived signals (e.g., CXCL12 cytokines and vascular cell adhesion protein 1/osteopontin adhesion molecules) promote B-ALL quiescence through NF-κB pathway activation, elucidating heterogeneous chemoresistance in patient-derived samples. Crucially, this model advances preclinical applications by demonstrating niche-cotargeting therapies for personalized screening, addressing translational gaps not captured in animal systems.

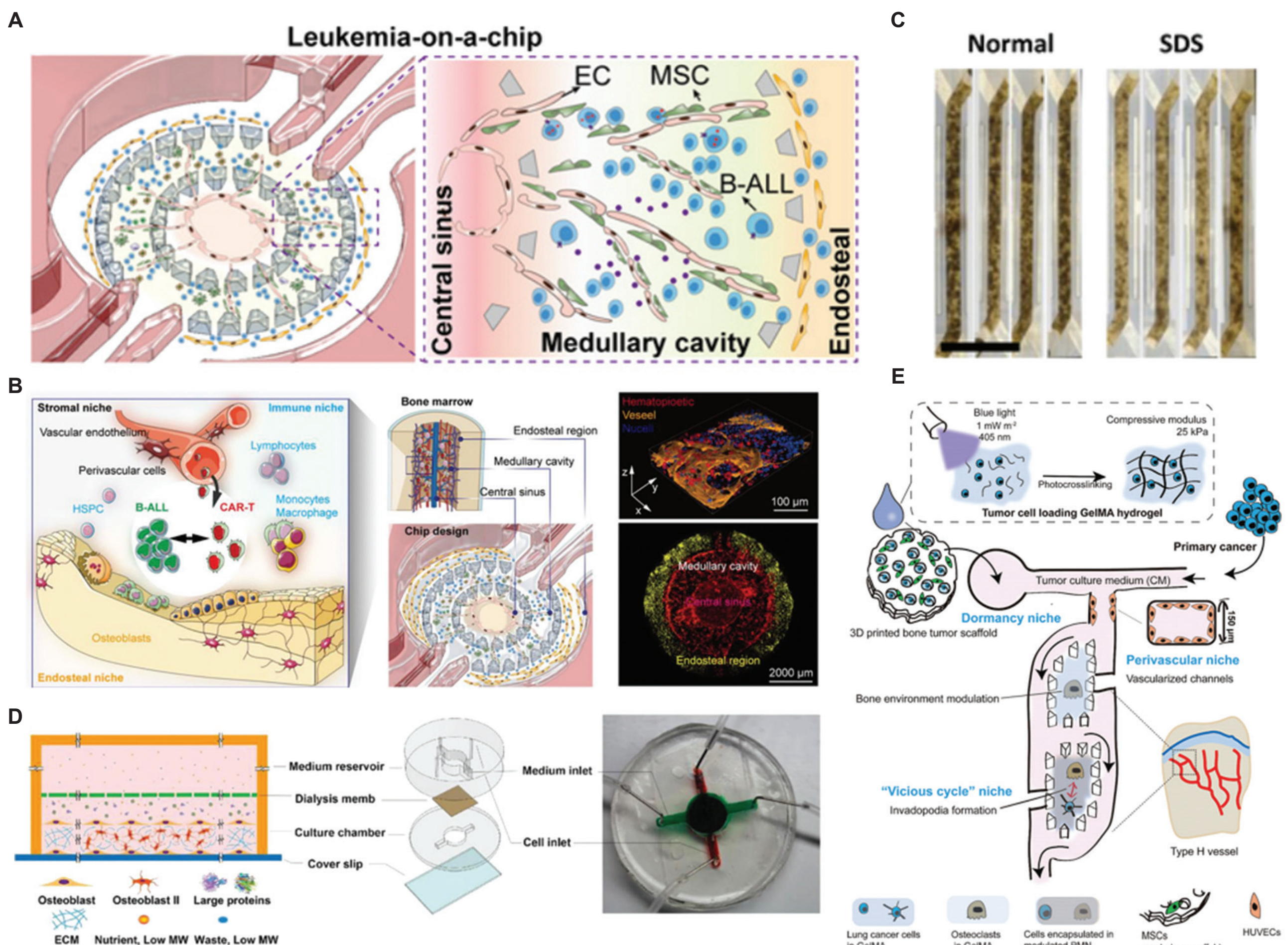

Figure 6. BMOC platforms for modeling human-specific bone marrow pathologies and metastatic niches. (A) Leukemia-on-a-chip architectural schematic featuring three functionalized compartments. (B) The bone marrow-on-a-chip (center) was seeded with human bone marrow-derived cells (right) to emulate the physiological environment found *in vivo* (left). (C) Comparative cluster of differentiation 34-positive ($CD34^+$) hematopoietic cell culturing in bone marrow chips: Healthy donors versus Shwachman-Diamond syndrome patients (2-week culture). (D) Schematic diagram of the design and construction of self-mineralizing bone-on-a-chip. (E) Design and application of a pre-metastatic niche-mimicking bone-on-a-chip platform. Images reprinted with permission from: (A) Ma *et al*.,[63] licensed under CC BY-NC 4.0; (B) Ma *et al*.,[70] licensed under CC BY 4.0; (C) Chou *et al*.[64] Copyright 2020, Springer Nature; (D) Hao *et al*.[61] Copyright 2018, Wiley-VCH; and (E) Ji *et al*.[71] Copyright 2023, Wiley-VCH.

Abbreviations: B-ALL: B-cell acute lymphoblastic leukemia; CAR-T: Chimeric antigen receptor T-cell; EC: Endothelial cell; ECM: Extracellular matrix; GelMA: Gelatin methacrylate; HSPC: Hematopoietic stem and progenitor cell; HUVEC: Human umbilical vein epithelial cell; MSC: Mesenchymal stem cell.

Complementing earlier models, Ma *et al.*[70] engineered an organotypic immunocompetent BMOC to address the critical translational gap in chimeric antigen receptor T-cell (CAR-T) therapy development (Figure 6B). Conventional preclinical platforms—limited by poor physiological relevance to human immunity—fail to predict clinical outcomes such as resistance or relapse. By recapitulating the stromal-immune niche dynamics of leukemia bone marrow, this chip enabled unprecedented real-time monitoring of CAR-T cell functions: from extravasation and target recognition to immune synapse formation and tumor killing. Beyond observation, the platform modeled diverse clinical responses (remission, resistance, and relapse) and identified key failure drivers through a novel matrix-based analytical index. This index quantitatively demarcated functional heterogeneity across CAR designs and donor sources, transforming the chip into a predictive "(pre-)clinical-trial-on-chip." Crucially, this approach provides a physiologically relevant framework for optimizing personalized CAR-T products—a capability unattainable with animal models or static co-cultures.

The BMOCs demonstrate transformative potential in modeling human-specific bone marrow pathophysiology by overcoming conventional culture limitations for studying myelotoxic injury and genetic disorders.[64] This vascularized platform uniquely captures dynamic injury-recovery cycles—unlike static 3D cultures or animal models—faithfully recapitulating key aspects of marrow dysfunction, including clinically relevant myeloerythroid toxicity following chemotherapeutic/radiation exposure, endogenous recovery after drug-induced myelosuppression, and crucially, disease-specific hematopoietic defects through patient-derived cells. For example, when modeling Shwachman–Diamond syndrome (SDS), the chip revealed a previously unreported neutrophil maturation defect through co-culture of SDS patient cluster of differentiation 34-positive ($CD34^+$) cells with stromal components (Figure 6C), thereby validating its human pathophysiology-mirroring capability while enabling the discovery of disease mechanisms inaccessible to animal studies. As a human-specific alternative, BMOCs bridge critical gaps in toxicity testing and rare disease research, accelerating therapeutic development while circumventing translational and ethical constraints of traditional approaches.

4.2. Metastatic niche recapitulation: From tumor colonization to dormancy

With the development of microfluidic technology and bioengineering, the microfluidic organ chip system has become an important platform for studying tumor metastasis.[87] Although the bone organ-on-a-chip platform was introduced late in bone metastasis research, its contributions to this field are similar.[88] Using microfluidic organ chip systems, researchers co-cultured multiple cells and studied many biological processes related to tumor bone metastasis, such as invasion,[89] intravasation,[90] extravasation,[91-94] and angiogenesis.[92,94,95] These studies provide deeper insights into the mechanism of tumor bone metastasis and important information that can help develop new treatment methods. For example, BMOC platforms uniquely address the clinical imperative of breast cancer bone metastasis research—where about 70% of metastatic cases involve skeletal colonization—by providing human-relevant models to decipher tumor-stromal crosstalk during metastatic progression.[91,94] Unlike traditional models hampered by sampling limitations, these systems enable longitudinal observation of metastatic cascades. Exemplifying this, Hao *et al.*[61] engineered a self-mineralizing bone-on-a-chip that spontaneously develops 85-μm-thick osteoid tissue mimicking human bone matrix without exogenous differentiation agents (Figure 6D). When co-cultured with DTCs, the platform revealed rapid cancer invasion into mineralized matrices, capturing unique colonization hallmarks previously only observable *in vivo*. Critically, this humanized model overcomes species barriers that undermine animal studies, while its miniaturized design enables high-throughput screening of metastasis-inhibiting therapeutics.

The BMOC platforms uniquely overcome traditional modeling limitations by enabling precise recapitulation of the bone perivascular niche's hemodynamic regulation—a critical determinant of tumor dormancy induction previously unmodelable in static systems. This capability is exemplified through the strategic integration of endothelial cells and MSCs to reconstruct physiologically relevant microvascular networks, faithfully mirroring human BMME.[92,94] Building on this foundation, Marturano-Kruik *et al.*[60] demonstrated a human triculture model integrating endothelial cells, BMSCs, and breast cancer cells within 3D bone matrices. By precisely controlling interstitial flow velocities and oxygen gradients, they established self-sustaining capillary networks without angiogenic supplementation—mimicking slow-flow conditions that maintain nutrient/signaling exchange in native perivascular niches. Crucially, cancer cells exposed to these physiological flows adopted a slow-cycling phenotype, directly linking hemodynamic parameters to dormancy-priming microenvironments. This vascularized platform thus provides unprecedented access to spatiotemporal regulation of tumor colonization and quiescence transitions.

Ji *et al.*[71] pioneered a multi-niche integrated BMOC platform by converging 3D bioprinting and microfluidics, enabling comprehensive recapitulation of metastatic progression from pre-metastatic niche (PMN) establishment to dormancy escape—a continuum previously unmodeled in single-niche systems. This platform synchronously

reconstructs three functionally interdependent metastatic microenvironments within a unified chip architecture (Figure 6E): (i) a biomimetic dormancy niche where 3D-printed bone matrices with MSCs replicate cortactin-regulated tumor–stromal interactions governing quiescence; (ii) a physiologically accurate perivascular niche featuring engineered H-type[96] microchannels lined with endothelial cells to simulate bone-specific vascular dynamics; and (iii) a vicious cycle niche demonstrating osteoclast-mediated reactivation of dormant cells. Crucially, quantitative cortactin or invadopodia analysis validated the platform's capacity to track dormancy-escape dynamics, revealing MSC/osteoclast crosstalk as a master regulator of metastatic reawakening. By simultaneously capturing these three PMN transition phases in a human-relevant system, this approach overcomes a fundamental limitation of prior models. It establishes a novel framework for identifying therapeutic targets against dormancy escape mechanisms.

4.3. Engineering next-generation BMOC technique: Precision blueprints for dormancy breakthroughs

Although BMOC platforms have achieved groundbreaking advances in dormancy mechanism research, significant limitations persist in modeling the dynamic monitoring and biological complexity of metastatic dormancy and reactivation. The primary challenge lies in dormant cell visualization—current systems typically rely on cell morphology or dormancy-associated protein detection, lacking real-time monitoring capabilities. This deficit in monitoring technology may oversimplify critical dynamic processes during dormancy–awakening transitions, such as inflammatory bursts or hemodynamic fluctuations triggered by bone remodeling. Static or simplified flow regimens in existing chips struggle to replicate such physiological variability, potentially masking key reactivation triggers (e.g., immune surveillance or hormonal signaling cascades). Notably, Correia *et al.*[26] leveraged lentiviral delivery of mVenus-p27K$^-$ reporters (specifically labeling quiescent tumor cells) to enable real-time microscopic tracking of dormant cells, offering a novel strategy to overcome this limitation.

Cellular fidelity bottlenecks similarly constrain translational value. Despite significant interpatient heterogeneity in dormancy behaviors, most BMOC studies employ immortalized cell lines or engineered stem cells rather than patient-derived primary cells. This reductionist approach may compromise clinical relevance, leading to misrepresentation of key dormancy-regulating pathways (e.g., p38 MAPK-dependent cell cycle arrest) in artificial co-culture systems. The integration of organoid technology with BMOC is driving the evolution of multidimensional modeling systems: patient-derived organoids (PDOs) generated from primary or metastatic lesions faithfully retain the genetic/phenotypic heterogeneity of parental tumors.[97] When coupled with BMOC platforms, this integrated system enables dynamic, stage-resolved analysis of tumor–stroma interactions.[98] For example, the feasibility of co-culturing PDOs with fibrotic matrices/immune cells in microfluidic chips has successfully established complex organotypic tumor microenvironments.[99] To further enhance physiological relevance, Du *et al.*[100] developed vascularized PDO chips featuring stratified microvascular networks, demonstrating that metastatic cells drive angiogenesis through Notch signaling, with vascular density positively correlating with clinical metastatic potential—providing a novel strategy for metastasis risk assessment.

Collectively, these technological gaps demand resolution through next-generation BMOC platforms. Future designs must integrate three strategic directions: longitudinal monitoring technologies for dormant cell dynamics, a PDO–BMOC hybrid multidimensional modeling framework for clinical translation, and precision-engineered vascularized organoid microenvironments. Only through such systematic innovations can BMOC substantially bridge the discrepancy between *in vitro* models and dormancy biology research.

5. Limitations and future directions

Although BMOCs can be used to simulate some characteristics of the BMME and study cell–cell/matrix interactions under specific physiological and pathological conditions, the complexity found *in vivo* cannot be fully replicated, such as medullary cavity structures, vascular network, and neuronal network. These factors may significantly affect the development of tumor dormancy, but their effects and mechanisms cannot be investigated because simulating them in organ chips is difficult. Crucially, the bone marrow exists in a state of hypoxia or low oxygen concentration, with significant oxygen gradients across its niches. Vascular niches exhibit higher oxygen levels, while endosteal niches are profoundly hypoxic.[101] This critical parameter regulates hematopoietic cell behavior,[102] including potential effects on dormancy. Its faithful representation within BMOCs is essential for achieving physiologically relevant models. While studies such as Chou *et al.*[64] have explored manipulating oxygen to study HSC behavior, and Houshmand *et al.*[103] identified the absence of hypoxia as a key limitation in niche modeling, robust integration and control of physiological oxygen gradients in complex BMOCs remain a significant challenge.

The 3D printing technology has been used to effectively construct complex microstructures and simulate the 3D structure and cell arrangement of the tumor microenvironment.[73,95,104] However, other hematopoietic

and immune cells associated with BMME niches may also have potential effects on the regulation of tumor dormancy, as they participate in tumor dormancy and invasion.[105] For example, immune niches are also involved in tumor metastasis. The presence of relevant macrophage subsets in the primary tumor is associated with the dormancy or dissemination of DTCs.[106] Therefore, future BMOC designs must prioritize the integration of critical immune components to build functional immune niches capable of interrogating their specific roles in regulating dormancy. Furthermore, to model the systemic aspects of metastasis and dormancy (e.g., dissemination from primary sites, homing to bone marrow, and awakening in distant organs), the development and integration of multi-organ chip platforms linking BMOCs with other relevant organ modules (e.g., liver and lung) hold immense potential but present substantial engineering challenges.[107] In the future, researchers need to include more metastasis-related niches in the design of BMOCs.

The BMME is a highly dynamic system, and researchers find it challenging to simulate processes, such as incomplete simulation of blood circulation, cell migration, and cross-organ communication, using current organ chip technology. However, incorporating these factors is necessary to elucidate mechanisms underlying diseases, predict pharmacokinetic parameters of drugs, and assess drug pharmacokinetics. Significant bottlenecks in BMOC development and adoption persist. These include achieving standardized vascularization (e.g., size, maturity, and perfusion stability across models), faithfully replicating complex inter-niche signaling (e.g., soluble factors, direct cell contact, and ECM cues), and ensuring high reproducibility both within and between laboratories.

Researchers have proposed a flexible approach, which is similar to modular platforms like Tetris, for integrated human chip systems. The platform offers the flexibility to be constructed and deconstructed according to analytical requirements. It enhances user convenience in pharmacological studies and mitigates leakage hazards. Nonetheless, ongoing challenges include restricted processing capacity, inter-module communication complexities, and issues with compatibility.[108,109] To overcome these limitations, the integration of cutting-edge tools is paramount. Artificial intelligence can accelerate design optimization, predict cellular responses, and analyze complex multimodal data streams generated by chips. Incorporating PDOs or other patient-specific cells into BMOCs offers a powerful path towards personalized medicine applications and better modeling of human-specific dormancy mechanisms.

In contrast to conventional cell culture platforms and animal-based models, BMOC systems have many advantages, such as physiological correlation, high-throughput drug screening, and real-time monitoring and imaging. However, their application also has several problems, such as data collection, integration, and analysis. A variety of manufacturing methods, such as 3D printing, machining, injection molding, and imaging processes, can be used to fabricate an integrated and accessible platform designed for visualization and sampling.[108]

The rapid development of big data and artificial intelligence technology provides new perspectives for research on bone-related pathophysiology, and deep information mining methods can be used to understand bone structure and function. Artificial intelligence-assisted manufacturing technology can be used to optimize the manufacturing process of bone marrow organ chips and improve their performance, including accuracy, resolution, and cell viability. With continuous technological advancements, more detailed tumor sample data, including information on the genome, transcriptome, proteome, and metabolome, can be obtained. Comprehensive analysis of multi-omics data using methods such as machine learning and artificial intelligence can be performed to identify new biomarkers and predict the potential and prognosis of tumor dormancy. To summarize, integrating microfluidic systems with these emerging technologies can provide new and effective ways to develop advanced BMOC platforms, facilitating further research on tumor dormancy.

6. Conclusion

The BMME plays a pivotal role in tumor dormancy, with significant implications for cancer metastasis and recurrence. The innovative BMOC technology has emerged as a valuable tool for modeling and investigating the complex interactions within the BMME, offering novel insights into the regulatory mechanisms of tumor cell dormancy. Despite the current limitations in replicating the full complexity of the BMME using BMOCs, ongoing advancements promise to refine the understanding of tumor dormancy. Future research should focus on enhancing the physiological fidelity of BMOCs and leveraging this technology to develop more effective therapeutic strategies for managing cancer dormancy and preventing relapse.

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Ethics approval and consent to participate

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Consent for publication

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Availability of data

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