

REVIEW ARTICLE

Toward precision oncology: Leveraging bone metastatic organoid models for mechanistic, translational, and therapeutic discovery

Chencong Lv^{1†}, Xiao Chen^{2†}, Hongjing Dou³, Zhenping Cao⁴, Jiaca Su^{2*}, and Tong Meng^{1*}

¹Department of Orthopedics, Shanghai General Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

²Department of Orthopedics, Xinhua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

³The State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai, China

⁴The State Key Laboratory of Systems Medicine for Cancer, Shanghai Cancer Institute, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

⁵Shanghai Key Laboratory for Nucleic Acid Chemistry and Nanomedicine, Institute of Molecular Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

*Corresponding authors: Jiaca Su (drsujiacan@163.com); Tong Meng (mengtong@medmail.com.cn)

[†]These authors contributed equally to this work.

Citation: Lv C, Chen X, Dou H, Cao Z, Su J, Meng T. Toward precision oncology: Leveraging bone metastatic organoid models for mechanistic, translational, and therapeutic discovery. *Organoid Res.* 2026;2(1):026050004. doi: 10.36922/OR026050004

Received: January 28, 2026

Revised: February 20, 2026

Accepted: March 2, 2026

Published online: March 26, 2026

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Abstract

Bone metastasis represents a frequent late-stage complication in cancers such as lung, breast, and prostate, severely affecting patients' quality of life. Conventional two-dimensional cultures and animal models fail to recapitulate the complex bone microenvironment. Patient-derived organoids (PDOs) offer a physiologically relevant three-dimensional platform to recapitulate bone metastasis by preserving native tumor features and modeling tumor–bone interactions. This review systematically outlines the current methodologies for constructing bone metastatic organoid models, evaluates their applications, and identifies future directions. We describe the essential components of culture systems and critically discuss their strengths and limitations in modeling bone-specific signaling. Furthermore, we highlight the capacity of PDOs to elucidate key aspects of bone metastasis, including tumor cell adaptation to the osseous niche, bidirectional remodeling of the microenvironment, and the dynamic monitoring of disease progression. Bone organoids are also discussed as a means of establishing a standardized bone microenvironment, offering a controllable in vitro platform for investigating interactions between tumor cells and the bone matrix. Furthermore, we present the translational potential of organoids for informing individualized therapy selection, evaluating clinical drug sensitivity, and facilitating the development of organoid biobanks. Looking forward, the development of patient-specific “bone metastasis-on-a-chip” systems with artificial intelligence-driven digital twins may transform the research paradigm from experimental simulation to precision prediction, ultimately advancing personalized therapeutic strategies for bone metastatic disease.

Keywords: Bone metastasis; Patient-derived organoids; Bone organoids; Tumor microenvironment; Precision medicine

1. Introduction

Bone represents the third most common site of distant cancer metastasis, predominantly observed in malignancies including lung, breast, prostate, liver, renal, and colorectal cancers.¹ Bone metastasis is often associated with a series of skeletal-related events, including pathological fractures, bone pain, hypercalcemia, and bone loss, that severely compromise patients' quality of life.^{2,3} Despite advances in cancer management, current therapeutic strategies for bone metastasis (including radiotherapy, chemotherapy, and immunotherapy) continue to fall short in efficacy. Under most conditions, the treatment of bone metastasis is largely palliative, focusing on symptom alleviation rather than curative outcomes.⁴⁻⁶

These therapeutic challenges, compounded by an incomplete understanding of the underlying mechanisms of bone metastasis, largely stem from a lack of research models capable of recapitulating the genetic heterogeneity and intricate microenvironment of the disease.⁷ Conventional two-dimensional cell culture systems fail to recapitulate the native three-dimensional (3D) architecture of bone tissue. Consequently, they exhibit substantial disparities in critical cellular processes, including migration, invasion, and pharmacological responses, compared to *in vivo* conditions.⁸ Moreover, as life sciences research advances, the limitations of animal models have become increasingly apparent. Their ability to faithfully recapitulate human tissue and organ physiology is fundamentally constrained by interspecies differences, prohibitive costs, and limited translational value.^{9,10} These structural and physiological shortcomings collectively hinder the precise modeling of the dynamic and complex interactions between tumor cells and the bone microenvironment.

Patient-derived organoids (PDOs) from patient tissue samples effectively overcome many constraints inherent to conventional models. These 3D cultures stably retain the genomic, proteomic, and morphological profiles of original tumors *in vitro*, and more accurately recapitulate the interactions between the bone microenvironment and tumor cells, including key processes such as angiogenesis and bone remodeling.^{11,12} Moreover, bone organoids are 3D biomimetic constructs that provide a promising platform for recapitulating the intricate architecture of bone and for bone disease modeling.¹³ Although their application in bone metastasis research is still in its early stages, they offer an unparalleled platform for systematically elucidating the spatiotemporal dynamics of bone metastasis and for developing novel therapeutic approaches.

This review delineates fundamental principles and methodologies for constructing bone metastatic organoids (Section 2) and addresses current limitations in simulating niche-specific signaling pathways. We highlight how these

systems have explored the mechanisms underlying tumor cell adaptation to the bone milieu, bidirectional remodeling at the tumor–bone interface, and real-time monitoring of metastatic dissemination (Section 3). We also critically evaluate the translational potential of organoids for drug sensitivity profiling, personalized treatment prediction, and biobanking, while exploring the complementary potential of engineered bone organoids (Sections 3.4–3.5). This discussion is contextualized within the current landscape of challenges, including limited reproducibility, incomplete representation of microenvironmental complexity, and the need for robust clinical validation, which are systematically examined in Section 3.6. Finally, we discuss how integrating bone organoids with single-cell sequencing, organ-on-a-chip systems, genome editing, and artificial intelligence (AI) may address existing bottlenecks (Section 4), offering a new framework for deciphering bone metastasis biology and advancing precision oncology (Section 5).

2. Engineering bone metastatic organoids to recapitulate the osseous niche

2.1. Processes and mechanisms of bone metastasis

Bone metastasis of solid tumors constitutes a multistage biological cascade, classically delineated as a sequential progression encompassing colonization, dormancy, reactivation, and bone remodeling.¹⁴ The conceptual framework for this process traces back to Stephen Paget's¹⁵ 1889 “seed and soil” hypothesis, which proposed that tumor cells (“seeds”) thrive only within permissive organ microenvironments (“soil”). Compelling evidence suggests that tumor cells undergo metabolic reprogramming to facilitate adaptation to distant microenvironments, a process that may be potentiated by the pre-metastatic niche.^{16,17}

Colonization is initiated when primary tumor cells undergo epithelial–mesenchymal transition, intravasate into circulation as circulating tumor cells, and eventually lodge within bone tissue as disseminated tumor cells.^{5,18} A subset of disseminated tumor cells may subsequently enter a dormant state, maintaining quiescence within the osseous niche under the regulation of osteoblasts, osteoclasts, and immune cells (such as myeloid-derived suppressor cells), mediated through signaling pathways including transforming growth factor beta, Wnt, and Notch.¹⁹⁻²¹ Upon exposure to permissive microenvironmental cues, dormant cells can undergo reactivation through mesenchymal–epithelial transition and proliferate into clinically detectable metastases.⁶ Concurrently, bone metastasis disrupts the physiological bone homeostasis. In osteolytic lesions, tumor-derived factors, such as parathyroid hormone-related protein, stimulate the production of osteoblast-derived receptor activator of nuclear factor κ B ligand (RANKL), which further activates osteoclast-mediated

bone resorption. The subsequent release of bone matrix-sequestered nutrients (including amino acids and fatty acids) and growth factors (including insulin-like growth factor 1 and transforming growth factor beta) further augments tumor proliferation and parathyroid hormone-related protein secretion, establishing a self-amplifying “vicious cycle” that drives metastatic progression and bone destruction.^{6,22}

2.2. Source of organoids and culture systems

Bone metastatic organoids are typically derived from metastatic lesions harvested from either patient specimens or animal models (Figure 1). As illustrated in Figure 1, the workflow initiates with the acquisition of metastatic tissue, which is then processed into single-cell suspensions. Subsequent embedding in a 3D matrix supports the establishment of organoids that faithfully retain the genomic and phenotypic hallmarks of the original tumor. These organoids subsequently serve as platforms for drug screening, mechanistic studies, and biobanking, ultimately informing personalized treatment strategies. Nevertheless, the establishment and maintenance of such organoid cultures necessitate systematic optimization of culture conditions to suppress the over-proliferation of resident non-malignant stromal cells.²³⁻²⁶

Specialized culture medium and tailored extracellular

matrix (ECM) scaffolds constitute the two core elements of organoid culture systems. The composition of the medium critically governs cell viability, proliferation, and phenotype maintenance, representing the fundamental determinant of organoid cultivation. To accommodate the distinct biological requirements of various tumor types, media are systematically supplemented with defined combinations of growth factors and cytokines. Currently, the culture conditions for bone metastatic organoids rely predominantly on the rational design and optimization of bioactive components. Table 1 summarizes the essential components of the medium used to develop organoids from common types of bone metastases.

In vitro culture of these organoids typically relies on basal media such as Dulbecco's Modified Eagle Medium, RPMI 1640, or Minimum Essential Medium to supply essential nutrients. To support sustained tumor cell expansion, these media are fortified with proliferation-promoting factors, specifically including epidermal growth factor, fibroblast growth factor, insulin-like growth factor, and B-27 (without vitamin A). Nutritional supplementation should also be provided by adding fetal bovine serum or other serum substitutes, while antibiotics such as penicillin and streptomycin are used to maintain an aseptic environment. To mitigate oxidative stress, antioxidants, including nicotinamide and N-acetylcysteine, are often

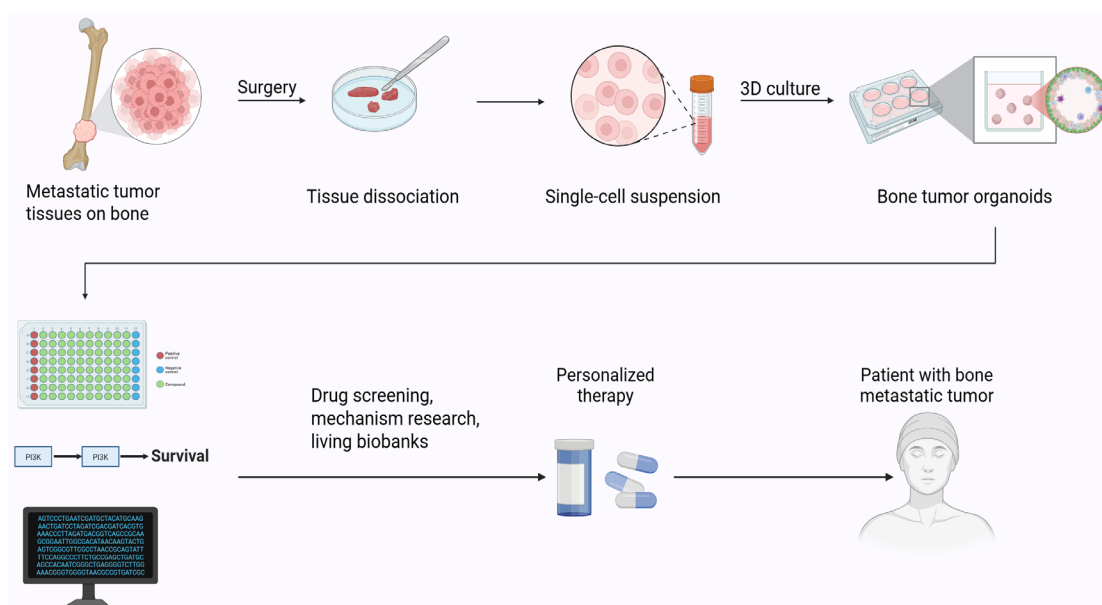


Figure 1. Workflow for establishing bone metastatic organoids and their potential applications in precision oncology research. This schematic outlines the process of generating bone metastatic organoids and their translational application in precision oncology. Bone metastatic tissue obtained via surgery is dissociated into single-cell suspensions and cultured in a three-dimensional (3D) matrix to establish organoids that retain key genomic, phenotypic, and functional features of the original tumor. These organoids serve as a versatile platform for high-throughput drug screening, mechanistic studies, and the development of biobanks. Based on experimental data generated from organoid models, personalized treatment strategies can be designed, thereby closing the translational loop from clinical sample acquisition through *in vitro* modeling to personalized treatment planning for bone metastatic diseases. Created in BioRender. Jiang, C. 2026. <https://BioRender.com/cqlbeb4>.

Table 1. The essential components of the medium for organoids of bone metastases

Origin	Sample source	Basic ingredients	Scaffold type	Bone-specific additions	Primary tumor additions	Success rate (%)	References
Breast cancer	Multiple sources, including bone	Heparin, cortisone, Mammocult™ Human Medium Kit (comprehensive base), penicillin (100 U/mL), streptomycin (100 µg/mL), nystatin (20 µg/mL)	Matrigel matrix	Not reported	Not reported	Not reported	27
Prostate cancer	Orthopedic surgical specimens	Advanced D-MEM/F-12, primocin, GlutaMAX, HEPES, Y-27632, penicillin/streptomycin, nicotinamide, R-spondin 1, N-acetylcysteine, B-27, SB-202190, Noggin, HGF, EGF, FGF-10, FGF2, PGE ₂ , A83-01, Wnt3a, fetal calf serum	Scaffold-free suspension culture system	None	DHT	Not reported	28
Lung cancer	Spinal tumors	Advanced D-MEM/F-12, GlutaMax, B27, N2, Y-27632, FGF-10, EGF, N-acetylcysteine, Noggin, A83-01	Matrigel matrix	None	None	77.8	29

Abbreviations: DHT: Dihydrotestosterone; DMEM: Dulbecco's Modified Eagle Medium; EGF: Epidermal growth factor; FGF: Fibroblast growth factor; HEPES: *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid); HGF: Hepatocyte growth factor; PGE₂: Prostaglandin E2.

added, and buffering agents such as *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) and bicarbonate are used to maintain physiological pH stability. Collectively, these components establish an integrated *in vitro* system that sustains the viability and proliferation of bone tumor cells.^{30,31}

Nevertheless, such media are predominantly composed of basic nutrients and broad-spectrum growth factors, designed to establish a generalized niche that supports tumor cell growth. They are deficient in critical signaling mediators, such as RANKL, bone morphogenetic proteins, high concentrations of calcium ions, or bone matrix-derived factors, that are essential for mimicking the bone microenvironment and inducing or sustaining metastatic phenotypic traits. Consequently, while organoids cultured under these conditions retain intrinsic tumor cell properties, they fail to recapitulate the dynamic crosstalk between tumor cells and the bone metastatic “soil.” This lack of “soil-specific signaling” constrains deeper exploration of the biological mechanisms underlying bone metastasis and hampers the development of drug screening platforms that mirror clinical disease.

Current technologies for culturing cancer organoids still face challenges in controllability and reproducibility, with the lack of standardized applicable media protocols being a major contributor. However, achieving such standardization constitutes a complex and demanding endeavor in itself.³² To advance culture system optimization, some researchers have pursued strategies to streamline media composition

from a nutritional perspective. For example, Lyra-Leite *et al.*³³ developed a minimal basal medium comprising only 39 components, aiming to reduce redundancy and improve culture efficiency. Although this design has not yet been directly applied to bone metastatic cancer organoids, its conceptual framework offers valuable insights for optimizing such media in this context.³³ Indeed, studies have systematically outlined rational strategies for formulating organoid culture media and have particularly emphasized the need to tailor medium composition according to individual patient differences.³² Furthermore, in the long-term culture of tumor epithelial cells, conditioned medium often outperforms fully defined chemical media in maintaining cell viability and function, underscoring the irreplaceable role of secreted microenvironmental cues in supporting organoid physiology.³⁴

In addition to the culture medium, supportive ECM is another essential component in the cultivation of bone metastatic cancer organoids. Its composition and properties critically govern organoid morphogenesis and function. By providing structural support and synergizing with soluble cytokines, the ECM can partially simulate the microenvironmental cues of the bone metastatic “soil,” thereby modulating tumor cell proliferation, behavior, and stem-like properties. Conventionally, dissociated tumor cell suspensions are embedded within ECM materials, such as Matrigel or gelatin, to mimic the 3D structure of bone *in vivo*.^{35,36} In recent years, synthetic biomaterial scaffolds have shown considerable potential in emulating

the microarchitecture of bone. Compared to natural matrices, synthetic scaffolds offer enhanced controllability and tunability of physical parameters, such as porosity, stiffness, and degradation kinetics, enabling more precise reproduction of the complex 3D organization of bone. This approach provides a promising strategy for constructing bone metastatic organoid models with higher physiological relevance.³⁷ Notably, conventional natural matrices, such as Matrigel, are often limited by significant batch-to-batch variability and ill-defined compositions, leading to inconsistent outcomes in organoid establishment. Critically, the flexibility of cultivation strategies is underscored by the ability of matrix-free systems to preserve both tumor heterogeneity and patient-specific transcriptional profiles in prostate cancer organoid cultures.³⁸

3. Bone metastatic organoids as a platform for deconstructing metastatic mechanisms

The tumor microenvironment (TME) is indispensable for the establishment and progression of bone metastases. Currently, organoids have been employed in 3D culture systems to recapitulate key aspects of the TME, offering a transformative platform to dissect dynamic tumor–stroma crosstalk.³⁹ This organoid platform systematically deciphers the core biological mechanisms of bone metastasis in malignancies with high skeletal tropism, such as prostate, breast, and lung cancers.⁴⁰

3.1. Tumor cell adaptability in bone metastasis (Seed properties)

Organoid models preserve the genomic and functional heterogeneity of primary and metastatic tumors, thereby serving as powerful experimental tools for deconvoluting the intrinsic characteristics of the “seed.” Ding *et al.*²⁸ established organoids from breast cancer metastases to the left pelvis and right tibia. Single-cell sequencing revealed the molecular evolutionary landscape and targetable signaling pathways in bone metastasis. Notably, bilateral bone lesions consistently retained the primary tumor driver mutations *PIK3CA-E545K* and *BRCA1-D1834H*, suggesting that phosphatidylinositol 3 kinase–protein kinase B–mechanistic/mammalian target of rapamycin (mTOR) inhibitors and poly (ADP-ribose) polymerase inhibitors may represent effective shared therapeutic strategies.⁴¹ In prostate cancer, organoids derived from bone metastatic sites stably recapitulated the resistance to anti-androgen therapies, directly reflecting the adaptive evolution of the “seed” under therapeutic pressure.⁴² Further investigations demonstrated that the bivalent mTOR complex 1/2 inhibitor, rapalink-1, markedly suppressed the growth of PDOs *in vitro* and xenograft tumors *in vivo*. Beyond durably blocking mTOR complex 1/2 signaling, rapalink-1 reshaped tumor metabolism, notably downregulating

key enzymes involved in glutamine metabolism and lipid synthesis. These findings reveal a functional adaptive dependency in advanced prostate cancer cells, suggesting a potential metabolic dependency on the mTOR signaling pathway.²⁸

3.2. Bidirectional remodeling of the tumor–bone microenvironment (soil modification)

Organoid co-culture systems provide a powerful experimental platform for modeling the dynamic dialogue and reciprocal reprogramming between tumor cells and constituents of the bone microenvironment. Early studies using 3D co-cultures revealed that prostate cancer cells can irreversibly reprogram bone-derived stromal cells into a tumor-promoting phenotype, substantially accelerating cancer growth and invasive potential. The work provided foundational insights into the role of the 3D microenvironment in cancer progression and serves as a classic example of the “soil” modified by the “seed.”⁴³ Furthermore, by establishing heterotypic 3D organoid models that co-culture prostate cancer cells with preosteoblasts, researchers have simulated tumor–bone matrix interactions within the metastatic niche. These models demonstrated that tenascin C activates Src signaling to promote the expression of the androgen receptor splice variant AR-V7. The same platform was used to investigate the bidirectional regulation of the tenascin C–AR-V7 axis by hormonal cues and anti-androgen therapies, elucidating its key role in the progression of castration resistant prostate cancer.⁴⁴ This study exemplifies the utility of organoid models in recapitulating key features of the TME and in decoding intercellular signaling crosstalk.

In breast cancer bone metastasis, an engineered human bone marrow model was established and integrated with patient-derived breast cancer organoids. This system simulated the colonization of breast cancer cells in the marrow niche and enabled the tracking of hematopoietic differentiation trajectories during tumor invasion.⁴⁵ Additionally, co-culture of these organoids with matched cancer-associated fibroblasts directly uncovered specific stroma-mediated mechanisms underlying therapy resistance.⁴⁶

3.3. Dynamic tracking of metastasis and therapy

Organoid models exhibit dynamic and experimentally tractable features that allow them to simulate metastatic processes and monitor therapeutic responses in real time. At the translational level, a retrospective analysis demonstrated that therapy guided by drug sensitivity testing of metastatic breast cancer organoids significantly prolonged patient progression-free survival compared with empirically chosen regimens, highlighting their value in dynamically predicting personalized therapeutic outcomes.²⁷ In mechanistic and

therapeutic discovery research, patient-derived lung cancer bone metastatic organoids have been used to dynamically model the evolution of bone metastasis and temporally track responses to denosumab *in vitro*. Downregulation of RANKL in this model correlates with the early onset of clinical osteogenic efficacy in patients, suggesting potential utility for predicting therapeutic response in bone metastasis. Transcriptomic analysis revealed that denosumab progressively remodeled the TME, specifically by modulating tumor necrosis factor signaling, Th17 cell differentiation, and zinc finger/homeobox transcription factors, thereby providing new insights into its dynamic mechanism of action.²⁹ Similarly, high-throughput screening conducted with breast cancer organoids identified the small-molecule compound S6, which blocks sclerostin–signal transducer and activator of transcription 3 interaction, and validated its efficacy in inhibiting bone metastasis in mouse breast cancer organoids. The study further underscores the potential of organoid models for *in vitro* drug discovery and efficacy validation.⁴⁷ To more precisely mimic physiological processes, cutting-edge research has successfully reconstructed a perivascular niche within the core of microspheres and a functional endosteal niche in their outer shell, offering a novel engineered tool for tracking early metastatic events.⁴⁸

3.4. Translational potential of bone metastatic organoids

Bone metastatic cancer organoids hold significant promise as a preclinical resource, primarily by providing an *in vitro* platform that captures the biological features of a patient's metastatic disease (Figure 1). This capability advances precision medicine across multiple fronts. Organoids facilitate high-throughput drug screening, enabling rapid assessment of sensitivity to standard therapies, experimental agents, and combination regimens, thereby accelerating the identification of candidate therapies for subsequent preclinical and clinical evaluation.⁴² Furthermore, they function as a predictive tool for personalized treatment planning. Drug sensitivity assays using PDOs serve as a platform for hypothesis generation regarding the effectiveness of therapy in individual cases. In research settings, this approach can inform the design of tailored treatment strategies, pending prospective clinical validation before routine implementation²⁹, helping to avoid ineffective therapies and reduce trial-and-error costs. For instance, PDOs have been used to guide personalized therapy selection for metastatic breast cancer through customized drug panel testing.²⁷ Additionally, the scalability and cryopreservation compatibility of bone metastatic cancer organoids enable the establishment of high-quality, functional living biobanks. These repositories not only preserve the genetic, phenotypic, and microenvironmental

features of the primary tumors but also allow for continuous expansion and long-term passage, offering a stable and renewable precious resource for translational research.⁴⁹

3.5. Bone organoid models

Although patient-derived bone metastatic organoids can closely recapitulate established metastatic niches, their microenvironment reflects a tumor-modified state and exhibits heterogeneity across samples. To longitudinally investigate how tumor cells shape the bone niche from its inception and establish a more standardized control system, bone organoids offer an essential complementary and foundational research platform. Bone organoids are engineered to reconstruct a 3D bone microenvironment with mineralized matrix, multicellular composition, and physiologically relevant functions through a “bottom-up” approach.^{50–56} This is accomplished by combining bone-derived cells, such as bone marrow-derived mesenchymal stem cells (BMSCs) and osteoblast/osteoclast precursors, with biomimetic biomaterials, thereby establishing a model for studying bone development, homeostasis, and disease.^{57,58} For example, digital light processing 3D bioprinting technology was employed to integrate BMSCs with a hydroxyapatite-containing composite hydrogel, creating organoids capable of spontaneous mineralization, vascularization, and multicellular bone tissue formation in mice, thereby establishing a high-fidelity platform for bone biology research.⁵⁸ The fabrication strategies and potential applications of bone organoids are summarized in Table 2.

Bone organoids and PDOs are complementary in both construction logic and research objectives. Bone organoids focus on engineering a standardized “soil,” whereas PDOs concentrate on preserving the patient-specific “seeds” along with their adapted “soil” complex. The integration of these two models opens new investigative paths forward. On one hand, patient-derived circulating tumor cells or primary tumor cells at different stages could be introduced into standardized bone organoids constructed from the same patient's or a healthy donor's cells, enabling longitudinal observation of the entire process of colonization, dormancy, and reactivation. Such an approach would help dissect early metastatic events and organ-specific mechanisms. On the other hand, technological advances in the bone organoid field, such as vascularization, innervation, and biomechanical integration, could be directly applied to augment the physiological complexity and functional relevance of bone metastatic organoid models, thereby improving their utility in drug screening and mechanistic exploration of bone metastasis.⁵⁷ This combined strategy of “engineered soil” and “patient-derived seeds” will enable more systematic and precise investigation of bone metastasis biology.

Table 2. The fabrication strategies and potential applications of bone organoids

Organoid type	Cell type	Medium composition	Scaffold type	Construction strategy	Potential application	Reference
Woven bone organoid	hBMSCs	DMEM, FBS, antibiotic/antimycotic, ascorbic-acid-2-phosphate, dexamethasone, β -glycerophosphate	Silk fibroin scaffold	Self-organization	Drug testing, mechanobiology research, tissue engineering	50
	BMSCs	L-DMEM or H-DMEM with 1% penicillin/streptomycin, sodium pyruvate, insulin, transferrin, selenium, dexamethasone, ascorbic acid, TGF- β 3	GelMA hydrogel microspheres	Digital light processing-based bioprinting + stepwise induction	Repair and regeneration of critical-sized long bone defects	51
Callus organoids	hPDCs	Expansion medium (DMEM, FBS, antibiotic-antimycotic, sodium pyruvate); chondrogenic induction medium (LC-DMEM, antibiotic-antimycotic, ascorbate phosphate, dexamethasone, proline, Rho kinase inhibitor Y27632, ITS+ premix, BMP2, GDF5, TGF- β 1, BMP6, bFGF-2)	Scaffold-free microspheroids	Self-assembly and timed chondrogenic induction	Repair and regeneration of critical-sized long bone defects	52
	BMSCs	α -MEM, 10% FBS, 1% penicillin-streptomycin, ascorbic acid, β -glycerophosphate	GelMA/AlgMA/HAP hybrid bioinks	3D bioprinting	Bone tissue engineering, regenerative medicine	53
Bone marrow organoids	iPSCs	STEMdiff APEL2 medium, StemFlex medium, RevitaCell, StemPro-34 SFM, KO serum replacement, chemically defined lipids, BMP4, VEGFA, VEGFC, FGF2, hSCF, Flt3, TPO, EPO, G-CSF, IL3, IL6, CHIR99021, heparin, GlutaMax	Mixed-matrix hydrogel (collagen I, collagen IV, and Matrigel)	Self-organizing hiPSC-derived bone marrow organoids in hydrogels via staged differentiation	Disease modeling, drug discovery	54
	iPSCs	Aggregation medium: knockout DMEM/F12 + 20% knockout serum replacement + 1% L-glutamine + 1% NEAA + 1% pen/strep + 100 μ M β -mercaptoethanol, mTeSRplus, essential 6 medium, Stempro-34 SFM, cytokines/growth factors: BMP4, VEGF, CHIR99021, SB431542, bFGF, SCF, IL-3, Flt-3L, TPO, Y-27632 (ROCKi)	Collagen I / Matrigel mixture	Self-organization	Modeling hematopoietic development, disease modeling	55
Cartilaginous organoids	iPSCs	DMEM, ITS-X, FBS, non-essential amino acids, sodium pyruvate, penicillin-streptomycin, ascorbic acid, BMP2, TGF- β 1, GDF5	Scaffold-free	Self-assembly	Repair of articular cartilage defects	56

Abbreviations: AlgMA: Alginate methacrylate; APEL2: Albumin polyvinyl alcohol essential lipids 2 medium; bFGF: Basic fibroblast growth factor; BMP: Bone morphogenetic protein; BMSCs: Bone marrow-derived mesenchymal stem cells; DMEM: Dulbecco's Modified Eagle Medium; EPO: Erythropoietin; FBS: Fetal bovine serum; FGF2: Fibroblast growth factor 2; Flt3: Fms-like tyrosine kinase 3 ligand; Flt-3L: Fms-like tyrosine kinase 3 ligand; G-CSF: Granulocyte colony-stimulating factor; GDF5: Growth differentiation factor 5; GelMA: Gelatin methacrylate; HAP: Hydroxyapatite; H-DMEM: High-glucose Dulbecco's Modified Eagle Medium; hBMSCs: Human bone marrow-derived mesenchymal stem cells; hPDCs: Human periosteum-derived cells; hSCF: Human stem cell factor; IL: Interleukin; iPSCs: Induced pluripotent stem cells; ITS: Insulin transferrin selenium; KO: Knockout; L-DMEM: Low-glucose Dulbecco's Modified Eagle Medium; LC-DMEM: Low-calcium Dulbecco's Modified Eagle Medium; MEM: Minimum Essential Medium; mTeSRplus: Maintenance medium for human pluripotent stem cells; NEAA: Non-essential amino acids; ROCKi: Rho-associated protein kinase inhibitor; SCF: Stem cell factor; SFM: Serum-free medium; TGF- β : Transforming growth factor beta; TPO: Thrombopoietin; VEGF: Vascular endothelial growth factor.

3.6. Limitations of bone metastatic organoids

Despite their considerable promise, current bone metastatic organoid models are constrained by key limitations that must be addressed to fully realize their translational potential. A primary limitation is their incomplete recapitulation of the native TME; most models lack essential components such as immune cells, functional vasculature, and neural networks, thereby restricting studies of critical processes like immune evasion and neurovascular invasion.⁹ Furthermore, challenges in reproducibility and standardization persist, driven by variable culture media formulations, batch-dependent extracellular matrices (e.g., Matrigel), and the absence of unified quality control metrics.

Additional technical hurdles include: (i) sampling bias in surgical specimens, which often capture only specific tumor regions and thus fail to represent intratumoral heterogeneity; (ii) the static nature of conventional cultures, which cannot replicate dynamic biomechanical cues or support ECM reconstruction; and (iii) the inherent difficulty of modeling the multi-step metastatic cascade from colonization to dormancy and reactivation within a single culture system.³¹ Recognizing these constraints is essential for interpreting model-based data and

underscores the need to integrate advanced technologies to evolve these platforms into more physiologically complete and predictive systems.

4. Integrating innovative technologies to address current bottlenecks

Bone metastatic organoid models have demonstrated substantial promise in simulating tumor complexity. However, to further elucidate the dynamic, multifactorial pathological process of bone metastasis, it is essential to address several persistent limitations, including resolving intratumoral heterogeneity, simulating the physiological microenvironment, validating causal mechanisms, and enabling high-throughput prediction. The integration of advanced technologies, such as single-cell multi-omics, organ-on-a-chip systems, clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing, and AI, is systematically enhancing the physiological fidelity and predictive capacity of these organoid systems (Figure 2). Figure 2 provides a schematic overview of how these technologies synergistically enhance organoid-based models derived from primary tumors, including prostate, breast, and lung cancers, thereby bridging the gap between mechanistic discovery and clinical translation.

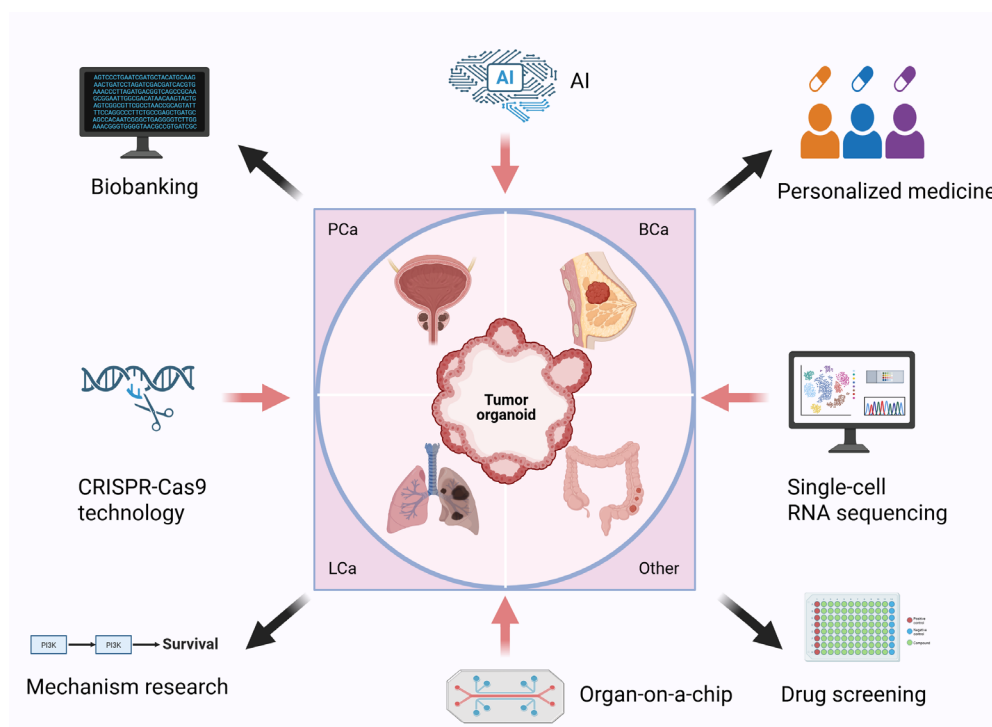


Figure 2. Multidimensional application and technological integration of bone metastatic organoids. The integration of advanced technologies, such as single-cell multi-omics, organ-on-a-chip systems, gene editing, and artificial intelligence (AI), enhances the physiological fidelity and predictive capacity of bone-metastatic organoids derived from various primary tumors, such as prostate cancer (PCa), breast cancer (BCa), and lung cancer (LCa). Thus, these bone metastatic organoids may provide platforms to promote mechanistic investigation toward translational utility. Created in BioRender. Jiang, C. 2026. <https://BioRender.com/n9mg2jc>.

Abbreviation: CRISPR: Clustered regularly interspaced short palindromic repeats.

4.1. Single-cell sequencing: Deconvoluting intratumoral heterogeneity and validating model fidelity

Single-cell multi-omics technologies represent a gold standard for assessing the fidelity of organoid models. A foundational conceptual and methodological advance in single-cell RNA sequencing (scRNA-seq) was first reported by Tang *et al.*⁵⁹ This technology enables the classification, characterization, and discrimination of individual cells at the transcriptomic level, uncovering rare but functionally pivotal cell subsets and potential therapeutic targets.⁶⁰ For instance, one study successfully established PDOs from bone metastatic lesions and performed scRNA-seq on both the original metastases and the corresponding PDOs. A direct comparison of their single-cell atlases demonstrated that PDOs faithfully preserved the cellular heterogeneity of the source tissue. Specifically, PDOs retained the composition and transcriptional profiles of epithelial cell subpopulations present in the original bone metastases, confirming their reliability as representative experimental models.⁴¹ Furthermore, scRNA-seq analysis revealed persistent *PI3K* and *BRCA1* mutations in breast cancer bone metastases. These findings not only shed light on the evolutionary trajectory of the metastatic “seed” but also directly guided the design of targeted therapeutic strategies, such as those targeting the phosphatidylinositol 3 kinase pathway, which were subsequently functionally validated in PDO models. In summary, the synergistic integration of high-resolution scRNA-seq with the dynamic, experimentally tractable organoid platform has deepened our understanding of the heterogeneity and evolution of cancer bone metastasis, while directly pointing toward potentially effective precision treatment strategies for patients.

4.2. Organ-on-a-chip: Introducing hydrodynamics and spatial dimensions

Organ-on-a-chip technology introduces critical physiological dynamics, such as fluid shear stress, mechanical cues, and spatially organized cell–cell interactions, into static organoid models via engineered microfluidic systems. An organ-on-a-chip is defined as a microfabricated cell culture device that leverages precisely controlled fluid flow, biophysical and biochemical cues, and dynamic cell–cell, cell–matrix, and tissue–tissue interfaces to mimic key architectural and functional features of human organs.^{61,62} For instance, a breast cancer bone metastasis-on-a-chip model was established using a 3D co-culture system to mimic the interactive microenvironment among cancer cells, osteoclasts, and osteocytes. This model not only recapitulated key hallmarks of the *in vivo* metastatic niche but also exhibited a transcriptomic profile highly congruent with that of mouse *in vivo* bone metastasis models. The

study further identified interleukin 6 as a central mediator of the tripartite crosstalk among osteocytes, osteoclasts, and cancer cells, offering new insights into cellular communication during bone metastasis.⁶³ In contrast to the stochastic architecture of conventional organoids, organ-on-a-chip platforms allow precise control over cellular organization and microenvironmental gradients, thereby more faithfully simulating the spatial invasion and niche remodeling of tumors within bone. This approach addresses a major limitation of traditional models: their inability to replicate complex physiological dynamics.

Furthermore, in the field of bone organoids, the convergence of 3D bioprinting technology with organ-on-a-chip systems offers a powerful tool for engineering bone microenvironments with precise geometry and functional zonation. For instance, projection-based photopolymerization can be employed to fabricate bone organoid scaffolds with biomimetic porous structures using composite bioinks containing BMSCs and hydroxyapatite, enabling spatiotemporal control over mineralization and vascularization both *in vitro* and *in vivo*.⁵⁸ Coupling such engineered bone constructs with microfluidic platforms not only simulates the nutrient and drug gradients present in bone but also introduces physiologically relevant mechanical stimuli, thereby offering an unprecedented experimental system for investigating tumor cell behavior within a dynamic and architecturally defined bone microenvironment.

4.3. Clustered regularly interspaced short palindromic repeats-Cas9 technology: Establishing causal links and engineering disease models

Clustered regularly interspaced short palindromic repeats-Cas9, as the foremost genome editing tool to date, provides an efficient, precise, and modular system for targeted genomic modifications. It has been widely implemented across diverse fields, including genetic disease research, gene therapy, drug development, and regenerative medicine.⁶⁴ The application of CRISPR-Cas9 in organoid models enables the systematic evaluation and validation of key driver genes in tumorigenesis and tumor reprogramming.⁶⁵ The integration of CRISPR-based genome editing with organoid technology marks a transition from correlative observation to causal investigation. Researchers can directly interrogate the necessity and sufficiency of a given gene in driving malignant transformation, mediating therapeutic resistance, or facilitating adaptation to bone metastasis by selectively knocking out, activating, or mutating the target locus in normal or tumor organoids. For example, Duarte *et al.*⁶⁶ employed genetically engineered organoids to explore drug resistance in *BRCA*-deficient mouse mammary tumors, demonstrating that orthotopically transplanted organoids retained the drug-response profiles of the

original cancers.⁶⁶ Moreover, genetic ablation of genes such as *PTPN22* or *MLL3* in mammary stem cells permitted the direct engineering of breast cancer organoids, thereby enabling investigation of tumorigenesis mechanisms.⁶⁷ In bone metastasis research, this technological platform holds substantial promise. It enables the generation of engineered organoids carrying specific mutational combinations, thereby dissecting how these alterations cooperatively drive skeletal colonization and therapy resistance. This approach not only bypasses certain ethical constraints but also provides mechanistically defined experimental models and therapeutic targets for precision oncology.

4.4. Artificial intelligence: From data analysis to virtual organoids

Artificial intelligence is comprehensively empowering organoid research by shifting the paradigm from an experiment-driven to a data-driven, intelligently designed approach. AI has been widely adopted across biomedical investigations, spanning disease diagnosis, gene editing, drug development, protein structure prediction, and organoid engineering, significantly enhancing the ability to analyze complex biological systems.⁶⁸ In organoid studies, data-driven AI methods accelerated the optimization of bone organoids, particularly in improving spatial precision and structural complexity.⁶⁴ While AI techniques have been extensively applied to organoid research of the pancreas, lung, colon, and other organs, their use in bone metastatic organoids remains relatively limited.³¹ In future research on bone metastasis, AI can assist in intelligent image analysis, high-throughput drug screening, and highly sensitive biosensing, thereby advancing the dynamic monitoring and mechanistic understanding of bone metastasis.⁶⁹ AI can further guide the optimization of organoid culture, for example, by algorithmically screening for optimal matrix materials or cellular compositions.⁷⁰

Concurrently, one of the most transformative advances is the realization of the “AI virtual organoid” concept.⁷¹ Serving as a digital twin of physical organoids, AI virtual organoids enable the simulation of thousands of drug combinations in virtual screening assays, drastically shortening development timelines and reducing experimental costs. In bone metastasis research, this enables potential therapies to be rapidly pre-screened *in silico* before precise validation in physical models, thereby facilitating the design of efficient, personalized treatment strategies. This progression marks a paradigm shift from retrospective data analysis to prospective, predictive digital modeling in oncology.

5. Future perspectives

Despite their considerable promise, bone metastatic organoid models confront several fundamental challenges.

Addressing these limitations will require integrating engineering and biological strategies, progressing toward standardized protocols and rigorous clinical validation, and developing more sophisticated, multifunctional model systems—all of which are essential to realizing their full translational potential.

Current organoid models remain constrained by inadequate vascularization, oversimplified representations of the microenvironment, and morphological and functional deviations from native tissue.^{12,72} To address vascularization challenges, engineering strategies such as organ-on-a-chip platforms and 3D bioprinting are crucial. Microfluidic chips enable precise control over perfusion dynamics, simulation of hemodynamic shear stress, and guidance of endothelial network self-assembly, while bioprinting permits the pre-defined patterning of vascular architectures for spatially regulated delivery of nutrients and oxygen.^{61,72} The bone and TMEs are essential regulators of metastatic progression; however, existing organoid models frequently exhibit an oversimplified and low-throughput TME⁷³, limited recapitulation of its dynamic nature⁷⁴, and insufficient complexity to accurately emulate the *in vivo* tumor ecological niche.⁷⁵ Integrating single-cell multi-omics analyses enables continuous validation and iterative refinement of these co-culture systems, thereby ensuring that cellular composition and molecular profiles faithfully reflect physiological conditions.

Emerging advances in bone organoid engineering, particularly in the construction of sophisticated bone microenvironments, promise to directly address these existing bottlenecks. For example, 3D bioprinting can be used to fabricate bone organoid scaffolds with biomimetic mineralized matrices and channel-like architectures, which may serve as an ideal physical and biochemical foundation for bone metastatic organoids.⁵⁸ Further integration of patient-derived tumor cells and immune components into such engineered bone organoids offers a promising strategy for constructing patient-specific “bone metastatic niche” models. Such systems would better recapitulate tumor progression, drug resistance, and immune evasion within an individualized bone microenvironment.

Beyond these technical challenges, broader barriers related to standardization and regulation must be addressed to facilitate clinical translation of organoid technologies. Inter-laboratory variability, driven by differences in tissue processing, culture media formulations, and matrix materials such as Matrigel, compromises reproducibility and hinders cross-study comparisons. Furthermore, consensus remains lacking on quality control metrics for organoid characterization, including criteria for genomic stability, cellular composition, morphological fidelity, and functional responsiveness. From a regulatory perspective, while agencies such as the Food and Drug Administration

have demonstrated growing acceptance of these models, their integration into drug discovery and development pipelines remains nascent.⁷⁶ Although regulatory adoption of organoid technology has progressed, its full integration into formal review processes requires further evidentiary support. Prospective, multicenter “organoid-informed clinical trials” are therefore imperative to establish the necessary evidence base for organoid-based data, thereby facilitating broader regulatory acceptance.

The establishment of bone metastatic organoids is still in its early phase, and the absence of consistent standards for their generation, culture, and evaluation poses a fundamental barrier to comparative analysis and clinical translation.¹¹ A future standardization framework should encompass standardized protocols for sample acquisition and processing, reference formulations for core media and supplements, defined culture systems (e.g., matrix selection), and evaluation criteria for critical quality attributes, including histomorphology, genomic stability, and biomarker expression. Building upon this foundation, it will be essential to initiate prospective “organoid-informed clinical trials.”⁷⁷ This method can directly correlate drug response profiles from patient-derived organoids with clinical outcomes, serving as a gold standard validation of the models’ predictive utility. Promoting the incorporation of functionally validated organoid-derived evidence into clinical practice guidelines will be an essential step toward transitioning organoids from experimental tools into clinical decision-support instruments. Nevertheless, these models are well-positioned as experimental platforms for dissecting metastatic mechanisms and as preclinical tools for drug sensitivity testing and biomarker discovery. However, while retrospective studies have demonstrated correlations between organoid drug responses and patient outcomes, prospective clinical trials are warranted to validate their predictive utility.

The next paradigm shift will involve transitioning from isolated organoid models toward highly integrated, personalized, and intelligently orchestrated research systems. This vision finds its ultimate manifestation in the construction of a patient-specific “bone metastasis-on-a-chip”: a micro-engineered system integrating tumor organoids, vascular networks, bone-matrix cells, and immune components—all derived from the same patient—to faithfully recapitulate a dynamic and individualized metastatic niche. Complementing this physical platform, AI-driven digital twin systems will establish a synergistic “physical-digital” dual-paradigm framework.⁷¹ Such an integrated strategy is poised to significantly accelerate drug discovery and personalized therapeutic decision-making, ultimately advancing the realization of patient-centered precision oncology.

6. Conclusion

Patient-derived bone metastatic organoids have emerged as a powerful platform that bridges the gap between conventional models and *in vivo* biology, uniquely preserving tumor heterogeneity while enabling mechanistic dissection of tumor–bone crosstalk, drug sensitivity profiling, and biobanking. Nevertheless, current iterations remain constrained by incomplete microenvironmental fidelity, a lack of standardization, and inadequate vascularization. Addressing these barriers requires integrating advanced engineering and analytical technologies. The convergence of engineered bone organoids with patient-derived tumor cells holds promise for constructing personalized “bone metastasis-on-a-chip” systems, thereby advancing both mechanistic understanding and therapeutic precision for bone metastatic disease.

Acknowledgments

None.

Funding

This work was supported in part by the National Natural Science Foundation of China (grant numbers: 82522063, 82472786, 82173168).

Conflict of interest

Jiacan Su serves as the Editorial Board Member of the journal but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Other authors declare they have no competing interests.

Author contributions

Conceptualization: Jiacan Su, Tong Meng

Visualization: Chencong Lv, Tong Meng

Writing—original draft: Chencong Lv, Xiao Chen

Writing—review & editing: Hongjing Dou, Zhenping Cao

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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