

Review Article

Innovative strategies for bone organoid: Synergistic application and exploration of advanced technologies

Xu Lou^{a,1}, Qirong Zhou^{d,1}, Zhenglin Dong^{d,1} , Long Bai^{b,c,e,f,*},
Jiacan Su^{b,c,d,f,**} , Hua Yue^{a,***}

^a Department of Osteoporosis and Bone Disease, Shanghai Clinical Research Center of Bone Disease, Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200233, China

^b Organoid Research Center, Institute of Translational Medicine, Shanghai University, Shanghai, 200444, China

^c National Center for Translational Medicine (Shanghai) SHU Branch, Shanghai University, Shanghai, 200444, China

^d Department of Orthopedics, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200092, China

^e Wenzhou Institute of Shanghai University, Wenzhou, 325000, China

^f MedEng-X Institutes, Shanghai University, Shanghai, 200444, China

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ABSTRACT

Bone organoids, as three-dimensional (3D) biomimetic constructs, have emerged as a promising platform for studying bone development, disease modeling, drug screening, and regenerative medicine. This review comprehensively explores innovative strategies driving bone organoid advancements, emphasizing the integration of cutting-edge technologies such as bioprinting, artificial intelligence, assembloids, and gene editing. While 3D bioprinting enhances spatial precision and structural complexity, artificial intelligence accelerates organoid optimization through data-driven approaches. Assembloids enable the assembly of multicellular systems to better replicate bone tissue microenvironments, whereas gene editing refines disease modeling and functional modifications. Despite these advancements, challenges remain, including the lack of vascularization, insufficient mechanical stimulation, and standardization issues across different models. Also, the clinical translation of bone organoids necessitates the establishment of rigorous evaluation frameworks, ethical guidelines, and regulatory policies to ensure their reproducibility and safety. Looking ahead, interdisciplinary convergence will be critical for constructing physiologically relevant “ex vivo skeletal systems”, advancing bone biology, precision medicine, and biomaterial testing. This review highlights the transformative potential of bone organoid technology and its future applications in personalized orthopedics and bone disease intervention.

The Translational Potential of this Article: This review provides a comprehensive overview of cutting-edge strategies for constructing bone organoids, emphasizing their integration with advanced technologies such as bioprinting, artificial intelligence, assembloids, and gene editing. By systematically discussing their applications in bone development, disease modeling, drug screening, and regenerative medicine, this article bridges the gap between experimental models and clinical translation. The insights into vascularization, skeletal patterning, and high-throughput screening platforms offer a foundation for developing physiologically relevant bone organoids with enhanced fidelity and functionality. These advancements hold significant potential for accelerating personalized medicine, facilitating preclinical evaluation of therapeutics, and ultimately improving treatment outcomes for skeletal diseases.

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* Corresponding author. Organoid Research Center, Institute of Translational Medicine, Shanghai University, Shanghai, 200444, China.

** Corresponding author. Organoid Research Center, Institute of Translational Medicine, Shanghai University, Shanghai, 200444, China.

*** Corresponding author.

E-mail addresses: bailong@shu.edu.cn (L. Bai), drsujacan@163.com (J. Su), yueyinglonghua@163.com (H. Yue).

¹ These authors contributed equally to this work.

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1. Introduction

1.1. The origin of organoids and an overview of bone organoids

Organoids, as three-dimensional (3D) *in vitro* miniature organ models, originate from the self-organizing ability of pluripotent stem cells or tissue-specific progenitor cells [1]. Through recapitulating key cellular components, spatial architecture, and partial physiological functions within a biomimetic microenvironment, organoids provide an advanced platform for studying tissue development and disease mechanisms. In 2009, the research team led by Hans Clevers in Netherlands successfully cultured Lgr5⁺ intestinal stem cells into murine intestinal organoids, accurately recapitulating the crypt-villus structure *in vitro*, making a new era in organoid-based biomimetic research [2]. Following this breakthrough, various tissue-specific organoid models were developed: in 2011, Sasai et al. generated retinal organoids with a retinal cup-like structure from embryonic stem cells, and in 2013, Knoblich et al. reported human brain organoids capable of modeling early neurodevelopment and microcephaly pathogenesis [3,4]. Over the past decade, organoid technology has rapidly expanded to encompass lung, kidney, pancreatic, cardiac, breast, ovarian, and colorectal models, establishing itself as an indispensable platform for recapitulating complex tissue-specific architecture and physiological functions, enabling in-depth investigation of disease mechanisms, and significantly accelerating the discovery, testing, and optimization of novel therapeutic strategies in preclinical research [5–12] (Fig. 1).

In recent years, bone and cartilage organoid research has also achieved significant progress. Bone and cartilage organoids refer to 3D biomimetic constructs formed through the self-organization of stem cells under defined *in vitro* culture conditions [13]. Bone organoids, in particular, have demonstrated the ability to recapitulate the intricate 3D architecture and multicellular composition of native bone tissue, partially exhibiting its functional characteristics. Studies have reported that bone organoids can mimic bone mineralization and mechanical responses, incorporating key cell types such as osteoblasts, osteoclasts, and bone progenitor cells [14,15]. Compared to conventional two-dimensional (2D) cell cultures and animal models, bone organoids provide a more physiologically relevant *in vitro* platform for studying

bone development, homeostasis, and disease mechanisms. Currently, bone organoids have been employed in diverse applications, including bone disease modeling, drug screening, and bone regeneration therapy development. Similarly, in the field of cartilage organoids, recent studies have leveraged induced pluripotent stem cells (iPSCs) to generate cartilage-like structures, facilitating research on articular cartilage regeneration and osteoarthritis (OA) pathogenesis [16] (Fig. 2). Collectively, the establishment of bone and cartilage organoids offers a transformative tool for precisely modeling skeletal development and disease processes *in vitro*, opening unprecedented avenues for bone biology and bone-related disease research [17].

1.2. Challenges and technical barriers in bone organoid construction

Despite the great potential of bone organoids, their construction remains challenging due to several technical bottlenecks. Bone is a mineralized connective tissue composed of osteoblasts, osteocytes, osteoclasts, and stromal cells, embedded within a hierarchical structure ranging from nanoscale collagen fibrils to trabecular and cortical architectures [18]. This complex organization confers exceptional mechanical strength and load-bearing capacity, supporting locomotion, organ protection, and calcium-phosphate homeostasis. Additionally, bone exhibits dynamic remodeling regulated by mechanical cues, which profoundly influence cell differentiation, matrix deposition, and tissue maturation [19]. These biomechanical factors are largely absent in conventional organoid culture platforms, contributing to the difficulty in replicating bone-specific features *in vitro*. Unlike conventional organoids derived from tissues such as the intestine or liver, bone organoids are particularly difficult to cultivate due to the unique anatomical and physiological characteristics of bone tissue [20]. Current bone organoid models still exhibit significant morphological and functional differences from human skeletal tissue, making it difficult to fully recapitulate the complexity of native bone.

One of the major limitations is the lack of vascularization, which restricts the size of bone organoids and impairs their ability to mimic large-scale bone structures [21,22]. Native bone tissue is highly mineralized and well-vascularized, yet existing bone organoids typically lack mature vascular networks, resulting in inadequate oxygen and

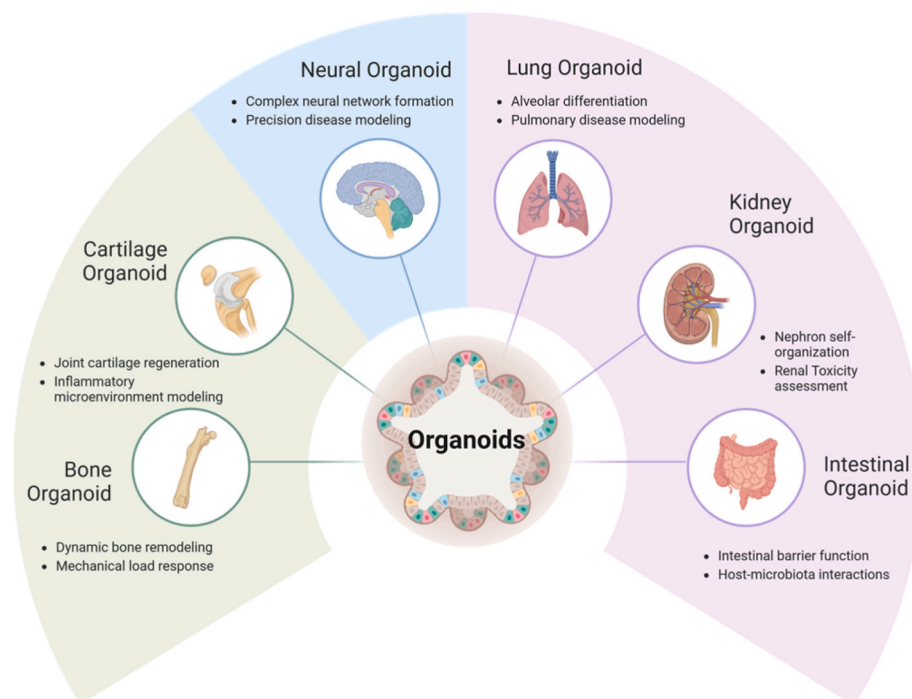


Fig. 1. Organoid models of key human organs.

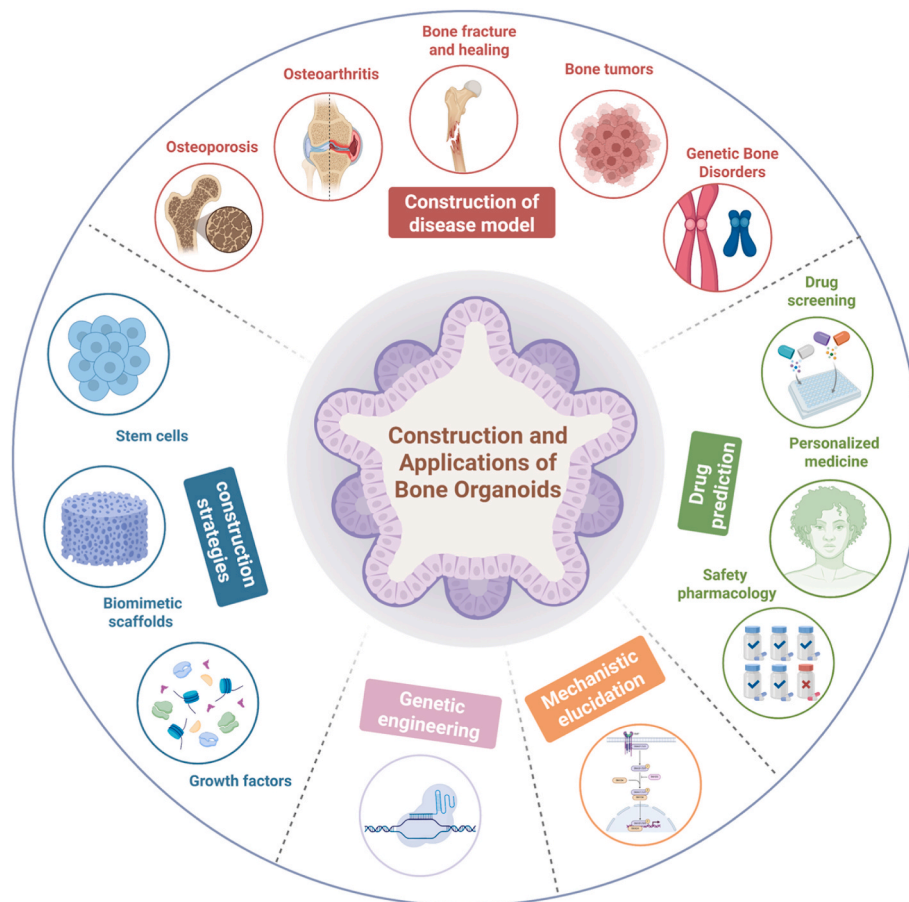


Fig. 2. Construction and application of bone organoids.

nutrient supply, which limits further cellular differentiation and long-term viability [23]. Insufficient vascularization has been recognized as a critical bottleneck not only for bone organoids but also for large organoid systems in general. Recent studies have attempted to introduce microvascular endothelial cells into the culture medium or employ microfluidic systems to provide fluid perfusion, partially alleviating nutrient transport limitations [24]. Nevertheless, a fully functional vascular network has not yet been established in bone organoids. Such vascularization deficiency not only restricts nutrient exchange but also interacts with other technical bottlenecks, including cellular complexity and biomechanical responsiveness, thereby collectively impeding the maturation and physiological relevance of bone organoids.

In parallel with the need for vascular integration, reconstructing the biomechanical environment of bone tissue presents equally significant challenges [25]. Mechanical forces play a critical role in bone development by regulating cellular behaviors such as proliferation, differentiation, and matrix mineralization through mechano-transduction pathways. Dynamic loading can promote osteogenic lineage commitment of MSCs and enhance endothelial network formation, thereby supporting the coordinated development of vascularized bone tissue. In fact, most organoid cultures are maintained in static suspension or hydrogels, lacking mechanical cues, which may lead to deviations in osteogenic differentiation from physiological conditions. Recent studies have begun employing bioreactors to apply cyclic stress or vibrational forces to bone organoids, attempting to mimic the mechanical environment of native bone tissue [26,27]. However, these approaches remain at an early stage and require further refinement.

Complementing the above biological limitations, the material and technical basis for *in vitro* culture, particularly scaffold selection and

protocol standardization, also exerts a decisive influence on bone organoid fidelity. The choice of culture media and scaffold materials significantly impacts the maturation and reproducibility of bone organoids. Most organoid models rely on Matrigel, a basement membrane extract, as a 3D scaffold [28]. Nevertheless, Matrigel is derived from mouse tumors, exhibits batch-to-batch variability, and contains murine proteins, which may compromise the fidelity of human bone organoid differentiation and hinder clinical translation [29]. Moreover, Matrigel possesses limited mechanical strength and fails to provide a rigid microenvironment comparable to native bone. Alternative scaffolds such as collagen-based hydrogels have been explored, yet they often suffer from poor mechanical stability and uncontrolled degradation rates [30]. Developing biomaterials that simultaneously provide appropriate mechanical properties and support osteogenic differentiation remains a major challenge in bone organoid engineering. Additionally, the absence of standardized protocols across different laboratories, including variations in cell sources such as embryonic stem cells, induced pluripotent stem cells, and adult stem cells, as well as in differentiation factor combinations and culture media formulations, has led to substantial batch-to-batch variability, limiting the comparability of results and posing challenges for clinical translation [31]. In summary, overcoming the limitations of vascularization, integrating multiple cellular components, optimizing scaffold materials, and establishing standardized culture protocols are critical steps toward improving the physiological relevance and practical utility of bone organoids [32]. Addressing these challenges will pave the way for more advanced *in vitro* models that can better mimic bone development, disease progression, and regenerative processes.

1.3. Advanced technologies driving bone organoid construction and applications

Despite persistent challenges, emerging advanced technologies are creating novel opportunities for the construction and application of bone organoids. These innovations can be broadly classified into two complementary categories: engineering-driven advances such as bioprinting and artificial intelligence (AI), which enhance structural fidelity and culture optimization, and biology-oriented advances such as assembloid technology and gene editing, which enrich cellular complexity and genetic programmability. These approaches correspond to complementary facets of organoid engineering: bioprinting enables precise spatial patterning and scaffold fabrication, AI facilitates data-driven optimization of induction conditions and culture environments, assembloids mediate the integration of heterogeneous cell populations to reconstruct physiological complexity, and gene editing confers programmable control over lineage specification and disease modeling.

As the foundation for precise structural reconstruction, bioprinting lays the groundwork for subsequent optimization and integration enabled by AI and assembloids. The rise of 3D bioprinting in tissue engineering has provided a powerful tool for organoid fabrication. Bioprinting enables the precise spatial deposition of cells and biomaterials based on predesigned 3D architectures, allowing for the high-fidelity reconstruction of tissue structures [33]. In the context of bone organoids, this technology has the potential to overcome the stochastic nature of traditional self-organization methods by introducing anatomical features resembling native bone tissue, such as porous trabecular-like structures and vascular channels. Through refined structural design, bioprinting presents a novel approach to enhancing the scale and biomimetic complexity of bone organoids [34].

Building upon this structural foundation, AI contributes a powerful layer of optimization and analysis that accelerates the iterative advancement of organoid research. Machine learning (ML) and deep learning (DL) techniques are being applied to optimize organoid culture conditions and enable high-throughput, high-precision analysis [35]. By integrating AI-driven algorithms with organoid experiments, large-scale experimental data can be analyzed to extract optimal differentiation protocols, facilitating the rapid identification and refinement of induction parameters. These AI-based approaches significantly improve the efficiency of bone organoid research and enhance data output, allowing researchers to derive biologically meaningful insights more rapidly. AI is expected to become an indispensable tool in bone organoid research, shifting experimental design from an experience-based approach to a data-driven paradigm.

While AI refines internal conditions, assembloid technology complements these efforts by expanding intercellular and intertissue complexity. The emergence of assembloid technology offers new possibilities for constructing complex *in vitro* tissue systems. This approach involves the controlled assembly or fusion of different organoids or cell aggregates to create multi-tissue constructs that simulate inter-organ or inter-tissue interactions [36]. In bone organoid research, assembloid technology holds great promise for integrating bone organoids with cartilage, vasculature, and bone marrow to replicate the physiological connections between bone and surrounding tissues. The incorporation of vascular organoids could provide blood supply, improving bone organoid survival and maturation, while the integration of hematopoietic bone marrow organoids may facilitate the reconstruction of the hematopoietic function of bone [37].

To further increase biological fidelity and disease relevance, gene editing provides genetic precision and functional interrogation, playing a crucial role in the construction of bone organoids and the development of disease models [38]. The CRISPR/Cas9 system, widely employed in human stem cell and organoid engineering, enables targeted modifications of source cells to establish bone disease organoid models with well-defined genetic backgrounds. Gene editing can also be utilized to introduce fluorescent reporter genes into organoids, facilitating

real-time tracking of osteoblast differentiation and osteoclast activity, thereby providing valuable insights into cellular behavior [39–41]. Collectively, bioprinting, AI, assembloids, and gene editing each contribute distinct yet complementary advantages to the development and application of bone organoids. The integration of these advanced technologies is anticipated to markedly enhance the biomimetic complexity, functional fidelity, and physiological relevance of bone organoids. This convergence not only supports deeper insights into skeletal biology but also establishes a multidisciplinary foundation for future organoid-based innovations in regenerative medicine, disease modeling, and precision therapeutics.

2. Application of bioprinting technology in bone organoid construction

2.1. Overview of bioprinting technology development

2.1.1. Fundamental principles and classification of bioprinting

Bioprinting represents a sophisticated convergence of computer-controlled deposition, materials science, and cell biology, enabling the precise 3D fabrication of tissue-like and organoid structures [42]. Its core objective is to precisely control the spatial arrangement of cells, microenvironmental factors, and biomaterial properties to mimic the complex architecture and physiological functions of native tissues. Based on printing mechanisms and material deposition strategies, bioprinting technologies are primarily categorized into inkjet bioprinting, extrusion bioprinting, and light-based bioprinting [43]. Inkjet bioprinting operates through pressure-driven or thermal bubble-controlled droplet ejection, enabling the deposition of low-viscosity bioinks with high precision and rapid printing speed. Nevertheless, its applicability is constrained by strict requirements on bioink rheology and potential thermal or mechanical stress that may compromise cell viability [44,45] (Fig. 3A). Extrusion bioprinting relies on mechanical force or pneumatic pressure to continuously extrude bioinks, making it suitable for high-viscosity materials such as hydrogels and bioceramic composites [46]. This technique facilitates the printing of high-cell-density constructs and is widely applied in bone tissue engineering (BTE), but its relatively low resolution and shear-induced cell damage remain challenges [47] (Fig. 3B).

Light-based bioprinting utilizes laser or projection-based photopolymerization to selectively crosslink photosensitive biomaterials, allowing for the fabrication of high-resolution, multi-scale structures [48]. This approach is particularly advantageous for constructing intricate microarchitectures and vascular networks, yet it demands stringent control over the biocompatibility and phototoxicity of the materials (Fig. 3C). Each bioprinting technology presents unique advantages and limitations, necessitating careful selection and optimization based on specific requirements for bone organoid construction, such as scaffold mechanical properties, cell viability, and growth factor release kinetics. A rational integration of these parameters is critical to ensuring structural stability, functional maturation, and long-term viability of bone organoids *in vitro*, thereby enhancing their utility in disease modeling, drug screening, and regenerative medicine applications [49,50].

2.1.2. Bioprinting within BTE: unique advantages for bone organoid fabrication

BTE integrates biomaterial scaffolds, stem or progenitor cells, and bioactive factors to promote bone regeneration. As an important and rapidly evolving subset of BTE, bioprinting introduces spatial control and architectural complexity into engineered constructs [51]. Traditional BTE strategies often face limitations in recapitulating the native bone microenvironment due to inadequate spatial precision, limited capacity to recreate extracellular matrix (ECM) organization, and challenges in vascularization. Bioprinting technology, with its high precision, programmability, and ability to construct multicellular systems,

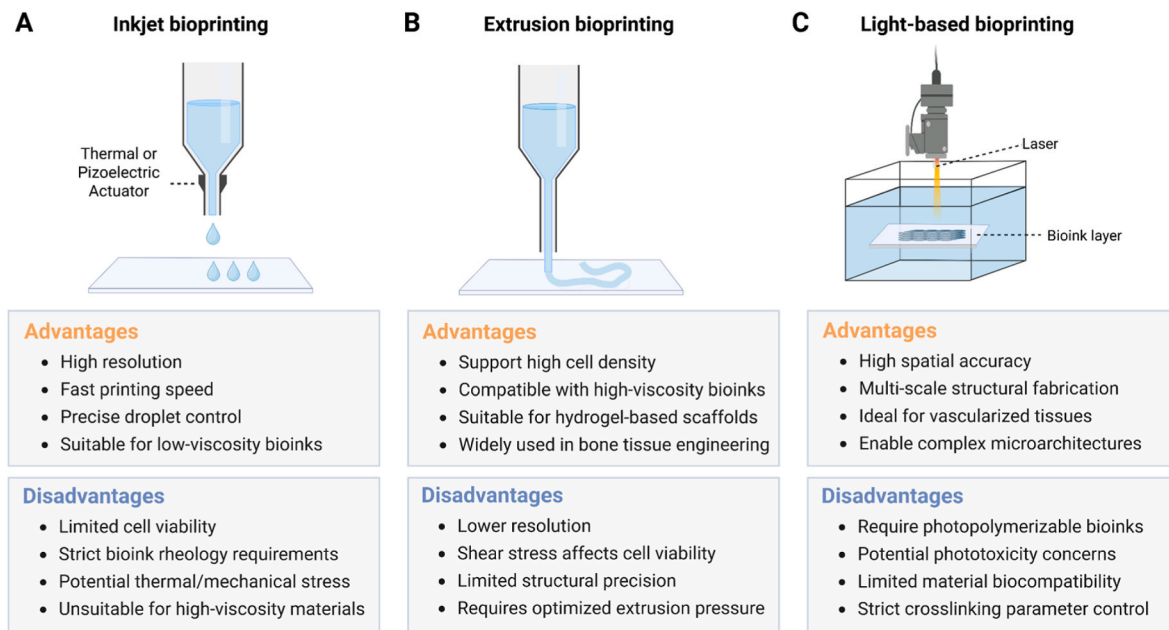


Fig. 3. Schematics of common bioprinting techniques for organoids.

offers a transformative solution for bone organoid fabrication, significantly enhancing the accuracy and functional integrity of bone tissue regeneration.

Firstly, bioprinting enables precise layer-by-layer deposition of bioinks, allowing accurate reconstruction of the intricate 3D microarchitecture of bone tissue, thereby establishing a biomimetic microenvironment that promotes spatially organoid cell adhesion, proliferation, and lineage-specific differentiation essential for bone organoid development [52]. Secondly, in contrast to traditional static cell-seeding methods, bioprinting improves cellular distribution, enhances intercellular crosstalk, and facilitates tissue maturation [53]. Additionally, it enables the incorporation of angiogenic factors or microvascular structures, thereby promoting prevascularization and improving post-implantation viability [54].

By combining structural fidelity with functional customization, bioprinting expands the capacity of BTE to model bone diseases, screen therapeutics, and fabricate transplantable constructs tailored to patient-specific needs. Its high programmability and reproducibility enable precise spatial control over cellular organization and microenvironment cues, thereby enhancing the physiological relevance, functional stability, and scalability of bone organoids. Moreover, bioprinting facilitates the integration of osteogenic and angiogenic elements within a single construct, supporting vascularized bone tissue formation and long-term viability. As a result, bioprinting holds significant promise for advancing both fundamental research and clinical applications, offering a versatile platform for disease modeling, high-throughput drug screening, and personalized bone regeneration therapies [55,56].

2.2. Optimization of bioprinting for bone organoid construction and maturation

2.2.1. Bioink formulation and printing parameter optimization for microstructural reconstruction

Bioink serves as the fundamental component in bioprinting-based fabrication of organoids, with its physicochemical properties critically influencing printing resolution, cell viability, as well as the structural stability and functional maturation of the resulting constructs [43]. An ideal bioink should exhibit excellent biocompatibility to support cell adhesion, proliferation, and differentiation, while maintaining appropriate mechanical strength to preserve the 3D architectural integrity of

the organoid. To achieve controlled construction, careful coordination of key parameters such as cell type, bioink composition, printing method, crosslinking mechanism, and spatial resolution is essential, as these factors collectively define the structural fidelity, physiological functionality, and translational potential of bioprinted bone organoids (Table 1).

Given that bone tissue formation relies on a suitable ECM, the optimization of bioink requires a balance between its rheological properties and degradation rate. Currently, natural biopolymers such as collagen, gelatin methacrylate (GelMA), sodium alginate, and chitosan are widely utilized for bone organoid construction due to their superior biocompatibility and biodegradability [61]. These biomaterials provide an optimal adhesion interface for osteoblasts and MSCs, while their porosity and mechanical properties can be finely tuned to better recapitulate the native bone microenvironment. Additionally, incorporating osteoinductive growth factors and angiogenic factors into bioinks enhances osteogenic differentiation and vascularization, thereby improving the physiological relevance and maturation of bone organoids [62].

Beyond bioink composition, printing parameters, such as printing pressure, nozzle diameter, layer thickness, crosslinking strategy, and printing speed, profoundly impact the microstructure and cellular behavior within bone organoids [63]. For instance, optimizing the porosity and mechanical properties of printed constructs facilitates the reconstruction of bone organoids with biomechanical characteristics closely resembling native bone tissue, thereby enhancing their post-implantation stability and long-term functional reproducibility [64]. Collectively, precise bioink optimization and refined printing parameter adjustments are essential for engineering physiologically relevant bone organoids, providing a robust *in vitro* platform for bone regeneration research and disease modeling [65].

2.2.2. Structural guidance for osteogenic differentiation and prevascularization for enhanced vascularization

Bone tissue maturation is highly dependent on osteogenic differentiation and vascularization, with precise microenvironmental regulation playing a crucial role in modulating cellular behavior and tissue remodeling [66]. Bioprinting technology enables the construction of biomimetic tissue structures that closely replicate the native bone microenvironment, providing precise mechanical and biochemical cues

Table 1
Representative applications of bioprinting.

Cell type	Bioink composition	Printing method	Crosslinking method	Functional outcome	Reference
human keratinocytes, fibroblasts, and endothelial cells	GelMA	Extrusion-based bioprinting	Photo-crosslinking	Successfully constructed skin organoids to accelerate wound healing	[57]
Patient-derived organoid cells	Alginate hydrogel	Embedded-based bioprinting	Thermal and calcium dual crosslinking	Platform recapitulating tumor heterogeneity and predicting drug responses	[58]
Hepatic parenchymal HepaRG cells, stellate cells and HUVECs	Sodium alginate-based bioink 1 and dipeptide-based bioink 2	3D droplet-based bioprinting	Calcium ions-alginate crosslinking, electrostatic interaction and spontaneous self-assembly	<i>In vitro</i> construction of biomimetic liver organoids with preserved structure and enhanced hepatic function	[59]
MSCs and HUVECs	GelMA	Extrusion-based bioprinting	Photo-crosslinking	In situ regeneration of vascularized bone organoids for cranial defect repair	[60]

at the cellular level to optimize osteogenic differentiation. For instance, the design of multilayer gradient scaffolds can spatially regulate osteoblast proliferation and differentiation, thereby enhancing bone matrix deposition and mineralization. Additionally, insufficient vascularization remains a major bottleneck in maintaining the physiological functionality and long-term viability of bone organoids [67]. Traditional *in vitro* culture systems fail to effectively induce vascularization, whereas bioprinting facilitates prevascularization strategies by co-depositing endothelial and osteogenic cells during organoid construction, thereby simulating the natural crosstalk between vascular and bone tissues to enhance blood supply and functional integration. For example, bioinks embedded with vascular endothelial growth factor (VEGF)-releasing microspheres can promote early-stage angiogenesis while ensuring long-term vascular network stability, thereby improving the physiological relevance and maturation of bone organoids [67,68]. Furthermore, integrating bioprinting with dynamic bioreactor culture can further optimize the vascularization process by promoting the formation and maturation of microvascular networks, enhancing structural stability and functionalization of vascularized bone constructs [69]. Collectively, these advances lay a robust foundation for the downstream application of bioprinted bone organoids in disease modeling and regenerative medicine, as elaborated in the subsequent discussion.

2.3. Utilization of bioprinting in bone organoid disease modeling and regenerative medicine

2.3.1. *In vitro* modeling of bone diseases using bioprinted pathological organoids

Building on the structural and vascular complexity achieved through advanced bioprinting approaches, bioprinting technology has revolutionized *in vitro* bone disease modeling by providing a precise and highly controllable platform for constructing pathological bone organoids. Traditional 2D cell cultures fail to recapitulate the 3D microenvironment of bone tissue, making it difficult to accurately model cell–matrix interactions and biomechanical signaling during disease progression [70]. Although animal models partially replicate the pathological features of bone diseases, species differences, individual variability, and ethical concerns limit their ability to faithfully simulate the onset and progression of bone disorders [71]. Leveraging its high-precision cell deposition, spatial tissue reconstruction, and microenvironmental regulation capabilities, bioprinting enables the fabrication of disease-specific bone organoids with enhanced physiological relevance and reproducibility. Furthermore, by incorporating microenvironmental cues such as inflammatory factors and mechanical stimuli, bioprinted organoids can more accurately respond to disease-related microenvironmental changes, increasing their applicability in drug screening and personalized medicine [72,73].

In osteoporosis modeling, bioprinting facilitates precise control over the ratio of osteoclasts to osteoblasts, dynamically regulating the RANKL/OPG signaling axis to recreate the disruption of bone remodeling [74]. This approach allows for a more accurate representation of

pathological features such as trabecular bone loss, ECM degradation, and bone microstructure fragility. Additionally, bioprinted models incorporating biodegradable scaffold materials and mechanical stimuli, such as fluid shear stress and cyclic compression, better simulate mechanoadaptive degeneration observed in osteoporosis [75]. These models serve as valuable platforms for investigating bone loss mechanisms and screening anti-resorptive drugs. In OA models, bioprinting enables spatially controlled deposition of chondrocytes, synovial cells, and inflammatory factors, thereby reconstructing the pathological OA microenvironment [76]. This approach induces cartilage degradation, mimics synovial inflammation, and replicates matrix metalloproteinase (MMP) dysregulation [77]. Compared to traditional co-culture systems, bioprinted OA organoids provide a more physiologically relevant model of subchondral bone sclerosis and joint microenvironment deterioration. These organoids can serve as high-throughput platforms for evaluating chondroprotective agents, anti-inflammatory drugs, and MMP-13 inhibitors. In summary, bioprinting technology enables precise cell patterning and microenvironment control, advancing bone disease modeling and regenerative medicine with enhanced physiological relevance and therapeutic precision.

2.3.2. Bioprinting-based bone defect repair and personalized regenerative medicine

As a logical continuation from disease modeling, this segment explores the transition of bioprinting from *in vitro* studies toward translational *in vivo* regenerative medicine. The repair of bone defects imposes stringent requirements on the structural precision, biocompatibility, and osteoinductive capacity of tissue-engineered scaffolds [78, 79]. Bioprinting technology, with its ability to precisely control cellular spatial distribution and biomaterial mechanical properties, offers a promising approach for bone regenerative medicine. Unlike conventional bone repair strategies that rely on xenografts, autografts, allografts, or artificial implants, bioprinting enables the fabrication of personalized, highly controllable bone tissue constructs, thereby significantly improving repair outcomes. Through integrating patient-specific imaging data (CT and MRI) with computer-aided design (CAD), bioprinting allows for the precise replication of bone defect geometries [80]. Via layer-by-layer deposition of bioinks containing osteogenic cells, growth factors, and biomaterials, bioprinted constructs can closely mimic the 3D architecture of native bone tissue. Unlike traditional static cell-seeding methods, bioprinting facilitates precise spatial control over cell distribution during the fabrication process, optimizing cell–matrix interactions and enhancing osteoblast adhesion, proliferation, and mineralization. Furthermore, by incorporating biodegradable biomaterials and osteoinductive factors, bioprinted scaffolds provide a biomimetic microenvironment that supports functional bone remodeling and long-term structural stability.

In the realm of personalized precision medicine, bioprinting, when combined with patient-derived stem cells such as autologous MSCs, enables the generation of immunocompatible bone organoids or tissue grafts, thereby minimizing immune rejection and enhancing clinical

feasibility [81]. For various pathological bone defects, bioprinting can be tailored to specific patient microenvironmental needs by fine-tuning bioink composition, printing parameters, and mechanical properties to achieve individualized tissue constructs. Additionally, bioprinting facilitates the development of *in vitro* bone organoid platforms that mimic disease-specific bone microenvironments, serving as high-fidelity models for personalized drug screening and the evaluation of therapeutic responses at the patient level. Looking ahead, advancements in bioprinting technology, combined with AI-driven optimization of printing parameters, biosensors for real-time monitoring, and multi-omics data integration for individualized treatment strategies, are expected to drive a new era of precision and efficiency in bone defect repair and regenerative medicine [82]. In particular, the bioprinting-enabled construction of bone-vascular co-culture systems holds great potential for improving vascularization and functional integration post-implantation, thereby offering transformative solutions for complex bone injuries and personalized regenerative therapies.

3. AI empowering bone organoid research: construction, analysis, and applications

3.1. Overview of AI

3.1.1. Core concepts and technological evolution of AI

AI is a computational framework designed to simulate human cognitive functions, including data processing, pattern recognition, reasoning, decision-making, and autonomous optimization [83,84]. It encompasses multiple subfields, such as ML, DL, computer vision (CV), and natural language processing (NLP) [85–87]. Since the concept of AI was first introduced at the Dartmouth Conference in 1965, the field has evolved through three major paradigms: Symbolism, Connectionism, and Behaviorism [88]. With the advent of big data, high-performance computing, and algorithmic advancements, AI has entered a new era of intelligence and efficiency. Currently, deep neural networks (DNNs), leveraging multi-layer neural architectures such as convolutional neural networks (CNNs) and graph neural networks (GNNs), have significantly enhanced the processing of unstructured data, positioning AI as a powerful tool in biomedical research [89].

3.1.2. AI applications in biomedical research

AI has been extensively applied in biomedical research, covering disease diagnosis, gene editing, drug development, protein structure prediction, and organoid engineering, significantly enhancing the analytical capabilities for complex biological systems [90]. In genomics, AI enables precise gene regulatory network analysis and improves CRISPR-Cas9 target recognition accuracy. In 2024, the first AI-designed editor for precise genome editing, OpenCRISPR-1, successfully modified human DNA, ushering in a new era of algorithm-driven gene editing [91]. In protein structure prediction, AI-driven algorithms such as AlphaFold2 efficiently resolve 3D protein conformations, providing crucial insights for drug discovery. A study by Wallner and Kalakoti introduced AFsample2, a method integrating multiple sequence alignment (MSA) column masking into AlphaFold2 predictions, which reduces constraints imposed by co-evolutionary signals and enhances structural diversity [92]. Through leveraging AI-driven optimization strategies, AFsample2 improves the accuracy of alternative conformational state predictions across various protein systems and significantly expands conformational space coverage, offering a novel tool for investigating protein dynamics and AI-powered drug discovery.

Furthermore, AI demonstrates immense potential in drug development, where DL combined with high-throughput screening (HTS) and virtual screening (VS) facilitates the prediction of small-molecule bioactivity and pharmacokinetics, accelerating the drug discovery pipeline. In organoid engineering, AI optimizes culture media composition, regulates growth factor concentrations, and models cell differentiation pathways to enhance organoid physiological relevance. For

instance, reinforcement learning (RL) algorithms dynamically adjust osteogenesis-angiogenesis coupling conditions in bone organoids, while CV techniques analyze microscopic structural features such as trabecular thickness and vascular network connectivity, establishing a quantitative framework for organoid maturation assessment [93]. Additionally, the integration of AI with automated robotics (Lab-on-a-Chip) is advancing the standardization of high-throughput organoid fabrication and phenotypic screening, providing high-fidelity biological models for personalized disease modeling, regenerative medicine, and precision drug screening [94].

3.2. AI-driven optimization strategies for bone organoid construction

3.2.1. AI-assisted biomaterial and growth factor selection

The successful construction of bone organoids relies on the coordinated regulation of multiple factors, including biomaterial scaffolds, cell types, growth factors, and mechanical stimulation. Precise control over these parameters is crucial for achieving functional maturation and facilitating clinical translation [95]. However, traditional screening strategies predominantly rely on experience-driven experimental approaches, which often involve trial-and-error methods for material selection and culture condition optimization. These conventional techniques are constrained by long screening cycles, high costs, and limited reproducibility, posing significant challenges for high-throughput and high-precision parameter optimization [96]. Bone tissue formation is an inherently dynamic process, encompassing intricate cellular interactions, ECM deposition, and vascularization, all of which contribute to the complexity of bone development. The integration of AI into bone organoid engineering introduces a data-driven optimization framework, leveraging high-throughput computation and ML algorithms to refine biomaterial properties, biological signaling pathways, and microenvironmental factors, thereby enhancing both the efficiency and stability of organoid formation.

In the selection of biomaterial scaffolds, DL algorithms facilitate high-throughput data mining, molecular dynamics simulations, and biomaterial property predictions, enabling precise evaluation of biocompatibility, degradation kinetics, and mechanical adaptability. This computational approach aids in identifying optimal scaffolding materials for bone organoid culture, such as collagen and GelMA [97]. In the context of growth factor optimization, AI-driven methodologies, including Bayesian Optimization and RL, enable dynamic prediction of the optimal composition and concentration of key osteogenic factors, such as BMP-2 (bone morphogenetic protein-2), TGF- β (transforming growth factor-beta), and VEGF-A (vascular endothelial growth factor-A). Through integrating osteogenic differentiation pathways and signaling network dynamics, AI enhances the precision of dose–response relationships, ensuring optimal spatiotemporal control of growth factor signaling. The application of AI-driven parameter optimization facilitates the dynamic adaptation of biomaterial scaffolds and growth factor conditions, promoting tissue maturation and functional fidelity, thereby advancing its translational applications in regenerative medicine and disease modeling.

3.2.2. ML for culture media and environmental optimization

The *in vitro* functional maturation of bone organoids relies on the precise coordination of multiple factors, including cell differentiation timing, metabolic homeostasis, and biomimetic microenvironment construction. Traditional experience-driven culture systems fail to precisely regulate these variables, resulting in insufficient organoid maturation, limited mineralization capacity, and compromised physiological functionality. ML-based intelligent optimization strategies integrate multi-omics data (e.g., single-cell transcriptomics, metabolomics, and proteomics), dynamic sensor signals (e.g., bioreactor mechanical parameters and physicochemical microenvironment indicators), and high-content imaging information to systematically establish the relationship between culture medium composition and cell differentiation states,

which enables closed-loop optimization of culture medium formulations and microenvironmental parameters. For instance, DL models, such as random forests and support vector machines (SVMs), can predict the effects of varying concentrations of BMP-2, dexamethasone, and ascorbic acid on bone organoid maturation [98,99]. These insights allow for the optimization of growth factor combinations and sequential administration, significantly enhancing osteogenic induction efficiency. Furthermore, transfer learning algorithms can integrate datasets across metabolic networks and protein interaction networks to predict the temporal release patterns of factors such as VEGF-A, thereby promoting the synchronized mineralization and vascularization of bone organoids [100]. In summary, ML-driven optimization enables precise, data-integrated regulation of culture conditions, significantly enhancing the functional maturation.

The bone microenvironment is highly responsive to mechanical stimuli, which not only regulate osteoblast proliferation and differentiation but also influence ECM synthesis and mineralization processes. ML, combined with microfluidic chips and dynamic bioreactors, enables the optimization of shear forces, tensile strain, and hydrodynamic parameters to induce type I collagen synthesis and enhance matrix mineralization [101]. Leveraging biomechanical modeling and deep reinforcement learning (DRL), AI can analyze real-time cellular mechanotransduction responses and dynamically adjust culture conditions to upregulate osteogenic gene expression, thereby improving the structural integrity and functional maturity of bone organoids. Additionally, AI-driven feedback systems, coupled with high-resolution spectroscopic analysis (e.g., Raman spectroscopy and Fourier-transform infrared spectroscopy) and non-invasive imaging techniques (e.g., multiphoton microscopy and optical coherence tomography), enable precise monitoring and regulation of the culture environment. Generative adversarial networks (GANs) further establish mapping relationships between microenvironmental parameters (e.g., oxygen concentration, pH levels, nutrient gradients, and growth factor release dynamics) and cellular metabolic states, enabling adaptive microenvironmental regulation [102]. This AI-powered optimization strategy not only enhances the elastic modulus of bone organoids, making them more mechanically comparable to native bone tissue, but also improves vascular infiltration following *in vivo* transplantation, thereby increasing their translational potential in regenerative medicine and disease modeling.

3.2.3. Predicting cell differentiation for stable production

The scalable construction and engineering applications of bone organoids rely on precise regulation of cell differentiation to ensure correct spatial distributions of distinct cellular subtypes. Bone tissue formation involves complex cell fate determination processes, including the differentiation of MSCs into osteoblast, chondrocytes, and adipocytes [103]. Traditional culture methods struggle to dynamically capture key transition points in these lineage conversions, leading to increased tissue heterogeneity, compromised physiological functionality, and reduced reproducibility in bone organoid production. The integration of AI, particularly DL-based cell fate prediction models, offers a data-driven strategy to elucidate multicellular differentiation trajectories, optimize culture conditions, and enhance production stability [104].

ML algorithms leveraging single-cell RNA sequencing (scRNA-seq) data can accurately analyze the temporal dynamics of cell differentiation and establish a comprehensive lineage trajectory from undifferentiated MSCs to mature osteoblasts [105]. For instance, recurrent neural networks (RNNs) and long short-term memory (LSTM) models can be trained on large-scale differentiation datasets to predict the dynamic expression patterns of key transcription factors such as RUNX2, SOX9, and PPAR γ , thereby inferring the probabilistic distribution of distinct differentiation pathways. Additionally, GANs, when integrated with temporal data, can synthesize high-fidelity simulated differentiation states, enhancing the generalization capability of the model for

predicting rare cellular transitions. These AI-driven predictive models enable the early identification of differentiation biases, facilitating the timely optimization of growth factor administration schedules or mechanical stimulation strategies to direct MSC differentiation preferentially toward osteogenesis rather than adipogenesis or chondrogenesis. This approach significantly enhances the structural stability and functional consistency of bone organoids, promoting their reliability in regenerative medicine and disease modeling applications.

The physiological functionality of bone organoids depends not only on accurate cell lineage differentiation but also on the spatial organization of distinct cell populations. DL integrated with spatial transcriptomics (ST) enables the analysis of intra-organoid cellular subtype distribution, optimizing bioprinting strategies and cell seeding densities. Raphael et al. introduced GASTON (Gradient Analysis of ST Organization with Neural Networks), an unsupervised DL algorithm designed to autonomously learn topological structures and spatial gene expression gradients from ST data [106]. By constructing an isodepth coordinate system, GASTON precisely captures continuous expression gradients and abrupt transcriptional transitions, demonstrating superior performance in neuronal differentiation and tumor microenvironment analysis. This method provides a novel computational tool for investigating spatially regulated biological processes. Furthermore, the integration of GNNs facilitates the inference of cell–cell communication networks, predicting intercellular interactions and optimizing signaling pathway activation states to reconstruct a bone microenvironment that closely mimics physiological conditions. Cang et al. proposed scGeom, a computational framework that combines DL and GNNs to quantify the high-dimensional structure of scRNA-seq data through topological data analysis [107]. This approach enables the unsupervised identification of transitional cells and the inference of cellular differentiation potential. Collectively, these AI-driven optimization strategies not only enhance the stability of cell differentiation within bone organoids but also improve physiological consistency across different organoid batches, laying the foundation for large-scale production and clinical applications (Table 2).

3.3. AI assisting in bone organoid disease modeling and drug development

3.3.1. AI-driven precision disease modeling

The integration of AI in disease modeling has overcome the limitations of traditional experimental approaches, enabling bone organoids to more accurately and efficiently recapitulate the pathological progression of complex bone diseases. The pathogenesis of osteoporosis, OA, and genetic bone disorders is influenced by multiple factors, including inflammation, metabolic dysregulation, and mechanical stress [108]. Nevertheless, existing *in vitro* models often fail to capture the dynamic nature of disease progression. AI-driven approaches, incorporating computational simulations, big data analytics, and multi-omics integration, facilitate the construction of high-fidelity disease-specific bone organoids, optimizing modeling efficiency while enhancing physiological relevance.

Table 2
AI-augmented strategies for optimizing bone Organoid construction.

Optimization target	AI techniques applied	Improved parameters
Biomaterial scaffold selection	Deep learning, molecular dynamics stimulation	Biocompatibility, degradation rate, mechanical strength
Growth factor regulation	Reinforcement learning, Bayesian optimization	Dose, combination, and release timing of factors
Culture medium formulation	Support vector machines, random forests	Nutrient composition, metabolic homeostasis
Cell differentiation prediction	Recurrent neural networks, spatial transcriptomics with graph neural networks	Lineage trajectory, transcriptional transition, spatial pattern

In osteoporosis organoid modeling, AI can integrate patient-specific bone metabolic data, imaging-based omics information, and clinical biomarkers to identify key regulatory factors governing the balance between osteoblasts and osteoclasts through DL algorithms. Furthermore, RL algorithms can be employed to optimize the biomechanical parameters of the culture environment, such as shear stress and simulated microgravity, to more accurately replicate mechanically induced bone resorption. Similarly, in OA organoid modeling, AI-driven multi-omics data mining enables precise analysis of inflammatory signaling pathways involving IL-1 β , TNF- α , and MMP-13. By leveraging single-cell ST, AI can predict key drivers of cartilage degradation, facilitating the optimization of OA disease modeling strategies. These AI-driven methodologies dynamically refine the microenvironment of bone organoids, ensuring greater pathological fidelity and improving the reproducibility of *in vitro* disease models.

3.3.2. Predicting drug actions and optimizing screening

The application of AI in drug development provides a precise and efficient screening and evaluation system for bone organoids, overcoming the limitations of traditional large-scale experimental screening. Conventional drug discovery is time-consuming and costly, relying on animal models or 2D cell cultures, which fail to accurately replicate the bone tissue microenvironment. AI-driven *in silico* screening, drug response prediction, and molecular optimization strategies leverage high-throughput data and computational simulations to accelerate drug discovery and enhance the precision of bone disease treatments [109, 110]. Also, DL integrated with multi-omics data analysis enables AI to accurately decipher molecular interactions within the bone microenvironment, predict synergistic or antagonistic effects of drug combinations, and provide theoretical insights for personalized treatment strategies.

In drug screening for bone organoids, AI can be employed to train DDNs on large-scale experimental datasets to predict the specific regulatory effects of candidate compounds on osteogenic, osteoclastic, and chondrogenic cells. For instance, GNNs can analyze the topological

structure of drug molecules and protein–protein interaction networks to optimize target affinity while minimizing adverse effects. Additionally, AI-driven molecular dynamics (MD) simulations and quantum chemistry calculations can predict the diffusion kinetics, metabolism, and stability of drugs within the bone organoid microenvironment. In osteoporosis drug development, AI can model the mechanisms of bisphosphonate derivatives and RANKL inhibitors and employ RL to generate novel compounds with enhanced efficacy and selectivity [111, 112]. In the field of bone tumor therapy, AI can leverage molecular dynamics (MD) simulations and free energy calculations to precisely analyze the binding affinity and interaction mechanisms between anti-cancer drugs and bone matrix proteins. By integrating GNNs and DRL, AI can predict the targeted distribution of small-molecule compounds within the bone microenvironment and optimize their chemical structures to enhance bone affinity and improve drug selectivity [113]. Furthermore, AI-driven *in silico* screening can incorporate pharmacokinetic (PK) and pharmacodynamic (PD) parameters to model drug retention time and metabolic pathways within bone tissues, thereby optimizing dosage regimens and delivery strategies. This computational approach not only enhances the targeted delivery efficiency of anti-bone tumor agents but also minimizes their accumulation in non-target tissues, reducing systemic toxicity and providing a novel AI-driven strategy for precision therapy in bone metastases and primary bone tumors (Fig. 4).

Looking ahead, AI is poised to play a central role in bone organoid research and drug development, driving advancements in disease modeling, drug screening, and personalized therapies. With the integration of ST, automated cell culture systems, and real-time imaging analysis, AI can consolidate multi-dimensional datasets to enable dynamic monitoring and precise regulation of bone organoids [114,115]. For example, CV coupled with DL can assess organoid morphology and physiological states in real time, automatically identifying structural alterations induced by drug interventions. RL can further optimize culture conditions to facilitate intelligent decision-making in drug screening. The convergence of these technologies will not only enhance

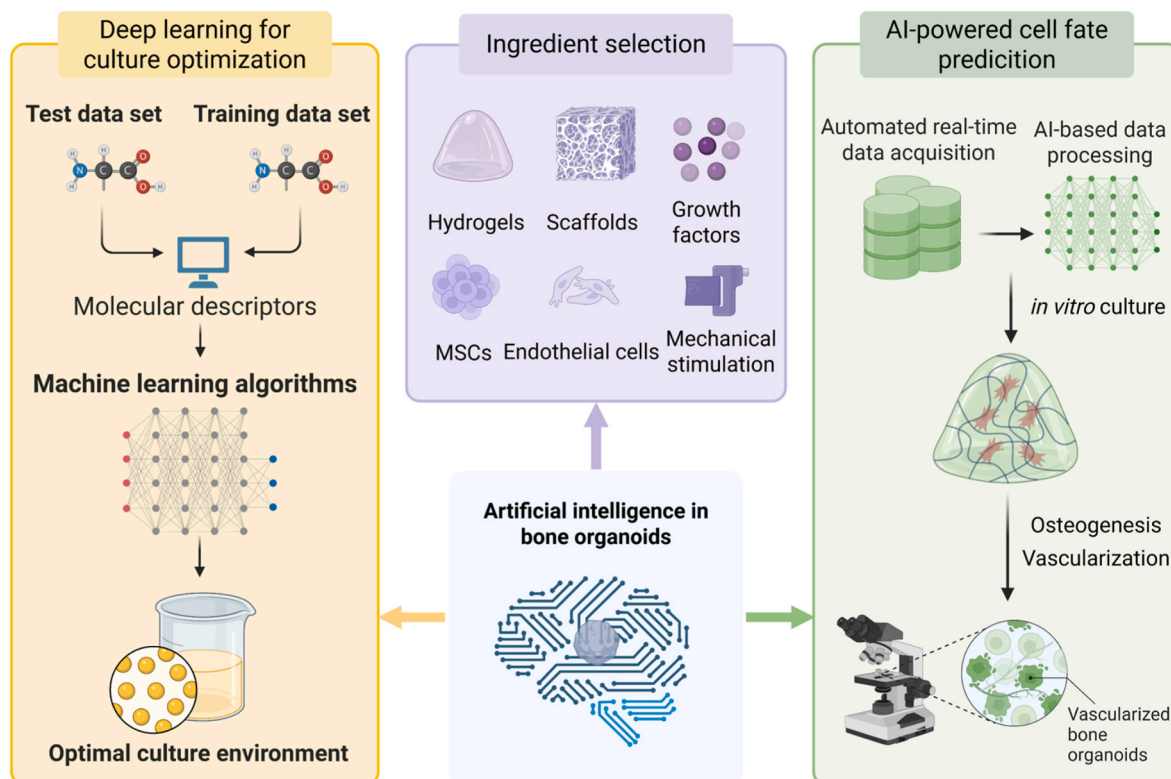


Fig. 4. Applications of AI in bone organoid construction.

the physiological relevance of bone organoids but also accelerate the transition from fundamental research to clinical applications, offering novel strategies for bone regenerative medicine and disease treatment.

4. Assembloids enhance physiological complexity of bone organoids

4.1. Overview of assembloids

4.1.1. Introduction to assembloids

Assembloids, as an advanced 3D cell culture model, enable the precise spatial organization of multiple cell types or organoids, allowing for a more accurate recapitulation of the structural and functional complexity of native tissues, thereby providing a sophisticated platform for investigating intricate biological processes with greater depth and precision [116,117]. Compared with conventional single-type organoid models, assembloids achieve a higher level of physiological complexity in bone organoids by precisely controlling the spatial arrangement and interactions of multiple organoid types or functionally heterogeneous cell populations. Pasca et al. developed 3D cortico-motor assembloids through integrating cortical, spinal, and muscle organoids, successfully reconstructing a human cortico-spinal-muscle multi-synaptic circuit *in vitro* [118]. This system remains stable over long-term culture and provides a physiologically relevant platform for studying motor circuit development, disease mechanisms, and therapeutic interventions for neuromuscular disorders.

The approach via assembloids, holds promise for effectively reconstructing the intricate and dynamic intercellular and intertissue networks of native bone tissue, not only recapitulating the localized

structural features of individual tissues but also extending to the coordinated interactions and functional integration among multiple tissues. This advanced bioengineering strategy effectively overcomes the limitations of conventional organoid culture systems, including restricted cellular diversity, disorganized spatial architecture, and inadequate functional maintenance [119]. Moreover, through the refinement of the cellular microenvironment, this strategy facilitates efficient nutrient diffusion and metabolic waste clearance, thereby alleviating prevalent issues such as cell necrosis in traditional organoid cultures and ultimately enhancing the functional integrity and long-term stability of organoid systems.

4.1.2. Advantages of assembloids in bone organoid research

Owing to their superior capability in enhancing model complexity and functional maturation, assembloids have emerged as a powerful platform for deciphering organ developmental mechanisms, enabling precise disease modeling, and advancing regenerative medicine research. By precisely orchestrating the spatial arrangement of diverse cell types, assembloids establish a standardized and functionally integrated 3D cellular network, thereby faithfully recapitulating the complex and dynamic interactions between cells and between cells and the ECM within native bone tissue [120]. Furthermore, via incorporating endothelial or vascular cells and establishing a physiologically functional vascular-like network, assembloids enhance nutrient diffusion and promote efficient metabolic waste clearance, which significantly improves cell viability and maintains functional stability in long-term bone organoid cultures.

Additionally, assembloids integrate immune cells to establish a finely tuned local immune microenvironment, enabling a more comprehensive

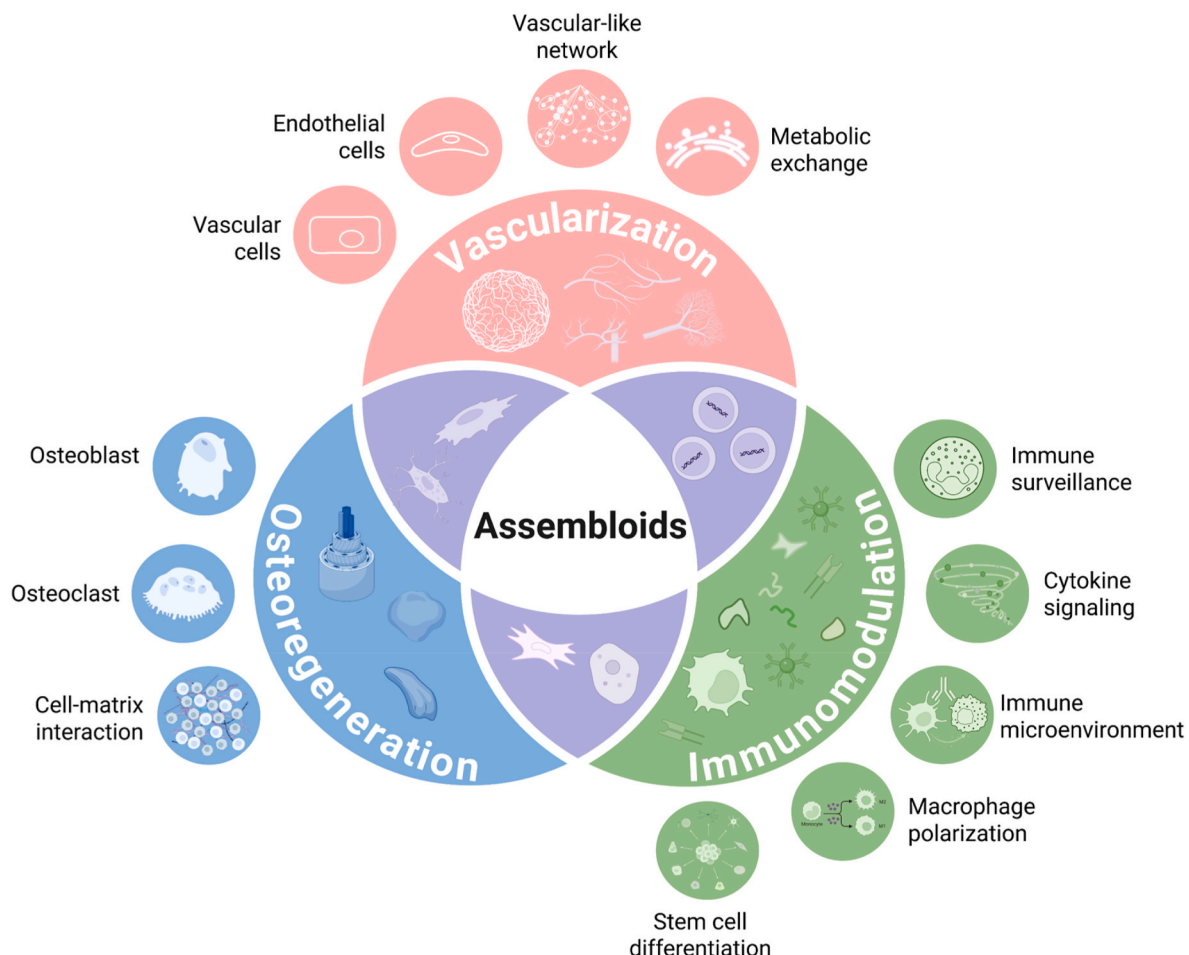


Fig. 5. Multifaceted functional advantages of assembloids in bone organoids.

and in-depth simulation of the immunoregulatory properties of native bone tissue and further enhancing the physiological relevance of the model (Fig. 5). In summary, assembloids demonstrate significant potential in bone organoid research by leveraging biomimetic structural design, precise multicellular spatial organization, and the construction of a finely regulated immune microenvironment, offering new opportunities to drive groundbreaking advancements and expand the frontiers of this field.

4.2. Future perspectives of assembloids in bone organoid research

4.2.1. Integrating biophysical and biochemical cues into bone assembloids

Despite the remarkable advantages of assembloids in bone organoid construction, several challenges remain, including incomplete cellular differentiation, insufficient tissue maturation, and limited *in vitro* long-term culture stability. Bone organoids formed solely through self-organization of cells often fail to accurately recapitulate the *in vivo* biomechanical environment, ECM architecture, and biochemical signaling of native bone tissue, thereby restricting their applicability in bone development research, disease modeling, and regenerative medicine. To address these limitations, integrating biophysical stimulation, functional biomaterials, and growth factor delivery into the assembloid microenvironment has emerged as a critical strategy for enhancing bone organoid stability, maturation, and physiological functionality [121]. These external regulatory approaches not only enhance the osteoinductive capacity of assembloids by promoting cell proliferation, differentiation, and matrix mineralization but also maintain long-term structural stability and dynamically modulate the bone tissue microenvironment, enabling a more precise simulation of physiological and pathological bone states.

Biomechanical signaling serves as a fundamental regulatory mechanism in bone tissue development and remodeling, orchestrating cellular differentiation, ECM synthesis, and the maintenance of bone homeostasis [122]. Osteocytes are continuously exposed to mechanical stress *in vivo*, and such stimuli regulate critical biological processes that drive tissue adaptation and reconstruction. However, conventional organoid culture systems lack physiologically relevant biomechanical microenvironments, failing to accurately recapitulate the mechanosensitive responses of bone tissue and thereby limiting their applicability in mechanobiology research and bone disease modeling. To address this limitation, the incorporation of dynamic fluid shear stress, cyclic tensile strain, and low-frequency vibration can effectively activate mechanotransduction pathways, enhance osteogenic differentiation, promote ECM mineralization, and sustain the long-term structural stability of bone organoids. Furthermore, the integration of bioreactors can further refine the functional properties of engineered bone models, improving their physiological relevance and enabling more precise simulation of *in vivo* mechanical cues.

The bone tissue microenvironment is primarily composed of highly specialized ECM, which plays a crucial role in maintaining structural stability, regulating cellular behavior, and mediating signal transduction [123]. However, conventional organoid culture systems often lack appropriate ECM support, leading to the collapse of 3D structures, loss of cell polarity, and impaired signal transmission. To effectively alleviate these challenges, the incorporation of functional biomaterials, such as biomimetic scaffolds, nanocomposite materials, and hydrogel-based carriers, provides essential mechanical support for bone organoid construction while enabling surface modification with bioactive moieties to enhance cell adhesion, promote differentiation, and optimize matrix synthesis. For instance, nanofibrous scaffolds engineered via biomimetic mineralization can precisely replicate the mechanical properties of native bone ECM, thereby enhancing osteogenic cell proliferation and differentiation. Additionally, smart hydrogels loaded with growth factors or ECM proteins enable the spatially and temporally controlled release of bioactive signals, thereby refining the dynamic microenvironment of organoids.

Growth factors are critical signaling molecules that regulate bone tissue differentiation and remodeling, playing an essential role in maintaining bone homeostasis and promoting regeneration [124]. Nevertheless, conventional bone organoid culture systems lack dynamic and controllable growth factor regulation mechanisms, resulting in asynchronous cell differentiation, limited matrix mineralization, and insufficient microenvironmental modulation, which compromise the physiological relevance of the model. The development of precisely controlled growth factor delivery systems, such as biodegradable microspheres, nanocarriers, and programmable hydrogels, enables the spatiotemporal release of osteoinductive factors, including BMP-2, TGF- β , and VEGF-A. This approach enhances osteogenic differentiation and vascularization in bone organoids, providing a more physiologically relevant *in vitro* platform to model the developmental and reparative processes of native bone tissue.

Assembloids represent a cutting-edge platform for bone organoid research, offering unprecedented opportunities to model bone development, disease progression, and regenerative processes [125]. Assembloids integrate biomechanical stimulation, functional biomaterials, and controlled growth factor delivery to enhance the physiological relevance of bone organoid models. Biomechanical cues regulate mechanotransduction pathways essential for osteogenesis, while biomimetic scaffolds and engineered hydrogels provide mechanical support and biochemical signaling, promoting cell adhesion, differentiation, and matrix mineralization. Also, precisely tuned growth factor delivery systems enable spatiotemporal control over osteoinductive signaling, optimizing bone organoid function and vascularization. These advancements collectively contribute to a more biomimetic and functionally robust bone organoid system, facilitating applications in bone biology research and regenerative medicine.

4.2.2. Advancing the applications of assembloids in bone disease modeling

Assembloids, with their highly biomimetic 3D architecture, precise multicellular spatial organization, and dynamically regulated microenvironment, offer distinct advantages in faithfully recapitulating the complex physiological characteristics of bone tissue *in vitro*. This approach provides a novel strategy for the precise construction of bone disease models. The onset and progression of bone diseases involve intricate intercellular signaling, dynamic regulation of the inflammatory microenvironment, and disruptions in bone remodeling mediated by osteoblasts and osteoclasts [126]. However, conventional *in vitro* models exhibit limitations in cellular heterogeneity, tissue architecture, and functional maintenance, making it challenging to accurately replicate the pathophysiological processes of bone diseases. Therefore, the development of high-fidelity and reproducible *in vitro* bone disease models is of significant practical importance for elucidating disease mechanisms, identifying potential therapeutic targets, and advancing personalized precision medicine. Via leveraging multicellular co-culture systems, controlled microenvironmental remodeling, and optimized cell–matrix interactions, assembloids enable a more comprehensive reproduction of key pathological features of bone diseases, effectively addressing the shortcomings of traditional *in vitro* models. This approach provides an advanced bone organoid platform for preclinical research, precision drug screening, and personalized therapeutic strategies while reinforcing its applicability for clinical translation [127].

In the construction of osteoporosis models, osteoporosis is a systemic metabolic bone disorder characterized by an imbalance between bone resorption and bone formation, leading to decreased bone mineral density, deterioration of bone microarchitecture, and increased bone fragility. The onset and progression of osteoporosis involve dysregulated osteoblast and osteoclast activity, as well as an altered bone matrix microenvironment, ultimately resulting in excessive osteoclast-mediated bone resorption surpassing osteoblast-mediated bone formation and disrupting the dynamic equilibrium of bone remodeling [128]. Nevertheless, conventional *in vitro* models have inherent limitations in cellular heterogeneity, tissue architecture, and dynamic

microenvironmental regulation, making it challenging to accurately recapitulate the pathophysiological mechanisms of osteoporosis. Assembloids enable the construction of highly biomimetic pathological bone organoid models by integrating osteoblasts, osteoclasts, adipocytes, macrophages, and matrix-producing cells through multicellular co-culture strategies, combined with biomechanical stimulation and cytokine modulation. In this model, reduced mechanical loading suppresses osteoblast differentiation, while an elevated RANKL/OPG ratio and increased levels of pro-inflammatory cytokines enhance osteoclast activation, leading to excessive bone resorption [129]. This disruption in bone homeostasis induces progressive bone mass loss and microstructural deterioration, faithfully recapitulating the core pathological features of osteoporosis. The assembloid-based osteoporosis model provides a physiologically relevant *in vitro* platform for investigating bone metabolism disorders, screening novel therapeutics targeting bone remodeling, and evaluating biomaterials for bone loss repair, ultimately advancing personalized precision strategies for osteoporosis treatment.

For the development of OA models, OA is a chronic degenerative joint disease characterized by cartilage degeneration, synovial inflammation, and alterations in the subchondral bone [130]. Its pathogenesis involves complex interactions among chondrocytes, synoviocytes, immune cells, and the ECM, ultimately leading to cartilage degradation, activation of pro-inflammatory cytokines, and structural abnormalities at the osteochondral interface [131]. Conventional *in vitro* models fail to accurately recapitulate these multilayered pathological processes. Assembloids, through employing multicellular co-culture strategies, precisely integrates chondrocytes, synoviocytes, and bone marrow-derived mesenchymal stem cells (BMSCs) while incorporating pro-inflammatory cytokine regulation, mechanical stress stimulation, and ECM remodeling to establish a pathologically relevant OA organoid model [16]. This system enables the controlled modulation of TNF- α and IL-1 β levels, activating synoviocytes and upregulating the expression of matrix metalloproteinases such as MMP-13, thereby promoting cartilage degradation. Additionally, it replicates mechanical damage and cellular stress responses observed in OA progression. Furthermore, the inclusion of subchondral bone-derived cells facilitates the dynamic reconstruction of the osteochondral interface, further enhancing the pathophysiological relevance of the model. This assembloid-based platform not only provides a dynamic multicellular system for evaluating the role of the inflammatory microenvironment in OA progression but also enables the investigation of intercellular interactions within the joint. The OA assembloid model serves as a physiologically relevant *in vitro* system for elucidating cartilage degradation mechanisms, screening novel anti-inflammatory and chondroprotective therapies, and offering a reliable experimental framework for precision-targeted OA interventions.

Assembloids provide a highly biomimetic and physiologically relevant *in vitro* platform for constructing bone disease models, overcoming the limitations of conventional *in vitro* systems through precise multicellular spatial organization, dynamic microenvironmental regulation, and controlled biomechanical stimulation [132]. This approach enables the accurate recapitulation of key pathological features of osteoporosis and OA, thereby facilitating disease mechanism exploration, accelerating high-throughput drug screening, and offering an advanced tool for regenerative medicine. Looking ahead, the integration of bone organoids with assembloids will further enhance the physiological fidelity of models, drive advancements in BTE, disease modeling, and personalized therapeutic strategies, and accelerate the clinical translation of bone disease research.

5. Gene editing technology facilitates precision modeling of bone organoids

5.1. Overview of gene editing technology

5.1.1. Fundamental principles and advancements of gene editing

As a powerful molecular biotechnology, gene editing enables precise manipulation of genetic information by targeting and modifying specific genomic sequences, with broad applications in functional genomics, disease modeling, and genetic disorder therapy. The core mechanism of gene editing relies on engineered nuclease systems that recognize specific DNA sequences and induce double-strand breaks (DSBs), which are subsequently repaired via either non-homologous end joining (NHEJ) or homology-directed repair (HDR) to achieve gene knockout, insertion, or replacement. Since the emergence of recombinant DNA technology in the 1970s, gene editing techniques have undergone three major generations of innovation, transitioning from zinc finger nucleases (ZFNs) to transcription activator-like effector nucleases (TALENs) and ultimately to the CRISPR-Cas9 system [133]. Both ZFNs and TALENs rely on sequence-specific protein-DNA interactions, which improve site-specific editing precision but suffer from high design complexity and complicated operation, limiting their widespread adoption. In contrast, CRISPR-Cas9 has revolutionized the gene editing landscape with its ease of design, low cost, and high editing efficiency, emerging as the predominant genome-editing tool and demonstrating broad applications in biomedical research, gene therapy, drug development, and regenerative medicine.

5.1.2. Core advantages and multifaceted applications of CRISPR-Cas9

CRISPR-Cas9, as the most representative gene-editing tool to date, exhibits exceptional advantages in precise genome manipulation due to its high efficiency, precision, and modularity. The CRISPR-Cas9 system consists of Cas9 nuclease and single-guide RNA (sgRNA), where Cas9 induces DSBs at target DNA sequences, while sgRNA facilitates sequence-specific recognition through complementary base pairing, enabling highly precise gene modifications. Compared with conventional ZFNs and TALENs technologies, CRISPR-Cas9 offers superior target specificity, reduced off-target effects, and enhanced scalability, with an editing cycle reduced to less than two weeks and significantly lower costs, greatly improving the feasibility and applicability of gene editing [134]. A key advantage of CRISPR-Cas9 lies in its modular design, wherein the nuclease activity of Cas9 and the targeting specificity of sgRNA are functionally independent. Through simply altering the sgRNA sequence, the system can be rapidly reprogrammed to target different genomic loci, providing unparalleled editing flexibility. This technology not only overcomes the cumbersome design limitations of earlier gene editing approaches but also facilitates high-throughput genome-wide screening, offering a transformative platform for functional genomics research and the mechanistic investigation of genetic diseases [135].

Owing to its exceptional editing efficiency and high programmability, CRISPR-Cas9 has been widely applied across various fields, including genetic disease research, gene therapy, drug development, and regenerative medicine, driving advancements in precision medicine. In genetic disease research, CRISPR-Cas9 has been employed to construct disease models, enabling the elucidation of molecular mechanisms underlying genetic disorders and facilitating gene correction for monogenic diseases such as β -thalassemia. In drug development, this technology has been integrated into genome-wide HTS to identify potential therapeutic targets, thereby significantly enhancing the precision of drug discovery. Farin et al. developed an organoid-based CRISPR-Cas9 pooled screening platform, enabling high-throughput gene editing in human colorectal organoids to systematically identify tumor driver genes and assess their clonal selection advantages [136]. The efficacy of this screening strategy was validated in both *in vitro* 3D cultures and *in vivo* xenograft models, establishing the feasibility of utilizing organoids

for precise functional genomic studies in oncology. Furthermore, in regenerative medicine, CRISPR-Cas9 facilitates the optimization of stem cell genome editing strategies, enhancing their differentiation potential and improving tissue repair and functional reconstruction. Huangfu et al. conducted genome-wide CRISPR-Cas9 screening to systematically dissect the genetic regulatory network governing human pluripotent stem cell self-renewal, revealing that pluripotency maintenance and cellular adaptability are mediated by distinct genetic modules [137]. They identified key chromatin regulators and metabolism-related genes, including the novel regulator OTUD5, providing a critical theoretical foundation for the application of gene editing in stem cell fate regulation and regenerative medicine. As gene editing technology continues to integrate with organoid engineering, CRISPR-Cas9 is emerging as a pivotal tool in organoid research, shaping the future of precision medicine.

5.1.3. Applications of gene editing in organoid research

The rapid advancement of gene editing technologies has endowed organoid research with unprecedented precision in genetic manipulation, enhancing their applicability in modeling organ development, deciphering disease mechanisms, and screening personalized therapeutic strategies. Compared to conventional 2D cell cultures or animal models, organoids more closely recapitulate *in vivo* physiological conditions. However, despite overcoming several limitations of traditional models, organoids still face challenges in genetic stability, pathological mutation modeling, and cell behavior analysis due to intrinsic variability in gene expression. The integration of gene editing has significantly improved the genetic tractability of organoid models, enabling precise manipulation at both single-cell and tissue levels. In tumor organoid research, gene editing facilitates the simulation of driver mutations or oncogene activation, allowing for the construction of personalized cancer models, in-depth elucidation of tumorigenesis, and assessment of targeted therapeutic strategies. A study by Clever and colleagues demonstrated that CRISPR-Cas9-mediated base editing enables the efficient generation of complex tumor models in adult stem cell-derived organoids, achieving precise mutagenesis of specific oncogenes [138]. This approach has been further validated in liver, endometrial, and colorectal organoids, underscoring its broad applicability in large-scale tumor modeling and precision oncology drug screening.

Additionally, in neural organoid research, gene editing has been employed to investigate the genetic basis and pathogenesis of neurodegenerative diseases such as Alzheimer's and Parkinson's disease, facilitating the identification of potential therapeutic targets. Artegiani et al. reported that human fetal brain tissue can self-organize into organoids (FeBOs) *in vitro* while maintaining cellular heterogeneity and regional specificity during long-term expansion, thereby establishing a novel central nervous system organoid platform [139]. Their study revealed that FeBO growth relies on tissue integrity and a specialized ECM microenvironment and leveraged CRISPR-Cas9-mediated gene editing to generate syngeneic mutant FeBO lines, providing an essential *in vitro* model for studying brain development, neurological disorders, and brain tumor biology. Furthermore, in organoids derived from the intestine, lungs, and kidneys, gene editing has been applied to model genetic disorders such as cystic fibrosis and renal tubulopathy, enabling the functional validation of disease-associated mutations and the exploration of gene therapy strategies. With the integration of single-cell sequencing, ST and HTS technologies, gene editing applications in organoid research will further propel the advancement of precision medicine, offering more refined and efficient *in vitro* models for disease modeling and personalized therapeutic development.

5.2. Applications of gene editing technology in bone organoid construction

5.2.1. CRISPR-Cas9-mediated gene regulation for enhancing bone organoid construction and development

CRISPR-Cas9-mediated targeted gene editing offers a revolutionary

strategy for constructing functionalized bone organoids by orchestrating osteogenic differentiation, ECM remodeling, and vascularization. Traditional bone organoid culture systems are hindered by low osteogenic efficiency, inadequate ECM mechanical properties, and the absence of vascular networks. CRISPR-Cas9 can specifically activate key osteogenic genes such as RUNX2, SP7 (Osterix), and BMP-2, significantly enhancing the osteogenic differentiation potential of MSCs and promoting bone matrix mineralization [140]. Concurrent regulation of COL1A1 and SPARC expression optimizes ECM topology and elastic modulus, thereby mimicking the biomechanical environment of native bone tissue. Additionally, editing pro-angiogenic factors such as VEGF-A and PDGFB facilitates the formation of functional microvascular networks by endothelial cells, thereby improving metabolic exchange within bone organoids.

Also, CRISPR-Cas9 can be integrated with epigenetic regulation strategies, such as DNA methylation modification, to achieve spatiotemporal coordination of osteogenic and angiogenic signaling pathways, ultimately enhancing the long-term stability of bone organoids [141]. Moreover, gene editing can be coupled with biomaterial-based and dynamic culture systems, such as engineering biomimetic scaffolds with osteogenic and angiogenic gene expression vectors, to further augment the structural maturation and functional fidelity of bone organoids. Collectively, this multi-scale integration strategy not only redefines bone organoid engineering paradigms but also optimizes the synergy between osteogenesis and angiogenesis, thereby enhancing physiological relevance and providing a robust foundation for bone regeneration research, disease modeling, and clinical translation.

5.2.2. Gene editing-driven personalized and disease-specific bone organoid engineering

Breakthroughs in gene editing technology have provided a fundamental driving force for constructing genetically defined and pathologically traceable disease-specific bone organoid models, significantly advancing research in bone disease mechanisms and precision intervention. For instance, Dai et al. demonstrated the gene editing of COL1A1 modulates Hif-1 α -mediated glycolytic metabolism, effectively reversing chondrocyte senescence and cartilage degeneration, thereby advancing the therapeutic potential of organoids for degenerative joint diseases [142]. Additionally, in the case of the monogenic disorder osteogenesis imperfecta (OI), characterized by pathogenic mutations in COL1A1/COL1A2 that disrupt type I collagen synthesis and impair bone matrix mineralization, CRISPR-Cas9 enables the introduction of patient-specific mutations into normal stem cells to generate OI organoid models that closely recapitulate clinical phenotypes. These models dynamically replicate key pathological features, including impaired osteoblast differentiation, abnormal collagen fiber organization, and increased bone fragility, thereby providing an effective platform for screening therapeutic agents targeting collagen post-translational modifications or mineralization pathways.

Beyond monogenic disorders, gene editing has also been extended to polygenic and multifactorial bone diseases such as osteoporosis, where precise modulation of the RANKL/OPG signaling axis enables the reconstruction of osteoclast-driven bone resorption and impaired osteoblast-osteoclast coupling within organoid models [143]. This approach enables the reconstruction of core pathological features of osteoporosis and, when combined with high-throughput sequencing and biomechanical analysis, facilitates the dynamic interrogation of molecular regulatory networks underlying bone metabolism, ultimately supporting the development of novel anti-resorptive and osteoanabolic therapeutics.

Additionally, CRISPR-Cas9 technology has been leveraged to engineer immune-compatible bone organoids by editing HLA genes to mitigate immune rejection, thereby enhancing their potential for personalized regenerative medicine. With the advent of single-base editing and epigenome-targeting approaches, disease modeling has progressed from single-gene correction to multidimensional regulation,

offering deeper insights into the heterogeneous mechanisms of bone disorders. This technological advancement paves the way for developing cross-scale precision diagnostics and therapeutic evaluation systems, providing an innovative paradigm for the individualized treatment of bone-related diseases and translational medicine research.

5.3. Applications of gene editing technology in bone disease research and drug development

5.3.1. Gene editing-driven mechanistic studies in bone organoid models

The construction of bone organoids not only serves as a model for simulating physiological bone development but also provides a powerful platform for elucidating the pathological mechanisms underlying bone diseases [144]. Bone formation and disease progression are tightly regulated by the dynamic balance between osteogenesis, osteoclastogenesis, and ECM remodeling, processes governed by key signaling pathways such as Wnt/ β -catenin, BMP/TGF- β , Notch, and Hedgehog signaling pathway. Nevertheless, traditional research approaches face challenges in selectively modulating these pathways at the tissue level and dissecting their roles in bone pathophysiology. CRISPR-Cas9-mediated genome editing enables precise genetic interventions, including targeted knockout, gene activation, and point mutations, allowing for a mechanistic understanding of bone metabolic disorders, genetic bone diseases, and bone defect repair.

In osteoporosis research, disruption of the equilibrium between osteoclast-mediated bone resorption and osteoblast-mediated bone formation represents the central pathological feature [145]. CRISPR-Cas9-based gene editing enables precise modulation of the RANKL/OPG signaling axis in bone organoids, facilitating the reconstruction of osteoclast-osteoblast coupling and dynamic modeling of osteoporosis pathogenesis. Through selectively enhancing RANKL expression to promote osteoclast differentiation or upregulating OPG to antagonize bone resorption, this approach provides a high-fidelity *in vitro* system for dissecting the molecular cascades of bone resorption imbalance and identifying therapeutic targets for anti-osteoporotic strategies. Similarly, in osteosclerosis research, hyperactive osteoblast function and excessive ECM accumulation, commonly driven by mutations in COL1A1/COL1A2, SOST, or LRP5, lead to disorganized type I collagen fibrils and abnormal bone mineralization [146]. Introducing specific pathogenic mutations in bone organoids via CRISPR-Cas9 enables the faithful recapitulation of osteosclerosis-associated genetic and pathological features while providing a platform to investigate the roles of Wnt/ β -catenin and BMP/TGF- β signaling in pathological bone formation. Furthermore, targeting SOST and DKK1 within these models facilitates the development of therapeutic interventions aimed at suppressing aberrant osteogenesis.

Beyond bone remodeling disorders, vascular dysfunction is a critical factor in many bone pathologies, including osteonecrosis and diabetes-associated bone defects [147]. Key angiogenic regulators such as VEGF-A, FGF2, and ANGPT1 play pivotal roles in bone-vascular interactions. By precisely modulating these genes using CRISPR-Cas9, vascularized bone organoids with disease-relevant characteristics can be generated, enabling the study of how impaired blood supply and oxygenation affect bone regeneration while identifying novel targets for therapeutic intervention in vascular-associated bone diseases. Collectively, the integration of genome editing technologies with bone organoid models enhances their physiological relevance and establishes them as an indispensable tool for investigating bone disease mechanisms, ultimately advancing precision medicine and translational research.

5.3.2. Advancing drug screening applications of bone organoids through gene editing

The introduction of gene editing technology has significantly enhanced the precision and reproducibility of bone organoids in drug screening, making them a highly relevant *in vitro* model for osteoporosis

treatment, anti-inflammatory bone therapies, and bone repair materials [148]. Traditional drug screening for bone diseases has relied heavily on 2D cell cultures or animal models. However, 2D cultures fail to recapitulate the sophisticated 3D bone microenvironment, while species-specific differences in animal models limit their translational reliability. CRISPR-Cas9-mediated precise gene regulation enables the construction of genetically controllable bone organoids carrying disease-specific mutations, thereby faithfully recapitulating the core pathological features of osteoporosis and OA [149–151]. Targeted modulation of the RANKL/OPG axis using CRISPR-Cas9 allows dynamic reconstruction of bone homeostasis imbalances in bone organoids, facilitating the screening of novel anti-resorptive and osteoanabolic drugs. Furthermore, in OA research, gene knockouts or activations targeting inflammatory mediators such as MMP-13, IL-1 β , and TNF- α can precisely model cartilage degradation and joint inflammation, providing an *in vitro* platform for evaluating new anti-inflammatory and chondroprotective therapies. Additionally, CRISPR-based high-throughput genome-wide screening in bone organoids allows the systematic identification of key regulators of bone metabolism, inflammation, and regeneration, accelerating the discovery of innovative therapeutic targets for both common and rare bone diseases and advancing precision medicine applications.

Gene editing also plays a pivotal role in personalized drug screening and precision medicine [152,153]. In the context of genetic bone disorders such as OI and Marfan syndrome, CRISPR-Cas9 enables the development of patient-specific bone organoids carrying precise pathogenic mutations, allowing for the evaluation of gene correction strategies and bone matrix repair therapies while assessing their clinical feasibility. Marfan syndrome, a connective tissue disorder caused by mutations in the FBN1 gene, is characterized by skeletal abnormalities, excessive long bone growth, and ligament laxity [154,155]. CRISPR-Cas9-mediated gene editing in bone organoids facilitates the recreation of Marfan-associated pathological features, thereby refining targeted interventions for the TGF- β signaling pathway and advancing personalized therapeutic strategies [156]. Moreover, HLA gene knockout can mitigate immune rejection in organoid transplantation, enhancing the compatibility of bone grafts and expanding the application of personalized bone organoids in regenerative medicine and transplantation therapies [151]. Looking ahead, the integration of single-cell sequencing, ST, and AI-driven drug screening is expected to optimize the entire pipeline from target discovery to personalized therapy. Gene editing-empowered bone organoid-based drug screening will not only provide a robust and predictive *in vitro* model for precision medicine in bone diseases but will also accelerate the translation of personalized therapeutic strategies into clinical applications (Fig. 6).

6. Advances in bone organoid research: technological advantages, challenges, and future perspectives

6.1. Opportunities and limitations: technological advancements and challenges in bone organoid development and applications

While these emerging technologies have created unprecedented opportunities for bone organoid research, it is equally significant to recognize their current limitations, which necessitate further refinement and standardization. From a technical perspective, each technology has inherent challenges that must be addressed. In the context of 3D bioprinting, the achievable resolution remains fundamentally constrained by nozzle size and bioink properties, posing significant challenges in recapitulating the intricate microscale architecture of native bone tissue with high fidelity [157,158]. The simultaneous bioprinting of multiple cell types and their long-term viability remain significant challenges, as different cells exhibit varying tolerance to the printing process and culture conditions, necessitating further optimization to maintain their survival and functionality. Moreover, the selection of biomaterials for bioprinting remains limited, as achieving an optimal balance between

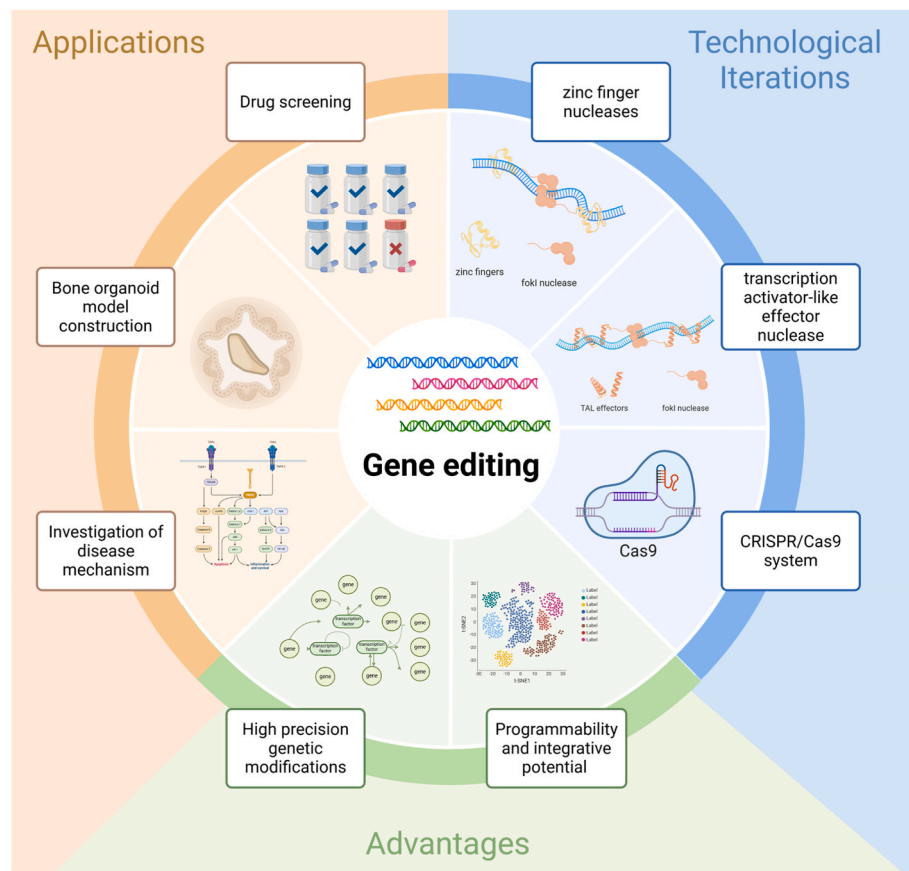


Fig. 6. Advancements, advantages, and applications of gene editing technology.

mechanical strength and biodegradability is a complex task. In the field of AI, challenges related to data availability and algorithmic limitations pose significant hurdles. The effective training of DL models relies on extensive high-quality datasets, yet the field of bone organoids currently lacks standardized, large-scale datasets [159]. Variability in cell sources and culture methodologies across different laboratories results in inconsistencies in organoid data, which can introduce bias when integrated for AI model training. Furthermore, many AI-based decision-making processes function as “black boxes”, making it difficult to directly interpret how predictions are generated. This lack of transparency may undermine confidence in AI-driven conclusions within scientific research. To address these issues, the development of AI algorithms specifically tailored for organoid applications is essential, along with the integration of explainable AI approaches to enhance the interpretability and reliability of computational predictions.

Although assembloid hold great promise, challenges remain in reproducibility and controllability. Bone organoids from different batches may exhibit variations during fusion, leading to structural and functional inconsistencies, which complicates experimental reproducibility. Additionally, the fusion of two or more organoids currently relies primarily on self-organization, limiting the ability of researchers to control the spatial configuration and cellular distribution at the fusion interface. This variability can result in structural heterogeneity among assembloids. Therefore, strategies such as microfabricated scaffolds, external magnetic fields, or mechanical forces need to be developed to guide organoid fusion in a controlled manner, ensuring the formation of standardized structures. Similarly, the application of gene editing in organoids presents certain limitations. A major concern is the potential introduction of off-target mutations or DNA damage, which may compromise organoid stability and functionality [160]. Moreover, when editing human-derived cells, clonal selection and expansion are often

required, prolonging the experimental timeline and increasing the risk of phenotypic drift. In multicellular organoids, post-maturation gene editing can lead to mosaicism, where cells exhibit different degrees of genetic modification, thereby complicating data interpretation. Although gene editing serves as a powerful tool, careful consideration must be given to the timing and methodology to ensure clear and reproducible phenotypic outcomes at the organoid level.

From the perspective of bone organoids themselves, current models still lack full maturation. For instance, their degree of mineralization often does not reach that of adult bone, and their bone density and mechanical properties remain suboptimal. Furthermore, bone organoids generated in different laboratories exhibit considerable variability in cellular composition and phenotypic characteristics, lacking standardized evaluation criteria, which limits their broader application [161]. Beyond these technical limitations, ethical and privacy considerations are equally critical [162]. The research and application of bone organoids involve the use of human cells and genetic modifications, necessitating strict adherence to ethical guidelines. Since organoids retain the genomic information of donors, there is a theoretical possibility of inferring an individual genetic disease risk, making the protection of donor privacy particularly important [163]. Additionally, if genetically edited or long-term cultured organoids are intended for therapeutic use, potential risks such as tumorigenicity and pathogen contamination must be thoroughly assessed to ensure their safety. As bone organoid technology advances toward clinical applications, a key priority is the establishment of ethical and legal frameworks that clearly define the responsibilities of researchers, patients, and regulatory authorities. Public education efforts are also essential to clarify that organoids do not equate to fully functional human organs, thereby mitigating unrealistic expectations and ethical concerns. In conclusion, the development of bone organoid technology must integrate ethical regulations and

privacy protections alongside scientific progress, ensuring the legitimacy and integrity of both research and application. Addressing these concerns will be instrumental in gaining public trust and establishing a solid foundation for the translation of bone organoids from laboratory research to clinical practice.

6.2. Future directions: interdisciplinary integration and clinical translation potential

Looking ahead, the development of bone organoids will increasingly integrate multidisciplinary technologies, advancing toward the construction of more complex and physiologically relevant “*ex vivo* skeletal systems”. The convergence of multiple technologies will become a prevailing trend. The previously discussed approaches, including bioprinting, AI, assembloids, and gene editing, are not independent of one another. Future bone organoid fabrication is likely to involve the combined application of these methods. For instance, an envisioned workflow may involve AI algorithms optimizing the selection and timing of differentiation factors based on extensive literature and experimental data. Subsequently, 3D bioprinting could be used to spatially arrange MSCs, endothelial cells, and biomaterial scaffolds into a pre-organized bone-like structure. This construct would then be cultured in a bioreactor under mechanical stimulation to enhance maturation. During this process, gene editing techniques could introduce fluorescent reporter genes to enable real-time monitoring of osteogenesis and vascularization [164]. These interdisciplinary intersections provide a promising roadmap for the next generation of functional bone organoid systems.

If required, bone organoids may be further integrated with bone marrow or cartilage organoids to generate complex constructs encompassing mineralized bone matrix, vascular networks, and hematopoietic functionality. This multidisciplinary synergy will overcome the limitations of single-modality approaches, enabling the *in vitro* fabrication of bone tissues with architecture and functionality more closely resembling native bone. Moreover, such integration is expected to catalyze new technological advances, including organoid-on-a-chip systems. These platforms incorporate bone organoids into microfluidic devices that precisely regulate fluid dynamics and chemical gradients, thereby replicating the perfusion and nutrient delivery of native bone. They can also be interconnected with other organoid-on-a-chip models, such as muscle organoids, to construct multi-organ interaction systems. This “tissue cascade” strategy offers a promising avenue for exploring crosstalk between the skeletal system and systemic metabolism or immune response. Collectively, bone organoid research will advance beyond simple spherical constructs toward highly biomimetic complexes incorporating bone formation, hematopoiesis, nutrient exchange, and mechanotransduction.

Bridging the gap between laboratory research and clinical translation of bone organoids requires extensive exploration and refinement of application models and evaluation systems. Bone organoids hold significant potential as personalized medicine tools for lifelong health monitoring and disease intervention. Bone-related diseases such as osteoporosis, OA, and bone metastases often progress slowly and exhibit substantial inter-individual variability, making it challenging to capture real-time microstructural changes in bone tissue using conventional imaging and biochemical markers. Culturing patient-specific bone organoids derived from their own cells offers a promising approach to predicting individual responses to specific treatments, serving as an *ex vivo* surrogate for personalized therapeutic assessment [165]. Establishing a comprehensive biobank and data-sharing platform for bone organoids, integrating patient-derived organoids and their drug response data, would facilitate organoid-guided precision orthopedics and enhance the accuracy of clinical decision-making for bone disorders. Beyond applications in precision medicine, bone organoids also serve as human-derived *in vitro* testing platforms for evaluating the biocompatibility, osteogenic potential, and safety of orthopedic implants and biomaterials. This approach enables the optimization of material design

before animal experiments, improving clinical predictive accuracy while reducing the reliance on animal models. Also, the clinical translation of bone organoid technology necessitates the development of a standardized evaluation system that defines morphological, molecular, and functional benchmarks while incorporating high-throughput omics analysis to quantify organoid maturation and stability [166–168]. Simultaneously, establishing comprehensive regulatory policies and industry standards is essential to defining the regulatory classification of bone organoids across different applications and formulating standardized guidelines for cell sourcing, culture protocols, quality control, and safety assessment to ensure their reproducibility, safety, and clinical applicability.

In conclusion, bone organoids, as an emerging *in vitro* tissue model, are continuously evolving toward greater complexity and physiological relevance. Driven by interdisciplinary technological integration, they are expected to play an increasingly important role in investigating bone disease mechanisms, screening drugs and biomaterials, advancing personalized medicine, and facilitating tissue regeneration [169]. Achieving this vision requires addressing remaining technical and regulatory challenges. Nevertheless, with the establishment of standardized evaluation systems and regulatory frameworks, research and applications of bone organoids will undoubtedly advance to a new level [170]. In the future, bone organoids are anticipated to be fully integrated into clinical and biomedical research, providing innovative solutions for maintaining skeletal health across all stages of life, from childhood growth and development to age-related bone degeneration.

Ethics approval and consent to participate

This review is based solely on the synthesis and analysis of existing literature and does not involve any experiments with human subjects or animals. Therefore, ethical approval is not required, as no direct participation or consent from patients or healthy individuals is necessary.

Declaration of competing of interest

The authors affirm that no financial interests or personal relationships exist that could have influenced the findings presented in this paper.

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